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Review

How Diverse—CD4 Effector T Cells and their Functions

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CD4 effector T cells, also called helper T (Th) cells, are the functional cells for executing immune functions. Balanced immune responses can only be achieved by proper regulation of the differentiation and function of Th cells. Dysregulated Th cell function often leads to inefficient clearance of pathogens and causes inflammatory diseases and autoimmunity. Since the establishment of the Th1–Th2 dogma in the 1980s, different lineages of effector T cells have been identified that not only promote but also suppress immune responses. Through years of collective efforts, much information was gained on the function and regulation of different subsets of Th cells. In this review, we attempt to sample the essence of what has been learnt in this field over the past two decades. We will discuss the classification and immunological functions of effector T cells, the determinants for effector T cell differentiation, as well as the relationship between different lineages of effector T cells.

Keywords: Th1, Th2, Treg, Th17, Tfh, immune disease, inflammation, autoimmunity

Introduction

Since birth, our immune system is constantly bombarded with self-antigens, innocuous antigens and foreign pathogens, such as airborne and food-borne viruses, bacteria and parasites. To stay healthy, complex immune strategies have evolved in mammals to maintain self-tolerance and to defend against foreign pathogens. To accomplish such a daunting task, the immune system has developed mechanisms to efficiently cope with these insults in the living species. The two fundamental arms of the immune response, innate and adaptive immunity, jointly form a defensive front against pathogens. Innate immunity evolved earlier and is mediated by cells such as macrophages and dendritic cells (DC) and is specific only to a few classes of microorganisms. The later evolved adaptive immunity mediated by T and B cells and enhances pathogen eradication in vertebrates by adding antigen specificity and memory onto pre-existent innate immunity. A defining property of adaptive immunity is the antigen-driven differentiation of clonally restricted lymphocyte precursors into effector cells with enhanced functional potential.

Effector T cells are the key players in steering the immune responses to execute immune functions. While effector T cells were initially identified to be immune promoting, recent studies unraveled negative regulatory functions of effector T cells in modulating adaptive as well as innate immunity. The differentiation of naïve T cells into fully functional effector T cells is characterized by the acquisition of new profiles of cytokine production and is largely directed by cytokines produced by activated cells of the innate, as well as adaptive, immune system. Key advances in the understanding of effector T cells have been tied to cytokine biology—cytokine production is vital for the classification and function of effector T cells. Owing to their critical and diverse functions in controlling immune responses, the functions and regulation of effector T cells have been a subject of intense investigation since the Th1–Th2 paradigm was conceived and established in 1986. In the ensuing years, vast knowledge has been gained in identifying new classes of effector T cells and in understanding their functions and regulation.

Effector T cell lineages—old and ever increasing new players

In immunology, different lineages of immune cells were conventionally distinguished by the morphology and expression of a cluster of differentiation markers on the surface. In 1986, a seminal study by Coffman and Mosmann revealed that cytokine profiles can be used to categorize CD4 effector T cells, namely helper-T-cell-1 and -2 (Th1 and Th2), which possess different immunological functions (Coffman and Carty, 1986; Mosmann et al., 1986).

