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Pathophysiology of T follicular helper cells in humans and mice

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Follicular helper T cells (T_{FH} cells) compose a heterogeneous subset of CD4$^+$ T cells that induce the differentiation of B cells into plasma cells and memory cells. They are found within and in proximity to germinal centers in secondary lymphoid organs, and their memory compartment also circulates in the blood. Our knowledge on the biology of T_{FH} cells has increased significantly during the past decade, largely as a result of mouse studies. However, recent studies on human T_{FH} cells isolated from lymphoid organ and blood samples and recent observations on the developmental mechanism of human T_{FH} cells have revealed both similarities and differences between human and mouse T_{FH} cells. Here we present the similarities and differences between mouse and human lymphoid organ–resident T_{FH} cells and discuss the role of T_{FH} cells in response to vaccines and in disease pathogenesis.

A number of seminal discoveries made in mice and humans led to the description of B follicular helper T (T_{FH}) cells in the early 2000s. The requirement of T cell help for the development of antibody responses was first described in the 1960s (ref. 1). CD4$^+$ helper T cells (T_H cells) were then found to be necessary for the development of germinal centers, discrete structures in secondary lymphoid organs where the selection of high-affinity B cells and the development of B cell memory occur[2–4]. *In vitro* studies in the 1980s, mostly involving CD4$^+$ T cell clones and recombinant cytokines, showed that T_H2 cells are the major T_H subset engaged in helping B cells by secreting interleukin 4 (IL-4) and IL-10 (refs. 5,6). In mouse, T_H1 cells also contribute to the regulation of antibody responses by inducing B cell class switching toward IgG2a. However, for almost two decades it was unclear how the T_H1 and T_H2 cells engaged in B cell help in lymphoid organs were biologically and developmentally distinct from those that exit lymphoid organs and migrate into peripheral tissues. The chemokine receptor CXCR5 was discovered in 1993 as a G protein–coupled receptor expressed primarily by B cells[7], and in 1996 it was shown to be critical for the migration of B cells into follicles in lymphoid organs in mice[8]. In 1999, CD4$^+$ T cells activated in lymphoid organs of immunized mice were found to express CXCR5, which was required for the cells' migration into follicles[9]. In the early 2000s, studies on CD4$^+$ T cells in human tonsils showed that cells expressing CXCR5 have a superior capacity to induce immunoglobulin production in B cells *in vitro* relative to CD4$^+$ T cells lacking CXCR5 expression. On the basis of their localization and functions, tonsillar CXCR5$^+$ CD4$^+$ T cells were designated as T_{FH} cells[10–12]. A similar CD4$^+$ T cell subset was found in mouse lymph nodes[13]. Profiling of cytokine production and gene expression in human and mouse T_{FH} cells showed that these cells are distinct from T_H1 and T_H2 cells[14–16] and help B cells mainly by delivering activating signals with the TNF family molecule CD40L and the cytokine IL-21 (refs. 14,17–20). In 2009, the transcription repressor Bcl-6 was discovered to be an essential factor for T_{FH} cell generation *in vivo* in mice[21–23], and since then T_{FH} cells have been recognized as an independent T_H subset distinct from T_H1, T_H2 and T_H17 cells.

Our knowledge of the biology of T_{FH} cells has increased significantly during the past decade (reviewed in refs. 24,25). Like in other fields of immunology, important biological features of T_{FH} cells have been learned of from studies in mouse models, whereas studies of the ontogeny and function of T_{FH} cells in humans have remained relatively limited, mainly because of difficulties in investigating and manipulating T_{FH} cells from human secondary lymphoid organs. Furthermore, there are only two main sources of human T_{FH} cells for research: tonsils from children who have experienced recurrent throat infections but are otherwise healthy, and spleens, generally from cadaveric organ donors. This poses a challenge in investigations of human T_{FH} cells' association with human diseases such as cancer and autoimmunity. Over 60 million years of independent evolution have introduced significant differences in the immune systems of humans and mice. Thus, it is important to address whether conclusions drawn in mouse T_{FH} studies also hold true for human T_{FH} cells. Recent progress in our understanding of the biology of blood-circulating T_{FH} cells in humans has provided clues on how to determine whether alteration of T_{FH} responses contributes to human diseases. Furthermore, analyses of blood memory T_{FH} cells (and also lymph node cells in some instances) from patients with primary or acquired immunodeficiencies have also provided important insights regarding the development and/or maintenance of T_{FH} cells

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in humans. Together with *in vitro* studies aiming at determining the developmental mechanisms of human T_{FH} cells, these studies have started revealing similarities as well as differences between humans and mice.

In this review, we first summarize our current knowledge of the biology of lymphoid organ T_{FH} cells and discuss the similarities and differences between these cells in mice and humans. Then we discuss recent findings on the circulating memory compartment of T_{FH} cells in human blood (hereinafter called blood memory T_{FH} cells). Last, we summarize recent insights into the role of T_{FH} cells in disease pathogenesis and discuss how T_{FH} cells participate in or contribute to both beneficial and aberrant immune responses observed in various human diseases.

T_{FH} subsets and dynamics in mice

Recent studies in mice and humans show that T_{FH} lineage cells in lymphoid organs are composed of subsets that differ in their localization, phenotype and function. The circulating memory compartment of T_{FH} cells in human blood also contains subsets that differ in phenotype and function.

Studies in mice have shown that after interaction with dendritic cells (DCs) in the T cell zones of secondary lymphoid organs, a fraction of activated $CD4^+$ T cells migrate toward B cell follicles by upregulating the chemokine receptor CXCR5 (refs. 9,26) (mediated by increased expression of the transcription factors Bcl-6 (refs. 27,28) and Ascl2 (ref. 29)) while downregulating the chemokine receptor CCR7 (ref. 30) and the cell adhesion molecule PSGL-1 (ref. 31). These $CXCR5^+Bcl\text{-}6^+CD4^+$ T cells (hereinafter called T_{FH} precursors) interact with antigen-presenting B cells at the border of the B cell follicle and T cell zone[9,32], a required process for the generation of germinal center (GC)-resident T_{FH} cells (GC T_{FH} cells) and the differentiation of primed B cells along both GC and extrafollicular pathways[33]. Bcl-6 expression in $CD4^+$ T cells is a prerequisite for GC formation[21–23]. The origin of T_{FH} cells is not restricted to naive cells, and there is some evidence suggesting that other T_H subsets including T_H1, T_H2, T_H17 and regulatory T (T_{reg}) cells may become T_{FH} cells in GCs (reviewed in ref. 25). This is consistent with the heterogeneity in cytokine expression patterns among GC T_{FH} cells developed under different immunization protocols and by different types of infectious agents (reviewed in ref. 34).

