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Th cell functional subsets have unique transcriptional programs that form the molecular basis for T cell differentiation and functions. T follicular helper (T_{FH}) cells have emerged as the main providers of T cell help to B cells during the germinal center (GC) reaction, where B cells undergo selection events through competition for Ag and for access to GC T cell-mediated prosurvival and differentiation signals. Because T cell help is one limiting factor for GC B cells, the molecular mechanisms controlling T_{FH} cell abundance and functionality are central to the GC reaction and generation of long-term humoral immunity. Two signaling pathways are absolutely critical for T_{FH} cells: phosphoinositide-3–kinase pathway and the signaling lymphocyte activation molecule-associated protein. In this review, the molecular mechanisms constituting the signaling network in T_{FH} cells will be explored. The Journal of Immunology, 2010, 184: 6563–6568.

T cell help to B cells mediated by sustained T–B interactions is critical for Ig class switching, the maintenance of the germinal center (GC) reaction, and the selection of high-affinity B cell clones. The effector T cells that are specialized in providing help to B cells are termed T follicular helper (T_{FH}) cells, and they are characterized by expression of the chemokine receptor CXCR5, which enables migration into the B cell follicle (1). The T_{FH} cells reside in the GC in contrast to pre-GC T_{FH} cells that interact with B cells in the T-B border (2) or CXCR5-negative Th cells that can support an extrafollicular autoreactive Ab response (3). T_{FH} cells can be considered to represent a separate functional subset of Th cells on the basis of recent work that established that T_{FH} cells have a unique requirement for high expression of the transcription factor B cell leukemia/lymphoma-6 (Bcl-6) (4–7). This distinguishes them from Th1, Th2, or Th17 lineages, which do not share such a requirement, and are dependent on the expression of T-bet, Gata-3, and RORγT, respectively (8). However, T_{FH} cells are highly versatile in their ability to produce cytokines and, depending on the immunological setting, have been shown to produce IL-4 (9–11), IFN-γ (9), and IL-17 (12), in addition to their hallmark cytokine IL-21 (13). Several mouse models that have T_{FH} cell defects display concomitant reduction in the GC reaction and long-term humoral immunity (8). The provision of help to GC B cells has been suggested to be a limiting factor in the GC reaction as B cells compete for access to GC T cells (14). Therefore, to understand the underlying mechanisms of the GC reaction, the regulation of GC T_{FH} cell formation and functions is an essential area of research.

T_{FH} signaling requirements

The “follicular hypothesis” suggests that activated T cells undergo migration into the B cell follicle where they receive specific signals that support their proliferation and differentiation (15). Consistent with this, T_{FH} cells require B cells with the relevant Ag specificity that can capture and then present cognate peptide Ag on MHC class II (5) and mutations that impair the duration of T_{FH} residency in the GC lead to T_{FH} cell defects (2). The T_{FH} cell subset expresses an array of cell surface receptors that is required for efficient T cell priming, such as CD28:CD80/86 (16), inducible costimulator ICOS–ICOS ligand (ICOSL) (8), and CD40L:CD40 (10) interacting with counterreceptors on dendritic cells or B cells. In addition, T_{FH} cells also express receptors that underlie their specialized localization, function, and ability to respond to the local cytokine milieu. These receptors include signaling lymphocyte activation molecule (SLAM) family members CD84 and Ly108 (17) and the chemokine receptor CXCR5 (18). Cytokines that are involved in T_{FH} cell development are type I IFNs acting on dendritic cells to secrete IL-6 (19) and IL-21 that promote T_{FH} long-term survival (8, 20). Together with high-affinity TCR specificity (4), these receptors provide the molecular basis for initiating T_{FH} cell signaling. Recent exploration of the molecular mechanisms regulating T_{FH} cell differentiation has revealed the key importance of the transcriptional repressor Bcl-6 (4–7). However, there are at least two signaling pathways that are crucial and nonredundant for generation of functional T_{FH} cells that display otherwise moderate effects in T cell development and the generation of the other subset. These two pathways are as follows: 1) the PI3K pathway, which functions downstream of ICOS and regulates the abundance and effector functions of T_{FH} (21), and 2) the adaptor molecule SLAM-associated protein (SAP), which functions downstream of SLAM family receptors to prolong physical contact between B and T cells (Fig. 1) (22).

