The origins, function, and regulation of T follicular helper cells

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The generation of high-affinity antibodies (Abs) plays a critical role in the neutralization and clearance of pathogens and subsequent host survival after natural infection with a variety of microorganisms. Most currently available vaccines rely on the induction of long-lived protective humoral immune responses by memory B cells and plasma cells, underscoring the importance of Abs in host protection. Ab responses against most antigens (Ags) require interactions between B cells and CD4+ T helper cells, and it is now well recognized that T follicular helper cells (Tfh) specialize in providing cognate help to B cells and are fundamentally required for the generation of T cell–dependent B cell responses. Perturbations in the development and/or function of Tfh cells can manifest as immunopathologies, such as immunodeficiency, autoimmunity, and malignancy. Unraveling the cellular and molecular requirements underlying Tfh cell formation and maintenance will help to identify molecules that could be targeted for the treatment of immunological diseases that are characterized by insufficient or excessive Ab responses.

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Cognate interactions between Ag-specific B cells, CD4+ T helper cells, and DCs in response to foreign Ag lead to the formation of germinal centers (GCs). GCs are specialized structures in B cell follicles of secondary lymphoid tissues where somatic hypermutation of Ig variable (V) region genes and selection of high-affinity B cells occur, followed by differentiation of long-lived memory or plasma cells (PC). This process ensures the development of long-lived humoral immunity after infection or vaccination with T cell–dependent (TD) Ag and is a unique feature of the mammalian immune system (Tangye and Tarlinton, 2009; Goodnow et al., 2010).

It has been known for decades that CD4+ T cells are required for the formation of productive GCs, as well as for generating Ag-specific memory and PCs (Miller et al., 1965; Tangye and Tarlinton, 2009; Goodnow et al., 2010; Crotty, 2011). However, the exact nature of the CD4+ T cell subset that provides help to B cells remained enigmatic. Early studies implicated Th2 cells, as they produce IL-4, which induces isotype switching and Ig secretion. However, mice lacking key regulators of Th2 development are still able to form GCs and elicit TD Ab responses (Nurieva et al., 2008; O'Shea and Paul, 2010; Crotty, 2011).

In recent years, T follicular helper (Tfh) cells have emerged as the key cell type required for the formation of GCs and the generation of long-lived serological memory (Vinuesa et al., 2005b; King et al., 2008; Crotty, 2011). Similar to other CD4+ T cell lineages (Th1, Th2, Th17, and regulatory T [T reg] cells), the generation of Tfh cells requires signaling pathways activated downstream of cytokines and cell surface molecules, and the subsequent activation of specific transcription factors. Here, we discuss recent advances in understanding the requirements for the generation and acquisition of effector function by Tfh cells.

A distinct subset of effector CD4+ T cells

The term "Tfh cell" was first used in the year 2000 to describe a subset of CD4+ T cells present in human lymphoid tissues (tonsils) that expresses the chemokine receptor CXCR5 and functions primarily to provide help to B cells (Breitfeld et al., 2000; Schaerli et al., 2000). Expression of CXCR5, together with loss of

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the T cell zone–homing chemokine receptor CCR7, allows Tfh cells to relocate from the T cell zone to the B cell follicles, where they are positioned to directly support B cell expansion and differentiation (Ansel et al., 1999; Hardtke et al., 2005; Haynes et al., 2007; Fig. 1).

Tfh cells can be distinguished from other CD4$^+$ T cell lineages by their low expression levels of cytokines (IFN-γ, IL-4, and IL-17) and transcription factors (T-bet, GATA3, and RORγt) characteristic of Th1, Th2, and Th17 cells, respectively (Chtanova et al., 2004; Kim et al., 2004; Rasheed et al., 2006; Nurieva et al., 2008; Vogelzang et al., 2008; Ma et al., 2009). Furthermore, Tfh cells express a unique combination of effector molecules that are critical for their development and function, including high levels of the surface receptors ICOS, CD40 ligand (CD40L), OX40, PD-1, BTLA and CD84, the cytokine IL-21, the cytoplasmic adaptor protein SLAM-associated protein (SAP), and the transcription factors Bcl-6 and c-Maf (Breitfeld et al., 2000; Schaerli et al., 2000; Chtanova et al., 2004; Vinuesa et al., 2005a; Rasheed et al., 2006; Haynes et al., 2007; Lim and Kim, 2007; Johnston et al., 2009; Ma et al., 2009; Deenick et al., 2010; Kroenke et al., 2012). These molecules play critical roles in promoting the activation, differentiation, and survival of B cells and/or CD4$^+$ T cells. For instance, CD40L rescues B cells from apoptosis and promotes their proliferation (Tangye et al., 2012), and IL-21 enhances the differentiation of CD40L-stimulated human B cells, inducing secretion of all Ig isotypes (Bryant et al., 2007; Avery et al., 2008; Ettinger et al., 2008). Engaging ICOS on CD4$^+$ T cells induces the production of B helper cytokines, such as IL-10 and IL-21 (Hutloff et al., 1999; Bauquet et al., 2009; Chevalier et al., 2011), and SAP is necessary for CD4$^+$ T cells to form stable contacts with cognate B cells to promote their activation and differentiation (Qi et al., 2008; Cannons et al., 2010). The importance of these molecules in Tfh function is underscored by the finding that targeted deletion of *Cd40/Cd40l*, *Icos*, *Il21/Il21r*, or *Sh2d1a* (encoding SAP) in mice, or loss-of-function mutations in *ICOS*, *CD40L*, and *SH2D1A* in humans (Al-Herz et al., 2011; Crotty, 2011; Tangye et al., 2012), severely

