New insights into the differentiation and function of T follicular helper cells

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Abstract | The seminal studies characterizing T follicular helper (T_{FH}) cells described a non-polarized $CD4^+$ T cell population with a unique ability to home to B cell follicles and to induce antibody production by B cells. In the past few years, the study of T_{FH} cells has enjoyed a renaissance and there has been a surge of research activity aimed at understanding the function and differentiation of these important cells. This Review focuses on the current progress in T_{FH} cell biology and the important questions that remain unanswered. Particular attention is paid to recent studies that support the idea that T_{FH} cells are a separate T cell lineage and those that probe the relationship of T_{FH} cells to other T helper cell subsets.

Somatic hypermutation
A unique mutation mechanism that is targeted to the variable regions of rearranged immunoglobulin gene segments. Combined with the selection for B cells that produce high-affinity antibody, somatic hypermutation leads to affinity maturation of B cells in germinal centres.

Class switch recombination
The process by which proliferating B cells rearrange their DNA to switch from expressing IgM (or another class of immunoglobulin) to expressing a different immunoglobulin heavy-chain constant region, thereby producing antibody with different effector functions.

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The production of high-affinity, class-switched antibody is important for the clearance of pathogens following infection, for the establishment of long-term humoral immunity and for the efficacy of vaccines. To make high-affinity, class-switched antibody, B cells must receive cognate help from $CD4^+$ T cells during a germinal centre reaction[1]. Germinal centres are discrete structures within the B cell follicles of secondary lymphoid organs in which the processes of somatic hypermutation, class switch recombination and affinity maturation of activated B cells occur accompanied by the production of memory B cells and plasma cells[2,3]. The $CD4^+$ T cells that are responsible for providing help migrate into the germinal centre[4–7] where, according to evidence from intravital microscopy, T cell–B cell interactions are weighted towards intense B cell competition for a small number of $CD4^+$ T cells[8]. The absence of T cell help during B cell priming leads to B cell apoptosis, rather than differentiation into germinal centre B cells or plasma cells[9].

The requirement of T cell help for B cell antibody production was first shown in the 1960s[10,11]. However, it took more than three decades before the subset of $CD4^+$ T cells that provided this help was identified in germinal centres. Termed T follicular helper (T_{FH}) cells[12–14], these cells were found to have a unique ability to home to B cell follicles owing to their expression of CXC-chemokine receptor 5 (CXCR5) and to induce antibody production during co-culture with B cells[12–14]. In contrast to other T cell subsets, T_{FH} cells were poor cytokine secretors, which led to their description as a non-polarized subset. However, cytokine production in those early studies was examined in $CXCR5^+$ memory $CD4^+$ T cells from peripheral blood, and the relationship of these cells to bone fide T_{FH} cells still requires clarification. Several studies have since shown that $CXCR5^+$ T_{FH} cells isolated from secondary lymphoid tissue can produce cytokines[46,76]. In conjunction with their unique localization in germinal centres, the capacity of T_{FH} cells to provide help to B cells depends on their expression of molecules that influence T and B cell collaboration. T_{FH} cells are defined by high levels of expression of inducible T cell co-stimulator (ICOS), programmed cell death 1 (PD1), the transcriptional repressor B cell lymphoma 6 (BCL-6) and cytokines that influence B cell differentiation and antibody production such as interleukin-21 (IL-21) and IL-4. These features are present in both humans and mice; however, human T_{FH} cells also express IL-10 (REFS 14,15), which has an important role in the differentiation of human B cells, and high levels of the chemokine CXCL13 (REF. 16).

In this Review, I discuss our current understanding of T_{FH} cell biology. Particular attention is given to recent studies designed to probe the relationship of T_{FH} cells to other T helper (T_H) cells and to determine whether T_{FH} cells are a distinct lineage. I also review the definition of the T_{FH} cell phenotype in the context of increasing heterogeneity observed in the T_{FH} cell subset.

Differentiation of T_{FH} cells

The interaction of interdigitating dendritic cells (DCs) bearing peptide–MHC class II complexes and naive $CD4^+$ T cells bearing T cell receptors (TCRs) with high affinity for antigen[17] initiates the expression of a B cell follicle homing programme that leads to the recruitment of T_{FH} cell precursors to the T cell–B cell border in

