Human T Follicular Helper Cells: Development and Subsets

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Abstract

Antibody response constitutes one of the key immune protection mechanisms. T follicular helper (Tfh) cells represent the major CD4+ T cell subset that provides help to B cells to induce antibody response. How Tfh cells develop and how Tfh cells or their associated subsets regulate antibody response in humans remains largely unknown. In this review, we will summarize the recent discoveries on the biology of Tfh cells, with a particular focus on human Tfh cells.

Keywords

Human • T follicular helper cells • CD4+ T cell subsets • Antibody response • Tonsils • Blood • CXCR5 • Autoimmune diseases

10.1 Introduction

T follicular helper (Tfh) cells are the major CD4+ T cell subset that provides help to B cells to induce antibody response [1, 2]. Tfh cells are essential for the formation of germinal centers (GCs), the site of the selection of high-affinity B cells, and their differentiation into memory B cells or long-lived plasma cells. Similar to other CD4+ T cell subsets such as Th1, Th2, and Th17 cells, the magnitude and the duration of Tfh response need to be controlled by the immune system, because exaggerated Tfh response causes autoimmunity [3]. Recent studies including ours identified similarities and differences in the biology of Tfh cells between humans and mice. Determining the biology and developmental pathways of human Tfh cells is of great significance, as it will provide direct insights regarding the development of novel vaccine design and therapeutic strategies for human autoimmune diseases.

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10.2 T Follicular Helper Cells: The CD4+ T Cell Subset Specialized for B Cell Help

In secondary lymphoid organs, CD4+ T cells primed by dendritic cells (DCs) loaded with antigens interact with antigen-primed naïve B cells at extrafollicular sites [4], typically at the border of T cell zone and primary follicles [5]. This interaction initiates the B cell differentiation process towards two different paths: extrafollicular plasma cells and cells forming GCs [6–8]. Extrafollicular plasma cells contribute to the early generation of specific antibodies after antigen exposure [9]. Germinal center B (GC-B) cells subsequently differentiate into either high-affinity long-lived plasma cells or memory B cells after an extensive selection step [10, 11] (Fig. 10.1).

Together with GC-B cells and follicular dendritic cells, Tfh cells constitute essential cell compartments for GC formation. In GCs, Tfh cells play an important role in the selection of high-affinity B cells and in the induction of the differentiation of selected B cells. While no unique markers have been reported, Tfh cells can be identified by the combination of several markers that are directly associated with their functions. The chemokine (C-X-C) receptor 5 (CXCR5) is important for their initial migration into B cell follicles [12–15]. Tfh cells express PD-1, which was shown to play a role in the selection of high-affinity B cells in GCs [16]. Inducible co-stimulator (ICOS) is critical for the development [17, 18] and functions [15, 19, 20] of Tfh cells. CD40 ligand (CD40L) expressed by Tfh cells provides essential signals to B cells through CD40 for their differentiation and class-switching [21]. Tfh cells and their precursors secrete IL-21 [22–24], a γc-family cytokine which potently promotes the growth, differentiation, and class-switching of B cells [25]. IL-21 delivers activation signals to B cells via STAT3 (signal transducer and activator of transcription 3), and accordingly STAT3-deficient

