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Follicular Helper CD4 T Cells (T_{FH})

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Abstract

T cell help to B cells is a fundamental aspect of adaptive immunity and the generation of immunological memory. Follicular helper CD4 T (T_{FH}) cells are the specialized providers of B cell help. T_{FH} cells depend on expression of the master regulator transcription factor Bcl6. Distinguishing features of T_{FH} cells are the expression of CXCR5, PD-1, SAP (SH2D1A), IL-21, and ICOS, among other molecules, and the absence of Blimp-1 (*prdm1*). T_{FH} cells are important for the formation of germinal centers. Once germinal centers are formed, T_{FH} cells are needed to maintain them and to regulate germinal center B cell differentiation into plasma cells and memory B cells. This review covers T_{FH} differentiation, T_{FH} functions, and human T_{FH} cells, discussing recent progress and areas of uncertainty or disagreement in the literature, and it debates the developmental relationship between T_{FH} cells and other CD4 T cell subsets (Th1, Th2, Th17, iTreg).

INTRODUCTION

Follicular helper CD4 T (T_{FH}) cells in humans were initially described in 2000 and 2001, when several groups reported that a large proportion of CD4 T cells in tonsils have a unique phenotype, expressing high levels of CXCR5 (1–3). Tonsils are a secondary lymphoid organ similar to lymph nodes but with constant exposure to antigens via the throat and upper respiratory tract that have, as a result, large and active germinal centers (GCs), which makes tonsils an appealing organ for the study of GCs. GCs are histologically distinct structures that develop within B cell zones (follicles) of secondary lymphoid tissues, and it is within GCs that the interrelated and multifaceted processes of B cell affinity maturation (hypermutation and selection), class switch recombination (CSR), plasma cell differentiation, and memory B cell differentiation predominantly occur. These features make the GC key to protective immunity against many pathogens. GCs depend on CD4 T cells (4–6), and many tonsillar CD4 T cells are located inside GCs (1–3). The chemokine receptor CXCR5 (Bln1) expressed by B cells is required for migration and responsiveness to CXCL13 (formerly B lymphocyte chemoattractant, or BLC) to form follicles (7). CD4 T cells that express CXCR5 can migrate in response to CXCL13 (2, 8) and relocate to follicles. This colocalization of CD4 T cells with B cells is critical for T-B interactions, as T cell receptor–major histocompatibility complex class II (TCR-MHCII) engagement is pivotal to the restriction of cognate B cell help. CD40L and other important surface molecules are crucial components of T cell help to B cells and require direct cell-cell contact.

While the original descriptions of CXCR5⁺ T_{FH} cells were important first steps (1–3), they were insufficient to convincingly establish T_{FH} cells as a full CD4 T cell program, and the scientific literature generally did not acknowledge T_{FH} cells as a distinct differentiation lineage of CD4 T cells at the level of Th1, Th2, Treg, or Th17 cells (9–11). There were multiple reasons for this. First and foremost,

differentiation of CD4 T cells is heavily controlled by a small number of master regulator transcription factors that powerfully determine Th1, Th2, Th17, and iTreg differentiation (Tbet, GATA3, ROR γ t, Foxp3) (12), and there was no known master regulator transcription factor required for T_{FH} differentiation. In addition, it was not sufficiently convincing that T_{FH} cells possess specialized attributes beyond expression of CXCR5. Recently, the identification of Bcl6 as a master regulator of T_{FH} differentiation (13–15), the demonstration of the requirement of T_{FH} cells for B cell help in vivo (13, 15, 16), and the identification of the importance of IL-21 for T_{FH} function (16–20) have now established T_{FH} cells as a distinct CD4 T cell type, and one of great importance for protective immunity. Understanding T_{FH} differentiation and function is of central importance for rational vaccine design, as nearly all licensed human vaccines function on the basis of protective T cell–dependent antibody responses (21). Furthermore, T_{FH} cells can play important roles in common autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (22–24). This review covers recent T_{FH} cell findings in the context of the broader CD4 T cell literature and highlights important areas of uncertainty or disagreement.

T_{FH} DIFFERENTIATION

Bcl6 as Master Regulator of T_{FH} Differentiation

Bcl6 was recently identified as a master regulator of T_{FH} differentiation (13–15). In the absence of Bcl6 (*Bcl6*^{-/-}), T_{FH} differentiation does not occur in vivo (13–15). Differentiation of other CD4 T cell subsets is relatively unaffected by the loss of Bcl6 (13–15). Constitutive expression of Bcl6 drives T_{FH} differentiation in vivo (13). T_{FH} cells express high levels of Bcl6, and non- T_{FH} cells (activated antigen-specific CD4 T cells that are not T_{FH} , i.e., Th1, Th2, Th17, or other subsets) express high levels of Blimp-1 (13, 31–32). Blimp-1 is an antagonist



of Bcl6, and Bcl6 is an antagonist of Blimp-1 (31, 33). CD4 T cells that constitutively express Blimp-1 suppress the expression of Bcl6 and fail to generate T_{FH} cells (13). Importantly, the expression of Blimp-1 does not inhibit differentiation of non- T_{FH} CD4 T cell subsets (13). These findings are best explained as a bimodal fate decision made by activated CD4 T cells to become either $Bcl6^+$ T_{FH} cells or $Blimp-1^+$ non- T_{FH} effector cells (13, 31).

Bcl6 is a transcriptional repressor (31, 34, 35) originally identified as the master regulator of GC B cell differentiation (34). Bcl6 controls GC B cell differentiation by regulating cell cycle genes, regulating DNA damage response genes, and suppressing a host of signaling pathways, including B cell receptor (BCR) signaling (31, 34–36). While Bcl6 is required in both GC B cells and T_{FH} cells, it appears to control T_{FH} differentiation largely by regulating genes separate from those it controls in GC B cells (31). This likely occurs because of the ability of Bcl6 to dimerize with a host of different corepressors through its BTB, RDII, and Zn finger domains (31, 35). Our understanding of the mechanisms by which Bcl6 controls T_{FH} differentiation is currently limited. Bcl6 can bind to thousands of genes in B cells (36, 37). What is currently known in CD4 T cells is that Bcl6 can modulate microRNA expression (15), and Bcl6 can inhibit differentiation of other CD4 T cell subsets (14) (**Figure 1**). Bcl6 can antagonize transcription factors important for Th1 (14, 15), Th2 (14, 41, 42), or Th17 (14, 43) differentiation (**Figure 1**). There is evidence that Bcl6 inhibits murine ROR γ t function but not expression (14), whereas in humans Bcl6 can bind the ROR γ t promoter (15). It is fascinating that Bcl6 interacts with these differentiation pathways at different levels, and it will be interesting to determine the mechanisms for how this regulation occurs. Bcl6 antagonism of Blimp-1 is one key mechanism by which Bcl6 inhibits non- T_{FH} differentiation (13) (**Figure 1**). Blimp-1 directs effector cell differentiation in CD8 T cells (38–40) and non- T_{FH} CD4 T cells (13, 31). By repressing Blimp-1, Bcl6 can limit all non- T_{FH} differentiation.

ON SEMANTICS: NOMENCLATURE AND MARKERS

Differences in nomenclature are common in an active and rapidly moving area of biology. As Keith Yamamoto has wryly observed, “Scientists would rather use each others’ toothbrushes than use each others’ nomenclature.” Although the situation is less dramatic now than it was 20 years ago in the heyday of gene cloning, nomenclature disagreements still occur. Use of different nomenclatures is entirely appropriate while fields mature. What is critical is that differences in nomenclature—differences in semantics—do not obscure the underlying biological processes. In the field of T_{FH} cells, there are currently three or more T_{FH} nomenclatures used, and variations are continuing to evolve. The original T_{FH} nomenclature was used to define all $CXCR5^+$ CD4 T cells as T_{FH} cells and to equate $CXCR5$ expression with germinal center (GC) or follicular localization and B cell help (1, 3). In one current terminology, T_{FH} cells localized in GCs (or presumed to localize in GCs) are referred to as T_{FH} cells. All predecessors of those cells with T_{FH} -like characteristics (including $CXCR5$ expression) are termed pre- T_{FH} cells. In a second terminology, T_{FH} cells localized in GCs are referred to as GC T_{FH} cells, and predecessors of those cells are termed pre-GC T_{FH} cells. In a third terminology, all $CXCR5^+$ $Bcl6^+$ cells are termed T_{FH} cells, and T_{FH} cells localized to GCs are referred to as GC T_{FH} cells. The third terminology is used in this review, given that T_{FH} and GC T_{FH} cells are clearly related [both express $CXCR5$ and $Bcl6$ (25) and appear to be able to interconvert] and are distinguishable from other CD4 T cell subsets. Neither T_{FH} nor GC T_{FH} cells exist in the absence of Bcl6 (13–15). Furthermore, both T_{FH} and GC T_{FH} cells provide enhanced B cell help (25, 26), and B cell help *in vivo* occurs both within GCs and elsewhere in follicles and neighboring regions.

IL-21-producing cells, *in vitro* or *in vivo*, without further characterization are of indeterminate origin and should not be classified as T_{FH} cells without characterization of Bcl6, $CXCR5$, and/or additional T_{FH} differentiation markers. This is because many CD4 T cells that are not T_{FH} cells can express substantial IL-21, including Th17 cells (27–30).

Although Bcl6 can antagonize other CD4 T cell differentiation pathways, this antagonism is partial in many situations, and strong Th1, Th2, or Th17 inductive signals generate T_{FH} cells with dual characteristics. It has been known for years that CD4 T cells within GCs can express



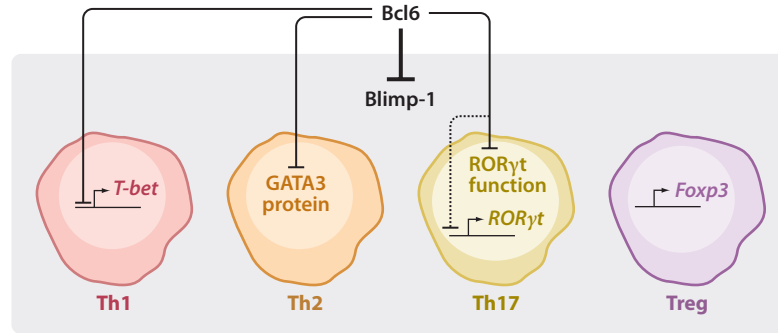


Figure 1

Bcl6 inhibition of other CD4 T cell differentiation pathways. Bcl6 can cause a generalized inhibition of other CD4 T cell differentiation pathways by blocking Blimp-1 expression (13, 31). Bcl6 can bind the *T-bet* gene (15), can inhibit Th2 differentiation without inhibiting GATA3 mRNA expression (41), can inhibit Th17 differentiation by inhibiting ROR γ t activity (14), and can bind the human ROR γ t promoter (15), but Bcl6 does not inhibit ROR γ t expression in murine CD4 T cells (14). Antagonism of Tregs by Bcl6 has not been reported, but gut Tregs can lose Foxp3 and differentiate into Bcl6⁺ T_{FH} cells under inflammatory conditions (67).

canonical cytokines of different CD4 T cell subsets, such as IFN- γ (13, 25, 44, 45) or IL-4 (2, 46) [and more recently IL-17 (47)]. It was also known that CD4 T cells polarized to Th1 or Th2 in vitro could support antibody responses in vivo after transfer (48, 49); however, those experiments suffered from the classic criticisms of polarization experiments: that a small population may have remained unpolarized in vitro and that that population experienced extensive outgrowth after adoptive transfer. Other work demonstrated that T_{FH} differentiation in vivo did not depend on Th2, Th1, or Th17 differentiation pathways and that T_{FH} cells expressed low levels of Th2, Th1, or Th17 cytokines (16). Although that work helped to establish T_{FH} cells as an independent differentiation pathway (16), the lack of IFN- γ - or IL-4-producing T_{FH} cells conflicted with previous work (2, 48, 49) and resulted in a CSR conundrum. It is well accepted that CD4 T cell help to B cells includes the central decision of B cells to class switch to the appropriate Ig isotype for maximal antibody effector functions against the pathogen, be it IgG2a complement fixing and Fc receptor binding in the context of viral infections (50, 51) or IgE in the context of parasite infection (52). Although CSR can occur before GC

development, GCs are major sites for CSR (44, 53). Furthermore, even T-dependent CSR before GC development is primarily T_{FH} dependent (13). One can argue that the cytokines necessary for directing CSR may originate from non-T_{FH} cells, with diffusion of the cytokine through the lymph node or spleen to the antigen-specific B cells in the GC. However, the body of evidence favors focal expression of cytokines for maximal effect in cell-cell interactions (44, 54, 55). Although IFN- γ expression by GC T_{FH} cells is important for murine B cell IgG2a class switching and IL-4 expression by GC T_{FH} cells is important for murine B cell IgG1 class switching, T_{FH} cells may only need to express low levels of these cytokines for induction of CSR during T-B interactions.

More recently, single cell analysis by multiparameter flow cytometry and other experiments demonstrated that murine T_{FH} cells can produce IFN- γ (13, 25, 44, 45), IL-4 (25, 32, 44, 56, 57) [or the more restricted Th2 cytokines, IL-5 and IL-13 (56)], or IL-17 (45, 47). T_{FH} cells responding to an acute viral infection express less IFN- γ than do non-T_{FH} effector CD4 T cells (13), which is consistent with different levels of T-bet expression (13) and with the different roles of IFN- γ produced by these CD4

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T cell types. While there is quantitatively less T-bet or GATA3 in T_{FH} versus non- T_{FH} cells in vivo (13, 25, 44, 56), consistent with the fact that Bcl6 can negatively regulate those differentiation pathways (14), there is not an absolute separation between Bcl6 expression and the expression of other master regulator transcription factors, or between T_{FH} and other CD4 T cell subsets in mice (13, 44). Phenotypic plasticity is also seen in other CD4 T cell differentiation pathways (58–60). These results can be interpreted in multiple ways, as is discussed below.

Our understanding of GC T_{FH} cells that express non- T_{FH} cytokines was refined by recent elegant work of Locksley and colleagues (44), who showed that most IL-4-producing cells in lymph nodes 14–21 days after *Leishmania major* or *Nippostrongylus brasiliensis* infection were GC T_{FH} cells, not canonical Th2 cells. These IL-4-producing cells express high levels of Bcl6, SAP (SLAM-associated protein), CXCR5, ICOS (inducible costimulator), and IL-21 and localize to GCs (44). The IL-4 is functional, as conjugates between IL-4-secreting CD4 T cells and GC B cells undergoing IgG1 CSR are isolated (44). T_{FH} cells' ability to secrete non- T_{FH} cytokines is not limited to IL-4. T_{FH} cells with IFN- γ -producing GC T_{FH} cells were also observed in *L. major* infection (44). IFN- γ -secreting CD4 T cells and GC B cells undergoing IgG2a CSR were observed, confirming that Th1 or Th2 cytokine-secreting CD4 T cells play a role within GCs in instructing GC B cell immunoglobulin CSR (44). This was interpreted as evidence that T_{FH} cells can produce cytokines associated with canonical helper T effector subsets depending on environmental conditions, and those cytokines are critical in mediating the function of T_{FH} cells. The observation that T_{FH} cells produce IL-4 after parasite infection was confirmed by two additional studies, using *Schistosoma mansoni* and *Heligmosomoides polygyrus* (56, 57).