Abbreviations used in this paper: Bcl-6, B cell leukemia/lymphoma-6; BLIMP-1, B lymphocyte-induced maturation protein 1; GC, germinal center; ICOSL, ICOS ligand; PD-1, programmed cell death-1; PI3K, phosphatidylinositol-3,4,5-tris-phosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SAP, signaling lymphocyte activation molecule-associated protein; SLAM, signaling lymphocyte activation molecule; T_{FH}, T follicular helper.

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ICOS and PI3K signaling in T\(_{FH}\) cells

ICOS is highly homologous to CD28 (23) and is expressed on the surface of T cells following activation through the TCR (24). Thus, ICOS signaling takes place during the effector T cell stage rather than during initial priming (25). ICOS is required for protective immunity in Th1- and Th2-mediated responses to infection (26, 27), regulation of CD40L (23, 28), IL-4 production (29), and clonal expansion of primed T cells (30). The ICOS–ICOSL pair is a central requirement for humoral immunity (31). Consistent with the “follicular hypothesis,” B cell-specific expression of ICOSL is required for T\(_{FH}\) cell accumulation (8). ICOS deficiency mediated by genetic deletion or Ab inhibition in vivo causes a reduction in T\(_{FH}\) cells in both humans and mice (32, 33). By contrast, enhanced expression of ICOS in the sarco/ge mouse strain with a mutation in the Roquin gene is associated with expansion of T\(_{FH}\) cells and is sufficient to rescue T\(_{FH}\) cells in CD28 knockout mice (34, 35). Further evidence for the crucial importance of ICOS in T\(_{FH}\) cells and their provision of help to B cells was revealed by the study of mice with a mutation of Tyr\(_{181}\) in the cytoplasmic tail of ICOS (21). This tyrosine is critical for recruitment of the regulatory subunits of PI3K (36).

The class Ia PI3Ks consist of three catalytic subunits p110\(\alpha\), \(\beta\), and \(\delta\) that form heterodimers with a family of adaptor proteins that constitute the regulatory subunits: the p85\(\beta\) or p55\(\gamma\), which are separate gene products, and p85\(\alpha\), p55\(\alpha\), and p50\(\alpha\), which arise from a single gene. The regulatory subunits are recruited to phosphorylated tyrosine residues in the intracellular tail of receptors, and they control the stability, location, and enzymatic activity of p110 subunits. PI3K catalytic subunits mediate the phosphorylation of the D-3 position of the inositol ring of the cell membrane phospholipid phosphatidylinositol-4,5-bis-phosphate to generate phosphatidylinositol-3,4,5-tris-phosphate (PIP\(_3\)). These lipids function as second messengers often by interacting with specific domains of proteins to modulate their location and/or activity. PIP\(_3\) is metabolized by phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a lipid phosphatase that directly opposes PI3K, and by SHIP-1 and SHIP-2, which remove the phosphate from the 5’ position of the inositol head group (37). ICOS signaling is extremely dependent on the activity of PI3K and differs from CD28, which recruits additional signaling adaptor proteins (36, 38–41). ICOS generates high PIP\(_3\) levels upon activation (26), and the recruitment of the p50\(\alpha\)-PI3K regulatory subunit may underlie the extraordinary capacity of ICOS to induce PI3K signaling (42), in accordance with p50\(\alpha\)-associated PI3K activity being greater than that of p85\(\alpha\) or p55\(\alpha\) after insulin stimulation (43). In mice, the mutation of ICOS Y181 (ICOS-YF) resulted in abrogation of PI3K recruitment and led to reduction in T\(_{FH}\) cells after immunization and, importantly, the ICOS-YF mutant were phenotypically similar to ICOS knockout mice. In addition, lack of ICOS Y181 in T cell blasts reduced the accumulation of IL-21 and IL-4 mRNA after restimulation, directly implicating the PI3K pathway in ICOS-mediated induction of T\(_{FH}\) effector function (21). Thus, ICOS is critical for T\(_{FH}\) cell abundance and their cytokine production, and moreover, the PI3K pathway is absolutely required for ICOS signaling. These findings raise questions about the nature of the PI3K catalytic subunits essential for ICOS signaling and also the role PI3K enzymes play in integrating signaling and how this shapes T\(_{FH}\) cell identity and function.