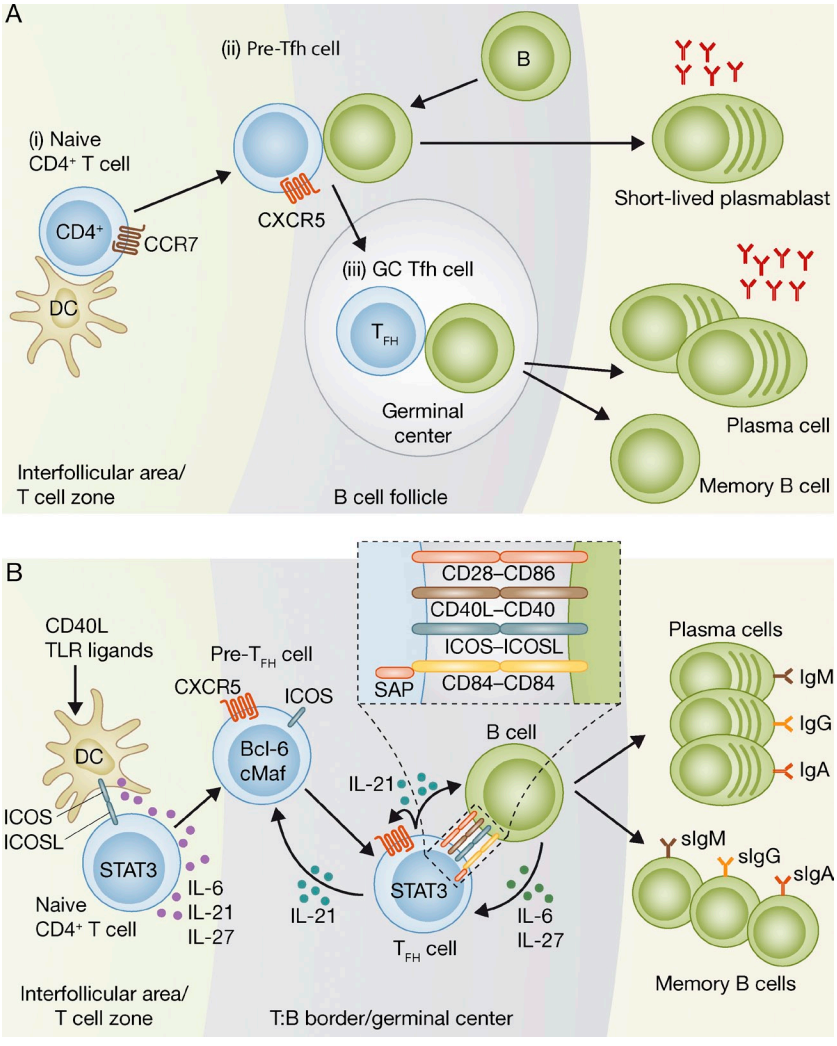

Figure 1. Anatomical localization and cellular requirements for Tfh cell generation. (A; i) Naive CD4$^+$ T cells are activated in interfollicular areas or T cell zones of lymphoid tissues after recognition of peptide–MHC class II complexes on DCs. (i) DCs provide signals that up-regulate CXCR5 and down-regulate CCR7 on CD4$^+$ T cells allowing them to migrate to B cell follicles. (ii) At the T cell–B cell border, pre–Tfh cells interact with activated B cells presenting cognate Ag. This results in the pre–Tfh cells delivering help to the B cells, resulting in their differentiation into short-lived extrafollicular plasmablasts or their migration into follicles to form GCs. Ongoing stimulation and Ag presentation provided by B cells drives the full development of Tfh cells. (iii) Within GC, Tfh cells continue to provide help to the B cells, supporting the GC reaction and facilitating the generation of long-lived plasma cells and memory B cells. Reciprocal signals provided by the B cells are also crucial for sustaining the Tfh cells. (B) Initial priming of naive CD4$^+$ T cells by DCs induces expression of Bcl-6 and CXCR5; this requires ICOS/ICOS-L interactions. DCs may produce IL-6 and IL-27, which promote Bcl-6 and c-Maf expression, as well as IL-21 production by CD4$^+$ T cells, in a STAT3-dependent fashion. CXCR5-mediated relocation of Bcl-6$^+$CXCR5$^+$ pre–Tfh cells to the T cell–B cell border allows subsequent interactions with Ag-specific B cells. The Tfh program is imprinted after subsequent interactions with B cells in the GC. The interactions are dependent on the formation of stable T cell–B cell conjugates, which requires CD4$^+$ T cell–intrinsic signaling via SAP-associating receptors (CD84) and involves CD40L/CD40, ICOS/ICOS-L, and CD28/CD86. IL-21 produced by Tfh cells can act in an autocrine manner to maintain Tfh cells at various stages of differentiation. Similarly, B cell–derived IL-6, and possibly IL-27, could contribute to the maintenance of Tfh cells. Tfh cells mediate differentiation of GC B cells into memory and plasma cells via the provision of CD40L and IL-21.

compromise the generation of robust GC reactions and subsequent long-lived serological memory against TD Ags (Table 1).

Subsets of Tfh cells

Provision of help to B cells during TD B cell responses requires that $CD4^+$ T cells be in close proximity to cognate B cells. This occurs at the T cell–B cell border/interfollicular zones and within GCs (Fig. 1 A). Because of its role in directing cells toward B cell areas, CXCR5 was an early defining marker of Tfh cells. More recently, Bcl-6 has been described as a key molecular feature of these cells. It is now clear, however, that there is marked heterogeneity among $CD4^+$ $CXCR5^+Bcl\text{-}6^+$ T cells with respect to phenotype, function, and anatomical location. Indeed, the $CXCR5^+Bcl\text{-}6^+$ population comprises at least two subsets: early Tfh cells, which most likely provide help at the T-B border or in interfollicular zones before GC formation (Kerfoot et al., 2011; Kitano et al., 2011; Lee et al., 2011), and later-arising Tfh cells that are found in the GC (Yusuf et al., 2010; Choi et al., 2011).

In human tonsils, there are two major $CD4^+CXCR5^+Bcl\text{-}6^+$ T cell populations that can be distinguished based on high versus low expression of CXCR5. Those expressing the highest levels of CXCR5 also express the most abundant PD-1, ICOS, CD40L, and Bcl-6, and contain the highest frequencies of cells producing IL-21 and the CXCR5 ligand CXCL13 (Lim and Kim, 2007; Ma et al., 2009; Bentebibel et al., 2011; Kroenke et al., 2012). These cells also express the highest levels of IL-4 (Ma et al., 2009; Bentebibel et al., 2011; Kroenke et al., 2012). Although this finding seems to contradict initial findings showing that IL-4 was not prominently expressed by Tfh cells, these earlier studies examined bulk $CD4^+CXCR5^+$ T cells, rather than distinct subsets (Chtanova et al., 2004; Kim et al., 2004; Rasheed et al., 2006). CD57 is expressed by ∼30% of tonsillar $CD4^+CXCR5^{hi}$ T cells (Rasheed et al., 2006; Lim and Kim, 2007; Ma et al., 2009), and this population localizes to GCs (Banerjee and Thibert, 1983; Hutloff et al., 1999; Kim et al., 2001). Importantly, $CD4^+CXCR5^{hi}$T cells are capable of supporting Ig class switching and Ab secretion by naive, memory, and GC B cells in vitro (Kim et al., 2001; Avery et al., 2008; Ma et al., 2009; Bentebibel et al., 2011). Thus, in humans the $CXCR5^{hi}CD57^+$ subset of $CD4^+$ T cells correspond to GC Tfh cells. The exact relationship between the $CD57^-$ and $CD57^+$ subsets of $CXCR5^{hi}$ $CD4^+$ T cells remains to be determined.

$CXCR5^{lo}Bcl\text{-}6^+$ $CD4^+$ T cells also express low levels of ICOS and are found outside of GCs. These cells provide help to naive and memory, but not GC, B cells (Bentebibel et al., 2011) and may function during the initial phase of B cell activation at the T cell–B cell border. It is also possible that these cells migrate to the GC after initial interactions with naive B cells and mediate the differentiation of GC B cells—a possibility consistent with the finding that $CXCR5^{lo}ICOS^{lo}$ $CD4^+$ T cells up-regulate PD-1 and ICOS to levels approximating that on GC Tfh cells after co-culture with naive B cells (Bentebibel et al., 2011). Thus, $CD4^+$ T cells expressing low levels of CXCR5 not only provide help to B cells early in the response, but probably also contain precursors from which GC Tfh cells emerge.

Similarly, murine $CXCR5^{hi}Bcl\text{-}6^+$ $CD4^+$ T cells include those that are found in the follicle or within GCs. GL7 has been reported to mark a subset of murine Tfh cells expressing the highest levels of CXCR5, Bcl-6, PD-1, ICOS, CD200, and IL-21 that localize to GCs (Yusuf et al., 2010). Another study, however, found GL7 expressed by $CD4^+$ $CXCR5^+$ T cells in the interfollicular zone (Kerfoot et al., 2011). Additional studies are required to definitively show whether GC Tfh cells can be differentiated from non-GC Tfh cells based on phenotype rather than localization alone. Another subset of murine $CD4^+$ T cells provides the requisite help for the early differentiation of B cells into extrafollicular plasmablasts producing low-affinity Ig (Lee et al., 2011). Reminiscent of Tfh cells, these extrafollicular Th cells express Bcl-6 and CXCR5 and provide help to B cells via the production of IL-21. However, they can be distinguished from GC Tfh cells by lower levels of PD-1 and their localization to the T cell–B cell border (Lee et al., 2011). It is likely that these cells represent progenitors of Tfh/GC Tfh cells, and they may well correspond to human $CXCR5^{lo}ICOS^{lo}Bcl\text{-}6^+$ $CD4^+$ T cells that localize outside of GCs (Bentebibel et al., 2011; Fig. 1 A).