One important means of discovery is to approach old questions with new techniques to explore new explanations. Immunology has thrived through the processes of discovery and re-discovery. The dawn of the Th1–Th2 dogma is a good example of such a principle in practice. In the studies published in 1980s (Coffman and Carty, 1986; Mosmann et al., 1986), Coffman and Mosmann and, independently, Bottomly (Kim et al., 1985) proposed a model to answer two older questions: are delayed-type hypersensitivity and B cell help mediated by different types of helper T cells? And how are allergic response and IgE production regulated? Based on the studies conducted on mouse CD4 T cell clones using state-of-art techniques for that time, they proposed that two types of Th cells exist to promote reciprocal patterns of immunity through the production of distinct cytokines: Th1 cells that make IL-2 and IFN-γ are important for classical delayed-type hypersensitivity reactions, whereas Th2 cells that make IL-4 promote IgE production and allergic reactions. In addition, they postulated that each subset of Th cell promotes its own development and inhibits the other via secreted cytokines; such that the induction of one type of response suppresses the other. This hypothesis established a new paradigm that Th cells are functionally heterogeneous and cytokines are important for Th cell function and can be used to distinguish different classes of Th cells. Such a notion fueled effector T cell research ever since and tremendous progress has been made in identifying new types of effector T cells and in characterizing their functions and regulation. Several types of effector T cells have been documented with distinct biological functions. Th1, Th2, Th17, Tfh and Th9 cells are involved in inflammatory responses while regulatory T cell (Treg) including naturally occurring Treg (nTreg), induced Treg (iTreg) and class 1 Treg (Tr1) engage in immune suppression (Table 1).

Th1 cells

Th1 cells are defined based on the production of pro-inflammatory cytokines IFN-γ, TNF-α and TNF-β to stimulate innate and T cell immune responses. Th1 cells also promote IgG2a or the equivalent IgG2c in other mouse strains production by B cells. The Th1 response tends to be dominated by cell-mediated forms of immunity characterized by cellular cytolytic activity. Th1 cells are important to protect the host from the obligate intracellular pathogens. A classic example is its role during infection by *Leishmania major*, a protozoal parasite. Such infection is lethal to genetically susceptible but not to genetically resistant mouse strains (Sadick et al., 1987). Protection of a resistant mouse strain, C57BL/6, correlated with an appropriate IFN-γ-mediated Th1 response, and susceptibility of BALB/c mice correlated with an inappropriate IL-4-mediated Th2 response (Heinzel et al., 1989). The Th1 response accounts for such protection because susceptible BALB/c mice become resistant upon transfer of a Th1 cell line specific to an immunodominant *L. major* antigen (Scott et al., 1988). Th1 response and IFN-γ production were also found to be critical to protect hosts from infections by intracellular bacteria, such as *Mycobacterium avium* (Kobayashi et al., 1997), *Salmonella typhimurium* (Mastroeni et al., 1999) and *Listeria monocytogenes* (Buchmeier and Schreiber, 1985), by fungi, such as *Cryptococcus neoformans* (Zhang et al., 1997), and by viruses, such as herpes simplex virus (HSV) (Fujioka et al., 1999), influenza A virus (Sareneva et al., 1998) and vaccinia virus (Tanaka-Kataoka et al., 1999). In addition to the beneficial effects of clearing foreign pathogens, Th1 response also helps tumor immune rejection. For example, Th1 $CD4^+$ T cells were shown to be important in maintaining the host as tumor free in a transferred sarcoma model (Micallef et al., 1997).

On the one hand, the pro-inflammatory properties of Th1 cells are suited for pathogen clearance and anti-tumor immunity; on the other hand, they can cause tissue damage and elicit unwanted inflammatory disease and self-reactivity. Th1 cells and IFN-γ contribute to inflammatory diseases such as inflammatory bowel disease (IBD) (Davidson et al., 1996; Parronchi et al., 1997) and graft-versus-host disease in recipients following bone marrow transplantation (Hu et al., 1999), as well as autoimmune disorders such as insulin-dependent diabetes mellitus (also known as type-1 diabetes) (Wang et al., 1997; Pakala et al., 1999) and rheumatoid arthritis (RA) (Leung et al., 2000).

Th2 cells

Th2 cells are defined as producers of IL-4, IL-5, IL-9, IL-10 and IL-13. Th2 cells promote IgG1 and IgE class-switching and eosinophil recruitment (Arthur and Mason, 1986; Coffman and Carty, 1986; Mosmann et al., 1986; Paliard et al., 1988; Firestein et al., 1989). Unlike the Th1 response, the Th2 response is often associated with humoral responses during which high levels of pathogen-

Table 1 Category, differentiation and function of effector CD4 Th cells.