We have recently addressed this question by creating a T cell–specific deletion of the p110\(\alpha\) catalytic subunit of PI3K and found that this mouse model lacked T\(_{FH}\) cells. The defect in T\(_{FH}\) cell formation resulted in impairments in the GC reaction, affinity maturation, and the generation of memory B cells. The magnitude of PI3K activity was crucial for T\(_{FH}\) cells, because deletion of PTEN, the phosphatase that restricts PIP\(_3\) accumulation, resulted in an expansion of T\(_{FH}\) cells. Moreover, there was a block in induction of Akt phosphorylation and cytokine gene expression following ICOS restimulation (J. Rolf and M. Turner, submitted for publication). These results show a crucial role for p110\(\alpha\) in T\(_{FH}\) cells, consistent with data revealing the T cell intrinsic requirement of the PI3K regulatory subunits in T cell help to B cells (44).

Many of the receptors important for T\(_{FH}\) cells, such as the costimulatory molecules ICOS (26, 32), OX40 (45, 46), and CXCR5 (47), are known to signal through the PI3K pathway. Integration of signals from receptors signaling through PI3K generates the high levels of PIP\(_3\) required for T\(_{FH}\) formation and maintenance. By contrast, the negative regulator programmed cell death-1 (PD-1), which is highly expressed by T\(_{FH}\) cells (48), inhibits PI3K activity. The mechanisms for PD-1 suppression of the PI3K signaling are suggested to involve recruitment of SHIP enzymes (49) or enhanced expression of the phosphatase PTEN (50). The negative regulation of the PI3K pathway by PD-1 might limit excessive T\(_{FH}\) stimulation.

Vav1 signaling in T\(_{FH}\) cells

Vav proteins are guanine nucleotide exchange factors for the Rho/Rac family of GTPases and play an essential role in regulating T cell activation (51, 52). Vav-1 has been shown to have an important, nonredundant role in T cell development and activation and has been implied in signaling in the T\(_{FH}\) subset. Defective Ab production by Vav-1 mutant B cells can be rescued by the provision of T cell help. In Vav-1 mutant mice, Ag-specific proliferation of T cells in the T zone was found to be normal, but the induction of IL-4 mRNA and Ig class switching...
was impaired (53). Moreover, c-Maf expression and IL-4 production were defective when Vav1−/− T cells were activated in vitro (54), and these two molecules are important both in Th2 and T FH cells. Vav1 phosphorylation was shown to be stimulated by IL-21 in CD4+ T cells, suggesting that Vav1 plays a role in signaling in T FH cells (13). Vav1 was also phosphorylated upon engagement of the SLAM family receptor Ly108 and may sustain the interactions between B and T FH cells necessary for the GC reaction (17, 55). Vav1 also contributes to the activation of the PI3K pathway in T cells (56). Thus, the microenvironment in the GC contains cues that initiate signaling through Vav1 and PI3K that contribute to the T FH-specific signaling network.