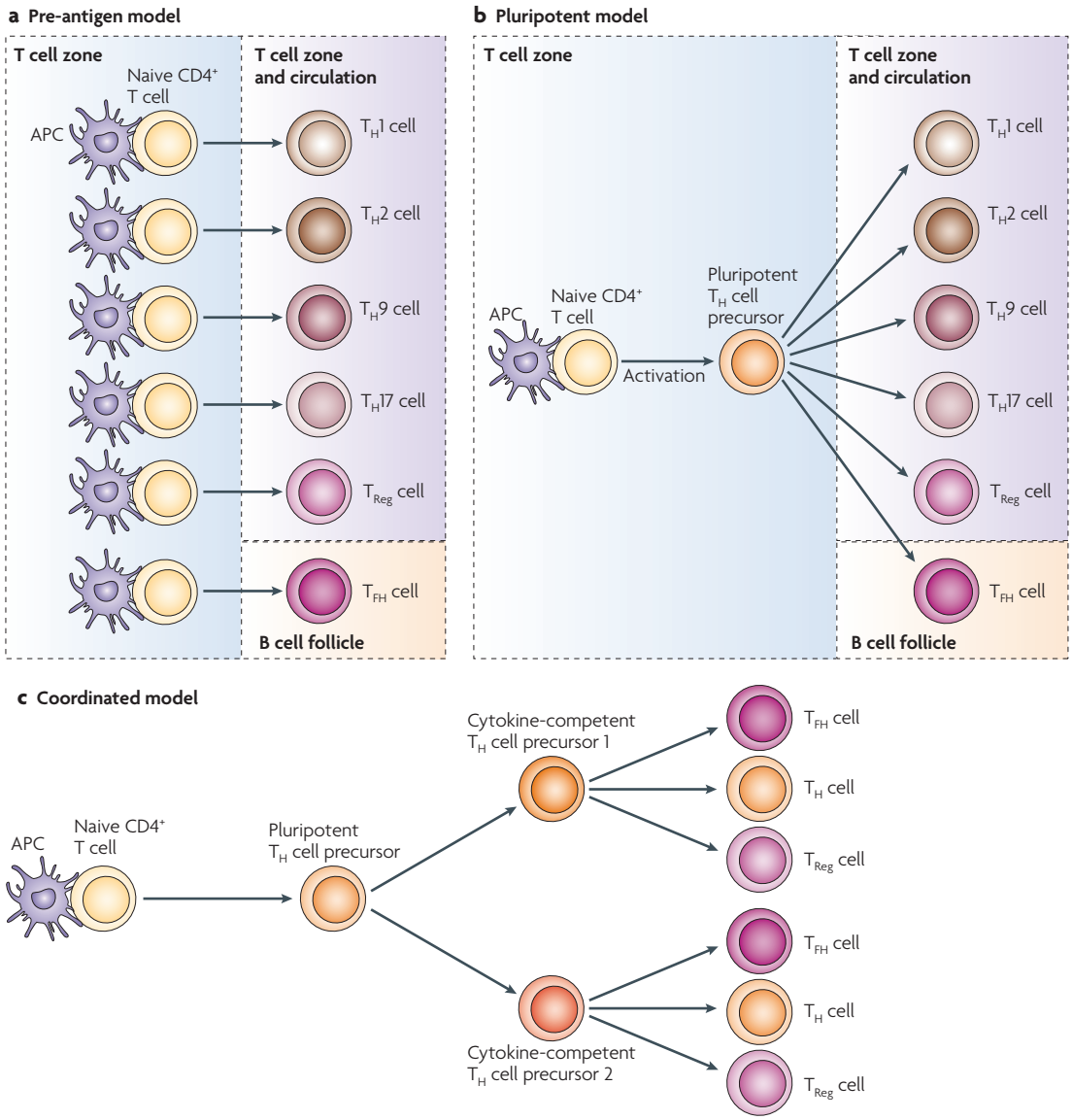

Figure 1 | **Three models for the differentiation of T follicular helper cells.** T follicular helper (T_{FH}) cells and differentiated T helper (T_H) cell subsets (such as T_H1 cells, T_H2 cells, interleukin-9 (IL-9)-producing T_H9 cells, IL-17-producing T_H17 cells and inducible regulatory T (T_{Reg}) cells) are derived from naive $CD4^+$ T cells. **a** | The pre-antigen model suggests that T_{FH} cells and other T_H cell subsets arise from distinct naive $CD4^+$ T cell precursors. **b** | The pluripotent model suggests that all T_H cell subsets arise from a common pluripotent T_H cell precursor. **c** | The coordinated model suggests that T_{FH} cells and T_H cells arise from distinct cytokine-competent T_H cell precursors. In this model, IL-4-producing T_{FH} cells and T_H2 cells would have a common precursor. APC; antigen-presenting cell.

Asymmetrical cell division
A type of division that produces two daughter cells with different properties. This is in contrast to normal cell divisions, which give rise to equivalent daughter cells. Notably, stem cells can divide asymmetrically to give rise to two distinct daughter cells: one copy of themselves and one cell programmed to differentiate into another cell type.

the white pulp of secondary lymphoid organs. T_{FH} cells migrate into the B cell follicle, where they may continue their differentiation programme[18]. T_H cells that complete interactions with antigen-presenting cells or fail to establish interactions with B cells at the T cell–B cell border either leave the secondary lymphoid organ or participate in the stimulation of antibody production by B cells in extrafollicular foci. T_{FH} cell differentiation is influenced by several factors in the local environment, including the interaction with B cells[15,19] and the cytokine milieu (which in turn is determined by the pathogen or antigen encountered), and is likely to be finalized within the B cell follicle during the germinal centre reaction.

There are several possible scenarios for the generation of T_{FH} cells: T_{FH} cell fate may be decided at the first encounter of a naive $CD4^+$ T cell with antigen (FIG. 1a), or at increasingly later points during T_{FH} cell differentiation (FIG. 1b, c). However, these models are not mutually exclusive and indeed might overlap, especially in the context of T_H cell plasticity[20] and asymmetrical cell division[21]. Model 1 suggests that all T_H cell subsets (that is, T_H1, T_H2, T_H17, inducible regulatory T (T_{Reg}) cells and T_{FH} cells) arise independently from distinct naive $CD4^+$ T cells immediately following the initial encounter with peptide–MHC class II complexes. In this model, T_{FH} cells, but not other T_H cell subsets, would arise exclusively from naive $CD4^+$

T cells bearing TCRs with the highest affinity for antigen[17]. Model 2 suggests that T_{FH} cells share T_H cell precursors with other differentiated T_H cell subsets that migrate to non-lymphoid tissue sites. In this model, T_H cells and T_{FH} cells would emerge from the same pluripotent activated CD4+ T cell precursor and T_{FH} cells would be subsequently selected from cells with the highest affinity for antigen[17] through preferential interactions with B cells. In model 3, T_{FH} cells and their 'paired' T_H cell subset arise in a cytokine-competent manner[22] from cytokine-polarized T_H cell precursors that may include the coordinate generation of inducible T_{Reg} cells. However, precisely how T_H cell fate is determined remains unknown.

Early events in the T cell zone

Antigen and TCR affinity. Several recent studies have focused on understanding how T_{FH} cells are generated, but the exact timing and context of T_{FH} cell differentiation are subject to ongoing investigation. The initial events in the differentiation of T_{FH} cells are common to all T_H cell subsets. Naive T cells that express the CD4 co-receptor mature in the thymus and are phenotypically characterized by the absence of T cell activation surface markers. The first division of a naive CD4+ T cell occurs 25–30 hours after TCR recognition of peptide antigen presented in the context of MHC class II molecules on the surface of interdigitating DCs in the T cell zone of secondary lymphoid tissues. The progeny of this initial clonal expansion is temporarily sequestered in the secondary lymphoid organ through the upregulation of expression of the early activation marker CD69 and the transient downregulation of expression of the receptor sphingosine 1-phosphate receptor 1 (S1PR1), which regulates egress of cells from lymphoid tissues[23]. Subsequently, within 2–3 days following appropriate exposure to differentiation signals, activated T cells then re-express S1PR1, allowing them to migrate out of the secondary lymphoid tissue into the circulation[23]. These emigrants include effector T_H cells that are destined for non-lymphoid tissue sites. By contrast, T_H cells that retain cell surface expression of CD69 remain in the lymph node[12,24]. Among these are CXCR5+ cells with the potential to become T_{FH} cells[6,7,25].

