The good, the bad and the ugly — T_{FH} cells in human health and disease

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Abstract | Antibody production is an important feature of the vertebrate immune system. Antibodies neutralize and clear pathogens, thereby protecting against infectious diseases. Such humoral immunity has great longevity, often persisting for the host's lifetime. Long-lived humoral immunity depends on help provided by $CD4^+$ T cells, namely T follicular helper (T_{FH}) cells, which support the differentiation of antigen-specific B cells into memory and plasma cells. T_{FH} cells are stringently regulated, as aberrant T_{FH} cell activity is involved in immunopathologies such as autoimmunity, immunodeficiencies and lymphomas. The elucidation of the mechanisms that regulate T_{FH} cell differentiation, function and fate should highlight targets for novel therapeutics.

Germinal centres
The structures that are formed by the expansion of antigen-activated B cell blasts that have migrated into the follicles of lymph nodes. The B cells in these structures proliferate and the immunoglobulin genes undergo somatic hypermutation before the cells leave as plasma cells or memory cells.

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Following infection or vaccination, the induction of protective immunity against invading pathogens depends on the generation of an appropriate type of immune response. This relies on the flexibility of naive $CD4^+$ T cells, which differentiate into diverse subsets with specialized effector functions to protect against infection by distinct pathogens[1] (TABLE 1). The importance of having distinct subsets of $CD4^+$ T cells is evident in disease states that arise from the perturbed differentiation or function of specific effector T cell populations[2] (TABLE 1).

The generation of these effector T cell subsets depends on the stimulatory cytokines that are present in the microenvironment during activation; these cytokines induce transcription factors that prime naive precursor cells for differentiation. Interleukin-12 (IL-12) induces the T-box transcription factor T-bet (also known as TBX21) in the case of T helper 1 (T_H1) cells, IL-4 induces GATA-binding protein 3 (GATA3) in the case of T_H2 cells and IL-6 or IL-23 induce retinoic acid receptor-related orphan receptor-γt (RORγt) in the case of T_H17 cells[1] (TABLE 1). However, the concept of 'master regulators' for lymphocyte differentiation is an overly simplified idea, as numerous transcription factors are required for the commitment of $CD4^+$ T cells to specific effector lineages (TABLE 1). Thus, the ultimate outcome of T_H cell differentiation depends on the coordinated functions of several important molecular regulators that operate to control gene expression and effector function.

T_H cell subsets have been identified and characterized for immunity against specific pathogenic threats (TABLE 1), but the production of neutralizing antibodies is required for the development of protective immunity to most infectious diseases. In this case, the differentiation of B cells to antibody-secreting cells is dependent on instructive signals that arise from T follicular helper (T_{FH}) cells. The importance of antibody production by antigen-specific B cells is exemplified not only by our ability to establish serological memory that provides long-lasting protection against pathogen infection but also by the success of most vaccines, which rely on antibody responses for their efficacy. The fundamental role of T cells in B cell differentiation was first reported nearly 50 years ago[3]. However, it is only in the past decade that we have gained a clear understanding of the biology of T_{FH} cells.

T_{FH} cells were first described in humans as $CD4^+$ T cells in secondary lymphoid tissues that expressed the B cell zone-homing chemokine receptor CXC-chemokine receptor 5 (CXCR5) and therefore localized to B cell follicles, including germinal centres (GCs). $CD4^+CXCR5^+$ T cells were more efficient than $CD4^+CXCR5^-$ T cells at inducing class switching and antibody secretion in B cells[4–7]. Further studies showed remarkable similarities between human and murine T_{FH} cells[8–10], which allowed elucidation of the mechanisms by which T_{FH} cells drive affinity maturation as well as antibody and autoantibody production in GCs (reviewed in REFS 11–13) (BOXES 1,2).

The most accurate definition of T_{FH} cells relates to their function as cells that migrate to follicles and interact with antigen-specific B cells to support B cell differentiation. However, using this empirical definition to isolate and study T_{FH} cells makes detailed analysis of this subset of cells challenging owing to the inherent difficulty in isolating cells from anatomically discrete regions of lymphoid tissues. For this reason, T_{FH} cells are more commonly defined

Table 1 | **Effector subsets of CD4⁺ T cells: ontogenic and functional requirements, and roles in disease**

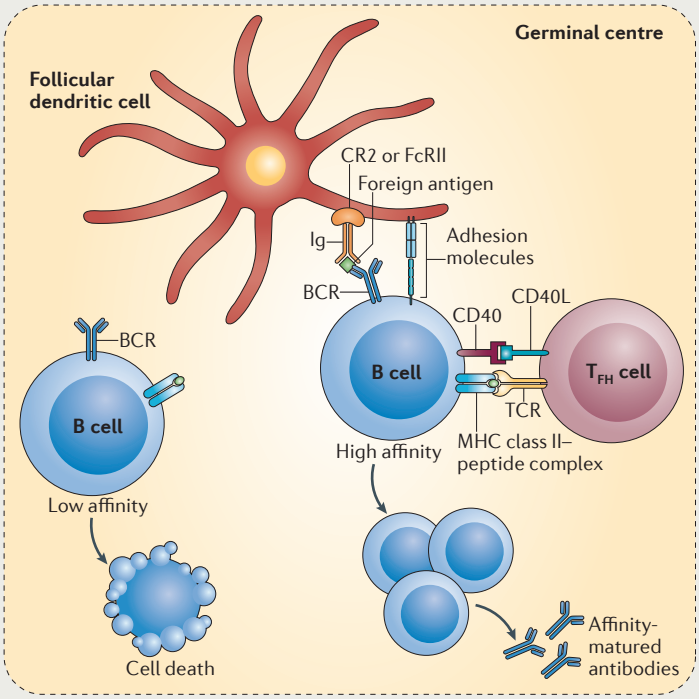
CD4⁺ T cell subset	Inducing cytokines	Activated STATs	Transcription factors	Suppressing cytokines	Canonical cytokines produced	Roles in host protection	Associated pathologies	Refs
T_H1 cells	• IL-12 • IFNγ	• STAT4 • STAT1	T-bet	IL-4 and IL-10	IFNγ	• Antiviral and antimicrobial immunity • Cell-mediated immunity	• Mendelian susceptibility to mycobacterial disease (decrease in T_H1 cells) • Multiple sclerosis (increase in T_H1 cells)	1,121, 122
T_H2 cells	• IL-4	• STAT6	GATA3 and MAF	IFNγ	IL-4, IL-5 and IL-13	• Immunity to extracellular parasites	• Allergy, asthma or eczema (increase in T_H2 cells)	1,123
T_H17 cells	• IL-23 and IL-1β • IL-6 and IL-1β • TGFβ	• STAT3	RORγt and RORα	• IL-4, IFNγ, IL-27 and IL-2 • TGFβ (suppresses IL-22 expression)	IL-17A, IL-17F, IL-21, IL-22 and IL-26*	• Protection at mucocutaneous sites • Antimicrobial immunity (for example, against *Candida* spp. and *Staphylococcus* spp.) • Inflammatory bowel disease	• Inflammatory bowel disease (increase in T_H17 cells) • Susceptibility to fungal infections (decrease in T_H17 cells)	1,121, 122,124
T_H9 cells	• TGFβ • IL-4	• STAT6	PU-1 and IRF4	IFNγ and IL-27‡	IL-9	• Protection against helminth infections	• Allergy (atopic dermatitis) and asthma (increase in T_H9 cells)	125
T_H22 cells	• TNF • IL-6	• STAT1 • STAT3 • STAT5	RORγt and AHR	High doses of TGFβ	IL-22	• Barrier immunity (skin, intestines and airways) • Enhancement of innate immunity • Tissue regeneration	• Allergy (atopic dermatitis) (increase in T_H22 cells) • Inflammation at joints and barriers (increase in T_H22 cells in mice)	126
T_{Reg} cells	• TGFβ and IL-2	• STAT5	FOXP3	IL-6	TGFβ and IL-10	• Immune suppression	• IPEX syndrome (decrease in T_{Reg} cells)	75
T_{FH} cells	• IL-6, IL-21 and/or IL-27 • IL-12	• STAT3 • STAT4 • STAT1	BCL-6, IRF4, MAF and BATF	IL-2 and IL-10	IL-21, IL-4 and IL-10	• Help for B cell activation or differentiation • Generation of long-lived antibody responses	• Humoral immunodeficiency (decrease in T_{FH} cells) • Autoimmunity (increase in T_{FH} cells) • T cell lymphoma (increase in T_{FH} cells) (see TABLE 2)	23–25, 45

AHR, aryl hydrocarbon receptor; BATF, basic leukine zipper transcriptional factor ATF-like; BCL-6, B cell lymphoma 6; FOXP3, forkhead box P3; GATA3, GATA-binding protein 3; IFNγ, interferon-γ; IL, interleukin; IPEX, immunodysregulation, polyendocrinopathy and enteropathy X-linked; IRF4, interferon-regulatory factor 4; ROR, retinoid-related orphan receptor; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor-β; T_{FH}, T follicular helper; T_H, T helper; TNF, tumour necrosis factor; T_{Reg}, T regulatory. *Human-specific cytokine. ‡Reported in mice.

on the basis of their surface phenotype. Consequently, in both humans and mice[8–10,14–17], T_{FH} cells are considered to be CD4⁺ T cells that express the highest levels of CXCR5, together with the surface receptors inducible T cell co-stimulator (ICOS) and programmed cell death protein 1 (PD1; also known as PDCD1), the transcriptional repressor B cell lymphoma 6 (BCL-6) and the cytokine IL-21 (BOX 3), and that have downregulated the T cell zone-homing receptor CC-chemokine receptor 7 (CCR7) and IL-7 receptor-α (IL-7Rα) (TABLE 1).

This phenotypic approach to defining T_{FH} cells has facilitated their molecular and cellular characterization. However, it needs to be appreciated that, just as B cells undergo important differentiation events at the T cell–B cell border (such as the formation of extrafollicular plasmablasts) and in GCs (such as the formation of memory and plasma cells), CD4⁺ T cells can differentiate into T_{FH} cell subsets that are strategically located at these regions to facilitate distinct phases of a T cell-dependent B cell response[6,7,18,19]. These T_{FH} cell subsets probably provide early B cell help at the T cell–B cell border, and/or they represent precursor cells that differentiate into GC T_{FH} cells following receipt of appropriate signals from inside the active B cell follicle and that guide the differentiation of GC B cells into memory or plasma cells. In this Review, we discuss recent developments in the investigation of the mechanisms that underlie T_{FH} cell development and function, the discovery of specialized subsets of T_{FH} cells and how perturbations to T_{FH} cells potentially contribute to numerous human diseases.

Box 1 | **T_{FH} cells and affinity maturation of GC B cells**



Affinity maturation of the antibody response is based on the selective perpetuation (known as 'positive selection') of germinal centre (GC) B cells that have acquired increased affinity for foreign antigens through somatic hypermutation (SHM) of their immunoglobulin variable-region genes. Whereas SHM itself takes place in B cells that occupy the GC dark zone, high-affinity GC B cells only undergo positive selection after returning to the light zone where they gain preferential access to foreign antigens that are expressed on the surface of follicular dendritic cells (see the figure). However, the precise mechanisms by which preferential access to antigens translates into positive selection of high-affinity GC B cells have not been clearly established.

The specific provision of T follicular helper (T_{FH}) cell-derived helper signals to high-affinity GC B cells is proposed to be one of, if not the only, major drivers of antibody affinity maturation[12,13]. In theory, the greater propensity of high-affinity GC B cells to access foreign antigens should augment their ability to internalize, process and present foreign peptides to the T_{FH} cells that reside in the light zone of GCs (see the figure). However, although it is clear that T_{FH} cells are required to support the GC response, it has been difficult to establish their precise role in driving affinity maturation. In a recent study, a GC response was manipulated in mice such that antigen presentation by GC B cells to T_{FH} cells was decoupled from B cell receptor (BCR)-mediated antigen recognition. This resulted in the delivery of T_{FH} cell help to all GC B cells and the proliferative expansion of the population of T_{FH} cells regardless of their affinity for antigen[12]. This result does not prove that preferential delivery of T_{FH} cell help is the fundamental driver of affinity maturation but it clearly demonstrates that T_{FH} cells have the potential to perform this role if high-affinity GC B cells are indeed superior at presenting antigen to T_{FH} cells.

CD40L, CD40 ligand; CR2, complement receptor 2; FcRII, low affinity Fc receptor for immunoglobulin; Ig, immunoglobulin; TCR, T cell receptor.

Requirements for T_{FH} cell formation

T_{FH} cell differentiation requires input from several surface receptors (including CD28, ICOS, CD40 ligand (CD40L) and signalling lymphocytic activation molecule (SLAM) family members), as well as from cytokines and their associated signalling pathways (for example, signal transducer and activator of transcription 3 (STAT3) or SLAM-associated protein (SAP; also known as SH2D1A)), which all culminate in the induction of BCL-6, an important regulator of the T_{FH} cell lineage[20–22]. However, additional transcription factors (including interferon-regulatory factor 4 (IRF4), basic leucine zipper transcriptional factor ATF-like (BATF) and MAF) and microRNAs also have important regulatory functions during T_{FH} cell development[22–25] (FIG. 1; TABLE 1; Supplementary information S1 (table)). The requirements for T_{FH} cell formation have been extensively reviewed in the literature[23–26] and are summarized in Supplementary information S1 (table). In this Review, we discuss the most recent studies that have identified pathways that positively and negatively influence T_{FH} cell generation and function, and that clarify previous inconsistencies.