The issue of the T_{FH} cell relationship to other CD4 subsets is further complicated by the finding that IL-4 represents a special case, as GC T_{FH} cells can express IL-4 independently of Th2 characteristics (see the discussion on

IL-4 below in the section entitled T_{FH} Functions) (25). Therefore, although T_{FH} cells can clearly express IFN- γ during immune responses to infections that induce a Th1-biased condition (13, 25, 45) and IFN- γ expression can be used as a surrogate marker of additional Th1 features (e.g., T-bet) (13, 25), IL-4 expression is not a direct demonstration that T_{FH} cells have additional Th2 characteristics. IL-5 or IL-13 in combination with GATA3 is a better direct demonstration, which was done in the context of schistosoma egg-injected mice (56), thereby confirming that the T_{FH} program can coexist with the Th2 program. The capacity of T_{FH} cells to exhibit characteristics of Th2 cells was interpreted differently by different laboratories.

Note that abundance of cytokine mRNAs does not necessarily correlate well with actual amounts of cytokine secretion by T_{FH} cells, and in vitro it commonly takes strong stimulation (PMA plus ionomycin) to induce significant cytokine production by T_{FH} cells. This is likely because of the low levels of Blimp-1 expressed by T_{FH} cells (13) and the resultant low levels of active XBP-1, which is a major regulator of endoplasmic reticulum and Golgi secretion capacity in lymphocytes (61–63). The high level of PD-1 on T_{FH} cells also likely limits proximal TCR signaling in T_{FH} cells and abrogates cytokine secretion, although this has not yet been experimentally demonstrated.

Early Models of T_{FH} Differentiation

Three models of T_{FH} differentiation have been put forward in the literature (Figure 2). After discussing these models here and their relationships to other CD4 T cell subset differentiation, I propose an integrated model in the next section based on recent findings (see Figure 3). Note that Th1, Th2, and Th17 subsets are frequently called lineages. However, the term lineage should be reserved for cells with irreversible developmental changes, such as the development of B cells, or CD4 versus CD8 T cells. Given the mounting evidence of plasticity in Th1, Th2, Th17, and iTreg commitment (58, 60, 64–68), it is



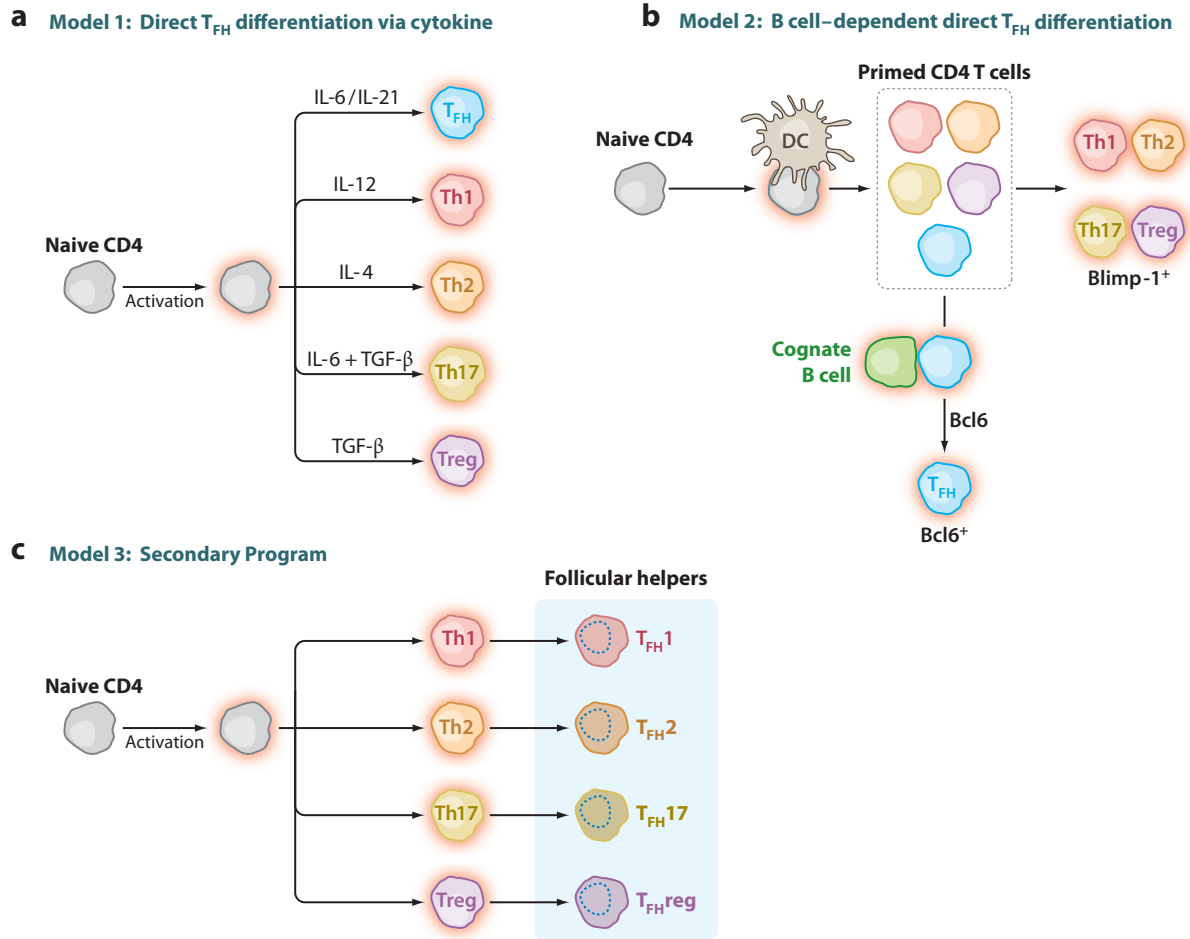


Figure 2 Three early models for T_{FH} differentiation: (a) Model 1: Direct T_{FH} differentiation via cytokine; (b) Model 2: B cell–dependent direct T_{FH} differentiation; (c) Model 3: Secondary program. (See text for details.)

more appropriate to refer to these important differentiated cell types as different CD4 T cell subsets, not lineages, and the term subsets is used herein.

Purified CD4 T cells cultured in the presence of IL-12 and TCR stimulation begin expressing T-bet and IFN- γ and differentiate into Th1 cells in the absence of any additional external signals. Analogous inductions can be done for Th2, Th17, or iTreg differentiation, using isolated CD4 T cells in the presence of one or two appropriate cytokines. Exposure of purified CD4 T cells to IL-6 or IL-21 induces

CD4 T cells to produce IL-21 (16, 27, 69). Nurieva, Dong, and colleagues (16) have shown that CD4 T cells cultured in the presence of spleen antigen-presenting cells (APCs) and IL-21 express CXCR5 mRNA, express some Bcl6 mRNA, and, upon transfer into host mice, have enhanced B cell help capacity compared with unbiased CD4 T cells (Th0, with Th1, Th2, Th17, and Treg pathways inhibited). It was further shown that IL-6 or IL-21 could induce Bcl6 and CXCR5 mRNA in vitro (14). IL-6 and IL-21 both signal through STAT3 (signal transducer and activator of



Integrated model of T_{FH} differentiation: multistage and multifactorial

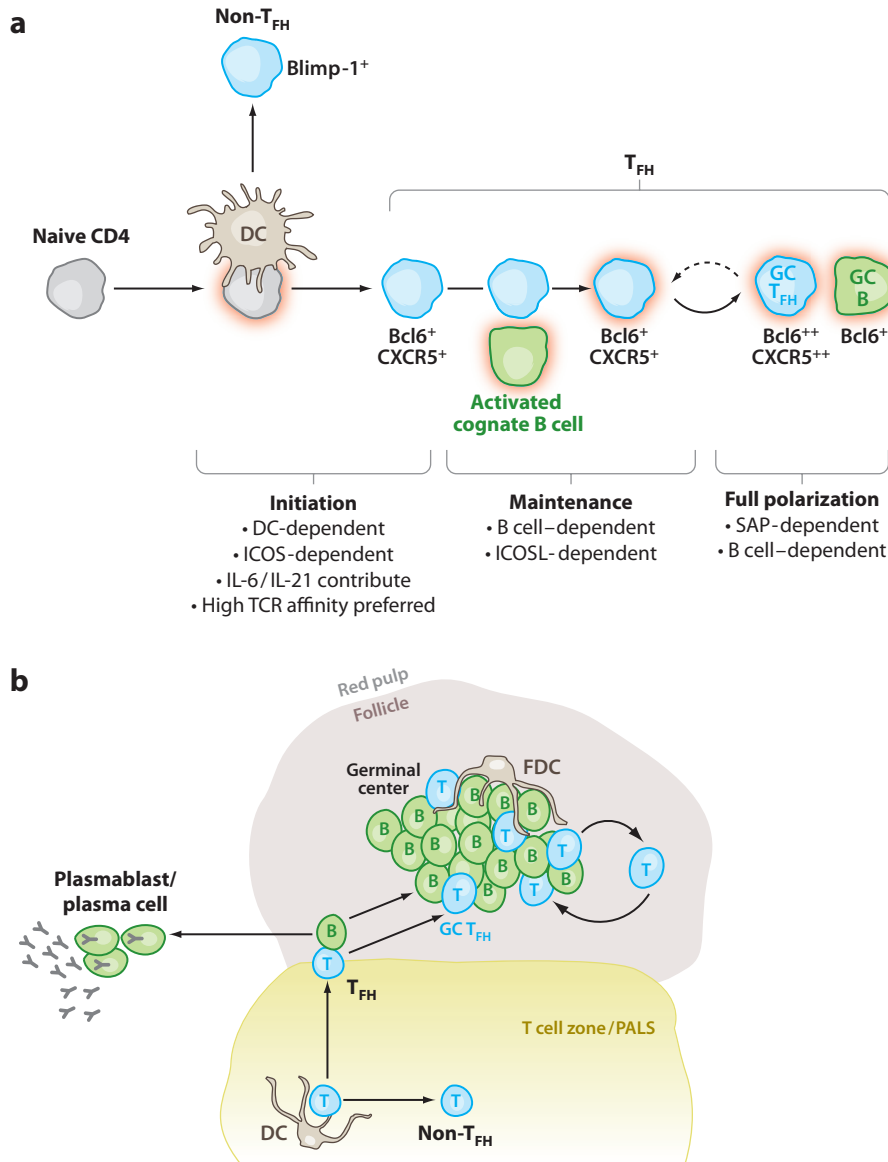


Figure 3

Proposed integrated model of T_{FH} differentiation: multistage and multifactorial. (a) Multiple stages of T_{FH} differentiation, including initiation, maintenance, and full polarization. Required signals are indicated. T_{FH} cells are defined as Bcl6⁺ CXCR5⁺ CD4 T cells. T_{FH} differentiation is independent of (not dependent on) Th1/Th2/Th17 differentiation. See text for details. (b) The geography of T_{FH} differentiation within spleen or lymph node.



transcription 3), among other pathways, and STAT3-deficient CD4 T cells fail to differentiate into T_{FH} cells (16). Those data support a model of T_{FH} differentiation analogous to Th1, Th2, or Th17 differentiation, where exposure of CD4 T cells to a single cytokine (IL-6 or IL-21) drives differentiation of a distinct and independent T_{FH} cell subset (**Figure 2a**) (16).

This model has been controversial, however, for several reasons. Neither Bcl6 nor CXCR5 were induced by IL-6 *in vitro* in two other studies, which did show potent induction of IL-21 by IL-6 (69, 70). IL-21 can be produced by Th17 or Th2 cells *in vitro* (27–30, 71), making it unclear how T_{FH} differentiation specificity would arise. Purified CD4 T cells cultured in isolation with IL-21 and anti-CD3 and anti-CD28 do not upregulate Bcl6 protein or CXCR5 protein (D. Eto and S. Crotty, manuscript submitted). IL-21^{-/-} or IL-21R^{-/-} mice can develop normal or near normal levels of T_{FH} cells after protein immunization (17–19), after viral infection (72; D. Eto and S. Crotty, manuscript submitted), or in autoimmunity (22). Note that although IL-21 is not required for T_{FH} differentiation, IL-21 production by T_{FH} cells is an important function of T_{FH} cells, as GC B cells are usually aberrant in the absence of T_{FH} production of IL-21 (17–19). IL-6^{-/-} mice can develop normal levels of T_{FH} cells after protein immunization (19) or viral infection (19). Additionally, one study showed no loss of CXCR5⁺ CD4 T cells in mice with STAT3-deficient CD4 T cells (70). This model also fails to account for the loss of T_{FH} cells in the absence of T-B interactions (13, 56, 73), discussed below.

A second model of T_{FH} development has proposed that T_{FH} cells are a distinct CD4 T cell subset that can differentiate independently of Th1, Th2, and Th17 but that direct differentiation of T_{FH} cells is dependent on B cell interaction (**Figure 2b**, Model 2: B cell-dependent T_{FH} differentiation) (31). Strikingly, T_{FH} cells are not observed in the absence of B cells after protein immunization (73), viral infection (13), or parasite infection (56). Given that B cell-deficient mice have various

immunological abnormalities—most notably, lymphoid architecture defects—CD19^{-/-} mice were used to demonstrate that the absence of T_{FH} cells was not an epiphenomenon caused by tissue architectural abnormalities (73). Subsequently, antigen-specific B cells were determined to be required, as BCR transgenic mice with a BCR specific for the irrelevant antigen hen egg lysozyme (HEL) (MD4/μMT) were incapable of developing virus-specific T_{FH} cells after infection with lymphocytic choriomeningitis virus (13). In addition, constitutive expression of Bcl6 in antigen-specific CD4 T cells *in vivo* could overcome the requirement of B cells for T_{FH} differentiation, as Bcl6-expressing lymphocytic choriomeningitis virus-specific CD4 T cells could differentiate into T_{FH} cells even in completely B cell-deficient mice (13). The simplest interpretation of these findings was that T_{FH} and GC B cell development is tightly interrelated; not only are T_{FH} cells required for B cell responses, but B cells are required for T_{FH} cell responses. Indeed, B cells and T_{FH} cells reciprocally induce the same master regulator transcription factor in each cell type: T_{FH} cells induce Bcl6 expression in B cells to induce and sustain GC B cell differentiation (13–15), and B cells induce Bcl6 expression in CD4 T cells to induce and sustain T_{FH} differentiation (**Figure 2b**) (13).