	Th1	Th2	Th9	Th17	Tfh	Treg
signature cytokines	IFN-γ TNF-α; TNF-β STAT4;	IL-4; IL-5; IL-10; IL-13 STAT6;	IL-9	IL-17; IL-21; IL-22 ROR-α	IL-21	TGF-β IL-10 Foxp3
lineage-specific transcription factors	T-bet; Hlx	GATA3; c-maf; IRF4; Gfi-1		ROR-γt		
inducing cytokines	IL-12; IL-18; IL-27; IFN-γ	IL-4	TGF-β/IL-4	IL-6/TGF-β IL-21/TGF-β IL-23	IL-21	TGF-β IL-10; IL-2; TSLP
pathogens cleared	intracellular bacteria; protozoal parasites; fungi; viruses	extracellular pathogens including helminthes and nematodes	helminthes	similar to Th1, but with certain specific functions (see text)		negatively regulate pathogen clearance
immune pathology involved	IBD; GVHD; IDDM (T1D); RA	atopic asthma; allergies		MS (EAE); RA; psoriasis; IBD; allergies		maintain self-tolerance and homeostasis

Th1, Th2, Th17, Th9, Tfh and Treg effector CD4 T cells are defined based on the cytokines produced. Lineage-specific transcription factors critical for a particular Th cell differentiation and function are activated following inducing cytokine stimulation. Fully functional Th cells contribute to the clearance of specific types of pathogens and are involved in certain inflammatory and autoimmune diseases.

specific immunoglobulin are generated to neutralize foreign organisms. Thus, the Th2 response is important to resist extracellular forms of pathogens, such as helminthes and nematodes (Finkelman et al., 1991; Sher and Coffman, 1992). Th2 cells are also important for mucosal immunity (mucus hyper-secretion and increased contractility) in the lung. An over-exuberant Th2 response leads to pathological changes in the host. Chronic inflammatory airway diseases, such as atopic asthma and allergy characterized by local infiltration with allergen-specific CD4 T cells, were attributed to Th2 cells (Wierenga et al., 1990; Kay et al., 1991; Parronchi et al., 1991; Durham et al., 1992; Robinson et al., 1992; Yssel et al., 1992; Ebner et al., 1993). Because the Th2 response is considered to combat pathogens of more recent origins compared with the Th1 response, it is thought that Th2 immunity evolved after Th1 immunity in vertebrates.

Th17 cells

Th17 cells are a newly identified class of effector T cells attracting much attention recently. As indicated by the name, Th17 cells produce IL-17A, E and F among the six members of IL-17 family, IL-17A, B, C, D, E (or IL-25) and F (Fort et al., 2001; Kolls and Linden, 2004; Langrish et al., 2005). It is now becoming clear that Th17 cells also produce IL-21 and IL-22 (Liang et al., 2006; Nurieva et al., 2007; Korn et al., 2007; Zheng et al., 2007). The Th17 response seems to share commonality with both Th1 and Th2 responses. Th17 cells have been suggested to contribute to the resistance to *Listeria*, *Salmonella*, *Toxoplasma*, *Cryptococcus*, *Leishmania* and *Francisella* (Lieberman et al., 2004; Chackerian et al., 2006; Kleinschek et al., 2006). A few infection models have shown a significant and specific role for the Th17 response, including *Klebsiella* infection in the lung (Happel et al., 2005), intravenous *Candida albicans* infection (Huang et al., 2004), and infection of the natural rodent pathogen *Citrobacter rodentium* in the gut (Mangan et al., 2006). In addition, the preferential production of IL-17 by T cells during infection with *Bacteroides fragilis* (Chung et al., 2003), *Borrelia burgdoferi*, *Mycobacterium tuberculosis* (Infante-Duarte et al., 2000) and fungal species (LeibundGut-Landmann et al., 2007) suggests that Th17 responses are triggered by specific pathogens and are required for their clearance. In addition to controlling infection, Th17 cells play an important role in the induction and propagation of autoimmunity. IL-17 expression has been associated with autoimmune diseases such as multiple sclerosis (MS), RA, psoriasis, IBD, as well as allergic responses (Langrish et al., 2005; Yen et al., 2006). In particular, Th17 cells are critical for the development of MS/EAE (experimental autoimmune encephalomyelitis) and RA (Bush et al., 2002; Nakae et al., 2003; Hofstetter et al., 2005; Langrish et al., 2005; Komiyama et al., 2006), whose development was originally attributed to Th1 cells before the discovery of Th17 cells. While it is accepted that Th17 is a T cell lineage distinct from Th1 and Th2, its evolution in relation to Th1 and Th2 is under debate.

