



Insights Into the Molecular Mechanisms of T Follicular Helper-Mediated Immunity and Pathology

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T follicular helper (Tfh) cells play key role in providing help to B cells during germinal center (GC) reactions. Generation of protective antibodies against various infections is an important aspect of Tfh-mediated immune responses and the dysregulation of Tfh cell responses has been implicated in various autoimmune disorders, inflammation, and malignancy. Thus, their differentiation and maintenance must be closely regulated to ensure appropriate help to B cells. The generation and function of Tfh cells is regulated by multiple checkpoints including their early priming stage in T zones and throughout the effector stage of differentiation in GCs. Signaling pathways activated downstream of cytokine and costimulatory receptors as well as consequent activation of subset-specific transcriptional factors are essential steps for Tfh cell generation. Thus, understanding the mechanisms underlying Tfh cell-mediated immunity and pathology will bring into spotlight potential targets for novel therapies. In this review, we discuss the recent findings related to the molecular mechanisms of Tfh cell differentiation and their role in normal immune responses and antibody-mediated diseases.

Keywords: T follicular helper cells, germinal center, transcription, autoimmunity, cancer

INTRODUCTION

Germinal centers (GCs) are secondary lymphoid structures within B cell follicles where B cells go through affinity maturation and class-switch recombination to generate high-affinity antibodies (1, 2). GC reactions play a critical role in the invasion of pathogens, while abnormal GC reactions are implicated in systemic autoimmune diseases, chronic inflammation, allergic responses, and B cell malignancies. The GC reaction is initiated and amplified by GC B cell and CD4⁺ T cell interactions followed by T follicular helper (Tfh) cell help to B cells which leads to the generation of long-lived serological memory (2, 3). Exaggerated expansion of Tfh cells results in excessive GC reactions, self-reactive B cell proliferation, and increased long-lived plasma cell differentiation, as well as an overproduction of high-affinity pathogenic autoantibodies (4). Understanding the development and function of Tfh cells is important for generating new vaccine strategies against pathogens as well as targeted approaches to abrogate the inappropriate activity of these cells in patients with various autoimmune diseases.

T follicular helper cell development occurs in a stepwise process (1, 2). Contact of naïve CD4⁺ T cells with antigen-presenting dendritic cells (DCs) within T cell follicles is the first step of commitment toward Tfh cell differentiation (**Figure 1**). This step of Th cell development, named as pre-Tfh is reflected by upregulation of CXC chemokine receptor 5 (CXCR5) expression as well as key genes in the Tfh pathway such as B-cell lymphoma 6 protein (Bcl6), Achaete-scute homolog 2 (Ascl2), ICOS, programmed cell death-1 (PD-1), and Batf, and the downregulation of CC chemokine receptor 7 (CCR7) expression. These changes guide the pre-Tfh cells to the T/B cell border where proper signals received from B cells trigger a further increase in the Tfh-associated gene expression pattern (Bcl6, PD-1, ICOS, and CXCR5), commitment to the functional GC Tfh cell program and subsequent GC formation. While in mice, IL-6, IL-21, and Bcl6 are essential for Tfh formation, in humans, Tfh generation relies on TGF- β , IL-12, IL-23, and Activin A signaling (5–9). In addition, Tfh cell differentiation is negatively controlled by cytokines (IL-2 and IL-7) costimulatory molecules [cytotoxic T lymphocyte antigen 4 (CTLA4) and PD-1], and transcriptional factors [signal transducers and activators of transcription (STAT)5, Blimp-1, FOXO1, Foxp1, and Krüppel-like factor 2 (Klf2)] (10–16).

In this review, we have discussed positive and negative regulation mechanisms of mouse and human Tfh differentiation including costimulation and cytokine driven signaling pathways and subsequent activation of downstream transcriptional factors. We also elaborate on the cellular

requirement of other follicular T cells within GCs for Tfh cell development and GC formation. Moreover, we will review the current advances in Tfh cell biology in various disease settings.

Tfh CELLS IN MICE AND HUMANS

Several seminal discoveries made in humans and mice in the early 2000s led to identification of B follicular helper T cells that are indispensable for GC formation and B cell function (17, 18). These cells express high levels of CXCR5 and low CCR7 in both humans and mice (17–20). CXCR5 expression is indispensable for T cell migration from T zones toward CXCL13-rich B-cell follicles, where they interact with B cells for further maturation and then provide help for the generation of high-affinity antibodies and long-lived plasma cells (21–24). Thus, based on their localization and function, CXCR5⁺ CD4⁺ T cells were designated as Tfh cells.

Profiling of cytokine and gene expression patterns provided the evidence that mouse and human Tfh cells are distinct from Th1 and Th2 subsets and help B cells by delivering activating signals with CD40L and the cytokine IL-21 (3, 6, 18, 25). Moreover, these mouse and human gene-profiling experiments helped to identify key molecules including Bcl6, Ascl2, IL-21, PD-1, and ICOS which play a substantial role in Tfh cell development, migration, homeostasis, and function in both species. In 2009, three independent groups identified the essential function of the transcription factor (TF) Bcl6 for Tfh cell development and

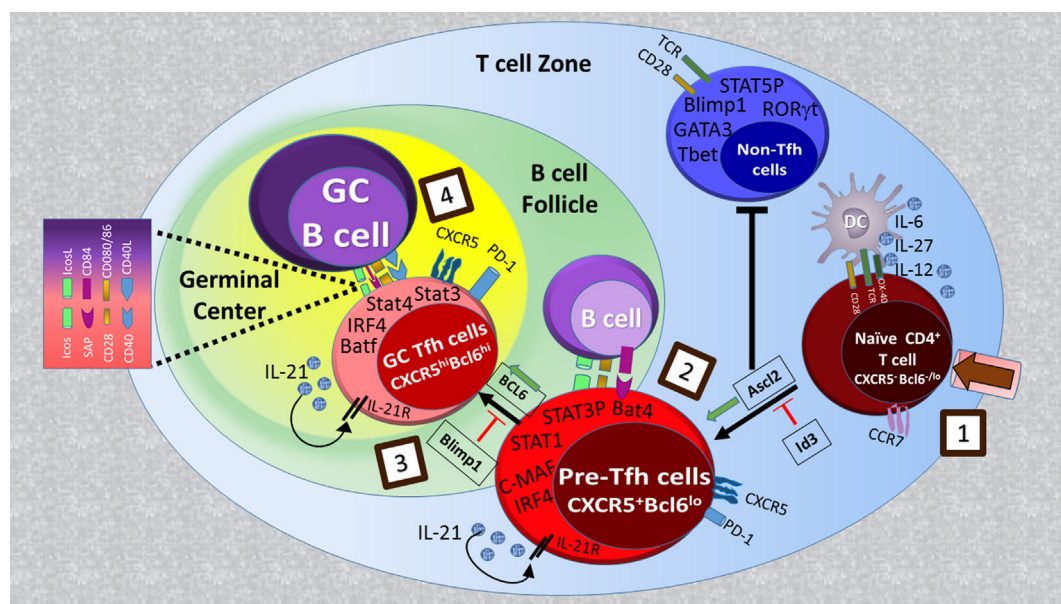


FIGURE 1 | Regulatory signaling for T follicular helper (Tfh) cell development: naïve CD4⁺ T cell priming by MHC/antigen interaction on DCs (step 1) leads to the generation of CXCR5⁺Bcl6^{lo} pre-Tfh cells with increased activity of transcriptional factors such as Achaete-scute homolog 2 (Ascl2), signal transducers and activators of transcription (STAT)1, STAT3, IFN-regulatory factor 4 (IRF4), and Batf (step 2). Ascl2 expression in activated T cells orchestrates them to migrate toward B cell follicles by upregulation of CXC chemokine receptor 5 (CXCR5) expression and repressing IL-2R-Blimp1 pathway as well as Th1, Th2, and Th17 cell differentiation. Upon interaction with cognate B cells at T-B border (step 3), CXCR5⁺ T cells begin to upregulate B-cell lymphoma 6 protein (Bcl6) expression, which in cooperation with other transcription factors determines the final differentiation state of Tfh cells within germinal centers (step 4).

function (7, 26, 27). Since then Tfh cells have been recognized as a distinct lineage of T helper cells.