Substantial heterogeneity among Tfh cells was recently revealed by the description of IL-21 reporter mice (Lüthje et al., 2012). Approximately 20–40% of Tfh (i.e., $CXCR5^+PD\text{-}1^+$ $CD4^+$) cells expressed IL-21 during immune responses against peptide Ag, and these cells also expressed substantially higher levels of IFN-γ, IL-10, and *Tbx21* (encoding T-bet) than did $IL\text{-}21^-$ Tfh cells. On the other hand, *Bcl6*, *GATA3*, and *Il4* expression, as well as ex vivo proliferation, were comparable between $IL\text{-}21^+$ and $IL\text{-}21^-$ Tfh cells. Transfer studies demonstrated that $IL\text{-}21^-$ Tfh cells could give rise to $IL\text{-}21^+$ Tfh cells after reexposure to the initiating Ag, demonstrating a precursor/product relationship, at least in the setting of a recall response.

Requirements for naive $CD4^+$ T cell–to–Tfh differentiation

Cytokines. The key features of Tfh cells—production of IL-21, elevated expression of CXCR5, ICOS, and BCL-6, and provision of B cell help—have been used as surrogates to identify cytokines that potentially contribute to their generation.

Several cytokines, including IL-6, IL-21, and IL-27, induce IL-21 production by naive murine and human $CD4^+$ T cells in vitro in a STAT3-dependent manner (Huber et al., 2008; Nurieva et al., 2008; Suto et al., 2008; Dienz et al., 2009; Batten et al., 2010; Eto et al., 2011; Lu et al., 2011; Ma et al., 2012). Interestingly, IL-12 also induces IL-21 in human and mouse $CD4^+$ T cells (Schmitt et al., 2009; Ma et al., 2009, 2012; Nakayamada et al., 2011; Eto et al., 2011; Diehl et al., 2012). Although IL-12 is often associated with activation of STAT4, its ability to induce IL-21 expression was also largely STAT3 dependent (Nakayamada et al., 2011; Ma et al., 2012). Activation of $CD4^+$ T cells with IL-21–inducing cytokines

also resulted in elevated expression of CXCR5, ICOS, and Bcl-6, and improved Ab production by co-cultured B cells (Nurieva et al., 2008; Dienz et al., 2009; Ma et al., 2009, 2012; Schmitt et al., 2009; Eto et al., 2011; Nakayamada et al., 2011; Diehl et al., 2012). Collectively, these findings suggested that, akin to Th1, Th2, and Th17 lineages, cytokines present within the stimulating microenvironment in vivo influence the commitment of naive $CD4^+$ T cells to the Tfh lineage. However, the in vitro conditions used do not completely recapitulate physiological Tfh differentiation, as in vitro–generated Tfh cells express lower levels of CXCR5, Bcl-6, and ICOS than GC Tfh cells and lack expression of other Tfh-associated molecules (Ma et al., 2009, 2012; Schmitt et al., 2009; Eto et al., 2011). Thus, the differentiation state of in vitro–derived Tfh cells more closely corresponds to pre–Tfh than to GC Tfh cells.

Table 1. Effects of gene deficiency on Tfh cell formation and identification of regulators of Tfh formation

Gene/protein	Effect of KO on Tfh cells	References
Cytokines		
IL-6	Mild/moderate decrease/no effect	Nurieva et al., 2008; Poholek et al., 2010; Eto et al., 2011; Harker et al., 2011
IL-21, IL-21R	Mild/moderate decrease/no effect	Vogelzang et al., 2008; Linterman et al., 2010; Poholek et al., 2010; Eto et al., 2011
IL-27Ra	Decreased	Batten et al., 2010
Signaling		
SH2D1A (SAP)[a]	Absent	Qi et al., 2008; Linterman et al., 2009; Cannons et al., 2010; Deenick et al., 2010
STAT3[a]	Decreased	Nurieva et al., 2008; Lu et al., 2011
STAT5	Increased	Johnston et al., 2012
Transcription factors		
Bcl-6	Absent	Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009; Poholek et al., 2010
c-Maf	Decreased	Bauquet et al., 2009; Pot et al., 2009; Hiramatsu et al., 2010; Kroenke et al., 2012
BATF	Absent	Betz et al., 2010; Ise et al., 2011
IRF4	Decreased/absent	Kwon et al., 2009
PRDM1 (Blimp1)	Increased	Johnston et al., 2009
Tbx21 (T-bet)	Increased	Nakayamada et al., 2011; Oestreich et al., 2012
Surface receptors		
CD40/CD40L[a]	Decreased/absent	Akiba et al., 2005; Bossaller et al., 2006
ICOS[a]/ICOS-L	Decreased/absent	
CD28	Decreased/absent	Akiba et al., 2005; Salek-Ardakani et al., 2011
CD80	No effect	
CD86	Decreased	
CD84	Decreased	Cannons et al., 2010

[a]Mutations in *STAT3*, *CD40L*, and *ICOS* in humans results in reduced frequencies of circulating $CD4^+CXCR5^+$ T cells in the peripheral blood (Bossaller et al., 2006; Ma et al., 2012), while mutations in *SH2D1A* compromise Tfh-like function of $CD4^+$ T cells (Ma et al., 2005). These mutations are associated with impaired humoral immune responses in affected individuals.

Conflicting results have been obtained regarding the relative importance of these cytokines for Tfh cell induction in vivo. IL-6 or IL-21 deficiency has been reported to have mild, moderate, or no effect on Tfh cell numbers (Nurieva et al., 2008; Vogelzang et al., 2008; Linterman et al., 2010; Poholek et al., 2010; Zotos et al., 2010; Eto et al., 2011; Harker et al., 2011). Despite conjecture regarding the individual roles of IL-6 and IL-21 in driving Tfh formation, these cytokines are likely to collaborate, as concomitant blockade of both cytokines was required to cause a significant decrease in Tfh cells in vivo (Eto et al., 2011). This finding points to redundancy among cytokines in Tfh development such that deficiency in IL-6 or IL-21 is compensated by other factors. Indeed, Tfh generation is also impaired in mice lacking IL-27R (Batten et al., 2010). Thus, in vivo and in vitro findings provide firm evidence for an important role for STAT3 and STAT3-activating cytokines in regulating Tfh development (Table 1).

Receptor–ligand interactions. The findings that cytokines could induce some, but not all, features of Tfh cells indicated that additional signals are required for full Tfh differentiation. Obvious candidates for these signals are specific receptor/ligands pairs on $CD4^+$ T cells and APCs.

