Molecular Control of Follicular Helper T cell Development and Differentiation

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Follicular helper T cells (Tfh) are specialized helper T cells that are predominantly located in germinal centers and provide help to B cells. The development and differentiation of Tfh cells has been shown to be regulated by transcription factors, such as B-cell lymphoma 6 protein (Bcl-6), signal transducer and activator of transcription 3 (STAT3) and B lymphocyte-induced maturation protein-1 (Blimp-1). In addition, cytokines, including IL-21, have been found to be important for Tfh cell development. Moreover, several epigenetic modifications have also been reported to be involved in the determination of Tfh cell fate. The regulatory network is complicated, and the number of novel molecules demonstrated to control the fate of Tfh cells is increasing. Therefore, this review aims to summarize the current knowledge regarding the molecular regulation of Tfh cell development and differentiation at the protein level and at the epigenetic level to elucidate Tfh cell biology and provide potential targets for clinical interventions in the future.

Keywords: Tfh, Bcl-6, Blimp-1, transcription factors, epigenetics

INTRODUCTION

A subset of CD4+ T cells, which help B cells and are a resident in B follicles, has been described in the early 1990s (1–4). The existence of follicular helper T (Tfh) cells was proposed in 2000 (5, 6). However, the existence of these cells was not widely accepted until the identification of the Tfh cell lineage-specific transcription factor, B-cell lymphoma 6 protein (Bcl-6), in follicular T cells in 2009 (7, 8). High expression of CXCR5 and low expression of CCR7 enable T cells to enter and stay in germinal centers (GCs) (6, 9–11). Bcl-6 deficient T cells have been shown to fail to differentiate into follicular helper T cells (8), indicating the importance of Bcl-6 in the determination of Tfh cell fate. Under the effects of CCL19 and CCL21, expression of the receptor CCR7 on naive CD4+ T cells enables these cells to migrate into T cell zones in the secondary lymph nodes (9, 12). With stimulation from antigens and CD80, CD86 and ICOSL expressed on dendritic cells (DCs), these cells differentiate into pre-Tfh cells with high expression of PD-1, CXCR5 and signaling lymphocytic activation molecule adapter protein (SAP) (13) and low expression of CCR7 and P selectin glycoprotein ligand 1 (PSGL1) (14, 15) (Figure 1). Generally, Tfh cells provide signals for B cell maturation, differentiation and survival via ICOS, CD40L, IL-4, and IL-21 (16, 17). ICOS and...
Under the effects of CCL19 and CCL21, expression of the receptor CCR7 on naïve CD4^+ T cells allows these cells to migrate into T cell zones in the secondary lymph nodes. With stimulation from antigens and CD80, CD86 and ICOSL expressed on dendritic cells (DCs), these cells differentiate into pre-Tfh cells, with high expression of CXCR5, PD-1 and signaling lymphocytic activation molecule adapter protein (SAP) and low expression of CCR7 and P selectin glycoprotein ligand 1 (PSGL1). Generally, Tfh cells provide signals for B cell maturation, differentiation and survival via ICOS, CD40L, IL-4, and IL-21 (16, 17). ICOS and ICOSL ligation is involved in Tfh-B cell interactions, which promotes calcium spikes in T-cells and CD40-CD40L signaling in B cells.

ICOS ligation is involved in T-B cell interactions, further promoting calcium spikes in T-cells and CD40-CD40L signaling in B cells (18). ICOS-deficient T cells fail to express CXCR5 and are unable to migrate into follicles, a finding also observed during antibody-blockade of ICOS-ligand (19, 20). PD-1 has been found to limit the number of Tfh cells (21). More evidence is needed to address the role of PD-1 in the migration and function of Tfh cells. SAP has been found to stabilize the interaction between B cells and Tfh cells (22). Therefore, Tfh cells can be distinguished from Th1, Th2 and Th17 cells using surface markers with a profile of CCR7^lo^PSGL1^lo^CXCR5^hi^PD-1^hi^ICO5^hi^.

The discovery of Bcl-6 in Tfh cells is a hallmark for the identification of Tfh cells. The essential role of Bcl-6 has been confirmed in a mice study, indicating that CD4^+ T cells deficient in Bcl-6 fail to differentiate into Tfh cells (8). Forced expression of Bcl-6 in CD4^+ T cells promotes the expression of CXCR5, CXCR4, and PD-1 (8). Bcl-6 can bind to the promoters of Th1 and Th17 cell transcriptional regulators T-bet and RORγT, thereby repressing the production of IFN-γ and IL-17 (8). The key role of Bcl-6 in Tfh cell fate determination has been further confirmed in subsequent studies (23, 24), and one of them reveals that Bcl-6 regulates Tfh cell early differentiation in an IL-21- and IL-6-independent manner (24). Conversely, Bcl-6 can bind to the promoters and enhancers of several migration-related genes, such as CCR7, CCR6, PSGL1, CXCR5, CXCR4, PD-1, and SAP (24, 25). In addition, Bcl-6-targeted genes are enriched in the MAPK and JAK-STAT signaling pathways and cytokine-cytokine receptor ligations, which are involved in cell activation, metabolism and maintenance (26).
Bcl-6 and STAT5

Similar to the Bcl-6-Blimp-1 axis, Bcl-6 and STAT5 also inhibit each other due to their overlapping binding sites in many Tfh cell-related genes, including Socs2, Il7r, and Tcf7. In a mouse study, Bcl-6 has been found to repress both IL-7R and STAT5 expression, as well as inhibiting IL-2-induced STAT5 activation (32). This inhibitory effect on STAT5 by Bcl-6 is due to the abrogation of STAT5 phosphorylation (32). In contrast, signals through IL-2-CD25 activate STAT5 and inhibit Bcl-6 and CXCR5 via inducing Blimp-1 (20, 33, 34), and lack of IL-2R signaling leads to Bcl-6 expression (35). A high concentration of IL-2 has been found to inhibit Bcl-6 expression in polarized Th1 cells, in which the Bcl-6 DNA-binding domain is masked by the T-bet-Bcl-6 complex and normally shows low levels of Bcl-6 expression in response to limited IL-2 (36). However, in response to low IL-2, besides increased Bcl-6 and IL-6R, Th1 cells can also increase the expression of IL-7R, which can repress Tfh-related genes, including cxcr5 and bcl6 via IL-7-dependent STAT5 activation (37). In addition, Bcl-6 in Tfh cells has been observed to have a decreased level of 5-hydroxymethylcytosine (5hmC), which might explain the markedly high level of Bcl-6 in Tfh cells (32). Conversely, Bcl-6 deficiency results in increased STAT5 signaling and promotes the differentiation of non-Tfh effector T cells. The inhibitory effects of STAT5 have been found to be Blimp-1-independent. In addition, inhibition of IL-2 results in the reduction of Blimp-1 expression (38), indicating that IL-2, STAT5 and Blimp-1 collaboratively inhibit Tfh cell differentiation (39).

