Follicular Helper T Cells: New Insights Into Mechanisms of Autoimmune Diseases

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ABSTRACT

Background: Autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes, etc) are characterized by the production of autoantibodies against one's own cell components, resulting in the dysfunction of normal organs. At present, therapies for autoimmune diseases involve a variety of nonspecific antiinflammatory and immunosuppressive agents with significant side effects. Current studies have suggested that the germinal center (GC) may be the pathogenic hot spot for the production of autoantibodies in autoimmune disease. Events occurring in the GC—such as the selection of high-affinity B cells, proliferation of B cells, and differentiation of B cells into plasma cells—all depend on T cells. Follicular helper T (Tfh) cells are a recently identified T-cell subset, named for their location in GCs. Tfh cells are characterized by their signature transcription factor (B-cell lymphoma 6), surface molecules (CD40 ligand, chemokine [C-X-C] receptor 5, inducible T-cell costimulator, programmed cell death protein-1, etc), and cytokines (interleukin [IL]-21, IL-6, IL-10, etc). Through these signals, Tfh cells help B cells form GCs and drive B cells to differentiate into memory B cells and plasma cells that produce antibodies. However, uncontrolled generation of Tfh cells in the GCs or peripherals could lead to autoimmunity. Recent studies from our group and others have shown that Tfh cells are expanded in the peripheral blood of patients and in the lymphoid tissues of mice with lupus or rheumatoid arthritis and play an important role in promoting pathogenic autoantibody production.

Methods: In this review, we summarize the latest immunologic findings regarding the characteristics and development of Tfh cells, their relation to other CD4^{+} T-cell subsets, and the function of Tfh cells in normal immune response and autoimmune diseases.

Conclusion: A clear understanding of the mechanisms of Tfh cell–mediated immunity and pathology may lead to the development of novel therapeutic targets in autoimmune diseases.

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INTRODUCTION

Follicular helper T (Tfh) cells, a special CD4^{+} T-cell subset localized in the B-cell follicle, were first reported in tonsils[1] where immune cells are constantly exposed to foreign antigens, resulting in the expansion of immune cells and the formation of germinal centers (GCs). The GC is a discrete lymphoid anatomic structure in secondary lymphoid organs (tonsils, lymph nodes, spleen, etc) where clonal expansion, somatic hypermutation, affinity maturation, and the development of B-cell memory and long-lived plasma cells occur, thus playing a key role in the protective immunity against pathogens.[2-4]

Recently Tfh cells have attracted close attention for their role in providing critical help to B cells and contributing to autoimmunity.[5-8] Although Tfh cells and other CD4^{+} T-cell subsets share some phenotypic and functional properties, Tfh cells bear their specific identity via signature surface markers, cytokines, and transcription factors. Through these specific molecules and cytokines, Tfh cells play an important role in the selection of B-cell clones with high affinity toward foreign antigens in favor of developing a robust humoral immune response, while preventing the selection of B cell clones with weak

affinity or affinity toward self-antigens to maintain self-tolerance.

Autoimmune diseases are currently thought to develop in genetically susceptible individuals from environmental exposure that triggers errant immune responses, causing the loss of tolerance to ubiquitous self-antigens and the generation of autoreactive B cells.[9] Then these autoreactive B cells obtain excess help from the uncontrolled generation of Tfh cells, leading to increased production of pathogenic auto-antibodies, inflammation and tissue injury, the onset of clinical symptoms, continued immune amplification, and eventually irreversible tissue damage. It was believed that Tfh cells may shape the outcome of B cell differentiation and be involved in the pathogenesis of autoimmune diseases. Dysregulation of Tfh cells is associated with the development of several autoimmune diseases, such as systemic lupus erythematosus (SLE),[10,11] Sjögren syndrome,[10,12] juvenile dermatomyositis,[13] and rheumatoid arthritis.[14,15]

In this review, we summarize the latest immunologic findings regarding the characteristics and development of Tfh cells, their relation to the other CD4$^+$ T cell subsets, and the function of Tfh cells in normal immune response and autoimmune diseases.