Treg cells

Naturally occurring regulatory T cells (nTreg) are a subset of CD4 T cells that develop in the thymus and constitutively express high levels of IL-2 receptor α chain (CD25), CTLA-4 and GITR. nTreg comprises 5–10% of peripheral CD4 T cells. They produce increased levels of IL-10 and membrane-bound forms of TGF-β (Nakamura et al., 2001). Strictly speaking, nTreg may not qualify as an effector T cell according to the traditional definition in that they do not actively secrete large amounts of effector cytokines in order to engage in immune regulation. However, unlike naïve T cells possessing little immune activity, nTreg produces inhibitory effector cytokines and actively modulates immune responses with potent activities. Thus, in a broader sense, nTreg is a type of effector T cell. The induced regulatory T cell (iTreg) is differentiated from naïve T cells in the presence of TGF-β following T cell receptor (TCR) stimulation. These cells produce large amounts of IL-10 and TGF-β (Weiner, 2001; Stassen et al., 2004). Unlike Th1, Th2 or Th17 cells, iTreg displays immune-suppressive activity with minimal antigen specificity. Tr1 is another regulatory T cell subset producing IL-10 and TGF-β but distinct from iTreg according to other molecular signatures, such as Foxp3 expression (Vieira et al., 2004).

The most prominent function of Treg cells is to maintain self-tolerance and immune homeostasis. nTreg cells were initially identified to be critical for self-tolerance, because disruption of their function invariably results in a systemic lymphoproliferative autoimmune syndrome in both mice and humans (Sakaguchi et al., 1995; Shevach, 2000, 2002; Sakaguchi, 2004). Disruption of Treg function contributes to a plethora of autoimmune and inflammatory pathologies. Treg are also suggested to be important for tempering immune responses against infectious agents and in re-establishing immune homeostasis following pathogen clearance (Belkaid, 2008). Interestingly, a recent study suggests that Treg can also facilitate immune response against HSV infection by recruiting natural killer (NK) cells, DC and T cells to the site of infection (Lund et al., 2008).

Follicular helper T cells

Tfh (follicular helper T cells) are found enriched in the edge of the B cell zones and follicular regions and germinal centers. Tfh cells express high levels of CXCR5, the receptor for chemokine CXCL13 that is abundant in B cell zones (Ansel et al., 2000). Tfh-promoted generation of high-affinity antibodies and memory B cells were initially ascribed to Th1 and/or Th2 mechanism. However, recent evidence suggests that Tfh is a unique effector T cell subset expressing B cell-promoting cytokine IL-10 and IL-21 that are not typically associated with Th1 or Th2 cells (Chtanova et al., 2004; Nurieva et al., 2008; Suto et al., 2008; Vogelzang et al., 2008). With IL-21 expression, Tfh is nevertheless distinct from Th17 cells (Nurieva et al., 2008), because Tfh can differentiate independent of TGF-β and retinoid-related orphan receptors that are essential for Th17 differentiation.