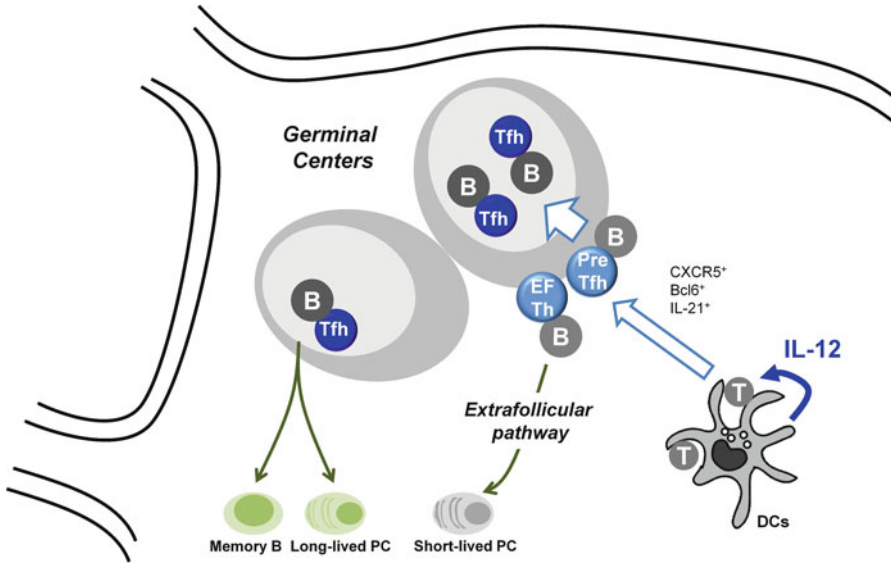

Fig. 10.1 Generation of human Tfh cells. In secondary lymphoid organs, naïve CD4+ T cells interact with DCs loaded with antigens. IL-12 secreted by the DCs induces naïve CD4+ T cells to initiate the program to differentiate into the Tfh lineage. These cells migrate towards B cell follicles, and interact with B cells. T and B cell interaction initiates the B cell differentiation process towards two different paths: extrafollicular plasma cells and cells forming GCs. How Tfh precursors and extrafollicular helper (EF) T cells overlap remains unclear in humans. Inside the GCs, Tfh cells provide help to high-affinity B cells and support their differentiation into long-lived plasma cells or memory B cells

patients show severely impaired antibody responses including a decreased generation of memory B cells [26].

10.3 Development of Tfh Cells

Differentiation of naïve CD4+ T cells towards conventional helper T cell (Th) subsets (including Th1, Th2, and Th17 cells) is regulated by the signals that they receive from DCs and from microenvironment [27, 28]. Recent mouse studies indicate that this is also the case for Tfh cell generation. Tfh cells express large amounts of B cell lymphoma 6 (Bcl-6) [23], the transcription repressor that is necessary and sufficient for Tfh cell generation in vivo [29–31]. Initial commitment towards Tfh cells occurs by upregulation of Bcl-6 expression when CD4+ T cells encounter with DCs [32–35]. The subsequent interaction between T and B cells appears to be important for the maintenance of Bcl-6 expression in Tfh cells. However, Bcl-6 does not regulate IL-21 secretion in mouse [30, 31] or human CD4+ T cells [36]. This is in contrast to other transcription factors engaged in the differentiation of other conventional Th subsets, regulating the secretion of cytokines typical of each subset. Thus, Tfh cell generation occurs through a highly orchestrated process, and likely requires other transcription factors in addition to Bcl-6.

Which types of DCs do promote Tfh cell generation? DCs are endowed with enormous functional plasticity, which permits them to induce different immune responses according to the microenvironment. In addition, the DC system is composed of subsets associated with the induction of different types of immunity [37, 38]. Our study on human skin DC subsets demonstrated that CD14+ dermal DCs are one of the most efficient DC subsets at inducing human naïve CD4+ T cells to become Tfh-like cells in vitro [39]. CD4+ T cells primed by CD14+ dermal DCs, but not by epidermal Langerhans cells, strongly induce naïve B cells to become antibody secreting plasma cells producing IgM, as well as to switch isotypes towards IgG and IgA [39]. Furthermore, in vitro-generated DCs sharing properties with CD14+ dermal DCs induce the differentiation of CD40-activated naïve B cells into IgM-producing plasma cells through direct DC and B cell interactions [40]. These observations suggest that CD14+ dermal DCs display unique properties to promote the development of antibody responses in humans. Notably, in mice, activated dermal DCs migrate into the outer paracortex just beneath the B cell follicles, whereas LCs migrate into the T cell rich inner paracortex [41]. This suggests that also in mice, dermal DCs, rather than LCs, are one of the major DC subsets associated with the development of humoral immunity.

Mouse studies showed that STAT3 signaling delivered by IL-6 and IL-21 contributes to Tfh cell development [42–45]. Similarly, STAT3-deficient human subjects (Hyper IgE syndrome) were shown to have altered Tfh response [46], although whether this is T cell intrinsic or secondary to defective B cell response [26] remains unclear. We and others showed that the IL-12-STAT4 pathway is one of the major pathways in humans by which DCs promote the development of IL-21-producing Tfh-like cells [47, 48]. IL-6 and IL-21 are much less potent than IL-12 in vitro at inducing human naïve CD4+ T cells to express Tfh-associated molecules, including IL-21, CXCR5, ICOS, and Bcl-6 [47, 49, 50]. Indeed, dermal CD14+ DCs, but not LCs, express IL-12 upon CD40L stimulation [39], which explains at least in part why dermal CD14+ DCs are efficient at inducing Tfh-like cells. Interestingly, there is evidence that the IL-12-STAT4 pathway contributes to the development of Tfh cells also in mice [51, 52]. Thus both STAT3 and STAT4 are involved in the generation of Tfh cells in mice and humans, while the extent of contribution by each pathway and/or cytokine might be different. It will be important to address whether IL-12 and/or IL-23 indeed contribute to in vivo Tfh and GC response in humans. Also it will be important to determine how the initial lineage commitment towards Th1 and Tfh cells is regulated in humans, because the IL-12/STAT4 axis also potently promotes Th1 cell generation through the upregulation of T-bet [53]. In mice, the effect of IL-12 on CD4+ T cells for the expression of Tfh-associated molecules is short-lived and eventually dominated by T-bet-driven Th1 cell generation [51].

