Review Article

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Essential Transcription Factors and Functional Roles of Follicular Helper T Cells in Human Autoimmune Diseases

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ABSTRACT

Follicular helper T (TFH) cells are a subset of effector CD4+ T cells that support the differentiation of antigen-specific B cells in the germinal center. TFH cells are distinct from other established CD4+ T cell subsets and possess a list of transcription factors, including BCL6, IRF4, c-Maf, Batf, NFAT1-2, and STAT3. The mentioned factors direct several activities such as cell differentiation, migration to the follicles, cell-to-cell interaction, as well as cell programming. Given that TFH cells are essential for the germinal center formation, affinity maturation and the development of most high-affinity antibodies. TFH cells may play crucial roles in different pathologic conditions, particularly autoimmune diseases. However, the mechanisms that cause functional differences of TFH cell responses are not exactly defined. In this review first the immunological profile of TFH cells will be discussed then attempts will be made to give a broad picture on the role of this key subset of T cells in autoimmune diseases.

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INTRODUCTION

CD4$^+$ T follicular helper (TFH) subsets participate in B cell development by providing signals directed to B cells located in the germinal center (GC) (1). TFH-B cell interaction occurs in the inter-follicular regions of GC and is crucial for efficient immune responses. Moreover, it

can promote autoimmune diseases. TFH cells express different transcription factors, which are critical for directing them to the GC and presenting high-potential humoral responses (2). These different transcription factors potentially provide distinct cell-to-cell interactions, modulating the nature of immune responses in physiological and pathological conditions (3). Heterogeneities in the TFH populations and their cognate cells will delineate the fates of the humoral immune response, which may develop a wide spectrum of autoimmunity since TFH cells are the dominant cells in GCs to help B cells synthesize antibodies. On the other hand, autoantibodies induce autoimmune cells by a tissue-specific manner; thus, there is a close relationship between TFH cells and autoimmune cells. In this paper, we look at the data that specifies the transcription pattern of different subsets of TFHs, including the significance and functionality of these cells in GC formation and the creation of high-affinity antibody-producing B cells. Furthermore, we will explore how these subsets may be engaged in diverse autoimmunity illnesses. Finally, we will present how TFH subsets can be used as a target cell in the treatment of various autoimmune disorders.

Search Strategy

We searched Scopus, PubMed, ScienceDirect, EMBASE, and Web of Science to retrieve studies conducted on TFH cells in all autoimmune diseases from 1 March 2010 to 1 Dec 2021. Three authors (S.I., S.T., and F.A.) independently assessed the titles and abstracts of all selected papers, and then all retrieved papers were screened again by two authors, focusing on the complete text of the articles. Two authors (A.S. and Sh.Kh.) addressed the conflicts on the eligibility of articles, and if they could not reach an agreement, a third author (MS) assisted in building a better conclusion.

Crucial Transcription Factors Necessary for the Development of TFH Cells

TFHs, a subset of $CD4^+$ T cells located in follicles, were discovered in the early 2000s. However, TFHs were not globally welcomed until the TFH cell lineage-specific transcription factor, B-cell lymphoma 6 protein (BCL6), was identified. BCL6–deficient T cells are reported not to be able to differentiate into follicular helper T cells, indicating the importance of BCL6 in determining TFHs fate. In addition, CXCR5 high expression and CCR7 low expression make T cells enter and locate in the germinal centers (GCs). Dendritic cells (DCs) contribute to differentiation into pre-TFH cells with phenotype $PD\text{-}1^{high}CXCR5^{hig\ h}SAP^{high}CCR^{low}PSGL^{low}$ influenced by antigen stimulation and expression of CD80, CD86, and ICOSL. However, the ultimate step of TFHs' differentiation is controlled by a complicated cocktail of transcription factors, e.g., the BCL6-Blimp1 axis, signal transducer, and activator of transcription (STAT) proteins, e.g., STAT1 and STAT3-5b, B-cell activating transcription factor (Batf), c-Maf, interferon regulatory factor 4 (IRF4), Achaete-scute homolog 2 (ASCL2), T-cell-specific transcription factor 1 (TCF-1), and lymphoid enhancer-binding factor (LEF-1) (4, 5).

The transcription factors complex is involved in the process of cell differentiation of TFH cells, all of which contribute to the determination of the fate of T cells between the TFH and non-TFH groups. However, other transcription factors are introduced that may affect TFH cell differentiation. A new study revealed that deficiency in nuclear factor of activated T cells (NFAT) proteins, including NFAT1 and NFAT2 proteins in CD4+ T cells, might result in an impaired germinal center formation upon viral infection because of reduced TFH cell differentiation and defective expression of proteins such as PD-1, ICOS, and SLAM family receptors. ChIP-seq data indicated that NFAT proteins possibly regulate the genes crucial to TFH cell differentiation and activities (6). Another study demonstrated that NFAT proteins may regulate TFH cell differentiation through IRF4 expression and metabolic reprogramming of $CD4^+$ T cells (7). Moreover, OX40/OX40L

axis may regulate TFH cell differentiation, maintain cell survival, and promote the helper function of TFH cells for B cells (8). Some of the key transcription factors contributing to the differentiation of the TFH cells are described in Table 1.

BCL6

BCL6 is a major factor in the formation and evolution of TFH cells, B cells, the formation of the germinal centers and plays a role in differentiating TFH cells and inhibiting differentiation towards other T cell lines (9). BCL6 decreases P selectin glycoprotein ligand 1 (PSGL1), the binding protein to CCL19 and CCL21, resulting in a decrease in the expression level of this molecule, and this change in the expression pattern of PSGL1 is associated with the increase of PD1 and CXCR5 molecules, which have a key designation in TFH development (10). BCL6 influences the movement of TFH cells through the effect on the promoter of genes involved in the migration of T cells (9, 11, 12). It increases the expression level of PD1 and CXCR5. Differentiation of TFH cells is independent of the expression of IL-6 and IL-21 cytokines. Although these two cytokines are important for the evolution of $BCL6^+$ TFH cells *in vivo*, it is not required that they be expressed. (11).