Studies in mice and humans show that Tfh cells are localized in lymphoid organs and are composed of subsets that differ in their localization, phenotype, and function (28). In mice, after priming with DCs in the T zones of secondary lymphoid organs, a fraction of naive CD4⁺ T cells acquire CXCR5, PD-1, and Bcl6 expression, downregulate CCR7, and migrate toward B cell follicles (**Figure 1**) (21, 29–32). These CXCR5⁺Bcl6⁺CD4⁺ T cells, called Tfh precursors (pre-Tfh), interact with antigen-presenting B cells and further differentiate to fully programmed GC Tfh cells, which provide help to B cells within GCs. GC Tfh cells can be distinguished from Tfh precursors by high expression levels of CXCR5 and PD-1 (28). Sustained expression of Bcl-6 in GC Tfh cells is essential for GC formation (7, 26, 27). The origin of Tfh cells is not restricted to naive cells, and there is some evidence suggesting that other Th subsets including Th1, Th2, Th17, and regulatory T cells (Tregs) may become Tfh cells in GCs (3). This is consistent with the heterogeneity in cytokine expression patterns among GC Tfh cells developed under different immunization protocols and by different types of infectious agents (33). In human tonsils, CXCR5^{lo}ICOS^{lo} pre-Tfh cells (or extrafollicular helper T cells) express multiple Tfh molecules including CD40L, IL-21, and CXCL13 but not Bcl6 and are localized outside of GCs where they help naive B cells to become immunoglobulin-producing cells (34). By contrast, CXCR5^{hi}ICOS^{hi}PD-1^{hi} GC Tfh cells provide help to GC B cells and promote their survival, proliferation, and differentiation into immunoglobulin-producing cells (28). In human tonsils, GC Tfh cells contain subsets coexpressing Bcl-6 and ROR γ t, and Bcl-6 and T-bet (8). This suggests that other Th subsets may be able to differentiate into Tfh cells or that Tfh cells share the same developmental path with Th1 and Th17 cells as observed in mice (35, 36).

A CXCR5⁺ subset of CD4⁺ T cells can also be identified in the peripheral blood of mice and humans, subsequently referred to as circulating Tfh (cTfh) cells. Although these CCR7^{lo}PD-1⁺ cells express lower levels of ICOS and PD-1 and seldom express Bcl6 they are closely related to Tfh cells (37). Their differentiation depends on ICOS and Bcl6 but not SLAM-associated protein (SAP), suggesting that cTfh cells are primary Tfh precursors or early memory Tfh cells. The cTfh cell subset undergoes active Tfh differentiation into mature Tfh cells in secondary lymphoid organs upon antigen reencounter and their presence correlates with autoimmune diseases such as lupus and rheumatoid arthritis (RA) (37). In addition, based on the expression of CXCR3 and CCR6, human cTfh cells can also be subdivided into three main subsets, namely, CXCR3⁺CCR6⁻ (Tfh1), CXCR3⁻CCR6⁻ (Tfh2), and CXCR3⁻CCR6⁺ (Tfh17) cells (38, 39). Tfh2 and Tfh17 cells, but not Tfh1 cells, represent efficient B cell helper cells to regulate immunoglobulin isotype switching (38). A recent data showed that in addition to cTfh memory, Tfh cells could be local in the draining lymphoid organs and sustain B cell responses after reactivation (40). In contrast to cTfh cells, local memory Tfh cells promote plasma cell differentiation and could be released to the circulating memory compartment over time.

Tfh CELL DIFFERENTIATION

T follicular helper cell differentiation is a complex process, which is tightly regulated (41) (**Figure 1**). It begins during naive CD4⁺ T cell priming by DCs in the T cell zone of secondary lymphoid tissues and continues through the first cognate T cell:B cell interaction at the T–B junction until Tfh cells differentiate into mature GC Tfh cells when they enter follicles (2, 3, 42, 43). At each of these stages, Tfh cell development is influenced by signaling pathways downstream of cell surface molecules including the T cell receptor (TCR), costimulatory molecules, and cytokine receptors leading to the activation of specific transcriptional machinery (2, 3, 42, 43).

Costimulatory Molecules

T follicular helper cell differentiation from naive CD4⁺ T cell precursors is a multistep process which requires costimulatory signals. Positive [CD28, ICOS, SAP, glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR), etc.] and negative [CTLA4, PD1, and B and T lymphocyte attenuator (BTLA)] costimulation works together with TCR signaling during Tfh cell development to guide activation, proliferation, differentiation, migration, survival, and effector functions (1–3, 33, 44). Imbalance between positive and negative costimulation signals leads to increased Tfh cell number and consequently to Tfh-driven autoimmunity (45, 46).

Positive Costimulation

CD28 and ICOS

CD28 and ICOS are two structurally and functionally related costimulatory molecules that are critical for Tfh cell differentiation (2, 47, 48). CD28, which is constitutively expressed on both naive and resting T cells, specifically binds to its ligands CD80 or CD86 and regulates T-dependent B cell responses (49, 50). While previous studies suggest the overlapping function of CD80 and CD86 in antibody responses, during virus infection CD86 expression on B cells but not CD80 is critical for Tfh cell generation and function (51). CD28 signaling regulates (i) early key events of Tfh differentiation, especially the expression of PD-1, ICOS, OX-40, Bcl6, and CXCR5 (47); (ii) the late stage of Tfh differentiation as B7 ligand blockade during ongoing infection impairs Tfh cell response (52, 53); and (iii) Tfh cell survival (53).

ICOS, another CD28 family member, is highly expressed on Tfh cells and is critical for Tfh cell generation and GC formation (2, 48). Unlike CD28, ICOS does not regulate any of the early differentiation steps of naive T cells into Tfh cells, particularly Bcl6 expression (47); however, ICOS is important to maintain the phenotype of already differentiated Tfh cells (47). Mice in which the ICOS–ICOSL interaction is disrupted as well as ICOS-deficient patients have fewer Tfh cells and smaller GCs (6, 47). ICOS exerts its costimulatory function through phosphoinositide 3-kinase (PI3K) signaling which results in the activation of Akt (47). Akt phosphorylates the transcriptional factor FOXO1 which thereby stays in the cytoplasm and becomes functionally inactive (47). This is critical because FOXO1 suppresses Tfh cell differentiation through the negative regulation of Bcl6 and positive regulation of Klf2 expression (15). Klf2 plays a negative role

in Tfh differentiation by binding to promoter regions of CXCR5, CCR7, CD62L, PSGL-1, and S1pr1, which leads to the suppression of CXCR5, relocation of Tfh cells from B cell follicles back to T cell zone and their phenotype reversion to non-Tfh cells (44). Besides the ICOS–Klf2 axis, ICOS promotes interaction of the p85a regulatory subunit of PI3K with osteopontin (OPN-i), followed by translocation of OPN-I into the nucleus, its interaction with Bcl6 and protection of Bcl6 from proteasome degradation; thus, sustain responses by Tfh cells (54).

SLAM-Associated Protein

SLAM-associated protein is an intracellular adaptor protein essential for the function of SLAM family receptors (SFRs) to regulate immune responses (55, 56). SFRs consist of nine members, four of which are highly expressed on Tfh and B cells: CD150/SLAM, CD229/Ly9/SLAMF3, CD84/SLAMF5, and NTB-A/Ly108/SLAMF6 (57–59). High intrinsic SAP expression in both human and mouse GC Tfh cells, but not in B cells, is important for the development of long-term humoral immunity: particularly formation of GCs, long-lived plasma cells, and memory B cells (57, 60, 61). Bcl6 is required for SAP expression in GC Tfh cells (62). SAP through SFRs regulates T:B cell adhesion, cytokine production, and TCR signaling strength (63). Patients with X-linked lymphoproliferative disease (XLP) caused by a SAP gene mutation as well as mice lacking SAP expression, display defects in GC Tfh cell generation and GC reactions (3, 55). While SAP-deficient CD4⁺ T cells express normal levels of CXCR5, ICOS, and Bcl6, they have an impaired ability to stably interact with cognate B cells and sustain GC reactions (58, 61, 64, 65). Thus, SAP is not required for early Tfh cell differentiation (Bcl6^{hi}CXCR5⁺), but is indispensable for sustained T:B cell interactions and the full polarization to GC Tfh cells (Bcl6^{hi}PD1^{hi}) mainly by the following mechanisms: (1) SAP binds phosphotyrosines of the SLAM immunotyrosine switch motifs (ITSM) and mediates positive signaling by recruiting Src family kinase Fyn and PKC θ (66); (2) SAP competes with the tyrosine phosphatase SHP-1 for Ly108 ITSM binding, signaling through which contributes to reductions of T:B contacts and to an impairment in GC Tfh cell generation and GC responses (58). In addition to regulating T:B adhesion, positive signaling through SAP and PKC θ regulates cytokine secretion by GC Tfh cells, particularly IL-4 (60, 67). Moreover, Sap/Ly108 engagement sustains the TCR signaling that is required for establishing the Tfh cell:B cell synapse and thus allowing Tfh cells to provide help to B cells (68).

Glucocorticoid-Induced Tumor Necrosis Factor Receptor-Related Protein

Glucocorticoid-induced tumor necrosis factor receptor-related protein (TNFRSF18, and CD357), a member of the tumor necrosis factor receptor superfamily, is expressed at high levels in Tregs and activated T cells (69). Activation of GITR by its natural ligand GITRL enhances proliferation and effector T cell responses and inhibits Treg mediated suppression (70, 71). GITR is highly expressed on Tfh cells compared with non-Tfh cells in the spleens of collagen-induced arthritis (CIA) mice (72). GITR signaling promotes Tfh cell expansion and survival. Administration of

GITR-Fc protein greatly reduces CIA severity by suppressing Tfh development and thereby humoral immune responses. In addition, in the chronic lymphocytic choriomeningitis virus (LCMV) infection model, a Tfh cell intrinsic role for GITR in sustaining Tfh cell responses and LCMV-specific antibody production has been identified (73). Thus, GITR signaling is considered as a positive regulator of Tfh generation (44).

