T Helper Cell Differentiation, Heterogeneity, and Plasticity

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Naïve CD4 T cells, on activation, differentiate into distinct T helper (Th) subsets that produce lineage-specific cytokines. By producing unique sets of cytokines, effector Th subsets play critical roles in orchestrating immune responses to a variety of infections and are involved in the pathogenesis of many inflammatory diseases including autoimmunity, allergy, and asthma. The differentiation of Th cells relies on the strength of T-cell receptor (TCR) signaling and signals triggered by polarizing cytokines that activate and/or up-regulate particular transcription factors. Several lineage-specific master transcription factors dictate Th cell fates and functions. Although these master regulators cross-regulate each other, their expression can be dynamic. Sometimes, they are even coexpressed, resulting in massive Th-cell heterogeneity and plasticity. Similar regulation mediated by these master regulators is also found in innate lymphoid cells (ILCs) that are innate counterparts of Th cells.

Cytokines are the central mediators of immune responses, and CD4 T helper (Th) cells are the professional cytokine-producing cells. By producing effector cytokines, Th cells play critical roles during adaptive immune responses to infections; distinct Th subsets are involved in protective immunity to different pathogens (Murphy and Reiner 2002; Zhu and Paul 2008; Zhu et al. 2010). There are different types of Th cells based on their cytokine profiles. Initially, type 1 T helper (Th1) and type 2 T helper (Th2) cell clones that preferentially produce interferon γ (IFN- γ) and interleukin (IL)-4, respectively, were reported (Mosmann et al. 1986; Mosmann and Coffman 1989; Paul and Seder 1994). A third major CD4 Th effector cell population Th17 that produces IL-17 was not discovered until decades later (Park

et al. 2005; Acosta-Rodriguez et al. 2007; Weaver et al. 2007; Korn et al. 2009). All of the Th1, Th2, and Th17 cells are differentiated from naïve CD4 T cells when they are activated through T-cell receptor (TCR)-mediated signaling. Not only are the Th effector cells important for protective immunity, they can also induce inflammatory responses to self-antigens or to nonharmful allergens, resulting in autoimmunity or allergic diseases, respectively (Paul and Zhu 2010; Zhu et al. 2010). Interestingly, naïve CD4 T cells can also develop into regulatory T cells (Tregs), which are important for maintaining immune tolerance and for regulating the magnitude of immune responses (Shevach 2000; Chen et al. 2003; Sakaguchi 2004; Josefowicz et al. 2012; Abbas et al. 2013).

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Th cells can produce the majority of the known cytokines. In addition to the signature effector cytokines, IFN- γ , IL-4, and IL-17A, Th cells may preferentially express many other important cytokines, such as lymphotoxin α for Th1; IL-5, IL-9, IL-13, and IL-24 for Th2; and IL-17F and IL-22 for Th17 cells. In addition, all of the Th subsets are capable of producing IL-2, IL-6, IL-10, IL-21, tumor necrosis factor α (TNF- α), and granulocyte macrophage colony-stimulating factor (GM-CSF). Furthermore, some regulatory functions of Tregs are mediated through production of anti-inflammatory cytokines such as transforming growth factor (TGF)- β , IL-10, and IL-35. Not only are Th cells professional cytokine producers, they can also respond to a variety of cytokines, including IL-1, IL-7, IL-12, IL-15, IL-18, IL-23, IL-27, IL-33, and type 1 IFNs, etc., that are produced by accessory cells. During differentiation, Th cells can also respond to their own cytokines, including IFN- γ and IL-4, resulting in powerful positive feedback or cross-inhibitory effects.

In this review, I will mainly focus on effector Th-cell differentiation, heterogeneity, and plasticity regulated by cytokines and transcription factors. Because innate lymphoid cells (ILCs), an innate equivalent of Th cells) are also professional cytokine-producing cells (Artis and Spits 2015), the relationships between conventional Th cells and ILCs will also be discussed.

DISTINCT FUNCTIONS OF Th-CELL SUBSETS

Different Th-cell subsets have distinct immune functions in protective immunity. Th1 cells (Szabo et al. 2003) are mainly important for host defense against intracellular pathogens, including viruses, protozoa, and bacteria; they are also responsible for the development of certain forms of organ-specific autoimmunity. One of the major functions of Th1 cells is to activate macrophages through IFN- γ production.

Th2 cells are critical for mediating immune responses against extracellular parasites, including helminthes. These cells are also responsible for the pathogenesis of inflammatory asthmatic and allergic diseases. By producing IL-4, Th2

cells induce B-cell immunoglobulin (Ig) switching to IgG1 and IgE (Kopf et al. 1993); by producing IL-5, Th2 cells recruit eosinophils (Coffman et al. 1989); and by producing IL-13, Th2 cells can induce the movement of smooth muscle cells and mucus production by epithelial cells (Urban et al. 1998; Kuperman et al. 2002; Wynn 2003). IL-4 and IL-13 produced by Th2 cells can also induce alternatively activated macrophages (Gordon 2003).

