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Cytokine Regulation and Function in T Cells

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Abstract

T lymphocytes, the major effector cells in cellular immunity, produce cytokines in immune responses to mediate inflammation and regulate other types of immune cells. Work in the last three decades has revealed significant heterogeneity in $CD4^+$ T cells, in terms of their cytokine expression, leading to the discoveries of T helper 1 (Th1), Th2, Th17, and T follicular helper (Tfh) cell subsets. These cells possess unique developmental and regulatory pathways and play distinct roles in immunity and immune-mediated pathologies. Other types of T cells, including regulatory T cells and γδ T cells, as well as innate lymphocytes, display similar features of subpopulations, which may play differential roles in immunity. Mechanisms exist to prevent cytokine production by T cells to maintain immune tolerance to self-antigens, some of which may also underscore immune exhaustion in the context of tumors. Understanding cytokine regulation and function has offered innovative treatment of many human diseases.

1. INTRODUCTION

$CD4^+$ T cells regulate immune and nonimmune cells in immune responses via production of cytokines, so they are called helper T (Th) cells. Upon activation, T cells differentiate into effector cells that produce cytokines and other immune mediators. According to their cytokine expression profiles and immune regulatory functions, effector Th cells are classified into distinct functional subsets whose generation is differentially regulated by cytokines and transcription factors. Since the discovery of Th1 and Th2 cells in the late 1980s, two additional major subtypes of Th cells, i.e., Th17 and Tfh (T follicular helper) cells, have been discovered and characterized in detail. Effector differentiation of T cells is regulated by the innate immune system, in part via production of proinflammatory cytokines.

Cytokine regulation and function not only are a central theme of T cell biology and immune regulation but also have important implications in immunopathology. Targeting various cytokines has become powerful in treating immune disorders, from autoimmunity to allergy, as well as immunotherapy against cancer.

In this article, I summarize the cytokine regulation and function underlying the biology of $CD4^+$ T cells and discuss the latest knowledge on negative regulation of T cell function in immune tolerance.

2. Th1 AND Th2 CELLS

$CD4^+$ T cells are essential regulators of adaptive immunity. It has long been postulated that they have two fundamental functions—promoting inflammation and antibody production by B cells. In the late 1980s, Mossman & Coffman (1) noticed that based on cytokine secretion, there existed two patterns among $CD4^+$ T cells, and they named these cells T helper 1 (Th1) and Th2 cells (**Figure 1**). Th1 cells produce IFN-γ to enhance antigen presentation and facilitate bactericidal function by macrophages. They are therefore critical players in cellular immunity. Th2 cells, on the other hand, secrete IL-4, -5, and -13. IL-4 promotes B cell proliferation and IgE production. Th2 cells were thus thought to mediate humoral immunity and control extracellular pathogens.

2.1. Regulation of Th1 and Th2 Differentiation by Innate Cytokines

Effector differentiation of $CD4^+$ T cells not only depends on T cell receptor (TCR) and costimulatory receptors but also is differentially regulated by the cytokines produced by innate immunity, which provide cues to the nature of the infection or environmental assault.

2.1.1. IL-12. Th1 differentiation is regulated by IL-12, consisting of p35 and p40 subunits (2). IL-12 expression is upregulated as a result of innate activation, for example, via Toll-like receptors (TLRs). The receptor for IL-12 is composed of IL-12Rβ1 and IL-12Rβ2. IL-12Rβ2 is selectively expressed by Th1 cells and promotes not only Th1 commitment but also Th1 propagation and maintenance.

2.1.2. IL-33. In contrast to Th1 cells, Th2 cells selectively express the receptors for IL-25 and IL-33, both produced by epithelial cells. IL-33, belonging to the IL-1 cytokine family, is an alarmin molecule expressed first as a full-length protein that functions as a protease sensor (3). When encountering various allergen proteases, full-length IL-33 was rapidly cleaved, leading to its activation to promote Th2 differentiation; preventing the cleavage process reduced allergic airway inflammation. IL-33 receptor consists of a ubiquitously expressed IL-1R accessory protein

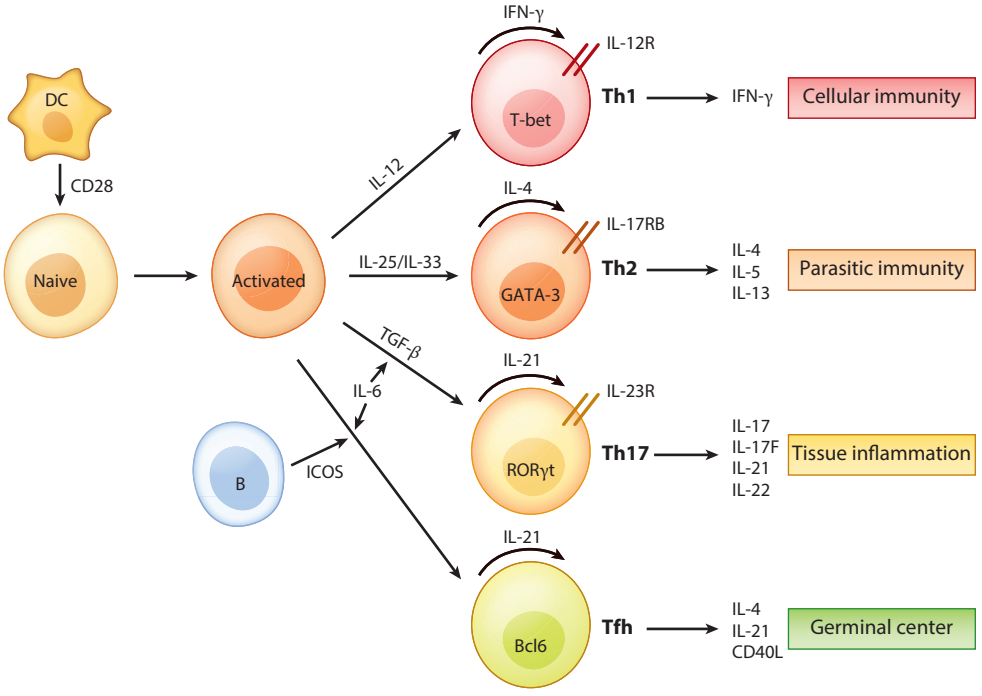

Figure 1

Development of Th subsets. In the presence of distinct antigen-presenting cells and innate cytokines, naive $CD4^+$ T cells differentiate into effector Th cells that are characterized by their expression of distinct cytokines and transcription factors. These cells have differential immune functions. Abbreviations: DC, dendritic cell; RORγt, RAR-related orphan receptor gamma; Tfh, T follicular helper; Th, T helper.

(IL1RAcp) and ST2, which is selectively expressed by Th2 cells (4). ST2 upregulation accompanies Th2 differentiation and reinforces IL-5 and IL-13 production (5, 6).