STAT3

IL-21 and IL-6/STAT3 are first described to be essential for Th17 cell differentiation (40). Next, STAT3 has found to be critical for Tfh cell differentiation. The evidence come from the fact that reduced IL-21 production is reported in mouse STAT3-deficient T cells, and only a STAT3 mutation, rather than Il12rb1, reduce the frequency of Tfh cells in vivo (41). Similarly, in CD4+ T cell-conditional STAT3 knockout mice, fewer Cxcr5+ Tfh cells, as well as defective GCs and reduced IgG and IgM antibody production, have been observed after KLH immunization (42, 43). In another study, the gene expression of Cxcr5 and Icos is shown to be downregulated in STAT3-deficient mice, while the expression of Blimp-1 is increased (44). More importantly, cluster analysis showed that STAT3-deficient Ly6C+ Psgl-1+ T cells in the T cell zone more closely resemble Th1 cells, with a high expression of IFN-induced genes (44). More direct evidence is that STAT3 can form a complex with Ikaros zinc finger transcription factor Aiolos to regulate Bcl-6 expression (45). In a human study, rather than in a mouse system, TGF-beta has been found to provide critical additional signals for STAT3 and STAT4 to initiate Tfh cell differentiation (46), emphasizing the important role of STAT3 in Tfh cell development. Unlike the critical role of IL-6 in early Tfh cell differentiation, STAT3 deficiency fails to recapitulate the impaired Tfh frequency. However, in this study, STAT1 activity has been found to be required for Bcl-6 induction and initiating Tfh cell differentiation (47). In addition, STAT3 can suppress type 1 IFN induced CD25 expression and can compete with STAT5 to bind to the Bcl6 locus (48). However, it might be difficult to distinguish whether the effects of STAT3 is intrinsic to the Tfh cell or a reflection of diminished capacity for other cell subset differentiation. The forced overexpression of STAT3 in T cell may provide an explanation to this issue, which is still lacking at this moment.

TCF-1 and LEF-1

TCF-1 and LEF-1 belong to the TCF-LEF subfamily and have been well-documented to be necessary for the maturation of double negative T cells to the double positive stage in thymus. In addition, TCF-1 has been reported to restrain mature T cell-mediated Th17 responses via suppressing IL-17 expression (49). TCF-1 and LEF-1 have been reported as critical transcription factors in Tfh cell differentiation by two independent studies published in the same year (50, 51). The loss of either TCF-1 or LEF-1 in mice leads to defects in Tfh cells, and the depletion of both TCF-1 and LEF-1 results in the impairment of Tfh cell differentiation and GC formation. In addition, the important role of LEF-1 has been emphasized by the observation that forced LEF-1 expression promotes the differentiation of Tfh cells (51). In another study, TCF-1 and LEF-1 are revealed to regulate the Bcl-6/Blimp-1 axis. TCF-1 has been identified as a positive regulator for Bcl-6 and it displays negative effects on Blimp-1 via directly binding to the Bcl-6 promoter to form a complex and regulatory region known as intron 3 of Prdm1 (51). In addition, TCF-1 has been found to upregulate IL-6R expression and inhibit IL-2R expression (51), indicating that TCF-1 might be upstream of STAT3 and STAT5. The exact function of LEF-1 in Tfh cells remains unclear. However, evidence shows that LEF-1 synergistically works with TCF-1 to regulate Tfh cells, and TCF-1 can inhibit LEF-1 expression (51). Furthermore, TCF-1 and LEF-1 have been found to promote early Tfh cell differentiation by maintaining the expression of IL-6Rα and gp130 and enhancing ICOS and Bcl-6 expression (52).
Ascl2
Ascl2 is a basic helix-loop helix (bHLH) transcription factor that has been reported to initiate Th cell differentiation via upregulating CXCR5 but not Bcl-6 in T cells in vitro (53). In addition, in vivo, Ascl2 can promote T cell migration to the border of B cell follicles and can promote Th cell differentiation by inhibiting Th1 and Th17 signature genes and upregulating Th17 cell-related genes (53). In other studies, Ascl2 has been shown to be responsible for low CD25 expression on regulatory follicular T cells (Trf) (54). Ascl2 displays the active chromatin marker trimethylated histone H3 lysine 4 (H3K4me3), which has not been observed in other T cell subsets. In contrast, other Th cell-related genes, such as Bcl-6, Maf, Batf, and Irf4, are associated with H3K4me3 in all T-cell subsets (55).

C-Maf
C-Maf, a member of the activator protein 1 (AP-1) transcription factor family, has been found to be highly expressed by Th17 and mature Th cells compared with CD4^+ICOS^+CXCR5^- or CD4^+ICOS^-CXCR5^- non-Th cells. During Th17 cell differentiation, IL-6 plus TGF-β or IL-21 plus TGF-β can increase the expression of c-Maf, which is ICOS-dependent (56). As mentioned before, Bcl-6 controls the expression of migration genes that are important for the migration of T cells to the follicles. However, the introduction of Bcl-6 cannot alter the production of IL-21 and IL-4, which are the key cytokines produced by Th17 cells. c-Maf has been found to affect the production of IL-21 and CXCR5 (57). In addition, c-Maf and Bcl-6 have been reported to cooperate in the expression of Th1 cell-related genes, such as CXCR4, PD-1, and ICOS (57). The selective loss of c-Maf expression in T cells leads to the inhibition of Th1 cell differentiation in response to vaccinations and bacteria, and it is also critical for high-affinity antibody secretion in vaccinated animals (58). In addition, in Th1 cells, c-Maf has been shown to positively regulate IL-4 production via binding to the conserved noncoding sequence 2 (CNS2) region of the IL-4 locus and via the induction of Irf4 (59–61); however, this effect is c-Maf-independent (61).

Batf
Batf is also a member of the AP-1 family, which lacks transcriptional activation domains (TADs). Batf has been found to be highly expressed by Th17 cells and directly regulates the expression Bcl-6 and c-Maf (62). The expression of Batf has been observed to be regulated by IL-4-STAT6 in Th9 cells and IL-6-STAT3 signaling in M1 mouse myeloid leukemia cells (63–65). In Batf-deficient mouse T cells, the expression of Bcl-6 and c-Maf decreased dramatically, and Bcl-6 alone is not sufficient for Th1 cell differentiation in the absence of Batf (62). In addition, Batf can cooperate with Irf4 along with STAT3 and STAT4 to promote IL-4 production in Th17 cells via binding to the CNS2 region in the IL-4 locus. Batf does not impair IL-4 in Th2 cells but only Th1 cells (61). However, other studies show that the loss of Batf impairs IL-4 production in both Th1 and Th2 cells (66, 67).