While B cells are important APCs in numerous contexts, they are generally not available as the initial APC for CD4 T cells during responses to infections or protein immunizations because naive antigen-specific B cells are exceedingly rare. Dendritic cells (DCs) are required for most CD4 T cell priming because they are potent APCs that are not restricted by an antigen-specific receptor, and DCs colocalize with naive CD4 T cells in the T cell zone (periarteriolar sheath) (74) (**Figure 2b**). In the “B cell-dependent T_{FH} differentiation” model, CD4 T cell priming occurs on a DC, but that initial priming does not induce T_{FH} differentiation (**Figure 2b**) (31). After initial recognition of antigen, some CD4 T cells and B cells migrate to the T cell zone–B cell zone border (T-B border) (73, 75–79). In this second round



of interactions, primed T cells that interact with cognate B cells at the T-B border are induced to express Bcl6 and become T_{FH} cells. High-level CXCR5 expression by T_{FH} cells then retains these cells in the B cell follicle, maximizing their interactions with B cells, driving GC B cell differentiation, and sustaining T_{FH} differentiation (13, 19, 25). SAP (*SH2D1A*) is required for GCs (80) and is an absolutely critical signal transduction molecule for T-B interactions (81). SAP is therefore essential for GC T_{FH} differentiation in most contexts (25). However, the B cell-dependent T_{FH} differentiation model is challenged by recent data showing that B cells are not uniquely capable of inducing T_{FH} differentiation, as mice lacking MHCII on B cells could develop T_{FH} cells in the presence of repeated antigen injection (82), and T_{FH} differentiation is present at early time points after an acute viral infection, before fading later in the absence of B cells (Y. Choi and S. Crotty, manuscript submitted). These data indicate that B cells are normally critical APCs for the maintenance of T_{FH} differentiation but are not required to initiate T_{FH} differentiation (82; Y. Choi and S. Crotty, manuscript submitted).

A third proposed model is that T_{FH} cells are not a distinct CD4 T cell subset and that the T_{FH} program is a secondary program more akin to central versus effector memory differentiation (12). This “Secondary program” model posits that T_{FH} differentiation is a phenotypic state achieved by CD4 T cells that have already undergone Th1, Th2, or Th17 differentiation (Figure 2c, Model 3) (56). If one subscribes to an absolutist model of CD4 T cell differentiation, in which Th1, Th2, Th17, and iTreg differentiation programs are completely distinct from each other and individual cells cannot possess overlapping attributes of these subsets in vivo, then one may conclude that the T_{FH} differentiation program is not analogous to Th1, Th2, Th17, and iTreg differentiation, as T_{FH} cells can clearly possess overlapping attributes with these other CD4 T cell differentiation states (13, 25, 44, 45, 56). In that interpretation, the T_{FH} program is a phenotypic state achieved by a CD4 T cell that

has already been primed and differentiated to a Th1/Th2/Th17 cell. The key feature of this model is that a T_{FH} cell cannot exist independently of Th1, Th2, or Th17 cells; a cell must become a Th1/Th2/Th17 cell before differentiating to a T_{FH} cell (56, 83, 84). This model also implies that T_{FH} differentiation would not antagonize Th1, Th2, or Th17 differentiation.

A variation on the T_{FH} cell as a secondary program phenotypic state model revisits the following question: If T_{FH} cells are not a fully distinct subset, then are T_{FH} cells simply CD4 T cells that express CXCR5? The answer to this question is an emphatic “No.” The gene expression changes in T_{FH} cells are at least as extensive as the gene expression changes that distinguish Th1, Th2, and Th17 cells. The T_{FH} gene expression program involves changes in numerous transcription factors, cell surface receptors, cytokines, and additional molecules well beyond CXCR5 (13, 16, 25, 85–87). Indeed, the T_{FH} program is especially rich in cell surface receptor changes, making T_{FH} cells relatively easy to phenotypically identify by flow cytometry by surface stains of cells directly ex vivo, in contrast to Th1, Th2, or Th17 cells. This extensive set of cell surface receptor changes is reflective of the importance of cell-cell interactions between T_{FH} and B cells for the specialized B cell help functions of T_{FH} cells. Bcl6 binds between 1,700 and 4,000 gene promoters in GC B cells (36, 37) and can regulate perhaps as many genes in T_{FH} cells, although this has yet to be experimentally determined.

A Multistage Multifactorial Model of T_{FH} Differentiation

T_{FH} differentiation is controversial, and these controversies center on multiple different essential aspects of T_{FH} biology, resulting in disparate models of T_{FH} differentiation. How do we resolve the controversies of these models? I propose a multistage, multifactorial model of T_{FH} differentiation (Figure 3).

Priming. Bcl6 is the central regulator of T_{FH} differentiation and is the key to understanding



development of this cell type. A causal relationship between Bcl6 expression and T_{FH} cells has been demonstrated (13–15). Bcl6 protein expression level is closely correlated with CXCR5 expression level throughout T_{FH} differentiation, with the highest levels of Bcl6 mRNA and protein and CXCR5 mRNA and protein expression in GC T_{FH} cells (25, 26, 86, 88). To understand the underlying mechanisms of Bcl6 induction, we recently examined requirements for Bcl6 expression by CD4 T cells in vivo, with a particular focus on Bcl6 protein, as Bcl6 mRNA does not consistently reflect Bcl6 protein levels in B cells (89–91). Strikingly, development of Bcl6⁺CXCR5⁺ CD4 T cells occurred as early as day 2 in vivo in response to an acute viral infection, with further increases in Bcl6 protein expression levels and Bcl6⁺CXCR5⁺ CD4 T cell frequency as the adaptive immune response matured (Y. Choi and S. Crotty, manuscript submitted). Normal early induction of Bcl6 and CXCR5 by CD4 T cells at days 2–4 in vivo was observed in the absence of B cells (Y. Choi and S. Crotty, manuscript submitted). However, T_{FH} differentiation was lost by day 8 in vivo in the absence of B cells (13), demonstrating that although DCs initially prime T_{FH} differentiation, those signals are transient, and B cells are required as a second stage of APC–CD4 T cell interaction to sustain and complete T_{FH} differentiation in vivo, as B cells rapidly become the primary APCs available (13, 56, 73, 82) (**Figure 3a,b**).

Higher TCR affinity may result in prolonged T cell–DC interactions during the extended periods of CD4 T cell–DC interaction in the first 24 h of priming in vivo (74, 92–94). Higher TCR affinity has been associated with a preference for T_{FH} differentiation over non-T_{FH} differentiation (32), which may be due to higher TCR affinity causing extended T–DC interactions (93) and resulting in prolonged costimulatory receptor engagement and/or cytokine exposure at the time of DC priming. ICOS expression by CD4 T cells at the time of DC priming is required for expression of Bcl6 and T_{FH} differentiation

(**Figure 3**) (Y. Choi and S. Crotty, manuscript submitted).

Work from multiple laboratories shows that IL-21 influences T_{FH} cells. IL-21 is essential for GC B cell survival and proliferation by direct action on IL-21 receptor (IL-21R)-expressing B cells (17, 18), at least in part via enhancement of Bcl6 expression in the GC B cells (17). However, IL-21^{-/-} and IL-21R^{-/-} mice had modest (17) if any (19, 22, 72) T_{FH} development defect. Given that cognate B cells are required for maintenance of T_{FH} cells under most conditions, one explanation for the importance of IL-21 for T_{FH} cells in some conditions is that IL-21 is indirectly required for maintenance of T_{FH} cells via the requirement of IL-21 for B cell survival, particularly at later times. In the absence of surviving cognate B cells, differentiated T_{FH} cells would be lost. While this is likely occurring, IL-21 also has direct effects on CD4 T cells, most clearly demonstrated in the context of Th17 differentiation (28–30). Furthermore, Nurieva, Dong, and colleagues (14) reported induction of Bcl6 by IL-21 in vitro (**Figure 4**). A confounding factor for understanding the influence of IL-21 on T_{FH} cells is that IL-6 also primarily signals through STAT3 and may compensate for the absence of IL-21. Naive and activated CD4 T cells express both IL-6R [IL-6R + gp130 (IL-6st)] and IL-21R [IL-21R + common gamma chain (CD132, *IL2RG*)], and IL-6 and IL-21 may have redundant functions. Whereas DCs are well known producers of IL-6, no APCs are known to produce IL-21.

We have now generated evidence that whereas the absence of either IL-6 or IL-21 does not limit T_{FH} differentiation (19), the absence of both IL-6 and IL-21 substantially abrogates T_{FH} cells (IL-21^{-/-} + anti-IL-6) (D. Eto and S. Crotty, manuscript submitted). Nevertheless, purified CD4 T cells cultured in isolation with IL-21 and anti-CD3 and anti-CD28 do not significantly upregulate Bcl6 protein or CXCR5 protein (D. Eto and S. Crotty, manuscript submitted). This leads to a conclusion that IL-6 and IL-21 redundantly contribute to T_{FH} differentiation, but these



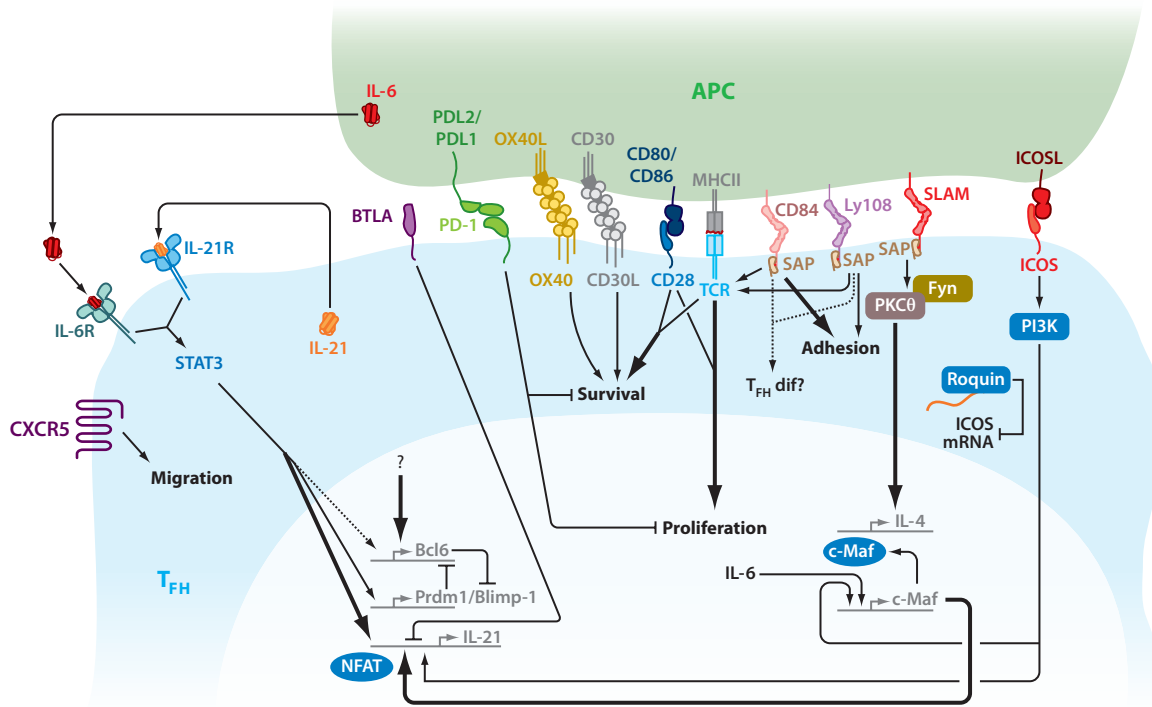


Figure 4
Signals to T_{FH} cells. See text for details.

cytokine signals by themselves are insufficient for instruction of T_{FH} differentiation. It is likely that multiple signals acting in concert, including ICOS, are required for initiation of T_{FH} differentiation (Bcl6⁺CXCR5⁺) at the time of DC priming, similar to the multifactorial requirements for Th17 differentiation (95).

As with other CD4 T cell effectors, the capacity of a naive CD4 T cell to differentiate into a T_{FH} cell requires costimulation in addition to TCR stimulation. GCs do not develop in CD28^{-/-} mice, presumably due to a lack of T_{FH} cells (96, 97). OX40 is a TNF receptor family member important for survival of activated T cells (Figure 4) (98). OX40 was reported to induce CXCR5 mRNA expression (99) and to be involved in induction of CD4 T cell migration to the T-B border after priming (100, 101). However, these studies did not determine total antigen-specific CD4 T cell numbers and could not distinguish between T

cell survival effects versus T_{FH} differentiation effects. In addition, OX40^{-/-} mice had near normal GC and antibody responses (102), indicating normal T_{FH} differentiation. More recent work using anti-OX40L monoclonal antibody (mAb)-treated mice or OX40L^{-/-} mice has shown normal CXCR5 expression, normal T_{FH} differentiation, normal GC development, and normal antibody titers in the absence of OX40 signals (103). In the only published study that directly examined antigen-specific CXCR5⁺ CD4 T cell responses in vivo in the absence of OX40 signaling, CXCR5⁺ CD4 T cell numbers were normal, as were GCs (104).

Commitment: the T-B border phase. Geography is of great importance for T_{FH} differentiation and function. T and B cells first interact in the T-B boundary region (6, 76, 105). CXCR5 is the canonical T_{FH} marker. T_{FH} cells migrate in response to CXCL13 (1, 2, 13, 73). In

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the absence of CXCR5, T_{FH} frequencies are reduced in vivo (73, 78, 79, 106). CCR7 downregulation may also be sufficient for this migration to the T-B boundary region (73, 79). Although CXCR5 expression is sufficient for localization to the T-B border, CXCR5 expression is not sufficient for T_{FH} localization inside follicles (8). High levels of CCR7 expression can block CD4 T cell migration to the B cell zone (73). PSGL1 (P-selectin glycoprotein ligand 1) can bind CCR7 ligands CCL19 and CCL21 and may affect T cell migration; PSGL1 can also enable T cell migration to inflamed sites via P-selectin and E-selectin binding (107). In either scenario, Bcl6 downregulates PSGL1 expression and thereby prevents PSGL1-dependent migration (19). In the absence of CXCR5 expression, a CD4 T cell is unlikely to encounter a cognate B cell, due to location, and is likely to have non- T_{FH} differentiation programs reinforced by other signals in the T cell zone, including upregulation of Blimp-1 (13, 31, 32, 83).