Unlike Blimp-1, which acts as an antagonist of BCL6, it prevents the differentiation of TFH cells, thereby preventing the formation of B cell response and antibody production in GC. So BCL6 and Blimp-1 have the opposite effects and play a key role in the differentiation of TFH cells and subsequent follow-up of the response of B cells (13, 14). During the TFH differentiation process, BCL6 has an

Table 1. Transcription factors that are involved in differentiation, development, and signaling pathways of TFH cells

Transcription Factors	Mechanisms	References
NFATs	*Germinal center formation upon viral infection *Regulate the expression of proteins such as ICOS, PD-1, and SLAM family receptors *Regulate IRF4 expression and metabolic reprogramming of $CD4^+$ T cells	(6-7)
BCL6	↓STAT5/IL7R signaling → Differentiate info TFH cells ↑PD1, ↑CXCR5, ↓PSGL1 → Differentiate info TFH cells	(12-14)
ASCL2	↑CXCR4, ↑CXCR5, ↓CCR7 → Differentiate info TFH cells ↓PSGL1, ↓IL21 → Differentiate info TFH cells *Interaction with Id3: Role in onset of the evolution of TFH cells	(16)
c-Maf	*Increasing the production of IL-21 *Increasing the expression of CXCR5, PD1, ICOS, CXCR4 *Affecting on expression of IL-4 and allergic responses	(19)
TCF-1 and LEF	↑IL6Rα, ↑gp130, ↑ICOS, ↑BCL6 → Differentiate info TFH cells *Response of CD4+ naïve cells to signals of TFH *Promote the TFH differentiation during acute viral infections	(21, 23)
BatF	*Controlling the expression of BCL6 and c-Maf Differentiate into TFH cells *Affecting on expression of IL-4 and allergic responses	(22-24)
FOXP1	*IL21 → Regulation the process of TFH differentiation, but no essential for the evolution of TFH *Increase the maintenance of the silent of naïve cells by affecting on ERK/ MEK signaling	(25-26)
IRF-4	*Forming complexes with other transcription factors including BatF, BCL6, and STATs *Formation of germinal centers and TFH differentiation	(32, 34)

Achaete-scute homolog 2 (ASCL2; B-cell activating transcription factor (Batf); B-cell lymphoma 6 protein (BCL6); interferon regulatory factor 4 (IRF4); T-cell-specific transcription factor 1 (TCF-1); forkhead box P1 (FOXP1); lymphoid enhancer-binding factor (LEF-1)

antagonist effect on the STAT5/IL-7R, so the gene deletion of BCL6 in T cells leads to an increase in STAT5/IL-7R signaling and significant expression of $CD127^+$ in non-TFH cells (14, 15). STAT5 and BCL6 both bind to a similar locus. Non-TFH cells are dependent on IL-2, IL-7, and P-STAT5, which bind to their receptors, including SOCS2, IL-7R, and TCF-7, respectively; nevertheless, BCL6 can decrease the expression of STAT5 in TFH cells because BCL6 can bind to this locus (14, 15). Therefore, the expression of Blimp-1, STAT5, and IL-2 has a negative regulatory impact on the differentiation of TFH cells.

ASCL2

Although BCL6 is an important and impressive molecule in TFH function, it does not provoke migration, but rather increases the CXCR5. Instead, the ASCL2 molecule, a basic helix-loop-helix (bHLH) structure, has a pivotal impact on the migration of TFH cells (16). Both BCL6 and ASCL2 play a key role in the differentiation of T cells towards TFH and inhibit the differentiation of Th1, Th2, and Th17, thereby increasing the expression of CXCR4 and CXCR5, while CCR7, PSGL1, and IL-21 signaling declined. ASCL2 promotes the expression of CXCR5, whereas reduces CCR7. ASCL2 is shown to increase the expression of Cxcr5-mRNA (up to about 60-fold) but has no significant effect on the expression of Cd40Ig, Sh2d1a, BatF, Prdm1, Btla, Pdcd1, BCL6, IL-21, and ICOS genes. Meanwhile, the expression of ASCL2 in $CD4^+$ T cells decreases CD122, CD25, PSGL-1, and CCR7expression (16).

TCF1 and LEF

TCF1 and LEF proteins are transcription factors containing conserved high-mobility group (HMG) sequences and binding regions. Also, these two molecules exert a prominent role in the early stages of the evolution of the T cells, including the double negative and double-positive T cells; ($CD4^-$ $CD8^-$ (17, 18) and $CD4^+$ $CD8^+$ (19, 20), respectively. The elimination of these two transcription factors leads to a defect in the differentiation of TFH cells and disturbance in the formation of the germinal centers. However, the mandatory expression of LEF-1 enhances the differentiation of TFH cells (21). In addition to the function of TCF1 as a substantial transcription factor in triggering the differentiation of TFH cells, this molecule is established to increase the activity of differentiated TFH cells during acute viral infections. TFH cells highly depend on TCF1-intrinsic HDAC activity to suppress CTLA4 and guard B cell function. Further, TCF1 and LEF1 transcription factors have prominent roles in preventing further induction of CTLA4 and LAG3 coinhibitory receptors in TFH cells, thereby preserving the activation of B cells (22).

It expresses BCL6 and inhibits the expression of Blimp-1 by direct connection to the promoter BCL6 and regulatory region 5' of the Prdm-1 gene (23). In general, the effect of these two transcription factors on cellular differentiation is accomplished by two common mechanisms: first, they promote the response of $CD4^+$ naive cells to signals from TFH cells. Second, they express the continuous expression of IL-6Rα, gp130 cytokines, and the high expression of ICOS, the growing expression of BCL6, which enhances the differentiation of TFH cells in the early stages (23, 24). In addition, the virus-specific Th1 and TFH cells in viral infection, express TCF1 and Blimp-1 in the early stages even before the expression of the CXCR5 expression, the expression of TCF1 had a negative effect on the expression of IL-2 and Blimp-1 that play an essential role in the preservation, development, and differentiation of TFH cells (24).