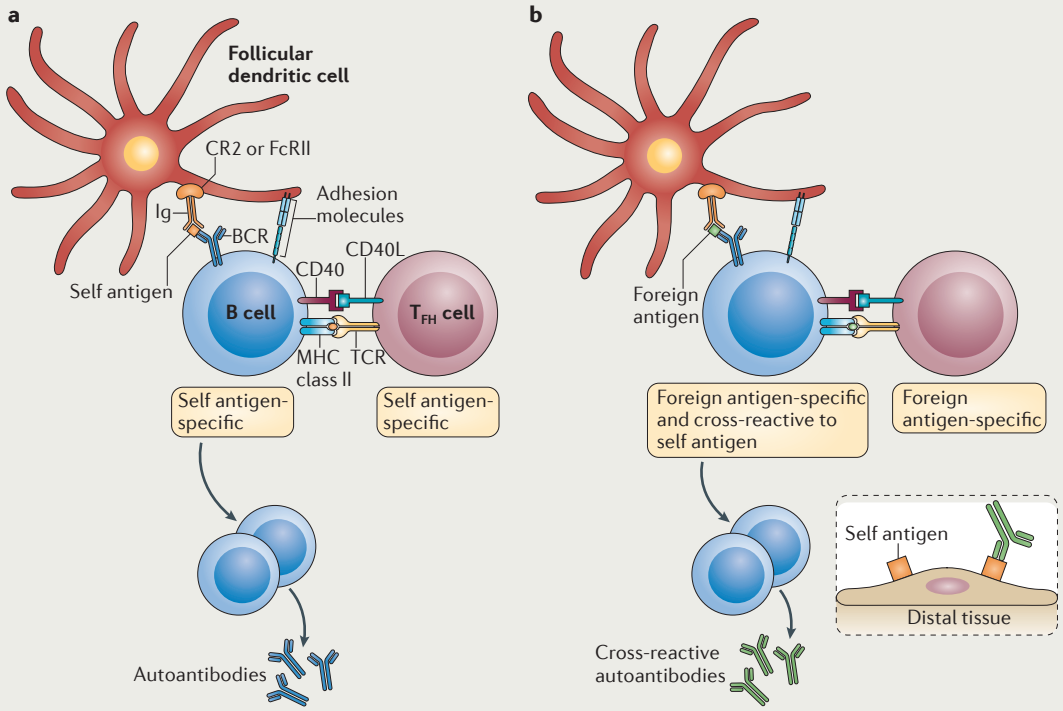
B cell lymphoma 6 (BCL-6). A transcriptional repressor identified as being crucial for the formation of T follicular helper (T_{FH}) cells. Several mechanisms have been proposed for the role of BCL-6 in T_{FH} cell commitment, including suppression of the expression of transcription factors that are required for the generation of alternative T_H fates, suppression of microRNAs and cooperation with other transcriptional regulators to induce the expression of important T_{FH} cell-related genes.

The TNF receptor superfamily. B cell-activating factor (BAFF; also known as TNFSF13B) and a proliferation-inducing ligand (APRIL; also known as TNFSF13) are ligands of the tumour necrosis factor (TNF) superfamily that regulate B cell survival and differentiation[11]. Both ligands bind to the common receptors transmembrane activator and CAML interactor (TACI; also known as TNFRSF13B) and B cell maturation antigen (BCMA; also known as TNFRSF17), and BAFF also binds to the BAFF receptor (BAFFR; also known as TNFRSF13C); receptors which are predominantly expressed on B cells[11]. NF-κB-inducing kinase (NIK; also known as MAP3K14) is a component of the BAFFR signalling pathway[11]. Expression of NIK and BAFFR by B cells was found to be required for their constitutive expression of ICOS ligand (ICOSL). The significance of this is that NIK deficiency in B cells compromises T_{FH} cell induction[27]. Thus, sustained BAFF–BAFFR–NIK signalling in B cells maintains ICOSL expression, thereby facilitating cognate ICOS–ICOSL interactions between activated $CD4^+$ T cells and B cells, which result in optimal T_{FH} cell differentiation.

Box 2 | **T_{FH} cells and autoantibody production at the germinal centre**

Although the generation of high-affinity antibodies directed against foreign pathogens is the '*raison d'être*' of the germinal centre (GC), the random nature of the somatic hypermutation (SHM) process can lead to the generation of antibodies that recognize self antigens with high affinity. Given the fact that many pathogenic autoantibodies are both somatically hypermutated and of high affinity[13], it has long been presumed that T follicular helper (T_{FH}) cell-driven selection of self-reactive GC B cells can make an important contribution to autoimmune disease. Indeed, it has recently been demonstrated that in some circumstances T_{FH} cells are required to drive systemic autoimmunity[34].

A failure of T cell self-tolerance in the T_{FH} cell repertoire is no doubt an important driver of autoimmunity (see the figure, panel **a**), but recent data indicate that T_{FH} cell responses that are directed against foreign antigens can also support an autoantibody response under certain circumstances[70]. Thus, if a somatically mutating GC B cell acquires self-reactivity but also maintains its cross-reactivity with foreign antigens, then foreign antigen-specific T_{FH} cells can support B cell maturation and drive autoantibody production (see the figure, panel **b**). This mechanism selectively operates against peripheral tissue-specific self antigen targets, as cross-reactivity with self antigens that are present in the GC microenvironment results in the deletion of the self-reactive GC B cells[70]. Thus, these data indicate that pathogen-specific T_{FH} cells might drive the production of peripheral tissue-specific cross-reactive autoantibodies that are found in post-infectious autoimmune diseases such as rheumatic fever and Guillain–Barre syndrome[13].



BCR, B cell receptor; CD40L, CD40 ligand; CR2, complement receptor 2; FcRII, low affinity Fc receptor for immunoglobulin; Ig, immunoglobulin; TCR, T cell receptor.

In contrast to BAFFR, TACI negatively regulates B cell function[11]. In keeping with this, *Taci*$^{-/-}$ mice have expanded populations of T_{FH} cells and GC B cells[28]. In the absence of TACI, greater ICOSL expression on B cells results in an increase in the number of T_{FH} cells[28]. These observations demonstrate that an important extrinsic determinant of T_{FH} cell formation is the balance between positive and negative signalling that is provided by BAFF and APRIL through BAFFR and TACI, respectively. These interactions cooperatively regulate ICOSL expression on antigen-presenting B cells (FIG. 1). These studies highlight the crucial role for ICOS–ICOSL signalling in T_{FH} cell formation, particularly as the phenotype of NIK-deficient mice recapitulates that of mice that are globally deficient in ICOS[29] or mice with B cells that lack expression of ICOSL[30].

X-linked lymphoproliferative disease
A rare, often fatal, primary immunodeficiency disease that is characterized by an inability to mount an effective immune response against Epstein–Barr virus, as well as a susceptibility to developing lymphoma and/or hypogammaglobulinaemia.

The SLAM family of surface receptors. The SLAM family of receptors includes SLAM (also known as SLAMF1), CD84 (also known as SLAMF5), natural killer cell receptor 2B4 (also known as CD244), T lymphocyte surface antigen LY9 and NTBA (natural killer, T and B cell antigen; also known as SLAMF6 and LY108 in mice). These receptors recruit SAP, thereby allowing $CD4^+$ T cells to activate signalling intermediates, including protein kinase Cθ (PKCθ), BCL-10, nuclear factor-κB (NF-κB) and FYN, that are important for receptor function[31]. Naive SAP-deficient $CD4^+$ T cells fail to form stable conjugates with cognate B cells[32,33], and are therefore unable to differentiate into T_{FH} cells and to help T cell-dependent B cell responses[17,18,23,34]. This explains why individuals with X-linked lymphoproliferative disease (XLP) caused by mutations in *SH2D1A* (the gene encoding

Box 3 | **IL-21 is a potent differentiation factor for human B cells**

Interleukin-21 (IL-21) was discovered in 2000 as a pleiotropic cytokine that is capable of activating most lymphocyte populations[116]. The initial description reported that IL-21 strongly induced the proliferation of CD40-stimulated human B cells, but that it inhibited IL-4-induced B cell proliferation[116]. Since then, many studies have established the potency of IL-21 as a growth and differentiation factor for human B cells. When human B cells are primed with T cell help in the form of a CD40-specific monoclonal antibody or CD40 ligand (CD40L), IL-21 induces robust B cell proliferation as well as the expression of activation-induced cytidine deaminase (*AICDA*; required for immunoglobulin class switching), B lymphocyte-induced maturation protein 1 (*BLIMP1*; also known as *PRDM1*) and X box-binding protein 1 (*XBP1*)[83,117,118], all of which mediate the differentiation of B cells into plasma cells. Consequently, at least *in vitro*, IL-21 efficiently induces subsets of activated B cells to undergo class switching either to become IgG- or IgA-expressing cells, or to become plasmablasts secreting IgM, IgG, IgA or IgE[83,117–120]. IL-21 predominantly induces switching to IgG3, IgG1 and IgA1 subclasses[117,119,120], whereas IL-21-stimulated naive, GC or memory B cells produce large quantities of immunoglobulins[83,117,119]. IL-21 can also induce the expression of B cell lymphoma 6 (*BCL6*) in human naive B cells[117], which is consistent with IL-21 having a role in establishing GCs. Before the discovery of IL-21, it was well recognized that class switching by human B cells was regulated by IL-4 (which promotes class switching to IgG4 and IgE), IL-10 (which promotes class switching to IgG1 and IgG3), IL-13 (which promotes class switching to IgG4 and IgE) and transforming growth factor-β (TGFβ; which promotes class switching to IgA), and IL-10 was also considered to be a strong inducer of immunoglobulin secretion. Furthermore, B cell survival was shown to be positively regulated by IL-4 or IL-10 (reviewed in REF. 82). Studies over the past decade have highlighted the importance of IL-21 in humoral immunity in humans by demonstrating that it has the remarkable ability to exert all of these functions on human B cells.

SAP) have poor humoral immunity[31]. A role for CD84 in SAP-dependent T_{FH} cell generation following immunization with protein antigen was recently demonstrated[33]. However, the T_{FH} cell deficiency in *Cd84*$^{-/-}$ mice was less severe than in SAP-deficient mice[33], and T_{FH} cell formation following viral infection was unaffected by the absence of CD84 (REF. 35).

Analysis of gene-targeted mice has failed to show a requirement for SLAM family receptors other than CD84 in T_{FH} cell formation (reviewed in REF. 23), although SLAM has been shown to be required for IL-4 expression by GC T_{FH} cells[18]. One interpretation of these observations is that the severe effect of SAP deficiency in T_{FH} cells reflects a requirement for numerous SLAM receptors during the differentiation of T_{FH} cells. Alternatively, as SLAM receptors can also recruit inhibitors of signalling (such as lipid and tyrosine phosphatases), SAP deficiency might exacerbate negative signals that are delivered through one or more of the SLAM receptors. Consistent with this idea, loss of LY108 reversed the inability of SAP-deficient $CD4^+$ T cells to form T_{FH} cells and to support B cell responses[35]. This was due to a reduced recruitment of SH2 domain-containing protein tyrosine phosphatase 1 (SHP1; also known as PTPN6) to the immune synapse by LY108 (REF. 35). Thus, LY108 functions as a rheostat that is capable of delivering positive SAP-dependent and negative SHP1-dependent signals that dynamically regulate T_{FH} cells (FIG. 1).

The role of PD1. An important phenotypic determinant of T_{FH} cells is their high expression of PD1 (REF. 14). PD1 has an inhibitory role in T_{FH} cell differentiation, as mice with impaired PD1 function have more T_{FH} cells ($CCR7^{low}ICOS^{hi}$ cells in *Pd1*$^{-/-}$ mice) as a result of increased proliferation and reduced apoptosis[36–39]. Expression of PD1 ligand 1 (PDL1; also known as CD274), rather than PDL2, on B cells constrains T_{FH} cell formation via the PD1 pathway[38] (FIG. 1). Although these studies showed that ablating PD1 signalling increased T_{FH} cell numbers, they yielded conflicting results about how this affected the outcome of the GC response[36–39]. For instance, some groups reported increased antigen-specific antibody responses in PDL1-deficient mice[38] or in *Plasmodium*-infected mice that had been treated with a PDL1-specific mAb[39]. These findings in mice are consistent with data demonstrating that ligation of PD1 suppresses the proliferation, activation and function of human T_{FH} cells *in vitro*[40]. However, other groups have reported impaired plasma cell and GC responses in the absence of PD1 signalling[36,37]. These differences might reflect nuances in the experimental systems that have been used, but they might also result from distinct functions of PD1 in the development and function of not only T_{FH} cells but also follicular T regulatory cells (follicular T_{Reg} cells), which express higher levels of PD1 than T_{FH} cells[41].