In mice, GC T_{FH} cells and T_{FH} precursors have been defined largely on the basis of differences in cell surface markers, particularly the expression of CXCR5 and PD-1, which are more highly expressed in GC T_{FH} cells than in their precursors outside GCs. During the acute or early phase following immunization, IL-21 is expressed almost exclusively by $CXCR5^{hi}PD\text{-}1^{hi}$ GC T_{FH} cells in lymphoid organs[35]. Investigation of T_{FH} cells is, however, no longer restricted to their identification on the basis of phenotypic markers and cytokine secretion; it is now possible to visualize and track GC T_{FH} cells directly with intravital imaging. Such imaging studies have revealed that GC T_{FH} cells display unique cell dynamics: GC T_{FH} cells continuously immigrate and redistribute to other follicles and neighboring GCs[36], in a manner dependent on high expression of CXCR5 and the trafficking molecule sphingosine-1-phosphate receptor 2 (S1PR2)[37]. Signals derived from interacting B cells induce GC T_{FH} cells to increase intracellular Ca^{2+} and their expression of IL-4 and IL-21, which enhance their capacity to promote the growth and differentiation of B cells[38]. A dynamic exchange of GC T_{FH} cells among multiple GCs and bidirectional signals between GC T_{FH} cells and B cells during their cognate interactions likely represent important mechanisms associated with the efficient selection and expansion of high-affinity B cells during GC responses. GC T_{FH} cells exiting GCs rarely remain in the T cell zone or enter the bloodstream (at least while GC responses are actively in progress)[36], suggesting that GC T_{FH} cells and their precursors display different cell dynamics.

Mouse studies also identified cell subsets important for the suppression of GC response[34]. These suppressors contain $Foxp3^+$ T follicular regulatory (T_{FR}) cells[39–41]. Current evidence shows that, at least in mice, T_{FR} cells are differentiated from thymus-derived $Foxp3^+$ regulatory T cells. T_{FR} cells dampen GC responses by limiting the numbers of both T_{FH} and B cells in GCs[39–41]. Given that mice lacking functional T_{FR} cells favor the accumulation of non-antigen-specific B cells[40], T_{FR} cells might be specialized in repressing self-reactive B cells in GCs. T_{FR} cells are also likely to be responsible for terminating the GC response[39]. In either case, the balance between T_{FH} and T_{FR} cells in the GC environment likely represents a key factor in the generation of both high-affinity protective antibodies and pathogenic autoantibodies.

T_{FH} subsets in human tonsils

Studies in the early 1980s showed that $CD4^+$ T cells in human tonsillar GCs express CD57 (ref. 42) and that these $CD57^+$ T cells display a limited ability to express IL-2 (ref. 43). Although early studies suggested that CD57 is a marker for functionally mature GC T_{FH} cells in humans, CD57 is expressed by only approximately 30% of GC T_{FH} cells, and it is also expressed by a fraction (~10%) of $CD4^+$ T cells localized outside GCs[15,18]. T_{FH} cells in human GCs are currently defined by their high expression of CXCR5, inducible costimulator (ICOS) and T cell inhibitory receptor PD-1 (refs. 15,18,44). Of note, ICOS is not a useful marker for defining GC T_{FH} cells in mice, as ICOS expression is largely similar between GC T_{FH} cells and T_{FH} precursors[36]. In human tonsils, $Foxp3^+$ T_{FR} cells within GCs are much rarer than in mice (unpublished observations), and current knowledge on human T_{FR} cells is very limited.

While Bcl-6 is well recognized as a transcription factor defining the T_{FH} lineage, mouse studies show that GC T_{FH} cells contain cells coexpressing Bcl-6 and T-bet (the transcription factor typically expressed by T_H1 cells)[35,45]. In humans, GC T_{FH} cells in tonsils also contain a subset coexpressing Bcl-6 and RORγt (the transcription factor typically expressed by T_H17 cells), in addition to a subset coexpressing Bcl-6 and T-bet[46]. It remains to be established whether these GC T_{FH} cell subsets and GC T_{FH} cells lacking expression of T-bet or RORγt have distinct functions; nevertheless, this observation suggests that other T_H subsets might be able to differentiate into T_{FH} cells in humans, similar to cells in mice. An alternative explanation is that there are developmental paths that are shared between T_H1 and T_{FH} cells and between T_H17 and T_{FH} cells in humans, as also proposed in mice[47,48].

Recent data point to the possibility that the development of T_{FH} cells might differ between mice and humans. In humans, the cytokine TGF-β acts with IL-12 and IL-23 to promote the expression of multiple T_{FH} molecules, including CXCR5, IL-21 and Bcl-6 (ref. 46), on activated naive $CD4^+$ T cells (**Fig. 1**). Furthermore, human $CD4^+$ T cells cultured under conditions used to generate T_H17 cells (for example, a combination of TGF-β, IL-23, IL-6 and IL-1β) coexpress T_{FH} molecules and T_H17 molecules[46]. This is in stark contrast to mouse $CD4^+$ T cells, in which TGF-β signals suppress the expression of T_{FH} molecules including IL-21, ICOS and Bcl-6 (refs. 21,46,49,50).

In human tonsils, $CD4^+$ T cells expressing low amounts of CXCR5 and ICOS ($CXCR5^{lo}ICOS^{lo}$ cells) are exclusively localized outside GCs[18]. $CXCR5^{lo}ICOS^{lo}CD4^+$ T cells in tonsils appear to be T_{FH} precursors (or extrafollicular helper T cells), as these cells express multiple

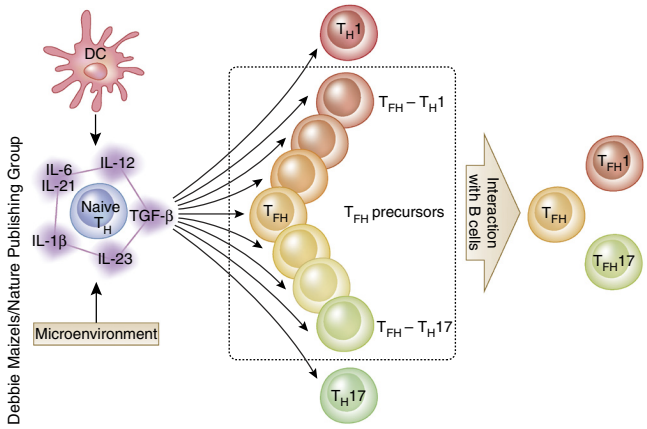

Figure 1 Potential mechanism in the generation of human T_{FH} subsets. As with other T_H subsets, signals derived from antigen-presenting cells (including DCs) and the microenvironment instruct naive CD4$^+$ T cells to differentiate into the T_{FH} lineage. The major cytokines driving the early T_{FH} differentiation process in humans are IL-12 and IL-23, and TGF-β synergizes with these cytokines. Other STAT3-activating cytokines, including IL-6, IL-21 and IL-1β, also support this process in the presence of IL-12, IL-23 and TGF-β. The differentiation of human naive CD4$^+$ T cells is regulated by the balance of signals derived from these cytokines, and activated CD4$^+$ T cells differentiate into precursors of variable T_H subsets such as T_H1, T_H17 and T_{FH} cells. Some T_{FH} precursors share properties of T_H1 and T_H17 cells (dotted rectangle in middle panel); interactions with B cells promote their differentiation into mature T_{FH} cells, including $T_{FH}1$ and $T_{FH}17$ cells. The mechanism associated with the generation of $T_{FH}2$ cells is currently unknown.