Th17 cells are essential for orchestrating immune responses to extracellular bacteria and fungi. They are also responsible for different forms of autoimmunity, including psoriasis, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases (Ouyang et al. 2008; Korn et al. 2009). Th17 cells are also involved in severe asthma (McKinley et al. 2008). Th17 cells produce three major cytokines: IL-17A, IL-17F, and IL-22. IL-17A and IL-17F have redundant functions in diseases, but they may also have unique functions (Iwakura et al. 2011). The major function of IL-17A and IL-17F is to recruit and activate neutrophils. They can also stimulate different cell types to produce inflammatory cytokines, including IL-6. IL-22 is a critical cytokine for stimulating cells at mucosal barriers to produce antimicrobial peptides and proinflammatory cytokines and chemokines (Liang et al. 2006).

Treg cells include thymus-derived Treg (tTreg) cells and peripherally derived Treg (pTreg) cells (Shevach 2009; Sakaguchi et al. 2010; Josefowicz et al. 2012; Abbas et al. 2013). Together with the tTreg cells, pTreg cells play important roles in maintaining immune tolerance and regulating the magnitude of immune responses by controlling the differentiation and functions of T effector cells (Korn et al. 2009; Zhu et al. 2010; Crotty 2011; Bilate and Lafaille 2012).

CYTOKINE-MEDIATED SIGNAL TRANSDUCERS AND ACTIVATORS OF TRANSCRIPTION (STATs) ACTIVATION IS CRITICAL FOR Th-CELL-FATE DETERMINATION

Th-cell differentiation involves T-cell activation. Indeed, the strength of TCR signaling has a ma-

jor impact on Th-cell-fate determination (Tao et al. 1997). Sometimes the TCR signaling strength may even have a dominant effect over adjuvants that usually create the cytokine environments that drive T-cell differentiation (Tubo et al. 2013; Nelson et al. 2014; van Panhuys et al. 2014). In particular, stimulation with low-dose peptide favors Th2-cell differentiation (Yamane et al. 2005), consistent with the finding that resting dendritic cells (DCs) promote Th2-cell differentiation (Stumbles et al. 1998; Everts et al. 2009; Steinfelder et al. 2009). TCR costimulation mediated by CD28, which is required for optimal T-cell activation, is also involved in Th-cell differentiation (Seder et al. 1994). In contrast, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) negatively regulates T-cell differentiation (Oosterwegel et al. 1999; Bour-Jordan et al. 2003).

It is well known that, besides TCR-mediated signaling, cytokine-mediated signals are critical for the differentiation of Th cells. For example, IL-12, mainly produced by antigen-presenting cells, including macrophages and DCs, induces Th1-cell differentiation (Hsieh et al. 1993). IL-12 activates a transcription factor, STAT4, in differentiating CD4 T cells (Hsieh et al. 1993; Kaplan et al. 1996b; Thierfelder et al. 1996). IFN- γ , which is produced by Th1 cells themselves, also promotes Th1-cell differentiation through activating STAT1 (Lighvani et al. 2001; Afkarian et al. 2002; Martin-Fontecha et al. 2004). Indeed, either IL-12 or IFN- γ is capable of inducing Th1-cell differentiation at least in vitro (Zhu et al. 2012). However, during *Toxoplasma gondii* infection, which elicits a robust Th1 response, IFN- γ signaling seems to be dispensable for generating IFN- γ -producing T cells, whereas IL-12 is essential.

IL-4 induces Th2-cell differentiation by activating STAT6 (Kaplan et al. 1996a; Shimoda et al. 1996; Takeda et al. 1996). A constitutively active STAT6 mutant is sufficient to replace IL-4 in inducing Th2-cell differentiation (Kurata et al. 1999; Zhu et al. 2001). However, under certain circumstances, Th2-cell differentiation in vivo particularly in response to parasite infection may occur in an IL-4/IL-4R α /STAT6-independent manner (Finkelman et al. 2000;

Jankovic et al. 2000; Min et al. 2004; Voehringer et al. 2004; van Panhuys et al. 2008).