CHARACTERISTICS OF Tfh CELLS

Tfh cells have been identified as a distinct T helper cell subset based on their characteristic surface phenotype and cytokine profile, as well as their signature transcription factor.[16,17] Several surface molecules expressed by Tfh cells (discussed below) are necessary for both the development and maintenance of Tfh cells and are critical to the interaction between Tfh cells and B cells that exerts the B cell response against pathogens.

Chemokine Receptor 5

Chemokine receptor 5 (CXCR5) is involved in Tfh cell homing to the B cell follicles. During GC formation, Tfh cells with strong expression of CXCR5 are attracted to the gradient expression of CXCR5 cognate (C-X-C motif) chemokine ligand 13 (CXCL13) in GCs, allowing Tfh cells to migrate and form stable contacts with antigen-primed B cells in the B cell follicles.[18] The homing and colocalization of Tfh cells with B cells set up a center stage for T-B cell interaction, as T cell receptor (TCR) major histocompatibility complex class II (MHC-II) engagement is pivotal to the restriction of cognate B cell help.[19]

Inducible T-Cell Costimulator

Inducible T-cell costimulator (ICOS, or CD278) is a costimulatory molecule that belongs to the CD28 superfamily. It interacts with its ligand (ICOSL) expressed on antigen-presenting cells or B cells.[20] ICOS plays an important role in the regulation of Tfh cell development, T-cell–dependent antibody response, and GC reactions.[21] Mice deficient in ICOS exhibit reduced number of Tfh cells,[22] impaired GC formation, and profound defects in B cell maturation and immunoglobulin (Ig) isotype switching.[23] Recent evidence showed that ICOS supported Tfh cell formation and maintenance via the production of interleukin (IL)-21 and c-Maf expression.[24]

Programmed Cell Death Protein-1

Programmed cell death protein-1 (PD-1, or CD279) is generally considered a potent inhibitory receptor expressed by T cells, and associated with T-cell tolerance and CD8 cytotoxic T-cell exhaustion during chronic virus infection and cancer, thus playing a negative role in immune response.[25,26] Compared to other T-cell subsets, Tfh cells express the highest levels of PD-1.[27] Through the interaction between PD-1 on Tfh cells and its ligand (PD-1L) on GC-B cells, Tfh cells deliver survival signals to GC-B cells.[28] The regulation of PD-1 may affect GC magnitude and output, such as the high affinity of long-lived plasma cells.[29] Taken together, during Tfh cell activation PD-1 delivers a survival signal to GC-B cells, whereas ICOS functions as a costimulating molecule, thus determining the outcome of the Tfh-cell–mediated B cell response.

CD40 Ligand

Tfh cells also interact with B cells through the expression of surface molecules such as the CD40 ligand (CD40L), a member of the tumor necrosis factor family. CD40L is well known for its role in Ig isotype switching and B-cell survival via its interaction with CD40 on B cells.[30] Patients with CD40L deficiencies or mutations have a severe lack of memory B cells and an absence of GCs.[31] Of note, CD40L is widely expressed by CD4$^+$ T helper cells and is not limited to Tfh cells.

Other Surface Molecules on Tfh Cells

CD57,[32] B and T lymphocyte attenuator (BTLA),[33] the members of the signaling lymphocytic activation molecule-associated (SLAM) protein family[34] (SAP), CD84, and natural killer T-B antigen also contribute to GC formation and immune homoeostasis.

Cytokines

Tfh cells also exhibit a unique expression profile of cytokines (such as IL-21, IL-4, and IL-10) and other soluble factors (CXCL13, etc).[35-37] IL-21 is a signature cytokine for Tfh cells. Reports from our research and

that of others showed that IL-21 plays a critical role in promoting B-cell somatic hypermutation, Ig isotype switching, and plasma cell generation.[38-40] Through the IL-21 receptor, IL-21 also induces Tfh-cell differentiation in an autocrine fashion.[41,42] Furthermore, IL-21 synergizes with IL-6 and B-cell activating factor to regulate Tfh-cell and B-cell differentiation.[43]

