

## Follicular helper T lymphocytes in health and disease

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### Abstract

A correct antibody response requires the participation of both B and T lymphocytes and antigen presenting cells. In this review we address the role of follicular helper T lymphocytes (T<sub>FH</sub>) in this reaction. We shall focus on the regulation of their development and function in health and disease. T<sub>FH</sub> can be characterized on the basis of their phenotype and the pattern of secretion of cytokines. This fact is useful to study their participation in the generation of antibody deficiency in primary immunodeficiency diseases such as common variable immunodeficiency, X-linked hyper IgM syndrome or

X-linked lymphoproliferative disease. Increased numbers of T<sub>FH</sub> have been demonstrated in several autoimmune diseases and are thought to play a role in the development of autoantibodies. In chronic viral infections caused by the human immunodeficiency virus, hepatitis B or C virus, increased circulating T<sub>FH</sub> have been observed, but their role in the protective immune response to these agents is under discussion. Likewise, an important role of T<sub>FH</sub> in the control of some experimental protozoan infections has been proposed, and it will be important to assess their relevance in order to design effective vaccination strategies.

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**Key words:** Follicular helper T (T<sub>FH</sub>) lymphocytes; T<sub>FH</sub> development; Chemokine (C-X-C motif) receptor 5; Interleukin-21; Programmed cell death-1/Programmed cell death ligand 1 (PDL-1) or PDL-2; Primary immunodeficiencies; Autoimmunity; Chronic viral infections; Protozoan infections

**Core tip:** Follicular helper T lymphocytes (T<sub>FH</sub>) are essential to establish a correct and protective humoral immune response. Correct regulation of their development and differentiation is necessary to achieve a normal antibody response. They can be characterized by their phenotype and function. It has been proposed that their role is important in the generation of immunodeficiency or autoimmunity, as well as in the control of chronic viral or protozoan infections. This review comments recent advances in human T<sub>FH</sub> research that may be useful in order to design adequate therapeutic or vaccination strategies.

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## INTRODUCTION

The assembly of a correct antibody response requires the participation of B and T lymphocytes, as well as that of antigen presenting cells from the myeloid lineage. It involves a complex system of interactions and regulatory mechanisms. Failure of this equilibrium at any level disturbs and impairs the generation of an efficient, long term antibody response.

A subset of helper T cells, follicular helper T lymphocytes (T<sub>FH</sub>) is necessary to provide help to B lymphocytes in the process of antibody synthesis and maturation. T<sub>FH</sub> encompass a heterogeneous group of cells with distinct gene expression profile and function<sup>[1]</sup>. Without T<sub>FH</sub> the protective antibody responses are largely diminished. Primary immune deficient patients with genetic defects that affect the synthesis of molecules essential for T<sub>FH</sub> generation or function, such as the inducible co-stimulator (ICOS) or the signaling adaptor SLAM-associated protein (SAP), lack an efficient antibody response and may suffer recurrent infections that compromise their health and survival<sup>[2,3]</sup>. Excessive or dysregulated T<sub>FH</sub> can also result in the generation of autoantibodies and are associated to autoimmune diseases<sup>[4,5]</sup>.

In this review we shall describe the nature and function of this T cell subset and we will focus on its role in the generation of immune deficiency or autoimmunity in humans. We will also address the importance of T<sub>FH</sub> in the assembly of an efficient humoral response for the control of chronic diseases caused by different infectious viral agents, *e.g.*, human immunodeficiency virus (HIV), hepatitis B virus (HBV) or C virus (HCV), as well as parasites or protozoa.

## T<sub>FH</sub> PHENOTYPE AND FUNCTION

CD4<sup>+</sup> T helper (Th) cells present in B cell follicles have been recognized as an important subset of helper T lymphocytes necessary for the assembly of the antibody response involving T-B cooperation and B cell memory<sup>[1,6,7]</sup>. T<sub>FH</sub> have a typical phenotype, appropriate transcription factors and exhibit surface molecules essential for helper function. They secrete interleukins (ILs) that promote growth, differentiation and class switching of B cells (IL-4, IL-10 and IL-21). Plasticity is a main characteristic of T<sub>FH</sub>. Thus, T<sub>FH</sub> can also express many transcription factors thought to be master regulators of T helper cell lineages, as GATA binding protein 3 (GATA-3) and the T-box transcription factor (T-bet)<sup>[7]</sup>.