The migration of T_{FH} cells to the T-B border allows the critical B cell-dependent phase of T_{FH} differentiation to occur (Figure 3a,b). Activated B cells express ICOSL, and the expression of ICOSL by B cells is required for T_{FH} cells (16). T_{FH} cells are lost in ICOS^{-/-} (103), anti-ICOSL blocking mAb-treated (44, 103), or ICOSL^{-/-} mice (16). T_{FH} cells also appear to be lost in ICOS-deficient humans (108–110). ICOS expression on CD4 T cells is induced and maintained by CD28-dependent and CD28-independent pathways (111, 112). ICOS-mediated PI3 kinase (PI3K) signaling is required for T_{FH} differentiation and is associated with reductions in IL-21 and IL-4 (Figure 4) (113). The PI3K subunit P110 δ is specifically required for ICOS downstream signaling and the production of IL-21 (114). New data also show that ICOS is required for T_{FH} differentiation at the time of DC priming. Roquin is a critical negative regulator of ICOS expression. Sanroque mice have a missense mutation in the Roquin gene (*Rc3b1*), resulting in constitutively increased ICOS expression, increased numbers of T_{FH} cells, hyperactive

GCs, and spontaneous lupus disease (115). Roquin is an RNA-binding protein that binds to ICOS mRNA and directs its degradation in a Dicer- and Argonaut-independent process (Figure 4) (116). ICOSL on B cells is also downregulated after interaction with ICOS as a negative feedback loop (117, 118). As a result, signals maintaining ICOSL are required to maintain ICOSL-ICOS interactions. ICOSL regulation in DCs is not well characterized.

Commitment over time is a critical component of CD4 T cell differentiation programs. It has long been known that multiple rounds of in vitro polarization are required for maximally stable Th1 or Th2 differentiation (12), and instability of CD4 T cell differentiation has been recently more widely recognized (65, 119). Changes to establish stability of differentiation are prominently manifested as epigenetic changes in the DNA of key genes involved in the differentiation program (120, 121). Initial T_{FH} phenotypes obtained at the DC priming stage are lost during further rounds of division in the absence of B cells (13, 73, 82). This demonstrates that T_{FH} differentiation is not fully committed after DC priming and requires the presence of cognate B cells for commitment (Figure 3a). Data regarding the epigenetics of Bcl6 and T_{FH} differentiation are currently lacking but would be of great interest. In addition to epigenetic modifications, the duration of sustained Bcl6 expression itself could have a strong influence on which genes further down the transcription cascade become sustainably expressed, due to repression of certain target genes long enough to allow downstream factors to interact and establish gene expression, analogous to that described for other transcriptional regulators (122).

In this model (Figure 3), T_{FH} differentiation is independent of Th1/Th2/Th17 differentiation. That is, T_{FH} differentiation is not dependent on Th1/Th2/Th17 differentiation. T_{FH} differentiation can occur at the time of DC priming (Y. Choi and S. Crotty, manuscript submitted) and is not dependent on the CD4 T cell first becoming

a Th1/Th2/Th17 cell, in contrast to the “Secondary program” model (**Figure 2c**). However, while T_{FH} differentiation is not dependent on Th1/Th2/Th17 differentiation, neither is it exclusive of Th1/Th2/Th17 differentiation. T_{FH} is best considered as an overlaid program, on top of (or parallel to) Th1/Th2/Th17 differentiation. Therefore T_{FH} cells and GC T_{FH} cells in GCs can possess T-bet or GATA3 or ROR γ t expression and secrete moderate levels of canonical Th1, Th2, or Th17 cytokines, which are important for CSR decisions by B cells. This lack of exclusivity is likely a central feature of T_{FH} . Non- T_{FH} are Th1/Th2/Th17 cells that further polarize into Blimp-1⁺ highly cytokine-secreting cells with limited proliferative potential (**Figure 3**), analogous to terminal effector CD8 T cells. T_{FH} differentiation antagonizes non- T_{FH} effector CD4 T cell differentiation by Bcl6 antagonism of Blimp-1.

This concept of T_{FH} as an overlaid, non-exclusive CD4 T cell program remains controversial, and some investigators prefer a model in which T_{FH} differentiation is completely exclusive of Th1, Th2, or Th17 differentiation, which is supported by findings that Bcl6 limits expression or function of other canonical master regulator genes (**Figure 1**).

Also note that this model does not exclude the possibility that a non- T_{FH} cell could become a T_{FH} cell. **Figure 3** demonstrates the simplest pathway by which T_{FH} differentiation occurs, starting with a naive CD4 T cell. T_{FH} differentiation may also be accessible to differentiated non- T_{FH} cells under certain circumstances (56), though there are caveats to cell purity and stability in such adoptive transfer experiments (119). The stability of Bcl6 expression is discussed below.

Note that in humans, there are minimal Th1, Th2, or Th17 characteristics evident in GC T_{FH} cells (15, 26, 86, 87), with the exception of IL-4 (2, 26), which is likely made in a T_{FH} -specific (Th2-independent) manner (25). Nevertheless, human T_{FH} (CXCR5^{int}Bcl6⁺) cells do have partial characteristics of Th1/Th2/Th17 cells.

Full polarization: germinal center phase. As discussed in the sidebar, two phenotypically distinct populations of Bcl6-expressing T_{FH} cells are readily identifiable in mice and humans. These are referred to as pre- T_{FH} and T_{FH} cells, or T_{FH} and GC T_{FH} cells in different nomenclatures. CXCR5⁺Bcl6⁺ CD4 T cells are T_{FH} cells and express elevated levels of ICOS and PD-1 (25, 73). GC T_{FH} cells are further differentiated T_{FH} cells that express the highest levels of Bcl6 and CXCR5 in mouse CD4 T cells (Bcl6⁺⁺CXCR5⁺⁺⁺) (25, 73) and the highest levels of Bcl6 and CXCR5 in human tonsillar CD4 T cells (CXCR5^{hi} versus CXCR5^{int}) (26, 86). GC T_{FH} cells are most frequently identified by coordinate expression of the highest levels of CXCR5 and PD-1 expression in mice (15, 73), though other markers such as CXCR5 and GL7 expression in mice (25) or CXCR5 and the highest ICOS expression in humans (1, 86) can be used. These markers identify GC T_{FH} cells as T_{FH} cells within GCs, whereas the remaining T_{FH} cells are predominantly in the follicle or in apposition to the follicle (25, 73). T_{FH} and GC T_{FH} cells are two stages of a stepwise progression (**Figure 3**). T_{FH} and GC T_{FH} cells have similar gene expression profiles, with GC T_{FH} cells being a further polarized state of T_{FH} cells. T_{FH} cells express elevated CXCR5, PD-1, ICOS, BTLA (B and T lymphocyte attenuator), CD200, and SAP compared with naive CD4 T cells or non- T_{FH} cells. Murine GC T_{FH} cells express levels of each of these T_{FH} markers at even higher levels than T_{FH} cells (**Table 1**) (25, 73). The T_{FH} differentiation state correlates with the amount of Bcl6 expressed (25, 86), with Blimp-1 expression repressed in both cases (25). Similar relationships exist for human CXCR5^{int} T_{FH} (or pre- T_{FH}) cells and CXCR5^{hi} GC T_{FH} cells (also called GC Th or T_{FH} cells in human nomenclature) (26, 86). Both T_{FH} and GC T_{FH} cells can provide B cell help in vitro (25, 26, 86). In vivo, T_{FH} cells that cannot become GC T_{FH} cells (e.g., *sb2d1a*^{-/-}) can only provide early B cell help, including help for some extrafollicular responses (22, 25), whereas GC T_{FH} cells are absolutely essential for GC development



Table 1 Markers for human and mouse T_{FH} cells

Marker	Human		Mouse		Naive CD4 T cell	Activated non-T _{FH} CD4
	T _{FH}	GC T _{FH}	T _{FH}	GC T _{FH}		
CXCR5	+	++	+	++	—	— ^a
Bcl6	+	++	+	++	—	—
PD-1	+	++	+	++	—	variable
ICOS	+	++	+	+	—	variable
SAP	normal	high	normal	high	normal	normal
IL-21	+	++	+	++	—	variable
CXCR4	normal	high	high mRNA	high mRNA	normal	normal
CXCL13	low	high	—	—	—	—
BTLA	normal	high	high	high	normal	low
SLAM	unknown	unknown	normal	normal	normal	variable ^b
CCR7	low	low	low	low	high	variable
CD200	-/+	+	+	+	—	—
IL-4	—	+	—	+	—	Th2 ⁺

^aIn vitro activated human, but not mouse, CD4 T cells transiently upregulate CXCR5 upon strong TCR + CD28 signaling (M. Kroenke and S. Crotty, unpublished data).

^bSLAM expression is high on most Th1 and Th2 cells.

and the generation of long-term humoral immunity (25, 80, 81, 123, 124). In summary, there are at least two levels of Bcl6 expression, which correlate well with different activity levels of T_{FH} and GC T_{FH} cell populations. Therefore, T_{FH} differentiation is a multi-stage, multifactorial process characterized by initial upregulation of Bcl6 at the priming stage, stabilization of Bcl6 expression at the T-B interaction phase, and maximization of Bcl6 expression in the GC phase (**Figure 3**).

In some respects, GC T_{FH} cells have the gene expression profile of a highly activated T_{FH} cell, commensurate with ongoing stimulation via TCR antigen recognition, with GC B cells presenting antigen and providing costimulatory signals. GC T_{FH} cells also have several distinct features. GC T_{FH} cells have heightened expression of SAP and a specialized ability to express IL-4 independent of Th2 differentiation (25, 44) (see the section entitled T_{FH} Functions for additional information).

SAP is an SH2 domain intracellular signaling protein required in CD4 T cells for GCs

(80). SAP is critical for GC T_{FH} development (**Figure 3a**) (25). SAP deficiency is the cause of the human genetic deficiency X-linked lymphoproliferative disease (XLP), characterized by poor antibody responses and an absence of GCs (discussed further in the section on Human T_{FH} Cells, below). SAP has a major role in T cell–B cell adhesion (81, 125). SAP is not required for sustained CD4 T cell adhesion to DCs, but it is required for sustained CD4 T cell adhesion to B cells (81). Adhesion signals are transduced from SLAM (signaling lymphocytic activation molecule) family receptors by SAP (**Figure 4**) (125). There are five SLAM family receptors capable of binding SAP, four of which are expressed on CD4 T cells. Given that no single SLAM family receptor deficiency directly phenocopies SAP deficiency, there is likely a redundancy between SLAM family members, although alternative explanations are possible (126; see also 127 in this volume). SLAM family receptors may primarily provide CD4 T cell adhesion to B cells, with GC T_{FH} differentiation induced by other signals originating from the B cells that necessitate prolonged T-B



conjugate times provided by SAP-dependent SLAM family adhesion. In that indirect requirement scenario, ICOS may be the primary GC T_{FH} differentiation signal requiring extended SAP-dependent T-B conjugate times, given the requirement for ICOSL expression on B cells. Sustained ICOS expression is required for GC T_{FH} cells (103, 128). Alternatively, given that adhesion is not the only process that SAP signaling regulates, signals from engagement of SLAM family receptors such as CD84 may also directly provide signals that drive $T_{FH} \rightarrow GC\ T_{FH}$ differentiation in a SAP-dependent manner (Figure 4).

IL-6 is expressed by activated follicular DCs (FDCs) (129, 130), which permeate the GC with particular density in the apical light zone (131). GC T_{FH} cells are also enriched in the light zone of the GC (4, 5, 73, 132). GC T_{FH} cells express IL-6R (25, 85, 86). IL-6 produced by FDCs may normally enhance Bcl6 expression in GC T_{FH} cells for maximal differentiation and function. IL-6^{-/-} mice have normal GC T_{FH} cells in most instances (5, 133, 134), again implicating a heavy degree of overlap in cytokine functions, and autocrine IL-21 is able to provide the necessary signals when IL-6 is absent.

Terminal differentiation? It is currently unclear whether GC T_{FH} cells are terminally differentiated cells, with no developmental progression available to them besides death, or whether instead GC T_{FH} cells cycle between GC T_{FH} and T_{FH} states (Figure 5). In one model, GC T_{FH} cells are terminally differentiated, cannot leave the GC, and are destined to die. This model is supported by the observation that GC T_{FH} cells express extremely high levels of PD-1 (25, 73, 86, 112) and are prone to apoptosis in vitro (86). However, susceptibility to apoptosis is not sufficient evidence of terminal differentiation, as GC B cells are also extremely susceptible to apoptosis in vitro and yet are highly malleable cells in vivo, capable of subsequently entering several different differentiation pathways, either short-lived or long-lived.

In the alternative model, T_{FH} and GC T_{FH} cells have a cyclical relationship in which GC T_{FH} cells cycle between GC T_{FH} and T_{FH} states as they migrate between a GC and a follicle (Figure 5b). This model highlights the relatedness of GC T_{FH} and T_{FH} cells (Figure 3a). As introduced above, the gene expression differences between Bcl6⁺CXCR5⁺ T_{FH} cells and Bcl6⁺⁺CXCR5⁺⁺ GC T_{FH} cells in mice

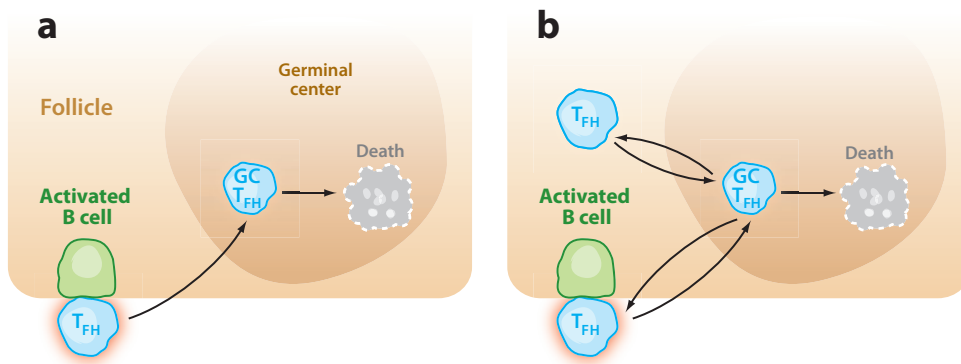


Figure 5

Models of the T_{FH} -GC T_{FH} cell relationship. (a) Unidirectional progression model from T_{FH} cell to GC T_{FH} cell, with GC T_{FH} being a terminal differentiated state ending in death. (b) Cyclical model. CD4 T cells can enter and exit GCs, resulting in interconversion between T_{FH} and GC T_{FH} . A T_{FH} cell can traffic into a GC, interact with GC B cells, and subsequently exit the GC and return to a T_{FH} cell state, staying in the follicle or the T-B border region due to CXCR5 expression.



(25) and humans (86) are evidence that GC T_{FH} cells are highly activated T_{FH} cells due to ongoing antigen and costimulatory signal stimulation with GC B cells. CD4 T cells in GCs are not sessile (135). Intravital microscopy studies of CD4 T cell behavior in GCs indicate that a substantial percentage of antigen-specific CD4 T cells regularly exit the GC (81). From these data, we can infer that GC T_{FH} cells move from a GC into the surrounding follicle. If the GC T_{FH} state is maintained by constant engagement with antigen-specific B cells (or near constant engagement), then the GC T_{FH} cells presumably return to a resting T_{FH} state upon exiting the GC. A T_{FH} cell is $CXCR5^+CCR7^{low}$ and will remain in the follicle or T-B boundary region if it exits the GC. Such a T_{FH} cell would still have access to the GC and, upon migration back to a GC, would reengage a cognate B cell, provide help to the bound GC B cell, and upregulate GC T_{FH} markers due to the engagement with the GC B cell, in a cyclical process (Figure 5b). These models remain to be further tested.