FOXP1

FOXP1 is a member of the family that has four different isoforms, FOXP1A, FOXP1B, FOXP1C, and FOXP1D, and are expressed in almost all the tissues. FOXP1 is a key regulator factor that plays a fundamental role in the development of thymocytes and the production of silent naïve cells, so the defect

in the FOXP1 factor leads to the abnormal development of cells (25). FOXP1 directly induces the expression of IL-21, balances the expression of ICOS and downstream signaling in the early stages of T cell activity. $TCD4^+$ cells with FOXP1 deficiency during the TFH evolution process have relative resistance to blockade of ICOSL; it may regulate TFH differentiation (26). The two FOXP1A and FOXP1D isoforms produce a "double-check" mechanism, induced by the stimulation of TCR and regulates the differentiation of TFH cells and humoral responses (27). In addition, the FOXP1 has partially retained the silence of naïve cells by the regulation of the MEK and ERK kinase pathways (28). The MEK-ERK signaling pathway has a major role in inducing ICOS expression, and this activity is triggered by TCR (26).

BATF

BATF is a protein of the family of activator protein-1 (AP1) that has the basic leucine zipper structure. This transcription factor forms a molecular complex consisting of BATF-JUN by building to the JUN factor, which plays a pivotal role in the formation of the germinal centers (28). In BATF-deficient mice, the number of B cells is normal; however, the number of subgroups of $TCD4^+$ cells, inducing Th2, Th17, and TFH, reduces. Finally, these mice are interrupted in the formation of the germinal centers resulting in an impaired maturation of B cells (28). BATF also controls the expression of BCL6 and c-Maf in T cells, both of which are essential for the development of TFH cells. In addition, BATF directly controls the expression of activation-induced cytidine deaminase (AID) and C_H-I_H in B cells and also controls the class-switching recombination (CSR) process *in vivo* (29).

On the other hand, the expression of IL-4 in TFH cells also depends on the BATF, so c-Maf and BATF signaling are the dominant factors for expressing IL-4 in TFH cells. Therefore, the impairment of BatF affects the production of IL-4 in TFH cells and defense in allergic asthma (29). BATF expression in TFH cells is induced by signaling IL-4-STAT6 and IL-6-STAT3 (30). Thus, IL-4 and IL-6 trigger the production of BATF in the TFH cells via the STAT6 and STAT3 pathways, respectively. The BATF functional mechanism in this process depends on the formation of the IRF4-BATF complex, which binds and activates the CNS2 locus on IL-4, then facilitates the production of IL-4 in TFH cells through STAT3 and STAT6 signaling (31). In addition, c-Maf has a positive influence on IL-4 production in TFH cells by attachment to CNS2 areas and inducing transcription of this region (15).

IRF4

IRF4 is a major transcription factor of the interferon regulatory factors expressed in most immune cells. IRF4 is known to be a lymphocytic-specific nuclear factor that leads to $TCD4^+$ cell differentiation toward Th2, Th9, Th17, and TFH, as well as in the differentiation of $TCD8^+$ naïve cells toward TC9 and TC17, producing IL-9 and IL-17, respectively. Additionally, regulatory T cells (Tregs) require IRF4 expression to perform their function. Therefore, IRF4 plays a chief role in guiding immune responses managed by T cells *in vivo* (32). The importance of IRF4 in controlling the differentiation of Th2 and Th17 cells is very impressive; thus, the differentiation of Th17 cells is impaired in $IRF4^{-/-}$ mice, resulting in a multiple sclerosis-resistant mouse model (33).

As mentioned above, IRF4 also interferes with the function of Tregs, thus the systemic autoimmune diseases are associated with defects in the expression of IRF4 in Tregs. Further, IRF4 interferes with the differentiation of TFH cells, the formation of the germinal center, and the survival of lymph nodes during the immune responses (32, 34). IRF4 forms complexes with other transcription factors, inducing BatF, BCL6, and STATs. Differentiation of TFH cells and the formation of the germinal centers were disturbed in the lymph nodes, and the

germinal centers were not formed in Peyer's patches of IRF4$^{-/-}$ mice (34, 35). Although IRF4$^{-/-}$ mice may express other transcription factors, these transcription factors cannot exert their effects by differentiating TFH cells, thus GC centers are not formed naturally.

c-Maf

c-Maf is another transcription factor highly expressed in Th17 and TFH cells. The genetic deletion of c-Maf leads to a defect in the production of IL-21 and a decrease in the number of Th17 and TFH cells. Moreover, it regulates ICOS production. The mechanism of the c-Maf function is that this factor, and through the MARE regions, is capable of activating IL-21P (19). C-Maf can interact with the BCL6 transcription factor. However, both c-Maf and BCL6, together, enhance the synergistic expression of CXCR5. In addition, the BCL6 and c-Maf factors have a synergistic effect on the expression of some genes belonging to the TFH (e.g., PD1, ICOS, and CXCR4). On the other hand, c-Maf has a positive effect on the production of IL-4 in TFH cells when linked to CNS2 regions, coupled with this region's induction of transcription (15).

Chemokine Pattern and Signaling of TFH Cells

Some chemokine receptors such as CXCR3 and CCR6 are used to identify Th1-like TFH (CXCR3^{+} CCR6^{-}), Th17-like TFH (CXCR3^{-} CCR6^{+}) and Th2-like TFH (CXCR3^{-} CCR6^{-}), all of which express transcription factors and cytokines such as IL-21 TBX21, IFNγ, GATA-3, IL-4, IL-5, IL-13, ROR-C, IL-17A and IL-22 (Figure 1). Th17-like, and Th2-like TFH cells (not Th1-like TFH) secret IL-21 (36). TFH cells express surface molecules that play a functional role concerning B and T cells. Moreover, some molecules, such as ICOS, PD1, IL-4, IL-21, and CD40-L that potentially induce differentiation, growth, and class switching of B cells (37), are secreted by TFH. Also, TFH needs to express simultaneously CXCR5 to leave the T-rich region and locate alongside the B cells of the follicle and reduce the expression of the