IRF4
IRF4 has been well-documented as an important transcription factor in the differentiation of helper T cells and B cells via promoting cell development (68). IRF4 expression in mouse T cells has also been found to promote GC formation by promoting Th1 cell differentiation (69). In IRF4 knockout mice, CD4^+ T cells in lymph nodes and Peyer’s patches failed to express Bcl-6 and Th17 cell-related genes. In addition, the adoptive transfer of wild-type T cells cannot rescue the failed IRF4^-/- T cell differentiation (69), indicating a critical role for IRF4 in T cell development. In wild-type mice, IRF4 can interact with JUN and Batf to form a heterotrimer that can bind to AP1-IRF4 complexes and regulate Th cell differentiation (69). In another study, IRF4^-/- CD4^+ T cells have impaired STAT3 binding and fail to differentiate into Th1 cells (70). In a recent study, the Irf4 locus is reported to “sense” the intensity of TCR signaling to determine the Irf4 expression level. The binding of IRF4 to divergent DNA sequences is regulated by the expression levels of IRF4 and controls Th cell fate determination (71). In Th2 cells, enhancers show a spectrum of occupancy by the Batf-IRF4 complex, which correlates with the sensitivity of gene expression to TCR signal strength (72). The adaptor molecule LAT has been revealed to export the repressor HDAC7 from the nucleus of CD4(+) T cells. The loss of LAT results in impaired TCR signal and the repression of HDAC7 targeted gene Nur77 and Irf4 (73). Furthermore, IRF4 has been reported to be induced in a TCR-affinity dependent manner, and it is critical for clonal expansion (74).

In addition, other transcription factors have also been reported to be involved in Th1 cell differentiation. Foxo1, which has been found to negatively regulate Th1 cell differentiation in the early stages of differentiation (75), has also been identified to positively promote Th1 cell differentiation in the late stage of this process (76). However, the molecular mechanism remains unclear. FOXP1 negatively regulates Th1 cell differentiation by directly inhibiting ICOS expression and IL-21 production (77). Kruppel-like factor 2 (KLF2), a transcription factor, has been found to be involved in T cell trafficking, survival and homeostasis. KLF2 deficiency has been linked with increased number of Th1 cells, and forced expression of KLF2 results in reduced Th1 cell differentiation and GC formation (78). KLF2 can negatively control Th1 cell differentiation by inhibiting the homing receptors, such as CXCR5, CCR7, S1PR1 and PSGL1 (79), via induction of negative regulators for Th1 cells, including Blimp-1, T-bet and GATA3 (78).

Other Proteins Regulating Tfh Cell Differentiation E3 Ubiquitin Ligase
Roquin is an RNA binding protein, which has been revealed to play a critical role in innate and adaptive immune systems. The lack of Roquin activity results in numerous autoimmune diseases, such as lupus and inflammatory bowel disease. It is well-known that samroque mice, which have the mutant ROQUIN M199R that promotes Th cells, show a spontaneous germinal center (GC) and accumulation of plasma cells (30, 80, 81). The ubiquitin E3 ligase Roquin-1 negatively regulates Tfh cell differentiation by recognizing and directly binding a cis-element in the 3′
untranslated region of ICOS mRNA, thereby repressing ICOS expression (82). The combined loss of Roquin-1 and 2 results in spontaneous Tfh cell and germinal center development (83). Other Tfh-related genes, such as Il6, Ifrd4, Ox40, (84, 85) and Ifng (86), are repressed by Roquin. The loss of the RUNG domain of Roquin has been found to reduce the number of Tfh cells, which might be a result of impaired mTOR signaling (87) and reduced Bcl-6 expression (88). In addition, the E3 ubiquitin ligase Itch has also been reported to regulate Tfh cells by regulating the ubiquitination and degradation of Foxo1 (89), and the effect of Itch has been revealed to be upstream of Bcl-6, which is validated by the fact that forced Bcl-6 in Itch deficient mice can restore Tfh cell differentiation (89). Moreover, the E3 ubiquitin ligase Cullin3 acts as a negative regulator by directly binding to Bcl-6 and regulating the ubiquitination of histone proteins (90). Furthermore, in transplantation, herpesvirus entry mediator/B- and T-lymphocyte attenuator (HVEM/BTLA) signaling pathway has been found to be dispensable for the expansion of Tfh cells and formation of de novo host anti-donor isotype-specific antibodies (91).

**Notch–1 and –2**
The T cell-specific deletion of Notch-1 and Notch-2 results in the reduced number of fully mature Tfh cells and the absence of high-affinity Abs (92). These mature Tfh cells produce low levels of IL-21 and displayed low expression of Bcl-6 and C-Maf. However, the effect of the loss of Notch on Tfh cell differentiation is in an IL-4-independent manner (92). In a recent study, Notch signaling has been identified as an early lineage-determining factor between Tfh and Th2 cell fate (93). In addition, Delta-L 1/4-mediated signals to Tfh cells occur from stroma cells, and follicular dendritic cells are not required (93, 94). Fasnacht et al. (94) shows DLL4 in stromal cells is important for Tfh development. In a previous study, fibroblasts, rather than hematopoietic or endothelial cells, as niche cells, support Notch-2 driven differentiation of marginal-zone B cells, ESAMDCs, and Tfh cells (94).

**Surface Molecule Regulation**

**CXCR5**
CXCR5 is a hallmark of Tfh cells that guides T cells to migrate to the B cell zone by binding to CXCL13 that is expressed by follicular dendritic cells (95). CXCL13 is expressed in the follicular mantle zone and not in the endothelial venules and paracortical T cell zone, where ligands for CCR7 exist. Unlike CCR7 ligands, CCL19 and CCL21, CXCL13 controls the segregation between T and B cells, rather than recruiting T cells and B cells to lymph nodes (96). Therefore, these CXCR5 hi T cells express a low level of CCR7, which helps these T cells to migrate to GCs (6, 9, 10, 97). Moreover, CXCR5 has been found to help the maintenance of PD-1 hi Tfh population in GCs (9). CXCR5-deficient mice have a low GC number and antibody production (95), which shows the important role of CXCR5 in Tfh cell differentiation. In addition to being controlled by Bcl-6, CXCR5 expression is also regulated by nuclear factor of activated T cells (NFAT2) (98).