In addition to IL-4, IL-2 through activating STAT5 is critical for the differentiation of Th2 cells in vitro (Zhu et al. 2003; Cote-Sierra et al. 2004). IL-2-mediated STAT5 activation is detectable 24 h after T-cell activation with a low dose of TCR stimulation, which induces Th2 differentiation (Yamane et al. 2005). CD4 T-cell proliferation and survival only need low levels of STAT5 activation (Moriggl et al. 1999; Cote-Sierra et al. 2004); however, Th2-cell differentiation requires high levels of STAT5 activation (Kagami et al. 2000, 2001; Zhu et al. 2003; Cote-Sierra et al. 2004). Other cytokines such as IL-7 and thymic stromal lymphopoietin (TSLP) can also activate STAT5 in T cells. Indeed, TSLP expression may trigger the initiation of Th2-cell differentiation in vivo (Ito et al. 2005; Zhou et al. 2005; Liu 2006; Sokol et al. 2008). Although TSLP mainly acts on DCs (Ito et al. 2005; Liu et al. 2007), it may also directly stimulate naïve CD4 T cells to become Th2 cells (Al-Shami et al. 2005; Omori and Ziegler 2007; Rochman et al. 2007; He et al. 2008). IL-7 can promote Th2-cell differentiation in vitro; however, its physiological function during Th2 differentiation in vivo is still elusive. Activated STAT5 directly regulates IL-4 production by binding to the *Il4/Il13* locus at different regulatory regions (Zhu et al. 2003; Cote-Sierra et al. 2004; Liao et al. 2008). Furthermore, IL-2-mediated STAT5 activation induces IL-4R α expression at the early stage of Th2-cell differentiation (Liao et al. 2008).

For Th17 and pTreg differentiation, TGF- β is involved (Chen et al. 2003; Korn et al. 2009). Together with IL-2-mediated STAT5 activation, TGF- β induces Treg differentiation; on the other hand, together with IL-6-mediated STAT3 activation, TGF- β induces Th17-cell differentiation (Bettelli et al. 2006; Veldhoen et al. 2006). IL-21 and IL-23 have a similar function as IL-6 in inducing STAT3 activation and, thus, Th17-cell differentiation. However, IL-6, IL-21, and IL-23 may be involved in the different stages of Th17-cell development and maintenance (Korn et al. 2009). IL-2 signaling is important for Treg generation. In the absence of IL-2, IL-2R α , or IL-2R β , Treg-cell numbers are reduced and mice

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or humans bearing mutations in the genes encoding IL-2, IL-2R α , or IL-2R β develop autoimmune disease (Fontenot et al. 2005; Caudy et al. 2007). In contrast, IL-2-mediated STAT5 activation suppresses Th17-cell differentiation (Laurence et al. 2007; Liao et al. 2011; Yang et al. 2011).

CYTOKINE-MEDIATED POSITIVE FEEDBACK DURING Th-CELL DIFFERENTIATION

The cytokine-mediated positive feedback mechanism is one of the basic principles of Th-cell differentiation. During Th1-cell differentiation, IFN- γ produced by developing Th1 cells may instruct IFN- γ nonproducers to produce IFN- γ . Similarly, IL-4 produced during Th2-cell differentiation may induce IL-4 expression by the previous IL-4 nonproducers. Furthermore, low amounts of IFN- γ or IL-4 derived from T cells may further induce these cells to produce high levels of IFN- γ or IL-4 in an autocrine manner. Therefore, Th1- and Th2-cell differentiation is enforced by the positive feedback loops. TGF- β produced by Treg cells may be important for the generation of pTreg cells (Andersson et al. 2008). Th17 cells are also capable of producing either TGF- β 1 or TGF- β 3, both of which may serve as positive feedback mechanisms for Th17-cell differentiation (Gutcher et al. 2011; Lee et al. 2012). Because TGF- β induces both Th17 and pTreg-cell differentiation and both cell types can produce TGF- β , how TGF- β exactly works in vivo may depend on other factors such as the presence of inflammatory cytokines and the stage of Th-cell differentiation.

MASTER TRANSCRIPTION FACTORS DICTATE T-CELL DIFFERENTIATION

Although networks of transcription factors regulate Th-cell differentiation (Zhu and Paul 2010b; Ciofani et al. 2012; Hu et al. 2013; Yosef et al. 2013), lineage-specific master transcription factors play the most critical roles during the differentiation process (Fig.1). T-bet is the master transcription factor for Th1-cell differentiation, and it directly regulates IFN- γ

production (Szabo et al. 2000, 2002). Because IFN- γ also induces T-bet expression, this explains the positive feedback for Th1-cell differentiation (Lighvani et al. 2001; Afkarian et al. 2002). IL-12 can also induce T-bet independent of IFN- γ signaling (Yang et al. 2007; Zhu et al. 2012). Thus, IL-12 and IFN- γ redundantly induce T-bet expression both in vitro and in vivo. In addition to IL-12 and IFN- γ , other cytokines such as IL-27 and type I IFNs are also capable of inducing T-bet, although their actual functions during Th-cell differentiation in vivo are still elusive (Zhu et al. 2012). T-bet is capable of inducing its own expression (Mullen et al. 2001); however, such autoregulation may not be required when either IL-12 or IFN- γ is present (Zhu et al. 2012). Nevertheless, T-bet collaborates with STAT4 to induce optimal IFN- γ production, and one of the main functions of T-bet in Th1-cell differentiation is to remodel the *Ifng* locus. Genome-wide study has shown that T-bet directly regulates a large number of Th1-specific genes (Zhu et al. 2012).