T_{FH} molecules such as CD40L, IL-21 and CXCL13 but lack the expression of Bcl-6 protein[18]. Functionally, isolated CXCR5^{lo}ICOS^{lo}CD4$^+$ T cells are more effective than T_{FH} cells *in vitro* at helping naive B cells to become immunoglobulin-producing cells, possibly because they produce large amounts of IL-21. In contrast, GC T_{FH} cells but not CXCR5^{lo}ICOS^{lo}CD4$^+$ T cells provide help to GC B cells and promote their survival, proliferation and differentiation into immunoglobulin-producing cells *in vitro*. The inability of CXCR5^{lo}ICOS^{lo}CD4$^+$ T cells to help GC B cells is due to their expression of Fas ligand, which induces the death of Fas-expressing GC B cells[18]. Thus, T_{FH} cells at different maturation stages differ in their location and biological functions in human tonsils[18]. The observation that human T_{FH} precursors help naive B cells is consistent with the presence of mouse T_{FH} precursors with low expression of PD-1 and Bcl-6 at the T-B border shortly after T cell priming and before the induction of GCs[33]. These T cells likely induce the differentiation of B cells that have recently engaged their antigen receptors.

Memory T_{FH} subsets in human blood

CD4$^+$ T cells expressing the chemokine receptor CXCR5 were first described in human blood in 1994 (ref. 51) and were considered to represent recently activated T cells[10,12,52]. More recent studies indicate that blood CXCR5$^+$CD4$^+$ T cells contain long-lived memory cells that share functional properties with T_{FH} cells[53]. Accordingly, blood CXCR5$^+$CD4$^+$ T cells are currently termed blood (or peripheral) memory T_{FH} cells. Unlike GC T_{FH} cells, blood memory T_{FH} cells—even those expressing ICOS and the proliferation marker Ki67—do not express the Bcl-6 protein[54–58], indicating that Bcl-6 is dispensable for their maintenance. The molecular mechanisms by which blood memory T_{FH} cells maintain their T_{FH} characteristics remain largely unknown and thus represent an important research topic.

Human blood memory T_{FH} cells actually include several populations with unique phenotypes and functions (see ref. 53 for a detailed review). Although staining strategies and markers selected to define blood T_{FH} subsets differ among laboratories, we propose that blood memory T_{FH} cells can be assessed on the basis of the following three sets of parameters: the presence of the chemokine receptors CXCR3 and CCR6; of the immunoregulatory molecule PD-1 and the chemokine receptor CCR7; and of the costimulatory molecule ICOS (**Fig. 2a**).

The first set of parameters defines three major subsets: CXCR3$^+$CCR6$^-$ cells that share properties with T_H1 cells (hereinafter called blood memory $T_{FH}1$ cells), CXCR3$^-$CCR6$^-$ cells resembling T_H2 cells (hereinafter called blood memory $T_{FH}2$ cells) and CXCR3$^-$CCR6$^+$ cells resembling T_H17 cells (hereinafter called blood memory $T_{FH}17$ cells)[55]. Experiments in which isolated T cell subsets were cultured with B cells in the presence of superantigen (such as staphylococcus enterotoxin B to induce cognate interactions between T and B cells) indicated that blood memory $T_{FH}2$ and $T_{FH}17$ cells can induce naive B cells to produce immunoglobulins and to switch isotypes through IL-21 secretion. In contrast, blood memory $T_{FH}1$ cells lack the capacity to help naive B cells[55,59,60]. Furthermore, whereas blood memory $T_{FH}2$ cells promote IgG and IgE secretion, blood memory $T_{FH}17$ cells induce IgG and IgA secretion[55]. Thus, $T_{FH}2$ and $T_{FH}17$ cells represent efficient B cell helper cells with a distinct capacity to regulate immunoglobulin isotype switching.

The second (PD-1 and CCR7) and third (ICOS) sets of parameters define three other subsets: one activated subset (ICOS$^+$PD-1^{2+}CCR7^{lo}) and two quiescent subsets (ICOS$^-$PD-1$^+$CCR7^{int} and ICOS$^-$PD-1$^-$CCR7^{hi}). In healthy subjects ICOS expression is limited to a small population of blood T_{FH} cells; this population substantially increases after vaccination, and thus defines activated memory T_{FH} cells. In mice these cells appear before the formation of GC T_{FH} cells, because deletion of the *Sh2d1a* gene, encoding the SAP, an adaptor protein essential for the functions of SLAM family receptors, does not affect the formation of this circulating subset but prevents the appearance of terminally differentiated GC T_{FH} cells[58]. ICOS$^-$ cells are further divided into PD-1$^+$ cells (~30% of blood memory T_{FH} cells) and PD-1$^-$ cells, both of which lack the expression of Ki67 and thus are in a quiescent state[58,59]. CCR7 expression by blood memory T_{FH} cells negatively correlates with that of PD-1 (refs. 52,53).

The matrix combination of the three parameters defines nine blood memory T_{FH} subsets (**Fig. 2a**). The activated ICOS$^+$PD-1^{2+}CCR7^{lo} populations within blood memory $T_{FH}2$ and $T_{FH}17$ cells might represent the most efficient helpers. Among quiescent $T_{FH}2$ and $T_{FH}17$ subsets, the ICOS$^-$PD-1$^+$CCR7^{int} population provides help to memory B cells more promptly than the ICOS$^-$PD-1$^-$CCR7^{hi} population[59,60], but the ICOS$^-$PD-1$^+$CCR7^{int} and ICOS$^-$PD-1$^-$CCR7^{hi} $T_{FH}2$ and $T_{FH}17$ subsets are equally capable of helping naive B cells.

In contrast, blood memory $T_{FH}1$ cells lack the capacity to help naive or memory B cells[54,55,59,60]. Therefore, whether blood memory $T_{FH}1$ cells represent a subset of memory T_{FH} cells remains controversial. First, upon T cell receptor stimulation, blood memory $T_{FH}1$ cells produce CXCL13, a chemokine that is highly expressed by GC T_{FH} cells and whose receptor, CXCR5, is expressed at high levels by B cells[18,61]. Second, blood memory $T_{FH}1$ cells contain antigen-specific memory cells[59]. Third, upon polyclonal stimulation with the phorbol ester PMA and ionomycin, blood memory $T_{FH}1$ cells produce IL-21 in amounts equivalent to those of blood memory $T_{FH}2$ and $T_{FH}17$ cells[59]. Finally, influenza vaccination transiently induces ICOS expression exclusively on blood memory $T_{FH}1$ cells[54]. The increase in ICOS$^+$ $T_{FH}1$ cells in blood is positively correlated with the generation