**ICOS/ICOSL**
With signals from MHC-antigen-TCR and CD28 stimulation, ICOS expression is induced on activated T cells. Therefore, ICOS is not a reliable marker for Tfh cells not only due to its expression on precursor Tfh cells but also due to its high expression on activated T cells. Signals through ICOS-ICOSL are critical for Tfh cell differentiation. B cell survival and activation, antibody class switching and GC formation (99). In human Tfh cells, ICOS is used as a marker of GC Tfh cells (100). However, ICOS is probably not a reliable marker for GC Tfh cells in mice due to its similar expression in Tfh cells and precursor Tfh cells (101). It has been found that initial DC priming is sufficient to differentiate CXCR5+ Bcl-6+ Tfh cells, but this process depends on consistent ICOS/ICOSL signaling from DCs (102). Further ICOSL signals from B cells are necessary for the complete differentiation and maintenance of GC-Tfh cells (14). ICOS has been found to be capable of regulating the migration of T cells to GCs via the induction of filopodia (17). Signals through ICOS/ICOSL activate phosphoinositide-3 kinase (PI3K) (103), which is also a critical kinase for Tfh cell differentiation via the AKT-mediated inactivation of FOXO (104). In addition, ICOS is able to maintain the Tfh cell phenotype via FOXO1-mediated KL2 expression (79), and FOXO1 is also inhibited by ICOS-induced mTORc2 (75). ICOS signaling can also affect IL-21 production via c-Maf, thereby regulating Tfh cell differentiation (56). The importance of ICOS in Tfh cells has been demonstrated by a study showing that ICOS-deficient mice have impaired GCs, a reduced level of CXCR5+ memory T cells (19, 105), impaired immunoglobulin class switching and low levels of IL-4 when primed in vivo and restimulated in vitro with a specific antigen (106, 107).

**OX40/OX40L**
OX40 belongs to the TNFR family and is transiently expressed by T cells during chronic virus infection (108). OX40 has been found to play a critical role in Tfh cell differentiation. Reinforcing OX40 stimulation promotes the expression of Blimp-1 in LCMV-specific T cells and inhibits the differentiation of Tfh cells (108). However, OX40-deficient mice display impaired generation of Tfh cells and GCs (109), indicating that OX40 is important for the Tfh cell differentiation. Indeed, OX40L has been reported to contribute to lupus by promoting Tfh cell responses (110). In addition, TSLP-activated dendritic cells have been found to be able to induce Tfh cell generation via OX40L (111). In addition, OX40 can cooperate with ICOS to amplify Tfh cell development during vaccinia virus infection (112).

**Other Important Surface Markers**
PD-1, which is usually expressed by exhausted T cells, is highly expressed on Tfh cells. PD-1/PD-Ls signals are generally considered as negative regulatory signals that dephosphorylate TCR signaling, thereby inhibiting activation and cytokine production by T cells (111). PD-1 expressed by Tfh cells is believed to balance the negative regulation from IL-2-mediated STAT5 signaling (114). In addition, Tfr cells also express PD-1, which regulates Tfr cells (115). In a recent report, PD-1 has been found to inhibit follicular T cell recruitment via limiting CXCR3 expression to confine Tfh cell localization in GCs and
increase the stringency of GC affinity selection through PD-1-PD-L1 ligation (116). Cytotoxic T lymphocyte antigen 4 (CTLA-4) is another negative regulator of T cells that has been reported to be expressed by Tfh cells and Tfr cells (117). Tfr and Treg cells regulate Tfh cells via B7-1 and B7-2 binding to CTLA-4. Loss of CTLA-4 in Tfh cells results in the promotion of B cell responses (118). Moreover, mice deficient in the SLAM-associated protein (SAP) show impaired GC formation and defects in T-B cell interaction (22, 119–121). Although SAP deficient T cells can express Tfh markers initially, reduced Tfh cells have been found in GCs from SAP deficient mice (30, 122), suggesting that SAP is required for the generation of functional Tfh cells and the differentiation of Tfh cell contains multiple steps.

Cytokine Regulation

Signals from follicular DCs and the cytokine milieu produced by DCs provide instructions for Tfh cell differentiation. Various cytokines, including IL-6, IL-21, IL-12, IL-23, IL-2, TGF-β, IL-1β, can regulate Bcl-6, STAT5, and Blimp-1 expression via the JAK-STAT signaling pathway (55). In addition, other STAT-activating cytokines, such as IL-1β and IL-6, support this process in the presence of IL-12, IL-23, and TGF-β. The precursors of Tfh cells share similarities with other T subsets and can further differentiate into Th1 and Th17 cells dependent on the balance of cytokines (123). Following interactions with B cells, precursor cells can differentiate into Th1-like Tfh cells and Th17-like Tfh cells (123). In addition, some reports have shown that Tfh cells can express IFN-gamma and IL-4, which provides help for cytokine-driven patterns of immunoglobulin class switching (124). In some autoimmune conditions, such as an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS), cells that display a Tfh cell phenotype produce IL-17 (56), and during helminth infection, Tfh cells in lymph nodes produce IL-4 (124–126). These IL-4 producing Tfh cells located in B cell follicles are found to be functionally different from Th2 cells found in peripheral region (124). These IL-4 producing Tfh cells express a low level of GATA3 and no IL-13 (127).

It has been well-established that IL-6-mediated STAT3 activation is critical for IL-21 expression in TCR stimulated mice and human T cells (40, 128). STAT3 can also respond to IL-21 and IL-23. Following cytokine stimulation, STAT3 is phosphorylated by JAK and binds to the Bcl-6 promoter to further promote Bcl-6 transcription (129). In addition, IL-12 has been reported to induce Bcl-6 expression in human T cells via STAT4 activation and has a greater effect on IL-21 production compared to IL-6 and IL-21 (130). IL-12 stimulates STAT4 pathway can also regulate CCR5, ICOS, c-Maf, and Batf expression in human T cells (131, 132). TGF-β has been found to enhance the function of STAT3-STAT4 to help T cells to express CCR5, ICOS, Bcl-6, c-Maf, IL-21, and Batf, as well as to repress the expression of Blimp-1 (42). However, in mice, TGF-β has been reported to have negative regulatory effects on Bcl-6 expression via mir-10a (133). The positive regulation of TGF-β might be restricted to human in vitro studies. However, in mice, cytokines and TCR stimulation are insufficient, and the T-B interaction is necessary to generate Tfh cells (134).

FIGURE 2 | Network of transcription factors, cytokines and surface markers in Tfh cell regulation. In addition to the signals from surface markers, Tfh cells have been found to be regulated by a complex network of transcription factors, including the Bcl-6-Blimp1 axis, STAT1, STAT3, STAT4, STAT5, B-cell activating transcription factor (Batf), v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (c-Maf), interferon regulatory factor 4 (IRF4), Achaete-scute homolog 2 (Ac2), and T-cell-specific transcription factor 1 (TCF-1)-LEF-1, FOXO-1, FOXP-1, and NFAT-2. Since the study of Tfh cells began, some proteins have been identified to participate in the development of Tfh cells. In addition, cytokines such as IL-1 beta, IL-2, IL-6, IL-12, IL-21, IL-23, and TGF-β have been reported to be involved in the differentiation and survival of Tfh cells. “+” means positively regulates Tfh cell differentiation and “−” means negatively regulates Tfh cell development.
Epigenetic Regulations

Epigenetic regulation refers to a modification that will not change the DNA sequence but alters the gene expression through several modifications, such as DNA methylation, histone modification and non-coding RNA-mediated regulations. Increasing evidence has shown the cooperation between epigenetic modifications with transcription factors to determine T cell fate (135).