GATA3 is the master transcription factor for Th2-cell differentiation (Zhang et al. 1997; Zheng and Flavell 1997; Pai et al. 2004; Zhu et al. 2004). Retroviral expression of GATA3 is sufficient to induce endogenous GATA3 expression (Ouyang et al. 1998, 2000); however, GATA3 may not be required for its own expression when IL-4 is present (Wei et al. 2011). Unlike T-bet, which is not expressed in naïve T cells, GATA3 is expressed in naïve CD4⁺ T cells at low levels, possibly because of its critical role during CD4 T-cell development in the thymus (Ho et al. 2009; Wei et al. 2011). STAT6 activation by IL-4 is one of the major inducers for GATA3 up-regulation. However, low-dose TCR stimulation is sufficient to up-regulate GATA3 expression independent of IL-4/STAT6 signaling (Yamane et al. 2005); this may offer a mechanism through which IL-4-independent Th2 differentiation may occur in vivo. Alternatively, the initiation of Th2-cell differentiation in vivo may occur with low amounts of GATA3 expression when STAT5 is highly activated (Zhu et al. 2003; Cote-Sierra et al. 2004; Rochman et al. 2009). Nevertheless, both IL-4-dependent and IL-4-independent Th2-cell differentiation re-

Figure 1. Combinatorial expression of master transcription factors determines the heterogeneity and functionality of effector T helper (Th) cells, follicular Th (Tfh) cells, regulatory T (Treg) cells, and innate lymphoid cell (ILC) subsets. T-bet, GATA3, and ROR γ t are the master transcription factors of distinct Th cell and ILC subsets. Within the Th effector compartment, T-bet/ROR γ t coexpressing cells have been found under inflammation conditions. These cells could be derived from either T-bet- or ROR γ t-single positive cells. During type 2 immune responses, GATA3/T-bet coexpressing cells are also found. Similarly, in the ILC compartment, NKp46⁺ ILC3s coexpress T-bet and ROR γ t. Bcl6 is the master regulator for Tfh cells. Subsets of Tfh cells may either express low levels of effector master regulators, T-bet, GATA3, or ROR γ t, or have expressed one of these factors during their development. Interestingly, all of these master regulators, T-bet, GATA3, ROR γ t, and Bcl6, can be expressed by subsets of Foxp3-expressing Treg cells albeit at lower levels. Furthermore, although it is not indicated in the figure, all of these lymphocytes can express GATA3 but at various levels.

quire GATA3 (Zhu et al. 2004). Interestingly, during Th1 and/or Th17 differentiation, GATA3 expression is down-regulated (Zheng and Flavell 1997; Wei et al. 2011).

GATA3 may promote Th2-cell differentiation via different mechanisms (Yagi et al. 2011). Genome-wide profiling of GATA3 binding indicates that it directly binds to the *Il4/Il13* gene locus at various regions (Wei et al. 2011). Through its binding to the promoters of the *Il5* and the *Il13* genes, GATA3 induces *Il5* and *Il13* transcription (Yamashita et al. 2002; Tanaka et al. 2011). However, GATA3 mainly affects *Il4* expression through regulating epigenetic modifications at the Th2 cytokine gene locus.

Therefore, while GATA3 is required for the acquisition of IL-4-producing capacity by Th2 cells, in fully differentiated Th2 cells, GATA3 is no longer important for IL-4 production but it remains essential for the transcription of the *Il5* and *Il13* (Zhu et al. 2004). In addition, GATA3 directly regulates many other Th2-specific genes including *Il1rl1*, which encodes T1/ST2, the IL-33 receptor (Wei et al. 2011).

ROR γ t is the master transcription factor for Th17-cell differentiation (Ivanov et al. 2006). ROR γ t loss-of-function mutations in human patients results in susceptibility to both *Candida* and *Mycobacterium* infections (Okada et al. 2015). The induction of ROR γ t expression de-

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depends on STAT3 activation by IL-6, IL-21, and/or IL-23 (Zhu and Paul 2008; Korn et al. 2009). ROR γ t directly regulates IL-17A and IL-17F expression. At the genome scale, ROR γ t binds to and regulates only a selected set of Th17-specific genes after BAF/IRF4 and STAT3 have initiated the Th17 differentiation program (Ciofani et al. 2012).