T cell activation requires engagement of the TCR, CD28, CD40L, and ICOS by peptide/MHC class II, CD80/CD86, CD40, and ICOS-L, respectively. In the absence of signaling though CD28, ICOS/ICOS-L or CD40/CD40L, $CD4^+$ T cells fail to up-regulate CXCR5, resulting in reduced numbers of Tfh cells and impaired GC formation (Akiba et al., 2005; Bossaller et al., 2006; Choi et al., 2011; Salek-Ardakani et al., 2011). This is consistent with reduced frequencies of circulating $CD4^+CXCR5^+$ T cells in patients with mutations in *ICOS* or *CD40LG* (Bossaller et al., 2006). Interestingly, deficiency of CD86, but not CD80, recapitulates the Tfh defect seen in *Cd28*-deficient mice (Salek-Ardakani et al., 2011), suggesting that ligation of CD28 by CD86, rather than CD80, has a preferential role in Tfh development (Table 1).

Another important set of molecules involved in T cell–B cell interactions are the SLAM family of receptors—SLAM, CD84, 2B4, Ly9, and NTB-A (Ly108 in mice; Ma et al., 2007)—which elicit intracellular signaling by recruiting SAP. Remarkably, SAP-deficient $CD4^+$ T cells cannot support TD B cell responses caused by an inability to form stable conjugates with cognate B cells and differentiate into Tfh cells (Crotty et al., 2003; Qi et al., 2008; Linterman et al., 2009; Cannons et al., 2010; Deenick et al., 2010; Yusuf et al., 2010). The gene encoding SAP (*SH2D1A*) is mutated in patients

with X-linked lymphoproliferative disease, who exhibit impaired Tfh-like function (Ma et al., 2005, 2007). CD84 is also required for Tfh differentiation, as $CD4^+$ T cells lacking CD84 generate fewer Tfh cells in vivo, although the deficit is less severe than in *Sh2d1a*-deficient mice (Cannons et al., 2010). Thus, although CD84 represents one of the SAP-associating receptors required for Tfh formation, SAP-dependent signaling downstream of additional SLAM family receptors, such as Ly108 (Cannons et al., 2010), is also important (Table 1).

Ag-presenting cells. Although Tfh cells direct the differentiation of Ag-specific B cells, it is clear that reciprocal signals from B cells are also important for Tfh formation. Specifically, mice lacking B cells or those with B cells deficient in functional molecules (CD19, CD40, MHC class II, ICOS-L, and CD86) exhibited decreased numbers of Tfh cells after immunization or pathogen infection (Haynes et al., 2007; Johnston et al., 2009; Zaretsky et al., 2009; Deenick et al., 2010; Poholek et al., 2010; Salek-Ardakani et al., 2011). This suggests that Tfh generation is critically dependent on interactions between activated $CD4^+$ T cells and Ag-presenting B cells. Indeed, the Tfh deficiency seen in the absence of SAP reflected the requirement for SAP in the formation of stable conjugates between $CD4^+$ T and B cells, but not DCs (Qi et al., 2008; Cannons et al., 2010). Moreover, ICOS expression specifically on B cells is required for GC function (Nurieva et al., 2008), and B cells are uniquely able to induce Tfh features in co-cultured $CD4^+$ T cells (Chevalier et al., 2011).

However, the absolute requirement for B cells in the formation of early Tfh cells has been questioned by the finding that provision of excess Ag facilitates Tfh cell formation in the absence of B cells, CD40, or MHC class II (Deenick et al., 2010). This suggests that sustained Ag presentation by non–B cell APCs can support Tfh generation, and the previous conclusion that B cells are required for this process may have simply reflected the fact that they become the predominant APC during a TD immune response. This alternate view of Tfh formation has been validated by several recent studies. First, Ag-specific $CD4^+CXCR5^+Bcl\text{-}6^+$ T cells can be detected within 3 d of immunization/infection, preceding the activation of cognate B cells (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). In addition, the early appearance of Tfh-like cells in lymphoid follicles can occur in the absence of Ag-specific B cells (Poholek et al., 2010; Baumjohann et al., 2011; Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). Formation of $CD4^+CXCR5^+Bcl\text{-}6^+$ pre–Tfh type cells is also independent of SAP (Choi et al., 2011), which is reminiscent of the SAP-independent priming of $CD4^+$ T cells by DCs (Qi et al., 2008). Finally, direct immunization with MHC class II–restricted Ag-pulsed DCs, which circumvents Ag presentation by B cells, resulted in early Tfh cell generation (Baumjohann et al., 2011; Choi et al., 2011; Goenka et al., 2011).

Collectively, these findings provided convincing evidence that the initial generation of Tfh cells is actually B cell–independent and is instead driven by DCs. Indeed, initial interactions between DC and naive $CD4^+$ T cells facilitate the relocation of primed $CD4^+$ T cells from the interfollicular and T cell zones to the B cell follicle to become pre–/early Tfh cells (Fig. 1, A and B). However, B cells are important for complete Tfh differentiation, as they provide signals that reinforce the Tfh phenotype and/or promote their survival (Fig. 1 B). Central to this is the expression of ICOS-L on B cells, which ligates ICOS on $CD4^+$ T cells and induces the expression of key Tfh molecules including IL-21, Bcl-6, and c-Maf (Bauquet et al., 2009; Gigoux et al., 2009; Chevalier et al., 2011; Choi et al., 2011). SAP and SAP-associating receptors are also critical for full Tfh formation and function (Fig. 1 B), as the short-lived interactions between SAP- or CD84-deficient $CD4^+$ T and B cells results in impaired help to B cells and precludes B cells from providing the signals necessary to maintain Tfh cells (Qi et al., 2008; Cannons et al., 2010; Deenick et al., 2010). Cytokines will also contribute to Tfh formation (Fig. 1 B); however, their roles are likely dictated by the type of immune responses generated and the availability of other requisite signals provided by APCs.

Transcription factors. A common tenet in immunology is the notion of "master regulators" of lymphocyte differentiation, whereby a single transcription factor is necessary and sufficient for the commitment of naive cells to a specific lineage of effector cells. This is exemplified by the requirement for T-bet, GATA3, RORγt, and FoxP3 for the differentiation of naive $CD4^+$ T cells into Th1, Th2, Th17, and T reg cells, respectively (O'Shea and Paul, 2010).

For Tfh cells, a similar "master regulator" function has now been ascribed to Bcl-6, which was originally found to be expressed by a subset of $CD4^+$ T cells in GCs in human tonsils (Cattoretti et al., 1995). Studies performed 10–15 yr later revealed that these $Bcl\text{-}6^+$ $CD4^+$ T cells were Tfh cells (Chtanova et al., 2004; Vinuesa et al., 2005a; Rasheed et al., 2006; Ma et al., 2009), and several studies have now shown that Bcl-6 deficiency inhibits, and overexpression promotes, the generation of Tfh cells (Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009; Diehl et al., 2012; Kroenke et al., 2012), as well as extrafollicular $CD4^+$ Th cells (Poholek et al., 2010; Lee et al., 2011). These findings firmly established a critical role for Bcl-6 in coordinating Tfh formation and validated the prescient observation by Cattoretti et al. (1995) that "Bcl-6 expression in T cells may also be associated with some GC-related function."

Bcl-6 functions as a transcriptional repressor. Thus, it was initially difficult to invoke a mechanism by which Bcl-6 actively drives a Tfh program in naive $CD4^+$ T cells. However, several scenarios have now been proposed. As Bcl-6 suppresses GATA3 expression and Th2 responses (Kusam et al., 2003), it could promote Tfh differentiation by interfering with the expression or function of transcription factors that control alternate Th cell fates (Fig. 2). Indeed, enforced expression of Bcl-6 in naive $CD4^+$ T cells cultured under Th1 or Th17 conditions reduces the proportion of IFN-γ– or IL-17–expressing cells (Nurieva et al., 2009; Yu et al., 2009), most likely caused by its ability to bind to

consensus sites in the promoters of *Tbx21* and *Rorc* (encoding RORγt), and thus reduce protein expression and/or function. This is consistent with the enhanced generation of Tfh cells from *Tbx21*-deficient CD4+ T cells both in vivo and in vitro (Nakayamada et al., 2011; Oestreich et al., 2012).