A central notion in the differentiation of CD4+ T cells is that the TCR can transmit differing degrees of activating signals to initiate different effector outcomes[26,27]. In an effort to determine the important early events in T_{FH} cell generation, a recent study showed that following the transfer of CD4+ T cells into immunized mice, those T cells expressing TCRs with the highest affinity to peptide–MHC class II complexes and the most restricted TCR diversity were selected into the T_{FH} cell pool[17]. Thus, T_{FH} cell differentiation requires strong signals through the TCR and/or sustained interactions with antigen-presenting cells.

The acquisition of cytokine competency. A range of antibody isotypes is typically produced in response to infection or immunization, with one or two isotypes predominating. The relative amounts of the different antibody isotypes that are produced are determined by cytokine signals that control immunoglobulin class switch recombination, which are still not completely understood[28]. Early after activation by antigen, CD4+ T cells can produce a wide range of cytokines, but this ability is progressively lost as they differentiate into discrete T_H cell subsets with specific effector functions.

The interaction between cytokine-competent T_H cell precursors and B cells is crucial for directing their differentiation into various T_H cell subsets and into antibody-producing cells, respectively. In mice, the production of interferon-γ (IFNγ) and IL-4 is a characteristic, but not exclusive, feature of T_H1 and T_H2 cells, respectively. IL-4 induces sequential class switching to IgG1 and then to IgE, whereas IFNγ is associated with class switching to IgG2a[29] (FIG. 2). Several studies have shown that cytokine competency is initiated through interaction with DCs, but the ability of activated CD4+ T cells to produce IL-4 and IFNγ is initiated at the T cell–B cell border of lymphoid tissues[22,30–32]. The acquisition of cytokine production by T_H cells at this site subsequently begins the process of isotype switching[31,33]. Additional signals from the microenvironment such as the cytokines IL-6 and IL-21 and the consequent activation of signal transducer and activator of transcription 3 (STAT3) downstream of cytokine receptor signalling enforce the generation of T cells with a helper phenotype that, at this early stage, maintain the ability to engage multiple differentiation programmes[34–36]. T_{FH} cells, which are distinguished from other T_H cell subsets by their high affinity for antigen and/or sustained interaction with B cells, migrate beyond the T cell–B cell border into the B cell follicle. Some or all of the T_{FH} cells migrate while attached to B cells, as mobile conjugate pairs[37], to participate in the germinal centre reaction[38].

T_{FH} cell interactions with B cells

Positioning of T_{FH} cells in the B cell follicle. T_{FH} cells are probably best defined by their expression of CXCR5, which, in conjunction with the loss of expression of CC-chemokine receptor 7 (CCR7), allows them to migrate into the CXCL13-rich B cell follicles of secondary lymphoid tissues, where they provide instructive cues for the differentiation of B cells[6,7,12–14,25,39] (FIG. 3). However, CXCR5 expression is not unique to T_{FH} cells and it has been estimated that ~50% of CD4+ T cells in antigen-stimulated lymphoid tissues, such as human tonsils, are CXCR5+, but only a subset of these cells localize to the germinal centre[12,13,39]. In addition, CXCR5 is expressed by the subset of circulating CD4+ memory T cells mentioned above[12,13,39]. CXCR5 is expressed only transiently by CD4+ T cells during interactions with peptide–MHC complexes on the surface of antigen-presenting cells and is dependent on co-stimulatory signals delivered through CD28, OX40 (also known as TNFSF4) and ICOS[7,40,41]. By contrast, persistent expression of CXCR5 distinguishes T_{FH} cells from other fully differentiated non-germinal centre CD4+ T cells with B cell helper activity, such as those that participate in the differentiation of B cells to the plasma cells that are responsible for secreting low-affinity antibody in extrafollicular foci or those that express other chemokine receptors and migrate to non-lymphoid tissue sites to carry out their effector functions[12–14,17,22].

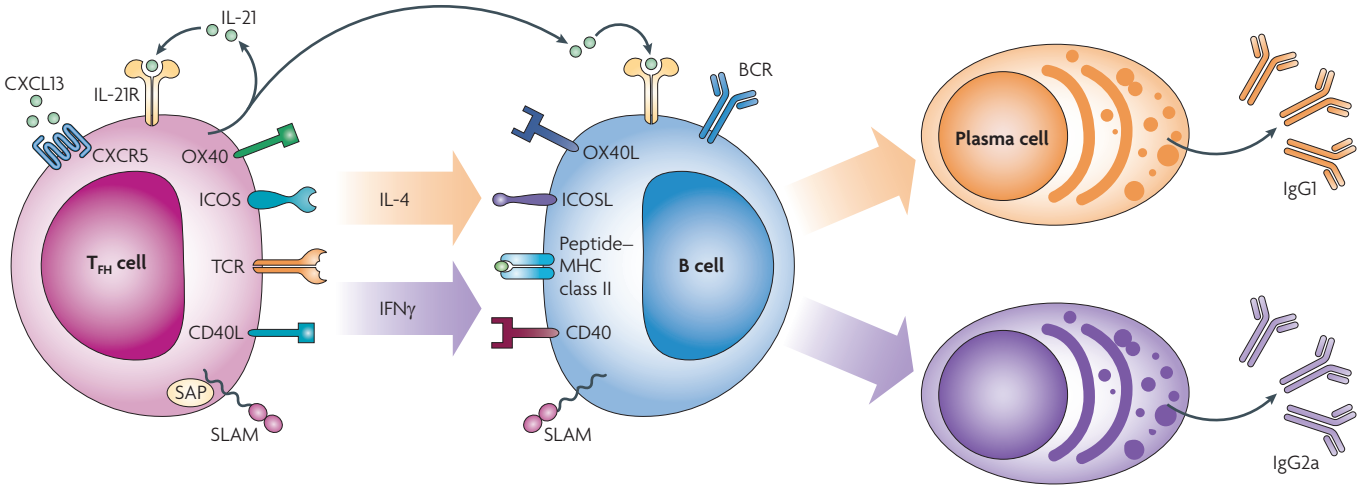

Figure 2 | **Antibody class switching is directed by cytokines.** A range of cytokines produced by T follicular helper (T_{FH}) cells can direct antibody class switching. The acquisition of T cell cytokine competency begins in the T cell zone and precursor T_{FH} cells have the capacity to induce class switching during their interaction with B cells at the border of the T cell zone and B cell follicle. Interleukin-4 (IL-4) induces the switch to IgG1 production (and then IgE production, not shown), and interferon-γ (IFNγ) induces the switch to IgG2a production. BCR, B cell receptor; CXCL13, CXC-chemokine ligand 13; CXCR5, CXC-chemokine receptor 5; ICOS, inducible T cell co-stimulator; IL-21R, IL-21 receptor; L, ligand; SAP, SLAM-associated protein; SLAM, signalling lymphocytic activation molecule; TCR, T cell receptor.