The master regulator for Treg generation and function is Foxp3 (Fontenot et al. 2003; Hori et al. 2003). Mutations in the human *FOXP3* gene are responsible for the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, and mutations in the *Foxp3* gene in mice, such as *Scurfy* mice, result in fatal autoimmune diseases. Genome-wide analysis indicates that Foxp3 directly binds to hundreds of genes, many of which are either positively or negatively regulated by Foxp3, especially in the thymus (Zheng et al. 2007). Some of Foxp3-regulated genes encode molecules that regulate gene expression and/or are involved in epigenetic modifications.

CROSS-REGULATION OF Th-CELL DIFFERENTIATION

During Th-cell differentiation toward a specific type, signals/pathways that induce such differentiation also repress the alternative lineage fates. At the transcriptional level, the transcription factors that are either activated or induced in one lineage often cross-regulate the expression and/or functions of the transcription factors that are involved in making other lineage decisions. For example, overexpression of T-bet suppresses *Gata3* transcription (Usui et al. 2006) and inhibits GATA3 function through direct protein–protein interaction (Hwang et al. 2005). Interestingly, T-bet and GATA3 binding sites colocalize at several critical Th1- or Th2-specific genes (Jenner et al. 2009; Kanhere et al. 2012; Zhu et al. 2012). It has also been shown that endogenous T-bet inhibits GATA3 function during Th1-cell differentiation, thus preventing the activation of a “default” program for Th2-cell differentiation (Zhu et al. 2012). On the other hand, during Th2-cell differentiation, GATA3 may suppress Th1-cell differenti-

ation by repressing STAT4 expression (Usui et al. 2003), and suppressing Runx3-mediation IFN- γ production (Yagi et al. 2010) as well as silencing the *Tbx21* locus (Wei et al. 2011). Cross-regulation has also been found between T-bet and ROR γ t (Lazarevic et al. 2011) and between Foxp3 and ROR γ t (Yang et al. 2008; Zhou et al. 2008). ROR γ t and Foxp3 may antagonize each other through protein–protein interaction (Yang et al. 2008; Zhang et al. 2008; Zhou et al. 2008).

NONCANONICAL Th SUBSETS THAT COEXPRESS MULTIPLE MASTER REGULATORS

Master regulators are usually expressed in a cell-type-specific manner (i.e., T-bet for Th1, GATA3 for Th2, ROR γ t for Th17, and Foxp3 for Treg cells) (Fig. 1). However, because both Th17- and Treg-cell differentiation require TGF- β , ROR γ t and Foxp3 are often coexpressed, presumably at early stages of Th17/Treg-cell differentiation. These cells may represent an intermediate stage before they eventually commit to either Th17 or Treg cells (Yang et al. 2008; Zhou et al. 2008; Komatsu et al. 2014). Interestingly, ROR γ t/Foxp3-coexpressing Treg cells, possibly representing a specialized Treg subset, are abundant in the large intestine (Ohnmacht et al. 2015; Sefik et al. 2015). Indeed, Treg-specific deletion of ROR γ t results in gut inflammation with an uncontrolled Th2 response (Ohnmacht et al. 2015).

In addition to ROR γ t, T-bet and GATA3 can be expressed by Treg subsets as well. Therefore, it has been suggested that Treg cells may hijack the master regulators of distinct T effector cells to specifically control a given type of immune response. According to this model, T-bet expression by Treg cells is critical for inhibiting Th1 responses (Koch et al. 2009). However, Treg-specific deletion of T-bet does not result in the development of Th1-related autoimmune diseases, indicating that T-bet is not required for Treg cells to control autoreactive Th1 cells at steady state (Yu et al. 2015). Because the functions of Treg cells during inflammation and/or immune responses may be different from their

functions at steady state, it remains to be determined whether T-bet expression in Treg cells is important for their ability to limit Th1-related immune responses (Oldenhove et al. 2009; Yamaguchi et al. 2011).

GATA3 is expressed in all T cells, albeit at different levels. Interestingly, some Treg cells can express high levels of GATA3 (Wang et al. 2011; Wohlfert et al. 2011; Rudra et al. 2012; Yu et al. 2015), potentially explaining why a slight reduction in Foxp3 expression results in a Th2 phenotype in these “Treg cells” (Wan and Flavell 2007). In fact, even with normal levels of Foxp3 expression, GATA3 may induce the expression of many “Th2-specific” genes such as *Il1rl1* and *Ccr8* in Treg cells (Wei et al. 2011). One study has shown that GATA3 deletion in Treg cells results in spontaneous Th2-like autoimmunity (Wang et al. 2011), but other studies show that the mice with GATA3 deletion specifically in Treg cells do not develop Th2-specific systemic diseases (Wohlfert et al. 2011; Rudra et al. 2012; Yu et al. 2015).