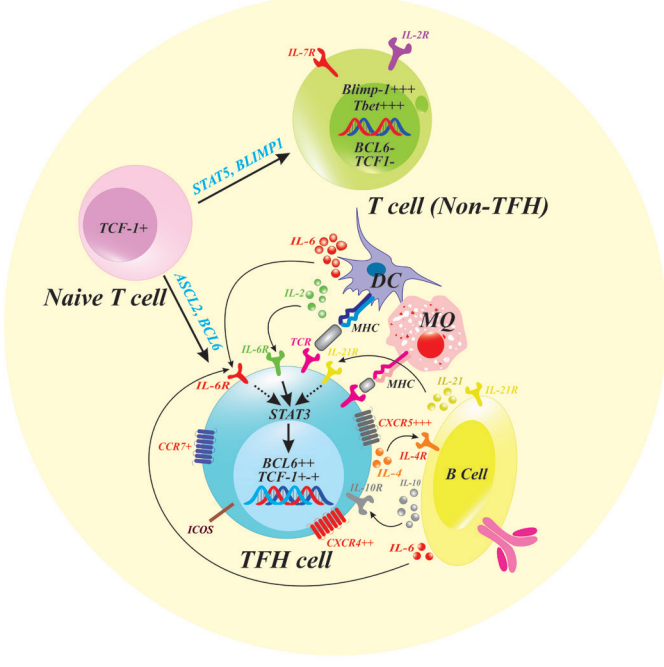

Figure 1. TFH cell is differentiated into TH-like TFH in the germinal center under the influence of IL-6, IL-21, ICOS, which is BCL6^{+}, CXCR3^{+} and, unlike TH1 expresses the low level of T-bet. TFH cells can also differentiate into TH2-like TFH under the influence of IL-6, IL-21, and ICOS, which is BCL6 $^{+}$. TH2-like TFH expresses less GATA3 than TH2 cells. TH17-like TFH is another differentiated cell derived from TFH and is BCL6^{+} and CCR6^{+} but low in RORg3. In addition, TH2-like and TH1-like TFH cells may produce high concentrations of IL-21 but TH1-like TFH cells do not.

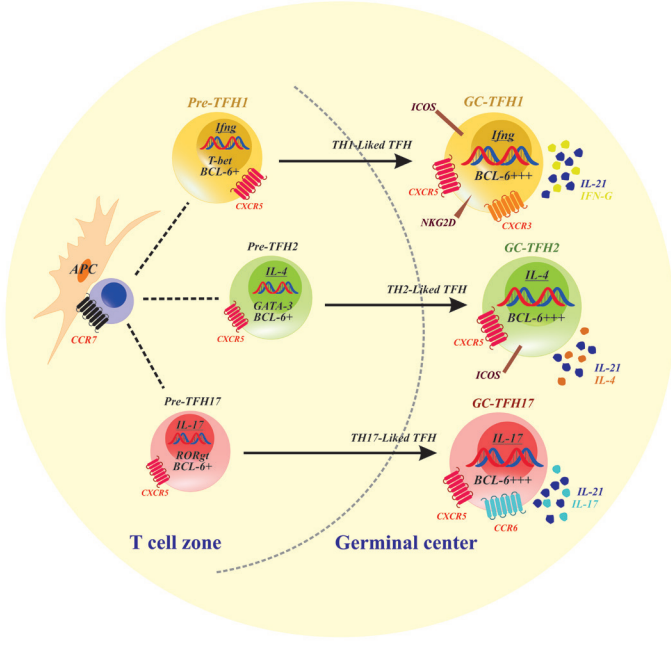

Figure 2. The naive T cells are differentiated into TFH cells in the germinal center under the influence of IL-21, IL-6 and the expression of ASCL2, BCL6, which is BCL6 $^{+}$, ICOS $^{+}$, PD1 $^{+}$, CXCR5^{hi}, CXCR4 $^{+}$, CCR7^{low}. However, naive T cells are differentiated into non-TFH cells under the influence of IL-7, Blimp1, and STAT5 and are characterized by IL-7R, IL-7, and IL-2R. Moreover, it represents molecules engaged in the differentiation of TFH cells, dendritic cells (DCs), and B cells that stimulate TFH cells, and crosstalk of TFH cells with both of them.

T-cell homing chemokine receptor (CCR7) (Figures 1, 2) (38-40). TCD4^{+}CXCR5^{+} is known as circulating TFH (41) which exhibits a high level of IL-21 and is more effective in inducing differentiation than TCD4^{+}CXCR5^{-} (36, 42). So in humans, TFH cells have two forms: the secondary lymphatic organ TFHs and circulating ones. Circulating Tfh (cTfh) cells are classified into two separate subgroups: effector memory Tfh cells (PD-1^{+}ICOS^{+}CCR7^{low}BCL6^{+}CXCR5^{+}CD4^{+}T cells) and central memory Tfh cells (PD-1-ICOS-CCR7^{high}BCL6^{+}CXCR5^{+}CD4^{+}T cells). Furthermore, regarding the expression of CXCR3 and CCR6, cTfh cells are further subdivided into four phenotypes, including Tfh2 (CXCR3^{-}CCR6^{-}), Tfh1 (CXCR3^{+}CCR6^{-}), [a transitional state of Tfh1/17 (CXCR3^{+}CCR6^{+})], and Tfh17 (CXCR3^{-}CCR6^{+}) cells that represent specific cytokines and T-cell specific transcription factors of Th2 (IL-4, IL-5, IL-13; GATA3), Th1 (IFN-γ; T-bet), and Th17 (IL-17 and IL-22; RORγt) cells, respectively (43).

These two cell forms have a key difference, namely, the lack of expression of BCL6 in peripheral blood TFH (42, 44, 45) (Figure 1). *In vitro* studies have shown that TCD4^{+}CXCR5^{+} derived from the circulatory system and the germinal center induce antibody production in co-culture with B cells (36, 42, 46).