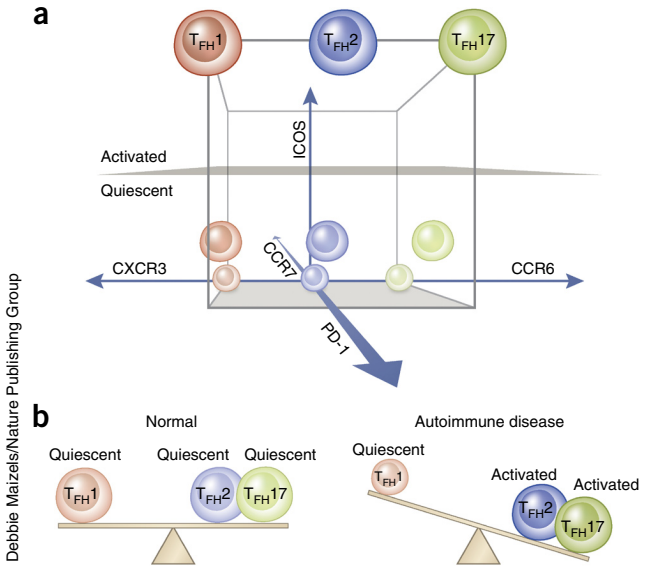

Figure 2 Alteration of blood memory T_{FH} subsets in human autoimmune diseases. (**a**) Three parameters—CXCR3 versus CCR6, PD-1 and CCR7, and ICOS—can subdivide human blood memory T_{FH} cells ($CD4^+CD45RA^-CXCR5^+$) into at least three $T_{FH}1$, $T_{FH}2$ and $T_{FH}17$ subsets (nine T_{FH} subsets in total). PD-1 and CCR7 define two quiescent subpopulations, PD-1^-CCR7^{hi} and PD-1^+CCR7^{int}, and ICOS defines the ICOS$^+$PD-1^{2+} activated population within the blood memory $T_{FH}1$, $T_{FH}2$ and $T_{FH}17$ subsets. The nine blood memory T_{FH} subsets are indicated in a three-dimensional scale. (**b**) $T_{FH}2$ and $T_{FH}17$ subsets represent efficient helpers among blood memory T_{FH} subsets. In the blood of patients with active autoimmune disease (such as SLE, juvenile dermatomyositis, Sjögren's syndrome or multiple sclerosis), the frequency of active (ICOS$^+$PD-1^{2+}) $T_{FH}2$ and $T_{FH}17$ subsets increases, whereas the frequency of $T_{FH}1$ subsets decreases. This alteration likely reflects the increase of functional T_{FH} cells in lymphoid organs and/or inflamed tissues.

of protective antibody responses[54]. Mechanistically, ICOS$^+$ blood memory $T_{FH}1$ cells help memory, but not naive, B cell differentiation into plasma cells via secretion of IL-21 and IL-10[54]. This observation suggests that blood memory $T_{FH}1$ cells also contribute to antibody responses, but only when they become ICOS$^+$PD-1^{2+}CCR7^{lo} activated cells. Collectively, blood memory T_{FH} cells can be subdivided into nonefficient helpers ($T_{FH}1$) and efficient helpers ($T_{FH}2$ and $T_{FH}17$). The differential expression of ICOS, PD-1 and CCR7 further defines developmentally and functionally distinct subpopulations within the subsets.

T_{FH} cells in primary immunodeficiencies

Primary immunodeficiencies are caused by defects in the expression of molecules involved in immune system development and/or function. Analyses of lymphoid tissue and blood samples from patients with primary immunodeficiencies have helped clarify the requirements for the development of normal immune responses in humans, including the generation of T_{FH} cells and GCs. With the success of whole exome sequencing in identifying gene variants responsible for primary immunodeficiencies (at least 34 new gene defects were identified in the past 4 years[62]), investigation of immune responses in affected patients is likely to accelerate understanding of human immunity in the near future.

Patients with ICOS deficiency suffer from common variable immunodeficiency and show severely impaired GC formation in lymphoid tissues and severely decreased blood memory T_{FH} cells, accompanied by a severe deficiency of memory B cells[63]. This demonstrates that ICOS is essential for the generation of T_{FH} and GC responses in humans, as shown in mice[64].

Multiple mouse models show that B cells play a fundamental role in the generation of GC T_{FH} cells by interacting with T_{FH} precursors[25]. This is also the case in humans, as patients with X-linked agammaglobulinemia, who lack mature B cells because of a deficiency of the tyrosine kinase Btk, and those with CVID, who have a significantly reduced number of B cells (<2% in lymphocytes), display significantly reduced frequencies of blood memory T_{FH} cells[65]. Patients with hyper IgM syndrome caused by a deficiency of functional CD40L also show severely impaired GC formation, as well as reduced blood memory T_{FH} cells[63] and memory B cells[66], which indicates the importance of CD40-CD40L interactions for GC and T_{FH} development in humans, as shown in mice[67].

SAP-deficient mice show profoundly altered GC reactions and generation of T_{FH} cells[68], as SAP is required for durable interactions between helper T cells and B cells, a process essential for the maturation of T_{FH} cells[69]. Consistently, patients with X-linked lymphoproliferative disease caused by SAP deficiency show impaired formation of GCs and impaired development of memory B cells[70]. Interestingly, both SAP-deficient mice and human patients with X-linked lymphoproliferative disease show normal frequencies of blood memory T_{FH} cells[58], suggesting that at least a subset of circulating blood T_{FH} cells develop before GC T_{FH} formation. Furthermore, migration of GC T_{FH} cells into the circulation is limited during the acute phase of the GC response[36,38]. It remains possible that GC T_{FH} cells and their descendent memory T_{FH} cells in lymphoid organs[39,40] might start circulating during the termination of the GC response.

In vitro studies have suggested that IL-12 promotes antibody responses in humans via two mechanisms. IL-12 produced by DCs ($CD14^+$ dermal DCs in human skin[71]) can induce the *in vitro* differentiation of CD40-activated naive B cells into IgM-producing plasma cells[72,73] and of naive $CD4^+$ T cells into T_{FH}-like cells[74,75]. Consistently, children deficient for the IL-12 receptor β1 (IL-12Rβ1) chain, which is shared by the IL-12 and IL-23 receptors, have reduced T_{FH} and GC responses[74]. Thus, in addition to the recent *in vitro* observation that TGF-β acts as a critical co-factor of IL-12 and IL-23 for the early T_{FH} cell differentiation process in humans[46], impaired T_{FH} response in IL-12Rβ1 deficiency *in vivo* provides evidence that IL-12 and IL-23 contribute to the development of T_{FH} responses in humans. Although the frequency of blood memory T_{FH} cells is significantly reduced in subjects with IL-12Rβ1 deficiency during childhood (less than 10 years old), it gradually increases with age and becomes normal in adults. This indicates that the development and/or maintenance of the T_{FH} cell response can be compensated through other pathways and/or cytokines, possibly IL-6, IL-21 and IL-27.

Mutations in the intracellular signaling molecule STAT3 cause most cases of hyper IgE syndrome. People with this syndrome are susceptible to a narrow spectrum of infections linked to defective T_H17 responses and show altered antibody responses with reduced blood memory T_{FH} cells[76,77], indicating that STAT3 is required for optimal T_{FH} cell generation in humans. Given that B cell responses to IL-10 and IL-21, cytokines delivering activation signals via STAT3, are severely altered in STAT3-deficient subjects[78], the reduced number of blood memory T_{FH} cells in STAT3-mutant subjects might be dependent on both B cell–intrinsic and T cell–intrinsic defects. Notably, upon exposure to IL-12, human STAT3 mutant naive $CD4^+$ T cells are induced to express high amounts of several T_{FH} molecules, such as CXCR5, ICOS and Bcl-6, despite lower expression of IL-21 (ref. 76). Thus, other molecules such as STAT4 might functionally compensate

for the lack of STAT3. This concept is supported by the finding that STAT3 and STAT4 play largely redundant roles in the expression of T_{FH} molecules by human naive $CD4^+$ T cells exposed to TGF-β[46].