10.4 Human Tfh Subsets

10.4.1 Tonsillar Tfh Subsets

Whereas Tfh cells are considered to help the selection and differentiation of B cells in GCs, the identity of $CD4^+$ T cells interacting with B cells outside GCs was unknown in humans. We recently identified a Tfh-committed subset that is exclusively localized outside GCs in human tonsils [24]. This subset can be identified by the expression of IL-7 receptor, and low levels of CXCR5 and ICOS ($CXCR5^{lo}ICOS^{lo}$). The expression of *BCL6* and *PRDM1* transcripts are comparable between $CXCR5^{lo}ICOS^{lo}$ $CD4^+$ T cells and $CXCR5^{hi}ICOS^{hi}$ GC-Tfh cells. Interestingly, these two Tfh-lineage subsets differentially help B cells. $CXCR5^{hi}ICOS^{hi}$ GC-Tfh cells are efficient at helping GC-B cells. Reciprocally, GC-B cells are able to maintain the survival of $CXCR5^{hi}ICOS^{hi}$ GC-Tfh cells. $CXCR5^{lo}ICOS^{lo}$ $CD4^+$ T cells are far more efficient than GC-Tfh cells at inducing naïve B cells to proliferate and differentiate into Ig-producing cells. Notably, $CXCR5^{lo}ICOS^{lo}$ $CD4^+$ T cells lack the capacity to help GC-B cells and induce the apoptosis of GC-B cells through the FAS/FAS-ligand (FAS-L) interaction. Thus, $CXCR5^{lo}ICOS^{lo}$ $CD4^+$ tonsillar $CD4^+$ T cells likely represent extrafollicular helper cells engaged in inducing the differentiation of B cells into extrafollicular plasma cells and/or represent precursors of GC-Tfh cells (Pre-Tfh cells).

10.4.2 Blood Circulating $CXCR5^+$ $CD4^+$ T Cell Subsets

Human tonsillar Tfh cells display distinct phenotype and gene profiles from other conventional Th subsets [15, 23, 54]. Discovery of Bcl-6 as a "master regulator" of Tfh cell generation further supports the concept that Tfh cells represent an independent $CD4^+$ T cell subset. However, mouse Tfh cells are indeed heterogeneous, and encompass distinct subsets secreting cytokines characteristic of Th1, Th2, and Th17 cells [55–59]. Furthermore, mouse Th2 [57] and T regs [60] were shown to be convertible into Tfh cells in vivo. Thus, the type of Tfh precursors also remains elusive and the relationship between Tfh cells and other Th subsets still remains unclear.

A fraction of human blood memory $CD4^+$ T cells expresses CXCR5 [61]. Several observations suggest the relationship between $CXCR5^+$ $CD4^+$ T cells and Tfh cells. For example, humans who show severely impaired GC formation through deficiency of CD40-ligand or ICOS display significantly less circulating $CXCR5^+$ $CD4^+$ T cells [18]. On the contrary, $CXCR5^+$ $CD4^+$ T cells expressing ICOS are present at a higher frequency in blood of lupus patients [62]. Our studies on human blood $CXCR5^+$ $CD4^+$ T cells conclude that they share functional properties with Tfh cells from secondary lymphoid organs, and likely represent their circulating memory compartment [63]. In concordance with Tfh cells, blood $CXCR5^+$ $CD4^+$ T cells induce naïve and memory B cells to become Ig-producing cells via IL-21, IL-10, ICOS, and secrete CXCL13. At variance with Tfh cells, blood $CXCR5^+$ $CD4^+$ T cells barely express CD69 and ICOS, and express PD-1 only at low levels [14, 48, 62], suggesting that they are in a resting state. Consistently, blood $CXCR5^+$ $CD4^+$ T cells required cell activation to provide help to B cells through cognate interaction. In contrast to GC-Tfh cells, blood $CXCR5^+$ $CD4^+$ T cells express CCR7 and CD62L, suggesting their capacity to migrate into secondary lymphoid organs.