Antigen presentation by dendritic cells (DC) is necessary to initiate T<sub>FH</sub> commitment<sup>[8-10]</sup>. As a consequence of this initial encounter, T<sub>FH</sub> express achaete-scute homologue 2 (Ascl2)<sup>[11]</sup>, B cell lymphoma 6 (Bcl-6), chemokine (C-X-C motif) receptor 5 (CXCR5) and ICOS triggering the T<sub>FH</sub> differentiation program<sup>[10,12,13]</sup>. These events take place outside the B-cell follicle in the absence of B cells<sup>[10,13,14]</sup>. SAP-deficient CD4<sup>+</sup> T cells, which fail to sustain prolonged interaction with B cells, but interact normally with antigen-presenting DC, upregulate

Bcl-6 and CXCR5 following activation<sup>[8,9,15]</sup>. Late cognate interactions with activated B cells are required to complete and sustain full differentiation of T<sub>FH</sub><sup>[15]</sup>. However, B cell-mediated antigen presentation can be overcome when antigen in excess is presented by DC<sup>[8,9]</sup>. Apparently, when provision of antigen is limited, B cells are more efficient than DC to capture antigen through their high affinity antigen-B cell receptor<sup>[10]</sup>. Therefore, antigen availability would dictate the transition of initially DC-primed-T<sub>FH</sub> towards B-cell primed-T<sub>FH</sub> as the differentiation program progresses in the interfollicular zone (Figure 1). The importance of DC in the induction of a full T<sub>FH</sub> response relies both on their ability to migrate to the B cell follicles through the upregulation of CXCR5 and downregulation of the chemokine (C-C motif) receptor 7 (CCR7) (providing a favorable spatial location for DC-B cell-T<sub>FH</sub> interactions), but also on their ability to release DC-derived cytokines that are necessary for T<sub>FH</sub> development<sup>[15,16]</sup>.

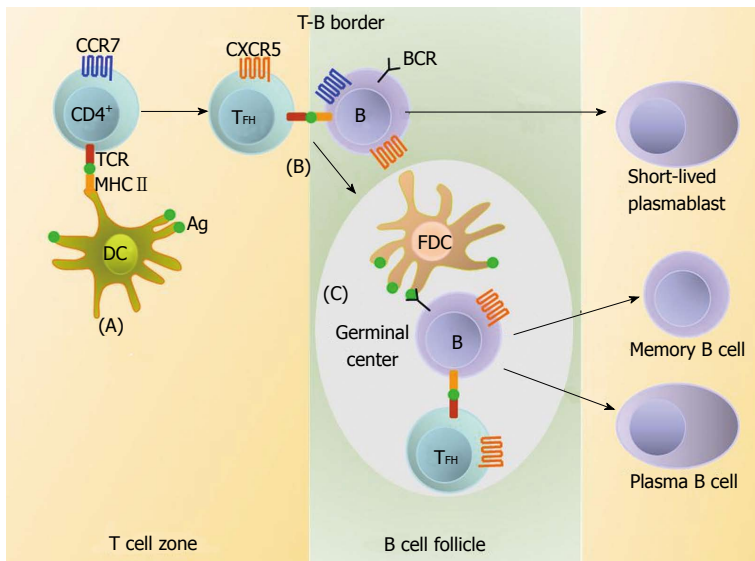
In addition to their presence in B cell follicles, T<sub>FH</sub> circulating counterparts have been identified in the blood stream<sup>[1]</sup> and share many of the phenotypic and functional characteristics of T<sub>FH</sub> residing in the follicles. The phenotypic hallmark of T<sub>FH</sub> is the surface expression of the chemokine receptor CXCR5, which enables their migration into B cell follicles, in response to the specific chemokine ligand CXCL13-rich follicular areas.

Deficiency of CXCR5 affects the antibody response. It impairs the germinal center (GC) response, reducing the frequency of GC B lymphocytes and isotype-switched antibody-secreting cells. ICOS is necessary for the induction of CXCR5 and for an efficient GC reaction<sup>[2]</sup>. In the absence of CXCR5, T cells cannot migrate to the follicles, but migration is not an absolute requirement for the formation of GC. CD40 ligand (CD40L), SAP and ICOS are other molecules expressed by T<sub>FH</sub> that are essential to ensure their ability to provide help to B cells<sup>[17]</sup>.

An increased expression of CXCR5, ICOS, the inhibitory receptor programmed cell death-1 (PD-1) and SAP characterize the T<sub>FH</sub> phenotype, as well as the downregulation of CCR7 and the IL-7 receptor (CD127)<sup>[1,6]</sup>.

The cytokine secreting profile of T<sub>FH</sub> includes the production of high amounts of IL-21. IL-10, IL-4 and IL-6 are also produced by T<sub>FH</sub>. All these cytokines are involved in the generation of an adequate antibody response by promoting growth differentiation and class switching of B cells. These characteristics of T<sub>FH</sub> have been demonstrated both in humans and in mice. Table 1 summarizes this information for human T<sub>FH</sub>.