Additional Transcription Factors Involved in T_{FH} Differentiation

Bcl6 does not act alone in controlling T_{FH} differentiation. Similar to other CD4 T cell subsets, a network of transcription factors is involved in controlling the various functionalities of T_{FH} . c-Maf, STAT3, Batf, and Bcl6-binding partners all have roles in T_{FH} differentiation and/or function.

Bcl6-binding partners. It would be unwise to forget that Bcl6 is a corepressor, acting in partnership with related Bcl6 family members, other transcription factors possessing BTB domains, or even proteins unrelated at the sequence level that can partner through alternative protein-protein interaction domains. As such, the function of Bcl6 in a cell—the genes regulated by Bcl6 by binding to specific chromatin sites—is almost certainly affected by the availability of corepressors in the nuclear milieu. Subsets of Bcl6-regulated genes can

presumably be differentially controlled by increased or decreased expression of specific Bcl6 corepressors. Known Bcl6-interacting factors include CtBP, BAZF, MTA3, SMRT, MIZ1 (*Zbtb17*), ETO (*Runx1t1*), PLZF, AP-1, BCoR, and NCoR, among others (136–149). The multifaceted interactions can allow for combinatorial Bcl6 targeting. Overlapping or neighboring DNA-binding motifs offer an additional layer of counterregulation, where Bcl6 competes with other transcription factors for occupancy of a gene regulatory site. Bcl6-binding motifs are overrepresented in proximity with STAT and IFN regulatory factor (IRF)4-binding motifs, which are thought to compete with Bcl6 for binding (36, 37).

STAT3. STAT3 is the major transducer of IL-21R signals in T cells and B cells (150). STAT3 is also the major transducer of IL-6R signals in T and B cells (151). Both IL-21 and IL-6 can influence Bcl6 expression in stimulated CD4 T cells (14, 16). The relationship between STAT3 and Bcl6 is complex. While STAT3 can bind the *Bcl6* gene and positively influence Bcl6 expression (152–154), STAT3 is a more potent inducer of Blimp-1 in B cells (20, 152, 155, 156) and CD4 T cells (153). Given that Bcl6 and Blimp-1 are direct antagonists, the induction of both Bcl6 and Blimp-1 by STAT3 [in an IRF-dependent manner (153)] indicates that the signaling regulation of Bcl6 versus Blimp-1 expression heavily depends on additional transcription factors. Of interest, STAT3 and IRF4 are both targets of repression by Bcl6 in GC B cells (36, 37, 157, 158). This blocks STAT3-IRF4-Blimp-1-dependent plasma cell differentiation. A loss of T_{FH} cells was reported in CD4-Cre STAT3^{fl/fl} mice (16), but it was not determined if this loss was T_{FH} -specific or due to a general defect in CD4 T cell activation, as neither T cell activation nor antigen-specific CD4 T cell responses were assessed. STAT3 regulates sustained proliferation in lymphocytes (154) as well as non-hematopoietic cells (159). Differentiation of $CXCR5^+$ CD4 T cells in vivo was not defective in the absence of STAT3 in another study (70).



Note that STAT3 does not appear to be important for human T_{FH} differentiation or function in a cell-intrinsic manner (160, 161) (see Human T_{FH} Cells section). This may be due to species-specific differences in the regulation of the *IL-21* gene or due to more complex issues in our understanding of T_{FH} differentiation. IL-21R can induce STAT5 phosphorylation (162), and it is controversial whether STAT3 or STAT5 signaling is more important for some biological processes affected by IL-21 signaling. While plasma cell differentiation induction by IL-21 is primarily controlled by STAT3 signaling (20, 155, 156, 163), GC B cell differentiation may be STAT3 and/or STAT5 regulated (20, 164, 165). In CD4 T cells, IL-21 or IL-6 signaling via STAT3 is required for Th17 differentiation (95).

c-Maf. c-Maf (*Maf*) specifically regulates IL-4 expression (12, 166) and IL-21 expression (45) (Figure 4), and IL-4 and IL-21 are major products of GC T_{FH} cells. c-Maf^{-/-} chimeric mice have defective T_{FH} differentiation in vivo (45). Investigators have suggested that this is due to defective induction or maintenance of IL-21 in the absence of c-Maf, in an ICOS-dependent manner (45). c-Maf has roles in the regulation of IL-4 in Th2 cells and IL-21 in Th17 cells (12, 45, 166). c-Maf is not required for Th17 differentiation (45) but does sustain Th17 cells (45). c-Maf most likely sustains Th17 cells by maintaining IL-21 expression over time (45), and IL-21 is an autocrine growth factor for differentiated Th17 cells (95).

c-Maf interacts with NFAT and JunB to induce IL-4 expression (12). Given that IL-4 is produced by GC T_{FH} independently of GATA3 induction (25), the difference between Th2 and GC T_{FH} regulation of IL-4 may be that Th2 IL-4 production is heavily GATA3 dependent, whereas GC T_{FH} IL-4 production is c-Maf and NFATc1 (NFAT2) or NFATc2 dependent (Figures 4, 6). GC T_{FH} cells express elevated NFATc1 (88) and express c-Maf (25, 45, 86). This is similar to IL-4 production by natural killer T (NKT) cells, which depends on NFAT (167) and c-Maf (166). This also distin-

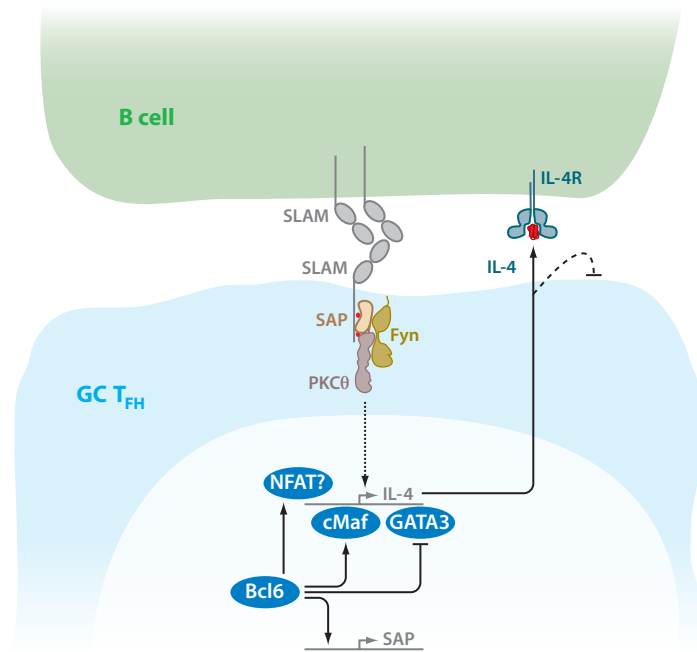


Figure 6

GC T_{FH} cell IL-4 production. GC T_{FH} cells express IL-4 via SLAM-SLAM engagement and signal transduction by SAP and PKCθ, with a likely contribution by Fyn. CD4 T cells constitutively express low levels of GATA3, and GC T_{FH} cells express elevated levels of c-Maf and NFATc1. IL-4 acts on GC B cells, which express the IL-4 receptor. GC T_{FH} cells have downregulated IL-4R and avoid an IL-4-to-GATA3 feedback loop (86, 88). (Abbreviations: GC, germinal center; NFAT, nuclear factor of activated T cells; PKCθ, protein kinase Cθ; SAP, SLAM-associated protein; SLAM, signaling lymphocytic activation molecule.)

guishes mechanisms for regulating IL-4 versus IL-4 plus IL-5 and IL-13, as expression of IL-5 and IL-13 by T cells is heavily GATA3 dependent and is specific to Th2 cells (12).

c-Maf directly binds to the IL-21 promoter (168–170). IL-6 induces c-Maf expression in CD4 T cells (170). Furthermore, constitutive c-Maf expression is sufficient to drive IL-21 expression in activated CD4 T cells, in the absence of any exogenous cytokines (170). NFAT proteins, highly active in stimulated CD4 T cells (171), also bind the IL-21 gene and induce IL-21 expression (172, 173).

Batf. Batf is an AP-1 superfamily member capable of partnering with Jun. Batf-deficient mice were recently reported to have defects in



Th17 (174), Th2 (175), and T_{FH} development (175) and to have reduced numbers of Tregs (175). Batf-deficient mice have a severe defect in GC development and B cell CSR, caused by a cell-intrinsic requirement for Batf in B cells as well as T cells (175). Batf is clearly required for several CD4 T cell functions in vivo, and its specific function in T_{FH} cells remains to be determined. However, Batf does bind to the IL-21 promoter and may be a key regulator of that cytokine (174).

IRF4. IRF4^{-/-} mice have been reported to be deficient in T_{FH} cells, but it was not determined if this was T_{FH} specific or due to a general defect in CD4 T cell activation, as neither T cell activation nor antigen-specific CD4 T cell responses were assessed (153). Most STAT3-dependent genes regulated by IL-21 are characterized by the presence of combined STAT3-IRF4-binding sites (153). Furthermore, IL-21 gene regulation by STAT3 is dependent on IRF4 being prebound to the gene of interest, including at the *Prdm1* gene (Blimp-1) (153). Because STAT3 is important for IL-21 induction and signal transduction, IRF4 is presumed to be important for T_{FH} differentiation or function. However, given that IRF4 is a potent inducer of Blimp-1 in CD4 T cells (153) and that IRF4 and Bcl6 are antagonistic factors in GC B cells known to potently cross-repress each other (157), it is unclear how IRF4 and Bcl6 interrelate in CD4 T cell gene expression regulation. Given the potential importance of IRF4 in various aspects of CD4 T cell biology and the known importance of IRF4 for B cells, an understanding of the role(s) of IRF4 in T_{FH} awaits conditional gene ablation studies.

Memory T_{FH} Cells

More research is required into memory T_{FH} cells in mice and humans. There is one clear report of memory T_{FH} cells in mice, but it was in the context of persisting antigen with ongoing antigenic stimulation of the T_{FH} cells (176). Clearly T_{FH} cells are present as long as GCs are present, but do T_{FH} cells survive past the

completion of the GC and become memory cells? Identification of memory T_{FH} cells will likely be complicated by this issue for a long time. A standard definition of a memory lymphocyte is a cell that is antigen experienced (has undergone an antigen-driven expansion) and then survives in the absence of antigenic stimulation for an extended duration and can respond to rechallenge (177, 178). Cells that received continuous ongoing antigenic stimulation may be maintained for extended periods as effector cells, but these cannot be defined as memory cells unless they can survive in the absence of antigen for a significant period of time (179). Given that GC T_{FH} cells localize to GCs, that GC reactions can persist for months (180), and that FDCs can retain antigen for many months (180, 181) or even for the lifetime of a mouse (182, 183), it may be challenging to establish whether T_{FH} cells develop into resting memory CD4 T cells. T_{FH} cells may not become antigen-independent memory cells, given that T_{FH} cells may require ongoing signals from B cells and FDCs to maintain the T_{FH} program. Alternatively, these highly antigen-experienced PD-1^{hi} GC T_{FH} cells may no longer be able to survive in the absence of antigen, as is the case with the PD-1^{hi} exhausted CD8 T cells found in high-antigen-load chronic viral infections (179).

It is intriguing that Bcl6 and Blimp-1 are antagonistic transcription factors that function as self-reinforcing switches for effector cell fate decision in CD4 T cells, CD8 T cells, and B cells (31). In B cells, Bcl6 expression is not a permanent state, as GC B cell differentiation is not a permanent state. Bcl6 is highly upregulated in GC B cells, and then downregulated in progeny prior to becoming memory B cells or plasma cells. Bcl6 regulation may be similar in CD4 T cells. Alternatively, Blimp-1 in CD8 T cells is associated with terminal effector cell differentiation (38–40), whereas Bcl6 is associated with CD8 T cell memory development (38, 184). This raises the question of whether Bcl6 expression in CD4 T cells facilitates memory development. Although some data associate Bcl6 with memory CD4 T cell development (185),



more work needs to be done, controlling for various factors, including allogeneic rejection.

Data in humans suggest that resting memory T_{FH} cells exist in peripheral blood as $CD40RO^+CXCR5^+$ cells, but this remains a contentious issue requiring more examination (see the section on Human T_{FH} Cells, below).

T_{FH} FUNCTIONS

The stated function of T_{FH} cells is to provide B cell help. However, this is an oversimplification of the complex tasks they perform. T_{FH} cells not only help B cells but also help themselves, either via autocrine cytokines or via interactions with B cells. In addition, T_{FH} cells likely provide signals to FDCs, the third cell type of the GC triumvirate. As for B cell help, this is not a singular molecular signal nor even a single process. T_{FH} cells are required for the formation and maintenance of GCs and for the generation of most memory B cells and plasma cells. $CD4$ T cell help is generally the primary limiting factor for GCs (114) and therefore for the generation of B cell memory. The control of these processes hinges on T_{FH} regulation of multiple B cell fate decisions, including cell death (Figure 7).

To initiate GC formation, T_{FH} cells must induce GC B cell differentiation by stimulating the expression of Bcl6 in activated B cells and provide cell survival and proliferation signals (Figure 8). Induction of Bcl6 in B cells is a complex process, and the signals involved are still incompletely understood (34, 36).

Once the nascent GC is formed, T_{FH} cells are required to provide cell survival and proliferation signals to GC B cells, stimulate the process of somatic hypermutation in the GC B cells, and then subsequently select successful GC B cells to proceed to the next round (6). This is an iterative process of induction of proliferation and selection of cells constantly performed by T_{FH} cells in the GC as the process of affinity maturation proceeds over days and weeks, through dozens of generations of GC B cells. T_{FH} cells provide survival signals to GC B cells via multiple pathways, including CD40L, IL-4, IL-21, PD-1, and BAFF, which

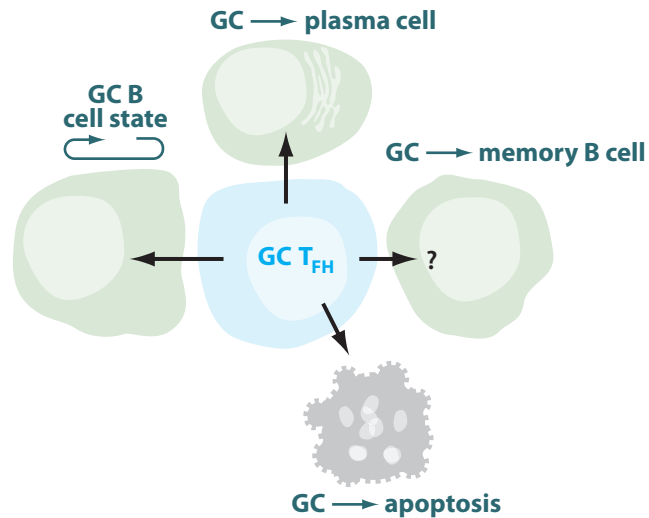


Figure 7

Regulation of B cell differentiation by GC T_{FH} cells. GC T_{FH} cells regulate multiple different B cell differentiation processes within the GC, via numerous molecular signals. GC T_{FH} cells are required for maintenance and proliferation of GC B cells (left). GC T_{FH} cells are also required for induction of differentiation of GC B cells into plasma cells (top). GC T_{FH} cells kill GC B cells that fail to present cognate antigen (bottom). GC T_{FH} cells may also signal GC B cells to differentiate into memory B cells (right). See text for details.

compete with Fas-FasL interactions (Figure 8). GC B cells constitutively express high levels of Fas (Figure 8). In the absence of prosurvival stimuli, GC T_{FH} cells will kill GC B cells via Fas-FasL. T_{FH} cells provide proliferation signals to GC B cells via multiple pathways, including CD40L and IL-21. Somatic hypermutation is fully dependent on expression of activation-induced cytidine deaminase (AID) (Aicda) by GC B cells (186, 187). AID is frequently coexpressed with Bcl6, but it is unclear how AID is regulated in the GC B cell (34) or what signals from T_{FH} cells most directly regulate AID. Overlaid on this process is CSR by the GC B cells, which requires AID (34, 187) and class switch factors produced by T_{FH} cells or other sources (34, 83).