Signature Transcription Factor B-Cell Lymphoma 6

B-cell lymphoma 6 (BCL-6), a protooncogene, encodes a poxvirus-zinc finger transcription factor that acts as a sequence-specific repressor of transcription through recruitment of a silencing mediator for retinoid and thyroid hormone receptors (SMRTs) and histone deacetylase-containing complex.[44] Previous studies showed that BCL-6 expression was largely confined in GC-B cells, and its function was closely associated with the fate of GC-B cells.[45] Recently BCL-6 has emerged as a master transcription factor for Tfh cells by affecting the differentiation of Tfh cells and the expression of Tfh cell signature molecules,[46] as well as controlling long-term CD4$^+$ T cell memory.[47] BCL-6–deficient mice lacked GCs, failed to develop Tfh cells, and consequently displayed defective T-cell–dependent antibody responses,[48,49] whereas other CD4$^+$ T cell subsets were relatively unaffected, indicating that BCL-6 is critical for programming Tfh cell differentiation and GC-B cell development. Because BCL-6 connects the differentiation of both Tfh cells and GC-B cells, it acts as a master transcription factor for T-cell–dependent antibody responses in GC.

Tfh CELL DEVELOPMENT AND FUNCTION

Tfh cells are generated and developed in the secondary lymphoid organs where naïve T cells and plasmoid dendritic cells locate at the T-cell zone in the paracortex and B cells stay at the B-cell follicles in the outer cortex (Figure 1).

Physiologically attracted to the T-cell zone chemokine gradient of C-C motif chemokine ligand 19 (CCL19) and CCL21, peripheral naïve CD4$^+$ T cells expressing the counterparts (CCR7 and P-selectin glycoprotein ligand-1 [PSGL-1]) of these chemokines migrate to the T-cell zone from the circulation.[50] Upon encountering the antigens provided by dendritic cells (DCs), naïve CD4$^+$ T cells gain expression of CXCR5 and transcription factor BCL-6 while losing expression of CCR7 and PSGL-1, are attracted to the chemokines (such as CXCL13) expressed by B cells, migrate to the B-cell follicle border, and become Tfh cells.[51] Meanwhile, the B cells encounter antigens presented by DCs or macrophages in the follicle and are activated through their B-cell receptor, which leads

to the upregulation of CCR7 and migration to the B-cell follicle border.[52,53] Thus, at the B-cell follicle border, Tfh cells and B cells migrate toward each other and finally engage in a stable cognate interaction, ultimately leading to a series of molecular events that shapes the humoral immune response, including the development of extrafollicular foci and GCs. First, the T-B cell interaction—via the TCR/MHC peptide and ICOS and its ligand (ICOSL)—promotes B-cell survival and then consequently leads B-cell migration and formation of the extrafollicular foci where B cells differentiate into short-lived antibody-producing plasmablasts.[54] Alternatively, with maintenance of CXCR5 expression for homing to the follicle[55] and greatest TCR signal strength on T cells,[56] both Tfh and B cells are selected and migrate into the follicle and lead to B-cell clonal expansion and GC formation. In GCs, Tfh cells then interact with GC-B cells though various pairs of molecules, such as ICOS-ICOSL, PD-1–PD-1L, CD40-CD40L, and IL-21–IL-21R, resulting in the generation of memory B cells and long-lived antibody-producing plasma cells (Figure 1).[57,58]

The location and mobilization of these immune cells are a dynamic process that was revealed by multiphoton intraviral imaging.[4] The studies showed that the interaction between Tfh and B cells in the GC is less frequent and far shorter compared to that in the T-B border, suggesting that cytokines are more efficient in T-B interaction in GCs and that cell-cell contact is critical in the T-B border. Indeed, our studies on GC-B cells showed that the cytokines secreted by GC-Tfh cells determine the fate of GC-B cell differentiation.[38,59] IL-4 directs GC-B cells to differentiate into memory B cells, whereas IL-10 and IL-21 steer them into plasma cells.

The preferential localization of Tfh cells in the GC suggests a unique, intimate relationship between Tfh and B cells. Events occurring in the GC, such as selection of high-affinity B cells, proliferation of B cells, and differentiation of B cells into plasma cells, all depend on the provision of help by Tfh cells, indicating that Tfh cells are a key participant in controlling B-cell peripheral tolerance and antibody production.