2.1.3. IL-25. It has been reported that multiple allergens also induced the expression of IL-25, a member of the IL-17 cytokine family also called IL-17E, by lung epithelial cells (7), though the underlying mechanism is unclear. In recent years, IL-25 has been found to be produced by a selective type of epithelial cell, called tuft cells (8). GPR91, the succinate receptor, and dietary succinate, but not acetate, may regulate IL-25 production by tuft cells (9). Airway brush cells, similar to tuft cells, serve as a dominant source of IL-25 in the airway. Allergen challenge expanded brush cells, dependent on LTC4 synthase or CysLT3R, the receptor for leukotriene E_4 (10). IL-25 promotes initial Th2 differentiation, via enhancing their IL-4 production (11). In addition, IL-25 receptor, consisting of IL-17RA and IL-17RB heterodimers, is highly expressed by Th2 cells (12). IL-25 has been reported to promote proliferation and function of effector Th2 cells via this receptor in both mouse and human (11, 13). Inhibition of IL-25 function attenuated allergic asthma models (11). IL-17RB can be bound by another IL-17 family cytokine, IL-17B. IL-17B-deficient mice developed more severe airway inflammation in an asthma model, consistent with IL-17B's role as an IL-25 antagonist (14).

2.2. Regulation of Th1 and Th2 Differentiation by Transcription Factors

IL-12-driven Th1 differentiation is mediated via a selective transcription factor, STAT4, whereas STAT6, downstream of IL-4, plays similar roles during Th2 differentiation (2). STAT4 and STAT6 directly bind to the signature cytokine genes for Th1 and Th2 cells, i.e., *Ifng* and *Il4*, respectively (15). Moreover, they also directly regulate the expression of Th1 and Th2 lineage-specific transcription factors, *Tbx21*, encoding T-bet protein, and *Gata3*, respectively (15).

2.2.1. T-bet. As a Th1 lineage transcription factor, T-bet drives Th1 differentiation and inhibits Th2 differentiation (16). *Tbx21*-deficient mice exhibited defects in IFN-γ expression in $CD4^+$ T cells (17). Mechanistically, T-bet directly binds to the *Ifng* gene, and interestingly, co-occupancy of T-bet and STAT4 was noticed (18), suggesting cooperation in their function.

2.2.2. GATA-3. GATA-3 is expressed by naive T cells, and GATA-3 expression is further enhanced in Th2 differentiation but diminished after Th1 differentiation (19). Reciprocal to the function of T-bet in Th1 cells, GATA-3 is necessary and sufficient in driving Th2 differentiation (19), whereas it represses Th1 differentiation (20). Mechanistically, GATA-3 binds to a regulatory element in the Th2 cytokine gene locus containing the *Il4*, *Il5*, and *Il13* genes (21), which is necessary for their proper expression (22). Chromatin-immunoprecipitation-coupled sequencing (ChIP-seq) analysis also revealed that GATA-3 functions as both a positive and negative regulator of gene expression (23). Conditional deletion of the *Gata3* gene in mature T cells consistently attenuated Th2 differentiation (24).

2.3. Th1/Th2 Cells in Immune-Mediated Diseases

Th1 and Th2 cells are important in immune disorders. While Th1 cells are frequently elevated in autoimmune diseases, Th2 cells have been well established as essential players in allergic diseases. In addition to IgE, Th2 cytokines are good targets for treating allergic asthma and atopic dermatitis. IL-5 is important for eosinophilia in allergy. Antibody drugs that inhibit IL-5 have been approved for treating patients with eosinophilic asthma (25, 26). Recently, dupilumab, an antibody binding to IL-4Rα and therefore inhibiting both IL-4 and IL-13 signaling, has been approved for treatment of allergic asthma and atopic dermatitis patients (27, 28). This encouraging progress in the clinic has provided proof-of-concept for the function of Th2 cells and their cytokines in human diseases. Additional cytokines, including TSLP, IL-25, and IL-33, are being evaluated at preclinical and clinical stages.

3. Th17 CELLS

In the 1990s, tremendous efforts were made to understand the regulation of Th1 and Th2 differentiation and the function of Th1 and Th2 cells in immune-mediated diseases. While Th2 cells have been linked with pathogenesis of allergic diseases, Th1 cells have been implicated in autoimmune disorders, considering that these cells are frequently found in inflamed tissues. However, it was recognized that a population of $CD4^+$ T cells secreting IL-17 may be more important than Th1 cells in the development of some autoimmune disease models. Deficiency in the *Icos* (29) or *Il23* (30) gene selectively impaired the function of these T cells, without affecting Th1 cells, but resulted in resistance to autoimmunity. Moreover, IL-23 expanded an IL-17-secreting novel T cell population with a distinct gene expression profile from that of IL-12-expanded Th1 cells (31). In 2005, two groups independently showed that naive T cells develop into IL-17-producing cells, so-called Th17 cells, via a lineage distinct from Th1 and Th2 cells (32, 33) (**Figure 1**).

3.1. Cytokine Regulation of Th17 Differentiation and Function

Like development of Th1 and Th2 cells, the development of Th17 cells is regulated by cytokines, and several cytokines have been shown to act at different stages of Th17 cell differentiation.

3.1.1. TGF-β/IL-6. Th17 cell differentiation was first shown to be induced by the combination of two cytokines: IL-6 and TGF-β (34–36). TGF-β is a pleiotropic cytokine belonging to the transforming growth factor superfamily and has three mammalian isoforms (TGF-β1, 2, 3). Transgenic overexpression of TGF-β in T cells increased Th17 response and the severity of experimental autoimmune encephalomyelitis (EAE) (34), a Th17 cell–dependent autoimmune disease model, whereas T cell–specific deficiency in *Tgfbr* caused defective Th17 cell differentiation and resistance to EAE (37). T cell–derived TGF-β appears important in supporting Th17 cell development, considering that T cell–specific ablation of *Tgfb* significantly dampened Th17 cell generation in vivo and resulted in resistance to EAE induction (38). On the other hand, blocking IL-6, another pleiotropic cytokine produced by many cells in the immune system, completely inhibited Th17 cell polarization in vitro, and T cell–specific deficiency in *Stat3*, coding the signaling molecule downstream of IL-6, impaired Th17 cell differentiation in vitro and in vivo and caused resistance to EAE (39, 40).

3.1.2. IL-21/IL-23. Interestingly, during Th17 cell development, two other STAT3-activating cytokines also participate at different stages. IL-21, induced by IL-6/STAT3 signaling, also activates STAT3 (41, 42). Moreover, acting as an autocrine cytokine, it could induce Th17 differentiation together with TGF-β, whereas IL-21 deficiency caused severe defects in Th17 cell differentiation in vitro and in vivo and resistance to EAE induction (41–43). IL-21 is thus proposed as a T cell–derived autocrine cytokine critically promoting the expansion and development of the Th17 cell program. IL-23, a member of the IL-12 cytokine family, is composed of a unique p19 subunit and a common p40 subunit shared with IL-12. Though IL-23 is required for Th17 cell response in vivo, it is dispensable for Th17 cell differentiation in vitro. IL-23 appears to function in the expansion and maturation of Th17 cells at a later stage, through its receptor IL-23R, induced by IL-6 and IL-21 (42, 44).

3.1.3. IL-1. Like IL-23R, the IL-1 receptor is induced after Th17 cell induction, in response to IL-6 signaling (45). IL-1 has an important role in reinforcing Th17 cell differentiation at both early and later stages. Deficiency in IL-1 receptor signaling not only significantly reduced Th17 cell induction but also greatly alleviated the disease symptoms in the EAE model (45). Notably, the combination of IL-1β, IL-23, and IL-6, in the absence of exogenous TGF-β, is sufficient to induce Th17 cells in vitro, which exhibited increased proinflammatory activity in an EAE model (46).