The products of the GC reaction are memory B cells and high-affinity plasma cells (both long-lived and short-lived). GC T_{FH} cells control all these processes (Figure 8). Therefore, GC T_{FH} cells are not statically providing a single input to GC B cells. GC T_{FH} cells must



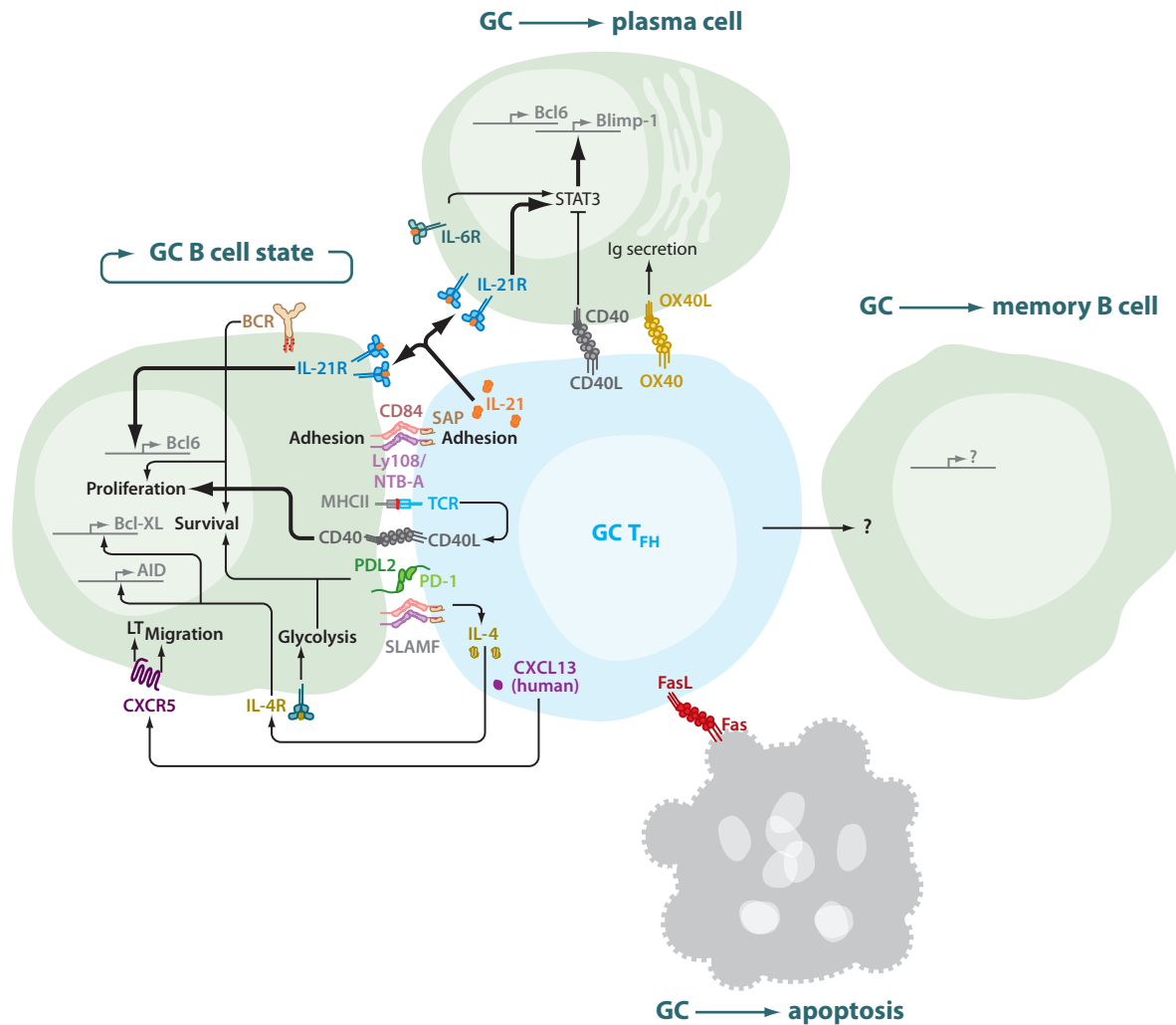


Figure 8

Molecular mechanisms by which GC T_H cells control germinal center B cell differentiation processes (see text for details).

instead provide different signals to maintain the GC B cell state and hypermutation, kill inappropriate GC B cells, and at some point signal GC B cells of appropriately high affinity to stop being GC B cells and instead differentiate into either plasma cells or memory B cells and exit the GC (**Figure 8**). Plasma cell differentiation is well understood (188, 189). Memory B cell differentiation is enigmatic (190, 191). Plasma cell differentiation is heavily controlled by cytokines, particularly those that induce Blimp-1 (**Figure 8**). IL-21 is a potent inducer of plasma

cell differentiation, particularly when CD40L signals are reduced (discussed further below). Numerous other cytokines enhance plasma cell differentiation, including IL-10, IL-6, and IL-4. Memory B cell differentiation is much less clear. There are no known signals that directly drive GC B cell to memory B cell differentiation (**Figure 8**) (190, 191). Memory B cell differentiation may occur as a default program in the absence of plasma cell differentiation signals (190, 191). Alternatively, plasma cell versus memory B cell differentiation may be a bimodal fate

decision for which the signals inducing memory B cell differentiation have not yet been identified (165, 192).

Given that the ability to rapidly evolve high-affinity antibodies via the GC process is the only way the body can compete with the rapid evolution of pathogens—particularly RNA viruses with high mutation rates and multiple generations per day (193)—and that the affinity maturation process depends on rapid GC B cell proliferation and abundant death, it is not surprising that a large proportion of T_{FH} functions are dedicated to B cell survival and proliferation. At the same time, the massive proliferation and mutation of GC B cells have immense potential for development of autoimmunity or lymphoma, and therefore both T_{FH} and GC B cell processes must be tightly regulated, with multiple layers of regulation and counter-regulation.

Functions of T_{FH} Proteins

T_{FH} cells selectively express a wealth of surface proteins (Figures 4, 8; Table 1). This is reflective of the two key features of T_{FH} cells: selective localization (requiring expression of specific chemokine receptors and absence of others) and direct physical interactions with B cells to provide B cell help. T_{FH} cells also exhibit a unique expression profile of cytokines and soluble factors, most notably IL-21 and IL-4 and the intriguing expression of CXCL13 by human T_{FH} cells (Figure 8, Table 1). The functions of these and other key T_{FH} molecules are discussed below. Importantly, there are overlapping functions of multiple T_{FH} molecules at each stage of B cell help, and therefore understanding T_{FH} function requires understanding the integration of multiple collaborative signaling processes that occur simultaneously or sequentially in T-B interactions. Furthermore, many of the surface protein interactions provide bidirectional signals to the GC B cell and the GC T_{FH} cell (Figures 4–8). CD4 T cells can also provide B cell help in extrafollicular regions and elsewhere (194), but those processes are not extensively covered here.

CD40L-CD40. CD40 is central to multiple stages and aspects of B cell activation and differentiation. There is no surface molecule more important to B cell function in mice than the BCR itself. CD40L is the only ligand for CD40, and CD40L is highly expressed by activated CD4 T cells, although surface expression is transient and can be difficult to quantify, as CD40 engagement results in immediate CD40L shedding or internalization (195). In the absence of CD40L or CD40, the block in GC development is absolute (196, 197). The block in plasma cell development in vivo is also extreme (198–200). CD40 is important for B cell activation, proliferation, and survival in vitro and in vivo. Of particular interest, CD40L-CD40 engagement is critical for the maintenance of GC B cells (201). GC B cells are highly proapoptotic and require constant survival signals (Figure 8) (6, 34). Provision of CD40L in vitro can rescue GC B cells from apoptosis (202–204), possibly by sustaining Bcl6 (205). Furthermore, CD40L inhibits plasma cell differentiation (204–206). GC B cell proliferation is maintained by continuous CD40L and IL-21 (207, 208) or CD40L and IL-4 (203–205, 209). Removal of CD40L but maintenance of IL-21 or IL-10 results in a dramatic switch, where the B cell differentiates to a plasma cell (204, 210; S. Crotty, unpublished data). This is likely a central signaling circuit for cell fate decisions in GCs, and both the CD40L and cytokine signals are provided by T_{FH} cells (Figure 8).

While CD40L is required to sustain GCs, CD40 signaling potentially drives proliferation of naive B cells in vitro without acquiring a GC B cell phenotype (209, 211). Furthermore, CD40 ligation induces NF- κ B and IRF4, which reduce Bcl6 expression (34). These differences in biological outcomes have yet to be reconciled. One possibility is that NF- κ B and IRF4 are not limiting factors for repression of Bcl6, though they participate. As such, the primary outcome of CD40 signaling would be proliferation, without Bcl6 induction, while providing the capacity to enhance Bcl6 repression signals from other inputs, such as cytokines. A second possibility is



that CD40 signals differently at different times on the basis of the changing availability of different TRAF proteins (212).

CD40L-CD40 is a bidirectional signaling complex. CD40L signaling to the CD4 T cell is critical for T cell priming and maintenance in most in vivo contexts (199, 213, 214), and CD40-CD40L signaling between T_{FH} and GC B cells almost certainly has significant signaling consequences within T_{FH} cells. Note that although CD40L is critical for T_{FH} function, murine GC T_{FH} cells do not express significantly higher levels of CD40L mRNA or intracellular protein compared with other CD4 T cell subsets. Instead, it is T_{FH} cells' capacity to colocalize with B cells and form extended T-B conjugates that confers their specialized capacity to provide CD40L signals to GC B cells and activated B cells.

SAP. SAP expression in CD4 T cells is indispensable for GC development (80, 81, 124). SAP performs at least three roles in T_{FH} function, and likely more. First, SAP is critically required for the formation of T-B conjugates (81, 125). In the absence of T-B conjugates, GC T_{FH} differentiation is aborted, as discussed above (in the subsection of T_{FH} Differentiation, "Full Polarization: Germinal Center Phase"). In addition, sustained T-B conjugate formation is a central feature and regulator of T cell help to B cells. More extended conjugation time translates into more signals from T_{FH} costimulatory molecules and cytokines, which directly impact B cell differentiation and survival. Therefore, SAP signaling acts as an overall rheostat for the magnitude of T cell help to GC B cells.

Second, SAP is required for induction of IL-4 production by GC T_{FH} cells. Strikingly, GC T_{FH} cells express IL-4 in a Th2-independent manner. IL-4 synthesis by GC T_{FH} cells requires SLAM, the prototypic SAP signaling receptor (25). SLAM is highly expressed by GC B cells (25, 123), allowing for SLAM-SLAM interactions between GC T_{FH} and GC B cells. The SLAM-dependent IL-4 production by GC T_{FH} cells requires signaling through SAP (25) and PKC θ (215). GC T_{FH} SAP-dependent

IL-4 production also almost certainly requires Fyn (215–217) (**Figure 6**).

Third, SAP may modulate crosstalk between SLAM family receptors and the TCR (**Figure 4**) (215, 217). Modulation of TCR signaling may be particularly important for T_{FH} cells, as T_{FH} cells are constantly reengaging with APCs (B cells). Antigen recognition is critical to T_{FH} function, as it is required for the T cell to recognize a cognate GC B cell and provide the necessary signals for GC B cell survival and/or differentiation. However, while massive GC B cell proliferation is intrinsically important for GC function—the purpose of a GC is rapid evolution of the BCRs to high affinity for the pathogen via massively parallel mutation, proliferation, and selection—CD4 T cell proliferation in the GC is not intrinsically important, as the overall size of GCs does not vary substantially over many days and the CD4 T cell frequency does not need to vary. Therefore, although massive GC B cell turnover occurs on a daily basis (>80% replacement), GC T_{FH} must be able to survive high-dose TCR stimulation without undergoing substantive proliferation. Minimal proliferation of GC T cells has been reported (218). This requires modulation of TCR signaling and is an issue worthy of substantial additional investigation. SAP is reviewed in detail by Schwartzberg and colleagues in this volume (127).

SLAM family receptors. CD4 T cells can express four members of the SLAM family receptors that bind SAP: SLAM (CD150), CD84, Ly108 (SLAMF6; NTB-A in humans), and Ly9 (CD229, SLAMF3). CD84 is a SAP-binding SLAM family receptor that is upregulated on activated murine CD4 T cells (125), activated human CD4 T cells (85), and GC B cells (125). CD84 forms high-affinity CD84-CD84 pairs (219) and facilitates prolonged SAP-dependent T-B conjugates (125). CD84^{-/-} mice have a partial defect in GCs, substantially less severe than in SAP-deficient mice (125; S. Crotty and P.L. Schwartzberg, unpublished data). CD84-mediated adhesion therefore contributes to GC T_{FH} differentiation and function and



collaborates with additional SLAM family receptors that also provide overlapping functions.

Ly108/SLAMF6 is a SAP-binding SLAM family member with similarities to CD84, but with somewhat lower binding affinity (220) and two distinct isoforms. The SLAM family locus has been identified as the SLE susceptibility locus *Sle1b* in mice (221) and as a lupus susceptibility locus in humans as well. Differential Ly108 isoform expression in both CD4 T cells and B cells is associated with a genetic disposition for autoantibody production by B6. *Sle1b* mice (221). There is a role for Ly108 in B cell negative selection (222), and it is currently unknown if Ly108 has a particular role in T_{FH} function. Ly108^{-/-} mice do not have a gross defect in GC or antibody responses (R. Kageyama and S. Crotty, unpublished data), but Ly108 does contribute to T-B adhesion (125). It remains an open question whether Ly108 is redundant with other SLAM family receptors for B cell help functions (125).

Ly9 is constitutively expressed at high levels on CD4 T cells. Ly9^{-/-} mice have no defect in T_{FH} differentiation, GCs, or antibody responses (223).