Cytokines. Numerous cytokines have been shown to be important for T_{FH} cell generation *in vivo* and *in vitro*. The initial cytokines that were identified to induce T_{FH} cell-like features in cultured $CD4^+$ T cells were IL-6 and IL-21 (TABLE 1). However, subsequent analyses of IL-6- and IL-21- or IL-21R-deficient mice yielded conflicting results regarding the necessity of these cytokines in regulating T_{FH} cell formation *in vivo* (reviewed in REFS 23,24). Furthermore, recent studies have provided greater insights into the roles of these cytokines in T_{FH} cell commitment. Using several mouse models of viral infection, investigators found varying — and transient — degrees of impairment in T_{FH} cell numbers in the absence of IL-6, but they observed consistently reduced levels of virus-specific IgG[42–45]. A more severe decrease in T_{FH} cell formation and protective IgG production occurred in the absence of both IL-6 and IL-21 (REFS 42,44) (FIG. 1). This requirement for IL-6 and IL-21 is consistent with reductions in the number of T_{FH} cells in the absence of functional STAT3 (REFS 30,46), which acts downstream of both cytokines (TABLE 1). Interestingly, the early reduction in the number of mouse T_{FH} cells in the absence of STAT3 was

SH2 domain-containing protein tyrosine phosphatase 1
(SHP1). A protein tyrosine phosphatase that is involved in suppressing intracellular signals delivered via numerous activating receptors, including T cell and B cell antigen receptors, as well as members of the signalling lymphocytic activation molecule (SLAM) family of surface receptors. One proposed mechanism of action is the direct or indirect dephosphorylation of components of the T cell receptor signalling pathway, such as CD3ζ, LCK, ζ-chain-associated protein kinase of 70 kDa (ZAP70) and phosphoinositide 3-kinase.

Follicular T regulatory cells
A subset of T regulatory (T_{Reg}) cells that co-opts the transcriptional machinery of T follicular helper (T_{FH}) cells to facilitate their migration to germinal centres, where they can appropriately restrain humoral immune responses, thereby potentially preventing overzealous antibody responses. Follicular T_{Reg} cells can be identified by the expression of typical T_{FH} cell surface markers (CXC-chemokine receptor 5 (CXCR5), inducible T cell co-stimulator (ICOS) and programmed cell death protein 1 (PD1)) along with the T_{Reg} transcription factor forkhead box P3. Their mechanism of action remains to be completely elucidated.

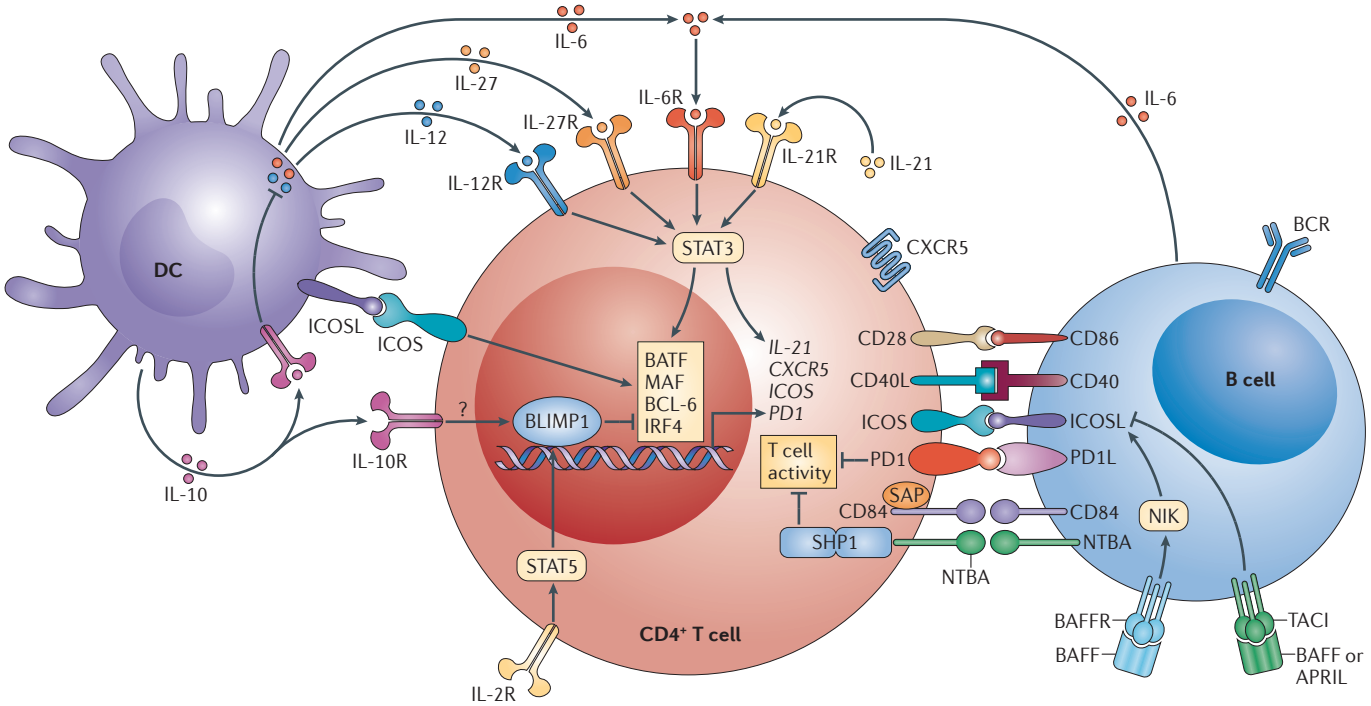


Figure 1 | **Cellular and molecular regulation of T_{FH} cell formation.** Naive CD4$^+$ T cells interact with antigen-presenting dendritic cells (DCs) in the interfollicular or T cell zones; DC-primed CD4$^+$ T cells acquire expression of CXC-chemokine receptor 5 (CXCR5) and B cell lymphoma 6 (BCL-6) to become early T follicular helper (T_{FH}) cells. These cells then migrate to the T cell–B cell border and — following interactions with cognate B cells — differentiate into germinal centre T_{FH} cells. This differentiation process is governed by signals provided by signal transducer and activator of transcription 3 (STAT3)-activating cytokines, including interleukin-6 (IL-6), IL-12, IL-21 and IL-27. These cytokines are secreted by DCs (which produce IL-6, IL-12 and IL-27), B cells (which produce IL-6 and possibly IL-27) and CD4$^+$ T cells (which produce IL-21). Cytokine-mediated activation of STAT1 might also contribute to this process (not shown). These cytokines operate individually or collectively to induce or enhance expression of the transcription factors BCL-6, MAF, basic leucine zipper transcriptional factor ATF-like (BATF) and interferon-regulatory factor 4 (IRF4), which then imprint the T_{FH} cell fate on a T cell by inducing the transcription of signature genes, including *CXCR5*, inducible T cell co-stimulator (*ICOS*), *IL21* and programmed cell death protein 1 (*PD1*). Cell–cell interactions among activated CD4$^+$ T cells, antigen-presenting DCs and B cells also promote T_{FH} cell formation. CD28–CD86, CD40 ligand (CD40L)–CD40 and ICOS–ICOS ligand (ICOSL) interactions are central to this process. Notably, ICOSL expression on B cells is controlled through the opposing effects of B cell-activating factor (BAFF) signalling via the distinct receptors BAFF receptor (BAFFR) and transmembrane activator and CAML interactor (TACI). In addition, signalling lymphocytic activation molecule (SLAM) family receptors have a dual role in T_{FH} cell generation: the recruitment of SLAM-associated protein (SAP) to these receptors facilitates and maintains conjugate formation between T and B cells, whereas recruitment of inhibitory phosphatases (such as SH2 domain-containing protein tyrosine phosphatase 1 (SHP1)) suppresses these interactions, thereby influencing the ability of CD4$^+$ T cells to form T_{FH} cells and to support B cell responses. T_{FH} cell generation is restricted by B lymphocyte-induced maturation protein 1 (BLIMP1), which is induced by IL-2 in a STAT5-dependent manner; BLIMP1 functions by repressing the expression of BCL-6. IL-10 also suppresses T_{FH} cell formation, but it is unknown whether this is also mediated via BLIMP1. APRIL, a proliferation-inducing ligand; BCR, B cell receptor; IL-10R, IL-10 receptor; NIK, NF-κB-inducing kinase; NTBA, natural killer, T and B cell antigen (also known as LY108 in mice); PDL1, PD1 ligand 1.

also transient and not as severe as that observed in IL-6-deficient mice, which indicates that there might be a requirement for an alternative signalling pathway downstream of IL-6R[45]. Indeed, the T_{FH} cell deficit in IL-6-deficient mice was recapitulated when STAT1 and STAT3 were deleted from CD4$^+$ T cells, which indicates that IL-6-mediated activation of both STATs is required for full T_{FH} cell development[45]. These results suggest that significant functional redundancy exists between IL-6 and IL-21, and that the relative importance of either cytokine is related to the level of its production at a given time and is inversely proportional to the levels of other compensatory cytokines.

Whereas IL-21 is likely to be produced by CD4$^+$ T cells themselves, IL-6 could be derived from activated B cells[44], from dendritic cells (DCs)[47] or from follicular dendritic cells[43]. IL-6 expression by B cells or DCs has been shown to promote IL-21 production by CD4$^+$ T cells that have been activated *in vitro*[44,47], and B cell-derived IL-6 restored T_{FH} cell numbers following transfer into mice that were deficient in both IL-6 and IL-21 (REF. 44). Thus, although interactions between DCs and naive CD4$^+$ T cells are important in initiating the T_{FH} cell programme[23], subsequent cognate interactions between these early T_{FH} cells and antigen-specific B cells are required to ensure their progression to a T_{FH} cell fate (FIG. 1).

Although it is known that numerous cytokines can promote T_{FH} cell formation (reviewed in REFS 23,24) (Supplementary information S1 (table)), much less is known about the factors that restrain this process. An important attribute of all T_H cell lineages is their ability to suppress the generation of cells with alternative effector fates[1] (TABLE 1). Thus, it is not surprising that T_{FH} cell formation can also be suppressed by several immunoregulatory cytokines. Impaired IL-10 signalling was shown to promote T_{FH} cell formation through at least two separate mechanisms. First, IL-10R deficiency enhanced T_{FH} cell function, as was shown by the increased antibody responses of cognate B cells following interactions with *Il10rb*$^{-/-}$ T_{FH} cells. Second, the inability of DCs to respond to IL-10 resulted in greater production of IL-6, IL-23 and IL-12; this, in turn, contributed to an increased frequency of T_{FH} cells in *Il10rb*$^{-/-}$ mice (FIG. 1). Furthermore, *Il10rb*$^{-/-}$ T_{FH} cells expressed higher levels of IL-17 and IL-21 than wild-type T_{FH} cells, which indicates that IL-10 regulates T_{FH} cells both quantitatively and qualitatively[48].

Importantly, the increased production of both IL-17 and IL-21 substantially shaped humoral immune responses in *Il10rb*$^{-/-}$ mice, as blockade of these cytokines not only impeded T_{FH} cell formation but also reduced the augmented antigen-specific antibody response[48]. Thus, consistent with its ability to suppress T_H1 and T_H17 cells[1] (TABLE 1), IL-10 also negatively regulates T_{FH} cells. However, the molecular mechanism through which IL-10 exerts its repressive effect is unknown. T_{FH} cell formation can also be limited by IL-2–STAT5 signalling, which induces B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1) to suppress BCL-6 function[49] (FIG. 1); therefore, IL-10-mediated BLIMP1-induction might also contribute to the inhibitory effect of IL-10 on T_{FH} cells.

Overall, positive and negative signals from numerous receptors and cytokines fine-tune T_{FH} cell formation, homeostasis and function. Consequently, perturbations in this balance might deregulate T_{FH} cell differentiation, thereby accelerating disease development in genetically susceptible hosts. The exact role of specific cytokines in the regulation of human T_{FH} cells remains unknown, but the identification of individuals with inactivating mutations in important cytokines and cytokine receptors will shed light on these requirements.

T_{FH} cell memory

Although T_{FH} cells are unquestionably important in establishing B cell memory, the fate of T_{FH} cells that are generated during GC reactions is unclear. Several recent studies in mice have investigated whether T_{FH} cells are short-lived effector cells or whether they differentiate into long-lived memory-type cells that can resume a T_{FH} cell state following re-exposure to the same initiating antigen. Although both CXCR5$^+$PD1^{low} T_{FH} cells and CXCR5^{hi}PD1^{hi} GC T_{FH} cells are generated following vaccination or infection, the number of GC T_{FH} cells decreases more rapidly than that of CXCR5$^+$PD1^{low} T_{FH} cells[50,51]. The decrease in GC T_{FH} cells is partly due to their loss of the T_{FH} phenotype rather than as a result of apoptosis. Thus, these 'former' T_{FH} cells most probably differentiate into memory cells that downregulate CXCR5, PD1 and BCL-6, that re-express CCR7, IL-7Rα and CD62L (also known as L-selectin), and that persist for a long time.