For example, differentiated CD4$^+$ T cells that express CCR6 tend to have a T_H17 cell phenotype[42], those that express CXCR3 are generally T_H1 cells and those with CCR4 expression are largely T_H2 cells[43]. Following immunization, antigen-specific T cells show a higher and more homogeneous expression of CXCR5 than do polyclonal T cells[7], but whether preservation of CXCR5 expression by T_{FH} cells reflects higher TCR affinity for antigen or sustained interaction with B cells remains unknown.

Co-stimulatory molecules. The expression of multiple co-stimulatory molecules is a feature of T_{FH} cells, which may reflect both the sustained multi-signal integration required for their generation and their unique association with B cells[44] (FIG. 3). This association begins at the T cell–B cell border and continues into the B cell follicle, where T_{FH} cells contribute to the germinal centre reaction. However, as numerous T_H cell subsets can help B cells produce antibody beyond the confines of the germinal centre, expression of these molecules *per se* does not adequately define a T_{FH} cell. Rather, T_{FH} cells have been shown to express higher levels of these molecules than any other T_H cell subsets, which correlates with an enhanced capacity to facilitate antibody production[12–14,45,46].

Co-stimulatory molecules positively regulate B cell differentiation, as indicated by the defective humoral immune responses in mice and humans with mutations in the genes *CD40LG* (which encodes CD40 ligand (CD40L)) and *ICOS*[47–50]. The interaction between CD40 (also known as TNFRSF5) on B cells and CD40L, which is transiently expressed by activated CD4$^+$ T cells, stimulates B cell proliferation and facilitates cytokine-induced class switching[51–53]. ICOS is expressed at high levels by T_{FH} cells (and T_H2 cells) and is induced following T cell activation[44]. Once engaged by its ligand (ICOSL) on B cells, ICOS induces the production of T_H cell-type cytokines such as IL-2, IL-4, IL-10 and IL-21 (REFS 44,54,55). Studies showing that ICOS deficiency is associated with a reduction in the germinal centre reaction and fewer T_{FH} cells in mice and humans implicate ICOS signalling in the maintenance and/or generation of T_{FH} cells[41,56]. In addition, the elevated expression of OX40, which is expressed by activated T cells, has been shown to denote T_{FH} cells, but its role in T_{FH} cell function remains unknown[17,57].

PD1. PD1 is an immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing inhibitory molecule that is highly expressed by T_{FH} cells[17,18]. The expression of inhibitory molecules by T_{FH} cells might reflect their role in the germinal centre as they favour strong cognate interactions with B cells[18]. PD1 expression by CD8$^+$ T cells is associated with chronically activated or exhausted cells, but whether this is true for CD4$^+$ T cells is not yet known[58].

IL-21. As discussed above, the generation of highly differentiated T_H cell subsets depends on the integration of multiple co-stimulatory signals that are received during the interaction of T_H cells with antigen-presenting cells; IL-21 seems to provide one of these signals (FIG. 3). However, the role of IL-21 in T_H cell differentiation remains controversial, which may reflect the fact that the production of IL-21 is common to both recently activated CD4$^+$ T cells and differentiated T_H cell subsets[46,59–65]. It is of interest that IL-21 is expressed most highly by T_{FH} cells, and the recent demonstration of greater levels of IL-21 in germinal centre T_{FH} cells than in T_H2 cells located in the lungs supports the idea that high IL-21 expression is a specialized feature of T_{FH} cells[17,22,46,63]. Analyses of mice in which IL-21–IL-21 receptor (IL-21R) interactions have been disrupted indicate that IL-21 is important for the generation of T_{FH} cells[34,65]

T_H17 cell
A subset of CD4$^+$ T helper cells that produce IL-17 and that are thought to be important in inflammatory and autoimmune diseases. Their generation involves IL-21 and IL-23, as well as the transcription factors RORγt and STAT3.