T_{FH} cells and autoimmunity

The generation of autoantibodies is a hallmark of autoimmune disease. Autoantibodies target a broad range of self-antigens, including nuclear components (such as double-stranded DNA), organ-specific antigens and soluble factors. These autoantibodies can profoundly dysregulate the function of multiple organs or systems through a variety of mechanisms. Autoantibodies result from a breakdown of tolerance mechanisms during B cell development[79], from T cell–dependent or –independent B cell activation, or as a consequence of somatic mutation and rogue selection within GCs[80,81]. Yet GCs can in some cases redeem self-reactive B cells[82]. There is now compelling evidence in mice and in humans that aberrant generation and/or activation of T_{FH} cells and extrafollicular helper T cells contributes to the pathogenesis of autoimmune diseases. Here we first describe evidence of aberrant T_{FH} responses in mouse and human autoimmunity, and then discuss how genetic factors are potentially associated with aberrant T_{FH} responses in human autoimmune diseases.

T_{FH} cells in autoimmune mouse models

The first evidence linking aberrant T_{FH} responses and autoimmunity came from studies of the *sanroque* mouse model, which carries a single-amino-acid mutation known as 'san' in the RNA-binding protein Roquin-1 (ref. 16). *Roquin*$^{san/san}$ (or *Rc3h1*$^{san/san}$) mice spontaneously develop lupus-like clinical symptoms and, in a genetically susceptible background, type 1 diabetes (T1D). The development of these diseases is accompanied by high amounts of antinuclear and anti-islet antibodies[16,83]. Mechanistically, the mutated Roquin in *Roquin*$^{san/san}$ mice displays an impaired ability to repress the expression of *Icos*[16] and *Ifng*[84] in activated helper T cells and promotes the generation of T_{FH} cells. *Ox40* (also known as *Tnfrsf4*), a co-stimulatory molecule highly expressed by T_{FH} cells[25], and *Tnf* are additional targets of Roquin and its paralog Roquin-2 (refs. 85–87). T_{FH} cells and myeloid cells accumulate and are overactive in *Roquin*$^{san/san}$ mice and in mice doubly deficient in Roquin and Roquin-2 (refs. 86,87). Genetic ablation of *Sh2d1a* (encoding SAP) in *Roquin*$^{san/san}$ mice prevents lupus pathology, whereas transfer of *sanroque* T_{FH} cells into wild-type mice promotes spontaneous GC formation, supporting a causal role for T_{FH} cell accumulation in the lupus-like disease[88]. These findings suggest that excessive T_{FH} cells in GCs corrupt positive selection, such as diminished competition for T cell help, and a lower threshold for selection allows the emergence of self-reactive clones. Excessive expression of ICOS[89] and, more prominently, of IFN-γ[84] was found to be an important contributor to *Roquin*^{san}-mediated T_{FH} cell accumulation. Of note, IFN-γ signaling blockade has been found not only to alleviate T_{FH} and GC B cell accumulation but also to virtually prevent all clinical manifestations associated with *Roquin*^{san}-mediated disease, unlike SAP deficiency, which did not correct the splenomegaly or hypergammaglobulinemia[88], or ICOS deficiency, which did not eliminate autoantibody formation[84]. Other mouse models have also revealed additional mechanisms that cause aberrant T_{FH} responses in autoimmune disease. In the BXSB-*Yaa* mouse model, which displays a duplication of the *Tlr7* gene, aberrant T_{FH} responses and the development of glomerulonephritis are completely dependent on IL-21 signals[90], in contrast to what occurs in *Roquin*$^{san/san}$ mice, in which IL-21 signals are not essential for pathology[91]. The generation of autoantibodies in the MRL/*lpr* lupus mouse model, which is characterized by deficiency of the proapoptotic molecule Fas, is dependent on the T and B cell interactions at extrafollicular sites[31]. Extrafollicular T_H cells that develop in MRL/*lpr* mice share features with GC T_{FH} cells: their development is dependent on ICOS and Bcl-6, and their helper function is dependent on IL-21 and CD40L[31]. These studies demonstrate that inhibiting the generation of pathogenic T_{FH} and extrafollicular helper T cells by blocking ICOS, CD40L, IFN-γ or IL-21 ameliorates the generation of autoantibodies and/or the development of glomerulonephritis, thereby providing a rationale for targeting these molecules for therapeutics.

Although T1D has long been thought to be a T cell–driven and organ-specific autoimmune disease, there is now evidence for a pathogenic role for both B cells and antibodies, as well as for T_{FH} cell dysregulation, in T1D etiopathogenesis. B cells are required for diabetes development in the nonobese diabetic (NOD) mouse model of T1D[92–94] and in the *Roquin*$^{san/san}$ model[83]. Direct evidence for a pathogenic role of autoantibodies comes from experiments demonstrating protection from diabetes in NOD mice made deficient for activating Fcγ receptors[95,96] and, conversely, diabetes induction upon passive transfer of antibodies against islet-expressed neo-self-antigens[83,96]. In line with these observations, it is not surprising that correlations are being described between T_{FH} cells and autoimmune diabetes. In the *Roquin*$^{san/san}$ model, aberrant T_{FH} response was directly linked with the development of high anti-islet autoantibody titers and T1D[83]. T_{FH} cells were increased in the pancreatic draining lymph nodes of mice that developed autoimmune diabetes[83], and cells sharing characteristics of T_{FH} cells, but expressing CCR9 instead of CXCR5, have been found in the pancreas of diabetic-prone NOD mice[97].

T_{FH} cells in human autoimmune diseases

Increased GC response in patients with systemic lupus erythematosus (SLE) was suggested in early studies by an increased frequency of $CD27^+CD38^{hi}$ somatically mutated antibody-producing plasmablasts in peripheral blood[98,99]. Because SLE patients display higher frequencies of self-reactive mature naive B cells than healthy controls owing to a defect in the early checkpoint of B cell repertoires[100,101], SLE patients seem predisposed to the development of a broader range of autoantibodies than healthy subjects. Whether and how T_{FH} cells contribute to the pathogenesis of human autoimmunity has been unclear, but recent progress in understanding the biology of blood memory T_{FH} cells has rendered the analysis of human T_{FH} responses in the context of autoimmunity feasible.