Negative Costimulation

Programmed Cell Death-1

Programmed cell death-1 (PDCD1 and CD279) is a member of the CD28 superfamily which is transiently expressed by activated conventional T cells and delivers an inhibitory signal by engaging its ligands PD-L1/CD274/B7-H1 and PD-L2/CD237/B7-DC that are expressed on activated B cells, DCs, and macrophages (74). PD1 shows high and sustained expression on exhausted T cells, Tregs, Tfh, and T follicular regulatory (Tfr) cells (75–78). In addition to conventional T cells, PD-1 is expressed by B cells, natural killer cells, and myeloid cells as well (79, 80). Early studies utilizing complete knockouts of PD1, PD-L1, or PD-L2, or their respective blocking antibodies tried to address the role of the PD-1 pathway in controlling humoral immunity. Interestingly, some studies have shown attenuated humoral immune responses upon PD-1 signaling blockade, whereas others have found enhanced responses (74, 81–85). Later work using cell-type specific deletion of PD1 and its ligand helped to delineate the function of PD1 signaling in GC responses and gain insight into the individual role of PD1 in Tfr and Tfh cells and PD1 ligands in DCs and B cells in the regulation of humoral immunity (77). The PD-1–PD-L1 pathway plays an immunoregulatory role in limiting the differentiation and suppressive function of Tfr cells (77). PD-1 expression can also modulate Tfh differentiation and function. Tfh cells from aged mice express higher levels of PD-1 compared with Tfh cells in young mice; PD-1 blockade in aged Tfh cells restores Tfh cell function, suggesting a cell-intrinsic role of PD1 in Tfh cells (12, 86). Interestingly, PD-L1 expression on DCs, but not B cells, inhibits Tfh and Tfr cell differentiation (87).

Cytotoxic T Lymphocyte Antigen 4

Cytotoxic T lymphocyte antigen 4 is a key checkpoint in immune tolerance (88–92). While CTLA4 and CD28 share the same ligands CD80 and CD86, CTLA4 interacts with them with higher affinity and avidity compared with CD28 (48). The central tenet of CTLA4 function is to regulate CD28 signaling, since fatal multiorgan inflammation as well as increased antibody levels in CTLA4 KO mice are prevented by the blockade of CD80 and CD86 (93–95). CTLA4 is constitutively expressed in Tregs and upregulated after activation in conventional T cells and plays a key role in mediating Treg function and in controlling conventional T cells (48). In fact, a Treg-specific deletion of CTLA4 recapitulates the phenotype of germline CTLA4 KO mice including increase in antibody production, indicating the role of CTLA4 in Tregs to control B cell responses (96). Recently, multiple groups assessed the role of CTLA4 in B cell responses and identified the function of CTLA4 on multiple T cell subsets including Tfh, Tfr,

and Tregs in regulating humoral immune responses (13, 97). While it was suggested that CTLA4-dependent suppression is the primary mechanism used by Treg and Tfr cells to control Tfh cell development and humoral immunity *via* CTLA4-dependent downregulation of CD80 and CD86 on B cells, Foxp1-dependent CTLA4 expression on non-Treg CD4⁺ cells has cell-intrinsic and negative regulatory functions in Tfh cell differentiation, maintenance, and function (13). CTLA4 controls Tfh cell differentiation by regulating the degree of CD28 engagement (52).

B and T Lymphocyte Attenuator

B and T lymphocyte attenuator (CD272) is an inhibitory receptor expressed on T and B cells that binds TNFR family member herpesvirus entry mediator and attenuates T and B cell activation and effector functions (98–100). Mice lacking BTLA exhibit increased antigen-specific IgG responses and with age gradually develop autoimmune hepatitis-like disease and autoantibody production to nuclear antigens (101), suggesting that BTLA negatively regulates humoral immune responses. BTLA is highly expressed in CXCR5⁺ Tfh cells compared with conventional CXCR5⁻ CD4⁺ T cells. While Tfh cell development is not affected in BTLA-deficient mice, BTLA expression in Tfh cells but not in B cells is critical to control GC B cell development and antigen-specific IgG2a and IgG2b production (102). Moreover, BTLA controls Tfh-mediated B cell responses by suppressing IL-21 production (102).

Cytokines

Along with antigen and costimulation signaling, specific cytokine-dependent cues play a central role in governing naive CD4⁺ T cell differentiation into specific effector T helper cell subsets. For example, IL-12 and IFN γ promote Th1 differentiation, whereas IL-4 drives Th2 differentiation (42). In addition, IL6 and IL-21 in combination with TGF β induce Th17 differentiation (42). There are multiple cytokines that exercise either positive or negative roles at different stages of Tfh development (1, 2). However, cytokine-dependent Tfh cell formation varies between mice and humans (1, 42). Particularly, while TGF β signaling opposes Tfh development in mice, it is required for human Tfh cell development (42).

Cytokines That Support Tfh Cell Formation in Mice and Humans

IL-6, IL-21, and IL-27

IL-6, IL-21, and IL-27 have all been implicated in Tfh cell development, although with differing roles (1, 2, 6, 7, 103, 104). IL-6 is mainly derived from activated B cells, DCs, and follicular DCs and is required in the initial stage of Tfh cell formation by inducing Bcl6 and IL-21 expression (5, 103, 105, 106). Mice deficient in IL-6 or IL-6R show reduced or delayed Tfh cell formation due to impaired signaling through STAT3 and STAT1 (5, 107). In addition, at the late stage of chronic viral infection, IL-6 derived from activated follicular DCs is crucial for maintenance of Tfh cell by upregulation of Bcl6 and viral control (3). Similar to mice, in humans, IL-6 derived from circulating plasmablasts is also a potent inducer of Tfh differentiation (108). IL-21 is primarily

produced by select CD4⁺ T cells including Tfh, Th17 cells, and natural killer T (NKT) cells and plays a more prominent role in sustaining Tfh cell identity and function (6, 7, 18, 36, 109). IL-21- and IL-21R-deficient mice display reduced numbers of Tfh cells after antigen immunization suggesting an autocrine role for IL-21 in the maintenance and augmentation of Tfh cell programming (6, 110). However, in mice deficient either in IL-6 or IL-21 signaling, Tfh cell development is only partially compromised, indicating that these cytokines may play redundant roles in Tfh cell development (5, 103). In fact, loss of both cytokines significantly diminished Tfh cell numbers compared with an IL-6 or IL-21 deficiency alone (5, 103). However, an IL-6/IL-21 deficiency does not cause the complete absence of Tfh cells, suggesting an existence of IL-6 and IL-21-independent mechanisms for Tfh cell generation. In fact, it has been reported that the cytokine IL-27 contributes to Tfh cell maintenance by promoting IL-21 expression (104). Mice deficient in IL-27 signaling show reduced IL-21 expression, Tfh cell number, and GC activity (104). Similar to mice, DC-derived IL-27 is critical for the induction of Tfh cell polarization, IL-21 secretion by Tfh cells, and Tfh-dependent production of IgG by B cells (111). In addition to IL-21 induction, it has been suggested that IL-27 may play an important role in Tfh cell development by antagonizing IL-2 signaling, which negatively regulates Tfh cell development (10, 112).

TGF- β , IL-12, IL-23, and Activin A

Recent data suggest that different groups of cytokines support Tfh cell formation in humans, with prominent roles for TGF- β , IL-12, and IL-23. While IL-12 and IL-23 are capable of inducing IL-21 expression in naive CD4⁺ T cells from human tonsils and peripheral blood, only IL-12 could augment expression of CXCR5, ICOS, CD40L, and Bcl6, thus IL-12 is likely to act at an early stage of human Tfh cell development. B cells co-cultured with IL-12-primed CD4⁺ T cells produce antibodies which in part is dependent on the expression of CD40L (113). In support of the importance of IL-12 and IL-23 for Tfh development, individuals with mutations disrupting the function of IL-12R β 1 (receptor for IL-12 and IL-23) have fewer circulating and GC Tfh cells and memory B cells. In mice, acting through STAT4, IL-12 induced a transitional stage in Tfh–Th1 cells, which express IL-21 and Bcl6. However, the IL-12–STAT4 pathway also promotes Tbet expression, which ultimately represses Bcl6 expression and Tfh cell programming. Since IL-12 is also linked to the generation of human Th1 cells, additional factors may also contribute to human Tfh cell generation. Surprisingly, TGF- β acts as an important cofactor for the early differentiation of human Tfh cells, but not in mice (7, 8, 114). TGF- β synergizes with IL-12 and IL-23, activating STAT4 and STAT3, and promoting Tfh cell development through induction of Tfh key molecules including CXCR5, ICOS, IL-21, Bcl6, Batf, c-Maf, and the downregulation of Blimp-1 expression (8). Recently, Activin A was identified as a novel inducer of Tfh cell programming in human and non-human primate cells, but not in mice by upregulating CXCR5, CXCL13, and PD-1 expression and repressing CCR7 and Blimp-1 expression (9). Activin A, in combination with IL-12, promotes the generation of Tfh like cells that have high expression levels

of CXCR5, Bcl6, PD-1, LAMF1, CXCL13, IL-21, LIF, LTA, and TNF (9). Activin-A-induced Tfh programming is dependent on signaling *via* SMAD2 and SMAD3 (9).