Bcl-6 also inhibits the regulatory effects of Blimp-1 and the expression of a cluster of microRNAs that suppress Tfh generation (Yu et al., 2009). *Bcl6* and *Prdm1*, which encodes Blimp-1, are reciprocally expressed in Tfh and effector CD4+ T cells (Fazilleau et al., 2009; Johnston et al., 2009; Ma et al., 2009; Choi et al., 2011), and deletion of *Prdm1* from murine CD4+ T cells enhances Tfh formation in response to lymphocytic choriomeningitis (LCMV). In contrast, *Prdm1* overexpression severely blunts Tfh genesis and attenuates GC reactions and Ab responses (Johnston et al., 2009). Thus, Bcl-6 and Blimp-1 function to control CD4+ T cell fate by promoting or inhibiting the generation of Tfh cells (Fig. 2 and Table 1).

A caveat to Bcl-6's role as a master regulator of Tfh cell differentiation is the inability of ectopic Bcl-6 to induce substantial expression of CXCR5 or IL-21—two of the defining features of Tfh cells—in human or murine naive CD4+ T cells in vitro (Nurieva et al., 2009; Yu et al., 2009; Diehl et al., 2012; Kroenke et al., 2012). Thus, other transcription factors must also be involved in Tfh differentiation. Indeed, Tfh cells are also diminished in mice lacking c-Maf (Bauquet et al., 2009), BATF (Betz et al., 2010; Ise et al., 2011), and IRF4 (Kwon et al., 2009; Table 1). Consequently, Bcl-6 may not be the "master regulator" of the Tfh lineage.

c-Maf induces IL-21 production in activated CD4+ T cells in vivo (Bauquet et al., 2009) and in vitro (Hiramatsu et al., 2010; Kroenke et al., 2012), and c-Maf expression itself is induced in CD4+ T cells by IL-6 (Hiramatsu et al., 2010) or IL-27 (the latter requiring IL-21; Pot et al., 2009). Furthermore, IL-27–mediated induction of IL-21 is c-Maf dependent (Pot et al., 2009), suggesting the existence of a positive feedback loop. Ectopic expression of c-Maf in human naive CD4+ T cells also yields a greater proportion of cells expressing CXCR5 compared with control-transduced cells, and co-transduction of Bcl-6 and c-Maf resulted in higher levels of expression of ICOS and PD-1 compared with either factor alone, providing evidence of cooperative interplay between these transcription factors (Kroenke et al., 2012; Fig. 2).

BATF deficiency also causes a cell-autonomous defect in Tfh generation that appears to result, at least in part, from a requirement for BATF in the induction of Bcl-6 and c-Maf (Betz et al., 2010; Ise et al., 2011; Fig. 2). Tfh formation and help for GC responses are defective in BATF-deficient CD4+ T cells in vivo; this could be improved by expressing Bcl-6 alone or together with c-Maf, but not by c-Maf alone. However, the level of rescue was only ∼60–70% of that achieved by overexpressing BATF in these cells (Ise et al., 2011), suggesting that other, as yet unidentified, BATF targets contribute to Tfh differentiation. It is also unclear which signals induce BATF. It will be important to determine whether IL-6 and IL-27 can induce BATF, and whether cytokine-driven c-Maf expression is secondary to the induction of BATF.

These studies have revealed a hierarchy of the transcriptional regulation of Tfh cells. BATF and STAT3-activating cytokines induce expression of Bcl-6 and c-Maf. Bcl-6 induces signature features of Tfh cells by suppressing

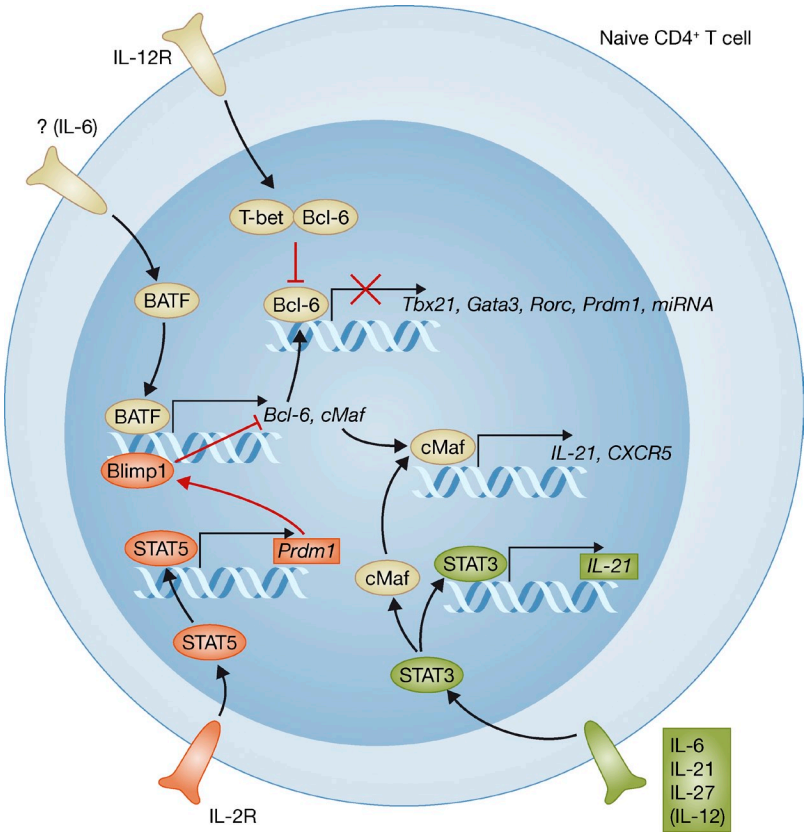

Figure 2. Molecular regulation of Tfh development. Tfh development requires the integrated and balanced function of numerous transcription factors. BATF acts proximally by inducing Bcl-6 and c-Maf. Bcl-6 imprints a Tfh fate by suppressing expression and/or function of transcriptional regulators of alternate effector fates (i.e., *Tbx21* [Th1], *Gata3* [Th2], and *Rorc* [Th17]), microRNAs that suppress Tfh generation, and *Prdm1* (encoding Blimp-1), which directly suppresses Bcl-6. c-Maf contributes to Tfh cells by inducing CXCR5 and IL-21. STAT3-activating cytokines IL-6, IL-21, and IL-27 can also induce c-Maf, and STAT3 can directly induce IL-21. IL-12 induces IL-21 in a STAT3-dependent manner, but it is unknown whether this involves c-Maf or is a direct effect of STAT3. Tfh generation is restricted by Blimp-1, which is induced by IL-2 in a STAT5-dependent manner and blocks Bcl-6 expression, and by T-bet (induced by IL-12), which physically associates with Bcl-6 and prevents Bcl-6–dependent repression.

both the function of other lineage-specific transcription factors, as well as Blimp-1 and multiple microRNAs, and c-Maf induces IL-21. STAT3 can also directly induce IL-21. The function of IRF4 in Tfh cell formation remains to be determined; however, it is required for IL-21– and IL-6–induced expression of IL-21 in murine $CD4^+$ T cells (Huber et al., 2008).