To add further support to the idea that T_{FH} cells can generate memory cells, it was recently shown that gene expression profiles of early T_{FH} cells share many similarities with precursor memory CD8$^+$ T cells[52]. Upon subsequent antigenic challenge, these memory-like cells form T_{FH} cells more quickly and promote GC formation and antibody production more effectively than naive or memory cells that originated from non-T_{FH} cells[50,51,53–55]. Interestingly, it was shown that not all of these cells adopted a T_{FH} cell phenotype, which indicates that cellular plasticity might allow them to differentiate into other T_H cell subsets. A caveat to these studies is the reliance on identifying T_{FH} cells by phenotype alone. In the future it would be useful to more precisely track T_{FH} cells in GCs and to determine their fate. However, taken together, these data demonstrate that, at least in mice, some T_{FH} cells can enter the memory pool and can provide long-term protection following reinfection.

Antigen-specific T cells have also been identified within the population of T_{FH}-like cells that is detected in the peripheral blood of humans. These cells seem to be more readily detected following recent antigen exposure, which indicates that they exist as T_{FH}-like cells or that their precursors adopt a T_{FH} cell phenotype in response to antigenic stimulation[24,56,57], as discussed below.

The expanding universe of T_{FH}-like cells

T_{FH} cells that provide B cell help during responses to 'conventional' T cell-dependent antigens have been well characterized, but 'unconventional' subsets of T_{FH} cells have also been identified, and these are discussed below.

Extrafollicular T_H cells. Humoral immune responses are initiated in extrafollicular areas of lymphoid tissues where B cells differentiate into short-lived plasmablasts following interactions with T_H cells[11,13,26]. Similar to T_{FH} cells, the formation of extrafollicular T_H cells requires BCL-6, and their function is mediated by CD40L, ICOS and IL-21 (REF. 26). However, extrafollicular T_H cells do not express high levels of CXCR5; rather, they are attracted to these sites by the CXCR4– CXCL12 (CXC-chemokine ligand 12) axis[26]. Extrafollicular T_H cells might also give rise to GC T_{FH} cells following interactions with cognate B cells[19,23,24].

NKT$_{FH}$ cells. Natural killer T (NKT) cells are innate-like T cells expressing a semi-invariant T cell receptor (TCR) that recognizes lipid antigens presented by the non-polymorphic MHC molecule CD1d. The ability of the NKT cell population to rapidly expand and to produce effector cytokines following encounter with an antigen — typically α-galactosyl ceramide (αGalCer) — links the innate and adaptive immune responses. NKT cells can initiate or enhance antibody responses to lipid and protein antigens via cognate[58–60] and non-cognate[61] mechanisms, respectively.

Follicular dendritic cells
Specialized non-haematopoietic stromal cells that reside in lymphoid follicles and germinal centres. These cells possess long dendrites and carry intact antigen on their surface. They are crucial for the optimal selection of B cells that produce antigen-binding antibodies.

Recent studies have reassessed the nature of NKT cells that provide B cell help. It was shown that following immunization with αGalCer–antigen conjugates, a small proportion of mouse NKT cells acquired a T_{FH}-like phenotype[62,63]. These NKT follicular helper (NKT_{FH}) cells were detected in human tonsils[62] and their development depended on the same factors that are important for conventional T_{FH} cells[62]. By producing IL-21, NKT_{FH} cells supported the rapid formation of GCs, yielding detectable levels of antigen-specific IgG, with some evidence of affinity maturation[62–64] (FIG. 2).

Although NKT_{FH} cells resembled T_{FH} cells, the most striking difference was their inability to invoke long-lived memory responses to lipid antigens (FIG. 2). Furthermore, the magnitude of the NKT_{FH} cell-induced antibody responses to lipid antigens was inferior to those driven by conventional T_{FH} cells[62–64]. Despite this, administering αGalCer as an adjuvant or as a conjugated component of immunizing antigens increased the production of antigen-specific antibodies. This probably reflects the direct actions of NKT_{FH} cells on antigen-specific $CD1d^+$ B cells, as well as the indirect actions of NKT_{FH} cells, such as cytokine production, on other cell types[58–61]. It might also indicate that there is synergy between NKT_{FH} and T_{FH} cells. Taken together, these findings support the inclusion of αGalCer in vaccine adjuvants.

γδ T_{FH} cells. The finding that mice and humans lacking the conventional αβ TCR have T_H cells that elicit humoral responses against T cell-dependent antigens led to the realization that γδ T cells can provide help to B cells to generate GCs[65]. Similar to conventional T_{FH} cells, some human γδ T_{FH} cells express CXCR5 and localize to follicles and GCs[66–68]. γδ T cells express a semi-invariant TCR repertoire and recognize non-peptidic phosphoantigens that are derived from microbial metabolites[65]. These antigens rapidly activate γδ T cells, which can subsequently acquire T_{FH} cell features[66,68,69]; the differentiation of γδ T cells to T_{FH}-like cells is increased by exogenous IL-21 (REFS 68,69) (FIG. 2). As γδ T cells do not produce IL-21 (REFS 68,69), they are dependent on extrinsic sources of this cytokine to differentiate into T_{FH}-like cells.

As conventional $CD4^+$ T cells, γδ T cells and NKT cells recognize different repertoires of microbial antigens, their ability to differentiate into T_{FH}-like effector cells provides a mechanism whereby humoral immunity can be generated against a broad range of pathogen-associated antigens (FIG. 2). This expands the number of molecular targets that initiate protective immunity, but it might also contribute to post-infection autoimmunity by generating cross-reactive autoantibodies[13,70] (BOX 2).

Follicular T_{Reg} cells. Recent studies have proposed that T_{FH} cells are controlled by follicular T_{Reg} cells — a specialized subset of T_{Reg} cells that colocalize within B cell follicles. Follicular T_{Reg}-like cells were first described in human tonsils in 2004 (REF. 71), but it took another 7–8 years for them to be examined in greater detail.

Follicular T_{Reg} cells comprise approximately 10–15% of the T_{FH} cell population in human and murine lymphoid tissues[72–74]. They show characteristics of both T_{FH} cells and T_{Reg} cells, but they lack expression of CD40L, IL-4 and IL-21 (REF. 73). Abrogating either follicular T_{Reg} cell development or their follicular localization was shown to enhance GC responses and subsequent antibody production[72,74]. Although follicular T_{Reg} cells show similar requirements to conventional T_{FH} cells for their development[73], they actually originate from thymus-derived T_{Reg} cells, rather than from peripherally derived T_{Reg} or T_{FH} cells. Similar to other specialized T_{Reg} cell subsets that have evolved to selectively control T_H1, T_H2 and T_H17 cells[75], follicular T_{Reg} cells seem to have co-opted the transcriptional machinery of T_{FH} cells to migrate into GCs to exert their regulatory effects[72–74].

PD1 is more highly expressed on follicular T_{Reg} cells than on T_{FH} cells. Interestingly, PD1 or PDL1 deficiency was shown to favour the development of follicular T_{Reg} cells but not T_{FH} cells[41]. Furthermore, PD1-deficient follicular T_{Reg} cells were more effective than normal follicular T_{Reg} cells at impeding T_{FH} cell-mediated B cell differentiation[41]. Thus, PD1 seems to negatively regulate not only the development and maintenance of follicular T_{Reg} cells but also their suppressive function. The increase in the number of follicular T_{Reg} cells compared with T_{FH} cells, together with the increased suppressive function of PD1-deficient follicular T_{Reg} cells[41], might explain some of the discrepancies in the findings regarding the consequences of PD1 deficiency on T_{FH} cell-dependent B cell responses[36–39].

The mechanisms by which follicular T_{Reg} cells attenuate humoral immunity remain unknown. Follicular T_{Reg} cells express *IL10* mRNA[73] (TABLE 1), and IL-10 can attenuate T_{FH} cell formation in normal[48] and autoimmune[76] settings. Thus, follicular T_{Reg} cell-derived IL-10 might be one means by which T_{FH} cell-mediated B cell responses are regulated. However, as follicular T_{Reg} and T_{FH} cells express similar levels of IL-10, it is difficult to predict how follicular T_{Reg}-derived IL-10 would affect T_{FH} cells in a different manner to their own endogenous IL-10. Although it is known that follicular T_{Reg} cells are likely to limit GC reactions by impeding both T_{FH} cells and GC B cells, therapeutically exploiting the immunoregulatory function of follicular T_{Reg} cells will require further delineation of their mechanism of action.

Human circulating T_{FH}-like cells

As access to human lymphoid tissues is limited, studies of human T_{FH} cells have exploited the fact that $CD4^+CXCR5^+$ T cells comprise a small subset of circulating lymphocytes[4,5]. However, there are clear differences between $CD4^+CXCR5^+$ T cells in the blood and those in the tonsils. For instance, $CD4^+CXCR5^+$ T cells in the blood do not express BCL-6 and their expression of ICOS and PD1 is substantially lower than that of T_{FH} cells[4,5,56,77,78]. Despite this, *in vitro*-cultured blood-derived $CD4^+CXCR5^+$ T cells produce more IL-21, IL-10 and CXCL13 — which are all features of T_{FH} cells[8,9] — and are more efficient at inducing B cell differentiation

γδ T cells
T cells that express the γδ T cell receptor. These T cells are present in the skin, vagina and intestinal epithelium as intraepithelial lymphocytes. Although the exact function of γδ T cells is unknown, it has been suggested that mucosal γδ T cells are involved in innate immune responses.