Cytokines Which Inhibit Tfh Cell Formation

IL-2, IL-10, and IL-7

The IL-2/STAT5 axis is shown to be inhibitory for Tfh cell formation. In an *in vivo* mouse model of influenza infection, it was shown that exogenous administration of IL-2 suppresses Tfh cell differentiation, GC formation, and neutralizes antibody production (10). T cell-intrinsic expansion of Tfh cells is mediated by loss of IL-2R α (115, 116). Mechanistically, activation of STAT5 by IL-2 enhances Blimp-1 expression and prevents binding of STAT3 to the Bcl6 locus (10). In addition, IL-2 inhibits the expression of IL-6 receptor α -chain (IL-6 α) and to a lesser extent, gp130 and thus negatively regulates Tfh differentiation (117). Moreover, a recent study depicts that IL-2 drives cells toward Th1 rather than Tfh differentiation through the activation of Akt and mTORC1 kinase (118). IL-7 negatively regulates Tfh differentiation by activating STAT5 and thereby repressing Bcl6 and CXCR5 (11). An additional study showed that IL-10, which has traditionally been viewed as a costimulator of antibody production, could also inhibit antibody responses indirectly by suppressing a subset of Tfh cells that produce IL-17 and IL-21 (119).

Transcriptional Regulation of Tfh Cells

The differentiation of naïve CD4⁺ T cells into Tfh cells is regulated by coordinated interplay between cell-extrinsic factors and cell-intrinsic transcriptional networks. Since the discovery of Bcl6 as a key factor for Tfh differentiation, several other transcriptional factors that either support [IFN-regulatory factor 4 (IRF4), c-Maf, Batf, STATs, Ascl2, TCF1, Lef1, etc.] or oppose [FOXO1, FOXP1, BLIMP-1, STAT5, KLF2, and peroxisome proliferator-activated receptor gamma (PPAR γ)] Tfh cell development, migration, and function have been defined (1–3). A dynamic balance between these multiple transcriptional factors is the main determinant of normal Tfh development and immunity, since dysfunction of negative transcriptional machinery triggers autoimmunity (120, 121).

TFs Positively Influencing Tfh Cell Differentiation

B-Cell Lymphoma 6 Protein

The Bcl6 has been recognized as a key transcriptional factor for Tfh cell development and for efficient GC responses (7, 26, 27). Expression of Bcl6 is driven by CD28 and IL-6/IL-21–STAT1/STAT3 signaling (47). Bcl6 overexpression leads to the upregulation of PD-1, CXCR5, CXCR4, and SAP which are essential for Tfh cell function in T and B cell interactions (106, 115). As a transcriptional repressor, Bcl6 functions to suppress genes that controls Tfh cell development by the following mechanisms: (i) Blimp-1 expression; (ii) genes encoding proteins that controls migration including EBI2, CCR7, CCR6, S1PR1, and PSGL1; (iii) Klf2 expression; (iv) genes that support Th1 (IFNGR1, T-bet, and STAT4), Th2 (Gata3), and Th17 (IL-17 and ROR γ t) cell development (7, 26, 27, 122–126).

c-Maf

c-Maf is a bZIP transcriptional factor that plays an important role in the regulation of cytokine production and Th2, Th17, and Tfh cell differentiation (87). Both Th17 and Tfh cells have higher expression of c-Maf, and loss of c-Maf in T cells results in a defect in IL-21 production and fewer Th17 and Tfh cells (36). Recent data further indicate an important and non-redundant role for c-Maf in the initiation of Tfh cell development and T-cell-mediated humoral responses. Loss of c-Maf expression in T cells leads to the decreased expression of key Tfh molecules, such as BCL6, CXCR5, and PD1 (127).

Batf

Batf is basic leucine zipper protein in the AP-1 family which was originally implicated in Th17 differentiation through direct regulation of transcription of ROR γ , IL-21, and IL-22 (128). While Batf expression is moderately increased in Tfh cells compared with other T helper subsets, Batf is an essential component for Tfh development through combined regulation of Bcl6 and c-Maf (129, 130). Batf-deficient mice fail to generate Tfh cells, and Bcl6 and c-Maf overexpression in Batf-deficient T cells improves Tfh cell development but not to the level of Batf reconstitution, suggesting that additional targets are required for complete Tfh cell induction (130). In addition, the Batf/IRF4 complex in cooperation with STAT3 and STAT6 is required for IL-4 expression in Tfh cells (120).

IFN-Regulatory Factor 4

Together with the well-known function of IRF4 in Th2, Th17, Th9, and Treg differentiation as well in plasma cell maturation (131–139), IRF4 also plays a critical role in Tfh cell development and GC formation (140). Mechanistically, IRF4 may cooperate with other transcriptional factors that determine T cell fate decision: (i) IRF4 interacts with Jun and Batf to form an IRF4–Jun–Batf complex that binds to AP-1–IRF4 composite elements (141); (ii) binds with STATs (142), and Bcl6 (143). Recently, it has been acknowledged that TCR signaling strength controls IRF-4 concentration and consequently cell fate choice between Bcl6-expressing Tfh and Blimp-1 expressing Teff cells (144). Increased TCR signaling leads to elevated IRF4 levels that function to coordinate Teff cell fate choice at the expense of Tfh cell fate (144).

Signal Transducers and Activators of Transcriptions

There are several members of the STAT family including STAT1, STAT3, and STAT4 that contribute to Tfh cell development (1, 6, 14, 106). It has been reported that STAT1 is required for early Tfh differentiation (107). Besides, IL-6 signaling during Tfh differentiation is mediated by both STAT1 and STAT3 TFs (107). It has also been noted that STAT1 directly regulates expression of key Tfh genes including Bcl6, CXCR5, and PD-1 by binding to their promoter loci (145). IL-6–STAT3 and IL-21–STAT3 signaling can promote Tfh cell differentiation by inducing Bcl6 expression (6, 7, 146, 147). STAT3 cooperates with the Ikaros zinc finger TFs, Aiolos, and Ikaros, to regulate Bcl6 expression (148). IL-6-mediated STAT3 activation restricts IL-2R α expression to

limit Th1 cell differentiation (107). In humans, functional STAT3 deficiency compromises Tfh cell generation (149). STAT4 can promote the expression of Bcl6 and the classical Tfh cell cytokine IL-21 in both mouse and human Tfh cells *in vitro* (35, 150). However, continued IL-12-driven Stat4 signaling can decrease the expression of Bcl6 and IL-21 and strengthen T-bet-derived Th1 differentiation at the expense of Tfh cells (35). Interestingly after acute viral infection, T-bet is co-expressed with Bcl6 in Tfh cells and is required alongside STAT4 to coordinate IL-21 and IFN- γ production in Tfh cells and for promotion of the GC response (151).

Notch1 and Notch2

Notch proteins belong to the family of evolutionary conserved transmembrane-bound receptors and play an important role in CD4⁺ T helper cell differentiation and/or function including Tfh cells (152). Mice with T-cell-specific deletions of Notch1 and Notch2 display impaired Tfh cell differentiation, IL-4 secretion by Tfh cells and GC reactions after immunization with T-dependent antigens or infection with parasites (153). Notch1- and Notch2-deficient Tfh cells express reduced levels of Tfh-associated molecules (CXCR5, PD-1, BTLA, and Bcl6), but normal levels of ICOS and increased Blimp-1 expression (153). Notch receptors 1 and 2 are required for Tfh cell generation and IL-4 expression by Tfh cells, but are dispensable for Th2 cell differentiation in response to parasitic helminth infection. Thus, Notch signaling is an important checkpoint in the bifurcation between Tfh and Th2 cell-driven hallmarks of type-2 immunity (154).

NFAT

Nuclear factor of activated T cells 2 (NFAT2) is highly expressed in Tfh cells, NFAT2 deficiency in T cells leads to enhanced GC reactions due to the impairment of Tfr cells to upregulate CXCR5 but not Tfh cells (155). A loss of both NFAT1 and NFAT2 in CD4⁺ T cells leads to impaired GC reactions due to reduced Tfh cell differentiation and decreased expression of proteins such as ICOS, PD-1, and SFRs which are important players in T/B interactions and B cell help (156).