DIFFERENTIATION AND REGULATION OF Tfh CELLS

The differentiation of the CD4$^+$ T cells depends on microenvironmental factors, including signals from B cells and the cytokines' milieu that influence their transcription factor expression and determine the type of T-cell subpopulations (T helper [Th]1, Th2, Th17, T regulatory, and Tfh cells) (Figure 2). Each T-cell subset produces specific cytokines and exerts its unique functions in host defense.[16,60] In contrast to
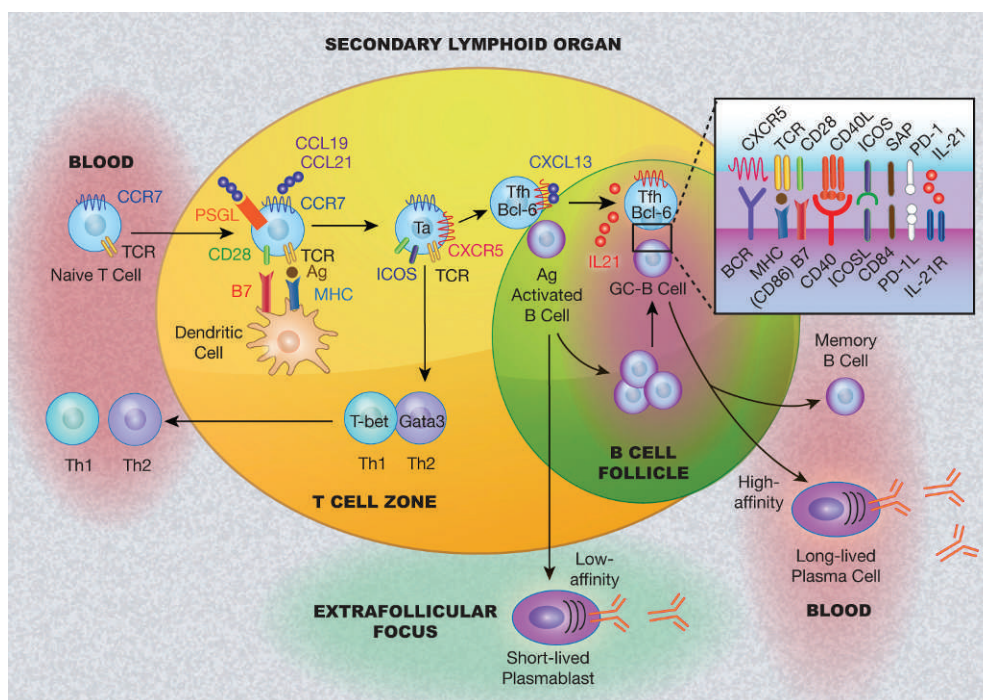
Figure 1. Schematic model of follicular helper T cell (Tfh) development and collaboration with B cells in secondary lymphoid organs. Naïve T cells in the peripheral blood migrate to the T-cell zone in the secondary lymphoid organs (tonsil, spleen, etc) following the gradient of (C-C motif) chemokine ligand (CCL) 19 and CCL21, by engaging to their counterpart (chemokine receptor type 7 [CCR7] and P-selectin glycoprotein ligand-1 [PSGL-1]). T cells become activated (Ta) by recognizing the antigen (Ag) provided by dendritic cells. Dendritic cells provide costimulatory signals to promote T-cell differentiation, resulting in the generation of T-cell subsets. With downregulation of CCR7 and PSGL-1 and upregulation of the expression of chemokine (C-X-C) receptor type 5 (CXCR5) and B-cell lymphoma 6 (BCL-6), Tfh cells are generated and migrate to the T-B border to interact with the antigen-activated B cells. The outcome for this interaction is the development of extrafollicular foci where B cells differentiate into short-lived plasmablasts and/or form germinal centers (GCs). In the GCs, Tfh cells promote B-cell maturation with clone expansion, somatic hypermutation, and class switching through pairs of surface molecules and cytokines (such as interleukin [IL]-21, etc). The interaction of Tfh and B cells leads to the generation of memory B cells and long-lived antibody-producing plasma cells. BCR, B cell receptor; CD40L, CD40 ligand; CXCL13, C-X-C motif chemokine 13; GATA3, GATA binding protein 3; ICOS, inducible T cell costimulator; ICOSL, ICOS ligand; IL-21R, IL-21 receptor; MHC, major histocompatibility complex; PD-1, programmed cell death protein-1; PD-1L, PD-1 ligand; SAP, signaling lymphocytic activation molecule-associated protein; TCR, T cell receptor; Th1, type 1 helper T cells; Th2, type 2 helper T cells.

the other T-helper subsets—such as Th1, Th2, and Th17 cells that are controlled by T-bet, GATA3, and RORγt, respectively—the differentiation of Tfh cells depends on transcription factor BCL-6.