As a group, T<sub>FH</sub> are heterogeneous. Despite the definition of a basic T<sub>FH</sub> profile, T<sub>FH</sub> have an inherent plasticity and they may convert to other cell subsets. Likewise, forkhead box P3 (Foxp3<sup>+</sup>) regulatory T cells that express CXCR5 and Bcl-6 (T<sub>FR</sub>) and migrate to human tonsils or murine lymphoid tissue have been described<sup>[18]</sup>. They are closely related by their phenotype to classic T<sub>FH</sub> and derive from T regulatory (Treg) cells<sup>[19]</sup>. In humans CXCR5<sup>+</sup> CD4<sup>+</sup> T cells are a circulating pool of memory



**Figure 1 Follicular helper T cells and the differentiation program of B lymphocytes.** (A) Naive CD4<sup>+</sup> T cells are activated following recognition of antigen (Ag) presented by dendritic cells (DC) in T cell zones. Upon antigen activation and co-stimulation by DC, nascent T<sub>FH</sub> upregulate CXC-chemokine receptor 5 (CXCR5), downregulate CC-chemokine receptor 7 (CCR7) and migrate towards B cell follicles; (B) At the T-B border TFH cells contact antigen-activated B cells that move to the T-cell zone after upregulating CCR7. T<sub>FH</sub> cells deliver help to B cells resulting in their differentiation into short-lived extrafollicular plasmablasts or their migration into B cell-follicles to form germinal centers (GCs); (C) Within GC, T<sub>FH</sub> cells promotes the B cell differentiation into long-lived plasma cells and memory B cells. T<sub>FH</sub>: Follicular helper T lymphocytes; FDC: Follicle dendritic cell.

**Table 1 Follicular helper T lymphocytes markers**

Marker	Human T <sub>FH</sub>		Naïve CD4 <sup>+</sup> T cell	Activated Non-T <sub>FH</sub> CD4 <sup>+</sup>
	T <sub>FH</sub>	GC T <sub>FH</sub>		
CXCR5	+	++	-	-
Ascl2	?	++	?	?
Bcl-6	+	++	-	-
Blimp-1	-	-	+/-	++/variable
PD-1	+	++	-	Variable
ICOS	+	++	-	Variable
SAP	+	++	+	+
IL-21	+	++	-	Variable
IL-4	-/+	++	-	Th2+
CCR7	-/+	-	++	Variable

T<sub>FH</sub>: Follicular helper T lymphocytes; GC: Germinal center; CXCR5: Chemokine (C-X-C motif) receptor 5; Ascl2: Achaete-scute homologue 2; Bcl-6: B cell lymphoma 6; Blimp-1: B lymphocyte-induced maturation protein 1; PD-1: Programmed cell death-1; ICOS: Inducible costimulator; SAP: Signaling adaptor SLAM-associated protein; IL: Interleukin; CCR7: Chemokine (C-C motif) receptor 7; Th: T helper.

cells that comprises three CD4<sup>+</sup> T helper subsets: Th1 T<sub>FH</sub> expressing CXCR5, CXCR3 and the transcription factor T-bet in the absence of CCR6; Th2 T<sub>FH</sub> expressing CXCR5 and the transcription factor GATA-3 in the absence of both CCR6 and CXCR3 and Th17 T<sub>FH</sub> expressing CXCR5, CCR6 and the transcription factor RORγt in the absence of CXCR3 (Table 2). These subsets of T<sub>FH</sub> have different helping abilities. While Th2 T<sub>FH</sub> and Th17 T<sub>FH</sub> can help naïve B cells to produce IgM, IgG and IgA, Th1 T<sub>FH</sub> cannot<sup>[1]</sup>.

Furthermore, a subgroup of CXCR5<sup>+</sup> CD4<sup>+</sup> circulating lymphocytes with low CCR7 and high PD-1 expression have been identified as an early memory subset of T<sub>FH</sub>, which upon antigen exposure differentiates into mature T<sub>FH</sub> capable to provide a prompt protective antibody response<sup>[20]</sup>.

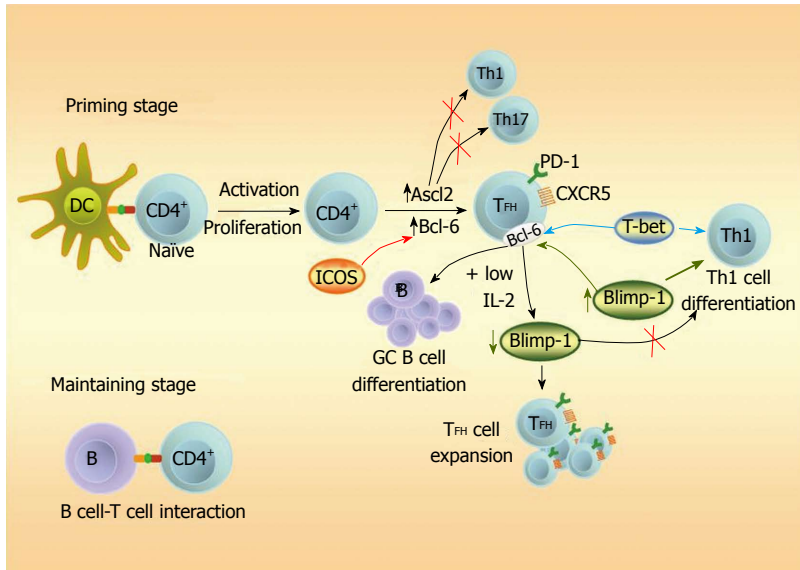
## REGULATION OF T<sub>FH</sub> DEVELOPMENT