Unsurprisingly, Tfh cell differentiation is also regulated by epigenetic modifications. DNA methylation refers to silencing gene expression, and demethylation/hydroxymethylation is related to gene reactivation. In Tfh cells, Bcl-6 binding to gene loci has been found to be associated with reduced recruitment of translocation methylcytosine dioxygenase 1 (TET1), which is a hydroxymethyltransferase. Bcl-6 binding is also observed to result in reduced 5-hydroxymethylcytosine (5-hmC) (32), which is a mechanism for DNA demethylation. In addition, our previous study, we found that IL-21 can increase TET2 enrichment on the promoter region of Bcl-6, which might explain the increased levels of Bcl-6 in lupus T cells (31). In addition, methylated H3K27 has been reported to prevent Tfh-related gene expression, while the H3K27me3 demethylase UTX sustains Tfh cells and antibody production (136). Positive histone modifications have been detected at the Bcl-6 locus in Tfh cells, but negative marks are present at Bcl-6 in other Tfh subsets (137). In addition, positive and negative histone modifications can be detected on Prdm1 in all Tfh cell populations. These positive and negative histone modifications might provide clues for Tfh cell plasticity. miRNAs, which are non-coding RNAs, regulate gene expression at the posttranscriptional and posttranslational levels. miRNAs silence gene expression by targeting the 3’-untranslated regions of mRNA, causing mRNA cleavage and translational repression. In Tfh cells, the miR-17-92 cluster has been observed to be downregulated, which might contribute to the overexpression of Bcl-6 (138). miR-155 can regulate Tfh cell accumulation in miR-146a-deficient mice, resulting in abnormal Tfh cell accumulation (138). miR-146a can directly targets ICOS and the overexpression of ICOS mediated by the loss of miR-146a results in spontaneous and cell-autonomous Tfh cell accumulation (139). The molecules regulating Tfh cell differentiation are summarized in Figure 2.

Increasing evidence has shown the plasticity of Tfh cells, which can be explained by epigenetic regulations. Tfh cells display repressive histone markings (H2K27me3) on Il4, Ifng, and Il17a, while permissive active chromatin H3K4me3 on Il21 locus (137, 140). Interestingly, the evidence that Tfh cells can produce effector T cell cytokines in response to the polarization cytokines and maintain the ability to produce IL-21 (137), can be explained by the fact that Tfh cells also display detectable H3K4me3 on Tbx21, Gata3, and Rorc locus (137). The positive H3K4me3 has been observed on the Bcl6 gene in Tfh cells from an in vivo and ex vivo system. Other in vitro differentiated Th cells also show permissive markers on Bcl6, which enables these cells to acquire Tfh cell phenotypes and the capacity to produce IL-21 (137).

CONCLUSIONS

Tfh cell differentiation is regulated by multiple transcription factors, receptors, cytokines, and epigenetic modifications. Unlike other Th cells, mouse Tfh cells are difficult to generate in vitro by cytokines and TCR stimulation, possibly reflecting a requirement for T-B cell interactions. ICOS/ICOSL signals might be an underlying explanation for the difficulty mentioned above. In addition, the cytokines driving differentiation in mouse and human systems are different; for example, TGF-β is a negative regulator in mice but a positive regulator in human Tfh cells. Tfh cells are heterogenic populations. Certain Th1, Th2, and Th17-like Tfh cells have been identified in GCs. In addition, Tfr cells have also been reported and regulate Tfh cell homeostasis. In addition, in certain inflammatory sites, such as synovium from rheumatoid arthritis patients, non-classic Tfh-like cells have been identified, which are CXCR5low but have high expression levels of Bcl-6, PD-1, and IL-21. Single-cell mRNA sequencing should facilitate studies aiming at dissecting Tfh cell subset heterogeneity and distribution in tissues and blood. Our understanding of epigenetic regulation of Tfh cells is limited. Due to the development of new technologies, new molecules might be identified in the near future.

AUTHOR CONTRIBUTIONS

HW wrote the manuscript. YD, MZ, JZ, MZ, LL, and GC edited the manuscript. ZH and QL revised the manuscript.

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REFERENCES

1. MacLennan IC, Gulbranson-Judge A, Toellner KM, Casamayor-Palleja M, Chan E, Sze DM, et al. The changing preference of T and B cells for partners as T-dependent antibody responses develop. *Immunol Rev.* (1997) 156:53–66. doi: 10.1111/j.1600-065X.1997.tb00958.x

2. Zheng B, Han S, Kelsoe G. T helper cells in murine germinal centers are antigen-specific emigrants that downregulate Thy-1. *J Exp Med.* (1996) 184:1083–91. doi: 10.1084/jem.184.3.1083

3. Bowen MB, Butch AW, Parvin CA, Levine A, Nahm MH. Germinal center T cells are distinct helper-inducer T cells. *Hum Immunol.* (1991) 31:67–75. doi: 10.1016/0198-8859(91)90050-J
19. Akiba H, Takeda K, Kojima Y, Usui Y, Harada N, Yamazaki T, et al. The role of ICOS in the CXCR5

17. Xu H, Li X, Liu D, Li J, Zhang X, Chen X, et al. Follicular T-helper cell and B-cell entanglement and

18. Liu D, Xu H, Shih C, Wan Z, Ma X, Ma W, et al. T-B-cell entanglement and B-cell follicles, and support immunoglobulin production. J Exp. Med. (2000) 192:1545–52. doi: 10.1084/jem.192.11.1545

23. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, et al. ICOSL-driven feed-forward regulation of germinal centre reaction. Nature (2008) 455:764–9. doi: 10.1038/nature07345

20. Barish GD, Yu RT, Karunasisir M, Ocampo CR, Dixon J, Benner C, et al. Bcl-6 and NF-kappaB cistromes mediate opposing regulation of the innate immune response. Genes Dev. (2010) 24:2760–5. doi: 10.1101/gad.1998010

22. Crotty S, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nat Immunol. (2001) 2:363–70. doi: 10.1038/colm0515

24. Poholek AC, Hansen K, Hernandez SG, Eto D, Chandele A, Weinstein JS, et al. In vivo regulation of Bcl6 and T follicular helper cell development. J Immunol. (2010) 185:313–26. doi: 10.4049/jimmunol.0904023