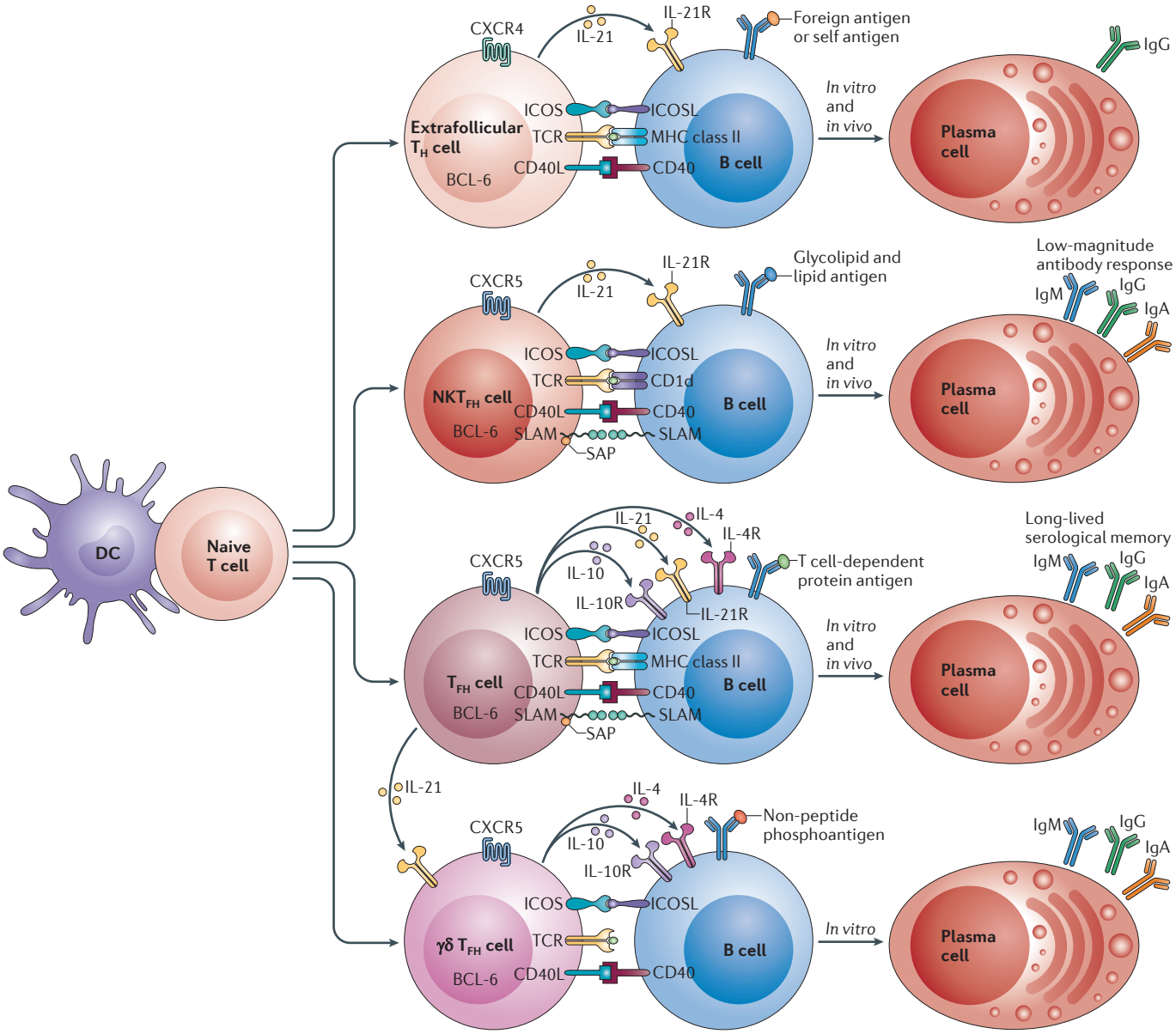


Figure 2 | **T_{FH} cell subsets with specialized effector functions.** Naive CD4$^+$ T cells, natural killer T (NKT) cells and γδ T cells can all differentiate into T follicular helper (T_{FH}) cells following instructive signals that are derived from antigen-presenting cells. These cells share many features, including their requirements for differentiation, their expression of transcription factors and their surface phenotype, which underlies their ability to induce B cell differentiation. Extrafollicular T helper (T_H) cells, NKT follicular helper (NKT_{FH}) cells and T_{FH} cells require inducible T cell co-stimulator (ICOS), B cell lymphoma 6 (BCL-6), SLAM-associated protein (SAP) and CD28 signalling for their differentiation, which occurs *in vitro* and *in vivo* in the presence of B cells. γδ T_{FH} cells also express ICOS, but the molecular requirements for their generation remain unknown. Extrafollicular T_H cells provide help to B cells outside the follicles; unlike other T_{FH}-type cell populations, these cells do not express CXC-chemokine receptor 5 (CXCR5). Rather, their localization to extrafollicular areas seems to be mediated by CXCR4. NKT_{FH} cells and T_{FH} cells support the rapid formation of GCs and the generation of antigen-specific antibodies in an interleukin-21 (IL-21)-dependent manner. T_{FH} and γδ T_{FH} cells might also support GC responses by secreting IL-4 and IL-10. γδ T_{FH} cells do not express IL-21 but might rely on exogenous IL-21 expression for their T_H cell function. T_{FH} cells respond to protein antigens and induce long-lived serological memory. Thus, they have a central role in pathogen-specific responses as well as in several immunopathologies. NKT_{FH} cells respond to glycolipid antigens and induce antibody responses of a lower magnitude than those induced by T_{FH} cells. NKT_{FH} cells might have a role in antibody responses against pathogens that express glycolipid antigens such as *Borrelia hermsii*, *Streptococcus pneumonia* and *Plasmodium falciparum*[62–64]. As γδ T cells recognize microbial metabolites, γδ T_{FH} cells might have an adjuvant role in responses to most infectious pathogens. Extrafollicular T_H cells have been most effectively characterized for their role in disease pathogenesis in murine models of human autoimmunity[26]. These cells might also represent precursors of T_{FH} cells[19]. CD40L, CD40 ligand; DC, dendritic cell; ICOSL, ICOS ligand; IL-21R, IL-21 receptor; SLAM, signalling lymphocytic activation molecule; TCR, T cell receptor.

than $CD4^+CXCR5^-$ T cells[56,78]. Thus, human circulating $CD4^+CXCR5^+$ T cells have some features of T_{FH} cells, which indicates that precursors of circulating $CD4^+CXCR5^+$ T cells might have experienced some aspects of a 'T_{FH} cell differentiation programme' *in vivo*.

Interestingly, subsets of circulating $CD4^+CXCR5^+$ T cells that have distinct effector functions also exist. By assessing the expression of CXCR3 and CCR6 — chemokine receptors that are associated with T_H1 and T_H17 cells, respectively — $CD4^+CXCR5^+$ T cells could be classified into T_H1-like, T_H17-like and T_H2-like ($CXCR3^-CCR6^-$) T_{FH} cell subsets[56]. Interestingly, the T_H2- and T_H17-like T_{FH} cell subsets were shown to produce higher levels of IL-21 and to induce naive B cell differentiation more efficiently than the T_H1-like T_{FH} cell subset. This showed that there is substantial heterogeneity among blood-derived $CD4^+CXCR5^+$ T cells and potentially clarified previous findings indicating that circulating $CD4^+CXCR5^+$ T cells might be inefficient 'helpers' for B cell differentiation[5].

Despite the improved characterization of circulating $CD4^+CXCR5^+$ T cells, their exact relationship with GC T_{FH} cells remains incompletely defined. It is also unclear what functional role BCL-6 has in blood-derived $CD4^+CXCR5^+$ T cells, as it does not seem to be required for their persistence, expression of CXCR5, production of IL-21 or for their ability to induce B cell differentiation. Kinetic analysis of T_H cell activation in mice showed that although BCL-6 is expressed by developing T_{FH} cells, it is downregulated at later time points[79]. Apart from BCL-6 expression, there seemed to be few differences between $BCL\text{-}6^+$ and $BCL\text{-}6^-$ T_{FH} cells with respect to their expression of important T_{FH} cell-related genes[79]. This indicates that although BCL-6 is required for T_{FH} cell formation, it might be dispensable for their maintenance following antigen clearance and the resolution of an immune response. Furthermore, $BCL\text{-}6^-$ T_{FH} cells upregulated *Il7r* and *Ccr7* and downregulated the cell cycle machinery[79].

Thus, T_{FH} cells probably yield a population of cells that exit the GC and lymphoid tissues, and that return to the circulation as a population of quiescent memory-type $CD4^+CXCR5^+$ T cells.

Human T_{FH} cells in immunity and disease

Dysregulated behaviour of T_{FH} cells and extrafollicular T_H cells has been found to contribute to autoimmune or immune-deficient states in several mouse models of human disease[10,24,26]. Consequently, there is great interest in determining the role of T_{FH} cells in human disease. Although the exact nature of circulating $CD4^+CXCR5^+$ T cells in humans remains unclear, investigation of this population of cells in various contexts has nonetheless provided important insights into their potential functions during normal immune responses and in immunopathologies (TABLE 2).

Circulating $CD4^+CXCR5^+$ T cells as biomarkers of effective humoral immunity. Long-lived T cell-dependent antibody responses underlie the success of most vaccines that are currently in use. For this reason, there is growing interest in identifying appropriate biomarkers of successful vaccination. Several publications reported the outcomes of immunization against influenza during the 2009 H1N1 epidemic. These studies included healthy individuals and a cohort of HIV-infected individuals. Whereas all healthy individuals generated protective levels of H1N1-specific IgG 4 weeks after vaccination, such a response was observed in only 50% of HIV-positive individuals. Successful induction of H1N1-specific IgG coincided with significant increases in both serum levels of IL-21 and frequencies of circulating $CD4^+CXCR5^+$ T cells and memory B cells[80,81]; this correlated with titres of serum H1N1-specific antibodies[81]. Thus, the ability of the H1N1 vaccine to induce protective antibody responses correlated with the induction of detectable features of T_{FH} cell-mediated immunity[80,81].

Another study found that the emergence of $CD4^+CXCR5^+CXCR3^+ICOS^+$ T cells in influenza-vaccinated individuals correlated with increased levels of neutralizing antibodies and — in adults — with the appearance of circulating plasmablasts[57]. This was unexpected, as it was previously found that circulating $CXCR3^+$ T_{FH}-like cells were poor inducers of naive B cell differentiation and that they did not produce IL-21 (REF. 56). However, $CD4^+CXCR5^+CXCR3^+ICOS^+$ T cells did promote the differentiation of memory, but not naive, B cells into plasmablasts *in vitro* through the production of IL-10 and IL-21 (REF. 57). This suggests that increased numbers of $CD4^+CXCR5^+CXCR3^+ICOS^+$ T cells could be used as an indicator of the development of protective antibody responses from pre-existing memory B cells, rather than of primary responses of naive B cells[57]. Despite this conclusion, it remains unknown which signals induce IL-21 expression in $CD4^+CXCR5^+CXCR3^+$ $ICOS^+$ T cells and why these cells fail to activate naive B cells even though they express CD40L, IL-10 and IL-21, which, in combination, can strongly induce the differentiation of naive B cells to plasmablasts[82,83].

Taken together, these studies show that quantifying the frequencies of circulating $CD4^+CXCR5^+$ T cells, or of their subsets, is a reliable predictor of vaccine success and of the magnitude of the induced response[57,80,81]. It also allowed for the separation of vaccine responders from non-responders[80,81]. Understanding why some individuals failed to elicit a substantial vaccine-specific T_{FH} cell-dependent antibody response might facilitate the development of improved vaccination strategies, particularly in individuals with suboptimal humoral immunity.

Autoimmune diseases. The detection of $CD4^+CXCR5^+$ T cells and CXCL13 in organs that are affected by autoimmune disorders, such as the salivary glands in Sjögren's syndrome, suggested that aberrant T_{FH} cell development can drive autoimmunity[84]. As a result of this finding, several studies have assessed the possibility of enumerating circulating $CD4^+CXCR5^+$ T cells as a potential biomarker of autoimmune diseases, especially given the difficulty of accessing and analysing

Sjögren's syndrome
A systemic autoimmune disease in which autoantibodies target and destroy exocrine glands such as the tear ducts and the salivary glands.

Table 2 | **Human diseases associated with aberrant T_{FH} cell function**

Human disease	T_{FH} cell-related phenotype	Correlated pathology	Refs
Autoimmunity			
Systemic lupus erythematosus	• Increased frequencies of circulating $CD4^+CXCR5^+PD1^{hi}$ cells, $CXCR5^+ICOS^{hi}$ cells or $ICOS^{hi}$ T cells • Increased serum levels of IL-21 and CXCL13	• Increased severity of end-organ damage • Higher serum levels of dsDNA-specific autoantibodies	77,85,94
Sjögren's syndrome	• Increased frequencies of circulating $CD4^+PD1^{hi}$ T cells, $CD4^+CXCR5^+ICOS^{hi}$ T cells or $CD4^+CXCR5^+CCR6^+$ (T_H17-type) T cells	• Higher serum levels of autoantibodies	77,93
Rheumatoid arthritis	• Increased frequencies of circulating $CD4^+CXCR5^+PD1^{hi}$ cells or $CD4^+CXCR5^+ICOS^{hi}$ T cells • Increased serum levels of IL-21	• Higher serum levels of CCP-specific autoantibodies • Higher disease score and serum levels of IL-21- and CCP-specific autoantibodies	90,92
Juvenile dermatomyositis	• Increased T_H2– and T_H17-like subsets among circulating $CD4^+CD45RO^+CXCR5^+$ T cells	• Higher disease score • Increased number of circulating plasmablasts	56
Autoimmune thyroid disease (Graves' disease or Hashimoto's thyroiditis)	• Increased frequencies of circulating $CD4^+CXCR5^+PD1^{hi}$ T cells or $CD4^+CXCR5^+ICOS^{hi}$ T cells	• Increased titres of serum autoantibodies against thyroid stimulating hormone receptor and thyroglobulin	91
Myasthenia gravis	• Increased frequencies of circulating $CD4^+CXCR5^+$ T cells or $CXCR5^+CD57^+$ T cells	• Increased disease severity	86,87
Immunodeficiency			
X-linked lymphoproliferative disease	• Normal frequency of circulating $CD4^+CXCR5^+$ T cells but impaired helper function *in vitro*	• Impaired cytokine production, ICOS expression and B cell help *in vitro*	17,96
CVID (ICOS deficiency) and hyper-IgM syndrome (CD40 or CD40L deficiency)	• Reduced frequency of circulating $CD4^+CD45RO^+CXCR5^+$ T cells	• Impaired development of T_{FH} cells owing to an inabilty to form GCs	29
AD-HIES (STAT3 deficiency)	• Reduced frequency of circulating $CD4^+CD45RO^+CXCR5^+$ T cells • Impaired generation of T_{FH}-like cells from naive precursors *in vitro*	• Inability of naive $CD4^+$ T cells to express IL-21 and to provide help to B cells in response to T_{FH} cell-inducing cytokines (for example, IL-12)	46
HIV	• Increased frequency of T_{FH} cells in lymph nodes of HIV-infected individuals • Enrichment of HIV-specific $CD4^+$ T cells in the T_{FH} cell compartment	• Positive correlations with plasma viraemia, numbers of GC B cells and plasma cells, hypergammaglobulinaemia and virus-specific antibodies	99, 101
Lymphoma			
AITL and FTCL	• Increased number of $CXCR5^+PD1^{hi}ICOS^{hi}OX40^+SAP^{hi}BCL\text{-}6^+MAF^+$ malignant cells • Detectable expression of CXCL13 and IL-21	• Might be correlated with aberrant humoral features such as follicular hyperplasia, hypergammaglobulinaemia and autoantibody production in these PTCLs	104–111

AD-HIES, autosomal dominant hyper-IgE syndrome; AITL, angioimmunoblastic T cell lymphoma; BCL-6, B cell lymphoma 6; CCP, cyclic citrullinated peptide; CCR6, CC-chemokine receptor 6; CD40L, CD40 ligand; CVID, common variable immunodeficiency syndrome; CXCL13, CXC-chemokine ligand 13; CXCR5, CXC-chemokine receptor 5; dsDNA, double-stranded DNA; FTCL, follicular T cell lymphoma; GC, germinal centre; ICOS, inducible T cell co-stimulator; IL, interleukin; PD1, programmed cell death protein 1; PTCL: peripheral T cell lymphoma; SAP, SLAM-associated protein; STAT3, signal transducer and activator of transcription 3; T_{FH}, T follicular helper; T_H; T helper.

secondary lymphoid organs in affected individuals. Early studies showed increases in the frequency of $CD4^+ICOS^+$ T cells in the peripheral blood of patients with systemic lupus erythematosus (SLE)[85] and in the frequency of $CD4^+CXCR5^+$ T cells in the peripheral blood of patients with myasthenia gravis. Furthermore, in myasthenia gravis, the frequency of $CD4^+CXCR5^+$ T cells in the circulation positively correlated with clinical disease scores[86,87]. Subsequent studies reported increases in circulating $CD4^+CXCR5^+ICOS^{hi}PD1^{hi}$ T cells in some patients with SLE or Sjögren's syndrome, and these were shown to correlate with autoantibody titres, frequencies of circulating GC B cells, plasma cells and disease severity[77,88,89]. Increased frequencies of circulating $CXCR5^+ICOS^{hi}$ or $CD4^+CXCR5^+PD1^+$T cells have also been observed in patients with rheumatoid arthritis and autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis), and have been shown to be accompanied by elevated levels of serum autoantibodies[90–92] (TABLE 2).