Since the description of an association between the frequency of $CXCR5^+ICOS^+$ and/or $CXCR5^+PD1^+$ circulating T_{FH} cells and the severity of SLE and Sjögren's syndrome[57], multiple studies have confirmed[102] or refined these findings, providing improved phenotypic characterization (for example, a reproducible association with the $CCR7^{lo}PD\text{-}1^{+/hi}$ subset[58]), and extended them to additional autoimmune diseases such as myasthenia gravis[103], rheumatoid arthritis[104,105], autoimmune thyroid diseases[106] and T1D[107]. An increase of $ICOS^+$ blood memory T_{FH} cells showed a positive correlation with serum autoantibody titers and disease activity and/or severity in these diseases[57,58,104,106–108]. These observations suggest that patients with active autoimmune disease display aberrant T_{FH} responses; this can be monitored through assessment of the increase of activated blood memory T_{FH} cells.

Accumulating evidence also indicates that patients with autoimmune disease display an alteration in the balance of blood memory $T_{FH}1$, $T_{FH}2$ and $T_{FH}17$ cells. In patients with juvenile dermatomyositis[55], adult SLE[109] and Sjögren's syndrome[102], $T_{FH}1$ cells are underrepresented among blood memory T_{FH} cells, whereas $T_{FH}2$ and/or $T_{FH}17$ cells are overrepresented. Such alterations were found

to correlate with disease activity, serum autoantibody titers and/or the frequency of blood plasmablasts[55,102,109]. Furthermore, although multiple sclerosis (MS) is not generally considered an autoantibody-mediated autoimmune disease, patients with MS also show the same alteration (low $T_{FH}1$ and high $T_{FH}17$) in the composition of blood memory T_{FH} subsets[110]. Two additional lines of evidence also support a pathogenic role of T_{FH} cells in MS. First, B cells are now thought to play a major pathogenic role in MS, as depletion of B cells with anti-CD20 significantly reduces the number of brain inflammatory lesions and halts the development of new lesions[111]. Second, ectopic B cell follicles are formed in the brain lesions of more than 40% of patients with secondary progressive MS, and the development of these structures correlates with disease severity[112].

Collectively, although definitive evidence is yet to be found, these observations suggest that an increase in activated $T_{FH}2$ and/or $T_{FH}17$ cell subsets and a decrease of $T_{FH}1$ cells within blood memory T_{FH} cells might be common across multiple autoimmune diseases (**Fig. 2b**). Such alterations in blood memory T_{FH} cells might reflect an overall increase of efficient helpers that promote the generation of antibodies in lymphoid organs and/or inflammatory sites in patients with autoimmune diseases. However, to date, studies linking human blood memory T_{FH} cells and T_{FH} cells in lymphoid organs and/or ectopic GCs in inflammatory tissues are lacking. T_{FH} cell responses in ectopic GCs might be of considerable importance, as discussed below.

T_{FH} cells in inflamed tissues

Inflammatory sites in autoimmune diseases often develop lymphoid cell aggregations including helper T cells and B cells, which leads to the formation of well-structured GCs (hereinafter called ectopic GCs). The mechanisms that control the initial development, cellular composition and functional maintenance of ectopic GCs seem to be largely shared with GCs in lymphoid organs[113]. For example, in lupus tubulointerstitial nephritis lesions, T_H cells found in lymphoid T and B cell aggregates are phenotypically similar to T_{FH} cells in lymphoid organs[114]. Although the precise mechanisms by which T_{FH} cells accumulate in inflammatory sites in humans remain largely unknown, studies in mouse models suggest the involvement of T_H17 cells[113]. In the experimental autoimmune encephalomyelitis mouse model, T_H17 cells induce the formation of ectopic lymphoid follicles in inflammatory brain lesions via IL-17 and the cell surface molecule podoplanin and show features of T_{FH} cells in these tertiary lymphoid structures[115]. Given that human patients with autoimmune diseases have increased activated memory $T_{FH}17$ cells in blood (as discussed earlier), that early developmental pathways for T_{FH} and T_H17 cells in inflammatory environments are shared in humans[46], and that in mice T_H17-derived IL-17 directly promotes B cell differentiation into GC B cells, which are thought to be the source of pathogenic autoantibodies[116], it is tempting to speculate that $T_{FH}17$ cells might be involved in the formation of ectopic GCs in human autoimmune diseases.

Among patients with autoimmune disease, those with ectopic GCs in inflammatory lesions often have higher disease activity and are refractory to treatment. Yet the formation of ectopic GCs does not simply reflect the extent or the duration of inflammation in the lesions, as only 20% to 40% of patients with chronic inflammation develop ectopic GCs[113]. The formation of ectopic GCs is preceded by aggregates of T and B cells in inflammatory sites. Therefore, at variance with B cell follicles in lymphoid tissues, where autoreactive B cells are excluded because of their reduced expression of CXCR5 (ref. 117), lymphoid aggregates in inflammatory tissues are likely to be permissive to the entry of autoreactive B cells. Encounters with T_{FH}-like cells at these sites[114] might induce their cell growth and/or differentiation into antibody-producing cells. This possibility is directly supported by the observation that B cells in lymphoid aggregates and/or ectopic GCs produce autoantibodies[113]. Moreover, organ-specific self-antigens are easily accessible by antigen-presenting cells, including B cells, because of the abundance of damaged and/or apoptotic cells caused by the inflammatory process. Therefore, ectopic GCs might represent a major site of autoantibody production in autoimmune diseases, and thus a potentially good target for therapeutics. The nature of T_{FH} cells in inflamed tissues requires further studies. Questions regarding subjects such as the developmental mechanism of T_{FH} cells at inflammatory lesions and how lymphoid aggregates develop into ectopic GCs can be addressed in mouse models, but an effort to determine whether the observations apply to humans will be required. Another important question is whether activated blood memory T_{FH} cells present in active autoimmune disease patients originate from inflammatory lesions. It will be critical to answer these questions in order to directly link the information obtained from the analysis of blood T_{FH} subsets with autoimmune disease pathogenesis.