Some studies show that human circulating TFH generally have CD4^{+}CXCR5^{+}, but other studies have introduced subtypes of T CD4^{+}CXCR5^{+}, including TCXCR5^{+}ICOS^{+}, TCXCR5^{+}ICOS^{high}, TCXCR5^{+}PD1^{+}, T CXCR5^{+}PD1^{high}, TCXCR5^{+}CD57^{+}, TCXCR5^{+}ICOS^{+}PD1^{+} (41, 42, 47, 48). While in another study, they did not use a CXCR5 marker to detect TFH in circulation and , but rather applied tissue TFH markers such as ICOS and IL-21 (49, 50). TCD4^{+}CXCR5^{+} is a peripheral blood cell being long-lived and has functional properties of TFH, so this cell is considered the memory TCD4^{+}CXCR5^{+} whose place is in the peripheral blood (51). Most of the blood memory TFH cells express CD62L and CCR7, which are central memory TCD4^{+}

markers (45, 52, 53) and have a high expression on the secondary lymphatic endothelial cells. These molecules on the peripheral blood memory TFH are possibly useful for the migration of these cells toward secondary lymphatic organs. In contrast, GC TFH does not express CCR7 (40). Besides, peripheral blood TFH does not express CD69 and ICOS markers (41, 54). However, as soon as these cells are activated, they rapidly express the CD69, and the ICOS induces the production of IL-10, IL-21, and CXCL13 (42, 44).

As previously stated, TFH can express ICOS, which is an essential molecule for the proliferation of TFH (11, 17, 55). The ICOS signal, received through dendrite cell in the T-rich region, induces BCL6 in TFH (11), while the ICOS signal received from the follicular B cells at the T-B interface induces the migration of TFH to the follicle (56). ICOS as a stimulant molecule is essential for inducing IL-21 production (57, 58). In humans and mice, ICOS defects result in a significant reduction in GC and TFH, followed by the disruption in antibody production and class-switching abnormalities (Figure 2) (11, 17). Individuals with an ICOS defect had impairment in maintaining and up-regulation of the expression of CXCR5 in T cells. Therefore, ICOS signaling is essential for the preservation and survival of $CXCR5^+$ TFH cells (55). Additionally, the SAP signaling (adapter SLAM-associated protein) plays an important role in the sustained interaction between B and T, which is necessary to differentiate TFH (59). The BCL6 signaling in human TFH induces the expression of CXCR5, ICOS, PD1, SAP, CD40L, and CXCL13 molecules, and interestingly, unlike TFH in the germinal center (GC TFH), BCL6 is not expressed in peripheral blood TFH (59, 60). Many studies have been conducted on the presence of circulating TFHs and their role as an important biomarker in various types of autoimmune diseases. The number of these cells or the level of autoantibodies is associated with the severity of these diseases (61). We shall continue to explain the contribution of TFH cells to the pathogenesis of autoimmune diseases.

Autoimmune Disease

Myasthenia Gravis

Myasthenia gravis is an autoimmune disease, affecting acetylcholine receptors that interfere with muscle and neuromuscular involvement. Acetylcholine receptors are attacked by auto-antibodies, leading to the blockade of neuronal transmission and anti-muscle Ab (antibody) tyrosine kinase Anti-musk Ab, following this incidence, symptoms including muscle weakness and early weariness emerge. Regarding the role of T cells in the production of antibodies, the use of thymectomy and disruption in T $CD4^+$ cell-mediated immunosuppression drugs have culminated in some beneficial therapeutic outcomes (62). Furthermore, there is indeed a significant body of proof that TFH cells play an important role in the development of MG. Functional deficiency in TFH cells may result in abnormal positive selection of self-reactive B cells, possibly leading to autoimmune diseases such as MG. Moreover, the frequency of circulating TFH cells directly correlates with the level of anti-AChR Ab (63, 64). TFH cells express other molecules, including BCL6, ICOS, PD1, IL-21, and CD57 as their major markers, which increase the number of these cells in autoimmune diseases such as MG (65, 66). However, the association between the population of $TCD4^+CXCR5^+$ cells in human peripheral blood and GC-TFH cells is still unclear.

Myasthenia gravis patients increased the number of $CD4^+$ cells that express CXCR5, and the severity of the symptoms will increase as the number of these cells increases (64). Simultaneously, the expression level of its chemokine receptor, CXCL13, also increased in the epithelial and endothelial cells of the thymus. Some T $CD4^+CXCR5^+$ T cells also express a high level of PD1 and ICOS in the bloodstream, introduced as functional TFH in the bloodstream. Additionally, the number of $PD1^{high}TCD4^+CXCR5^+$ and $TCD4^+CXCR5^+$

ICOS^{high} cells increase during the growing trend of the diseases and auto-antibody production. Following successful treatment and improvement of the disease, the abundance of these cells significantly decreases (67). On the other hand, the serum level of CXCL13 also increases in patients with MG, as the level of serum CXCL13 also enhances as the disease progresses. Therefore, CXCL13 is a key chemokine in the autoimmune MG and can be used as a therapeutic target to provide desirable results in controlling autoimmune diseases, especially MG (68, 69). In patients with MG, the expression of IL-21 mRNA in PBMCs consistently increases with serum CXCR5^{+}CXCL13^{+} CD4^{+} T cells, but no significant increase is observed in the expression of BCL6 in PBMC (63). A study found that the rate of TCD4^{+}PD1^{+} and TCD4^{+}ICOS^{+} cells in patients with Myasthenia gravis (MG) were not significantly different compared with the control subjects. However, previous studies demonstrated that the percentage of TCD4^{+}CXCR5^{+}PD1^{high} cells and TCD4^{+}CXCR5^{+}ICOS^{high} elevated in peripheral blood of patients with MG compared with the healthy controls (63).

Juvenile Diabetes Mellitus

Juvenile Diabetes Mellitus (JDM) is a rare chronic disease, especially in childhood, and is a systematic autoimmune disease that involves multiple organs, including muscle and gastrointestinal tract and other organs, but mainly affects the skin and the proximal muscles. These patients show some clinical manifestations, including muscle weakness, early fatigue, as well as skin rashes caused by autoimmune responses (64, 70). Extensive studies on JDM show several common mediators in JDM, including IFN-I and autoantibodies in the serum. Although there is limited information about JDM and its pathogenesis, some studies revealed that the frequency of Th1CD4^{+}CXCR5^{+} cells decreased significantly, while Th2 and Th17 cells increased, leading to an increase in Th2-liked and Th17-liked cells (Figure 1) (71). Following this differentiation, the number of CD19^{+}CD20^{+}CD27^{+}CD38^{++} plasma blasts increased in the circulation; consequently, the IgG serum antibody concentration increased (72).