T<sub>FH</sub> differentiation may be divided into two phases: the

priming and the maintaining stages. Priming depends on antigen-presenting signaling of DC, while maintaining is related to sustained B cell-T cell interaction and the consequent signaling events. While most studies have pointed out the role of the transcription factor Bcl-6 as an initiator of the T<sub>FH</sub> differentiation program during the priming stage, recent work by Liu *et al*<sup>[11]</sup> demonstrated that Ascl2, another transcription factor, is crucial for T<sub>FH</sub> development and function. Ascl2 is a basic helix-loop-helix (bHLH) transcription factor<sup>[21]</sup>. It directly regulates T<sub>FH</sub>-related genes and inhibits Th1 and Th17 signature genes. Upregulation of Ascl2 precedes that of Bcl-6, indicating that Ascl2 and not Bcl-6 may be the initial trigger for the T<sub>FH</sub> differentiation program.

Large amounts of Bcl-6 expressed by T<sub>FH</sub> can be counterbalanced by the repressor B lymphocyte-induced maturation protein 1 (Blimp-1). While Bcl-6 favors the development of T<sub>FH</sub> *in vivo*, Blimp-1 regulates the function of Bcl-6 and inhibits the generation of T<sub>FH</sub>. Bcl-6 controls GC B cell differentiation by regulating cell cycle genes, regulating DNA damage response genes and suppressing a host of signaling pathways, including B cell receptor (BCR) signaling<sup>[22]</sup>. It is a member of the BTB-POZ (bric-a-bric, tramtrack, broad complex-poxvirus zinc finger) family of transcriptional repressors. These repressors directly bind to specific DNA sequences through their zinc-finger DNA binding domains with the BTB-POZ domain mediating transcriptional repression<sup>[23]</sup>. In both GC B cells and T<sub>FH</sub>, Bcl-6 controls T<sub>FH</sub> differentiation by regulating genes separate from those it controls in B cells<sup>[22]</sup>. Molecular crosstalk between GC B cells and T<sub>FH</sub> influences the survival, proliferation and differentiation of each cell type<sup>[24]</sup>. In addition to promoting the expression of T<sub>FH</sub> signature genes, Bcl-6 represses *Prdm1* (the gene encoding the transcriptional repressor Blimp-1). Bcl-6 antagonism of Blimp-1 is one of the key mechanisms by which Bcl-6 inhibits non-T<sub>FH</sub> differentiation. Bcl-6-dependent suppression of Blimp-1 activity (by removal of the Blimp-1 “brake”) may favor the differen-





**Figure 2 Regulation of Follicular helper T cell development.** After antigen-presenting signaling of dendritic cells (DC) to CD4<sup>+</sup> T cells during priming, achaete-scute homologue 2 (Ascl2) and B cell lymphoma 6 (Bcl-6) induced by the inducible costimulator (ICOS), trigger for T<sub>FH</sub> differentiation program and inhibit Th1 and Th17 genes. Bcl-6 also controls germinal center (GC) B cell differentiation. B lymphocyte-induced maturation protein 1 (Blimp-1) and the T-box transcription factor (T-bet) regulate the function of Bcl-6 and favor the induction of a Th1 profile. Under low interleukin 2 (IL-2) conditions, excess of Bcl-6 counteracts Blimp-1 allowing expansion of the T<sub>FH</sub> and reduction of the Th1 programs of differentiation. Initial priming is sufficient to acquire the T<sub>FH</sub> markers but cognate B cells are needed for the subsequent maintenance stage. T<sub>FH</sub>: Follicular helper T lymphocytes; CXCR5: Chemokine (C-X-C motif) receptor 5.

**Table 2 Heterogeneity of follicular helper T lymphocytes in relation to other T helper cells**

	Markers				
	CD4	CXCR5	CXCR3	CCR6	Foxp3
Th1 T <sub>FH</sub>	+	+	+	-	-
Th2 T <sub>FH</sub>	+	+	-	-	-
Th17 T <sub>FH</sub>	+	+	-	+	-
T <sub>FR</sub> T <sub>FH</sub>	+	+	-	-	+

T<sub>FH</sub>: Follicular helper T lymphocytes; Th: T helper; CXCR5: Chemokine (C-X-C motif) receptor 5; CXCR3: Chemokine (C-X-C motif) receptor 3; CCR6: Chemokine (C-C motif) receptor 6; Foxp3: Forkhead box P3; T<sub>FR</sub>: Foxp3+ CXCR5+ Bcl-6+ regulatory T cells.

tiation program of Th cells towards the induction of T<sub>FH</sub> effectors<sup>[25]</sup>.