3.1.4. Pathogenicity. It is now generally accepted that Th17 cells can exist in different states with distinct gene expression patterns and physiological functions (46–49); this is supported by single-cell transcriptomic analysis (50). Th17 cells induced by IL-6, IL-1β, and IL-23 stimuli were highly pathogenic in driving autoinflammation and expressed increased amounts of T-bet, IFN-γ, GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-22, and IL-23R with diminished expression of IL-10 (46). Recently, it was shown that serum amyloid A proteins, together with IL-6, promote pathogenic Th17 cell development (51). However, it remains to be determined whether there is a reciprocal and reversible relationship between the nonpathogenic and pathogenic Th17 cell populations.

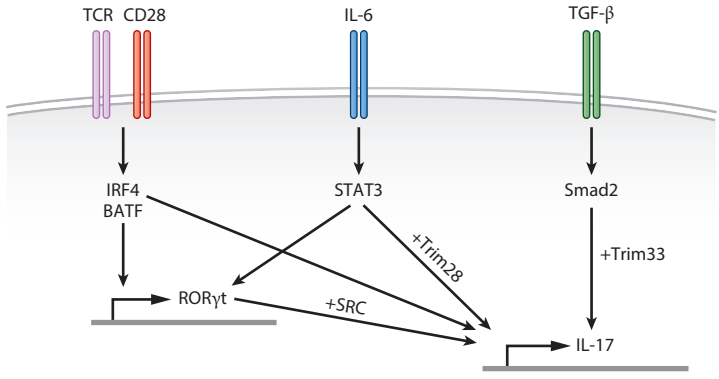

Figure 2

Signaling and transcriptional regulation of T helper 17 cell development. Signaling downstream of the TCR and CD28 as well as cytokines IL-6 and TGF-β induces activation of distinct transcription factors, which induces transcription of RORγt and IL-17 genes. Abbreviations: RORγt, RAR-related orphan receptor gamma; TCR, T cell receptor.

3.1.5. IL-2. IL-2 is a potent inhibitor of Th17 cell differentiation, via activation of STAT5 transcription factor (52). STAT3 and STAT5 were shown to bind to similar gene loci in a competitive manner, and each acted to antagonize the function of the other.

3.2. Regulation of Th17 Differentiation by Transcription Factors

Since the discovery of Th17 cells, numerous studies have been undertaken to reveal the signaling and transcriptional regulation of Th17 cell differentiation and their cytokine production (**Figure 2**).

3.2.1. RORγt/RORα. RAR-related orphan receptor gamma (RORγt), a steroid-type receptor of the nuclear receptor family, is the first transcription factor found to be selectively expressed by Th17 cells (53). RORγt is induced in the early stages of Th17 cell differentiation, up to 6 h after IL-6/TGF-β and TCR stimulation (54). RORγt deficiency impaired Th17 cell differentiation in vitro and in vivo and dampened many Th17 cell–related autoimmune diseases including EAE, while conversely, ectopic expression of RORγt in T cells drove Th17 cell differentiation (54). However, RORγt deficiency could not completely abolish IL-17 production, likely due to compensation by RORα, a family member of RORγt also highly and selectively expressed in Th17 cells (55). Similar to RORγt, RORα is sufficient to induce IL-17 expression, though it plays a less significant role than RORγt in Th17 cells, as RORα deficiency led to only ∼20–50% reduction of IL-17 expression under various culture conditions (55).

3.2.2. STAT3. Activated by IL-6, -21, or -23, STAT3 is absolutely required for Th17 cell differentiation, as well as upregulation of RORγt in response to TGF-β and IL-6 (40). Patients with hyper-IgE syndrome (HIES, or Job's syndrome) were found to have STAT3 mutations and also showed diminished IL-17 expression (56). ChIP-seq and RNA-seq studies revealed that STAT3 is crucial in the global induction of the Th17 transcriptional program, through binding to numerous Th17-associated gene loci (57). *Stat3* deficiency had a more profound effect on global epigenetic activation of the Th17 cell program than *Rorc* deficiency, and RORγt overexpression could not restore Th17 cell development in the absence of STAT3 (58, 59).

3.2.3. SMADs. TGF-β is essential for the induction of Th17 cells and Foxp3$^+$ regulatory T (Treg) cells. In the canonical TGF-β signaling pathway, binding of TGF-β to its receptor induces phosphorylation of R-SMAD proteins SMAD2 and SMAD3, which then interact with the common SMAD protein SMAD4 and translocate into the nucleus to induce downstream gene expression. Deletion of *Smad2*, *Smad3*, or *Smad4* partially reduced TGF-β-induced Foxp3 expression (60–62). SMAD4 is indispensable for the generation of Th17 cells, whereas *Smad3* deficiency enhanced IL-17 production (60, 62). In contrast, *Smad2* deletion led to substantially reduced IL-17 expression (61), while compound deficiency of both *Smad2* and *Smad3* almost completely abolished IL-17 expression (63). SMAD2 and SMAD3 interact not only with SMAD4 but also independently with a coregulator, Trim33, to mediate SMAD4-independent gene regulation. *Trim33* deficiency also reduced Th17 cell differentiation in vitro while increasing IL-10 production (64). Further analysis indicates that TRIM33 is recruited to both *Il17* and *Il10* gene loci, in a SMAD2-dependent manner. Thus, it appears that TGF-β signaling promotes the formation of a SMAD2/3/4 complex to induce Foxp3 expression, whereas in the presence of IL-6 signaling, TRIM33 interacts with SMAD2 and promotes *Il17* transcription in Th17 cells.

3.2.4. Others. A number of other transcription factors, including IRF4, BATF, c-Maf, IκBζ, and RUNX1, also regulate Th17 cell development, in addition to playing roles in other T cells (65–70). Interestingly, these transcription factors, like STAT3, bind large amounts of common targets in Th17 cells, including the *Il17-Il17f* gene locus (58). Integrating high-throughput ChIP-seq and RNA-seq data, researchers found that the binding of the IRF4-BATF complex, triggered by TCR signaling, served as the first step to initiate epigenetic changes and chromatin accessibility at key Th17-related gene loci. Together with STAT3, these pioneering factors initiate a transcriptional program that is then reinforced by RORγt (58). In addition, transcriptional profiling of Th17 cells revealed that Th17 cells adopt a dynamic regulatory network with three distinct transcription waves, from a naive state to a fully committed state of Th17 cell differentiation, that are coordinately regulated by two self-reinforcing but mutually antagonistic modules composed of 72 densely correlated regulators (71).

3.3. Regulation of Th17 Differentiation by Epigenetic Mechanisms

Epigenetic regulation plays a critical role in cell fate determination, including differentiation of effector Th cell subsets. High-throughput ChIP-seq studies have revealed that genes encoding Th lineage-specific signature cytokines, but not lineage-specific transcription factors, are generally marked with either permissive or repressive epigenetic markers following lineage specification (72, 73). In Th17 cells, the *Il17-17f* locus is enriched with permissive histone marker H3K4Me3, but not the repressive histone marker H3K27Me3, whereas the *Ifng* and *Il4* loci exhibit the opposite pattern. In contrast, the gene locus of *Rorc* is marked with H3K4Me3 in Th17 cells but with both H3K4Me3 and H3K27Me3 in Treg cells (73). Disruption of epigenetic programs results in defective Th cell differentiation or function. For instance, ablation of either Tet2 or Jmjd3, the enzymes catalyzing conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) or histone H3K27 demethylation, respectively, impairs Th17 differentiation (72, 74). The GOT1 enzyme was shown to catalyze transamination, leading to increased levels of 2-hydroxyglutarate in differentiating Th17 cells; inhibition of glutamate to α-ketoglutaric acid conversion prevents the production of 2-hydroxyglutarate and blocks the differentiation of Th17 cells (75). GOT1 inhibition with (aminooxy)acetic acid ameliorates EAE, providing a link between metabolism and epigenetic regulation of Th differentiation.