The adaptor protein SAP regulates T FH–B cell cognate interactions in the GC

SAP-deficient mice had a severe T cell-intrinsic impairment in long-term humoral immunity against viral infection (57). Further studies into the role of SAP have revealed that it is crucial for the provision of T cell help to GC B cells independently of its role in Th2 cytokine production (58) and that it regulates T FH cells (22). The initial priming of SAP-deficient T cells by dendritic cells progressed normally, but the late-phase interactions between SAP-deficient T cells and GC B cells was impaired. Elegant intravital imaging studies have revealed that the duration of T–B interactions was diminished when T FH cells lack SAP. Moreover, SAP-deficient T cells had decreased propensity to migrate into and dwell within the GC (59). The *sauroque* mutation, which causes enhanced T FH numbers and autoimmunity (34) as a result of dysfunctional posttranscriptional repression of ICOS (60), had reductions in T FH cell numbers and functions when crossed with SAP knockout mice, showing that SAP is necessary for T FH-mediated pathology (61). Although SAP signals through Fyn (62), the role of SAP in supporting the GC reaction was independent of its association with Fyn and SLAM (22). SAP harboring a mutation of amino acid Arg78 was no longer able to interact with Fyn but retained its ability to promote T FH development (22) and sustain adhesion between B and T cells (17). By contrast, mutation of Arg78 within the SAP Src homology 2 domain, which blocks the ability of SAP to interact with SLAM family receptors, abrogated the ability of T cells to form stable conjugates with B cells. Recently, the homophilic SLAM family members CD84 and Ly108 were shown to mediate the crucial adhesive interactions between T and B cells that allow sustained contact beyond the initial integrin-dependent phase (17). The SAP downstream signaling requirements in T FH cells remains unknown but may depend on other Src homology 3 domain-containing proteins that are associated with the TCR signalosome (63, 64). An alternative signaling mechanism initially thought to contribute to SAP downstream functions was competition with Src homology region 2 domain-containing phosphatase (65), such as SHIP phosphatases (62). Another intriguing possibility is that Ly108 signals through Vav, thereby interconnecting SAP and Vav pathways in T FH cells (55). When stimulated through the TCR complex, SAP-deficient mice show unimpaired PI3K activation and intact Vav phosphorylation (66); however, in the light of different Slam family members having unique downstream pathways (55), it is warranted to analyze Vav signaling in the context of CD84 and Ly108 homophilic interactions (17).

**T FH intracellular signaling and regulation of transcription factors**

The Foxo family of transcription factors, Foxo1, Foxo3, Foxo4 (AFX), and Foxo6, are implicated in regulation of cell proliferation, differentiation, metabolism, and survival (67). The PI3K pathway is an important negative regulatory axis for Foxo transcription factors mediated by Akt-dependent phosphorylation and nuclear exclusion of Foxo proteins. Because T cell-specific deletion of Foxo1 resulted in enhanced GC formation and autoantibody production (67), it is plausible that one function of Foxo1 is to negatively regulate T FH cell activity. This is in keeping with a general role of this transcription factor in T cell tolerance (68). In tumor cell lines, Foxo3a (69) and Foxo4 (70) have been shown to positively regulate transcription of Bcl-6, which is crucial for T FH cell differentiation.