As Ascl2<sup>[11]</sup>, Bcl-6 is responsible for the repression of a subgroup of signature genes in effector Th1 cells. It has been shown that Bcl-6 can interact with T-bet<sup>[26]</sup>, which is required for establishment of a Th1 gene expression profile<sup>[27]</sup>. Under low IL-2 conditions the Bcl-6/T-bet ratio increases and excess Bcl-6 represses *Prdm1* and counteracts Blimp-1-mediated inhibition of the T<sub>FH</sub> signature genes, allowing for expansion of the T<sub>FH</sub> and reduction of the Th1 programs of differentiation<sup>[26]</sup>. At the priming stage Bcl-6 expression is induced in CD4<sup>+</sup> T cells independent of CD40 or SAP signaling, while ICOS provides a critical early signal to induce Bcl-6 transcription<sup>[15]</sup>.

Both Ascl2<sup>[11]</sup> and Bcl-6 upregulate CXCR5 expression on T cells during priming and this facilitates their entry to the T/B border. This initial DC integrin-dependent priming is sufficient to acquire the T<sub>FH</sub> markers (CXCR5, PD-1, high levels of Bcl-6), but cognate B cells are needed for the subsequent maintenance and survival stage<sup>[28]</sup> (Figure 2).

## THE ROLE OF CYTOKINES IN T<sub>FH</sub> DEVELOPMENT AND FUNCTION

IL-21 has been recognized as an essential factor deter-

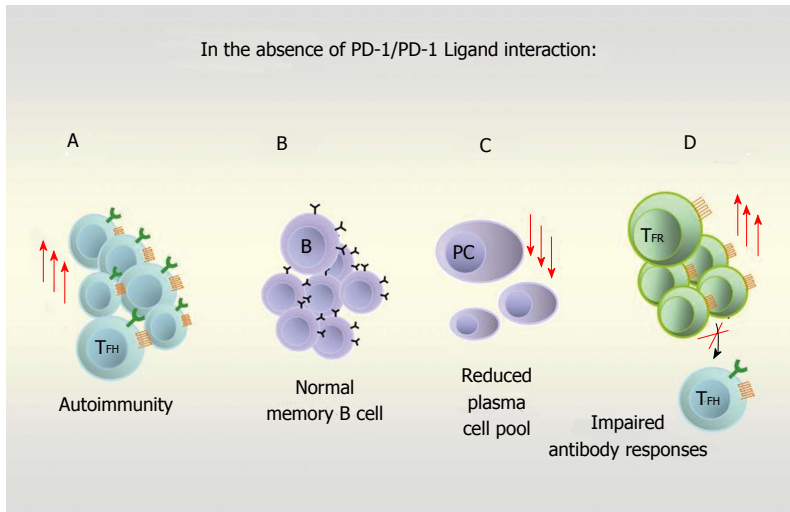
mining the maintenance of T<sub>FH</sub>. It is secreted by T<sub>FH</sub> and has an autocrine effect on them. Through interaction with the IL-21 receptor expressed on B lymphocytes, it promotes survival and proliferation of GC B cells. It has also direct effects on CD4<sup>+</sup> non-T<sub>FH</sub> T cells (Th17)<sup>[29]</sup> and may induce Bcl-6 in T<sub>FH</sub><sup>[30]</sup>. However, IL-21 requirement does not exclusively determine T<sub>FH</sub> differentiation, as IL-21<sup>-/-</sup> and IL-21R<sup>-/-</sup> mice can develop T<sub>FH</sub><sup>[31]</sup>. The combined absence of both IL-21 and IL-6 abrogates T<sub>FH</sub><sup>[6]</sup>. IL-6 and IL-21 redundantly contribute to T<sub>FH</sub> differentiation, but in the absence of other triggers as ICOS, these cytokine signals are insufficient for the instruction of T<sub>FH</sub> differentiation<sup>[6]</sup>.

In addition, IL-21 has been shown to prime human naïve B cells to respond to IL-2 by enhancing their differentiation into plasmablasts. This mechanism operates through STAT3 (signal transducer and activator of transcription 3) signaling and provides an adjunctive role to IL-21-induced B cell differentiation<sup>[32]</sup>.

## PD-1 AND ITS LIGANDS HAVE A CRITICAL ROLE IN THE ASSEMBLY OF THE HUMORAL RESPONSE

PD-1, a member of the CD28 family of costimulatory molecules, is highly expressed in T<sub>FH</sub>, while most human B cells do not express it<sup>[33]</sup>. In general, engagement of PD-1 by its ligands (Programmed cell death ligand 1 -PD-L1- or Programmed cell death 1 ligand 2 -PD-L2-, belonging to the B7 family) inhibits T cell proliferation and cytokine induction and leads to downregulation of T cell responses<sup>[34]</sup>.