Counter-regulation of Tfh cell formation

The eradication of infectious pathogens and the success of vaccines require Tfh cell–mediated B cell differentiation and Ab secretion. However, aberrant function of Tfh cells could trigger autoimmunity. Thus, processes must exist that restrict Tfh activity under normal circumstances.

Transcription factors and cytokines. The primary transcription factor that negatively regulates Tfh development is Blimp-1 (Johnston et al., 2009), which directly suppresses Bcl-6 expression (Fig. 2). Blimp-1 is induced by IL-2 in $CD4^+$ T cells (Gong and Malek, 2007), and CD25 (IL-2Rα) expression positively correlates with Blimp-1 and negatively correlates with Bcl-6 and CXCR5 (Choi et al., 2011). Consistently, IL-2 blockade or targeted deletion of STAT5, which is activated by IL-2, increased Tfh cell formation (Johnston et al., 2012; Oestreich et al., 2012), whereas overexpression of constitutively active STAT5 reduced Tfh generation in vivo (Johnston et al., 2012). Similar findings were recently reported in a model of influenza virus infection, whereby repeated administration of IL-2 reduced virus-specific humoral immunity by directly suppressing Tfh cell formation (Ballesteros-Tato et al., 2012). The inhibitory effect of STAT5 was Blimp-1–dependent, as it was overcome by deleting Blimp-1 from $CD4^+$ T cells (Johnston et al., 2012). STAT5 can also directly bind *BCL6* and suppress its expression (Walker et al., 2007). Thus, the IL-2–STAT5 axis is likely to control Tfh generation by suppressing Bcl-6 both directly and indirectly via induction of Blimp-1 (Fig. 2 and Table 1).

Regulatory cells. T reg cells are a subset of $CD4^+$ T cells that control the function of effector cells (Josefowicz et al., 2012). Remarkably, specific subsets of T reg cells can co-opt the differentiation programs of effector cells to selectively regulate distinct classes of immune responses. For instance, expression of T-bet, IRF4 or STAT3 in T reg cells results in cells that temper the activity of Th1, Th2 or Th17 cells, respectively (Josefowicz et al., 2012). The concept that T reg cells suppress Tfh cells is not novel, as T reg cells were identified in B cell follicles and GCs in 2004 (Lim et al., 2004). However, the nature of these T reg cells, their mechanisms of suppression, and their relevance to immune responses in vivo remained unexplored (Lim et al., 2004, 2005).

A subset of T reg cells that preferentially inhibits Tfh cells was suggested by the finding that ∼10–15% of the Tfh cells in murine and human lymphoid tissues express the T reg cell–specific transcription factor FoxP3 (Chung et al., 2011; Linterman et al., 2011; Wollenberg et al., 2011). These cells share features with both Tfh cells (expression of Bcl-6, CXCR5, PD1, and ICOS) and T reg cells (expression of GITR, CTLA4, KLRG1, CD25, and Blimp-1), but they differ from Tfh cells in that they lack CD40L, IL-4, and IL-21 (Linterman et al., 2011). Follicular T reg cells originate from thymic-derived (i.e., natural) T reg cells, rather than peripherally induced T reg cells or Tfh cells themselves, yet they exhibit requirements similar to conventional Tfh cells for their development and maintenance (Chung et al., 2011; Linterman et al., 2011; Wollenberg et al., 2011). Genetic ablation of Bcl-6 prevented the generation of follicular T reg cells, and CXCR5 deficiency disrupted their positioning within GCs. Both manipulations affected Tfh cell numbers only marginally but resulted in increased numbers of GC B cells and increased Ab production (Chung et al., 2011; Wollenberg et al., 2011). Thus, by hijacking the transcriptional machinery of Tfh cells and acquiring expression of CXCR5, follicular T reg cells can migrate into GCs to exert their regulatory effect (Fig. 3). Because human follicular T reg cells directly suppress Ab secretion by GC B cells, as well as Tfh cell function in vitro (Lim et al., 2004, 2005), it is likely that follicular T reg cells limit GC reactions by suppressing the in vivo responses of both Tfh and GC B cells. It will be interesting to determine whether some of the clinical symptoms in *FOXP3* mutant IPEX patients, such as the production of auto-Abs, are caused by an inability of follicular T reg cells to restrain GC responses.

The effects of follicular T reg cells may be complemented by the actions of both regulatory $CD8^+$ T cells and PCs. A subset of $CD8^+$ T cells restricted by the nonclassical MHC molecule Qa1 was found to acquire expression of CXCR5, migrate to B cell follicles, and attenuate the function of Tfh cells (Kim et al., 2010). Unlike conventional T reg cells, regulatory $CD8^+$ T cells lack expression of FoxP3 and CTLA4, and their suppressive capacity required their expression of perforin and a source of IL-15 (Kim et al., 2010). Preventing the interaction between Tfh cells and regulatory $CD8^+$ T cells resulted in a dramatic increase in Tfh cells and the development of a fatal lupus-like disease (Kim et al., 2010).

PCs also negatively regulate Tfh cells, as *Bcl6* and *Il21* expression was abrogated in co-cultures of PC and in vivo–derived Tfh cells (Pelletier et al., 2010). Intriguingly, although PCs presented peptides to naive Ag-specific $CD4^+$ T cells and induced T cell activation in vitro, expression of *Bcl6* and *Il21* (Pelletier et al., 2010) was not induced. This led to the proposal that PC limited the generation of Tfh cells, a scenario supported by increased numbers of Tfh cells in PC-deficient mice (Pelletier et al., 2010). However, this increase could also reflect the lack of Ab-mediated Ag clearance, thereby resulting in sustained T cell activation and Tfh formation. It also remains to be determined where and when PCs would interact with Tfh cells and how PCs would deliver such inhibitory signals.

Memory Tfh cells

In humans, a small subset of $CD4^+$ T cells in peripheral blood expresses CXCR5 (Förster et al., 1994; Breitfeld et al., 2000;