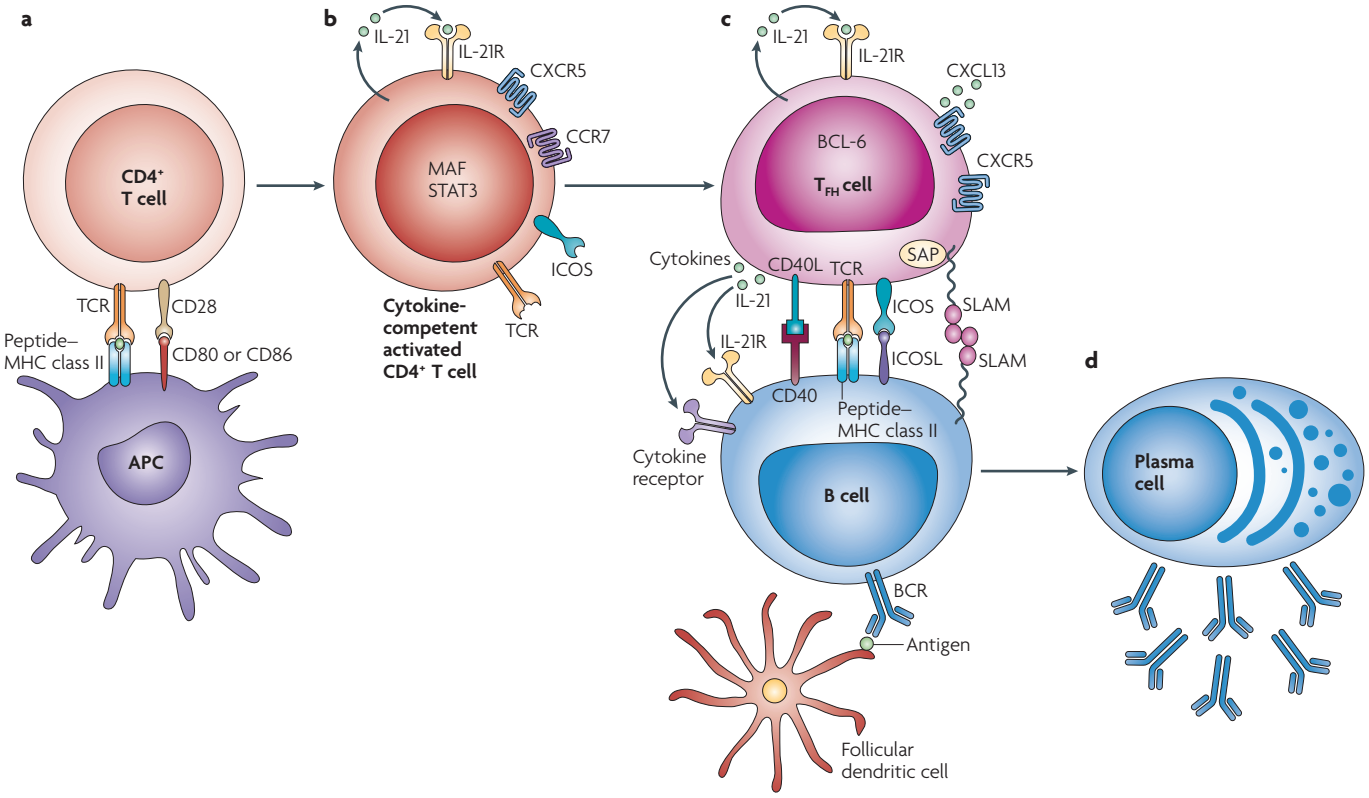

Figure 3 | **The multi-signal pathway for T follicular helper (T_{FH}) cell generation. a** | In the T cell zone of lymphoid tissues, mature dendritic cells expressing CD80 and CD86 present peptide–MHC class II complexes to the T cell receptor (TCR) of naive $CD4^+$ T cells, which constitutively express CD28. **b** | Activated $CD4^+$ T cells produce interleukin-21 (IL-21) and express inducible T cell co-stimulator (ICOS), MAF and signal transducer and activator of transcription 3 (STAT3) and begin their acquisition of cytokine competency (for example, IL-4 or interferon-γ (IFNγ) production) in the T cell zone. **c** | Sustained signalling of activated $CD4^+$ T cells through the TCR, ICOS and IL-21 receptor (IL-21R) at the T cell–B cell border leads to the modulation of cell surface molecules that are important for migration and T cell–B cell interactions, such as increased CXC-chemokine receptor 5 (CXCR5) expression and decreased CC-chemokine receptor 7 (CCR7) expression, and expression of ICOS, CD40 ligand (CD40L), OX40 and signalling lymphocytic activation molecule (SLAM) family members. Follicular dendritic cells bearing antigen interact with maturing B cells in the germinal centre reaction. **d** | Migration of functional T_{FH} cells to B cell follicles and delivery of T cell help to B cells support the selection of activated antibody-secreting plasma cells in germinal centres. APC, antigen-presenting cell; BCL-6, B cell lymphoma 6; BCR, B cell receptor; CXCL13, CXC-chemokine ligand 13; ICOSL, ICOS ligand; SAP, SLAM-associated protein.

Sanroque mice
An autoimmune strain of mouse that has a loss-of-function mutation in the gene roquin (also known as *Rc3h1*). These mice develop a T cell-mediated systemic lupus erythematosus-like syndrome and severe autoimmune diabetes when bred onto a susceptible genetic background.

X-linked lymphoproliferative disease
Individuals with X-linked lymphoproliferative disease have complicated immune dysfunctions, often triggered by infection with Epstein–Barr virus. Many patients develop fatal B cell lymphoproliferation. The gene that encodes SAP is mutated in these patients.

and has a crucial role in T_H cell differentiation before the acquisition of B cell follicle homing capacity by T_{FH} cells[65]. IL-21 also regulates expression of the T_{FH} cell transcription factor BCL-6, but the point at which IL-21 influences T_{FH} cell subset differentiation remains unclear.

IL-21 also has a well-established role in B cell proliferation and differentiation[46,66–68]. However, the observation that efficient IgG1 production by IL-21R-deficient B cells could be restored by IL-21R-sufficient $CD4^+$ T cells indicates that the role of IL-21 in B cell responses has a degree of redundancy, possibly with IL-4 (REF. 65). Similarly, IL-6 may be sufficient for the differentiation of some T_H cell subsets in the absence of IL-21, as IL-6 and IL-21 both have the ability to activate STAT3 (REFS 36,61). In addition to STAT3, IL-21 activates numerous pathways that are common to other co-stimulatory molecules such as ICOS. In the case of sanroque mice, in which a point mutation in a ubiquitin ligase results in the constitutive overexpression of ICOS, IL-21 is not required for the generation of T_{FH} cells[69].

SLAM-associated protein. Given that multiple proteins contribute to the interactions of $CD4^+$ T cells with DCs and B cells, it has been difficult to determine, at a T cell-intrinsic molecular level, which interactions are important for programming T_{FH} cell differentiation. However, a recent study has revealed that SLAM-associated protein (SAP; also known as SH2D1A) has an important role in the B cell-influenced differentiation of $CD4^+$ T cells into T_H cell subsets[19]. SAP is an adaptor molecule that is known to bind to signalling lymphocytic activation molecule (SLAM) and modulate both TCR signalling and T_H2 cell differentiation[19]. In human X-linked lymphoproliferative disease, which is caused by loss-of-function mutations in the gene encoding SAP (*SH2D1A*), and in an analogous gene-targeted mouse model there is a profound defect in germinal centre formation[70]. Live imaging of the dynamics of the interaction of $CD4^+$ T cells with DCs and B cells following immunization revealed that SAP expression by T cells was important for the stability of antigen-dependent T cell–B cell interactions but

dispensable for T cell–DC interactions[19]. Supporting a crucial role for SAP in T_{FH} cell generation, the introduction of a null allele of SAP into sanroque mice led to a reduction in T_{FH} cell numbers and abrogated germinal centre formation and autoantibody formation[69].