Dynamic epigenetic changes during Th cell development result from cytokine signaling and transcription factor binding. STAT3 activation triggers the recruitment of TRIM28, a coregulator also in the TRIM family, likely through protein-protein interactions (59). T cell–specific *Trim28* deficiency decreased Th17 cell differentiation and offered protection from autoimmunity. *Trim28*-deficient T cells exhibited reduced permissive epigenetic modifications, including H3K4Me3, H3K27Ac, and 5hmc at the *Il17-Il17f* locus. In addition, *Trim28* deficiency significantly impaired p300 and RORγt recruitment, suggesting that TRIM28 serves as an epigenetic activator to increase chromatin accessibility essential for RORγt recruitment. RORγt, as a nuclear receptor, interacts with coregulators to mediate epigenetic regulation. Recently, a role for SRC-3 in RORγt function was reported. SRC-3 was found to be recruited to the regulatory elements of the *Il17* and *Il1r1* genes, also bound by RORγt, and was required for their chromatin activation via recruiting p300 (76). Interestingly, *Src3* deficiency did not affect Th17 cell differentiation induced by IL-6 and TGF-β, but it selectively reduced pathogenic Th17 cell differentiation induced by IL-1, IL-6, and IL-23.

3.4. Th17 Regulation by Environmental Factors

Th17 cells are regulated not only by the immune system but also by the host environment.

3.4.1. Microbiota. Th17 cells are frequently found in the mucosal tissues. Work from Littman and his colleagues (77, 78) first demonstrated the importance of intestinal microbiota, in particular segmented filamentous bacteria, in the generation of mucosal Th17 cells. Subsequently, it was reported that the presence of these Th17-inducing bacteria determines the pathogenesis of a rheumatoid arthritis disease model (79). Long-chain fatty acids, lauric acid for example, favor Th1 and Th17 cell differentiation, and a diet rich in long-chain fatty acids increases Th17 cells in the lamina propria of the small intestine and aggravates EAE in mice (80). Moreover, the ratios of gut-associated Th17 and Treg cells are regulated by a high-fat diet through enhancing acetyl-CoA carboxylase 1–dependent de novo fatty acid synthesis, and bile-acid-derived 3-OxoLCA binds RORγt and inhibits RORγt-dependent Th17 cell differentiation (81). ATP derived from commensal bacteria was also reported to preferentially induce Th17 differentiation via $CD70^{hi}CD11c^{lo}$ cells in the lamina propria, which express IL-6, IL-23, and TGF-β-activating integrin $\alpha_5\beta_8$ (82). On the other hand, intestinal Th17 cell conversion to IL-10-expressing Treg cells was found using fate-mapping mouse models, which require aryl hydrocarbon receptor (AhR), which is likely regulated by local microenvironments (83).

3.4.2. Salt. Sodium chloride salt has been associated with human cardiovascular diseases. In addition, high salt uptake was shown to promote autoimmunity by increasing Th17 cell generation (84, 85). Mechanistically, Wu et al. (85) showed that the expression of salt-sensing kinase SGK1 can be induced by IL-23. Loss of SGK1 resulted in protection from the EAE model. A recent report suggests that a high level of salt promotes Th17 cell differentiation predominantly through its influence on gut-associated microbiota, via limiting *Lactobacillus murinus*, a gram-positive fermentative bacterium that suppressed Th17 cell differentiation and ameliorated salt-associated hypertension and EAE diseases (86).

3.4.3. Hypoxia. Hypoxia, a common physical condition in normal tissues and organs and under inflammatory conditions or in tumor, was reported to enhance Th17 but limit Treg cell differentiation (87). Hypoxia-induced Th17 cell differentiation is controlled by hypoxia-inducible factor 1α (HIF-1α), a key transcription factor and metabolic sensor induced by IL-6-STAT3, which binds

to the *Il17* gene promoter with RORγt and p300. Genetic ablation of *Hif1a* impaired Th17 cell differentiation, even under normal oxygen conditions, suggesting a broader role of HIF-1α. Interestingly, a study implicated that microRNA-210 (miR-210), induced by hypoxia, via interacting with HIF-1α, provides a negative-feedback mechanism in hypoxic Th17 cell differentiation and associated autoimmunity (88).

3.4.4. Fever. More recently, fever, an evolutionarily conserved physiological response to infection, was shown to selectively regulate Th17 cell differentiation in vitro (89). Th17 cells generated at febrile temperature (38.5–39.5°C), compared with those generated at 37°C, showed enhanced pathogenic gene expression with increased proinflammatory activities in vivo. Mechanistically, febrile temperature promoted SUMOylation of SMAD4 transcription factor to facilitate its nuclear localization; *Smad4* deficiency selectively abrogated the effects of febrile temperature on Th17 cell differentiation in vitro and ameliorated an autoimmune disease model.

3.5. Th17 Cells in Health and Disease

Th17 cells are an essential component in the immune system whose overactivation can also cause immune-mediated disorders.

3.5.1. Infection. Th17 cells are an essential component of the immune system. Defects in Th17 cell generation and function have been associated with immunological deficiency in humans. Patients with HIES, associated with recurrent bacterial and fungal infections, were found to carry a STAT3 gene mutation and displayed impaired Th17 cell generation (90). Moreover, certain patients with chronic mucocutaneous candidiasis had autosomal recessive deficiency of IL-17RA, autosomal dominant deficiency of IL-17F (91), or biallelic missense mutation in ACT1 (92), an essential signaling adaptor for IL-17 (93). All these findings demonstrate the importance of Th17 cells in host immunity.

3.5.2. Autoimmunity. Considering the important roles of Th17 cells in animal models of autoimmune diseases, it is not surprising that they are also important players in human chronic disorders. Upon the discovery of Th17 cells, *Il23r* was identified by genome-wide association studies as a susceptible gene in many human autoimmune diseases including inflammatory bowel disease and psoriasis (94–96). In animal models, inhibition of Th17 cell generation or function resulted in protection (33, 54). More importantly, clinical trials with antibodies to the Th17 pathway, including those for IL-23, IL-17, and IL-17RA, had success in treating human diseases (97). In particular, an antibody targeting IL-17 was shown to have efficacy in rheumatoid arthritis, uveitis, and psoriasis (98). RORγt is considered another desirable target, as multiple inhibitors of RORγt have been shown to be effective in an EAE model (99).

3.5.3. Cancer. Unlike in infectious and autoimmune diseases, the roles of Th17 cells in cancer are complex, with both pro- and antitumorigenic activities reported, depending on the exact tumor types and experimental settings (100). On one hand, Th17 cells may be required for chronic inflammation associated with the development of many types of tumors (101). On the other hand, Th17 cells and IL-17 can also promote immunogenicity via recruiting neutrophils, NK cells, and $CD8^+$ T cells into the tumor and activating their antitumor functions (102, 103). Whether Th17 cells can be targeted in immunotherapy of certain human cancers remains to be carefully considered.