Genetics of altered T_{FH} cell response

Autoimmune diseases result from complex interplay between genetic and environmental factors. Genome-wide association studies (GWAS) on multiple autoimmune diseases have yielded risk-associated loci[118] (summary at http://www.genome.gov/gwastudies). Variants in HLA class II alleles are at the top of the list of susceptibility loci in most autoantibody-mediated autoimmune diseases, supporting a requirement of $CD4^+$ helper T cells in disease pathogenesis. Importantly, multiple risk-loci identified in autoimmune diseases are potentially associated with the regulation of the development and/or the function of human T_{FH} cells (**Fig. 3**). The list includes genes associated with IL-12 and IL-23, as well as cytokines implicated in human T_{FH} cell differentiation[44,46,74,75], such as *IL12A*, *IL12B* (both in multiple sclerosis), *IL23R* (rheumatoid arthritis), *STAT3* (multiple sclerosis), *STAT4* (SLE, rheumatoid arthritis, Sjögren's syndrome), *IRF5* (SLE, rheumatoid arthritis) and *IRF8* (SLE, rheumatoid arthritis, multiple sclerosis). The transcription factors IRF5 and IRF8 positively regulate the production of IL-12 and IL-23 by macrophages and DCs[119,120]. IRF8 is essential for IL-12 production in humans. IRF8-deficient human subjects experience disseminated infection caused by bacille Calmette-Guérin vaccines, a clinical manifestation also seen in IL-12 receptor deficiency, due to a profound reduction of IL-12 production by monocytes[119]. IRF8 also contributes to signaling in TGF-β, another cytokine important for the generation of human T_{FH} cells[46], by promoting the expression of TGF-β-activating integrin $\alpha_v\beta_8$ on the surface of DCs[121]. Studies in SLE and Sjögren's syndrome suggest that the OX40 ligand (OX40L)-coding *TNFSF4* might be involved in the generation and/or homeostasis of T_{FH} cells: overexpression of OX40L causes the accumulation of T cells in B cell follicles[122], and OX40 receptor signals induce $CD4^+$ T cells to express CXCR5 in both mice[26,123,124] and humans (unpublished observations). Furthermore, GWAS identified multiple risk-loci encoding molecules expressed by T_{FH} cells, including *IL21* (SLE, rheumatoid arthritis) and *CXCR5* (multiple sclerosis, Sjögren's syndrome). A recent study showed that in addition to Blimp-1-encoding *PRDM1* (ref. 22), *PTPN22*, a risk locus identified in GWAS of SLE, rheumatoid arthritis, myasthenia gravis and T1D, negatively regulates the generation of T_{FH} and GC responses in mice[125]. However, whether and how most of these gene variants contribute to aberrant T_{FH} responses in autoimmune diseases remains unknown. The majority of risk loci consist of single-nucleotide polymorphisms within non-coding, putatively regulatory DNA in the proximity of genes[126],

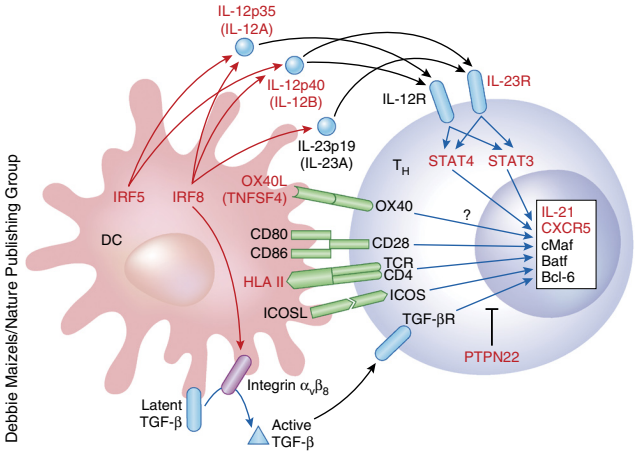

Figure 3 Risk loci of human autoimmune diseases associated with the T_{FH} developmental pathway. Multiple risk loci identified in GWAS in autoimmune diseases (indicated in red) are potentially associated with the regulation of the development and/or the function of human T_{FH} cells. At least seven risk loci—*IL12A*, *IL12B*, *IL23R*, *STAT3*, *STAT4*, *IRF5* and *IRF8*—are associated with IL-12 and IL-23. IRF8 also might contribute to TGF-β signaling by promoting the expression of TGF-β-activating integrin $\alpha_v\beta_8$ on the surface of DCs. Risk loci contain genes encoding T_{FH}-specific molecules (such as *IL21* and *CXCR5*), as well as genes associated with the inhibition of T_{FH} cell development (such as *PRDM1* and *PTPN22*). Whether and how these gene variants are associated with aberrant T_{FH} responses in autoimmune diseases remains to be established.

suggesting either that there is a disease-causing coding mutation in linkage disequilibrium with the GWAS-identified single-nucleotide polymorphism, or that alteration in pre- and/or post-transcriptional regulation is central to dysregulated immune responses in subjects with autoimmune disease traits. Recent studies aimed at mapping genetic variation contributing to transcriptional variation (termed expression quantitative trait locus mapping studies) through the use of purified immune cell subsets have started to reveal how gene variants regulate immune responses in different cell types[126–128]. The integration of expression quantitative trait locus mapping with blood memory T_{FH} cells and/or T_{FH} cells within inflammatory tissues and antigen-presenting cells obtained from patients with autoimmune disease might reveal how gene variants identified in GWAS contribute to the aberrant T_{FH} and GC responses.

T_{FH} cells in cancer

Given the demonstrated mutual dependence of T and B cells for growth and survival, it is not surprising that T_{FH} cells play important roles in supporting the growth and survival of follicular B cell tumors. Furthermore, T_{FH} cells themselves can give rise to a peripheral T cell tumor known as angioimmunoblastic T cell lymphoma (AITL). Other peripheral T cell tumors also present with phenotypic features and genetic abnormalities that suggest a T_{FH} origin. Recently, T_{FH} cells were found to infiltrate solid-organ tumors, where they might play both protective and pathogenic roles.

AITL, which accounts for ~20% of peripheral T cell lymphomas (PTCLs), is an aggressive tumor associated with a poor survival rate (33% 5-year survival)[129]. Most patients present with systemic disease associated with lymphadenopathy, hepatosplenomegaly, anemia and hypergammaglobulinemia and suffer from systemic illness. The neoplastic T cells account for only a small fraction of the lymphoid infiltrate and are mixed with a large number of reactive immune cell types, including small lymphocytes, eosinophils and plasma cells, and expansion of follicular dendritic cell networks. Genetic profiling of AITL and immunohistochemical analysis of neoplastic T cells have provided strong evidence that T_{FH} cells are the normal cellular counterpart of the neoplastic cells in AITL, which typically express BCL6, CD10 and other T_{FH} markers, including CXCL13 and PD-1 (ref. 130). In mice, heterozygosity for a *Roquin-1* mutation that causes T cell–autonomous T_{FH} expansion also leads to an AITL-like disease[131]. These mutations have not been found in humans, but mutations in *TET2*, *IDH2*, *DNMT3A* and *RHOA* are commonly found in AITL[132–134]. Although the first three genes are known to play a role in DNA methylation and epigenetic modification of gene expression, how these mutations selectively affect T_{FH} cells remains to be understood.

Up to 40% of other PTCLs (PTCLs not otherwise specified (NOS)) also express T_{FH} markers, and some share genetic mutations typically found in AITL, including in *TET2*, *IDH2* and *RHOA*. For this reason, these tumors are now being referred to as T_{FH}-like PTCL-NOS[135]. The neoplastic T cells of various types of primary cutaneous cell lymphomas also express various T_{FH} cell markers, with the exception of CD10, and in some cases also form rosettes with B cells, which typically occurs in AITL[136–139].