Achaete-Scute Homolog 2

Achaete-scute homolog 2, a BHLH-domain-containing TF, is selectively upregulated in Tfh cells and initiates Tfh cell development (157). Overexpression of Ascl2 can lead to a substantial induction of CXCR5 expression, but not Bcl6 and downregulation CCR7 expression *in vitro*, as well as accelerated T cell migration to the follicles and Tfh cell development *in vivo* in mice (157). Ascl2 inhibits expression of Th1 and Th17 signature genes (157). Ascl2 deletion as well as inhibition of its function with E-protein inhibitor Id3 leads to a total impairment of Tfh cell development and GC response (157).

T Cell Factor 1 (TCF-1) and LEF-1

T cell factor 1 is highly expressed in Tfh cells (158–160). TCF-1 plays an important role in the initiation of Tfh cell differentiation and the effector function of differentiated Tfh cells, as TCF-1 deficiency results in reduced generation of Tfh cells and impairs

their function to provide B cell help (159, 160). Similar to TCF-1, LEF-1 is also known for its essential role in early Tfh cell development (161, 162). LEF-1 and TCF-1 are upstream of Bcl6 induction and directly target Tfh signaling molecules (Bcl6, IL-6R, gp130, and ICOS) to promote Tfh cell differentiation (158). TCF-1 also suppresses Blimp-1 and IL-2Ra expression (159).

Early Growth Response Gene 2 (EGR2) and EGR3

Early growth response gene 2 and EGR3 can directly regulate the expression of Bcl6 and differentiation of Tfh cells (163).

Bob1

It has been reported that B-cell-specific octamer-binding protein 1, Bob1 in cooperation with TFs Oct1/Oct2 can directly bind to Bcl6 and BTLA promoters and promote their expression and Tfh cell development (164). However, at the same time, other groups have reported that the function of Bob1 is to mainly restrict the cellular frequency of Tfh cells (165, 166).

NF- κ B1

It has been reported that NF- κ B1 promotes Tfh cell responses by facilitating CXCR5 expression but no other Tfh-related molecules (Bcl6, IL-21, and PD-1), and the NF- κ B1-deficient T cells partially lose their ability to provide help to B cells *in vivo* (167). In addition, the non-canonical NF- κ B pathway may also play an essential role in Tfh development through regulation of ICOSL expression in B cells (168).

TFs Negatively Influencing Tfh Cell Differentiation

FOXO1 and FOXO1

Early studies suggest that a decreased expression of FOXO1 either because of increased expression of ICOS (15) or because of ITCH-mediated degradation (169) may increase Tfh cell differentiation. Analysis of mice with a specific deletion of Foxo1 in T cells revealed the requirement for Foxo1 in the suppression of Bcl6 expression and Tfh cell differentiation (15). In addition, enforced nuclear localization of Foxo1 prevents Tfh cell differentiation (15). However, Foxo1 is required during final differentiation to GC Tfh cells as Foxo1 deficient GC Tfh cells are substantially reduced (15). Foxp1 is an additional negative regulator of Tfh cell development. It mainly plays a role in dampening ICOS and IL-21 expression by regulating CCR7 and CTLA4 expression (170).

Krüppel-Like Factor 2

The TF, Klf2, regulates naïve T cell trafficking to secondary lymphoid tissues by promoting the expression of CD62L and S1PR1 (16). KLF2 expression impairs Tfh cell differentiation, whereas ablation of KLF2 expression enhances Tfh cell differentiation (171). This effect is related to the capacity of KLF2 to promote the expression of genes that oppose Tfh cell differentiation (Blimp-1, Gata3, and T-bet) and to repress the transcription of CXCR5 (171).

Blimp1 and STAT5

Blimp1, which is encoded by *Prdm1*, is a transcriptional repressor that has the ability to inhibit Bcl6 expression in B and T cells (122).

Blimp-1, induced by IL-2/IL-7 and STAT5 signaling, suppresses expression of Bcl6 and other Tfh-associated genes including CXCR5, c-Maf, Bcl6, Batf, and IL-21, thus preventing Tfh cell differentiation (14, 172).

Peroxisome Proliferator-Activated Receptor Gamma

Peroxisome proliferator-activated receptor gamma is a TF that regulates lipid and glucose metabolism (173). One-year-old T-cell-specific PPAR γ -deficient mice exhibited a moderate autoimmune phenotype with increased Tfh cells, GC B cells, glomerular inflammation, and enhanced autoantibody production. Mechanistically, PPAR γ by stabilizing the activity of Ikb α , Foxo1, and Sirt1 negatively regulates Bcl6 and IL-21 to inhibit Tfh cell differentiation and GC formation (173).

OTHER FOLLICULAR T CELLS

Several types of follicular T cells that have been found in GCs and characterized such as Tfr cells, follicular regulatory CD8⁺ T cells (CD8 Tfr), natural killer T follicular helper (NKTfh) cells, and follicular CD8⁺ T cells (fCD8).

Tfr Cells

T follicular regulatory cells, a newly identified subset of Tregs, have been found in GCs where they control GC responses (75–77, 86, 174–176). Tfr cells originate from thymic-derived Foxp3⁺ T cells as well as from Foxp3⁻ precursors rather than from Tfh cells (78, 177). They are different from Tfh cells and Tregs: on the one hand, Tfr cells express large amounts of Tfh-related factors including CXCR5, PD-1, Bcl6, CXCL13, and ICOS; on the other hand, they share numerous molecules that are expressed by Tregs, such as GITR, CTLA4, IL10, CD25, and Foxp3 (75, 76, 178).

T follicular regulatory cells similarly possess a multistage and multifactorial differentiation process (176) that requires CD28 and ICOS signaling (76, 77), Sap-dependent interaction with B cells (76), and expression of Bcl6 (178). TF NFAT2 contributes to the initial upregulation of CXCR5 in Tfr cells (155). In addition, recently it has been noted that the mTORC1–STAT3–TCF-1–Bcl6 axis and TRAF3 are essential for Tfr differentiation (179, 180). However, there are also some signals which inhibit Tfr-cell differentiation and function: PD-1, CTLA4, Blimp1, and helix-loop-helix proteins ID2 and ID3 (77, 78). In addition, the cytokine IL-21 can suppress Tfr cells through the upregulation of Bcl6 expression and downregulation of CD25 (103, 181, 182).

T follicular regulatory cells act to limit excessive GC responses by acting on both Tfh and GC B cells (13, 77, 86, 183). Moreover, Treg suppression is not limited to GC B cells and occurs at various steps during B cell differentiation, from B cell activation to class-switched B cells and plasma cells (78). The effect of Tfr cells to modulate GC reaction could be through several potential mechanisms. Tfr cells could suppress Tfh cells through CTLA-4, by dampening expression of CD28 ligands on GC B cells (13). Two immunosuppressive cytokines, IL-10 and TGF- β , may mediate the immune suppressive functions of Tfr within GCs (114, 119, 176, 184); however, a recent research showed that IL-10

is important for B cell survival and proliferation; thus, a detailed mechanism remains to be investigated (185). Interestingly, Tfr cells potentially have effects on antibody affinity, and their suppressive function could result in the selection of the highest affinity antigen-specific antibody and in the selection of higher affinity memory B cells (78).

Follicular Regulatory CD8 T Cells (CD8 Tfr)

In contrast to Foxp3⁺CD4⁺ Tregs, mouse CD8 Tregs do not constitutively express Foxp3 in the thymus and periphery (186), and the CD8⁺Foxp3⁺ T cells do not comprise CD8 Treg population due to lack of suppressive activity (187). Similarly, most of the human CD8 Tregs also lack Foxp3 (188). In mice, a specific subset of Qa-1-restricted CD8 Tregs with high expression levels of CXCR5 (named as CD8 Tfr) were found to possess the ability to limit GC size and prevent autoimmune disease in mice (189). Tfh cells are one of main targets of CD8 Tfr cells (189). In autoimmune-prone mice, CD8 Tfr cells can suppress the expansion of Tfh cells as well as autoantibody production (190). Recent data indicate on the importance of the TF STAT4 for CD8⁺ Tfr development, maintenance, and function toward Tfh and plasma B cells (191). Moreover, CD8 Tfr cells expressing IL-2R β are also shown to inhibit CD8 T cell function in an IL-10-dependent manner (192). Recently, cells with a CD8 Tfr phenotype (CD3⁺CD8⁺CXCR5^{hi}CD44^{hi}) have been identified in humans (193). In chronic CIV infection, CD8 Tfr cells localized in the follicles exhibit enhanced Tim-3 and IL-10 expression, but express less perforin compared with CD8 T cells. CD8 Tfr cells modestly limit HIV replication in Tfh cells by impairing IL-21 production *via* Tim-3 and inhibit B cell function (194). In addition, it has been reported that the KIR⁺CD8⁺ cells (KIR, killer cell immunoglobulin-like receptor, functional homolog of murine Ly49) exert inhibitory activity on CD4⁺CXCR5⁺ Tfh target cells in humans (193).