25. Hatzik N, Nance JP, Kroezen MA, Bothwell M, Haddad EK, Melnick A, et al. Bcl6 orchestrates Tfh cell differentiation via multiple distinct mechanisms. J Exp Med (2015) 212:539–53. doi: 10.1084/jem.20141380

26. Liu J, Havenar-Daughton C, Crotty S. Modulation of SAP dependent T:B cell interactions underlie germinal centre formation. Nature (2007) 448:480–3. doi: 10.1038/nature05969

27. Crotty S, Johnston RJ, Schoenberger SP. Follicular helper T cells are required for systemic autoimmunity. J Exp. Med. (2009) 206:561–76. doi: 10.1084/jem.20081886

28. Ochiai K, Muto A, Tanaka H, Takahashi S, Igarashi K. Regulation of the plasma cell transcription factor Blimp-1 gene by Bach2 and Bcl6. Int Immunol. (2008) 20:453–60. doi: 10.1093/intimm/dxn005

29. Wu H, Deng Y, Feng Y, Long D, Ma K, Wang X, et al. Epigenetic regulation in B-cell maturation and its dysregulation in autoimmunity. Cell Mol Immunol. (2018) 15:676–84. doi: 10.1038/cmi.2017.133

30. Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL, et al. Follicular helper T cells are required for systemic autoimmunity. J Exp. Med. (2007) 198:1742–52. doi: 10.1084/jem.20071237

31. Huang X, Wu H, Qiu H, Yang H, Deng Y, Zhao M, et al. The expression of Bcl-6 in circulating follicular helper-like T cells positively correlates with the disease activity in systemic lupus erythematosus. Clin Immunol. (2016) 173:161–70. doi: 10.1016/j.clim.2016.01.017

32. Liu X, Lu H, Chen T, Nallaparaju KC, Yan X, Tanaka S, et al. Genome-wide analysis identifies Bcl6-controlled regulatory networks during T follicular helper cell differentiation. Cell Rep. (2016) 14:1735–47. doi: 10.1016/j.celrep.2016.01.038

33. Gong D, Malek TR. Cytokine-dependent Blimp-1 expression in activated T cells inhibits IL-2 production. J Immunol. (2007) 178:242–52. doi: 10.4049/jimmunol.178.1.242

34. Shaffer AL, Lin KI, Kuo TC, Yu X, Hurt EM, Rosenwald A, et al. Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity (2002) 17:51–62. doi: 10.1016/S1074-7613(02)00335-7

35. Pepper M, Pagan AJ, Igyarto BZ, Taylor JJ, Jenkins MK. Opposing signals of the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. Immunity (2011) 35:583–95. doi: 10.1016/j.immuni.2011.09.009

36. Oestreich KJ, Mohn SE, Weimann AS. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TPH-like gene profile. Nat Immunol. (2012) 13:405–11. doi: 10.1038/imm.2011.224

37. McDonald PW, Read KA, Baker CE, Anderson AE, Powell MB, Ballesteros-Tato A, et al. IL-7 signalling represses Bcl-6 and the TFH gene program. Nat Commun. (2017) 8:10285. doi: 10.1038/ncomms10285

38. Nurieva RI, Podd A, Chen Y, Alexeev AM, Yu M, Qiu X, et al. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. J Biol Chem. (2012) 287:11234–9. doi: 10.1074/jbc.M111.324046

39. Johnston RJ, Choi YS, Diamond JA, Yang JA, Crotty S. STAT3 protein is a potent negative regulator of TFH cell differentiation. J Immunol. (2012) 209:243–50. doi: 10.4049/jimmunol.20111174

40. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature (2007) 448:480–3. doi: 10.1038/nature05969

41. Bell J, Bovey DT, Chan A, Batten M, Bostamante J, Boisson-Dupuis S, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. Blood (2012) 119:3997–4008. doi: 10.1182/blood-2011-11-392985

42. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity (2008) 29:158–69. doi: 10.1016/j.immuni.2008.05.009

43. Liu X, Nurieva RI, Dong C. Transcriptional regulation of follicular T-helper (Tfh) cells. Immunol Rev. (2013) 252:139–45. doi: 10.1111/imr.12040