Furthermore, it was found that the T_H2- and T_H17 (but not T_H1)-like $CD4^+CXCR5^+$ T cell subsets that provide B cell help are increased in juvenile dermatomyositis[56]. In light of this observation, circulating $CD4^+CXCR5^+$ T cells from patients with Sjögren's syndrome were re-examined with respect to their T_H-like phenotypes[93]. A positive correlation was found between the levels of serum autoantibodies and the numbers of circulating $CD4^+CXCR5^+$ T cells, particularly with regard to those that also expressed CCR6 (TABLE 2).

Systemic lupus erythematosus
(SLE). An autoimmune disease in which autoantibodies that are specific for DNA, RNA or proteins associated with nucleic acids form immune complexes. These complexes damage small blood vessels, especially in the kidneys. Patients with SLE generally have abnormal B and T cell function.

As these $CD4^+CXCR5^+CCR6^+$ T cells did not produce IL-17 (REF. 93), this study highlighted the possibility that $CD4^+CXCR5^+CCR6^+$ T cells are not strictly T_H17-like T_{FH} cells, but that they acquire CCR6 expression to facilitate migration to inflamed tissues.

Although some studies did not detect increases in T_{FH}-like cells in SLE[94] and rheumatoid arthritis[95], the concept has emerged that levels of circulating $CD4^+CXCR5^+$ T cells are generally increased in humoral autoimmune conditions. As this increase is usually correlated with clinical disease, the frequency of circulating $CD4^+CXCR5^+$ T cells or the serum levels of IL-21, which are both indicative of T_{FH} cell activity, seem to be useful biomarkers for predicting disease outcome. Furthermore, in patients with SLE, both the increased frequency of circulating $CD4^+CXCR5^+$ T cells and the clinical manifestations that are associated with this disease were reduced following corticosteroid treatment[88]. This indicates that T_{FH} cells are likely to contribute to disease pathogenesis, and it highlights the possibility of targeting T_{FH} cells to effectively treat antibody-mediated autoimmune conditions. However, it remains to be determined whether the association between autoimmunity and the expansion of the population of T_{FH} cells might reflect the activation of self-reactive T_{FH} cells or whether might reflect the increased stimulation of GC B cells producing cross-reactive autoantibodies as a result of increased T_{FH} cell help[13,70] (BOX 2).

Primary immunodeficiencies. Several primary immunodeficiencies have been associated with genetic defects that might affect T_{FH} cell differentiation and function. Mutations in the gene encoding SAP cause XLP[31], whereas mutations that affect the genes encoding CD40L and ICOS cause hyper-IgM syndrome and common variable immunodeficiency, respectively[2]. Important features of these immunodeficiencies include impaired humoral immune responses and a paucity of well-formed GCs in secondary lymphoid tissues[2]. The numbers of circulating $CD4^+CXCR5^+$ T cells are considerably decreased in CD40L- or ICOS-deficient individuals[29]. Although patients with XLP have normal numbers of $CD4^+CXCR5^+$ T cells, possibly as a result of persistent activation with viral antigens[17], their $CD4^+$ T cells fail to acquire features of T_{FH} cells *in vitro*[17,96] (TABLE 2).

Patients with autosomal dominant hyper-IgE syndrome — a primary immunodeficiency that is caused by mutations in *STAT3* — also show impaired functional antibody responses[2]. These patients have a partial deficiency in numbers of circulating $CD4^+CXCR5^+$ T cells, and their naive $CD4^+$ T cells fail to differentiate into T_{FH}-like cells *in vitro*[46]. STAT3 is likely to function downstream of several cytokines — including IL-6, IL-12, IL-21 and IL-27 — which indicates that signal integration among multiple cytokine receptors is required for T_{FH} cell formation[46] (TABLE 1). Patients with mutations in *IL12RB1*, which encodes IL-12Rβ1, also have fewer circulating $CD4^+CXCR5^+$ T cells[97]. This correlates with reduced numbers of memory B cells, abnormal GCs and low-avidity antibody responses to tetanus. Interestingly, antibody responses following natural infections or vaccination have been shown to be unaffected — or even enhanced — by IL-12Rβ1 deficiency, and the defect in circulating $CD4^+CXCR5^+$ T cells improved with age[97]. Thus, IL-12 signalling may only be required for T_{FH} cell formation and function early in life and for responses to only some antigens (for example, antigens that are derived from non-replicating pathogens)[97].

Taken together, these analyses of monogenic immunodeficiencies showed that *CD40L*, *ICOS*, *SH2D1A*, *STAT3* and possibly *IL12RB1* are required for T_{FH} cell formation, function and/or maintenance. These studies also provided further correlative evidence that circulating $CD4^+CXCR5^+$ T cells are related to bona fide T_{FH} cells, as a deficit in $CD4^+CXCR5^+$ T cells mimicked a deficiency of GCs and presumably lymphoid organ-resident T_{FH} cells. Importantly, the impaired formation and/or function of circulating $CD4^+CXCR5^+$ T cells is associated with compromised humoral immune responses, which highlights the contribution of T_{FH} cells to successful T cell-dependent antibody responses. As some immunodeficiencies are associated with mutations in *TACI* and *BAFFR*[2], and as studies in mice indicate that these receptors modulate T_{FH} cell formation[27,28], it will be interesting to assess the formation and function of circulating T_{FH}-type cells in individuals with these genetic lesions.

Acquired immunodeficiencies. $CD4^+$ T cells are targets of HIV infection and are therefore depleted in infected individuals. Paradoxically, several aspects of humoral immunity are augmented following HIV infection, including polyclonal B cell activation, the appearance of peripheral blood plasmablasts and hypergammaglobulinaemia[98]. To investigate the basis for these contrasting phenomena, T_{FH} cells have recently been assessed in individuals with HIV and in simian immunodeficiency virus (SIV)-infected macaques. A marked accumulation of T_{FH} cells was observed in the lymph nodes both of individuals with chronic HIV infection and of macaques that were chronically infected with SIV[40,99–102]. Interestingly, a substantially higher proportion of T_{FH} cells were specific for HIV compared with effector and memory $CD4^+$ T cell subsets[99,101] (TABLE 2). T_{FH} cells were found to be infected with SIV or HIV[100–102], and human T_{FH} cells were shown to contain substantially more copies of HIV DNA than naive, memory or effector T cell populations[101]. Strikingly, human T_{FH} cells were also better at supporting viral replication and infection of susceptible host cells[101]. Furthermore, T_{FH} cells that were present during chronic infection seemed to be persistently activated[99–101], unlike those in uninfected donors. Thus, ongoing antigenic stimulation of activated $CD4^+$ T cells in HIV-infected individuals might cooperatively drive these cells to a T_{FH} cell fate. Sustained signalling through IL-6R — which is highly expressed on T_{FH} cells derived from both normal donors and those with

Myasthenia gravis
A chronic autoimmune disease that involves the generation of T cell-dependent autoantibodies that are specific for the acetylcholine receptor. These antibodies interfere with the transmission of signals at neuromuscular junctions.

Rheumatoid arthritis
An immunological disorder that is characterized by symmetrical polyarthritis, often progressing to crippling deformation after years of synovitis. It is associated with systemic immune activation and the presence of acute-phase reactants in the peripheral blood, as well as rheumatoid factor (immunoglobulins that are specific for IgG), which forms immune complexes that are deposited in many tissues.

Graves' disease
A type of autoimmune disease, and the most common form of hyperthyroidism in humans. It results from activating antibodies that are specific for the thyroid stimulating hormone receptor (TSHR). In mouse models of Graves' thyroiditis, the disease is induced by immunization with the TSHR.

Hashimoto's thyroiditis
An autoimmune disease in which self-reactive B cells and T cells target the thyroid, resulting in hypothyroidism.

Juvenile dermatomyositis
A chronic, multisystem autoimmune and inflammatory disease involving muscle, skin, blood vessels, the gastrointestinal tract and other organs. Autoantibodies are often detected in these patients, but their specificities have not yet been completely defined.

chronic HIV or SIV infection[16,100,102] — in response to IL-6, which is abundantly produced during HIV infection[98], might also cooperatively drive these cells to a T_{FH} cell fate[24]. As IL-2 can suppress T_{FH} cell differentiation[49], it is possible that the loss of IL-2-producing T cells following HIV infection[102] also contributes to the increased numbers of T_{FH} cells in HIV-infected individuals.

The increase in T_{FH} cell numbers has functional consequences for humoral immunity during HIV infection. Thus, the frequency of T_{FH} cells positively correlates with plasma viraemia, the numbers of GCs and plasma cells, the levels of virus-specific IgG and the onset of hypergammaglogulinaemia[40,99–101] (TABLE 2). Furthermore, antiretroviral therapy has been shown to reduce the frequencies of T_{FH} cells, GC B cells and plasma cells, as well as the numbers of HIV-specific and HIV-infected T_{FH} cells[101]. These findings strongly indicate that the expansion of the T_{FH} cell population and the subsequent dysregulated antibody response might be driven by chronic viral infection.

It is possible that increases in HIV-specific T_{FH} cells would result in protective HIV-specific antibody responses; however, these are usually not broadly neutralizing[98]. It was recently found that although T_{FH} cells from uninfected and HIV-infected individuals show similar phenotypes and express comparable levels of cytokines, the T_{FH} cells from HIV-infected individuals were unable to promote B cell differentiation *in vitro*[40] as a result of heightened PDL1 expression on GC B cells, which inhibits T_{FH} cell function via PD1 (REF. 40). This extrinsically mediated functional impairment of T_{FH} cells might contribute to the inability of HIV-infected individuals to generate neutralizing HIV-specific antibodies. Elucidating mechanisms to promote the effector function of HIV-specific T_{FH} cells — for example, through PD1–PDL1 blockade — might facilitate the development of improved HIV vaccines.

Malignancies. Peripheral T cell lymphomas (PTCLs) are rare haematological malignancies constituting approximately 5–10% of all non-Hodgkin's lymphomas. PTCLs include angioimmunoblastic T cell lymphoma (AITL), follicular T cell lymphoma (FTCL) and PTCL-not otherwise specified (PTCL-NOS)[103,104].

Morphological, phenotypic and molecular characterization of malignant cells in AITL has shown that these cells have many of the features of T_{FH} cells[105–109] (TABLE 2). In a substantial proportion of cases of FTCL, which was previously considered to be a follicular variant of PTCL-NOS, the malignant cells were also found to show T_{FH} cell traits[104,108–111]. Importantly, the assessment of a set of T_{FH} cell-related genes in different malignancies led to the reclassification of several cases of PTCL-NOS as AITL[109], which demonstrates the robustness of molecular diagnoses of PTCLs. These studies established that numerous PTCLs probably arise from normal T_{FH} cells. Some of the cardinal features of AITL and FTCL include B cell activation, follicular hyperplasia, hypergammaglobulinaemia and autoantibody production[103,104,111]. These humoral immunity-associated features of AITL and FTCL probably reflect the dysregulated activity of malignant T_{FH} cells, which parallels the aberrant function of T_{FH} cells in autoimmunity. Thus, it might be possible to treat these features of AITL and FTCL by targeting T_{FH} cell-related molecules — such as PD1, CXCL13 and IL-21 — that are abundantly expressed in these diseases.

It is unknown why T_{FH} cells give rise to several types of PTCLs. Interestingly, T_{FH} cells undergo prompt apoptosis, at least *in vitro*[9], which indicates that the mechanisms that control their survival might be stringently regulated. Thus, the molecular lesions that override T_{FH} cell apoptosis may contribute to the malignant transformation of these cells.