The role of the PD-1/PD-L1 or PD-L2 axis in the generation of an adequate antibody response has been highlighted by Good-Jacobson *et al*<sup>[35]</sup>. Though PD-1 is commonly thought as a marker of "exhaustion", T<sub>FH</sub> cannot be considered as exhausted because they secrete abundant IL-21 and other cytokines during humoral response. In the absence of an operative PD-1/PD-L1



**Figure 3 Inhibitory receptor programmed cell death 1, its ligands and their role in humoral response.** In the absence of an operative programmed cell death 1 (PD-1)/PD-1 Ligand axis, follicular helper T lymphocytes ( $T_{FH}$ ) increase and autoimmunity may develop (A); memory B cells are formed normally (B); reduced plasma cells (PC) are found (C); Foxp3<sup>+</sup> CXCR5<sup>+</sup> Bcl-6<sup>+</sup> regulatory T cells ( $T_{FR}$ ) increase and have higher suppressive ability on  $T_{FH}$  function leading to impaired antibody responses (D).

**Table 3 Follicular helper T lymphocytes in autoimmune diseases**

Disease	$T_{FH}$	Ref.
SLE	Increased CXCR5 and function	[46,47,48]
Myasthenia gravis	Increased CXCR5 and function	[49]
RA	Increased CXCR5 and function	[1,50]
Juvenile Dermatomyositis	Mainly $T_{FH}$ with Th17 and Th2-like profile	[1]
ATD	Increased CXCR5, IL-21 high	[51]
Multiple Sclerosis	Increased CXCR5 IL-21 and IL-21R in neurons	[52,53]
Sjögren's syndrome	Increased CXCR5 $T_{FH}$ with Th17 and Th2-like profile IL-17?	[54,55]

$T_{FH}$ : Follicular helper T lymphocytes; SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; ATD: Autoimmune thyroid disease; CXCR5: Chemokine (C-X-C motif) receptor 5; IL: Interleukin; Th: T helper.

or PD-L2 axis,  $T_{FH}$  numbers increase and autoimmunity may develop. Memory B cells are formed normally but the plasma cell pool that depends on the late stage of the GC response is reduced<sup>[36]</sup>. There are conflicting reports about the function of the PD-1 pathway in controlling the humoral response. While some studies report attenuated antibody responses in conditions where the PD-1/PD-L1 and PD-L2 interactions were prevented<sup>[35,37]</sup>, others observed heightened humoral responses<sup>[38]</sup>. Recent work by Sage *et al*<sup>[19]</sup>, in which the contributions of  $T_{FH}$  devoid of contaminating  $T_{FR}$  could be analyzed<sup>[19]</sup> has clarified this question. In the absence of PD-1 and its ligand PD-L1,  $T_{FR}$  were increased and had higher suppressive ability on  $T_{FH}$  function, leading to impaired antibody responses. Thus, there is a dynamic control of antibody production by the balance between  $T_{FH}$  and  $T_{FR}$  cells and this equilibrium is tuned by PD-1/PD-L1 and PD-L2 interactions (Figure 3).

## $T_{FH}$ AND IMMUNODEFICIENCY

Defects in humoral immune response lead to immunodeficiencies, such as common variable immunodeficiency (CVID), X-linked hyper IgM syndrome (HIGM) or X-linked lymphoproliferative disease (XLP)<sup>[39]</sup>. Since ICOS, CD40L and SAP are highly expressed in  $T_{FH}$ , their role in the development of the humoral defects that characterize these diseases has been explored. In ICOS deficiency, which is a very rare condition, ICOS mutations are associated with CVID<sup>[40]</sup>. ICOS deficiency leads to a reduction of CXCR5<sup>+</sup> T cells (including  $T_{FH}$  and  $T_{FR}$ )<sup>[2]</sup>. However, most CVID patients do not have ICOS mutations and in these patients circulating CXCR5<sup>+</sup> CD4<sup>+</sup> T cells are not reduced<sup>[41]</sup>. In HIGM patients, lack of CD40L causes generalized dysfunction of CD4<sup>+</sup> T cells and inability to induce immunoglobulin switching<sup>[42]</sup>. It had been shown that peripheral CXCR5<sup>+</sup> T cells from XLP patients were unable to support immunoglobulin synthesis *in vitro*<sup>[43,44]</sup> and this led to the suggestion that  $T_{FH}$  were not functional in XLP. In fact, absence of SAP affects the stability of the  $T_{FH}$  B cell conjugates, necessary for the completion of an effective GC reaction and T-B cell cooperation<sup>[45]</sup>. However circulating  $T_{FH}$  in XLP patients could be induced to express ICOS, CD40L, IL-4, IL-10 and IL-21 upon stimulation, although the kinetics of expression was different to that of normal  $T_{FH}$ <sup>[46]</sup>. Nevertheless, the humoral response was impaired and the number of memory B lymphocytes was reduced in these patients<sup>[47]</sup>, leading to persistent hypogammaglobulinemia.