44. Edelmann SL, Heissmeyer V. Tfh cell differentiation: missing Stat3 uncovers interferons’ interference. Immunity (2014) 40:307–9. doi: 10.1016/j.immuni.2014.02.008
45. Read KA, Powell MD, Baker CE, Sreekumar BK, Ringel-Scaia VM, Bachus H, et al. Integrated STAT3 and Ikaros zinc finger transcription factor activities regulate Bcl-6 expression in CD4+ T cells. J Immunol. (2017) 199:2377–87. doi: 10.4049/jimmunol.1700106
46. Schmitt N, Liu Y, Bentebeul SE, Munagala I, Bourdery L, Venuparagusad K, et al. The cytokine TGF-beta co-opt signaling via STAT3 to STAT4 to promote the differentiation of human TFF cells. Nat Immunol. (2014) 15:856–65. doi: 10.1038/ni.2947
47. Choi YS, Eto D, Yang JA, Lao C, Crottty S. Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. J Immunol. (2013) 190:3049–53. doi: 10.4049/jimmunol.1203032
48. Ray JP, Marshall HD, Laidlaw BJ, Staron MM, Kaech SM, Craft J. RNAseq analysis of Th17 and Th1 cells reveals divergent regulatory circuits upstream of the transcriptional repressor Bcl6. Nat Immunol. (2015) 16:980–90. doi: 10.1038/ni.3226
49. Zhu L, Cao Y, Xie Z, Huang Q, Bai Q, Yang X, et al. The transcription factor IRF4 determines germinal center formation through follicular T helper cell differentiation. Proc Natl Acad Sci USA. (2012) 109:8664–9. doi: 10.1073/pnas.1205834109
50. Kroenke MA, Eto D, Locci M, Cho M, Davidson T, Haddad EK, et al. Stat3-dependent induction of BATF in M1 mouse myeloid leukemia cells. J Exp Med. (2015) 208:1749–56. doi: 10.1084/jem.201501017
51. Sengu T, Iwamoto T, Humphrey SE, Yokota T, Taparovski EJ, Hamaguchi M. Stat3-dependent induction of Batf in M1 mouse myeloid leukemia cells. Oncogene. (2002) 21:8186–91. doi: 10.1038/sj.onc.1205918
52. Jabeen R, Goswami R, Awe O, Kulkarni A, Nguyen ET, Attanasio A, et al. Th9 cell development requires a Batf-regulated transcriptional network. J Clin Invest. (2013) 123:1641–53. doi: 10.1172/JCI76499
53. Bao K, Carr T, Wu J, Barclay W, Jin J, Ciofani M, et al. Batf Modulates the Th2 loci control region and regulates CD4+ T cell fate during antileishmhm immunity. J Immunol. (2016) 197:4371–81. doi: 10.4049/jimmunol.1601371
54. Betz BC, Jordan-Williams KL, Wang C, Kang SG, Liao J, Logan MR, et al. Batf coordinates multiple axes of B and T cell function required for normal antibody responses. J Exp Med. (2010) 207:933–42. doi: 10.1084/jem.20091548
55. Huber M, Lohoff M. IRF4 at the crossroads of effector T-cell fate decision. Eur J Immunol. (2014) 44:1886–95. doi: 10.1002/eji.201344279
56. Ho W, Wu H, Chan V, Lau CS, Lu Q. Transcriptional and epigenetic repression of the Bcl6 gene by IRF4 is required for T follicular helper cell differentiation. J Immunol. (2014) 192:23–32. doi: 10.4049/jimmunol.1300357
57. Ise W, Kohyama M, Schraml BU, Zhang T, Schwer B, Basu U, et al. The transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. Nat Immunol. (2015) 16:991–9. doi: 10.1038/ni.3229
58. Myers DR, Lau T, Markegard E, Lim HW, Kasler H, Zhu M, et al. Tonic Lat-75a signals sustain Nur77 and Irf4 expression to tune naive CD4 T cells. Eur J Immunol. (2015) 45:540–54. doi: 10.1002/eji.201608017
59. Stone EL, Pepper M, Katayama CD, Kerdiles YM, Lai CY, Emslie H, et al. Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. Immunity (2009) 31:941–52. doi: 10.1016/j.immuni.2009.10.008
60. Krishnamoorthy V, Kannanganat S, Maiersson-Cline M, Cook SL, Chen J, Bahrroos N, et al. The IRF4 gene regulatory module functions as a read-write integrator to dynamically coordinate T helper cell fate. Immunity (2017) 47:481–97.e7. doi: 10.1016/j.immuni.2017.09.001
61. Zeng H, Cohen S, Guy C, Shrestha S, Neale G, Brown SA, et al. Quality of TCR signaling determined by differential affinities for the composite BATF-IRF4 transcription factor complex. Nat Immunol. (2017) 18:563–72. doi: 10.1038/ni.3714
62. Myers DR, Lau T, Markedag E, Lim HW, Kaser L, Zhu M, et al. Tonic LAT-HDAC7 signals sustain Nur77 and Irf4 expression to tune naive CD4 T cells. Cell Rep. (2017) 19:1538–71. doi: 10.1016/j.celrep.2017.04.076
63. Shi W, Man K, Smyth GK, Nutt SL, Kallies A. Whole transcriptome analysis for T cell receptor-affinity and IRF4-regulated clonal expansion of T cells. Genom Data (2014) 2:396–8. doi: 10.1016/j.gdata.2014.10.019
64. Wang H, Geng J, Wen X, Bi E, Kossenkov AV, Wolf AI, et al. The transcription factor Foxp1 is a critical negative regulator of the differentiation of follicular helper T cells. Nat Immunol. (2014) 15:667–75. doi: 10.1038/ni.2890
65. Lee JY, Skon CN, Lee YJ, Oh S, Taylor JJ, Malhotra D, et al. The transcription factor KLF2 restraints CD4(+) T follicular helper cell differentiation. Immunity (2015) 42:239–51. doi: 10.1016/j.immuni.2015.01.017
66. Heissmeyer V, Vogel KU. Molecular control of Th9-cell differentiation by Roquin family proteins. Immunity Rev. (2013) 253:273–89. doi: 10.1111/imr.12056
67. Bertossi A, Aichinger M, Sansonetti P, Lech M, Neff F, Pal M, et al. Loss of Roquin induces early death and immune deregulation but not autoimmunity. J Exp Med. (2011) 208:1749–56. doi: 10.1084/jem.20110578
68. Pratama A, Ramiscal RR, Silva DG, Das SK, Athanasopoulos V, Pitch J, et al. Roquin-2 shares functions with its paralog Roquin-1 in the repression of
of mRNAs controlling T follicular helper cells and systemic inflammation. *Immunity* (2013) 38:669–80. doi: 10.1016/j.immuni.2013.01.011

84. Tan D, Zhou M, Kiledjian M, Tong L. The ROQ domain of Roquin recognizes mRNA constitutive-decay element and double-stranded RNA. *Nat Struct Mol Biol.* (2014) 21:679–88. doi: 10.1038/nsmb.2857

85. Vogel KU, Edeleman SL, JohnSKM, Bertossi A, Heger K, Heinz GA, et al. Roquin paralogs 1 and 2 redundantly repress the Icos and Ox40 costimulator mRNAs and control follicular helper T cell differentiation. *Immunity* (2013) 38:655–68. doi: 10.1016/j.immuni.2012.12.004

86. Lee SK, Silva DG, Martin JL, Pratama A, Hu X, Chang PP, et al. Interferon-gamma excess leads to pathogenic accumulation of follicular helper T cells and germinal centers. *Immunity* (2012) 37:880–92. doi: 10.1016/j.immuni.2012.10.010

87. Ramiscal RR, Parish IA, Lee-Young RS, Babon JJ, Blagih J, Pratama A, et al. Attenuation of AMPK signaling by ROQUIN promotes T follicular helper cell formation. *Elife* (2015) 4:e08698. doi: 10.7554/eLife.08698

88. Ding Y, Li J, Yang P, Luo B, Wu Q, Zajac AJ, et al. Interleukin-21 promotes germinal center reaction by skewing the follicular regulatory T cell to follicular helper T cell balance in autoimmune BXD2 mice. *Arthritis Rheumatol.* (2014) 66:2601–12. doi: 10.1002/art.37835

89. Xiao N, Eto D, Elly C, Peng G, Crotty S, Liu YC. The E3 ubiquitin ligase Itch promotes Bcl6- expressing CD4+ Th cell differentiation outside germinal centers. *Nature* (2012) 491:906–11. doi: 10.1038/nature11516

90. Mathew R, Mao AP, Chiang AH, Bertozzi-Villa C, Bunker JJ, Sloman ST, et al. A negative feedback loop mediated by the Bcl6-cullin 3 complex limits Tfh cell differentiation. *J Exp Med.* (2014) 211:1137–51. doi: 10.1084/jem.20132267

91. Rodriguez-Barbosa JI, Fernandez-Renedo C, Moral AMB, Budler L, Del Rio ML. T follicular helper expansion and humoral-mediated reaction are independent of the HVEM/BTLA pathway. *Cell Mol Immunol.* (2017) 14:497–510. doi: 10.1038/cmi.2015.101