SLAM, the prototypic member of the SLAM family of receptors, forms relatively low-affinity SLAM-SLAM pairs (224) and may not have a significant role in T-B adhesion. SLAM is not required for T_{FH} or GC T_{FH} differentiation (25, 123). Nevertheless, SLAM plays an important nonredundant functional role in T_{FH} cells, as Th2-independent IL-4 production by GC T_{FH} cells is predominantly SLAM dependent (25). As a result of the loss of IL-4, SLAM^{-/-} GC T_{FH} cells have reduced B cell help capability (25). Ly108 may also contribute to T_{FH} cells' IL-4 production (225). The SLAM family receptors are reviewed in greater detail in Reference 127.

IL-21. IL-21 is highly expressed by T_{FH} and GC T_{FH} cells (16, 25, 85, 86, 115), although not exclusively expressed by T_{FH} cells (27–30). IL-21 is the most potent cytokine known for driving plasma cell differentiation in both mice (152) and humans (156, 207, 226, 227).

IL-21 induction of plasma cell differentiation is STAT3 dependent (20, 163) and involves induction of the plasma cell master regulator transcription factor Blimp-1 (the product of the *Prdm1* gene) (33, 189, 228). *Prdm1* is a STAT3 target gene induced by IL-21 (152, 153, 155). In the absence of STAT3, Blimp-1 is not induced, and plasma cell differentiation does not occur (20, 163). IL-21 is also important for optimal GC B cell proliferation (17, 18). GC B cell Bcl6 expression is somewhat reduced in the absence of IL-21 (17, 18).

A complexity of IL-21 is that it induces both Blimp-1 and Bcl6 in B cells (152, 153, 155). IL-21 may signal through both STAT5 and STAT3 to induce or sustain Bcl6 expression (20, 153, 155, 164, 229). Whether IL-21 sustains GC B cell proliferation primarily via Bcl6 or additional gene expression changes is unknown. Because IL-21 stimulates Bcl6 and Blimp-1, the different outcomes of IL-21 signaling—GC B cell maintenance versus plasma cell differentiation (**Figure 8**)—must depend on a combination of IL-21 with additional signals from GC T_{FH} cells to discriminate between these two pathways and cell fate decision. Combinatorial inputs from GC T_{FH} cells may consist of several different cell surface or cytokine signals (**Figure 8**) or may predominantly consist of CD40L + IL-21 maintaining the GC B cell state versus IL-21 alone driving plasma cell differentiation. This is an important issue and requires additional investigation.

In some conditions, IL-21 is not required for initial GC development (22, 72, 230). This implies substantial overlap between the functions of IL-21 and other T_{FH} cytokines for initiating GC B cell differentiation. IL-6 and IL-4 are two cytokines with overlapping GC B cell biology to IL-21, and there may be others. IL-6 also signals through STAT3 (151, 231) and STAT5 (232). IL-21R^{-/-} IL-4^{-/-} mice exhibit a much more severe GC defect than do IL-21R^{-/-} mice, indicating that IL-21 and IL-4 possess partially overlapping functions for GC B cells (230). The GC B cell proproliferative effects of IL-21 and prosurvival effects of IL-4 may intersect.

IL-4. The relationship of CD4 T cell subsets, IL-4 production, and B cell help has long been a conundrum. IL-4 was recognized early as a B cell survival and differentiation factor (233). The subsequent identification of Th1 and Th2 differentiation programs, with IL-4 as the canonical Th2 cytokine (12), led to a widespread association of Th2 cells with B cell help (234). However, GC B cell numbers and serum IgG responses were mostly unaffected in mice with genetic defects blocking Th2 differentiation, beyond alterations in Ig isotypes (5, 133, 235). It has now been determined that B cell help requires T_{FH} cells, not Th2 cells in vivo (13–16). Nevertheless, T_{FH} and GC T_{FH} cells have often been reported to produce IL-4 (2, 32, 44, 56, 57). It has now recently been determined that GC T_{FH} cells specifically produce IL-4 (25, 44), and this production depends on SAP and SLAM and can be independent of Th2 differentiation (25, 215). Production of IL-4 by GC T_{FH} cells is required for optimal B cell help (25, 57).

IL-4 has antiapoptotic activity on B cells (236, 237), which may be particularly crucial in GCs, as GC B cells are extremely susceptible to apoptosis (202). GC B cells express low levels of Bcl2 and high levels of Fas (238–241). IL-4 signaling in B cells prevents apoptosis by inducing the Bcl2 family member Bcl-XL (237). IL-4 signaling also potently increases glucose uptake and metabolism, enhancing cell survival and better poisoning GC B cells for additional proliferation (242). While it has been challenging to identify a nonredundant requirement for GC T_{FH} cell-produced IL-4 in GCs for most experimental systems (5, 14, 133, 235; S. Crotty, unpublished data), IL-4 does contribute to sustaining GCs in some experimental models (57), which may be due to the cumulative effects of the antiapoptotic activity of IL-4 over many rounds of GC B cell proliferation.

Class switch factors. Antibodies of different isotypes have different functionalities that are important for control of different classes of pathogens. Class switch factors are required to instruct isotype-specific B cell CSR. T_{FH} cells

produce cytokines that are class switch factors. As noted above, IL-4 is a class switch factor for murine and human IgG1 and IgE. Expression of IL-4 by GC T_{FH} cells is important for IgG1 CSR (44). IL-21 is a class switch factor for human IgG3, IgA, and IgG1 (243, 244) and murine IgG1 (230). Additional cytokines such as IFN- γ and IL-17 are expressed by GC T_{FH} cells in a context-dependent manner and control appropriate isotype switching to different pathogens (13, 25, 44, 245).

ICOS-ICOSL. As discussed above (T_{FH} Differentiation), ICOS is important for GC formation. ICOS-ICOSL engagement provides signals to CD4 T cells (**Figure 4**) required for initiation and maintenance of T_{FH} differentiation (**Figure 3a**). B cells express ICOSL, which is required for GC formation (16). However, whether ICOSL engagement provides any signals from T_{FH} cells to GC B cells remains unknown (**Figure 8**). ICOSL possesses a limited cytoplasmic tail with a single tyrosine and no known signaling motifs. There is no evidence of ICOSL signaling capacity within a B cell.

PD-1. PD-1 is a potent inhibitory receptor important for T cell tolerance and is associated with CD8 T cell exhaustion during chronic viral infections (246–248). PD-1 is highly expressed on GC T_{FH} cells (25, 73, 86, 112, 249). GC B cells express PD-L1 and elevated PD-L2 (250). PD-1 expression is induced by extended TCR signaling, and PD-1 expression is high on most activated CD4 and CD8 T cells (25, 246, 248, 251). As such, the high level of expression of PD-1 on GC T_{FH} cells is due in part to the repeated cognate T-B interactions that occur in the GC, resulting in long-term TCR stimulation. The strikingly high levels of PD-1 on GC T_{FH} cells may also be due to T_{FH}-specific regulation of PD-1 in addition to TCR activation.

PD-1 is expected to provide an inhibitory signal to GC T_{FH} cells, preventing excess CD4 T cell proliferation in a GC. PD-1-deficient (*Pdcd1*^{-/-}) and PD-1 ligand-double deficient (PD-L1 PD-L2, *Cd274*^{-/-} *Pdcd1lg2*^{-/-}) mice had higher frequencies of T_{FH} cells after



protein immunization (250). Unexpectedly, PD-1- or PD-1 ligand-deficient mice had worsened B cell responses instead of enhanced B cell responses. Although GCs formed normally, they were not sustained optimally because of increased GC B cell apoptosis (250). Reduced numbers of long-lived plasma cells were produced (though of normal affinity) (250). These outcomes were due either to reduced functionality of PD-1-deficient T_{FH} cells or to loss of signals to GC B cells via PD-1 ligands. Absence of PD-1 was associated with alterations in T_{FH} phenotypes such as reduced IL-4 and IL-21 (250), but those alterations remain inconclusive until antigen-specific T_{FH} studies are completed. PD-1 can provide bidirectional signaling through PD1-PDL1 or PD1-PDL2 complexes (248), and PD-1 may provide direct inductive signals from GC T_{FH} to GC B cells. PD-1 may also affect the duration of T-B conjugates by disrupting TCR-induced stop signals (252).

BTLA. BTLA is an inhibitory receptor expressed by B and T cells that binds HVEM and competes with several other receptors, including LIGHT (253). BTLA inhibits T cell proliferation (253). *BTLA*^{-/-} mice develop autoantibodies over time and fatal autoimmune hepatitis (254). BTLA is upregulated on T_{FH} cells, whereas BTLA is stable or downregulated on many other activated CD4 T cells (**Figure 4, Table 1**) (13, 16, 25, 86, 255). GC B cell numbers were increased in *BTLA*^{-/-} mice after protein immunization, as were serum antibody titers (255). Development of CXCR5⁺ T_{FH} cells in vivo was not altered in *BTLA*^{-/-} mice, indicating the T cell proliferation was not grossly altered and BTLA did not control T_{FH} differentiation (255). Interestingly, however, BTLA deficiency was associated with increased T_{FH} function. In particular, IL-21 production by CD4 T cells in vitro was increased in the absence of BTLA (255) (**Figure 4**). The mechanism by which BTLA influences IL-21 is unknown. BTLA on GC T_{FH} cells may also directly regulate GC B cells via HVEM or LIGHT (253).

Chemokine receptors. Chemokine receptors are important for appropriate localization of T_{FH} cells, a central attribute of this CD4 T cell subset. CXCR5 is the canonical T_{FH} marker, and its role in T_{FH} differentiation was discussed above. Downregulation of CCR7 was also discussed. For GC T_{FH} function within GCs, maintenance of CXCR5 expression is critical, as is lack of CCR7 expression to avoid relocalization to T cell areas. Furthermore, GC T_{FH} function in GCs may depend on the precise localization of the GC T_{FH} cell within the GC zones. In humans, GC T_{FH} cells express CXCR4 (1), which is important for localization of GC B cells to the dark zone of the GC (centroblasts) (256). GC T_{FH} cells are enriched in the light zone of GCs (4, 5, 73, 132), along with the highest density of FDCs (6, 131). FDCs induce CXCR4 expression on CD4 T cells (257) but also induce RGS proteins to inhibit GC T_{FH} cell responsiveness to CXCL12 (SDF1) (258), perhaps to transiently restrain GC T_{FH} cell migration to the dark zone until a second signal is received. The classic model of selection posits that T cell help to GC B cells and selection of GC B cells occurs in the light zone (centrocytes) (4, 6). However, GC T_{FH} cells were found in conjugates with B cells throughout GCs (81), and GC B cell proliferation is observed in light and dark zones (135). Modulation of CXCR4 expression on GC T_{FH} cells or modulation of CXCR4 functionality could provide a mechanism to shuttle GC T_{FH} cells between the light and dark zones of the GC to provide different help to GC B cells in each site, either qualitatively or quantitatively. Murine T_{FH} cells are not reported to differentially express CXCR4 protein (19), although CXCR4 mRNA is high in T_{FH} and GC T_{FH} cells (88), and CXCR4 is present on extrafollicular helpers in MRL^{lpr} mice (194).

T_{FH} cells need to avoid expression of inflammatory chemokine receptors that would recruit T_{FH} cells away from B cells and GCs and into the T cell zone, red pulp, or extralymphoid sites. T_{FH} cells have limited expression of CCR5, CCR2, CX3CR1, and related inflammatory cytokine receptors (13, 25).



Modulation of integrin expression and signaling is also probably important for T_{FH} cell localization, but this area of research has not been explored.

Additional surface molecules. A wealth of surface receptors expressed on T_{FH} cells likely have important functions in T_{FH} differentiation or T_{FH} help to B cells and need additional study. OX40 and CD30L are two that have received some attention. OX40 is important for activated T cell survival and is a potent inducer of antiapoptotic molecules (see T_{FH} Differentiation section). OX40 is expressed on many activated CD4 T cells (98), including GC T_{FH} cells, although levels vary depending on mouse strain (103). The development of normal GCs and long-term class switched antibody responses in OX40^{-/-} mice (102) is at least in part due to collaboration between OX40 and CD30L antiapoptotic signals in CD4 T cells (259). T_{FH} cells express elevated amounts of CD30L in both humans (85, 86) and mice (88). CD30^{-/-} mice form GCs but cannot sustain them, most likely because of a defect in CD4 T cell survival that was further exacerbated in CD30^{-/-}OX40^{-/-} mice (259). Bidirectional OX40-OX40L and CD30-CD30L signaling can occur (98, 260). Engagement of OX40L on activated B cells was reported to enhance Ig secretion by plasma cells (Figure 8) (261). It appears that OX40L does not increase the number of plasma cells but improves their maturation.

HUMAN T_{FH} CELLS

Mouse data are only useful insofar as they model the human condition. Understanding the generation of human GCs, memory B cells, and memory plasma cells is essential for understanding how to rationally design vaccines to generate immunological memory (177, 262, 263). Such an understanding is also important for understanding major human cancers (34) and autoimmune diseases (264).

T_{FH} cells were first identified in humans by studying CD4 T cell populations in tonsil and

identifying a large population of CXCR5⁺ CD4 T cells that predominantly localized within GCs (1–3). Human T_{FH} cells were demonstrated to be highly activated and to possess increased B cell help properties compared with CXCR5⁻ tonsillar CD4 T cells in vitro. A substantial population of CXCR5⁺ CD4 T cells was also identified within the memory CD4 T cell compartment (CD45RO⁺) in human peripheral blood (2, 3). However, the relationship between T_{FH} cells and blood CXCR5⁺ CD4 T cells has remained unclear and contentious (see below).

Human T_{FH} Markers

Human T_{FH} cells express markers that generally closely parallel murine T_{FH} cell markers and were, in fact, usually first identified in humans (85). The high percentage of close matches between human and mouse gene expression profiles for T_{FH} and GC T_{FH} cells bodes well for translating findings between the two species. Most T_{FH} markers directly correlate between the two species (Table 1) (16, 25, 26, 85, 86, 115). Tonsillar CXCR5⁺ CD4 T cells are T_{FH} cells (1, 3, 85). These T_{FH} cells can be subdivided into two populations, CXCR5^{int} and CXCR5^{hi} (or CXCR5^{int}ICOS^{int} and CXCR5^{hi}ICOS^{hi}) (26, 86), which have gene expression profiles and functionalities that correspond well with those of murine T_{FH} and GC T_{FH} cells (Table 1) (25, 26, 86; M. Kroenke and S. Crotty, unpublished data).

Regarding in vitro studies with human CD4 T cells, as noted above, IL-21-producing cells without further characterization are of indeterminate origin and should not be classified as T_{FH} cells without characterization of Bcl6, CXCR5, and/or additional T_{FH} differentiation markers. This is because many CD4 T cells that are not T_{FH} cells can express substantial IL-21, including human Th17 cells (95, 265, 266) and some Th1 cells (26, 160). CXCR5 is also a challenging marker in vitro for human CD4 T cells. Human T_{FH} cells rapidly lose CXCR5 expression in vitro (1, 2, 110).