Transcription factors. It remains unknown whether T_{FH} cells are fully differentiated once they migrate into the B cell follicle or whether they complete their differentiation programme during the germinal centre reaction, but recent studies have revealed that, like that of other fully differentiated T_H cell subsets, lineage commitment of T_{FH} cells is controlled by the expression of transcription factors[71]. The discovery of specific transcription factors that act as master controllers of cytokine gene expression to establish lineage-specific transcriptional programmes has greatly advanced our understanding of T_H cell lineage commitment. It is now established that T-bet (also known as TBX21) determines T_H1 cell lineage commitment and cytokine production, GATA-binding protein 3 (GATA3) drives T_H2-type cytokine production[72,73], retinoic acid receptor-related orphan receptor-γt (RORγt) directs the differentiation of the T_H17 cell subset[74], and the expression of forkhead box P3 (FOXP3) programmes T_{Reg} cell development and function[75] (FIG. 1). However, many transcription factors are not unique to distinct T_H cell subsets and may function in the generation of multiple T_H cell subsets. For example, the transcription factor MAF, previously shown to be crucial for IL-4 production, has an essential role in generating both T_{FH} cells and T_H17 cells[76]. MAF functions downstream of ICOS[76] and has been shown to transactivate IL-21 (REF. 77).

Transcriptional profiles obtained from microarray analysis identified BCL-6 among the transcription factors that were upregulated in T_{FH} cells but not other effector T_H cell subsets[63], and this finding has been confirmed by recent studies[17,22]. In BCL-6-deficient mice, T cell-dependent antibody responses are reduced owing to an absence of germinal centres, an observation that had been attributed to a B cell-intrinsic defect[78]. Consistent with this interpretation, BCL-6 expression is largely confined to B cells within germinal centres, where it acts as a master regulator of germinal centre lineage commitment, suppressing plasma cell differentiation by the extrafollicular pathway. Recently, however, BCL-6 has also been shown to act as a transcriptional repressor in T_{FH} cells, indicating that BCL-6 expression in both T and B cells is required for germinal centre reactions[79–81]. Forced expression of BCL-6 in $CD4^+$ T cells could suppress both T_H1 and T_H17 cell differentiation pathways while enhancing the development of characteristic features of T_{FH} cells[79,81]. In an analogous study, the analysis of BCL-6-deficient $CD4^+$ T cells and overexpression of BCL-6 in $CD4^+$ T cells showed that BCL-6 expression was both necessary and sufficient for *in vivo* T_{FH} cell differentiation[80]. By contrast, the transcriptional repressor B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1), which is highly expressed in the T cell zone but not in germinal centre T cells, was shown to inhibit T_{FH} cell generation, indicating reciprocal regulation of these two transcription factors during T_{FH} cell differentiation[17,80].

Despite the importance of BCL-6 for T_{FH} cell generation, expression of BCL-6 protein has been observed in only 10–15% of $CD4^+$ T cells located in the germinal centres of human tonsils[82]. Whether these BCL-6-positive cells are a functionally distinct population of T_{FH} cells in the B cell follicle or are terminally differentiated T_{FH} cells remains unknown. In this regard, it is possible that BCL-6 is expressed by T_{FH} cells during a defined period of time or in conjunction with antagonistic transcription factors[17], and the ability of BCL-6 to downregulate the secretion of cytokines such as IL-4 suggests that continuously high expression of BCL-6 in germinal centre T cells might be counterproductive[83].

The expression of master controller transcription factors is used to define T_H cell lineage commitment, but the unique expression of these factors may not be definitive for T_H cell fate. Epigenetic modifications influence the binding of transcription factors to the promoter regions of genes, contributing to the heritability of T_H cell lineage decisions, and evidence is emerging that these decisions remain open to revision. Through the analysis of the chromatin state in resting and effector T cells (including T_H1 cells, T_H2 cells, T_H17 cells cultured *in vitro* and T_{Reg} cells), a recent study has revealed the retention of both permissive and repressive transcription factor binding (bivalent) marks in T_H cell-specific genes, including those of transcription factors[20]. Transcription factor genes in a bivalent state have the potential for subsequent activation or silencing, suggesting that T_H cells retain the potential for functional revision[20]. Interestingly, examination of the *Il21* loci of T_H1, T_H2 and T_{Reg} cells indicated that *Il21* transcription is strongly suppressed in these cells[20], predicting a heritable distinction with T_{FH} cells that highly express IL-21. Future studies will be needed to determine whether T_H cells differentiated *in vivo*, including T_{FH} cells, have similar potential for plasticity at the level of epigenetic modifications.

The fate of T_{FH} cells

The fate of T_{FH} cells after resolution of the germinal centre reaction remains an important area of investigation. Live imaging of germinal centre cell dynamics indicates that B cells can move bidirectionally between the B cell follicle and the germinal centre, but there is currently no evidence to support bidirectional movement of T_{FH} cells[84,85]. Thus, it seems unlikely that T_{FH} cells give rise to the fully differentiated T_H cell subsets that migrate to non-lymphoid tissues (or vice versa). One possible outcome is that T_{FH} cells are terminally differentiated and die owing to the presence of high cell surface levels of CD95 (also known as TNFRSF6) and PD1 expression, which render them susceptible to apoptosis[45,86]. Alternatively, T_{FH} cells may remain *in situ,* either as bone fide memory T cells or as effector T cells that continue to survive owing to persisting antigen[87–89].

A recent study showed the existence of a population of $CD69^+CXCR5^+$ T_{FH} cells that remained in close proximity to $CXCL13^+$ follicular DCs for an extended period of time[24]. These cells could be rapidly reactivated to express effector molecules following secondary immunization and thus behave like antigen-specific memory T cells. T_{FH} cells were found in the vicinity of 'depots'

Follicular DCs
Specialized non-haematopoietic stromal cells that reside in the follicles and germinal centres. These cells have long dendrites, but are not related to dendritic cells, and carry intact antigen on their surface.

of peptide–MHC class II complexes in the draining lymph node after resolution of the germinal centre reaction, suggesting that local triggering of the TCR maintains the expression of CXCR5 and CD69 (REF. 24). Using IL-4-reporter mice, T_{FH} cells were also observed to make IL-4 protein more rapidly following secondary challenge with antigen than cells involved in the primary response[90]. Thus, antigen-specific T_{FH} cells can be detected for some time after an immune response and have the potential to interact with B cells to promote antibody production following secondary challenge or have some as yet unknown function. However, several studies indicate that $CD4^+$ T cells are not necessary for the long-term maintenance of memory B cells or IgG-producing plasma cells[91,92]. Further work will be needed to determine whether T_{FH} cells meet their demise at the resolution of the germinal centre reaction for which they were purposefully generated or whether the population of memory T_{FH} cells that persist in lymphoid organs after the resolution of the germinal centre reaction can improve the outcome of a reinfection.