Th9 cells

Th9 cells are a new type of helper T cells independently reported by Stockinger's and Kuchroo's groups (Dardalhon et al., 2008; Veldhoen et al., 2008). These cells generate high levels of IL-9 and IL-10. Th9 cells share commonalities with Th2 cells and yet are distinct by their IL-9 production. It appears that Th9 cells

are involved in intestinal responses to helminthes (Khan et al., 2003), which were traditionally thought to be mediated by the Th2 response. Whether cells producing IL-9 are fully differentiated effector cells and thus can be anointed with the appellation of Th9 remains to be validated. In addition, further studies are needed to reveal the function of this type of cells in pathogen clearance and inflammatory diseases.

Gene signatures and determinants: acquiring identities

In a host that has not experienced foreign antigens, effector T cells are rare. The differentiation of effector T cells requires antigen-driven clonal expansion of naive T cell precursors. Th cells may be generated through two mechanisms: 1) They arise from distinct precursors that are predisposed to develop into different lineages or 2) a common undifferentiated precursor can be instructed to differentiate into various lineages. With studies using TCR transgenic models, which provided uniform naïve T cell precursors with identical antigenic specificity, it is recognized that the latter is the dominant mechanism for Th cell differentiation. Many factors influence Th cell differentiation. TCR stimulation, co-stimulation, kinase cascades, cytokine signaling and transcriptional networks are all involved in Th lineage decision, with cytokine signaling being recognized as the predominant factor in driving Th differentiation. What signaling mechanisms, from the cell surface to the nucleus, instruct the establishment and stabilization of different types of effector T cells? (Table 1).

TCR stimulation and co-stimulation

TCR and major histocompatibility complex II (MHCII) interaction (signal I) is required for CD4 T cell activation. The nature of the antigenic stimulus influences Th polarization (Constant and Bottomly, 1997; Tao et al., 1997). Studies using altered peptide ligands showed that the affinity between TCR and peptide-bound MHCII, thus the strength of TCR stimulation, affects lineage commitment, potentially by controlling the duration or magnitude of Ca^{2+} fluxes (Sloan-Lancaster et al., 1997). The differential inclusion of specific membrane-bound factors into lipid rafts, known as SupraMolecular Activation Clusters (SMAC) (Cherukuri et al., 2001; Galbiati et al., 2001), in Th1 and Th2 cells may account for this phenomenon (Balamuth et al., 2001). The SMAC composition of resting effector Th1 and effector Th2 cells are indistinguishable. However, Th1 cells but not Th2 cells showed increased sensitivity to low-affinity stimulation (Balamuth et al., 2001), while they both responded efficiently to high-affinity antigens.

Besides TCR signal co-stimulation (signal II), mediated through CD28-B7 and other surface molecules, is essential for efficient T cell activation. Such molecules have also been suggested in influencing Th1 and Th2 differentiation. Compared with Th1, the Th2 response was more pronouncedly affected in CD28- or B7-deficient mice (Freeman et al., 1993; Green et al., 1994; Schweitzer et al., 1997). Inducible T-cell co-stimulator (Dong et al., 1999) and OX40 (Akiba et al., 2000) signaling were also shown to be preferentially required for Th2 differentiation. The interactions between LFA-1 and ICAM-1, -2 appear to inhibit Th2 response (Salomon and Bluestone, 1998; Smits et al., 2002). Depending on the types of ligands the receptor binds, Notch signaling that is known to be critical for T cell thymic development is also important in directing T cell differentiation; ligand Delta induces Th1, while ligand Jagged induces Th2 (Amsen et al., 2004).

The proximal event following T cell-APC synapse formation is immediate activation of several signaling molecules: The Src kinase phosphorylates and activates Tec tyrosine kinases, which then activate PLC-γ that is required for IP_3 generation to sustain intracellular calcium flux. Rlk (Txk in human) is a member of Tec family shown to be sufficient and required for IFN-γ but not IL-4 production (Kashiwakura et al., 1999; Takeba et al., 2002). In addition, RIBP, a Txk-binding protein is specifically required for IFN-γ production (Rajagopal et al., 1999). However, another Tec family member Itk appears to be required for IL-4 production and Th2 response upon infection with *L. major*, nematode *Nippostrongylus brasiliensis* or *Schistosoma mansoni* eggs (Fowell et al., 1999; Schaeffer et al., 2001). Therefore, Tec kinases are important for Th1–Th2 divergence by integrating antigen-stimulation signals. The roles of TCR stimulation, co-stimulation and proximal signaling events in the differentiation of recently characterized Th17, Th9 and Treg cells remain to be addressed.