NKTfh Cells

Recently, a subset of invariant NKT cells, recognized as follicular helper NKT cells (NKTfh cells), was discovered (195). NKTfh cells express CXCR5, PD-1, and Bcl-6 and support B cell responses (196). Similar to the development of conventional Tfh cells, the formation of NKTfh cells is dependent on CD28-mediated cognate interactions with B cells and Bcl6 expression (196). Studies utilizing CD4⁺ T cell-specific loss of Bcl6 determined that both Tfh and iNKTfh cells contribute to B cell help (197). However, unlike Tfh-derived B cell responses, those driven by NKTfh cells have no potential to generate long-lived plasma cells and memory B cells (196). NKTfh cells also possess the ability to boost memory B cell responses to T-dependent antigens but not T-independent lipid antigens (198). In addition, the NKTfh cells can induce limited GC B cell responses in the absence of CD4⁺ cell help (199). Further studies are still needed for a deeper understanding of the mechanisms of NKTfh cells in immunity and their roles in disease.

Follicular CD8⁺ T Cells (fCD8)

In contrast to the previous opinion that CD8⁺ T cells are restricted to extrafollicular areas (200), recent studies have showed that the

CD8⁺ T cells from human and non-human primates possess the ability to migrate to the lymphoid follicles and GCs to support B cells (201). CD8⁺ T cells localize in human tonsil follicles and possess follicular helper-like characteristics including high expression of Bcl6, CXCR5, ICOS, PD-1, CCR5, CD27, CD28, CD69, and CD95, but do not express CCR7, Blimp-1, Tim-3, and CD244 and are named as follicular CD8⁺ T cells (fCD8) (201–206). The current evidence suggests a crucial role of fCD8 in controlling intracellular pathogens and malignancies through the production of various cytokines (IFN- γ , TNF- α , and MIP1 β) in LCMV-infected mice, HIV-infected individuals, and cancer patients (203–205, 207–211). These cytokines activate APCs, promote polarization of naïve CD4⁺ T cells to Th1 cells, sustain activation of CD8⁺ T cells; thus, contributing in the control of viral infection and tumor growth. In addition, similar to Tfh cells, IL-21-producing fCD8 cells promote Ag-specific antibody responses by stimulating B cells, as well as generating and maintaining follicles and GCs (201).

THE ROLE OF Tfh CELLS IN DISEASES

The main function of Tfh cells is to control clonal selection of GC B cells and support B cell immunoglobulin synthesis, isotype switching, and somatic hypermutations. Pathological B cell activation and the production of autoantibodies is a hallmark of the defective immune response that accompanies autoimmunity. In the sections below, we discuss the role of Tfh cells in various disease settings (Figure 2).

Systemic Lupus Erythematosus (SLE)

Observations in animal models and in humans provide strong evidence that Tfh cells are important players in SLE pathology. Patients with SLE have an increased number of cTfh cells, which positively correlates with autoantibody titers (212). Furthermore, the proportion of peripheral blood T cells expressing ICOS is higher in patients with active SLE disease than in patients with inactive disease or in controls which probably leads to enhanced autoantibody production upon activation *via* ICOS–ICOSL interactions in these patients (45, 213). Production of high levels of IL-21 is a hallmark of Tfh cells and multiple studies have shown an increased frequency of CD4⁺IL-21⁺ T cells in SLE patients that was associated with disease severity (214). An increase in Tfh cells is also associated with a shift in the Th17/Treg populations with increases in Th17 cells and decreases in Tregs as well as an elevation of IgG⁺ class-switched memory B cells leading to a more inflammatory environment as observed in SLE (214–219). It is worthy to note that Tfh cells not only affect priming in secondary lymphoid tissues but can also impact local B cell activation and expansion. For example, within the tubulointerstitium of patients with lupus nephritis, Tfh-like cells are organized with B cells in structures resembling GCs (220, 221) suggesting that Tfh cells may also play a role in various complications that are associated with autoimmune pathologies.

Mice that have a single-amino-acid mutation in Roquin1 (a negative regulator of ICOS mRNA stability) demonstrate a spontaneous lupus-like phenotype that is accompanied by

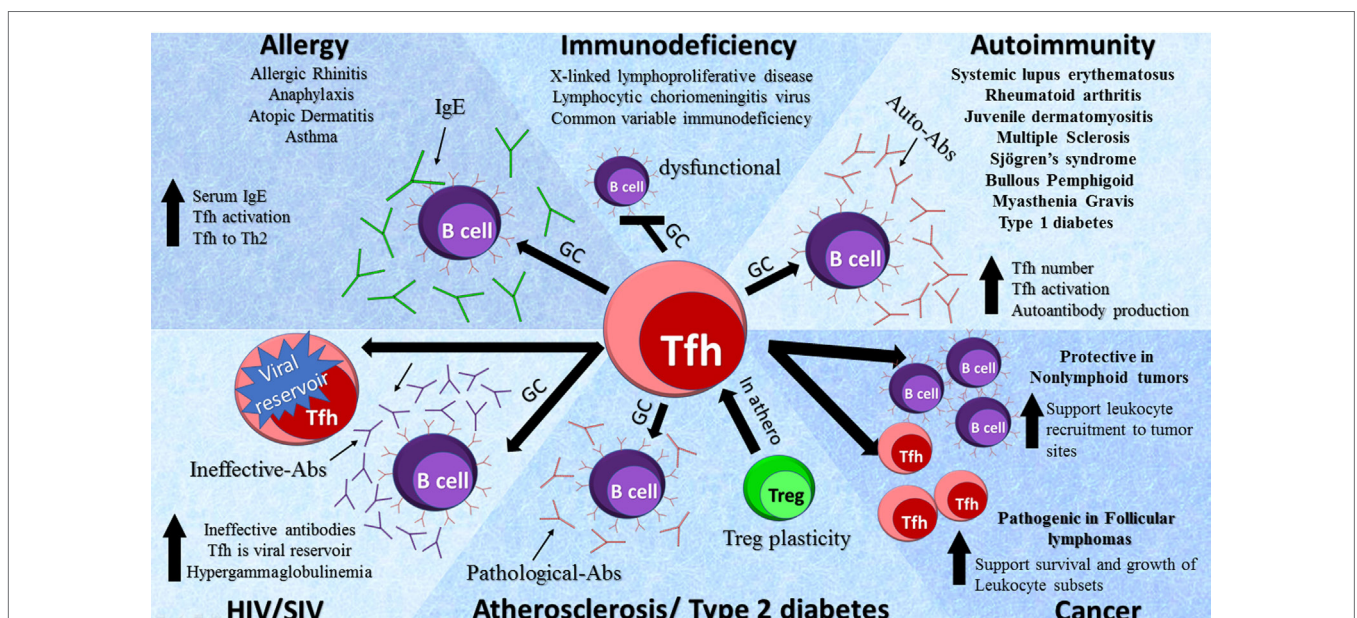


FIGURE 2 | Role of T follicular helper (Tfh) cells in diseases. Tfh cells are involved in a variety of pathologies by diverse mechanisms as depicted in the schematic diagram. Elevated Tfh activity leads to an increase in germinal center (GC) formation, activity, and subsequent production of auto-Abs in autoimmune diseases. In primary immunodeficiencies, a decrease in Tfh cells has been detected with dysfunctional GC formation, whereas in acquired immunodeficiencies such as HIV and SIV an increase in Tfh cells serves as viral reservoirs. In atherosclerosis regulatory T cells (Tregs) transition to Tfh cells *via* GCs leading to the production of pathological Abs. In allergy, there is an increase in Tfh cell activation and GC formation causing the production of IgE. The role of Tfh cells in cancers is complex and dependent on the types of tumors.

elevated numbers of Tfh cells expressing higher level of ICOS, IFN- γ OX40, and IL-21 and activated phenotypes of GCs (28, 222). Importantly, manipulation of Bcl6 expression and thus Tfh cell generation or adoptive transfer of lupus-associated Tfh cells from Roquin^{san/san} mice into healthy recipients induces the formation of GCs, highlighting a contributing role of Tfh cells in SLE (223). Another interesting model to study lupus-related pathophysiology is the BXSb mouse model, which displays lymphoid hyperplasia, monocytosis, immune complex-mediated glomerulonephritis, and an aberrant IL-21-dependent Tfh response (224). The MRL/lpr mouse model displays defective Fas signaling (225), which is characterized by high levels of autoantibodies and extrafollicular Tfh like cells that are dependent on ICOS, Bcl-6, and IL-21 signaling. The pathological functions of these T cells support extrafollicular B cell differentiation and plasmablast maturation (226). While increasing evidence suggests the importance of an extrafollicular Tfh-dependent response in the murine models of SLE (227–230), the importance of Tfh cells and extrafollicular sites in SLE patients is not well defined due to limitations in obtaining non-circulated follicular-resident Tfh cells.