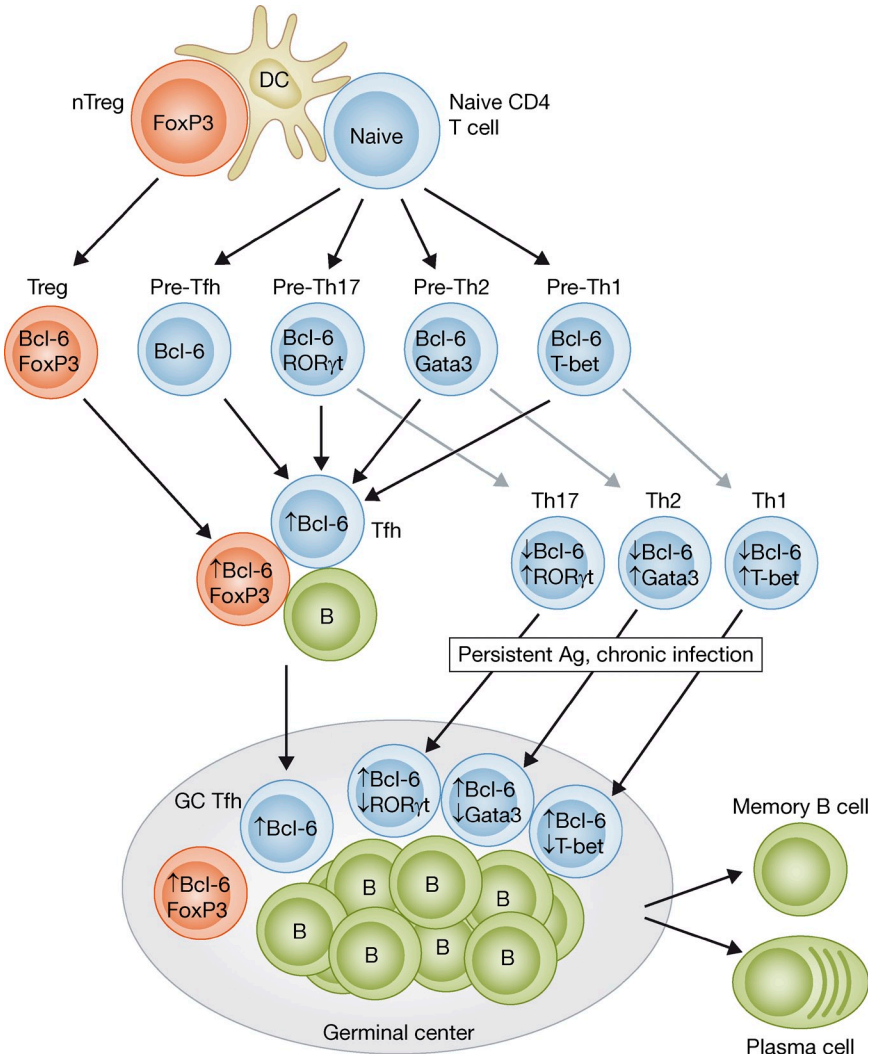

Figure 3. CD4⁺ T cell flexibility. Naive CD4⁺ T cells interacting with Ag-presenting DCs can acquire expression of Bcl-6. However, their commitment to specific effector lineages is governed by additional signals and the coordinated and antagonistic actions of different transcription factors. Sufficient levels of T-bet or GATA3, or potentially RORγt, will override the repressive effect of Bcl-6 to yield Th1, Th2, or Th17 cells, respectively. Yet some DC-primed naive CD4⁺ T cells will become pre–Tfh cells, defined by CXCR5 and Bcl-6 expression, which differentiate into GC Tfh cells after interactions with cognate B cells. Th1, Th2, and, presumably, Th17 cells retain flexibility in their differentiation programs such that under conditions of sustained Ag (e.g., persistent pathogen infection) they can down-regulate T-bet, Gata3, or RORγt, up-regulate Bcl6, and convert to Tfh cells. Regardless of their origin, Tfh cells drive the differentiation of cognate B cells into memory and plasma cells, which is necessary for long-lived protective Ab responses. The magnitude of Tfh-induced humoral responses is regulated by thymic-derived follicular T reg cells. Although pre–Th1, pre–Th2, pre–Th17, and pre–Tfh cells are illustrated as individual cell types, it is equally possible that Th1, Th2, Th17, and Tfh cells arise from a common CD4⁺ T cell precursor that acquires expression of T-bet, GATA3, RORγt, and Bcl-6, and then differentiates into bona fide effector cells after delivery of appropriate instructive cues. The scheme outlined for Th17 cells differentiating into Tfh cells is speculative. Although Th17 cells shares features with Tfh cells, it is unknown whether this reflects an intrinsic characteristic of Th17 cells or their conversion to Tfh cells.

Schaerli et al., 2000). These cells express low levels of Tfh markers (ICOS, OX40, IL-4, IL-21, Bcl-6, and c-Maf; Förster et al., 1994; Breitfeld et al., 2000; Schaerli et al., 2000; Chevalier et al., 2011) and are diminished in individuals unable to form GCs because of mutations in *CD40LG* or *ICOS* (Bossaller et al., 2006). For this reason, it has been inferred that circulating CD4⁺CXCR5⁺ T cells derive from GCs and are presumably Tfh cells. Most circulating CD4⁺CXCR5⁺ T cells express CD45RO (Förster et al., 1994; Breitfeld et al., 2000; Schaerli et al., 2000; Bossaller et al., 2006; Chevalier et al., 2011; Ma et al., 2012), which is typically associated with a memory cell phenotype. However, it is important to note that CD45RO expression does not discriminate between long-lived memory cells and recently generated effector cells. Interestingly, in healthy donors that received a tetanus booster >2 yr earlier, circulating tetanus toxoid–specific CD4⁺ T cells resided only in the CXCR5⁻ subset of CD4⁺CD45RO⁺ T cells (Breitfeld et al., 2000). In contrast, CMV or influenza-specific cells were present in both the CXCR5⁻ and CXCR5⁺ subsets (Morita et al., 2011). The presence of virus-specific CD4⁺ T cells in the CXCR5⁺ subset may reflect periodic encounter with Ag, as CMV establishes a persistent, latent infection and exposure to influenza may occur seasonally. Tetanus toxoid, in contrast, is likely to be cleared after vaccination. Thus, circulating CD4⁺CXCR5⁺ T cells in humans may represent recently generated effector cells that have exited lymphoid follicles rather than long-lived memory cells.

Fazilleau et al. (2007) described a similar population of CD4⁺CXCR5⁺ T cells in draining lymph nodes of mice that persisted after the peak of GC responses (30 d after immunization). As in humans, these cells had down-regulated expression of signature Tfh molecules (ICOS, OX40, IL-4, and IL-21). Although these murine CD4⁺CXCR5⁺ T cells were designated as "memory" Tfh cells, they may result from recent Ag encounter, as complexes of specific Ag–MHC class II were clearly still detectable 70 d after immunization (Fazilleau et al., 2007). IL-21⁺ Tfh cells isolated from mice 30–55 d after immunization or infection and transferred into naive mice gave rise to both IL-21⁺ Tfh cells and conventional CXCR5⁻PD-1⁻IL-21⁻ effector cells (Lüthje et al., 2012)

after reexposure to antigen. This suggests that Tfh cells can enter the pool of CXCR5$^-$ memory CD4$^+$ T cells, as described originally for tetanus-specific human CD4$^+$ T cells (Breitfeld et al., 2000). Although these studies have shed some light on Tfh memory, further investigation is required to provide a complete understanding of the relationship between, and kinetics of formation of, conventional memory, Tfh memory, and effector cells.

Plasticity of Tfh cells

T cell differentiation was once considered linear and irreversible, but it is now appreciated that this process is flexible, as so-called committed cells can acquire features of alternate effector fates (O'Shea and Paul, 2010). This has raised the question of whether Tfh cells are a distinct lineage of effector CD4$^+$ T cells or whether they instead represent a stage of activation of Th1, Th2, and Th17 cells. Initial studies demonstrated that Tfh cells could be generated in vivo under conditions that cripple the formation of Th1, Th2, and Th17 cells (Nurieva et al., 2008), suggesting that Tfh cells are a distinct lineage. However, Tfh cells can arise from Th1 (i.e., CXCR5$^-$T-bet$^+$IFN-γ^+) and Th2 (i.e., CXCR5$^-$PD-1$^-$ Bcl6^{lo}GATA3$^+$IL-4$^+$) precursors in response to infection with LCMV (Th1; Fahey et al., 2011), *Heligmosomoides polygyrus* (Th2), *Schistosoma mansoni* (Th2), or *Nippostrongylus brasiliensis* (Th2; King and Mohrs, 2009; Reinhardt et al., 2009; Zaretsky et al., 2009; Fig. 3). Tfh cells may also arise from Th17 cells. Indeed, Tfh and Th17 cells have several similarities, such as the molecular requirements for their generation (ICOS, c-Maf, IRF4, and STAT3), production of IL-21, and the ability to induce B cell differentiation (Breitfeld et al., 2000; Huber et al., 2008; Ma et al., 2008, 2012; Nurieva et al., 2008; Bauquet et al., 2009; Mitsdoerffer et al., 2010). This scenario, however, is speculative and requires examination in relevant in vitro or in vivo models.