**$T_{FH}$  AND AUTOIMMUNITY**

$T_{FH}$  emit instructive signals to B cells that favor the formation and maintenance of GC. Unwanted antibody responses may come together with the normal defensive antibody response against infectious agents, and in this scenario  $T_{FH}$  may play a crucial role. Several studies have addressed the contribution of  $T_{FH}$  to the generation of autoimmune diseases both in murine models and in humans<sup>[39,48]</sup>. Evidence involving  $T_{FH}$  in the generation of an autoantibody response has accumulated, in particular in systemic lupus erythematosus (SLE), both in humans and in mouse models (sanroque mice) as well as in other

autoimmune conditions. A deficit in the process of selection of GC B cells has been pointed out in SLE patients. GC are abundant in secondary lymphoid organs in SLE mice<sup>[49]</sup>. In human SLE, GC are overactive and it has been reported that expansion of T<sub>FH</sub> is causally related to the abundance of GC that form in the absence of foreign antigen, to the production of anti-double-stranded DNA antibodies and to end organ disease<sup>[49]</sup>. Although circulating T<sub>FH</sub> are expanded in sanroque mice and in SLE patients, their abundance appears to be a stable phenotype and not a marker of disease activity. A summary of reports on T<sub>FH</sub> activity or T<sub>FH</sub> role associated to autoimmune diseases is shown in Table 3. Increased numbers of circulating T<sub>FH</sub> have been reported associated to increased autoantibody titers in patients with SLE<sup>[49-51]</sup>, myasthenia gravis<sup>[52,53]</sup>, rheumatoid arthritis and juvenile dermatomyositis<sup>[1,54]</sup>, autoimmune thyroid disease<sup>[55]</sup>, multiple sclerosis<sup>[56,57]</sup> or Sjögren's syndrome patients<sup>[58,59]</sup>. T<sub>FH</sub> numbers increase correlating with titers of autoantibodies and the severity of end-organ involvement.

Autoimmune manifestations are encountered in many patients with CVID. In contrast to other patients with autoimmune manifestations, and no CVID, circulating immunoglobulin levels and plasma antibody titers were low in these patients, but co-existed with elevated circulating T<sub>FH</sub><sup>[41]</sup>. Expansion of T<sub>FH</sub> may play a key role in breakdown of the peripheral tolerance of autoreactive B cells. These cells, which are normally deleted during the GC reaction, may escape from the tolerance checkpoints due to the abundance of the survival help signals provided by excessive T<sub>FH</sub><sup>[60]</sup>.

## T<sub>FH</sub> IN VIRAL DISEASES

The role of T<sub>FH</sub> in HIV infection is not completely clear. Despite profound depletion of CD4<sup>+</sup> T cells during HIV infection, both the bulk T<sub>FH</sub> and HIV-specific T<sub>FH</sub> populations are expanded in HIV-infected patients<sup>[61]</sup>. This expansion correlates with changes observed in the B cell compartment, such as the increased frequencies of GC B cells and plasma cells and the decreased frequency of memory B cells<sup>[61,62]</sup>.

Furthermore, the increase of T<sub>FH</sub> associates with hypergammaglobulinemia in HIV-infected patients. However, the majority of these antibodies are not able to neutralize the virus. Even though there is an expansion of T<sub>FH</sub> in HIV-infected individuals, it seems that these cells are unable to provide appropriate B cell help<sup>[62]</sup>. On the other hand, a resting peripheral blood memory population of CXCR5<sup>+</sup> PD-1<sup>+</sup> CXCR3<sup>-</sup> CD4<sup>+</sup> T cells has been identified in rare HIV individuals that are able to generate broadly neutralizing antibodies. It has also been demonstrated that the frequency of this cell population correlates with the development of broadly neutralizing antibodies<sup>[63]</sup>. Lastly, it has been proven that T<sub>FH</sub> can be infected by HIV. Furthermore, it was suggested that these cells are a major reservoir that contributes to viral persistence<sup>[64]</sup>.

High frequency of peripheral blood T<sub>FH</sub> is also found

in HBV-infected individuals<sup>[65,66]</sup>. It has been reported that treatment with adefovir dipivoxil reduces the frequency of T<sub>FH</sub> and the concentrations of hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg), but increases the concentrations of serum anti-hepatitis B surface and "e" antigen antibodies (HBsAb, HBeAb), IL-2 and IFN- $\gamma$  in drug-responding patients, although the precise relationship between the frequency of peripheral blood CD4<sup>+</sup> CXCR5<sup>+</sup> T<sub>FH</sub> and these parameters requires further investigation<sup>[66]</sup>.

Peripheral blood T<sub>FH</sub> have also been associated with hypergammaglobulinemia in HBV-infected patients<sup>[67]</sup>.

HCV-infected patients also show an increased percentage of peripheral blood T<sub>FH</sub>. This high percentage of T<sub>FH</sub> was associated with low levels of HCV viremia<sup>[68]</sup>.