In these patients, the frequency of CD19^{+} plasma blasts is related to the differentiation of ThCXCR5^{+} cells toward Th2 and Th17 cells, while it has a negative correlation with the frequency of Th1CXCR5^{+} cells. This predominant Th1CXCR5^{+} cell population causes symptoms of JDM disease, including skin rash and muscle weakness (Figure 1) (71). Conversely, the frequency of plasma blasts is not associated with the frequency of CXCR5^{+} or CXCR5^{+}ICOS^{+} CD4^{+} T cells. Additionally, T cell CD4^{+}CXCR5^{-} and Th1CXCR5^{-} subtypes are not associated with the frequency of plasma blasts. In general, in JDM patients, alterations in the cell subtypes in ThCXCR5^{+} cells are associated with increased circulation of plasma blasts, and the occurrence of symptoms is associated with the disease.

Autoimmune Thyroid Disease (ATD)

Autoimmune thyroiditis is caused by an autoantibody against thyroid tissue, leading to hyperthyroidism. Graves and Hashimoto are two types of autoimmune thyroid disease in which TCD4^{+} cells contribute to B cells in the production of autoimmune antibodies. In Graves' disease, the production of antibodies against the TSH receptor leads to hyperthyroidism, whereas in Hashimoto's, autoantibodies act against the thyroid tissue, leading to its tissue destruction and hypothyroidism (62). A stimulating antibody against the thyroid results in hyperthyroidism due to Graves' autoimmune disease, while Hashimoto's disease produces antibodies against Thyroid peroxidase (TPO) and Thyroglobulin (Tg), along with inflammation and loss of function of the glands secreting Thyroid hormones. Although graves patients have a positive relationship with the percentage of TFH cells and serum free T3 and T4 levels in serum, the percentage of TFH

cells decreases after treatment. In Graves' patients, the number of $CXCR5^{+}ICOS^{high}$ $CD4^{+}$ T cells expressing BCL6 and IL-21 also increased. Interestingly, despite the different clinical symptoms of Hashimoto and Graves' diseases, the frequency of $CXCR5^{+}PD1^{high}$ $CD4^{+}$ T cells and $CXCR5^{+}ICOS^{high}$ $CD4^{+}$ T cells increase in both autoimmune diseases. In some patients with Graves, the number of $CXCR5^{+}ICOS^{high}$ $CD4^{+}$ T cells reduced six months after treatment with an autoantibody-based drug (63). Although the levels of $CXCR5^{+}ICOS^{high}$ $CD4^{+}$ TFH cells increase in patients with autoimmune thyroid, there is no significant increase in $CXCR5^{+}ICOS^{high}$ $CD4^{+}$ T cells. Thus, the population of $CXCR5^{+}ICOS^{high}$ $CD4^{+}$ T cells may be distinct from $CXCR5^{+}$ and $ICOS^{+}$ $CD4^{+}$ T cells, establishing the principal role of the TFH cells in autoimmune diseases such as ATD.

Sjogren's Syndrome

Sjogren's syndrome (SS) is known as a systemic autoimmune disease in which lymphocytes accumulate in secondary lymph nodes in the salivary glands and produce chemokines such as CXCL13 by epithelial cells of the gland. This chemokine must involve the homing of B cells and some of the subgroups of T cells activated in the secondary lymph nodes located in the salivary gland (65). Identifying CXCL13 and the CXCR5 chemokine receptor in the salivary glands demonstrates that the interaction between these molecules may participate in inflammation during the recruitment of B and T cells. In addition, the formation of the new germinal centers may be a cause of chronic inflammation in patients. Interestingly, in patients with SS, the level of autoantibody production increases with the frequency of $CXCR5^{+}$ $CD4^{+}$ T cells (65). In patients with SS, the frequency of Th17 cells with similar TFH-like properties increases in the peripheral blood, in which these cells have T $CD4^{+}CXCR5^{+}CCR6^{+}$ phenotype and uniformly express the high levels of BCL6 protein. Excessive expression of IL-21 in TFH cells distinguishes Th17-liked TFH cells from Th17 cells. Thus, despite the high expression of BCL6 and IL-21, Th17 cells can interfere with the proliferation of B cells and somatic mutations in the germ cells and can also affect antibody-related immune responses (73). Therefore, the frequency of TFH Th17-like cells, which have a $CD4^{+}CXCR5^{+}CCR6^{+}$ phenotype, is related to the synthesis of autoantibodies and SS progression; consequently it can be used as a biomarker to evaluate the various stages of SS or can be used as a targeted therapy (66). However, TFH Th17-like cells do not express IL-21 and cannot stimulate B cells to produce antibodies (Figure 1).

The spontaneous expression of PD1, ICOS, and IL-21 on the circulating T $CD4^{+}$ $CXCR5^{+}$ cells triggers the production of antibodies in B cells. Therefore, the frequency of the $CXCR5^{+}PD1^{high}$ TFH cells, $CXCR5^{+}$ ICOS high TFH cells, and $CXCR5^{+}$ TFH cells in the blood increase along with symptoms of SS (66). In addition, IL-12 production plays a very important role in increasing the number of TFH cells present in autoimmune diseases, such as SS, so that IL-12 production induces the expression of IL-21, CXCR5, ICOS, and BCL6 in naive $TCD4^{+}$ cells (68) and antibody production in B cells. Although the capacity of IL-12 to express IL-21 is much dependent on STAT3 (47, 69). SS patients expressing IL-12 have a higher TFH than IL-12-lacking patients. IL-21 increases the TFH survival rate through the activation of PI3K. In addition, IL-21 is necessary for the expression of the chemokine receptor CXCR5 that is involved in directing TFH cells to the germinal center (74, 75). On the other hand, in patients with SS, IL-21 has a very important role in differentiating B cells into plasma cells by the activation of STAT3 signaling.