3.5.4. Behavior. Recently, Th17 cells were shown to have unexpected roles in neuronal development and function. Th17 cells and IL-17 are required in mothers for maternal immune activation (MIA)-induced behavioral abnormalities in offspring (104). Gut commensal bacteria that can induce Th17 cells may increase the risk of neurodevelopmental disorders in the offspring of pregnant mothers undergoing immune system activation owing to infections or autoinflammatory syndromes (105). In response to Th17-dependent MIA, cortical abnormalities are preferentially localized to a region encompassing the dysgranular zone of the primary somatosensory cortex in the offspring (106). Moreover, activation of pyramidal neurons in this cortical region was sufficient to induce MIA-associated behavioral phenotypes in wild-type animals, whereas reduction in neural activity rescued the behavioral abnormalities in MIA-affected offspring. Recently, mice lacking IL-17 were shown to be deficient in short-term but not long-term memory, owing to the reduced plasticity of glutamatergic synapses in the absence of IL-17 (107). Interestingly, IL-17 came from a population of γδ T cells residing in the meninges. This line of work highlights the potential roles of Th17 cells in modulating neuronal activities and behavior, though the precise mechanisms require further investigation.

4. T FOLLICULAR HELPER CELLS

In addition to promoting inflammation, another fundamental function of $CD4^+$ T cells is to help B cells, the hallmark of which is the formation of the germinal center (GC) structure in lymphoid organs. In GCs, B cells hyperproliferate; undergo somatic hypermutation in their immunoglobulin genes, switch antibody isotypes; and differentiate into plasma and memory cells. Production of IL-21 and CD40L by T cells is important for the GC reaction (108, 109). GC T cells, or Tfh cells, were originally discovered to express the CXCR5 chemokine receptor (110, 111), also expressed by all B cells, to localize in B cell follicles. In 2008, it was postulated that Tfh cells represent a unique lineage of $CD4^+$ T cells (112). Indeed, Th1, Th2, and Th17 cells, including their signature cytokines and transcription factors, appear unimportant in GC development. Subsequently, three papers showed that Tfh cells highly express Bcl6 transcription factor, which is essential for their development and GC reactions (113–115), firmly establishing Tfh cells as a fourth subset of effector Th cells (**Figure 1**).

4.1. Extrinsic Regulation of Tfh Cell Development

T cell differentiation into effector cells is regulated not only by TCR signaling but also by costimulatory pathways, especially those in the B7-CD28 family. CD28 is necessary for differentiation of all effector Th lineages, whereas ICOS appears to have more important roles in Th2, Th17, and especially Tfh cells (116). ICOS, expressed by activated T cells, is required for GC reactions and Tfh cell development (117). How ICOS mediates Tfh generation was analyzed by three groups. Suh and his colleagues (118) first demonstrated a role for PI-3 kinase: Knock-in mice that selectively lost the ability to activate PI-3 kinase through ICOS have severe defects in Tfh generation and GC reaction. Wan et al. (119) demonstrated that the ICOS membrane proximal region recruits Lck to activate PI-3 kinase. However, Pedros et al. (120) also showed that the ICOS cytoplasmic region associates with TBK1 kinase, and this interaction is necessary for the Tfh response.

Cytokine signaling is vital in effector Th cell differentiation. IL-6 and IL-21, in the absence of TGF-β signaling, were reported to initiate Tfh cell development by activating Stat3 and inducing Bcl6 mRNA expression (112). However, in vitro, neither IL-6 nor IL-21 is sufficient to induce the full Tfh program, leading to Bcl6 and CXCR5 protein expression. IL-2 neutralization, as well as

addition of IL-6 and IL-21, was shown to derepress Bcl6 expression and Tfh development, since transcription factor Stat5, downstream of IL-2, may induce Blimp-1 to suppress Bcl6 expression (121, 122). IL-7 signaling, which also activates STAT5, has been shown to negatively regulate Tfh development in vivo (123). Interestingly, IL-7R and Bcl6 expression appear to be mutually exclusive. However, unlike other Th subsets, Tfh cells cannot be induced effectively in vitro using cytokine cocktails, suggesting additional mechanisms, including cell contacts, are involved in their generation.

When T cells, after their activation, are committed to the Tfh pathway, leading to Bcl6 expression, has not been resolved either. In an acute lymphocytic choriomeningitis virus (LCMV) infection model, Choi et al. (124) showed that Bcl6 induction occurs as early as the second cell division of antigen-specific $CD4^+$ T cells following dendritic cell priming. T cells with enhanced Bcl6 expression also exhibit CXCR5 expression, but not CD25 or Blimp-1 expression (124), suggesting an early separation of Tfh and non-Tfh lineages. Kitano and colleagues (125) developed a Bcl6-YFP reporter mouse expressing a Bcl6-YFP fusion protein and found that Bcl6-YFP is initially upregulated together with CXCR5 on all T cells as early as day 2 following immunization, peaks at day 3, and is then downregulated. Baumjohann et al. (126) specified two waves of Bcl6 expression during Tfh cell differentiation: Bcl6 expression is upregulated in all divided cells as early as the first division after $CD4^+$ T cell activation; a second wave occurs by the fifth cell division. By using a Bcl6-IRES-RFP mouse in which RFP expression represented Bcl6 protein expression, Liu et al. (127) remeasured the dynamic expression of Bcl6 and CXCR5 during Tfh differentiation in an immunization system. Their results revealed that, from day 2 to day 7, Bcl6 expression is gradually upregulated, following upregulation of CXCR5 expression, and high levels of Bcl6 expression require B cells. They thus postulated that Bcl6-independent CXCR5 expression guides activated T cells to migrate to interfollicular regions and that the subsequent T cell–B cell interaction at B-T borders instructs further Tfh cell commitment via upregulating or sustaining the expression of Bcl6 (**Figure 1**). In the meantime, the gradually increased Bcl6 expression acts together with multiple transcription factors to specify and/or stabilize Tfh cells, resulting in downregulation of Th1, Th2, and Th17 cell–associated genes.

4.2. Regulation of Tfh Cell Development by Transcription Factors

Identification of Tfh-regulating transcription factors has not only reinforced the unique genetic identity of Tfh cells but also revealed the complex regulatory network involved in their developmental regulation.

4.2.1. Bcl6. Bcl6 was originally identified as a transcriptional repressor in diffuse large B cell lymphomas (128). The function of Bcl6 in B cells has been extensively studied for almost two decades and shown to be obligatory for GC B cell generation (129). The functional importance of Bcl6 in Tfh cells was established in 2009 (113–115). Bcl6 was reported to be selectively expressed in Tfh cells, and ectopic expression of Bcl6 in activated $CD4^+$ T cells promoted Tfh cell development in vivo, while *Bcl6* $^{-/-}$ $CD4^+$ T cells failed to give rise to Tfh cells. These findings have defined Bcl6 as a critical transcription factor for Tfh cells.

The molecular targets of Bcl6 in T cells have been investigated in two studies. Hatzi et al. (130) utilized primary human GC Tfh cells and reported that BCL6 functions as a repressor in Tfh cells. Interestingly, they found that many BCL6-bound loci are characterized as sites for AP1 or STAT binding. Showing that BCL6 directly binds AP1, they suggested that BCL6 inhibits AP1 activity. Liu et al. (123) identified Bcl6 target genes in purified murine $Bcl6^+$ Tfh cells by analyzing genome-wide Bcl6 occupancy together with transcriptome profiling. Their study revealed that

Bcl6 functions as both an activator and repressor. Moreover, Bcl6 shares binding sites with STAT5, and it antagonizes the IL-7R (CD127)/STAT 5 axis, which is inhibitory to Tfh development.