The relationship between T_H cell subsets

T_{FH} cells can produce cytokines, such as IL-4, IL-10 and IL-21, that promote the survival, proliferation and differentiation of B cells[12,14,15,22,46]. However, the recent identification of IFNγ-producing T_{FH} cells coupled with evidence of their capacity to produce IL-17 shows that T_{FH} cells may have a more heterogeneous pattern of cytokine production than was previously appreciated[14,18,22,24,34]. There are several possible explanations that could reconcile these observations; for example, cytokine expression by T_{FH} cells has typically been measured relative to the abundance of cytokines produced by fully differentiated T_H cells[63] or by chronically activated tonsilar tissue following *ex vivo* re-stimulation and may lead to cytokine expression from cells that may or may not express cytokines *in vivo*[12,13].

T_{FH} cells and T_H2 cells. T_H2 cells have long been regarded as the main providers of help for antibody production by B cells. However, the observation that IL-4-deficient mice can generate T cell-dependent antibody responses suggested that IL-4 is not necessary for antibody production *per se*[93]. T_{FH} cells and T_H2 cells have a degree of commonality in that the targeted deletion of the genes encoding MAF, ICOS and IL-21R impairs the generation of both cell subsets[34,54,56,65,72,76,94,95]. Nevertheless, T_{FH} cells can be clearly distinguished from T_H2 cells by their high levels of expression of both CXCR5 and BCL-6 and by their reduced levels of expression of BLIMP1 and the T_H2 cell-associated chemokine receptor CCR4 (REFS 17,22,63,96).

Recently, several groups have analysed the relationship between IL-4-producing $CD4^+$ T cells and T_{FH} cells using IL-4-reporter mice[22,90,97]. These transgenic mice express either a single reporter gene marking cells that express *Il4* mRNA (IL-4-competent cells) or a dual reporter gene marking both cells that express *Il4* mRNA and those that actually secrete IL-4 protein. The analyses of T_H cell differentiation during infection of IL-4-reporter mice confirm previous reports showing that T_{FH} cells can express IL-4 (REFS 14,18,24,46) and show that most IL-4 production is localized to the B cell follicle[22,90]. The finding that $CXCR5^-PD1^-$IL-4-competent $CD4^+$ T cells from mice immunized with serum schistosome egg antigen (SEA) could upregulate CXCR5 and PD1 on adoptive transfer into naive hosts subsequently immunized with SEA suggests a relationship between T_H2 and T_{FH} cells[97]. However, whether fully differentiated T_H2 cells can migrate into the B cell follicle and become T_{FH} cells requires clarification. Similarly, in contrast to previous studies of cell transcriptional profiles[63], elevated levels of mRNA encoding GATA3 were reported in IL-4-competent $CD4^+$ T cells that also expressed PD1 (REF. 97). These findings offer important insights into the relationship between T_H2 cells and T_{FH} cells and support the notion that these cells may derive from a common precursor. However, they also emphasize the current limitations of the phenotypic characterization of T_{FH} cells on the basis of high expression of common $CD4^+$ T cell activation markers.

By contrast, during infection of the dual IL-4 reporter mice with *Leishmania major*, IL-4-producing T_{FH} cells could be distinguished from IL-4-producing T_H2 cells by their high expression of CXCR5 and IL-21 (REF. 22). The isolation of T cell–B cell conjugates from the lymph node draining the site of *L. major* inoculation showed that IgG1-producing B cells made contact with IL-4-producing T cells, whereas IgG2a-producing B cells made contact with IFNγ-producing T cells[22] (FIG. 3). IL-4-producing T cells were found conjugated to germinal centre B cells that expressed high levels of activation-induced cytidine deaminase (AID) and had hallmarks of somatic hypermutation[22]. These findings indicate that T_{FH} cells can produce cytokines that direct the production of different antibody isotypes and the affinity maturation of antibodies in the responding B cells.

T_{FH} cells and T_H1 cells. T_{FH} cells can be distinguished from T_H1 cells on the basis of their homing potential and tissue localization, which are largely due to their selective expression of CXCR5 and CXCR3, respectively. In contrast to IL-4, the typical T_H1-type cytokine, IFNγ, only weakly supports B cell survival and proliferation and was not originally detected at high levels in T_{FH} cells[63]. Recently, however, T_{FH} cells have been shown to express levels of the T_H1 cell-associated transcription factor T-bet that were equivalent to the levels observed in both non-T_{FH} $CD4^+$ T cells and effector T cells with non-lymphoid migratory capacity[17]. In addition, as noted above, IFNγ-producing T_{FH} cells were detected in the germinal centre and found conjugated to IgG2a-producing germinal centre B cells in the draining lymph node following *L. major* inoculation (FIG. 3). Interestingly, B cells conjugated with IFNγ-producing T_H cells had much lower levels of AID expression than those conjugated with IL-4-producing T_{FH} cells, suggesting an enhanced capacity of IL-4-producing T cells to direct antibody class switching[22]. However, as infection with *L. major* and other parasites, and also immunization with haptenated proteins in the presence of alum adjuvant, are expected to produce IL-4-dominant responses, additional studies will be required to determine the proportion of IFNγ-producing T_{FH} cells during an IFNγ-biased humoral response.

IL-4-reporter mice
Genetically engineered knock-in mice in which the gene encoding IL-4 has been replaced by sequences that encode a reporter molecule, such as green fluorescent protein (GFP). When the IL-4 promoter region is activated, GFP is expressed and GFP^+ cells can be seen by flow cytometry.

Activation-induced cytidine deaminase
An enzyme that is required for two crucial events in the germinal centre: somatic hypermutation and class switch recombination.