Multiple Sclerosis

Multiple sclerosis (MS) is a kind of progressive autoimmune disease that results

in the destruction of myelin and neuronal axons in the brain and the spinal cord, and ultimately, the demyelination of the nerves of this area occurs during enhanced aggressive humoral and cellular immune responses (76). As the disease progresses, the number of B cell follicles in the meningeal center increases, hence the humoral immunity hurts the cortical coherence. $CXCR5^+ICOS^+$ TFH cells increase in. patients with MS whose disease is progressing or who experience recurrent illness after remission. Following the increase of TFH-activated $ICOS^+$ TFH cells, the frequency of plasma blasts producing antibodies will also increase in the peripheral blood. Based on the general classification of TFH cells in MS patients, the frequency of $CCR6^+$ TFH cells (Th17-like) increased during the course of the disease as well as the frequent recurrence after recovery, while $CXCR3^+$ TFH cells (Th1-like) decreased compared with the healthy controls (77). IL-21 affects B and T cells as well as neurons in both acute and chronic stages and plays a central role in the pathogenesis of patients with MS (78). Collectively, it may be that the frequency of $CXCR5^+$ TFH cells and the number of increased germinal centers are associated with the progression of MS disease, higher plasma blast level promotes the process of demyelinating by producing more antibodies.

Rheumatoid Arthritis (RA)

Rheumatoid arthritis is a systemic chronic autoimmune disease that engages synovial joints, especially in the extremities (79). The autoantibodies, such as RF, Anti-CCP, and AKA (Anti-Keratin Antibody), are present in the peripheral blood, and the identification of these antibodies is useful in diagnosis and prognosis (80). In RA patients, the number of TFH cells increased, being characterized by $CD3^+CD4^+CXCR5^+$, $CD3^+CD4^+$ $CXCR5^+$ $ICOS^+$, $CD3^+CD4^+$ $CXCR5^+$ $PD1^+$, $CD3^+CD^+CXCR5^+$ $ICOS^{high}$, $CD3^+CD4^+CXCR5^+PD1^{high}$, and $CD3^+CD4^+$ $CXCR5^+PD1^+ICOS^+$ phenotypes. In addition, the frequency of naïve B cells characterized by $IgD^+CD19^+CD27^-$ and activated $CD4^+CD86^+$ and $CD19^+CD95^+$ cells also increased. This condition is significantly associated with a higher level of IL-21 and the presence of CCP in the serum of RA patients. Therefore, there is a positive relationship between $CD4^+CXCR5^+$ TFH cells and $CD19^+$ B cells in RA patients due to the role of multiple IL-21 (81). In addition, IL-21 is involved in the differentiation, proliferation, and activation of B cells, the production of antibodies in RA patients, and the increase in TFH-like cells in these patients (82). However, with the increase of IL-21 expression, the frequency of $IgD^+CD27^+CD19^+$ B cells, which later transform into memory B cells, reduced (81).

In patients with RA, the level of expression of BCL6-mRNA and the plasma concentration of IL-21 increased, followed by the increased presence of TFH cells, thus the production of Anti-CCP will enhance. Interestingly, a subgroup of TFH cells with $CD4^+CXCR5^+ICOS^+$ phenotype can be found in the peripheral blood during the aggravation of clinical manifestations and the presence of antibodies against CCP (83). In addition, there is a positive link between $CD95^+$ B cells and the frequency of TFH $PD1^+$ cells, while there is a negative correlation between $CD95^+$ B cells and $ICOS^+$ TFH cells (81). During the treatment procedure utilized to treat rheumatoid arthritis patients, the percentage of $PD1^+$ TFH cells and $CD86^+$ B cells decrease. These cell subtypes can be used to examine the effect of treatment on the progression or recovery of the disease. It is noteworthy that the frequency of $ICOS^+$ TFH cells remains unchanged during treatment in RA patients (81). Therefore, different TFH subtypes in RA patients have different effects on other cells, and the treatment of these patients also has different effects on the frequency of different subtypes of TFH.

Further studies on RA patients showed that the number of circulating TFH cells is normalized in the course of disease progression and the appearance of a chronic

form of RA disease due to the direction of these cells toward germinal centers; however, TFH cells increase highly in the early stages of the diagnosis (84). In addition, the IL-21 and IL-23 IL levels in RA elevate at the time of diagnosis, while IL-21 and IL-23 expression decrease in the chronic form of RA (85). Therefore, investigation of the effective cytokines in TFH cells or the study of the frequency of TFH cells in the bloodstream can aid in diagnosis, disease monitoring, and therapy success.

Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is referred to as a systemic autoimmune disease that influences different tissues. The tissue damage is caused by high levels of autoantibodies in the blood due to the interaction of B and T cells (86). In patients with SLE, there are different phenotypes of TFH cells, including $ICOS^{+}$ (44, 78, 79), $ICOS^{high}CXCR5^{+}$ (67), $CXCR5^{+}PD1^{high}$ (67), and $CXCR5^{+}PD1^{+}$ (87). In addition, the level of T cells with the phenotype $CD57^{+}CXCR5^{+}$ permanently maintains for 26 months (67). Of note, the ICOS, expressed on the surface of T cells, interacts with the ICOSL of B cells; therefore, the expression level of ICOSL reduces in B cells. This reduction is evident in patients with SLE (88); subsequently, following cell-cell interaction between TFH and B cells, B cells produce abundant antibodies against dsDNA (89). On the other hand, the high expression of ICOS leads to an increase in the ability of T cells to produce IFN-γ, IL-4, and IL-10, thereby leading to an increase in the IgG antibody level made in SLE patients against dsDNA.