92. Auderst F, Schuster S, Fasnacht N, Coutaz M, Charmoy M, Koch U, et al. Notch signaling regulates follicular helper T cell differentiation. *J Immunol.* (2013) 191:2344–50. doi: 10.4049/jimmunol.1300643

93. Dell’Angira M, Reinhardt RL. Notch signaling represents an important checkpoint between follicular T helper and canonical T helper 2 cell fate. *Muscol Immunol.* (2018) 11:1079–91. doi: 10.3185/101800-0012-9

94. Fasnacht N, Huang HY, Koch U, Favre F, Auderst F, Chai Q, et al. Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. *J Exp Med.* (2014) 211:2265–79. doi: 10.1084/jem.20132528

95. Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG, Cyter JG. In vivo-activated CD4+ T cells upregulate CXCL chemokine receptor 5 and reprogram their response to lymphoid chemokines. *J Exp Med.* (1999) 190:1123–34. doi: 10.1084/jem.190.11.1213

96. Ngo VN, Korner H, Gunn MD, Schmidt KN, Riminton DS, Cooper MD, et al. Lymphtoxin alpha/beta and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. *J Exp Med.* (1999) 189:403–12. doi: 10.1084/jem.189.2.403

97. Arnold CN, Campbell DJ, Lipp M, Butcher EC. The germinal center response is impaired in the absence of T cell-expressed CXCR5. *Eur J Immunol.* (2007) 37:100–9. doi: 10.1002/eji.200636486

98. Vaeth M, Muller G, Stass D, Dietz L, Kleiss-Hessing S, Serfling F, et al. Follicular regulatory T cells control humoral autoimmunity via NFAT2-regulated CXCR5 expression. *J Exp Med.* (2014) 211:545–61. doi: 10.1084/jem.20130604

99. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. *Nat Rev Immunol.* (2005) 5:853–65. doi: 10.1038/nri1714

100. Bentebibel SE, Schmitt N, Banchereau J, Ueno H. Human tonsil B-cell lymphoma 6 (BCL6)-expressing CD4+ T-cell subset specializes for B-cell help outside germinal centers. *Proc Natl Acad Sci USA.* (2011) 108:2488–97. doi: 10.1073/pnas.1010898108

101. Shulman Z, Gitlin AD, Targ S, Jankovic M, Pasqual G, Nussenzwieg MC, et al. T follicular helper cell dynamics in germinal centers. *Science* (2013) 341:673–7. doi: 10.1126/science.1241680
signaling lymphocytic activation molecule receptor (CD150). J Immunol. (2010) 185:190–202. doi: 10.4049/jimmunol.0903505

123. Ueno H, Banchereau J, Vinuesa CG. Pathophysiology of T follicular helper cells in humans and mice. Nat Immunol. (2015) 16:142–52. doi: 10.1038/ni.3054

124. Reinhardt RL, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. Nat Immunol. (2009) 10:385–93. doi: 10.1038/ni.1715

125. King IL, Mohrs M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. J Exp Med. (2009) 206:1001–7. doi: 10.1084/jem.20090313

126. Glatman-Zaretsky A, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. J Exp Med. (2009) 206:991–9. doi: 10.1084/jem.20090303

127. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nat Immunol. (2011) 13:58–66. doi: 10.1038/ni.2182

128. Yang Y, Ochando J, Yopp A, Bromberg JS, Ding Y. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4+ T cell activation. J Immunol. (2005) 174:2720–9. doi: 10.4049/jimmunol.174.5.2720

129. Eto D, Lao C, DiToro D, Barnett B, Escobar TC, Kageyama R, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. PLoS ONE (2011) 6:e17739. doi: 10.1371/journal.pone.0017739

130. Ma CS, Suryani S, Avery DT, Chan A, Nanan R, Santner-Nanan B, et al. Early commitment of naive human CD4+ T cells to the T follicular helper (T(FH)) cell lineage is induced by IL-12. Immunity Cell Biol. (2009) 87:590–600. doi: 10.1038/icb.2009.64

131. Nakayamada S, Kanno Y, Takahashi H, Jankovic D, Lu KT, Johnson TA, et al. Early Th1 cell differentiation is marked by a Th1 cell-like transition. Immunity (2011) 35:919–31. doi: 10.1016/j.immuni.2011.11.012

132. Wei L, Vahedi G, Sun HW, Watford WT, Takatori H, Ramos HL, et al. Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation. Immunity (2010) 32:840–51. doi: 10.1016/j.immuni.2010.06.003

133. McCarron MJ, Marie JC. TGF-beta prevents T follicular helper cell accumulation and B cell autoreactivity. J Clin Invest. (2014) 124:4375–86. doi: 10.1172/JCI76179

134. Kolenderaender B, Grewe B, Nemazee D, Uberla K, Temchura V. Generation of T follicular helper cells in vitro: requirement for B-cell receptor cross-linking and cognate B- and T-cell interaction. Immunology (2018) 153:214–24. doi: 10.1111/imm.12834

135. Kitagawa Y, Wing JB, Sakaguchi S. Transcriptional and epigenetic control of regulatory T cell development. Prog Mol Biol Transl Sci. (2015) 136:1–33. doi: 10.1016/bs.pmbts.2015.07.011

136. Cook KD, Shpargel KB, Starmer J, Whitfield-Larry F, Conley B, Allard DE, et al. T Follicular Helper cell-dependent clearance of a persistent virus infection requires T cell expression of the histone demethylase UTX. Immunity (2015) 43:703–14. doi: 10.1016/j.immuni.2015.09.002

137. Lu KT, Kanno Y, Cannons JL, Handon R, Bible P, Elkahloun AG, et al. Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. Immunity (2011) 35:622–32. doi: 10.1016/j.immuni.2011.07.015

138. Yang Y, Ochando J, Yopp A, Bromberg JS, Ding Y. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4+ T cell activation. J Immunol. (2005) 174:2720–9. doi: 10.4049/jimmunol.174.5.2720

139. Dong C, Chen CY, Su CD, Li L, Xie X, Zhang Y, et al. MicroRNA-146a regulates ICOS-ICOSL signalling to limit accumulation of T follicular helper cells and germinal centres. Nat Commun. (2015) 6:6436. doi: 10.1038/ncomms7436

140. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, et al. Global mapping of the miR-17 approximately 92 family are critical regulators of T(FH) differentiation. Nat Immunol. (2013) 14:849–57. doi: 10.1038/ni.2648

141. Pratama A, Srivastava M, Williams NJ, Papa I, Lee SK, Dinh XT, et al. MicroRNA-146a regulates ICOS-ICOSL signalling to limit accumulation of T follicular helper cells and germinal centres. Nat Commun. (2015) 6:6436. doi: 10.1038/ncomms7436

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