Even more, a study shows that liver T<sub>FH</sub> cells can be useful to predict the success of virological response following interferon-based treatment in HCV-infected patients. Tripodo *et al*<sup>[69]</sup> reported that the absolute number of liver T<sub>FH</sub> is lower in non-responders, intermediate in responding-relapsed patients and achieves a maximum in sustained virological response patients.

## T<sub>FH</sub> IN PROTOZOAN DISEASES

Reports about the involvement of T<sub>FH</sub> within human infections caused by obligate intracellular parasites are still required. We will focus on the findings achieved using experimental protozoan infection in mice models to study the function of different factors, receptors and cytokines involved in pathways related to T<sub>FH</sub>.

It is well known that experimental infections with *Toxoplasma (T.) gondii* display a model of Th1 cell response induction<sup>[70]</sup>. This model was useful to evaluate if T<sub>FH</sub> represented a temporary "state" of differentiation rather than a distinct lineage parallel to other subsets<sup>[71]</sup>. Also, to confirm the action of T-bet as a suppressor of both T<sub>FH</sub> and humoral responses *in vivo*<sup>[71]</sup>. The generation of parasite-induced Th1 responses by *T. gondii*, also served to understand the association of the T<sub>FH</sub> marker ICOS with T helper cytokine production *in vivo*. ICOS<sup>+</sup> CD4<sup>+</sup> T helper cells produce a variety of different effector cytokines and their pattern depends on the infection challenge. Infection with *T. gondii* leads to IFN- $\gamma$  production, while ICOS<sup>+</sup> CD4<sup>+</sup> T cells from the nematode *Schistosoma mansoni* (an inducer of Th2 responses) is associated with IL-10 secretion<sup>[72]</sup>.

According to these findings, experimental models using *Leishmania (L.) major* also demonstrated that ICOS is a critical regulator of both Th1 and Th2 immune responses *in vivo*<sup>[73]</sup>. Chronic infection with *L. major*, a model of prominent T-B cell interaction, was also used to evaluate the contribution of IRF-4 (member of IFN-regulatory factor family) to the interaction of T<sub>FH</sub> and GC B cells. Bolling *et al*<sup>[74]</sup> demonstrated the implication of this factor, since IRF4<sup>-/-</sup> mice lacked GC formation, differentiation of GC B cells and lymph node CD4<sup>+</sup> T cells from these mice expressed reduced amounts of the T<sub>FH</sub>-related molecules ICOS, IL-21 and Bcl-6. *L. major*



infection model also helped to demonstrate the relation of T<sub>FH</sub> and IL-4. All the IL-4 secreting cells in lymph nodes during infection with this parasite were T<sub>FH</sub> and these cells were distinct from conventional Th2 cells based on phenotype, location and function<sup>[75]</sup>.

Besides, analysis of the consequences of *in vivo* blockade of T cell inhibitory receptors indirectly associated with T<sub>FH</sub> were performed using blood-stage *Plasmodium (P.) yoelii* infection in mice. Butler *et al*<sup>[76]</sup> demonstrated that blockade of PD-L1 and LAG-3 (lymphocyte-activation gene 3) receptors led to improved parasite control associated with enhanced T<sub>FH</sub> numbers and substantial induction of plasma cell differentiation.

Experimental models in which mice were co-infected with *L. major* and *L. amazonensis* demonstrated that those mice that healed the lesions had more GC, more isotype switched GC B cells and more memory B cells than those who did not. A productive B cell response was required for healing a co-infection with these protozoans in this model<sup>[77]</sup>.

The development of T<sub>FH</sub> was also assessed in order to find strategies to enhance the efficacy of recombinant protein subunit vaccines using lipid-based nanoparticles (NPs). In this context, Moon *et al*<sup>[78]</sup> studied the impact of NP delivery on immune responses elicited by a candidate *P. vivax* subunit vaccine. They found that prolonged antigen presentation by this vaccine contributed to expand T<sub>FH</sub> and promote GC induction. The CD4<sup>+</sup> T<sub>FH</sub> subset provided critical cytokines and signals required to initiate somatic hypermutation and affinity maturation of B cells<sup>[79]</sup>, achieving broad antibody responses.

This information indicates that there is an association between protozoan infections, T<sub>FH</sub> and their related cytokines, receptors and B responses in the context of experimental mice models. Leishmaniasis, malaria, toxoplasmosis and other parasitic infections seriously affect humans. Reports about the implication of T<sub>FH</sub> and humoral responses are needed to better understand mechanisms involved in the progression and outcome of these diseases.

## CONCLUSION

Clearly, our research on T<sub>FH</sub> demonstrates that they are essential for the generation of a long-lasting humoral response. Their role in the assembly of the GC reaction explains why their dysfunction or their inability to interact correctly with B cells leads to immunodeficiency, to autoimmunity or to inefficient management of infectious diseases. It will be necessary to understand how the regulation of their function may be modified or restored in order to revert T<sub>FH</sub> deficiency or over activity, as well as to design adequate strategies for antibody production in vaccination programs.

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