4.2.2. STAT3/BATF/IRF4. In an immunization model, IL-21- and IL-6-induced STAT3 was reported important for Tfh cell differentiation (112). In support of this idea, *Stat3* functional deficiency in human compromised the generation of Tfh cells (131). However, in a viral infection model, $CD4^+$ T cells deficient in *Stat3* retained the ability to differentiate into Tfh cells (132). This may be due to the redundancy of STAT1, because Tfh commitment requires both STAT1 and STAT3. Mechanistically, STAT3 directly binds to the *Bcl6* promoter to regulate its transcription (133).

As stated above, STAT3 works together with IRF4 and BATF to regulate Th17 differentiation. It is not surprising to find the involvement of these two transcription factors in Tfh cell development. Using immunization and infection models, Bollig et al. (134) showed that IRF4 is a T cell intrinsic factor necessary for Tfh cell differentiation and GC formation. Ise et al. (135) reported that BATF is necessary for antibody class-switch recombination in vivo. In T cells, BATF directly controls expression of Bcl6, as well as c-Maf, a transcription factor important for IL-21 production (65).

4.2.3. Ascl2/E2A/Id3. Although Bcl6 is necessary for Tfh cell development, it does not directly bind to the *Cxcr5* gene. Instead, basic helix-loop-helix family member Ascl2, also highly expressed by Tfh cells, potently regulates CXCR5 gene expression and early Tfh cell migration and development (136). Overexpression of Ascl2 promoted T cell migration to the follicles and Tfh cell development in vivo (136). Combined analysis of gene expression and Ascl2-bound DNA demonstrated that Ascl2 acted to repress Th1, Th2, and Th17 cell differentiation (136). In addition, Ascl2 regulated CXCR5 and CXCR4 expression and suppressed the expression of CCR7, PSGL-1, and IL-2 receptors (both CD25 and CD122) to promote Tfh cell migration and development (136). *Ascl2* deficiency did not completely impair Tfh cell development, which was likely caused by a compensatory increase of E47, another E-box protein in T cells.

Ascl2 function is inhibited by native E-box protein inhibitor Id3. When Id3 was introduced into Ascl2-expressing T cells artificially, Tfh cell development was suppressed (136). Conversely, Id3-deficient T cells were observed to have enhanced Tfh cell generation in vivo, possibly undermining Sjögren syndrome in *Id3*-deficient mice (137). Similarly, it was reported that Id2, another Id family member, inhibited E-protein E2A in regulating Tfh cell development and favored Th1 differentiation (138).

4.2.4. Tcf7/LEF-1. Three groups simultaneously reported an important role of TCF-1, encoded by *Tcf7*, in regulation of Tfh development (139–141). Selective loss of *Tcf7* resulted in a Tfh cell defect, while compound mutation of both *Lef1* and *Tcf7* severely impaired the differentiation of Tfh cells and the formation of GCs. Forced expression of LEF-1 enhanced Tfh differentiation. Mechanistically, TCF-1 bound to the genes encoding Bcl6 and Blimp-1 (141, 142), a crucial negative regulator of Tfh development (113). Compound deletion of *Blimp1* with *Tcf1* restored Tfh cell frequency and numbers and generation of GC B cells.

4.2.5. Tox2. During characterization of Bcl6 target genes, Xu et al. (133) found that the transcription factor Tox2 was elevated in expression during Tfh cell differentiation, regulated by both STAT3 and Bcl6. Interestingly, enforced expression of Tox2 led to substantial Bcl6 expression and Tfh development. Tox2 bound directly to loci associated with Tfh cell differentiation, including the Bcl6 locus itself, and as a result increased chromatin accessibility at these sites. Tox2 was

required for optimal Tfh differentiation, and inhibition of both Tox2 and the related Tox abolished Tfh differentiation. Thus, a Tox2-Bcl6 axis establishes a transcriptional feed-forward loop that promotes the Tfh program.

4.2.6. STAT5–Blimp-1. Blimp-1 plays a negative role in Tfh cell differentiation. Overexpression of Blimp-1 in $CD4^+$ T cells inhibited Bcl6 expression and significantly reduced the differentiation of Tfh cells (113). Conversely, *Prdm1*$^{-/-}$ $CD4^+$ T cells showed enhanced ability to develop into Tfh cells (113). The mutual antagonism between Bcl6 and Blimp-1 may be central to Tfh versus non-Tfh cell development (113, 143).

In contrast to STAT3/STAT1 signaling, IL-2-induced STAT5 signaling plays a negative role in Tfh cell development (121, 122, 144). The constitutively active form of STAT5 inhibited Tfh differentiation and suppressed the expression of Tfh-associated genes (121). Importantly, STAT5 induced Blimp-1 expression (121, 122). Conversely, mice with STAT5-deficient T cells exhibited increased Tfh and GC B cells (121, 122).

4.3. Tfh Cell Function in Immunity and Diseases

Since T-dependent humoral immunity is an essential component of adaptive immunity, it is not surprising that Tfh cells have become more and more important in immune function as well as immune-mediated disorders.

4.3.1. Infection. A T cell–dependent antibody response is necessary for immune protection from a variety of microorganisms, in particular viruses. Tfh deficiency impaired virus clearance in mice infected with LCMV, vaccinia, or influenza (113, 136, 145). Interestingly, in viral infection, IgG2 response was characteristic (146), whereas IgG1 was the dominant antibody isotype in immunization, possibly due to the generation of IFN-γ^+ Tfh cells following viral infections (H. Feng & C. Dong, unpublished data). In human HIV infection, potent HIV-specific broadly neutralizing antibodies, existing in a minority of patients, had a high degree of somatic hypermutations, likely generated in GCs and regulated by Tfh cells (147). Strategies that elicit potent and appropriate Tfh responses therefore deserve more consideration in vaccine design.

4.3.2. Autoimmunity. Many human autoimmune diseases are characterized by autoantibody production (148). Animal models have highlighted the potential roles of Tfh cells in autoimmunity. An *N*-ethyl-*N*-nitroso urea-mutagenized *Rc3h1san/san* (sanroque) mouse developed spontaneous systemic autoimmunity, associated with enhanced generation of Tfh cells and spontaneous GC formation (149). *Icos* deficiency suppressed Tfh response and thus the disease progression in this model (150). In an experimental Sjögren syndrome model, ablation of *Bcl6* in T cells, leading to Tfh and GC defects, was shown to be protective (151).

When peripheral blood cells of autoimmune patients were analyzed, $CXCR5^+$ circulating Tfh-like cells were found, and expression of high levels of PD-1 but low levels of CCR7 appeared to correlate with disease progression (152). However, in contrast to what was found in mouse secondary lymphoid organs, these circulating Tfh-like cells lacked expression of Bcl6 and were less polarized in terms of their expression of Tfh-associated genes. Some groups even found subsets of these cells that are related to Th1, Th2, or Th17 cells (153). It is therefore crucial to understand human Tfh cell biology and to associate circulating T cell phenotypes with GC responses as well as disease states.