Interestingly, T-bet- and GATA3-expressing Treg cells do not represent stable Treg subsets. The expression T-bet and GATA3 in Treg cells is dynamic (Yu et al. 2015). Although deletion of either T-bet or GATA3 in Treg cells does not affect the overall function of these cells, Treg cells lacking expression of both T-bet and GATA3 up-regulate ROR γ t expression and acquire IL-17-producing capacity. These Treg cells are unstable and many lose Foxp3 expression over time. Thus, dynamic expression of T-bet and GATA3 together with cross-regulation among T-bet, GATA3, ROR γ t, and Foxp3 are important for maintaining Treg functions (Yu et al. 2015).

In addition to the heterogeneity of Tregs, within T effector populations, multiple master regulators may also be expressed in the same cell. For example, T-bet/ROR γ t coexpressing cells are found in the gut and inflamed brain (Ivanov et al. 2006; Hirota et al. 2011). Many of these cells are capable of expressing both IFN- γ and IL-17 (Lee et al. 2009; Ghoreschi et al. 2010; Lexberg et al. 2010; Hirota et al. 2011). T-bet/ROR γ t (IFN- γ /IL-17) coexpressing T cells, which are specific for *Candida albi-*

cans antigens, are also found in human patients (Zielinski et al. 2012). Interestingly, these cells may represent an important population that is involved in immune responses to *Mycobacterium tuberculosis* (*Mtb*) infection in humans. ROR γ t-deficient patients fail to control *Mtb* infections (Okada et al. 2015). Besides T-bet/ROR γ t coexpressing cells, GATA3/T-bet coexpressing cells have been detected in helminth-infected animals (Peine et al. 2013) and GATA3/ROR γ t coexpressing cells are found in asthmatic mice and patients (Cosmi et al. 2010; Wang et al. 2010).

EPIGENETIC MODIFICATIONS AND CELL PLASTICITY

Different modifications at the histones binding to the genomic regions correlate with gene activation or silencing (Barski et al. 2007). Trimethylation at the lysine position 4 of histone 3 (H3K4me3), particularly in gene-promoter regions, is usually associated with active gene loci. On the other hand, H3K27me3 is generally a marker of repressed genes. Epigenetic status at the effector cytokine loci is usually reflected by H3K4me3 in cells that express such cytokines, or by H3K27me3 in cells where these cytokine genes are silenced (Wei et al. 2009). However, bivalent modifications with both H3K4me3 and H3K27me3, which are indicative for the genes poised for expression, are found in the promoters of the master regulator genes, such as *Tbx21*, *Gata3*, and *Rorc*, etc., even in the cells that do not express such transcription factors (Wei et al. 2009). Such bivalent modifications may allow the induction of a master regulator expression in cells of alternative lineage fate, resulting in the generation of master regulator coexpressing cells and cell plasticity. Therefore, a transient signal that alters the balance of key master regulators, which will cross-regulate each other when coexpressed, may ultimately result in a lineage switch.

Although there are some reports showing that Th1 and Th2 cells may alter their lineage fates (Hegazy et al. 2010), these cells are relatively stable (Zhu and Paul 2010a). Cell plasticity is more common for Th17 and Treg cells (Zhou et al. 2009a). Th17 cells may acquire IFN- γ -

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producing capacity in a T-bet-dependent manner (Mathur et al. 2006; Bending et al. 2009; Lee et al. 2009). Through a fate-mapping study, it has been shown that IFN- γ -producing cells found in the brain of autoimmune mice are largely derived from the cells that express IL-17 (Hirota et al. 2011). Th17 cells may also *trans*-differentiate into Treg cells when inflammation is resolved (Gagliani et al. 2015). Although several studies have shown that Treg cells are stable even under inflamed conditions (Rubtsov et al. 2010; Sakaguchi et al. 2013), other studies have shown the possible switch from Treg cells to Th1, Th2, or Th17 cells (Xu et al. 2007; Komatsu et al. 2009, 2014; Oldenhove et al. 2009; Zhou et al. 2009b; Noval Rivas et al. 2015).

RELATIONSHIP BETWEEN T EFFECTOR CELLS AND CYTOKINE-PRODUCING Tfh CELLS

A critical function of CD4 T cells during immune responses is to help B cells produce antibodies and Ig class switching (Fig. 1). It has been shown that CD4 Th cells that are found in the B-cell follicle, termed as Tfh cells, are critical for exerting such functions (Crotty 2011). Th-cell effects on B cells rely on cytokine production; although IL-4 is important for the Ig class switching to IgE and IgG1 (Kopf et al. 1993; King and Mohrs 2009; Reinhardt et al. 2009; Zaretsky et al. 2009), IFN- γ induces Ig switching to IgG2a/IgG2c. Even though IL-21 produced by Tfh cells is important for helping B cells, there are at least two different types of Tfh cells, with one type producing IFN- γ and the other IL-4. In fact, most of the IL-4-producing Th cells in vivo have a Tfh phenotype (King and Mohrs 2009). Different from conventional Th2 cells, however, IL-4-producing Tfh cells do not express IL-13 (Liang et al. 2012).