Cytogenetic abnormalities occur infrequently in PTCLs[103,104]. However, mutations in isocitrate dehydrogenase 2 (*IDH2*) and *TET2* were recently detected in approximately 25% and 45% of cases of AITL, respectively, and *TET2* was also shown to be mutated in approximately 30% of cases of PTCL-NOS[112–114]. In AITL, there was no difference in clinical outcome between patients with *IDH2* mutations and those without *IDH2* mutations[113]. However, patients with *TET2* mutations were associated with more advanced disease and poorer clinical outcome than patients without *TET2* mutations[114]. How *TET2* mutations lead to the transformation of T_{FH} cells remains to be determined. Interestingly, a novel mouse model of AITL was recently reported in which 50% of mice that were heterozygous for the *Roquin* (also known as *Rc3h1*) allele developed AITL-like disease[115]. This model might provide important insights into the pathophysiology of AITL in humans and might facilitate preclinical testing of potential therapeutics. It also raises the possibility that heterozygous mutations in *ROQUIN* underlie AITL in some individuals.

Conclusions

Nearly half a century has passed since Jacques Miller first described the fundamental requirement for thymus-derived cells to support antigen-specific antibody production[3]. It has taken a substantial period of time to identify the T cell subset that is responsible for mediating B cell responses but in the past decade there have been important advances in our knowledge and understanding of the molecular and cellular biology as well as the function and regulation of T_{FH} cells. Indeed, we now have an appreciation of how these cells operate during normal immune responses and, perhaps more importantly, how they might underlie immunological diseases as diverse as autoimmunity, immunodeficiency and lymphomas. These rapid discoveries in T_{FH} cell biology should allow us to exploit these cells (as well as their associated receptors, cytokines, chemokines and biochemical pathways) for the development of novel therapeutics to treat these conditions, as well as for the development of next-generation vaccines to induce sustained protection against infection. Hopefully it will not take another 50 years before T_{FH} cell-targeted therapies are available to improve human health.

Isocitrate dehydrogenase 2 (*IDH2*). An enzyme that catalyses the oxidative decarboxylation of isocitrate to 2-oxoglutarate and is a component of the tricarboxylic acid cycle. Mutations in *IDH1* and *IDH3* have been detected in glioma, glioblastomas and acute myeloid leukaemia.

TET2
The *TET2* gene encodes an oxygenase that catalyses the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine to alter the epigenetic status of DNA. It is frequently mutated in human lymphomas.

Roquin
A RING-type ubiquitin ligase that represses the expression of inducible T cell co-stimulator (ICOS), thereby restraining the development and function of T follicular helper cells. A mutation in the *Roquin* (also known as *Rc3h1*) gene results in lupus-like disease in mice.

1. O'Shea, J. J. & Paul, W. E. Mechanisms underlying lineage commitment and plasticity of helper $CD4^+$ T cells. *Science* **327**, 1098–1102 (2010).
2. Al-Herz, W. *et al.* Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Front. Immunol.* **2**, 54 (2011).
3. Miller, J. F., De Burgh, P. M. & Grant, G. A. Thymus and the production of antibody-plaque-forming cells. *Nature* **208**, 1332–1334 (1965).
4. Breitfeld, D. *et al.* Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* **192**, 1545–1552 (2000).
5. Schaerli, P. *et al.* CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* **192**, 1553–1562 (2000).
6. Kim, C. H. *et al.* Subspecialization of $CXCR5^+$ T cells: B helper activity is focused in a germinal center — localized subset of $CXCR5^+$ T cells. *J. Exp. Med.* **193**, 1373–1381 (2001).
7. Kim, J. R., Lim, H. W., Kang, S. G., Hillsamer, P. & Kim, C. H. Human $CD57^+$ germinal center-T cells are the major helpers for GC-B cells and induce class switch recombination. *BMC Immunol.* **6**, 3 (2005).
8. Chtanova, T. *et al.* T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J. Immunol.* **173**, 68–78 (2004).
9. Rasheed, A. U., Rahn, H. P., Sallusto, F., Lipp, M. & Muller, G. Follicular B helper T cell activity is confined to $CXCR5^{hi}ICOS^{hi}$ CD4 T cells and is independent of CD57 expression. *Eur. J. Immunol.* **36**, 1892–1903 (2006).
10. Vinuesa, C. G. *et al.* A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* **435**, 452–458 (2005).
References 8–10 determine the molecular profile of human and mouse T_{FH} cells, and identify many of the defining characteristics of these cells.
11. Goodnow, C. C., Vinuesa, C. G., Randall, K. L., Mackay, F. & Brink, R. Control systems and decision making for antibody production. *Nature Immunol.* **11**, 681–688 (2010).
12. Victora, G. D. & Nussenzweig, M. C. Germinal centers. *Annu. Rev. Immunol.* **30**, 429–457 (2012).
13. Chan, T. D. & Brink, R. Affinity-based selection and the germinal center response. *Immunol. Rev.* **247**, 11–23 (2012).
14. Haynes, N. M. *et al.* Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1^{high} germinal center-associated subpopulation. *J. Immunol.* **179**, 5099–5108 (2007).
15. Kroenke, M. A. *et al.* Bcl6 and maf cooperate to instruct human follicular helper CD4 T cell differentiation. *J. Immunol.* **188**, 3734–3744 (2012).
16. Ma, C. S. *et al.* Early commitment of naive human $CD4^+$ T cells to the T follicular helper (T_{FH}) cell lineage is induced by IL-12. *Immunol. Cell Biol.* **87**, 590–600 (2009).
This study determines that IL-12 is important for the differentiation of human naive $CD4^+$ T cells to T_{FH} cells.
17. Deenick, E. K. *et al.* Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* **33**, 241–253 (2010).
18. Yusuf, I. *et al.* Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). *J. Immunol.* **185**, 190–202 (2010).
19. Bentebibel, S. E., Schmitt, N., Banchereau, J. & Ueno, H. Human tonsil *B-cell lymphoma 6* (*BCL6*)-expressing $CD4^+$ T-cell subset specialized for B-cell help outside germinal centers. *Proc. Natl Acad. Sci. USA* **108**, E488–E497 (2011).
20. Johnston, R. J. *et al.* Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* **325**, 1006–1010 (2009).
21. Nurieva, R. I. *et al.* Bcl6 mediates the development of T follicular helper cells. *Science* **325**, 1001–1005 (2009).
22. Yu, D. *et al.* The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* **31**, 457–468 (2009).
References 20–22 identify that BCL-6 is crucial for the generation of T_{FH} cells, and in reference 20 the authors elegantly demonstrate the opposing functions of BCL-6 and BLIMP1 in this process.
23. Crotty, S. Follicular helper CD4 T cells (T_{FH}). *Annu. Rev. Immunol.* **29**, 621–663 (2011).
24. Ma, C. S., Deenick, E. K., Batten, M. & Tangye, S. G. The origins, function, and regulation of T follicular helper cells. *J. Exp. Med.* **209**, 1241–1253 (2012).
25. Nutt, S. L. & Tarlinton, D. M. Germinal center B and follicular helper T cells: siblings, cousins or just good friends? *Nature Immunol.* **12**, 472–477 (2011).
26. Craft, J. E. Follicular helper T cells in immunity and systemic autoimmunity. *Nature Rev. Rheumatol.* **8**, 337–347 (2012).
27. Hu, H. *et al.* Noncanonical NF-κB regulates inducible costimulator (ICOS) ligand expression and T follicular helper cell development. *Proc. Natl Acad. Sci. USA* **108**, 12827–12832 (2011).
28. Ou, X., Xu, S. & Lam, K. P. Deficiency in TNFRSF13B (TACI) expands T-follicular helper and germinal center B cells via increased ICOS-ligand expression but impairs plasma cell survival. *Proc. Natl Acad. Sci. USA* **109**, 15401–15406 (2012).
29. Bossaller, L. *et al.* ICOS deficiency is associated with a severe reduction of $CXCR5^+CD4$ germinal center Th cells. *J. Immunol.* **177**, 4927–4932 (2006).
30. Nurieva, R. I. *et al.* Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* **29**, 138–149 (2008).
31. Ma, C. S., Nichols, K. E. & Tangye, S. G. Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules. *Annu. Rev. Immunol.* **25**, 337–379 (2007).
32. Qi, H., Cannons, J. L., Klauschen, F., Schwartzberg, P. L. & Germain, R. N. SAP-controlled T–B cell interactions underlie germinal centre formation. *Nature* **455**, 764–769 (2008).
In this study, the use of multiphoton *in vivo* imaging reveals the importance of SAP in sustaining conjugate formation between T_{FH} cells and B cells for the generation of GC and antibody responses.
33. Cannons, J. L. *et al.* Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* **32**, 253–265 (2010).
34. Linterman, M. A. *et al.* Follicular helper T cells are required for systemic autoimmunity. *J. Exp. Med.* **206**, 561–576 (2009).
35. Kageyama, R. *et al.* The receptor Ly108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. *Immunity* **36**, 986–1002 (2012).
36. Good-Jacobson, K. L. *et al.* PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nature Immunol.* **11**, 535–542 (2010).
37. Hamel, K. M. *et al.* B7-H1 expression on non-B and non-T cells promotes distinct effects on T- and B-cell responses in autoimmune arthritis. *Eur. J. Immunol.* **40**, 3117–3127 (2010).
38. Hams, E. *et al.* Blockade of B7-H1 (programmed death ligand 1) enhances humoral immunity by positively regulating the generation of T follicular helper cells. *J. Immunol.* **186**, 5648–5655 (2011).
39. Butler, N. S. *et al.* Therapeutic blockade of PD-L1 and LAG-3 rapidly clears established blood-stage *Plasmodium* infection. *Nature Immunol.* **13**, 188–195 (2012).
40. Cubas, R. A. *et al.* Inadequate T follicular cell help impairs B cell immunity during HIV infection. *Nature Med.* **19**, 494–499 (2013).
41. Sage, P. T., Francisco, L. M., Carman, C. V. & Sharpe, A. H. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nature Immunol.* **14**, 152–161 (2012).
42. Eto, D. *et al.* IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. *PLoS ONE* **6**, e17739 (2011).
43. Harker, J. A., Lewis, G. M., Mack, L. & Zuniga, E. I. Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. *Science* **334**, 825–829 (2011).
44. Karnowski, A. *et al.* B and T cells collaborate in antiviral responses via IL-6, IL-21, and transcriptional activator and coactivator, Oct2 and OBF-1. *J. Exp. Med.* **209**, 2049–2064 (2012).
45. Choi, Y. S., Eto, D., Yang, J. A., Lao, C. & Crotty, S. Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. *J. Immunol.* **190**, 3049–3053 (2013).
46. Ma, C. S. *et al.* Functional STAT3 deficiency compromises the generation of human T follicular helper cells. *Blood* **119**, 3997–4008 (2012).
This study establishes the importance of STAT3 and its related cytokines in generating human T_{FH} cells.
47. Kim, S. J., Zou, Y. R., Goldstein, J., Reizis, B. & Diamond, B. Tolerogenic function of Blimp-1 in dendritic cells. *J. Exp. Med.* **208**, 2193–2199 (2011).
48. Cai, G. *et al.* A regulatory role for IL-10 receptor signaling in development and B cell help of T follicular helper cells in mice. *J. Immunol.* **189**, 1294–1302 (2012).
49. Johnston, R. J., Choi, Y. S., Diamond, J. A., Yang, J. A. & Crotty, S. STAT5 is a potent negative regulator of T_{FH} cell differentiation. *J. Exp. Med.* **209**, 243–250 (2012).
50. Pepper, M., Pagan, A. J., Igyarto, B. Z., Taylor, J. J. & Jenkins, M. K. Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. *Immunity* **35**, 583–595 (2011).
51. Hale, J. S. *et al.* Distinct memory $CD4^+$ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. *Immunity* **38**, 805–817 (2013).
52. Choi, Y. S. *et al.* Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *J. Immunol.* **190**, 4014–4026 (2013).
53. Liu, X. *et al.* Bcl6 expression specifies the T follicular helper cell program *in vivo*. *J. Exp. Med.* **209**, 1841–1852 (2012).
54. Luthje, K. *et al.* The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nature Immunol.* **13**, 491–498 (2012).
55. Weber, J. P., Fuhrmann, F. & Hutloff, A. T-follicular helper cells survive as long-term memory cells. *Eur. J. Immunol.* **42**, 1981–1988 (2012).
References 50–55 examine the 'memory' capability of T_{FH} cells in murine models of disease. T_{FH} cells that are generated in response to protein antigen or pathogen infection can contribute to the long-term pool of memory $CD4^+$ T cells; however, these cells do not necessarily retain a T_{FH} cell phenotype, which demonstrates the plasticity that exists in this T cell subset in terms of their capacity to differentiate into alternative effector subsets.
56. Morita, R. *et al.* Human blood $CXCR5^+CD4^+$ T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* **34**, 108–121 (2011).
This study is a comprehensive analysis of circulating $CD4^+CXCR5^+$ T cells in humans, establishing that they exhibit hallmarks of tissue T_{FH} cells (they secrete IL-21, IL-10 and CXCL13, express ICOS and provide B cell help) and that they can be further divided into T_H1-, T_H2- and T_H17-type T_{FH} cells. Importantly, the T_H2 and T_H17 subset, but not the T_H1 subset, are over-represented in human autoimmunity.
57. Bentebibel, S. E. *et al.* Induction of $ICOS^+CXCR3^+CXCR5^+$ T_H cells correlates with antibody responses to influenza vaccination. *Sci. Transl. Med.* **5**, 176ra132 (2013).
58. Galli, G. *et al.* Invariant NKT cells sustain specific B cell responses and memory. *Proc. Natl Acad. Sci. USA* **104**, 3984–3989 (2007).
59. Barral, P. *et al.* B cell receptor-mediated uptake of CD1d-restricted antigen augments antibody responses by recruiting invariant NKT cell help *in vivo*. *Proc. Natl Acad. Sci. USA* **105**, 8345–8350 (2008).
60. Leadbetter, E. A. *et al.* NK T cells provide lipid antigen-specific cognate help for B cells. *Proc. Natl Acad. Sci. USA* **105**, 8339–8344 (2008).
61. Tonti, E. *et al.* NKT-cell help to B lymphocytes can occur independently of cognate interaction. *Blood* **113**, 370–376 (2009).
62. Chang, P. P. *et al.* Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses. *Nature Immunol.* **13**, 35–43 (2012).
63. Tonti, E. *et al.* Follicular helper NKT cells induce limited B cell responses and germinal center formation in the absence of $CD4^+$ T cell help. *J. Immunol.* **188**, 3217–3222 (2012).