Cytokines

The cytokine milieu is arguably the most dominant determinant of Th cell differentiation. To combat various types of pathogens, innate cells as well as T cells generate different sets of cytokines to skew T cell differentiation—different cytokine environments instruct T cells to differentiate into different Th lineages (Table 1 and Figure 1).

For Th1 differentiation

IFN-γ. IFN-γ is a signature cytokine for Th1 cells; it is also suggested to be important for the differentiation of these cells. The major sources for IFN-γ are NK, CD8 and CD4 Th1 cells. Naïve CD4 T cells and B cells were also shown to produce IFN-γ. Whether macrophages and DC make IFN-γ remains controversial (Munder et al., 1998; Yoshimoto et al., 1998; Ohteki et al., 1999; Fukao et al., 2000). Many lymphoid (including T cells) and non-lymphoid cell types express heterodimeric IFN-γ receptor-1 and -2, and thus can be stimulated by IFN-γ through the Jak1/Jak2-STAT1 signaling pathway (Bach et al., 1997; Leonard and O'shea, 1998). Impairment of this pathway leads to compromised immune responses against microbial pathogens and certain viruses in mice (Cooper et al., 1993; Dalton et al., 1993; Huang et al., 1993; Kamijo et al., 1993; Durbin et al., 1996; Meraz et al., 1996) and humans (Jouanguy et al., 1996; Jouanguy et al., 1999; Newport et al., 1996; Pierre-Audigier et al., 1997).
As a signature cytokine for Th1 cells, the exact role for IFN-γ in directing Th1 differentiation is, however, debatable. Early studies demonstrated an involvement of IFN-γ in Th1

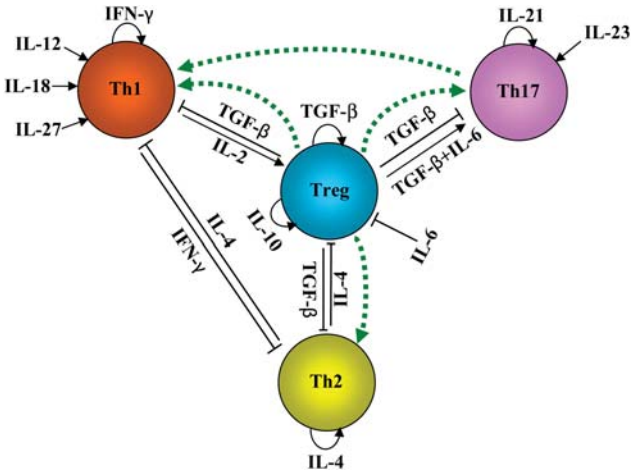

Figure 1 The balancing act of cytokines in Th differentiation and conversion. Largely through the effects of cytokines, different types of Th cells counterbalance each other to achieve immune homeostasis. Under certain circumstances, one type of Th cell converts into another (shown by dotted green lines) to facilitate desired immune responses.

differentiation *in vivo* during *L. major* clearance (Scott, 1991). Neutralization of IFN-γ protein and deficiency in IFN-γ gene led to abrogated Th1 development (Belosevic et al., 1989; Wang et al., 1994) in C3H and C56BL/6 mice. However, according to one study, Th1 cells were generated normally in IFN-γR1 deficient mice on a 129/Sv/Ev background (Swihart et al., 1995). *In vitro*, IFN-γ promotes Th1 differentiation of CD4 T cells from the C57BL/6 and B10.BR strains (Bradley et al., 1996). Such an effect was dependent on IFN-γR2 (Swihart et al., 1995). And yet, IFN-γ alone was shown to be insufficient for the induction of optimal Th1 differentiation of CD4 T cells from BALB/c mice *in vitro* (Macatonia et al., 1993; Wenner et al., 1996). These results strongly suggest that the discrepancies observed in IFN-γ effects on Th1 development can be largely attributed to mouse strain differences.