Tfh cells are considered as a fifth major Th-cell population that is different from conventional Th1, Th2, Th17, and Treg cells (Nurieva et al. 2008). Tfh-cell differentiation requires STAT3 activation, presumably by IL-21, but IL-2-mediated STAT5 activation suppresses Tfh-cell generation (Nurieva et al. 2008, 2012;

Johnston et al. 2012). The master transcription factor for Tfh cells is Bcl6 (Johnston et al. 2009; Nurieva et al. 2009). Interestingly, some Treg cells are also found in the B-cell follicle and these cells coexpress Foxp3 and Bcl6. These cells are named follicular regulatory T (Tfr) cells (Chung et al. 2011; Linterman et al. 2011). Tfr cells may play an important role in limiting the functions of Tfh cells in activating B cells.

Tfh cells do not express or express very low levels of T-bet and GATA3. However, IgE class switching completely depends on GATA3 expression, indicating that GATA3 is required for the development of IL-4-producing Tfh cells (Zhu et al. 2004). It is possible that low levels of GATA3 expression in Tfh cells are sufficient for these cells to produce IL-4 production (Yusuf et al. 2010; Liang et al. 2012). Alternatively, just like the dynamic expression of GATA3 found in Treg cells, as mentioned earlier (Yu et al. 2015), GATA3 may have been expressed at high levels at early stage of IL-4-producing Tfh-cell differentiation. Once the *Il4* locus is remodeled by GATA3, Tfh cells no longer require GATA3 for IL-4 production and thus do not need to express GATA3. This is consistent with the observation that in fully differentiated Th2 cells, *Gata3* deletion does not abolish IL-4 production (Zhu et al. 2004). Similarly, transient expression of T-bet during the early stage of Tfh-cell differentiation may allow these cells to acquire IFN- γ -producing capacity.

The developmental relationship between IFN- γ - or IL-4-producing Tfh cells and conventional Th1 or Th2 cells is still elusive. In vitro experiments suggest that conventional Th1 or Th2 cells can become IFN- γ - or IL-4-producing Tfh cells or differentiated Tfh cells can acquire IFN- γ - or IL-4-producing capacity (Lu et al. 2011). In vivo, activated IL-4-producing T cells may subsequently acquire a Tfh-cell phenotype through their interaction with B cells (Zaretsky et al. 2009). It is also possible that activated T cells may acquire IFN- γ - or IL-4-producing capacity and a Tfh phenotype simultaneously, and thus their fate to become either conventional Th1/Th2 effector cells or specific cytokine-producing Tfh cells may have been determined at very early stages of T-cell differentiation (Na-

kayamada et al. 2011; Johnston et al. 2012; Oestreich et al. 2012). It is interesting to point out that Tfh cells may be an important source for generating memory Th cells, which could subsequently give rise to conventional Th effector cells on reactivation (Luthje et al. 2012). In the future, genome-wide assessment of the transcriptomes and epigenomes of conventional Th cells, cytokine-producing Tfh-cell subsets and Th memory cells is necessary to further understand the relationship between these closely related cell types.

RELATIONSHIP BETWEEN Th CELLS AND INNATE LYMPHOID CELLS

During the past few years, ILCs have drawn much attention in the immunology field (Artis and Spits 2015). ILCs do not express antigen receptors, but they can respond to many inflammatory cytokines, such as IL-1, IL-12, IL-18, IL-23, IL-25, and IL-33, to produce their own cytokines, including IFN- γ , IL-5/13, and IL-17/22. ILCs express IL-7R α and at least partially depend on IL-7 and/or TSLP for their development. Because of their distinct cytokine-producing capacity, ILCs are classified into group 1 ILCs (ILC1s) that produce IFN- γ , group 2 ILCs (ILC2s) that produce IL-5 and IL-13, and group 3 ILCs (ILC3s) that produce IL-17 and IL-22. Not only do ILC subsets produce similar cytokines to those produced by Th-cell subsets, but ILCs also use the same set of master regulators, namely, T-bet, GATA3, and ROR γ t for their development and function. For example, the Th2 master regulator, GATA3, is also critical for ILC2 development and the functional maintenance of these cells (Hoyler et al. 2012; Mjosberg et al. 2012; Furusawa et al. 2013; Klein Wolterink et al. 2013; Yang et al. 2013; Yagi et al. 2014). Although some researchers have classified natural killer (NK) cells into ILC1s based on their cytokine production, NK cells are in fact the innate counterparts of CD8 T cells in the adaptive system (Cortez and Colonna 2016; Spits et al. 2016). Both NK and CD8 T cells express transcription factor Eomes. Developmentally, all non-NK ILCs (or Th-like ILCs, or IL-7R α -expressing ILCs) depend on GATA3,

whereas NK cells do not (Yagi et al. 2014); this mirrors the critical function of GATA3 in specifying CD4 but not CD8 lineage fate during T-cell development (Ho et al. 2009). In addition, GATA3 regulates IL-7R α expression in both T cells and ILCs (Wang et al. 2013; Zhong et al. 2016).