BCL-6 is an important arbiter of the Tfh-cell lineage fate. It acts as a transcription repressor by binding to the promoter region of T-bet, GATA3, and RORγt, thus suppressing Th1, Th2, and Th17 differentiation,[61,62] and it dominantly directs T cells toward Tfh cells. BCL-6 also affects the signature molecule expression on Tfh cells.[46] Blimp-1 is another transcription factor that acts as an antagonist of BCL-6. The balance between BCL-6 and Blimp-1 in T cells is critical for the fate of Tfh-cell differentiation.[63] By repressing Blimp-1 expression, BCL-6 directs CD4$^+$ T cells toward non-Tfh-cell (Th1, Th2, Th17) differenti-

ation. Of note, BCL-6 is also expressed on GC-B cells and controls GC-B cell differentiation with a set of regulating genes separate from those in Tfh cells.

Tfh CELLS IN THE PATHOGENESIS OF AUTOIMMUNITY

Autoimmune disease is characterized by the production of autoantibodies directing against the patient's own cells, contributing to tissue damage. Research studies have suggested that GCs may be the pathogenic hot spot for the production of autoantibodies in autoimmune diseases. Many lupus-prone murine models show that spontaneous generation of GCs positively correlates with the production of autoantibodies.[64,65] Within GCs, somatically mutated self-reactive B cells are normally
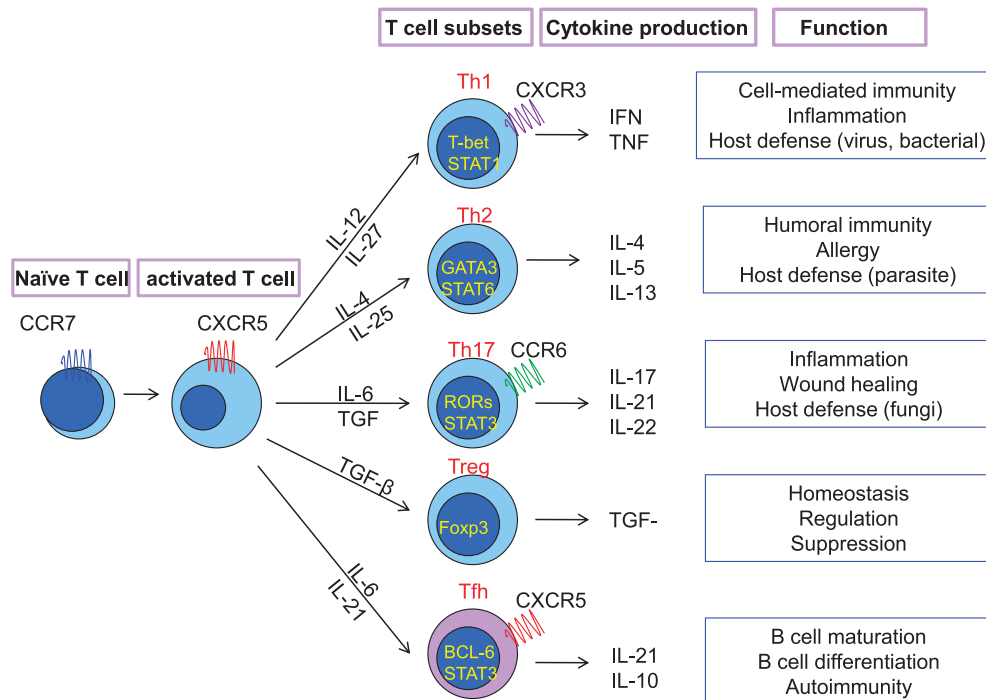
Figure 2. T helper cell lineage development and function. The cytokines produced by dendritic cells regulate the T helper cell lineage (Th1, Th2, Th17, T regulatory [Treg], and follicular helper T cells [Tfh]). Each of them exhibits a unique phenotype, cytokine, and transcriptional profile and exerts different functions in immune response. BCL-6, B cell lymphoma 6 protein; CCR, C-C chemokine recetor; CXCR, CXC chemokine receptor; Foxp3, forkhead box P3; IFN, interferon; IL, interleukin; RORs, retinoid-acid receptor related orphan receptors; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-beta; TNF, tumor necrosis factor.