IL-12. IL-12 is a soluble factor that potently induces Th1 differentiation (Manetti et al., 1993; Seder et al., 1993; Hsieh et al., 1993a, b; Manetti et al., 1994). IL-12 comprises two subunits, p35 and p40. Note that p40 is shared by another cytokine, IL-23, that is important for Th17 differentiation, which will be discussed in the later text. IL-12 is generated by innate cells following activation and IFN-γ stimulation (Kato et al., 1996; Koch et al., 1996; Ma et al., 1996). IL-12 binds to its receptor to activate Jak2/Tyk2-STAT1, 3, 4 signaling pathways (Gately et al., 1998).

CD4 T cells only upregulate IL-12R upon TCR stimulation (Presky et al., 1996). Functional IL-12R is maintained on Th1 cells, but diminishes on Th2 cells (Rogge et al., 1997; Szabo et al., 1997). IL-12 signaling appears to be necessary for the expression of IL-18Rα (Yoshimoto et al., 1998; Sareneva et al., 2000), a receptor for IL-18, which is an IL-1 family cytokine that serves as a cofactor for IL-12 in promoting IFN-γ production in CD4 T cells and Th1 differentiation (Robinson et al., 1997). Because Th1 cells maintain IL-12 and IL-18 receptor expression even in the resting state, IL-12 and IL-18 stimulation induces large amounts of IFN-γ by differentiated Th1 cells in the absence of TCR stimulation (Micallef et al., 1996; Kohno et al., 1997). Mice deficient in IL-12, IL-12R or STAT4 have profoundly diminished Th1 responses *in vitro* and *in vivo* (Thierfelder et al., 1996; Magram et al., 1996a; Kaplan et al., 1996b; Cooper et al., 1997; Wu et al., 1997; Wu et al., 2000). Humans with defective IL-12R signaling display compromised immune responses, especially to microbial infections (de Jong et al., 1998; Altare et al., 1998a, b; Gollob et al., 2000). As important as IL-12 signaling is for Th1 program, IL-12 is not the sole factor that controls Th1 responses (Thierfelder et al., 1996; Magram et al., 1996a; Kaplan et al., 1996b; Cooper et al., 1997; Wu et al., 1997; Wu et al., 2000). IFN-γ-producing T cells could still be detected in mice deficient for IL-12 signaling (Magram et al., 1996b; Kaplan et al., 1998; Schijns et al., 1998; Brombacher et al., 1999; Oxenius et al., 1999; Mullen et al., 2001; Jankovic et al., 2002). In addition, lymphocytic choriomeningitis virus, vesicular stomatitis virus, mouse hepatitis virus, *Toxoplasma gondii* and *M. avium* all elicited Th1 responses that appeared to be largely independent of IL-12 (Schijns et al., 1998; Oxenius et al., 1999; Jankovic et al., 2002). However, the long-term resistance to *T. gondii* and *L. major* were found to be dependent on IL-12 (Park et al., 2000; Stobie et al., 2000; Yap et al., 2000). Collectively, these findings suggest that IL-12 may not be required to initiate the Th1 program but rather be more important for sustaining optimal IFN-γ production following T cell activation.