Importantly, blood $CXCR5^+$ $CD4^+$ T cells comprise three subsets; Th1, Th2, and Th17 cells [63] (Fig. 10.2). These subsets can be defined according to the expression of chemokine receptors, expression of transcription factors, and the type of cytokine secretion patterns. Th2 and Th17 cells within $CXCR5^+$ compartment efficiently induce naïve B cells to produce immunoglobulins and to switch isotypes through IL-21 secretion. While $CXCR5^+$ Th2 cells promote IgG and IgE secretion, $CXCR5^+$ Th17 cells are efficient at promoting IgG and, in particular, IgA secretion. In contrast, $CXCR5^+$ Th1 cells lack the capacity to help naïve B cells. These findings suggest that Tfh cells associated with conventional Th subsets differentially shape the quality of human humoral immunity, with $CXCR5^+$ Th2 cells favoring IgE responses and $CXCR5^+$ Th17

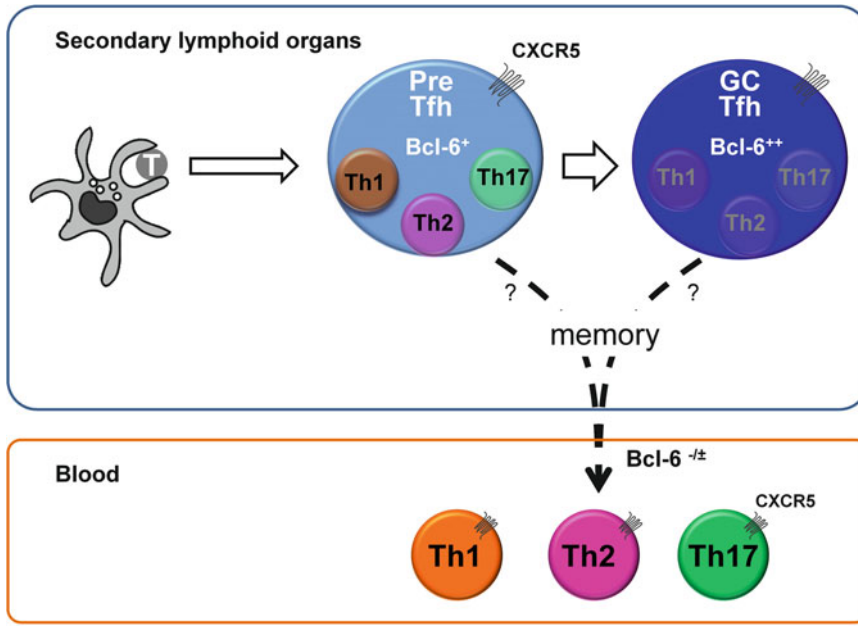

Fig. 10.2 Hypothetical model in the development of distinct Tfh subsets in humans. We surmise that Tfh-committed cells sharing properties with conventional Th1, Th2, and Th17 cells develop at the Pre-Tfh stage. During the maturation process towards GC-Tfh cells, transcription factor changes including Bcl-6 upregulation suppress the expression and/or function of transcription factors associated with conventional Th subsets. Once Tfh cells (or pre-Tfh cells) differentiate into memory cells, they decrease the expression of Bcl-6 and start to reveal the property of conventional Th subsets while maintaining the identity of the Tfh lineage

cells favoring protective mucosal antibody responses. Whether such Th1, Th2, and Th17-commited Tfh subsets are present in human secondary lymphoid organs is under investigation.

Importantly, alteration in the balance of blood CXCR5$^+$ CD4$^+$ T cell subsets, which likely reflects the type of Tfh cells in secondary lymphoid organs, was found to be associated with autoimmunity. Patients with juvenile dermatomyositis, a systemic autoimmune disease, display a profound skewing of blood CXCR5$^+$ CD4$^+$ T cell subsets towards Th2 and Th17 [63]. Significantly, the skewing of subsets correlates with disease activity and frequency of blood plasmablasts. Furthermore, a study on Sjogren's syndrome patient blood samples shows that CXCR5$^+$ Th17 cells are dominant in this disease, and the increase of these cells correlate with clinical characteristics including autoantibody titers and disease activity [64]. Thus, these studies provide a strong rationale that analysis on blood CXCR5$^+$ CD4$^+$ T cell subsets might provide diagnostic and/or prognostic biomarkers in human autoimmune diseases.

Whether blood CXCR5$^+$ CD4$^+$ T cells originate from cells that migrated out of GCs, Tfh precursors, or Tfh-committed extrafollicular helper cells [9, 65] is an important question, but will be challenging to address in humans.

10.5 Perspectives

Tfh cells in human secondary lymphoid organs and in blood are composed of functionally different subsets. Establishing the mechanisms whereby the DC system induces Tfh cells with different functions will facilitate the design of novel vaccines. In particular, establishing how DC system generates Tfh subsets associated with the induction of mucosal homing plasma cells will provide a significant insight in the development of novel mucosal vaccines.

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