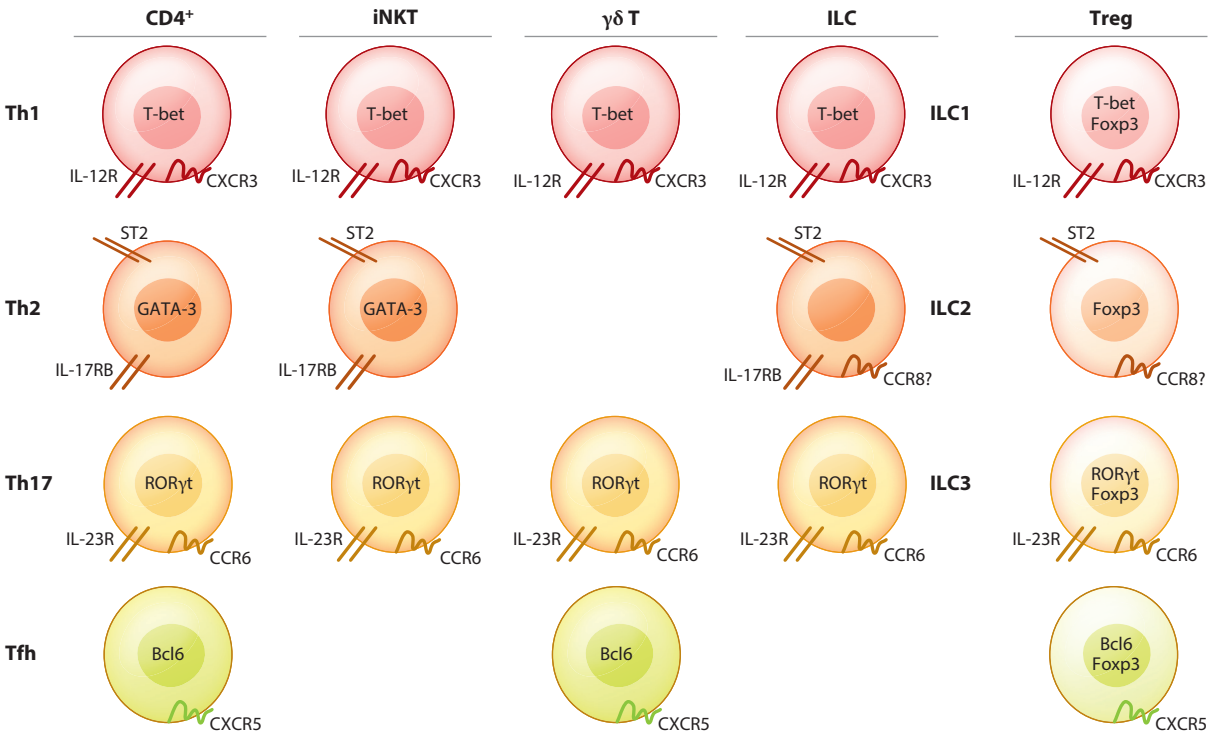

Figure 3

Subsets of immune cells. Classification of T helper cell subsets is also seen in other types of T cells and other lymphocytes. Abbreviations: ILC, innate lymphocyte; iNKT, invariant natural killer T cell; RORγ, RAR-related orphan receptor gamma; Tfh, T follicular helper cell; Th1, T helper 1 cell; Treg, regulatory T cell.

5. OTHER CYTOKINE-PRODUCING LYMPHOCYTES

In addition to the four well-established subsets of $CD4^+$ effector T cells, i.e., Th1, Th2, Th17, and Tfh cells, other populations of cytokine-producing $CD4^+$ T cells have been described in the literature, including Th9 and regulatory T type 1 (Tr1) cells. In addition, $Foxp3^+$ Treg cells have been shown to adopt some of the effector Th cell properties following activation. Lastly, γδ T cells and innate lymphocytes also exhibit distinct subsets, in a manner that resembles that of Th cells. We compare these cells to Th cells in this section (**Figure 3**); these cytokine-producing lymphocytes may provide a complex temporal-spatial immune regulation in health and disease.

5.1. Th9 Cells

IL-9 was originally found to be produced during gastrointestinal nematode infection and in allergic asthma and to enhance mast cell and eosinophil recruitment and function. Although IL-9 was originally classified as a Th2 cytokine, combined TGF-β and IL-4 induced IL-9-producing T cells that did not highly express the typical Th2 cytokines IL-4, IL-5, and IL-13; these were thus referred to as Th9 cells (154, 155). Th9 cells did not express high levels of transcription factors GATA3, FOXP3, and RORγt. Studies indicated that the ETS-family transcription factor PU.1 and IRF4 were essential for Th9 cell development (156, 157). Deficiency in PU.1 impaired Th9 cell generation but Th2 cell responses were normal, clearly indicating PU.1 as a crucial regulator

of Th9 cell differentiation (156). On the other hand, ectopic expression of PU.1 in Th2 cells drove the induction of IL-9 expression with low expression of Th2-type cytokines (156). However, PU.1 and IRF4 are also coexpressed by other Th cells and therefore may not be lineage-specific transcription factors. Whether Th9 cells represent a stand-alone Th cell lineage remains a subject of debate, because the regulation of IL-9 expression in vivo remains uncertain. More studies using mouse genetics as well as single-cell analysis of human T cells are needed to resolve this issue.

5.2. Tr1 Cells

$CD4^+$ T cells produce not only immune-stimulating cytokines to promote inflammation and humoral immunity but also regulatory or suppressive factors, including IL-10. A population of IL-10-secreting $CD4^+$ T cells was first described in human and called Tr1 cells (158). Subsequently, it was reported that a combination of the immunosuppressive drugs vitamin D_3 and dexamethasone induced human and mouse naive $CD4^+$ T cells to differentiate into Tr1-like cells in culture (159). More recently, cytokine IL-27, a member of the IL-12 family, was found to induce Tr1 cells in vitro, together with TGF-β (160). IL-27 was also reported to induce IL-10 expression by Th1, Th2, and Th17 cells (161). Tr1 cells lacked expression of Foxp3 but depended on other transcription factors for their generation. *Blimp1*-deficient $CD4^+$ T cells produced reduced levels of IL-10 compared to their normal counterparts, while overexpression of *Blimp1* promoted the Tr1 cell phenotype in effector T cells (162). IL-27 induced the expression of c-Maf, which, via interacting with AhR, promoted activation of *Il10* gene transcription (163). However, it remains unclear whether Tr1 cells in vivo represent a T cell lineage with a stable phenotype or a state of other types of effector T cells with regulatory features in response to environmental factors, such as those from gut microbiota. Gagliani et al. (83) reported that Th17 cells in the gut acquired an anti-inflammatory phenotype in a fashion dependent on canonical TGF-β signaling and AhR. Thus, Tr1 cells require further investigation to find their place in the landscape of T cell biology.

5.3. Treg Subsets

$Foxp3^+$ Treg cells are critical in maintaining immune hemostasis and in preventing immune overactivation. It is increasingly appreciated that Treg cells adopt Th-like transcriptional programs under inflammatory conditions, which may have important functional consequences. Treg cells were shown to upregulate T-bet during infection and in response to IFN-γ stimulation (164). T-bet promoted the expression of CXCR3, typically expressed on Th1 cells, to regulate Treg cell accumulation at sites of inflammation. Although deficiency of T-bet in Treg cells did not cause strong immunopathology, elimination of T-bet-expressing Treg cells resulted in severe Th1 autoimmunity (165).

Treg-specific deficiency of *Stat3* exacerbated Th17 responses, leading to fatal intestinal inflammation (166). *Stat3* deficiency reduced Treg expression of CCR6 and therefore the migration of Treg cells to the inflammatory sites. A subset of Treg cells in the intestine was shown to coexpress RORγt, regulated by microbiota (167, 168). Both dietary and microbial factors, via influencing the composition of the gut bile acids, contributed to the generation of these cells (169), likely derived from conventional naive T cells. In the absence of RORγt-expressing Treg cells, Th2-mediated immunity against helminths was potentiated (167). In addition, Kim et al. (170) reported that thymically derived Treg cells in lymph nodes upregulated RORγt following immunization and in an EAE model. IL-6/STAT3 appears important in this process, which may have functional roles in restricting autoimmunity.