64. King, I. L. *et al.* Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner. *Nature Immunol.* **13**, 44–50 (2012).
References 62–64 characterize NKT_{FH} cells and their contribution to responses to αGalCer antigen.
65. Vantourout, P. & Hayday, A. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nature Rev. Immunol.* **13**, 88–100 (2013).
66. Brandes, M. *et al.* Flexible migration program regulates γδ T-cell involvement in humoral immunity. *Blood* **102**, 3693–3701 (2003).
67. Caccamo, N. *et al.* CXCR5 identifies a subset of Vγ9Vδ2 T cells which secrete IL-4 and IL-10 and help B cells for antibody production. *J. Immunol.* **177**, 5290–5295 (2006).
68. Caccamo, N. *et al.* IL-21 regulates the differentiation of a human γδ T cell subset equipped with B cell helper activity. *PLoS ONE* **7**, e41940 (2012).
69. Vermijlen, D. *et al.* Distinct cytokine-driven responses of activated blood γδ T cells: insights into unconventional T cell pleiotropy. *J. Immunol.* **178**, 4304–4314 (2007).
70. Chan, T. D. *et al.* Elimination of germinal-center-derived self-reactive B cells is governed by the location and concentration of self-antigen. *Immunity* **37**, 893–904 (2012).
71. Lim, H. W., Hillsamer, P. & Kim, C. H. Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. *J. Clin. Invest.* **114**, 1640–1649 (2004).
72. Chung, Y. *et al.* Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nature Med.* **17**, 983–988 (2011).
73. Linterman, M. A. *et al.* Foxp3$^+$ follicular regulatory T cells control the germinal center response. *Nature Med.* **17**, 975–982 (2011).
74. Wollenberg, I. *et al.* Regulation of the germinal center reaction by Foxp3$^+$ follicular regulatory T cells. *J. Immunol.* **187**, 4553–4560 (2011).
References 72–74 characterize follicular T_{Reg} cells in human and mouse lymphoid tissues.
75. Josefowicz, S. Z., Lu, L. F. & Rudensky, A. Y. Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* **30**, 531–564 (2012).
76. Wu, H. Y., Quintana, F. J. & Weiner, H. L. Nasal anti-CD3 antibody ameliorates lupus by inducing an IL-10-secreting CD4$^+$ CD25$^-$ LAP$^+$ regulatory T cell and is associated with down-regulation of IL-17$^+$ CD4$^+$ ICOS$^+$ CXCR5$^+$ follicular helper T cells. *J. Immunol.* **181**, 6038–6050 (2008).
77. Simpson, N. *et al.* Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum.* **62**, 234–244 (2010).
This study demonstrates that numbers of circulating CXCR5$^+$CD4$^+$ T cells in human blood are increased in the autoimmune diseases SLE and Sjögren's syndrome.
78. Chevalier, N. *et al.* CXCR5 expressing human central memory CD4 T cells and their relevance for humoral immune responses. *J. Immunol.* **186**, 5556–5568 (2011).
79. Kitano, M. *et al.* Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity* **34**, 961–972 (2011).
80. Pallikkuth, S. *et al.* Upregulation of IL-21 receptor on B cells and IL-21 secretion distinguishes novel 2009 H1N1 vaccine responders from nonresponders among HIV-infected persons on combination antiretroviral therapy. *J. Immunol.* **186**, 6173–6181 (2011).
81. Pallikkuth, S. *et al.* Impaired peripheral blood T-follicular helper cell function in HIV-infected nonresponders to the 2009 H1N1/09 vaccine. *Blood* **120**, 985–993 (2012).
References 57, 80 and 81 establish the benefits of analysing circulating CD4$^+$CXCR5$^+$ T cells as a biomarker of successful vaccination.
82. Banchereau, J. & Rousset, F. Human B lymphocytes: phenotype, proliferation, and differentiation. *Adv. Immunol.* **52**, 125–262 (1992).
83. Bryant, V. L. *et al.* Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5$^+$ T follicular helper cells. *J. Immunol.* **179**, 8180–8190 (2007).
84. Salomonsson, S. *et al.* Expression of the B cell-attracting chemokine CXCL13 in the target organ and autoantibody production in ectopic lymphoid tissue in the chronic inflammatory disease Sjögren's syndrome. *Scand. J. Immunol.* **55**, 336–342 (2002).
85. Hutloff, A. *et al.* Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. *Arthritis Rheum.* **50**, 3211–3220 (2004).
86. Saito, R. *et al.* Altered expression of chemokine receptor CXCR5 on T cells of myasthenia gravis patients. *J. Neuroimmunol.* **170**, 172–178 (2005).
87. Tackenberg, B. *et al.* Clonal expansions of CD4$^+$ B helper T cells in autoimmune myasthenia gravis. *Eur. J. Immunol.* **37**, 849–863 (2007).
88. Feng, X. *et al.* Inhibition of aberrant circulating Tfh cell proportions by corticosteroids in patients with systemic lupus erythematosus. *PLoS ONE* **7**, e51982 (2012).
89. Terrier, B. *et al.* Interleukin 21 correlates with T cell and B cell subset alterations in systemic lupus erythematosus. *J. Rheumatol* **39**, 1819–1828 (2012).
90. Ma, J. *et al.* Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clin. Dev. Immunol.* **2012**, 827–480 (2012).
91. Zhu, C. *et al.* Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. *J. Clin. Endocrinol. Metab.* **97**, 943–950 (2012).
92. Liu, R. *et al.* A regulatory effect of IL-21 on T follicular helper-like cell and B cell in rheumatoid arthritis. *Arthritis Res. Ther.* **14**, R255 (2012).
93. Li, X. Y. *et al.* Role of the frequency of blood CD4$^+$ CXCR5$^+$ CCR6$^+$ T cells in autoimmunity in patients with Sjögren's syndrome. *Biochem. Biophys. Res. Commun.* **422**, 238–244 (2012).
94. Wong, C. K. *et al.* Elevated production of B cell chemokine CXCL13 is correlated with systemic lupus erythematosus disease activity. *J. Clin. Immunol.* **30**, 45–52 (2010).
95. Chakera, A. *et al.* The phenotype of circulating follicular-helper T cells in patients with rheumatoid arthritis defines CD200 as a potential therapeutic target. *Clin. Dev. Immunol.* **2012**, 948–218 (2012).
96. Ma, C. S. *et al.* Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4$^+$ T cells. *J. Clin. Invest.* **115**, 1049–1059 (2005).
This study determines that compromised humoral immunity in XLP is the result of a defect in CD4$^+$ T cell-mediated B cell help; this was the first study to implicate a T_{FH} cell defect as a cause of impaired antibody responses in primary immunodeficiencies.
97. Schmitt, N. *et al.* IL-12 receptor β1 deficiency alters *in vivo* T follicular helper cell response in humans. *Blood* **121**, 3375–3385 (2013).
98. Moir, S. & Fauci, A. S. B cells in HIV infection and disease. *Nature Rev. Immunol.* **9**, 235–245 (2009).
99. Lindqvist, M. *et al.* Expansion of HIV-specific T follicular helper cells in chronic HIV infection. *J. Clin. Invest.* **122**, 3271–3280 (2012).
100. Petrovas, C. *et al.* CD4 T follicular helper cell dynamics during SIV infection. *J. Clin. Invest.* **122**, 3281–3294 (2012).
101. Perreau, M. *et al.* Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J. Exp. Med.* **210**, 143–156 (2012).
102. Xu, Y. *et al.* Simian immunodeficiency virus infects follicular helper CD4 T cells in lymphoid tissues during pathogenic infection of pigtail macaques. *J. Virol.* **87**, 3760–3773 (2013).
103. de Leval, L., Gisselbrecht, C. & Gaulard, P. Advances in the understanding and management of angioimmunoblastic T-cell lymphoma. *Br. J. Haematol.* **148**, 673–689 (2010).
104. Hu, S., Young, K. H., Konoplev, S. N. & Medeiros, L. J. Follicular T-cell lymphoma: a member of an emerging family of follicular helper T-cell derived T-cell lymphomas. *Hum. Pathol.* **43**, 1789–1798 (2012).
105. Krenacs, L., Schaerli, P., Kis, G. & Bagdi, E. Phenotype of neoplastic cells in angioimmunoblastic T-cell lymphoma is consistent with activated follicular B helper T cells. *Blood* **108**, 1110–1111 (2006).
106. Roncador, G. *et al.* Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. *Haematologica* **92**, 1059–1066 (2007).
107. Marafioti, T. *et al.* The inducible T-cell co-stimulator molecule is expressed on subsets of T cells and is a new marker of lymphomas of T follicular helper cell-derivation. *Haematologica* **95**, 432–439 (2010).
108. de Leval, L. *et al.* The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and follicular helper T (T_{FH}) cells. *Blood* **109**, 4952–4963 (2007).
This study uses comprehensive gene array analysis to show that T_{FH} cells are the malignant cell type in AITL.
109. Iqbal, J. *et al.* Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. *Blood* **115**, 1026–1036 (2010).
110. Rodriguez-Pinilla, S. M. *et al.* Peripheral T-cell lymphoma with follicular T-cell markers. *Am. J. Surg. Pathol.* **32**, 1787–1799 (2008).
111. Miyoshi, H. *et al.* Clinicopathologic analysis of peripheral T-cell lymphoma, follicular variant, and comparison with angioimmunoblastic T-cell lymphoma: Bcl-6 expression might affect progression between these disorders. *Am. J. Clin. Pathol.* **137**, 879–889 (2012).
112. Quivoron, C. *et al.* TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell* **20**, 25–38 (2011).
113. Cairns, R. A. *et al. IDH2* mutations are frequent in angioimmunoblastic T-cell lymphoma. *Blood* **119**, 1901–1903 (2012).
114. Lemonnier, F. *et al.* Recurrent *TET2* mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. *Blood* **120**, 1466–1469 (2012).
115. Ellyard, J. I. *et al.* Heterozygosity for Roquinsan leads to angioimmunoblastic T-cell lymphoma-like tumors in mice. *Blood* **120**, 812–821 (2012).
116. Parrish-Novak, J. *et al.* Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* **408**, 57–63 (2000).
117. Ettinger, R. *et al.* IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. *J. Immunol.* **175**, 7867–7879 (2005).
118. Avery, D. T. *et al.* B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J. Exp. Med.* **207**, 155–171 (2010).
119. Pene, J. *et al.* Cutting edge: IL-21 is a switch factor for the production of IgG1 and IgG3 by human B cells. *J. Immunol.* **172**, 5154–5157 (2004).
120. Avery, D. T., Bryant, V. L., Ma, C. S., de Waal Malefyt, R. & Tangye, S. G. IL-21-induced isotype switching to IgG and IgA by human naive B cells is differentially regulated by IL-4. *J. Immunol.* **181**, 1767–1779 (2008).
121. Steinman, L. A brief history of T_H17, the first major revision in the T_H1/T_H2 hypothesis of T cell-mediated tissue damage. *Nature Med.* **13**, 139–145 (2007).
122. Annunziato, F., Cosmi, L., Liotta, F., Maggi, E. & Romagnani, S. Type 17 T helper cells — origins, features and possible roles in rheumatic disease. *Nature Rev. Rheumatol.* **5**, 325–331 (2009).
123. Afshar, R., Medoff, B. D. & Luster, A. D. Allergic asthma: a tale of many T cells. *Clin. Exp. Allergy* **38**, 1847–1857 (2008).
124. Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V. K. IL-17 and Th17 *Cells. Annu. Rev. Immunol.* **27**, 485–517 (2009).
125. Jabeen, R. & Kaplan, M. H. The symphony of the ninth: the development and function of Th9 cells. *Curr. Opin. Immunol.* **24**, 303–307 (2012).
126. Witte, E., Witte, K., Warszawska, K., Sabat, R. & Wolk, K. Interleukin-22: a cytokine produced by T, NK and NKT cell subsets, with importance in the innate immune defense and tissue protection. *Cytokine Growth Factor Rev.* **21**, 365–379 (2010).

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