Evidence for a pathogenic role of T_{FH} cells in B cell lymphoma first came from studies demonstrating that the number of T cells infiltrating B cell tumors was an important predictor of outcome[140]. Follicular lymphoma, the most frequent indolent non-Hodgkin lymphoma, is thought to originate from GC B cells[141]. The gene expression profiles of tumor-infiltrating T cells and myeloid cells, rather than that of malignant B cells, determine the prognosis for follicular lymphoma[142]. Furthermore, that prognosis worsens if T cells are localized within neoplastic follicles[143]. Tumor-infiltrating T cells have the phenotypic features of T_{FH} cells and overexpress IL-4 (ref. 144), TNF, IFN-γ and LT-α[145]. In particular, IL-4 produced by tumor-infiltrating T_{FH}-like cells causes follicular lymphoma tumor cells to secrete the chemokines CCL17 and CCL22, which attract the migration of T_{reg} cells and T_H2 cells[145]. $Foxp3^+$ T cells that resemble T_{FR} cells described in mice are also present in neoplastic follicles and are expanded during lymphomagenesis[146], although their significance is still unclear. While overall numbers of tumor-infiltrating $Foxp3^+$ T cells may be associated with improved survival[147], their follicular localization may be associated with worse survival and increased risk of transformation[148], perhaps through the dampening of the cytotoxic T lymphocyte–driven anti-tumor response[149]. Recent reports have described increased circulating T_{FH}-like cells in patients with chronic lymphocytic leukemia[150,151], particularly during the more advanced stages. These expanded T_{FH} cells may be of pathological relevance, given that the combination of IL-21 and CD40L induces robust chronic lymphocytic leukemia cell proliferation[150,152].

In nonlymphoid malignancies, T_{FH} cells are a component of the inflammatory infiltrate of breast cancer, and their presence, as assessed by an eight–T_{FH} gene signature, was associated with increased survival[153]. Moreover, a single T_{FH}-associated gene, CXCL13, conferred the dominant prognostic value. Extensively infiltrated tumors were found to contain tertiary lymphoid structures with visible GCs and CXCL13-producing T_{FH} cells. These findings suggest that T_{FH} cells, by virtue of their ability to secrete CXCL13 and organize ectopic lymphoid filtrates, may be important in coordinating the recruitment of immune cells that mediate the anti-tumor response.

In other tumor types, a high proportion and altered distribution of T_{FH} cells has been reported in colorectal cancer[154] and non–small cell lung cancer[155]. T_{FH} cells infiltrate thymomas, correlating with the severity of myasthenia gravis[156]. A recent comprehensive gene

analysis study on various immune cells isolated from human colorectal tumors revealed that the infiltration of B cells correlates with the infiltration of T_{FH} cells at tumor sites[157] and that the infiltration of B cells and T_{FH} cells, which is associated with the expression of *CXCL13* and *IL21*, positively correlates with patient survival. Whether and how T_{FH} cells might promote the development of colorectal tumors remains to be established.

T_{FH} cells in HIV and vaccine design

In the past couple of years an intriguing relationship was revealed between T_{FH} cells and both human and simian immunodeficiency virus (HIV and SIV, respectively). HIV eliminates the circulating memory $CD4^+$ T cells it infects, but several groups showed that T_{FH} cells accumulate in lymph nodes during chronic SIV[158,159] and HIV[160–163] infection. Furthermore, T_{FH} cells expand, despite old[164–166] and new[158,160] evidence that HIV infects T_{FH} cells, in which viral replication occurs. Reports from the late 1980s demonstrated that follicular dendritic cell networks in GCs represent a large reservoir of HIV virions[167], retained as immune complexes[163]. Thus, T_{FH} cells are constantly exposed to the virus during chronic infection. In contrast to the T_{FH} cell accumulation seen in lymph nodes, blood memory PD-1^+ $T_{FH}17$ cells capable of providing help to B cells are decreased in chronic HIV infection, but they recover after antiretroviral therapy[60].

The relationships among viral load, chronicity and numbers of T_{FH} cells are complex. Although most studies have failed to identify a correlation between lymph node T_{FH} cell numbers and viral load[158,159,161], there are possible explanations for the reduction in T_{FH} cells after antiretroviral therapy[160,162]: the antigen load might control the total number of T_{FH} cells, the compartmentalization of T_{FH} cells between secondary lymphoid tissues and the circulation, or the survival of the blood memory T_{FH} cells. Further work is clearly needed to explain the mechanisms behind these findings.

Chronic HIV infection is associated with an increased frequency of T_{FH} cells in lymph nodes together with increased expression of Bcl-6 (ref. 158). The expression of IL-6 receptor on T_{FH} cells is increased, which might mediate increased responsiveness to IL-6 (ref. 158). The importance of IL-6 signals for the maintenance of the T_{FH} response in chronic infection is also highlighted in a mouse model of chronic viral infection with LCMV clone 13 (ref. 168). Increased T_{FH} generation during chronic HIV infection may in turn affect the host's immune response not only against HIV, but also against other, unrelated viral and bacterial infections[169]. High T_{FH} numbers in HIV-infected individuals correlate with B cell dysregulation, including hypergammaglobulinemia, loss of memory B cells and, occasionally, production of autoantibodies and development of autoimmunity[170]. It is possible that the lowered threshold of B cell selection in GCs due to an excess of T_{FH} cells[171] might lead to the generation of antibodies with low affinities. Alternatively, T_{FH} cell function might be dampened by either T cell–intrinsic factors (e.g., increased sensitivity to IL-6 signaling)[158] or the microenvironment (e.g., increased PD-L1 expression by GC B cells that dampens IL-21 production by T_{FH} cells[162]).

Designing potent HIV vaccines remains a major challenge. It is now established that antibodies can be protective and prevent infection, as passive transfer of broadly neutralizing antibodies confers protection to HIV challenge in humanized mice infected with HIV[172,173]. However, most vaccine trials have failed to protect vaccinated individuals to any significant degree. A number of factors associated with HIV make the creation of a decently protective antibody-based vaccine difficult, such as fast mutation rates, structural properties of the envelope complex that make conserved epitopes relatively inaccessible to antibodies, and the need for B cells to undergo extensive somatic mutation to generate broadly neutralizing antibodies. The recent HIV vaccine RV144 "Thai" trial showed that binding of IgG antibodies to envelope proteins is associated with protection, whereas binding of IgA antibodies to envelope proteins correlates directly with the rate of infection[174]. These data suggest a need to better understand the nature and isotype of the antibody response to be elicited by vaccines for protection. There is encouraging evidence that numbers of blood $ICOS^-PD1^+$ $T_{FH}2$ and $T_{FH}17$ cells (but not $T_{FH}1$ cells) may serve as a useful biomarker for patients producing broadly neutralizing antibodies to HIV[175]. However, the significant overlap in measurements between high- and low-affinity groups calls for additional studies to further delineate the best correlates of protection. Although numerous studies have concluded that a potent T_{FH} response correlates with antibody titers and protective responses[54,58,59], there is also evidence that an excessive response might be deleterious. Thus, limiting of T_{FH} cells is required for optimal affinity maturation[176], and an excessive number of T_{FH} cells lowers the threshold for positive selection, allowing survival of low-affinity and self-reactive clones[88]. Furthermore, the balance between T_{FH} and T_{FR} cells is also likely to be important for the overall duration and quality control of GC responses[34]. So far, very little is known about how different adjuvants and prime-boost regime strategies influence the magnitude, longevity and quality of antibody responses. This knowledge will be important for the improved and rational design of protective vaccines.

Conclusions

In the past 5 years considerable progress has been made in the understanding of T_{FH} cells, particularly in humans. We predict that this progress will lead to improved vaccine designs, better management of autoimmune diseases and novel prognostic biomarkers for lymphoid and solid tumors.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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