The Genetics of Human T_{FH} Cells

Human genetic deficiencies provide crucial insights into human biology (186, 267, 268). There are excellent genetic data supporting central roles of SAP, CD40L, and ICOS in human T_{FH} biology.

SAP/SH2D1A and XLP. The human genetic disease XLP manifests as an increased susceptibility to a range of pathogens during childhood, with a particularly pronounced susceptibility to Epstein-Barr virus (EBV), which is frequently fatal in children with XLP. *Sb2d1a*, the gene encoding SAP, was identified as the defective gene causing XLP (127). Most human *Sb2d1a* mutations result in loss of expression of SAP (269). Using *sb2d1a*^{-/-} mice, SAP was demonstrated to be essential in CD4 T cells for GC responses and the generation of memory B cells and long-lived plasma cells (80). Subsequently, it was confirmed in humans that absence of SAP results in an extreme loss of GCs (in the two patients available for analysis) (270), aberrant CD4 T cell functions (271), and severe reductions in memory B cells (270–272). SAP binds to SLAM family receptors, and the SLAM family receptor locus has been identified as a murine autoimmune autoantibody susceptibility locus (221). Given the central role of SAP in antibody responses, it is likely that SAP-dependent signaling by SLAM family receptors is responsible for the increased autoantibody responses associated with certain SLAM family receptor alleles (221). However, additional mechanisms, such as B cell central tolerance, are potentially involved (222). These genetic findings support a central role for SAP in human T_{FH} cells. SAP and the SLAM family receptor functions are discussed further above (T_{FH} Differentiation and T_{FH} Functions) and by Schwartzberg and colleagues (127). Further studies are needed to determine mechanistic roles of SAP in human T_{FH} differentiation and function.

X-linked inhibitor-of-apoptosis (XIAP) mutations cause a human genetic disease with some overlapping phenotypes to *sb2d1a* mutations,

including lymphoproliferation (273). Whether XIAP patients have a defect in NKT cell development (273, 274), which is a signature of SAP deficiency in mice and humans, is controversial. Although both are encoded on the X chromosome, XIAP and SAP do not appear to interact in the same signaling pathways and appear to be phenocopies at the level of lymphoproliferation. *XIAP*^{-/-} mice have normal antibody responses to T-dependent antigens and normal NKT development (275).

CD40L and HIGM. CD40L is important in numerous aspects of T cell differentiation and function. A fraction of individuals with hyper-IgM syndrome (HIGM) have mutations in either CD40L or CD40 (186). The most pronounced phenotype of these individuals is the lack of circulating IgG and failure to mount isotype switched antigen-specific antibody responses, as the name implies (186). CD40L-deficient patients have a severe lack of memory B cells (276, 277), and the minimal and aberrant somatic hypermutation observed in those B cells indicates the absence of GCs (277). This is consistent with the critical requirement for CD40L at all T-dependent stages of antigen-specific B cell responses.

Note that CD40L is widely expressed by activated CD4 T cells and is not at all restricted to T_{FH} cells. This is consistent with murine CD4 T cell immunology and with the pleiotropic roles of CD40L in the immune system. Presumably, CD40L expression by T_{FH} cells undergoes some degree of specialized regulation, given the central importance of CD40-CD40L interactions in GCs; however, that regulation apparently does not occur at the transcriptional level, as T_{FH} cells express CD40L mRNA at levels comparable to other activated human CD4 T cell subsets (85, 86).

Although the bulk of the human CD40L-deficiency phenotype has been attributed to CD40L expression by CD4 T cells (and now presumably T_{FH} cells) engaging with CD40 on B cells and inducing proliferation and differentiation of B cells, it must be noted that

CD40L-CD40 interactions induce bidirectional signaling. In the absence of CD40L on CD4 T cells, defects in CD4 priming and CD4 memory are observed in humans (278, 279). This is consistent with comparable defects in mice and is due primarily to the importance of CD40 expression by DCs and B cells in priming and sustaining T cell responses (212).

ICOS deficiency. ICOS was first identified and cloned in humans (280) and was observed to be highly expressed on GC CD4 T cells (280). ICOS was then demonstrated to be important for CD4 T cell help to B cells in mice (281, 282). Based on those findings, ICOS became a candidate gene for human immunodeficiencies. It was determined that a subset of common variable immunodeficiency patients possessed no ICOS, due to a 1.8-kb genomic deletion (108, 283). Human ICOS deficiency causes a severe loss of memory B cells (109) and completely prevents all antigen-specific IgG responses to vaccines (109). While ICOS deficiency clearly affects human GCs, direct data are limited (109), which is unsurprising given the extreme rarity of individuals with ICOS mutations. The near complete absence of antigen-specific IgG responses, the occurrence of defective IgM responses, and the absence of memory B cells in ICOS-deficient patients was consistent with the high susceptibility of the individuals to various bacterial, viral, and fungal infections (109), which were largely treatable by regular injection of intravenous immunoglobulin (109, 284). The clinical features of ICOS deficiency also confirm the central role of T-dependent antibody responses in protective immunity in humans.

One interesting aspect of human ICOS-deficient patients is that the genetic defect is not a phenocopy of CD40L deficiency. ICOS-deficient patients have severe memory B cell, antigen-specific IgG, and total IgG defects comparable to CD40L-deficient patients. However, the canonical hyper-IgM phenotype associated with CD40L deficiency does not occur in ICOS deficiency (109).

STAT3. The human genetic deficiency hyper-IgE syndrome (HIES) is caused by heterozygous STAT3 mutations that act in a dominant-negative manner in STAT3 dimers (285, 286). STAT3 does not appear to be important for human T_{FH} differentiation in a cell-intrinsic manner. While STAT3 is required in humans for IL-6-mediated Th17 differentiation (161, 287), CXCR5⁺ CD4 T cells are present at normal frequencies in the blood of HIES patients (161). Furthermore, STAT4 has been implicated as the STAT responsible for IL-21 induction in human CD4 T cells (160). STAT3 signaling in B cells is important for the generation of memory B cells (20, 288) and long-lived plasma cells (20), although T-dependent antibody responses do occur in the absence of STAT3 (20, 288). IL-21 is a potent inducer of human plasma cell differentiation via STAT3-mediated Blimp-1 induction (152, 155, 156, 207, 226, 227). IL-21 signaling in B cells from HIES humans is severely reduced (20). This is consistent with the importance of IL-21 for GC B cells and plasma cells in mice (17, 18), although STAT3's role downstream of IL-21 may be less critical for GCs and memory B cells in mice (163). These data indicate that STAT3 is not needed for human T_{FH} differentiation or function, but STAT3 is important in the B cells that are the target of IL-21 produced by human T_{FH} cells.

Human T_{FH} Differentiation

The signals that specifically instruct the differentiation of human T_{FH} cells remain unclear. One approach has been to focus on the induction of IL-21 expression by CD4 T cells, as IL-21 is the most important cytokine expressed by T_{FH} cells. Two recent studies made the surprising finding that IL-12 induces IL-21 production in a STAT4-dependent manner (26, 160). In murine CD4 T cells, IL-6 (27, 69) or IL-21 (16, 27) signaling induces IL-21 expression, which is presumed to be STAT3 dependent, based on Th17 work (95)



and *in vivo* data (16). IL-21 does induce some expression of IL-21 in human CD4 T cells, but the expression is not sustained in comparison to IL-12 (26, 160). These differences are surprising given that mature human and murine T_{FH} cells have similar gene expression profiles.

What is unclear is the relationship between IL-21 expression and T_{FH} differentiation. There is not a one-to-one relationship between IL-21-expressing cells and T_{FH} cells. Therefore, while IL-21 is an important T_{FH} cytokine, expression of IL-21 is not exclusive to T_{FH} cells. One possibility is that IL-12-induced IL-21 production represents the earliest step of human T_{FH} differentiation (26) and that full T_{FH} differentiation occurs in a second stage following additional signals. In agreement with that model, ICOS is upregulated by IL-12 (26, 160). However, CXCR5 expression was not consistently enhanced by IL-12 (26, 160), and Bcl6 change was modest. Therefore, other signals may be involved. A second possibility is that T_{FH} differentiation is modular—consisting of multiple gene expression modules—and requires multiple parallel signals to induce the expression of each module in parallel. Examples of potential gene expression modules include an IL-21 expression module and a migration module (CXCR5, CXCR4, reduced CCR7). Whether Bcl6's role as a master regulator of murine T_{FH} differentiation (13–15) is conserved in human T_{FH} differentiation is also unknown. Mechanisms regulating human T_{FH} differentiation are an important issue in human immunology for enhancing vaccine development and for autoimmunity treatment. Understanding the signals instructing T_{FH} differentiation both in mice and humans is worthy of extensive further investigation to elucidate the molecular mechanisms of this differentiation process.

Compared with murine GC T_{FH} cells, human GC T_{FH} cells appear to be even more fully distinct from Th1, Th2, and Th17. Few Th1, Th2, or Th17 characteristics are evident in GC T_{FH} cells (15, 26, 86, 87), with the

exception of IL-4 (2, 26), which is likely made in a T_{FH}-specific (Th2-independent) manner (25).

Tonsil T_{FH} Cells, Blood CXCR5⁺ CD4 T Cells, and Memory T_{FH} Cells

A large population of CXCR5⁺ CD4 T cells is present within the memory CD4 T cell compartment (CD45RO⁺) in human peripheral blood (1–3). However, the relationship between T_{FH} cells and blood CXCR5⁺ CD4 T cells has remained unclear and controversial. Given that both tissue T_{FH} cells and blood CXCR5⁺ CD4 T cells express CXCR5, whereas tissue T_{FH} cells were highly activated and blood CXCR5⁺ CD4 T cells are resting cells with a memory phenotype, the simplest interpretation was that blood CXCR5⁺ CD4 T cells are memory T_{FH} cells (1). Three findings have cast doubt on this relationship. First, while tonsil T_{FH} cells exhibit heightened B cell help activity *in vitro*, blood CXCR5⁺ CD4 T cells do not (2). Second, microarray-based gene expression analysis showed large differences in gene expression patterns between the two cell types (86). Third, blood CXCR5⁺ CD4 T cells do not express elevated levels of Bcl6 protein (M. Kroenke and S. Crotty, unpublished data). An implied counterargument to those observations has been that tonsil T_{FH} cells are highly activated and are therefore not expected to be identical to resting CXCR5⁺ CD4 T cells in the periphery (1). More important evidence supporting the argument that CD45RO⁺ CXCR5⁺ CD4 T cells are memory T_{FH} cells is that humans with genetic deficiencies in ICOS have severely reduced GC responses (109) and blood CXCR5⁺ CD4 T cells are absent from those individuals (110). The loss of CXCR5⁺ memory CD4 T cells is selective, as ICOS-deficient humans have normal frequencies of CD4 T cells overall, whereas CD40L-deficient humans have an overall loss of memory CD4 T cells (110). Therefore, based on data from ICOS-deficient humans, blood CXCR5⁺ CD4 T cells appear to be memory T_{FH} cells that have passed



through a GC reaction (110). However, given the contradictory findings discussed above, the ontogeny of blood CXCR5⁺ CD4 T cells remains an open issue.

A related question is: Are there memory T_{FH} cells at all? This was discussed earlier for murine T_{FH} cells (see the T_{FH} Differentiation section), and the question obviously has greater implications in humans. In addition to the CXCR5⁺CD45RO⁺PD-1⁻ICOS⁻ cells normally found in peripheral blood, rare T_{FH} cells with a GC T_{FH} phenotype (PD-1^{hi}ICOS^{hi}) are found in the peripheral blood of a subset of humans with SLE (24).

Human T_{FH} Functions

Human T_{FH} functions largely parallel murine T_{FH} functions, to the extent that they have been assessed in vitro and to the extent that they can be inferred by their largely overlapping gene expression pattern with murine T_{FH} cells (Table 1) (16, 25, 26, 85, 86, 115). One notable exception is CXCL13, the ligand for CXCR5. In mice, CXCL13 is expressed by stromal cells (289) and is not expressed by murine T_{FH} or GC T_{FH} cells (88). In humans, CXCL13 is made at high levels by T_{FH} cells (86–87) and not by other CD4 T cells. CXCL13 is functional and able to attract B cells (87). FDCs bind and present large quantities of CXCL13 on their surface in GCs (131). Expression of CXCL13 by T_{FH} cells may enhance recruitment of GC B cells to the light zone, where most T_{FH} cells and FDCs reside. Alternatively, CXCR5 binding of CXCL13 can also provide signals to B cells distinct from migration, and so the expression of CXCL13 by T_{FH} cells may have multiple effects on GC B cells. CXCL13 binding to CXCR5 on naive B cells induces LTα1β2 expression (290) (Figure 8).

IL-10 is primarily associated with strongly immunosuppressive functions in mice, but in humans IL-10 is also a cytokine that promotes plasma cell differentiation (204). Human T_{FH} and GC T_{FH} cells do not express elevated levels

of IL-10 compared with other activated CD4 T cells (26, 85, 86).

OPEN ISSUES AND FUTURE DIRECTIONS

Much remains to be understood about T_{FH} cells in both mice and humans. Now that these cells are recognized as a clear subset of CD4 T cells with the critical function of B cell help and that Bcl6 is a central regulator of T_{FH} differentiation, it is important to understand the differentiation of these cells and their functions in detail. Regarding T_{FH} differentiation, what minimal signals are required to instruct T_{FH} differentiation? Are the critical signals from both cytokines and cell surface receptors? How would one enhance T_{FH} differentiation after immunization with a vaccine? How would one best block T_{FH} differentiation in the context of autoimmunity? How is T_{FH} differentiation distinguished from, and interrelated to, other CD4 T cell differentiation programs such as Th1, Th2, Th17, and Treg? What signals regulate Bcl6 induction? What signals regulate Bcl6 maintenance? What transcription factors other than Bcl6 are important for T_{FH} differentiation? What genes does Bcl6 regulate? What T_{FH} functions does Bcl6 directly regulate? Are there multiple subsets of T_{FH} cells? What do they do?

Many functions of T_{FH} cells have been identified or inferred from gene expression analysis. Are major T_{FH} B cell help functions still left unidentified? Which signals from T_{FH} cells are most important for which attributes of the multiple B cell differentiation pathways and survival and proliferation signals? What are the molecular mechanisms governing how those molecules signal to B cells?

In humans, much remains unknown about T_{FH} cells. What signals induce T_{FH} cells? Are IL-21-secreting CD4 T cells equivalent to T_{FH} cells? What is the role of Bcl6 in human CD4 T cells? Are there memory T_{FH} cells? Can T_{FH} or memory T_{FH} cells be identified in human peripheral blood for analysis after

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vaccinations? Given the importance of T-dependent B cell responses in protective immunity to a staggering variety of pathogens, further understanding of T_{FH} is necessary for improving public health by development of new and better vaccines.

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