eliminated at GC reaction via soluble antigen-induced apoptosis without T-cell help. In autoimmune disease, however, with dysfunctional B-cell selection and cognate help from Tfh cells, these self-reactive B cells could be able to survive, expand, differentiate, ultimately produce antibodies, and cause tissue or organ damage.

The contribution of Tfh cells in autoimmune disease has been mainly studied in murine models (Table) that show disease-prone mice to have an aberrantly expanded Tfh population.[66,67] The most striking evidence of Tfh involvement in autoimmunity is from the sanroque mouse model.[67] Sanroque mice have a single recessive mutation in the roquin gene that encodes a RING-type ubiquitin ligase protein that disrupts a repressor of ICOS expression. These mice demonstrated a spontaneous GC formation and SLE-like pathologies, such as anti-dsDNA antibodies, immune-complex mediated glomerulonephritis, and excessive Tfh cells with increased expression of ICOS and IL-21. Furthermore, deficiency of SAP molecules or deletion of an allele of BCL-6 in the sanroque mouse model resulted in significant reduction of Tfh cell numbers and IL-21 production, abrogated GC formation, and ameliorated SLE-like pathological

features.[68,69] Conversely, adoptive transfer of Tfh cells from the sanroque mouse into wild-type recipients induced spontaneous GC formation and autoantibody production,[70] providing direct evidence of the role of Tfh cells in the pathogenesis of autoimmune diseases. Thus, the data from autoimmune mice strongly suggest that excessive Tfh cells are responsible for autoimmunity and the dysregulation of the GC response.

In addition to the number of Tfh cells, the quality of help from Tfh cells also controls the extent of the immune response. Evidence has shown that excessive production of the Tfh signature cytokine IL-21 leads to autoimmunity.[71] Similar to the sanroque mouse model, BXSB mice—in which lupus-like autoimmunity is promoted by excessive signaling induced by self-RNA autoantigens as a result of duplication of the gene encoding toll-like receptor 7 (TLR-7)—also demonstrated spontaneous GC formation with significant Tfh cell expansion and IL-21 production.[72] Blocking IL-21 signaling in BXSB mice and lupus-prone MRL[lpr] mice led to diminished pathogenic autoantibody production and other lupus-like features,[73] indicating that IL-21 is required for

Table. Follicular Helper T (Tfh) Cells and Autoimmune Pathology in Mouse Models and Human Disease

Murine Model or Human Disease	Disease Manifestations	Phenotype of Tfh Cells	Dysfunction Related to Tfh Cells	Blockade or Treatment	Reference
Sanroque mouse (SLE)	Autoantibodies (such as anti-dsDNA Ab, ANA), hypergammaglobulinemia, glomerulonephritis	$CD4^+CXCR5^+PD\text{-}1^{hi}ICOS^{hi}$	GC expansion, increased IL-21 level	Null allele of SAP deletion	66, 67
BXSB-Yaa mouse (SLE)	Anti-ANA, dermatitis, hypergammaglobulinemia, glomerulonephritis	$CD4^+CXCR5^+PD\text{-}1^{hi}ICOS^{hi}$	GC expansion, increased expression of IL-21and ICOS	$IL\text{-}21^{-/-}$ mice	68
MRL^{lpr} mouse (SLE)	Autoantibodies, hypergammaglobulinemia, glomerulonephritis	$CD4^+CXCR5^+ICOS^{hi}$	GC expansion, increased expression of ICOS	IL-21R-Ig	70
NZB/WF1 mouse (SLE)	Anti-dsDNA, hypergammaglobulinemia, glomerulonephritis	$CD4^+CXCR5^+ICOS^{hi}$	Tfh expansion in the extrafollicular sites	ICOS-B7RP-1 Ab	71, 74, 75
Collagen-induced arthritis mouse (RA)	Anti-ANA, anti-dsDNA, hypergammaglobulinemia	$CD4^+CXCR5^+ICOS^{hi}$	GC expansion, increased expression of IL-21 and ICOS, increased GC-B cell number	CTLA-4-IgFc blockage costimulation signal	73
SLE patient	Anti-dsDNA, hypergammaglobulinemia, glomerulonephritis	$CD4^+CXCR5^+PD\text{-}1^{hi}ICOS^{hi}$	GC expansion, increased level of circulating Tfh cells		10, 11
SS patient	Anti-SSA, anti-SSB, hypergammaglobulinemia, glomerulonephritis	$CD4^+CXCR5^+PD\text{-}1^{hi}ICOS^{hi}$	Increased level of circulating Tfh cells, increased PD-1 expression in salivary lymphocytes		10, 12
JDM patient	Skin lesions	$CD4^+CXCR5^+$	Increased level of circulating Tfh cells		13
RA patient	Autoantibodies, joint inflammation	$CD4^+CXCR5^+ICOS^{hi}$	Increased level of circulating Tfh cells		14, 15