There have been several relatively successful attempts to reduce the severity of SLE in humans *via* blockade of Tfh-cell differentiation and activity. Early results from SLE therapies targeting T cells showed that patients had reduced serum anti-dsDNA titers, but presented minimal reduction of protective antibodies, increased complement markers, and reduced nephritis scores (231). Studies using monoclonal antibodies against ICOS-L inhibited the development of Tfh and GC B cells resulting in decreases in anti-dsDNA Igs and improved kidney function. For years, the main therapy for SLE has been broad spectrum immunosuppressants; however, a growing body of work shows that Tfh cells may be a more precise and attractive target for treating SLE.

Sjögren's Syndrome

Increased numbers of human peripheral blood CXCR5⁺ICOS⁺, CXCR5⁺PD-1⁺ Tfh cells and enhanced GC formation positively correlate with autoantibodies titers and severity of the primary Sjögren's syndrome (pSS) (227–230). IL-6 levels are increased in the serum, tears, and salivary gland epithelial cells of patients with pSS. Interestingly, co-cultures of salivary gland epithelial cells with T cells induce Tfh cell differentiation (3, 232) suggesting a critical role for epithelial cell-derived IL-6 in Tfh cell differentiation in pSS. There are several treatment options for patients with pSS that target Tfh cell functions and therefore the humoral response. Abatacept is a fusion molecule combining CTLA-4 with IgG Fc that binds to CD80/86 and consequently impairs CD28-mediated T cell costimulation (233). It has been shown that abatacept inhibits T cell-dependent B cell activation either *via* DC-defective Th activation or *via* blockade of T/B cell interactions (234). Abatacept treatment in pSS patients reduces circulating numbers of ICOS⁺ cTfh cells resulting in an attenuated Tfh cell-dependent B cell hyperactivity (235) providing a promising therapy for pSS and some other autoimmune pathologies.

Juvenile Dermatomyositis (JDM) and Autoimmune Myasthenia Gravis (MG)

Serum autoantibodies can be found in up to 70% of patients with JDM. Furthermore, these patients display changes in cTfh cells subsets with a decrease in cTfh1 cells, and an increase in the cTfh2 and activated memory cTfh17 cell subpopulations; resulting in an overall increase in cTfh subsets with efficient helper functions. Significantly, these changes in the composition of cTfh cell subsets positively correlate with disease activity and the frequency of circulating plasmablasts (38).

Elevated levels of circulating CXCR5⁺CD57⁺, CXCR5⁺ICOS^{hi}, and CXCR5⁺PD1^{hi} CD4⁺ T cells have also been reported in patients with MG (236). Functional studies demonstrate that cTfh cells from MG patients support autoantibody production, and thus, contribute to the development of disease. Interestingly, Tfh1 and Tfh17, but not Tfh2 cells, were found to be the major secretors of IL-21 (237). Thus, alterations in the composition of peripheral blood memory Tfh1, Tfh17, and Tfh2 subsets seems to be one of the distinct features of several autoimmune diseases including SLE (238), Sjögren's syndrome, multiple sclerosis (239), and MG (237). While an increasing body of evidence reports a shift in the Tfh1/Tfh2/Tfh17 balance in the peripheral blood of patients with autoantibody-mediated pathologies, it is not clear whether the same shift in the Tfh subsets occurs within the inflamed tissues and GCs in the secondary lymphoid tissues. It would be important to identify molecular mechanisms and microenvironmental stimuli that direct a preferential repopulation of Tfh17 and Tfh2 cells in autoimmune settings.

Rheumatoid Arthritis

One of the other examples for the implication of Tfh cells in autoimmune disease setting is RA, which is characterized by high levels of autoantibodies and abnormal GC B cell responses, which contribute to inflammation in the joints. Tfh cells have been detected in the synovial tissue of patients with RA, and along with IL-21 are found in higher frequencies in the periphery (240–242). The increase in Tfh cells in RA patients is positively correlated with elevated serum level of anti-CCP antibodies and consequently with disease severity (240). In animal models, a T cell-specific CXCR5 deficiency results in a significant reduction in GC formation, decreased levels of collagen-specific IgG1 antibodies and a resistance to CIA induction (243). As Tfh cells play a significant role in the progression of RA, therapeutic targeting of Tfh cells could be a valuable option for treating patients with RA.

Type 1 Diabetes (T1D)

Type 1 diabetes is caused by the autoimmune destruction of insulin-producing β -cells in the pancreas. T1D patients have higher frequencies of circulating CD4⁺CXCR5⁺ICOS⁺Tfh cells and higher levels of IL-21 which positively correlates with an increase in plasmablasts, serum autoantibodies, and C-peptide levels (244–246). It is possible that Tfh cells may play a critical role in the initial stages of T1D with an increase of activated CXCR5⁺PD-1⁺ICOS⁺ cTfh cells being found in both children with newly diagnosed T1D and in children at late stages of preclinical

T1D, characterized by impaired glucose tolerance (247, 248). Data from animal models also show that transfer of Tfh cells from a diabetic to a control animal induces elevated blood glucose levels and increased T cell infiltration into islets (244). Furthermore, Roquin^{san/san} mice that have overreactive Tfh cells display dramatically accelerated T1D induction. Collectively, there is direct evidence for a pathogenic role for Tfh cells in progression to T1D. Importantly, the increase in activated cTfh cells is strongly associated with positivity for multiple autoantibodies and could be used as a biomarker for the identification of a subgroup of patients with an active setting of T1D.

Type 2 Diabetes (T2D)

Evidence indicates a key contribution of the immune response in the manifestation of chronic low grade inflammation under the conditions of adipose tissue inflammation, islet β -cell dysfunction, and T2D. While the role of macrophages, CD4⁺, CD8⁺ cells, and follicular B cells is firmly established, there is limited knowledge about the Tfh cell role in obesity and T2D. Recently, Zhou and colleagues found that non-obese T2D patients vs BMI-matched healthy subjects have higher IgG levels and Tfh cells that are highly enriched in IFN- γ , but not IL-4 and IL-17 (249). Interestingly, overweight T2D patients (BMI \geq 24.0) had higher levels of cTfh and the balance of cTfh cell subsets was shifted toward the Th17 subtype (52). Importantly, patients with abdominal obesity had additional increases in cTfh compared with patients without abdominal obesity (52), suggesting that cTfh may play a critical role in the modulation of adipose tissue inflammation in obesity-induced T2D.

Atherosclerosis

Recent reports revealed a critical role for Tfh cells and consequently GC B cells in the development and progression of human and mouse atherosclerosis. Tfh cells support atherogenesis *via* the production of pathological Abs and the generation of highly active GCs. The loss of Tfh cells *via* a conditional knockout of *Bcl6* leads to a reduced atherosclerotic burden in atherosclerosis-prone mice (250, 251). Interestingly, a percentage of Tregs can switch the phenotype into pro-atherogenic Tfh cells but ApoAI can prevent Treg to Tfh cell conversion throughout atherosclerosis (250). It remains to be determined how atherosclerosis-prone conditions alter the memory pool of Tfh cells and how Tfh cells impact the processes of selection of high-affinity B cells and B cell memory development in atherosclerosis.

Allergy

Patients with allergic rhinitis (AR) and asthma preferentially have increased levels of cTfh cells that exhibit a Tfh2 cell phenotype (252). In patients with seasonal AR to ragweed pollen, activation of Tfh cells increases significantly during peak-season (253). It has been noted that the acute anaphylaxis response to peanut allergen is driven by IL-4⁺IL21⁺ Tfh cells and to a lesser extent by Th2 cells (254). Presence of Tfh cells but not Th2 cells is required for IgE production and for the development of an allergic response.

The increased levels of serum IgE is also a hallmark of atopic dermatitis (AD) (255–258). Peripheral Tfh cells in children with

AD have significantly increased levels of ICOS, PD-1, and IL-21 suggesting a highly activated phenotype. Furthermore, a strong positive correlation has been detected between the numbers of IL-21⁺ Tfh-like cells, activated memory B cell pool, and disease severity (259, 260). Interestingly, patients with asthma also display elevated levels of PD-1⁺ICOS⁺ Tfh2 cells and the ratio of Tfh2:Tfh1 cells positively correlates with the total IgE levels in the blood (46). In correlation with the data on human samples, mRNA and protein expression levels of CXCR5, ICOS, ICOSL, and IL-21 were also elevated in mouse models of asthma. Our recent findings also identify an important role of the TF Batf toward the generation of IL-4-expressing Tfh cells rather than Th2 and to their pro-allergic function (120). We further demonstrated that the IL-4-STAT6 signaling contributes to the Batf induction in Tfh cells and the Batf/IRF4 complex along with Stat3 and Stat6 aids IL-4 production in Tfh cells. Recent studies have also shown that Tfh cells can sense specific microenvironmental conditions and differentiate into Th2 cells after repeat exposure with house dust mite (HDM) (261). At the initial stage of disease, Tfh cells are preferentially differentiated, but a second exposure leads to the Tfh switch into Th2 cells which migrate to the lung and produce an inflammatory response. These results suggest that targeting Tfh cells may be a good therapeutic strategy to prevent Th2-cell-mediated immunity to HDM (261).