Because of similarities between ILC and Th subsets (Yagi et al. 2014; Koues et al. 2016; Shih et al. 2016), a specific type of ILCs may participate in the same class of immune responses in a similar manner to corresponding distinct Th subsets. Therefore, ILC2s are important players during type 2 immune responses, including immunity against helminth infections (Fallon et al. 2006; Moro et al. 2010; Neill et al. 2010; Price et al. 2010) and during allergic lung and skin inflammation (Chang et al. 2011; Monticelli et al. 2011; Halim et al. 2012; Roediger et al. 2013; Kim et al. 2014). Similarly, ILC1s may participate in type 1 immune responses and ILC3s are important for controlling extracellular bacteria, which induce Th17 responses (Qiu et al. 2013; Klose et al. 2014; Sano et al. 2015). As mentioned above, ILCs mainly respond to cytokine stimulation. Interestingly, in fully differentiated Th cells, IL-1 family receptors IL-18R, IL-33R, and IL-1R, which are preferentially expressed by ILC1s, ILC2s, and ILC3s, respectively, are also selectively expressed by Th1, Th2, and Th17 cells, respectively (Guo et al. 2009, 2012). As a result, just like ILCs, Th cells can also respond to cytokine stimulation, which is independent of TCR stimulation, to produce effector cytokines *in vivo* (Guo et al. 2015).

One major difference between ILCs and Th cells is antigen specificity. Because ILCs do not recognize specific antigen and they are already developed even before possible microbial threats, these cells provide the first line of host defense to infections. Their unique position in tissue sites fits quite well with their functionality. Interestingly, the same type of ILCs and Th cells may cross talk to each other. For example, IL-13 produced by ILC2s on activation can induce the migration of DCs to the draining lymph nodes, and these migratory DCs preferentially induce Th2-cell differentiation (Halim et al. 2014). In addition, some ILC2s, by expressing major

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histocompatibility complex (MHC) class II, can activate T cells, and IL-2 produced by T cells may then act back onto ILC2s to influence their activation and cytokine production (Mirchandani et al. 2014; Oliphant et al. 2014). Cross talk between ILC3s and Th17 cells has also been reported (Sano et al. 2015). Nevertheless, the functions of ILCs and Th cells may be substantially redundant. Optimal activation of ILCs is sufficient to control infections in the absence of Th cells, as shown in several mouse models. Interestingly, however, humans without ILCs, which can result because of the failure of ILC reconstitution after bone marrow transplantation, are capable of controlling infections (Vely et al. 2016). However, the collaboration as well as labor division between ILCs and Th cells may allow the host to survive severe infections. Because of the activation of either ILCs or Th cells alone is sufficient to induce many inflammatory diseases, investigating the development and functions of both ILCs and Th subsets is clinically relevant.

CONCLUDING REMARKS

CD4 Th cells are professional cytokine-producing cells. To acquire a unique cytokine-producing profile, naïve CD4 T cells need to go through a differentiation process. During Th-cell differentiation, TCR and cytokine-mediated signaling pathways induce activation of STAT proteins followed by up-regulation of lineage-specific master transcription factors. Activated STAT proteins collaborate with lineage-specific master regulators such as T-bet, GATA3, and ROR γ t in epigenetically remodeling the respective cytokine loci and regulating cytokine gene expression. Although these master regulators are usually expressed in a cell-type-specific manner, they can often be coexpressed. Furthermore, effector cell-related transcription factors can be expressed by Foxp3-expressing Treg cells, resulting in massively heterogeneous Th effector and regulatory populations. Bivalent histone modifications at the gene loci of master regulators may explain the coexpression of multiple factors, and the coexpression, dynamic induction, and cross-regulation of these master regu-

lators may determine the plasticity of Th cells. Some master regulator coexpressers are relatively stable, that is, T-bet/ROR γ t coexpressers. Importantly, these cells are found abundant in several inflammatory settings; they are considered as the most potent cells in inducing autoimmunity and they may be an important cell population to fight against *Mtb* infection in humans. How T-bet and ROR γ t, two mutually inhibitory transcription factors, may stably coexist in the same cell and what unique programs have been activated in these cells remain important questions. Investigation of Th- and ILC-cell heterogeneity and plasticity holds promise for finding specific treatments for a variety of human immunological diseases in the future. High-dimensional single-cell analyses, including single-cell RNAseq and CyTOF mass cytometry, may allow us to gain deeper insights into the immune responses mediated by Th cell and ILC subsets in autoimmunity, allergy, and infectious diseases.

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