T_{FH} cells and T_H17 cells. A key early discovery in the study of the T_H17 cell subset was the identification of the transcription factor RORγt, which specifies T_H17 cell lineage commitment. Interestingly, T_{FH} cells can produce IL-17 without expressing RORγt[34,65]. Nevertheless, the relationship between T_H17 cells and T_{FH} cells remains incompletely understood. For example, in autoimmune BXD2 mice, which spontaneously develop glomerulonephritis and erosive arthritis, IL-17-producing T_H cells localize in B cell follicles and promote germinal centre reactions[98]. Production of IL-17 by T_{FH} cells is not restricted to BXD2 mice; for example, it has been detected in $ICOS^{hi}CXCR5^+$ T_H cells from the spleen and draining lymph nodes in mice with experimental autoimmune encephalomyelitis (EAE)[76].

T_{FH} cells and FOXP3$^+$ T_{Reg} cells. New evidence that T_{FH} cells can be generated from T_{Reg} cells challenges the view that T_{FH} and T_{Reg} cells are distinct subsets[99]. Using reporter mice in which FOXP3 expression is marked by expression of enhanced green fluorescent protein ($FOXP3^{EGFP}$ mice), preferential generation of T_{FH} cells from $FOXP3^+$ T cells rather than $FOXP3^-$ T cells was observed in the Peyer's patches when donor cells were adoptively transferred into lymphopenic mice. The resulting T_{FH} cells were efficient at promoting germinal centre reactions and IgA production[99]. By contrast, $FOXP3^+$ T cells neither differentiated into T_{FH} cells nor supported germinal centre formation in the spleen or lymph nodes under the same experimental conditions, demonstrating a unique microenvironment for the generation of T_{FH} cells from $FOXP3^+$ T_{Reg} cells in the Peyer's patches[99]. Despite the detection of a small number of T_{FH} cells in the germinal centre, the physiological relevance of a population of germinal centre T_{Reg} cells that can suppress B cell responses remains unknown[100,101].

Clinical relevance

Understanding the ways in which T_{FH} cells are generated and regulated offers a unique challenge for both the improved design of protein vaccines and the treatment of antibody-mediated autoimmune diseases. Immunological tolerance among T cells is of paramount importance for the control of autoimmune antibody specificities, therefore it is likely that T_{FH} cells provide inappropriate helper signals to self-reactive B cells in cases of antibody-mediated autoimmune diseases. $CXCR5^+$ T cells that express increased levels of PD1 and ICOS can be found in the blood of patients with systemic lupus erythematosus (SLE) and Sjogren's syndrome[102,103]. Their activated phenotype indicates that they are distinct from circulating $CXCR5^+$ T cells that are found in normal subjects, suggesting some 'spill over' of T_{FH} cells from extra-lymphoid tissues in these autoimmune conditions. There are numerous examples of murine models of autoimmunity in which the inhibition of the function of T_{FH} cell-associated molecules, such as CD40L, ICOS, SAP and IL-21, results in reduced autoantibody production[104–109]. Blocking CD40–CD40L interactions similarly prevents autoantibody production and the aberrant accumulation of germinal centre-like B cells and plasmablasts in the peripheral blood of patients with SLE[110]. Mouse models of SLE are characterized by a T_{FH} cell-like transcriptome in their spleens[111,112], and in sanroque mice the expansion of T_{FH} cell populations resulting from excessive signalling through ICOS mediates increased autoantibody production and renal pathology[112].

A better understanding of the biology of T_{FH} cells could also aid our understanding of how to therapeutically target certain T cell lymphomas[113]. For example, the transcriptome of angioimmunoblastic T cell lymphoma (AITL) shares many similarities with that of T_{FH} cells, giving some insight into the cell of origin. Malignant AITL cells are $CD4^+$ T cells that express BCL-6 (REFS 82,114,115), CXCR5, CD40L, OX40 and PD1 (REFS 116,117) and produce CXCL13 (REF. 118) and are unique in their expression of CD10 (also known as neprilysin)[119]. Several studies have proposed that increased production of CXCL13 and constitutive expression of CD40L by malignant T_{FH} cells may lead to increased recruitment of B cells into follicles, their aberrant activation and subsequent hypergammaglobulinaemia[116,120]. T_{FH} cell markers are also observed in neoplastic cells of cutaneous $CD4^+$ small/medium-sized pleomorphic T cell lymphoma (CSTCL), suggesting that B cell stimulation by T_{FH} cells could also take place in some cutaneous T cell lymphomas[121].

Concluding remarks

Recent studies have advanced our understanding of T_{FH} cell biology, but several questions remain unanswered. First, is the heterogeneity of cytokine production from T_{FH} cells indicative of their pluripotency or are there distinct T_{FH} cell subsets as part of a broader T_{FH} cell family? Second, with regard to T cell plasticity, it is not known whether T_{FH} cells can differentiate into other T_H cell subsets outside of the germinal centre microenvironment or whether fully differentiated T_H cell subsets can become T_{FH} cells if given the correct signals. In this regard, it would be of interest to examine epigenetic modifications in T_{FH} cell-specific genes. Third, the ability of BCL-6 to suppress the production of IL-4, IFNγ and IL-17 suggests that *Bcl6* is either highly regulated and/or acts in conjunction with other, antagonistic transcription factors during T_{FH} cell differentiation, but how *Bcl6* expression is coordinated in T_{FH} cells remains unknown. Finally, it will be important to determine whether the T_{FH} cells that participate in the selection of higher-affinity B cell clones in the germinal centre are the same cells that initiate class switching.

Studies continue to emerge indicating that $CD4^+$ T cells have more functional plasticity than is generally appreciated[99,122,123]. These studies challenge our criteria for differentiated T_H cell subsets and question whether irreversible cell differentiation is achieved before cell death. Our Cartesian approach towards the classification of T_H cell subsets helps us to understand certain aspects of T_H cell differentiation but, unfortunately, this might be at the cost of understanding the remarkably flexible nature of $CD4^+$ T cells, which arises in response to their unique microenvironments.

Experimental autoimmune encephalomyelitis
An experimental model for the human disease multiple sclerosis. Autoimmune disease is induced in experimental animals by immunization with myelin or peptides derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord.

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DATABASES

UniProtKB: http://www.uniprot.org
AID | BCL-6 | CCR4 | CCR7 | CD10 | CD28 | CD40 | CD40L | CD69 | CD95 | CXCR3 | CXCR5 | CXCL13 | FOXP3 | GATA3 | ICOS | ICOSL | IFNγ | IL-4 | IL-6 | IL-10 | IL-21 | MAF | OX40 | PD1 | RORγt | S1PR1 | SAP | SLAM | STAT3 | T-bet

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