Recent studies have provided potential explanations for this flexibility. First, kinetic analyses of CD4$^+$ T cell responses have shown that CXCR5 and Bcl-6 are expressed within 1–3 d of Ag exposure (Kerfoot et al., 2011; Kitano et al., 2011). Early Bcl6 expression is likely facilitated by the open, active conformation of the *Bcl6* locus in naive mouse CD4$^+$ T cells, and permissive epigenetic marks (i.e., H3K4me3) in this locus in in vitro generated Th1, Th2, and Th17 cells (Lu et al., 2011; Nakayamada et al., 2011). In addition, in vitro stimulation of murine and human naive CD4$^+$ T cells under Th1-polarizing conditions (i.e., IL-12) yields a transitional cell population with features of both Th1 (IFN-γ^+T-bet$^+$) and Tfh (IL-21$^+$Bcl-6$^+$) cells (Schmitt et al., 2009; Nakayamada et al., 2011; Ma et al., 2012). However, Th1-type cells predominated over time under these conditions, as the majority of IL-21$^+$Bcl-6$^+$ cells were eventually lost because of sustained expression of T-bet (Nakayamada et al., 2011) and its ability to bind to and inhibit Bcl-6 (Oestreich et al., 2012). Indeed, overexpression of T-bet in vitro reduces Bcl-6 expression in naive CD4$^+$ T cells cultured with IL-12, whereas *Tbx21*-deficient CD4$^+$ T cells generated more Tfh-like cells in vivo and in vitro (Nakayamada et al., 2011; Oestreich et al., 2012). Collectively, these findings suggest that *Bcl6* is primed for rapid expression in activated CD4$^+$ T cells, thereby providing the molecular machinery to guide CD4$^+$ T cells to a Tfh fate (Fig. 3; Table 1).

The nature of the infection may also dictate the fate of Ag-specific CD4$^+$ T cells. For example, Tfh cells are preferentially generated under conditions of pathogen/Ag persistence, such as chronic LCMV infection, whereas both Tfh and Th1 cells are generated in similar numbers and with similar kinetics after acute LCMV infection (Fahey et al., 2011). This deviation from Th1 cells during chronic infection correlated with increased expression of *Bcl6* and reduced expression of *Tbx21* and *Prdm1*. Thus, T-bet can constrain Bcl-6 expression under conditions of limiting Ag, leading to reduced Tfh formation (Nakayamada et al., 2011). However, abundant Ag—resulting from persistent/chronic infection—overrides these constraints to promote the Tfh program (Fahey et al., 2011), most likely by maintaining high levels of Bcl-6, which then antagonizes T-bet function and Th1 cell formation (Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009; Oestreich et al., 2012; Fig. 3). This model is supported by the findings that high-affinity CD4$^+$ T cells preferentially develop into Tfh cells (Fazilleau et al., 2009), and that boosting with peptide Ag promotes Tfh formation (Deenick et al., 2010). In a similar fashion, IL-4–expressing Th2 cells may convert into Tfh cells during infection with Th2-inducing pathogens (King and Mohrs, 2009; Reinhardt et al., 2009; Zaretsky et al., 2009), in which persistent pathogen exposure induces Bcl-6 expression, which in turn suppresses GATA3. Although this has not been formally tested, it is consistent with the severe Th2-mediated inflammation seen in Bcl-6–deficient mice (Kusam et al., 2003).

These studies add to the concept of plasticity in T cell differentiation and infer that T cell differentiation is neither absolute nor solely driven by master regulators. Rather, the ultimate fate of activated CD4$^+$ T cells reflects the modular nature of the expression, function, and interplay of antagonistic and cooperative transcription factors.

Tfh-mediated diseases

Although Tfh cells are critical for the generation of effective long-lived protective Ab responses, dysregulation of Tfh function could manifest as various immune dyscrasias. This has indeed been established in both mice and men.

The first suggestion that Tfh cells could underlie immune-mediated diseases was their overabundance in several murine models of SLE (Vinuesa et al., 2005a; Subramanian et al., 2006) and the fact that preventing Tfh formation reduced disease (Hron et al., 2004; Linterman et al., 2009). Circulating Tfh-like cells are also found at increased frequencies in the blood of some patients with SLE, Sjogren's syndrome (Simpson et al., 2010), and juvenile dermatomyositis (Morita et al., 2011), which are autoimmune conditions characterized by production of pathogenic auto-Abs. Importantly, the expansion of Tfh-like cells in SLE correlated with auto-Abs titers

and tissue damage (Simpson et al., 2010).The flip-side to autoimmunity is immunodeficiency. Tfh cells are reduced in patients with mutations in *STAT3* (Ma et al., 2012), *CD40L*, and *ICOS* (Bossaller et al., 2006), as well as in corresponding gene-targeted mice (Akiba et al., 2005; Bossaller et al., 2006; Nurieva et al., 2008), and are associated with severe defects in protective humoral immunity (Al-Herz et al., 2011; Crotty, 2011; Tangye et al., 2012). Furthermore, although Tfh cells can be generated under some conditions in SAP-deficient humans and mice, they exhibit compromised function (Ma et al., 2005; Deenick et al., 2010; Lu et al., 2011). Thus, excessive generation of Tfh cells likely contributes to the production of pathogenic auto-Abs in several human autoimmune conditions, and insufficient Tfh generation underlies impaired humoral immune responses in primary immunodeficiencies.

Conclusions

Since their formal identification just over a decade ago, our knowledge of Tfh cells has grown exponentially, and we now have a much clearer understanding of the cells, molecules and signaling pathways required for their differentiation, as well as the mechanisms by which Tfh function is induced and constrained. The rapid influx of information has by no means abated, as witnessed by the recent descriptions of subsets of invariant NKT cells that resemble Tfh cells and provide help for primary B cell responses to lipid Ags in vivo (Chang et al., 2012; King et al., 2012; Tonti et al., 2012). Together with previous reports of populations of $CD8^+$ and γδ T cells with Tfh-like features in humans (Caccamo et al., 2006; Quigley et al., 2007), these findings illustrate that Tfh function is not restricted to classical TD B cell responses. Rather, subsets of Tfh cells appear to have evolved to promote Ab-mediated protection against specific microorganisms, such as glycolipid-rich pathogens (iNKT Tfh cells; Chang et al., 2012; King et al., 2012; Tonti et al., 2012) and intracellular pathogens such as mycobacteria (e.g., γδ Tfh cells; Caccamo et al., 2006). Mechanisms probably also evolved to ensure that the appropriate type of Tfh response is elicited while deleterious responses are suppressed. With such rapid advances in our understanding of Tfh cells, we are well placed to harness the power of these cells for therapeutic intervention and modulation. Thus, the identification of diverse subsets and functions of Tfh cells provides promise for novel strategies to improve vaccine development for inducing long-lived humoral protection against infectious diseases. Similarly, promoting or attenuating Tfh cell development or function may be an attractive rationale for treating immunological diseases such as immunodeficiency or autoimmunity. Hopefully, the next decade of Tfh research will see some of these possibilities realized.

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