ANA, antinuclear antibody; B7RP-1 Ab, B7-related protein-1 antibody; CTLA-4, cytotoxic T-lymphocyte antigen 4; CXCR, C-X-C chemokine receptor; GC, germinal center; GC-B, germinal center B cells; ICOS, inducible T-cell costimulator; IgFc, immunoglobulin Fc region; IL, interleukin; JDM, juvenile dermatomyositis; PD-1, programmed cell death protein 1; RA, rheumatoid arthritis; SAP, signaling lymphocytic activation molecule-associated protein; SLE, systemic lupus erythematosus; SS, Sjögren syndrome.

autoreactive B cell differentiation and that blocking IL-21 could be a potential therapy to treat SLE.

Studies from other lupus-prone mice give further insight into how Tfh cells help support autoantibody production.[70] Unlike the spontaneous GC formation observed in sanroque mice, plasmablasts in MRL[lpr] mice form extrafollicular foci lacking GCs and their Tfh cells are located in extrafollicular sites. Tfh cells in both extrafollicular and GC sites involving the lupus pathogenesis were observed in lupus-prone NZB/WF1 mice.[71] Their Tfh cells also expand beyond GCs to extrafollicular sites, exaggerating the lupus progress.

While the role of Tfh cells in autoimmunity has been established in mice, the association of Tfh cells with human autoimmune diseases remains largely unexplored. The fact that many pathogenic high-affinity anti-DNA antibodies detected in patients with autoimmune diseases exhibit heavy chain class switching and somatic hypermutation[72] suggests the involvement of GC-Tfh or Tfh-committed extrafollicular cells in the pathogenesis of autoimmune diseases. Recently, we and others discovered the expansion of circulating Tfh cells in patients with juvenile dermato-myositis,[13] rheumatoid arthritis,[14,15] SLE,[10,11] and Sjögren syndrome.[10] Furthermore, our recent study showed that the accumulation of circulating Tfh cells strongly correlated with high levels of plasmablasts, anti-dsDNA, and antinuclear antibodies, as well as the severity of disease activity in patients with SLE.[11] These observations suggest that the expanded circulating Tfh cells may act as the key mediators of autoantibody production and the pathogenesis of lupus. Our data also show that the circulating Tfh cells in patients with lupus and the synovial fluid in patients with rheumatoid arthritis shared similar phenotypic and functional properties with GC-Tfh cells in the follicles, indicating circulating Tfh cells derived from GC-Tfh cells and shuffling between peripheral blood and lymphoid tissues.

CONCLUSION

Progress has been made in understanding the immunobiology of Tfh cells. Tfh cells have emerged as distinct subsets of T-helper cells that provide critical help to B cells and play a critical role in GC generation and maintenance of humoral immune responses. Growing evidence also has implicated Tfh cells in the development of autoimmune disease. Currently, no effective anti-Tfh-cell antibody or inhibitor has been applied to the patients with autoimmune diseases. A deeper understanding of the role and the origins of Tfh cells will help advance translational research and lead to the development of a novel therapeutic protocol for autoimmune disease.

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