In patients with SLE, $TCD4^{+}CXCR5^{+}$, and $TCD4^{+}CXCR5^{-}$ cells express IL-21, which increases Th17 cells while decreasing Treg cells (90). Interestingly, the effect of different subtypes of T cells on cell types varies. Also, $CD4^{+}CXCR5^{+}$ IL-21-producing T cells are positively associated with the memory B cell population and negatively affect naive B cells (91). However, $CD4^{+}CXCR5^{-}$ IL-21-producing T cells correlate with the presence of Th17 cells and negatively affect Treg cells. Although TFH cells are associated with SLE disease, they do not play a determining role in assessing the severity of the disease or the level of autoantibodies. Furthermore, $CXCR5^{+}BCL6^{+}$ $CD4^{+}$ TFH cells elevate in patients with SLE, simultaneously $CXCR5^{+}BCL6^{+}$ B cells increase (91). Nevertheless, what determines the number of autoantibodies is the population of $CXCR5^{+}PD1^{+}$ $CD4^{+}$ TFH cells, wherein these cells have distinct functions in regulating disease progression

Treatment with methylprednisolone can significantly decrease the population of $CXCR5^{+}PD1^{+}$ $CD4^{+}$ TFH cells, followed by a decrease in the levels of circulating $CD138^{+}$ and $CD19^{+}$ plasma cells as well as autoantibodies constructed against nuclear dsDNA. Additionally, cells influenced by IL-21 were eliminated during dexamethasone inoculation (87). Another effective molecule in the progression of SLE is CXCL13 whose concentration and the level of chemokine receptor expression, CXCR5, decrease at the surface of Th and B cells of these patients. This refers to the migration of CXCR5-expressing TFH cells to lymphoid organs in which the rate of migration of T cells increases during the disease (87). Unlike $CXCR^{+}$ $PD1^{+}$ T cells, $CXCR5^{+}ICOS^{high}$ T cells do not play a role in determining the severity of the disease, and $CD4^{+}$ $CXCR5^{+}ICOS^{high}$ T cells increase during the final stages of organ damage , while the frequency of autoantibody titer is high (67). Despite numerous studies on the increase in peripheral blood $CD4^{+}$ $CXCR5^{+}$ TFH cells, a study has suggested this cell population decreases in people with SLE, which has led to a decrease in the migration of TFH cells to lymphoid organs that depend on the expression of CXCL13 (92). Overall, the characterization of various TFH subtypes is helpful in monitoring the conditions of the SLE and determining the level of autoantibodies made against dsDNA, as well as the severity of the disease.

Future Clinical Perspective

TFH cells play a significant role in autoimmune diseases, as well as in the germinal center generation and antibody responses. In a study on type 1 diabetes mellitus (DM), the effect of the anti-CD20 antibody (rituximab) on TFH was evaluated. The increase in TFH in circulation is accompanied by an increase in IL-21 expression, which confirms the association between circulating TFH and serum antibodies or C-peptide levels. It was observed that after the treatment with Rituximab, circulating TFH cells, BCL6 levels, IL-21, and IL-6 decreased. In addition, in 10% to 20% of patients, Beta-cell function improved. This information suggests that TFH cells may take part in the progression of type 1 DM (93). The miRNA family mir-17~92 plays a role in $TCD4^+$ migration toward B cell follicles by inhibiting the expression of AKT phosphatase and signaling ICOS-PI3K. miRNA family mir-17~92 plays an essential role in the differentiation of TFH and the stability of Phlpp2, which is the main mediator in this process. Thus, the manipulation of miRNA mir-17~92, or target genes and pathway *in vivo* facilitate the design of better vaccines for the treatment of autoimmune diseases (94). A tumorigenic study with TFH was performed by blocking PDL-1 and LAG3 in a malaria-like mouse models, with an increase in the number of TFH and B cells in the germinal centre, followed by the rapid evolution of protective antibodies and plasmodium cleaning. Thus vaccines incorporating monoclonal antibodies that disrupt the inhibitory pathway of TFH cells are thus likely suitable. (95). Interestingly, ICOS blockade decreases IL-6, IL-21, and TNF-α production in primary Sjögren's syndrome. In addition, blocking ICOS prominently decreases the germinal center reactions, while improving lupus development in mice. This issue suggests that ICOS may represent a therapeutic target in patients suffering from primary Sjögren's syndrome and SLE (96). MicroRNAs (miRNAs) are short (18-23 nucleotide) non-coding RNAs that post-transcriptionally control mRNAs. Dysregulation in the patterns of microRNAs may be beneficial to detect pathologic conditions, e.g., cancers and autoimmune disorders. Finding microRNAs related to TFH development in autoimmune diseases may provide novel biomarkers to diagnose or predict their outcomes (97-99). In addition, autophagy-related conditions may affect TFH development which needs further elucidation (100). Finally, because IL-21 and IL-6 both use STAT3, STAT inhibitors, Janus kinase (JAK)- STAT pathway inhibitors may be promising therapies for the treatment of autoimmune disease (101, 102). Because of the plasticity of T cells regarding the microenvironment, including cell to cell interaction, cell to matrix interaction, and soluble factors such as cytokines, TFH cell populations and sub-populations may be variable; therefore, *in vitro* identification of TFH cells may differ from *in vivo* studies. Single-cell investigations may help resolve the situation for implementation in personalized therapy.

CONCLUSION

Some lymphocytes may aggravate autoimmune diseases, while others may have ameliorating effects. Moreover, if cell subsets are overlooked, the total rise in their number may not imply a decided prognosis. Therefore, to achieve more tailored results, medicines should be directed on specific T cell populations and subpopulations, notably when customized or precision medicine is poised to conquer new therapeutic frontiers. For this purpose, the identification of each population and subpopulation may modulate the immune cells. To identify TFH cells, the identification of essential factors is necessary for differentiation, evolution, stimulation, and function of this cell population. Moreover, targeting IL-21 and IL-6 cytokines (by JAK-STAT inhibitors and monoclonal antibodies) and ICOS receptor

(by monoclonal antibodies) may be promising approaches for the treatment of autoimmune diseases, respectively. Therefore, a better understanding of the function of Tfh cells, their exact effect on antibody, cytokine production, and dysregulation in immune cells may shed light on the exact consequence of TFH cells on the autoimmune disease. This may open up new avenues for new treatment techniques to control the immune system.

AUTHORS CONTRIBUTIONS

S. I.: created the concept, studied the literature, and wrote the initial draft. Sh. Kh. and A. S. were in charge of reviewing the literature, supervising the study, editing the first document, and revising it . S. T., F. A., M. Sh., A.F., and F. S.: oversaw the research methodology, read the literature, gathered data, and authored the first draft. N. R.: portrayed the figures. F. S.: revised the manuscript for key intellectual content. The final manuscript was read and approved by all writers.

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