In 2011, a Treg cell subset expressing CXCR5 and Bcl6, markers for Tfh cells, was identified and named T follicular regulatory (Tfr) cells (171, 172). Localized to the GCs, Tfr cells are

generated from CXCR5$^-$ thymically derived Treg precursors, and they inhibit GC B cell expansion, affinity maturation of antibodies, and differentiation of plasma cells. Moreover, lack of Tfr cells was reported to potentiate autoimmune disorders, including Sjögren syndrome (151).

It has been suggested that some Treg cells resemble Th2 cells. *Irf4*-deficient Treg cells were shown to lack control of Th2 responses, resulting in heightened IL-4-dependent immunoglobulin isotype production and tissue lesions with pronounced plasma cell infiltration (173). However, GATA-3 expression did not seem to mark this type of Treg cell, and loss of *Gata3* in Treg cells did not specifically enhance Th2 immunity but rather affected Treg cell hemostasis (174). The identity of Th2-type Treg cells and the mechanisms underlying their generation thus need further investigation.

5.4. Invariant NKT Cells

A group of CD4$^+$ T cells with an invariant TCR α chain recognize CD1d-presented lipids. They also display subsets similar to Th1, Th2, and Th17 cells. These cells develop in the thymus and present as memory-type T cells in the periphery, which may produce cytokines in the innate phase of immune responses. These cells have been recently reviewed (175).

5.5. γδ T Cells

γδ T cells are a type of T lymphocyte carrying the γδ TCR. They differ from αβ T cells also in their ontogeny and their anatomic localization. These cells are typically derived from fetal or neonatal thymus and migrate into mucosal tissues. Thus, they have an important innate function to sense infection and tissue damage, and they act promptly. Both IFN-γ- and IL-17-expressing γδ T cell subsets have been identified (176), and there may be ones similar to Tfh cells (F. Rampoldi & C. Dong, unpublished data), though it is not clear whether there are ones similar to Th2 cells.

IL-17-secreting γδ T cells, or Tγδ17 cells, produce IL-17 in the early phase of immunity to infection (177). They may be activated in response to not only TCR engagement but also TLR and C-type lectin receptor signaling (178). Like Th17 cells, Tγδ17 cells express CCR6, IL-23R, and RORγt. High-mobility group transcription factors Sox4 and Sox13 have been reported to regulate RORγt expression (179).

5.6. Innate Lymphoid Cells

Work in the last 10 years has identified innate lymphoid cells (ILCs) as another innate source of cytokines in the early phase of infection (180). ILC1, 2, and 3 cells have been classified and resemble Th1, 2, and 17 cells, respectively, in their expression of effector cytokines and transcription factors. Bcl6- or CXCR5-expressing ILCs have been reported (181), though it is unclear whether they represent a distinct subset of ILCs.

ILCs develop from precursors in the bone marrow through a complex network of pathways. They typically reside in mucosal tissues and mount acute cytokine secretion in response to innate cytokines. Thus, they sense inflammation and serve as another source of effector cytokines, especially in the early phase of the immune response, when effector Th cells have not yet been developed and properly localized.

6. T CELL TOLERANCE AND DYSFUNCTION

T cell activation and differentiation into cytokine-producing effector cells are a major feature in adaptive immunity to various infectious agents. In contrast, T cells need to maintain tolerance to

self-antigens. Many cell-intrinsic and -extrinsic mechanisms prevent self-reactivity, or overproduction of cytokines.

6.1. T Cell Tolerance

When encountering antigen-presenting cells, T cells need to distinguish self-antigens from foreign antigens. Under infectious or inflammatory conditions, costimulatory molecules and cytokines regulate effector differentiation and cytokine expression in antigen-specific T cells. In contrast, T cells encountering self-antigens in the absence of costimulation and cytokine signaling are rendered tolerized, i.e., unable to produce cytokines in response to TCR signaling, and they become anergic (116, 182). Anergy is first characterized by a block of TCR signaling, associated with upregulation of negative regulators in multiple pathways (182, 183). It has also been shown that sustained calcium signaling in T lymphocytes could also drive T cell anergy in vitro (184). However, it became evident that T cells rendered tolerant, at least in vivo, are different from effector cells in their gene transcription programs (182, 185). Recently, Liu et al. (186) induced $CD4^+$ T cell tolerance in vitro using antigen-presenting cells lacking the *B7.1*, *B7.2*, and *B7h* genes and found that the tolerized T cells are distinct from effector and Treg cells in their transcriptional and epigenetic profiles. Notably, the transcription factor NR4A1 was shown to be stably expressed at high levels in tolerant T cells. Overexpression of NR4A1 inhibited effector T cell differentiation, whereas deletion of *Nr4a1* impaired T cell tolerance and exaggerated effector function in autoimmunity. Mechanistically, NR4A1 was preferentially recruited to binding sites of the transcription factor AP-1, where it repressed effector-gene expression by inhibiting AP-1 function.

6.2. T Cell Exhaustion

In addition to naive T cells becoming tolerant when they first encounter cognate antigens, activated T cells, $CD8^+$ T cells in particular, become dysfunctional in the context of chronic infection and a tumor microenvironment. The recent success of immune checkpoint blockade immunotherapy has been attributed with preventing T cell exhaustion and/or rejuvenating exhausted T cells in their effector function. In essence, $CD8^+$ T cells, under the influence of a tumor microenvironment and as a result of chronic antigenic stimulation, undergo transcriptional and epigenetic changes that result in their inability to produce effector molecules and in their gain of exhaustion-associated gene expression programs. Molecules involved in regulation of T cell tolerance, e.g., PD-1 and NR4A1, are important in this process. In addition, transcription factors Tox and Eomes have been defined as exhaustion-regulating transcription factors (187–189). This subject is rapidly evolving and has been recently reviewed in this journal (190). Future years will witness a complex molecular network involved in T cell exhaustion.

7. CONCLUSIONS AND PERSPECTIVES

Characterization of cytokine-producing $CD4^+$ effector T cells has led to the definition of Th1, Th2, Th17, and Tfh cells, which play distinct roles in immunity. Such division of labor is seen in other types of lymphocytes. Cytokines produced by the innate immune system provide a crucial signal to instruct T cell differentiation into distinct subsets, mediated by transcription factors in the STAT family, which work with other pioneering transcription factors downstream of the TCR to mediate chromatin changes. Lineage-specific transcription factors reinforce the effector cytokine expression and help maintain the stability of the Th differentiation program. Our

knowledge gained from T cell biology has greatly helped advance treatment of immune-related disorders.

There is growing evidence also for plasticity in cytokine expression in T cells. Those at the inflammatory sites may upregulate cytokines and transcription factors typically belonging to at least two classes of T cells. Transdifferentiation has also been reported for some T cells in certain contexts. Whether there are additional subsets of T cells is an issue of some discussion. It is anticipated that single-cell technologies, especially when applied to human specimens, may reveal further complexity in T cell biology. T cell lineage-tracing studies in animal models are also needed to investigate the plasticity and stability, as well as memory formation, of effector T cells. This will offer novel and precision therapy of human diseases—better understanding, better treatment.

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