Primary Immunodeficiency

There are several immunodeficiencies that directly affect the development and functions of Tfh cells and as a result alter B cell-dependent responses. XLP is a primary immunodeficiency caused by mutations in SH2D1A (encoding for SAP, signaling lymphocytic activation molecule-associated protein). A SAP deficiency does not impact CD4⁺ T cell development, but compromises Tfh cell differentiation (262). In line with this observation, XLP patients exhibited significant defects in GC formation, reductions in memory B cell responses, hypogammaglobulinemia, and impaired antigen-specific antibody responses (263). Mutations in CD40L, ICOS, and STAT3 also cause reduced number of CD4⁺CXCR5⁺ cells, defective GC formation, and impaired humoral immune responses. A recent study also highlighted the importance of the IL-12/IL-12R axis as patients with mutations in IL-12R β 1 demonstrate fewer circulating memory Tfh, memory B cells, and defective GCs compared with control subject (264).

HIV and SIV Pathologies

Proper activation of Tfh cells and their interactions with GC B cells are essential for an effective humoral immune response and the extermination of pathogenic human and simian immunodeficiency viruses (HIV and SIV, respectively). Paradoxically, HIV-infected individuals (265) and monkeys infected with SIV (266) display a significantly higher frequency of ICOS⁺ Tfh, PD-1⁺ Tfh, and ICOS⁺PD-1⁺ Tfh cells among total CD4⁺ T cells compared with non-infected controls. Furthermore, Tfh cell frequency is significantly higher in non-treated HIV⁺ patients compared with HIV⁺ patients treated with combination antiretroviral therapy, suggesting that an HIV viral persistence contributes to Tfh cell expansion (265–267). Interestingly, Tfh cells

in humans and macaques show preferential infection with HIV and SIV, respectively (200, 267, 268), likely *via* the chemokine receptor CCR5 as CCR5 is expressed on a precursor subset of Tfh cells and may potentially serve as a co-receptor for HIV (269). GC Tfh cells have been implicated in HIV persistence by supporting viral replication during treated infection and serve as an important cellular reservoir of HIV-1 DNA (270, 271). One explanation is that cytotoxic CD8⁺ T cells are CXCR5 negative and thus unable to migrate to follicles and target HIV-infected GC Tfh cells (200, 272, 273). In line with an increased number of Tfh cells, hypergammaglobulinemia is detected in HIV⁺ patients (274), but these antibodies are ineffective (265, 273, 275–278). Studies have proposed that PD-1 triggering by PD-L1 on GC B cells is a mechanism for the abnormal Tfh functions and defective B cell help (277). In addition, due to the increased number of Tfh cells, the exacerbated interactions of Tfh and GC B cells may lower the threshold for B cell selection resulting in the selection of B cells with low affinities (223). Thus, the relationship between viral entrance into Tfh cells, number and functions of Tfh cells, and B cell activation/maturation is complex and requires further investigations.

Chronic Infections

Lymphocytic choriomeningitis virus persistence results in extended TCR engagement and an IL-6-driven shift from a Th1-induced response toward a Tfh response (279, 280). In addition to studies that identified a key role of Tfh cells in HIV and SIV infections, studies with the LCMV disease model further highlight the importance of timely expansion and sustained functions of Tfh cells in the orchestration of a proper humoral response for control of persistent viral infection (279, 280). Moreover, annual influenza virus studies determined an increase in the number and activation levels of cTfh cells. These studies not only show that an increased cTfh cell frequency contributes to circulating plasmablast responses and infection clearance but can also be used as a marker to monitor the efficacy of influenza vaccination (281, 282). In other infections such as chronic hepatitis B virus patients have an increase in circulating regulatory Tfh cells (283). Interestingly, long-term disruptions of proper T cell-dependent Ab production have been detected in the case of parasitic infections such as *Leishmania* (284), *Litomosoides sigmodontis* (285), and *Plasmodium* infection (286) suggesting the negative regulation of the number and functions of Tfh cells under these conditions. To date, the precise mechanisms that regulate differentiations and functions of Tfh and Tfr cells in different infection settings remain to be determined. Particularly, further studies are required to assess the mechanisms governing Tfh cell development, persistence, and function at the different stages of various infection diseases.

Cancer

Accumulating evidence suggests that Tfh cells are involved in peripheral T cell and B cell-associated tumors due to their high impact on growth and survival of different leukocyte subsets. Angioimmunoblastic T cell lymphoma (AITL) is an aggressive tumor and isolated neoplastic T cells express CXCL13, ICOS, CD154, CD40L, and NFATC1 (287, 288), making these T cells

similar to Tfh cells (289). The expression of mutated RhoA G17V induces Tfh cell differentiation/activation; increased proliferation associated with ICOS upregulation, elevated PI3K, and mitogen-activated protein signaling (290). Loss-of-function mutations in epigenetic regulators such as TET2 and DNMT3A are frequent events in the pathogenesis of AITL. Interestingly, RhoA G17V expression accompanied with TET2 loss results in AITL development in mice. It is worth to note that altered RhoA GTPase activity has been linked with autoimmunity and studies in AITL further highlight a role of RhoA in shaping Tfh cell phenotype and response.

Like AITL, in follicular T cell lymphomas, infiltrating T cells resemble a phenotype of Tfh-like cells and express IL-4, TNF- α , IFN- γ , LT- α , CCL17, and CCL22 chemokines that play a role in the regulation of Treg and Th2 cell migration and modulate the activity of GC B cells within follicles as well. Not only Tfh-like cells but also Foxp3⁺ Tfr cells are found within neoplastic follicles and the number of Tfr cells is elevated during progression lymphomagenesis. Thus, there is a complex T cell response that is regulated by a delicate balance of CD4⁺, Tregs, and Tfh subsets. Probably one of the strongest predictors of survival would be the location pattern for T cell subsets, as accumulation within the follicles is linked with poor survival compared with a distribution pattern outside the follicle (291). To date, there is limited understanding in the functions of Tfh and Tfr subsets in lymphomagenesis and more detailed research in animal models and human samples will help to dissect the complex role of Tfh cells in cancer.

Unexpectedly, Tfh cells have protective roles in nonlymphoid tumors. Higher levels of Tfh cell infiltrates and their ability to organize tertiary lymphoid structures within tumors has been associated with increased survival and reduced immunosuppression which strongly correlate with an increased survival in breast cancer (292). Evidence suggest that IL-21 and CXCL13 may play a key role in the protective functions of Tfh cells *via* the modulation of local leukocyte recruitment. Infiltrating Tfh cells have also been reported in chronic lymphocytic leukemia, non-small cell lung cancer, osteosarcoma, and colorectal cancer (214, 293–297), where they positively correlated with patient survival (293). So far, very little is known about how Tfh cells impact the immune response involved in the suppression of tumor initiation and progression and further studies will be important for a better understanding of Tfh-related pathologies.

CONCLUSION

Since the identification of Tfh cells and the discovery of Bcl6 as a critical factor in their generation, there has been substantial progress made in understanding the molecular and cellular requirement for the development and function of mouse and human Tfh cells. To date, multiple cell-extrinsic and -intrinsic factors (costimulatory molecules, cytokines signaling, and transcriptional factors) have been determined to positively or negatively contribute to Tfh cell development. However, still many questions remain to be answered: (i) What is the exact composition and hierarchy of these factors and what are their

stage-specific requirements? (ii) Is Bcl6 required complete Tfh cell commitment? (iii) How Tfh-specific transcriptional factors impact epigenetic mechanisms governing Tfh cell generation? (iv) Which factors contribute to Tfh cell maintenance and memory formation? (v) What are the appropriate Tfh-specific target(s) for therapy in Tfh mediated autoimmune disorders and cancer?

T follicular helper cells mainly localize in secondary lymphoid organs and circulate in the blood and are beginning to emerge as crucial players in maintaining a healthy balance between protective and pathogenic immunity (Figure 2). However, due to the localization of Tfh cells in secondary lymphoid tissues, the study of human Tfh cell heterogeneity and function in normal and disease settings has been difficult. Further analysis and comparison of human circulating counterparts to tissue-resident Tfh cells in various disease settings is critical, since it will help to reveal the level of Tfh cell heterogeneity at various stages of diseases as well as will determine whether the disease-associated alterations in Tfh cells are the main cause or result of disorders. Thus, in view of an emerging role of Tfh cells in various disease settings, we believe that current progress and further understanding of the heterogeneity and regulation

of tissue-specific and cTfh cells in health and disease will lead to improved vaccine designs, better management of major autoimmune, inflammatory disorders, and cancer, and can be utilized as a novel prognostic biomarker for the identification of lymphoid and solid tumors.

AUTHOR CONTRIBUTIONS

All authors listed contributed significantly toward the preparation of the manuscript. LQ, TW, SB, EG, and RN wrote the manuscript. AS helped in writing, extensive editing, and revising the manuscript. HZ provided critical comments on the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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