It is also interesting to note that cytokine production by T FH cells may be induced by PI3K signaling because this pathway represses Foxo and thereby its target KLF-2, which inhibits cytokine production by T cells (71). Another transcription factor that plays a role in the regulation of cytokine gene transcription is NFAT. NFATc1 is highly expressed in T FH cells (72) and is regulated in an ICOS- and PI3K-dependent manner (29, 73). PI3K signaling inhibits the activity of glycogen synthase kinase 3, which promotes nuclear exclusion of NFATc1 (74), thus, the ICOS-PI3K axis may regulate cytokine gene expression through inhibition of glycogen synthase kinase 3 in T FH cells. NFAT binding sites are present in the IL-21 promoter where there appears to be redundancy among NFAT family members (75). NFATc1 cooperates with the AP-1 family transcription factor c-Maf to regulate IL-4 expression (76), and c-Maf has also been implicated in the function of T FH cells (12). Costimulation provided through ICOS signaling induces c-Maf in an IL-4–dependent manner (29), and c-Maf is important for production of IL-21 by in vitro differentiated T FH cells (12). The IL-21 production induced by c-Maf may signal in an autocrine manner and promote T FH survival (13), suggesting that ICOS and cytokine receptor signaling form an integrated network that sustains T FH cells. However, IL-21 produced by T FH cells primarily regulates the GC output through its effects on GC B cells, rather than through autocrine effects on T FH cells. IL-21 is required for GC B cell proliferation, differentiation into plasma cells and accumulation of somatically hypermutated memory B cell clones (20, 77). IL-21 positively regulates the levels of Bcl-6 in GC B cells (20) and during in vitro differentiation of T FH cells (6), but is dispensable for formation of T FH cells in vivo (20, 77). IL-6R and IL-21R both stimulate STAT3 in T cells and there may be redundancy between these cytokines in promoting T FH development which would explain the discrepancy between the results obtained in vitro and in vivo (8). The transcription factor STAT3, which is activated by tyrosine phosphorylation, plays a role in T FH cells (8) and for T cells to provide help to B cells (78). However, there is emerging evidence that IL-21 and STAT3 play different roles in GC B cells and T FH cells in terms of regulation of Bcl-6 and B lymphocyte-induced maturation protein-1 (BLIMP-1) expression. In B cells, IL-21-induced STAT3 interacts with IRF4 to promote transcription of *prdm1* (BLIMP-1) (79), however, it is improbable that this mechanism occurs in T FH cells as BLIMP-1 antagonizes Bcl-6 and inhibits T FH cell formation (5). Also, it is unclear whether the failure of T FH cells to develop in IRF4 knockout mice (79)
is a consequence of an intrinsic defect in T cells or the important role IRF4 plays in B cells (80).

The affinity of the TCR making contact with the peptide in the MHC class II groove can modulate differentiation of T cell effectors, driving cells either into Th1 or Th2 responses (81), and this observation is relevant also for the differentiation of T_{FH} cells. By using TCR transgenic models of varying TCR affinity, it was shown that high TCR affinity was preferentially associated with the T_{FH} lineage and that the resident effector T_{FH} cells expressed the highest levels of Bcl-6 (4). The well-characterized mutually antagonistic relationship between BLIMP-1 and Bcl-6 indicate that these two transcriptional repressors determine the fate of differentiating T cells (82). Bcl-6–deficient mice have drastc reductions in GC B cells and in generation of long-term memory (83, 84). These defects are caused by a requirement for Bcl-6 expression in both GC B cells and T_{FH} cells (6, 7). Bcl-6 is the key transcription factor in regulation of T_{FH} cell differentiation that is both necessary and sufficient for determining the T_{FH} cell phenotype (5) by repressing microRNAs (7) and other target genes. Ectopic expression of Bcl-6 can promote T_{FH} development in vivo in the absence of B cells, suggesting an essential role for the B:T interaction is the induction and/or maintenance of Bcl-6 expression (5). Although the signaling pathways regulating Bcl-6 expression and function in T_{FH} cells have not yet been elucidated, it seems that induction of Bcl-6 in T_{FH} cells is correlated with the ability of the TCR to form high-avidity interactions with peptide + MHC class II complexes presented by APCs (4). Because Bcl-6 gene-dosage effects are evident from the intermediate defects displayed in Bcl-6 heterozygous mice (61), it is probable that the activity of this transcriptional repressor is rate limiting and stringently regulated in T_{FH} cells. The molecular mechanisms controlling induction and maintenance of Bcl-6 expression in T_{FH} are likely to involve the signaling pathways PI3K, Vav1, and SAP, which have the potential to integrate transcriptional, posttranscriptional, and posttranslational processes.

Conclusions

The unique signaling requirements of T_{FH} cells are currently being delineated, and this T cell functional subset has been shown to require both the PI3K pathway and SAP for its formation and/or functions. In addition, Vav1 has been shown to be activated in T cells downstream of IL-21R and the SLAM family member Ly108, thus potentially providing a molecular association interconnecting the T_{FH} signaling network. However, the molecular mechanisms linking the intracellular signaling network to the T_{FH} transcriptional program mediated by Bcl-6 repression of its targets remains to be further elucidated.

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Disclosures

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