IL-27. IL-27 is a novel cytokine that shares several characteristics with IL-12 and IL-18 (Pflanz et al., 2002). Like IL-12, IL-27 induces Th1 differentiation from mouse and human naïve CD4 T cells; like IL-18, IL-27 synergizes with IL-12 to potently induce IFN-γ production (Pflanz et al., 2002). IL-27 is composed of EBI3 (Epstein-Barr virus-induced gene 3), a p40 homolog, and a novel subunit, p28. IL-27 binds to type I cytokine receptor family named WSX-1 (Sprecher et al., 1998) or T cell cytokine receptor (TCCR) (Chen et al., 2000) to activate STAT1 and promote expression of IL-12Rβ2 and IFN-γ (Hibbert et al., 2003; Takeda et al., 2003). IL-27R is highly expressed in T cells (Sprecher et al., 1998) but downregulated following activation (Chen et al., 2000). WSX-1- and TCCR-deficient mice exhibited impaired Th1 responses (Chen et al., 2000; Yoshida et al., 2001). Defective IFN-γ production in T cells from WSX-1-deficient mice was limited to the primary response but not secondary responses (Yoshida et al., 2001). In addition, both WSX-1- and TCCR-deficient mice demonstrated markedly increased susceptibility to infection with the intracellular pathogens *L. monocytogenes* (Chen et al., 2000) and *L. major* (Yoshida et al., 2001). Therefore, IL-27R may play an important role in early Th1 differentiation processes prior to IL-12R complex expression. The signal transduction pathway utilized by IL-27 warrants further study, which may lead to the identification of novel factors important for Th1 differentiation.

For Th2 and Th9 differentiation

IL-4 was initially identified and has remained as the most dominant, if not the only, cytokine known to have the greatest influence in driving Th2 differentiation (Le Gros et al., 1990; Swain et al., 1990). IL-4 binding to its receptor IL-4Rα triggers STAT6

activation and Th2 differentiation. The ability of primed $CD4^+$ T cells to produce IL-4 upon re-stimulation was directly correlated with the concentration of exogenous IL-4 added to the primary culture. In addition, neutralizing antibody-mediated IL-4 depletion abrogates the generation of Th2 cells, suggesting that the presence of some IL-4, even if endogenously derived, is essential for Th2 differentiation. Moreover, with diminished Th2 cytokine production, IL-4 deficient mice are defective in Th2-mediated pathogen clearance and develop less Th2-type autoimmune diseases, underscoring the critical role for IL-4 in programming Th2 differentiation (Kuhn et al., 1991; Grunewald et al., 1998; Urban et al., 1998; Kurup et al., 1999). IL-4 in combination with TGF-β has been shown to drive the differentiation of a newly identified Th9 cell lineage, revealing the diverse effect of this cytokine involving in multiple lineage determination (Dardalhon et al., 2008; Veldhoen et al., 2008).

For Th17 differentiation

TGF-β and IL-6. TGF-β and IL-6 act cooperatively and non-redundantly to promote Th17 commitment (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006a). When activated in the presence of LPS-stimulated antigen presenting cells and $CD4^+CD25^+$ nTreg, naïve T cells expressed high levels of IL-17A but diminished levels of Th1 or Th2 cytokines. TGF-β, a cytokine that will be discussed in the later text, is required for IL-17 production under such experimental settings (Veldhoen et al., 2006a). Importantly, in addition to TGF-β, differentiation of IL-17-producing effector cells required a soluble DC factor elicited by TLR- and MyD88-dependent signaling, which proved to be IL-6. Later study found that TGF-β together with IL-6, which alone induces IL-22 (Zheng et al., 2007), promoted the generation of Th7 cells and showed further that addition of IL-6 suppressed the TGF-β-induced generation of $Foxp3^+$ Treg while reciprocally promoting the generation of Th17 cells (Bettelli et al., 2006; Mangan et al., 2006). *In vivo*, mice deficient for TGF-β1 were essentially devoid of Th17 cells (Mangan et al., 2006). Similarly, blockage of TGF-β signaling through the over-expression of a dominant negative form of TGF-β receptor in T cells blocked EAE development and Th17 differentiation (Veldhoen et al., 2006b). Autocrine TGF-β appears to be the predominant mechanism for Th17 differentiation because mice with T cell-specific deletion of TGF-β1 were protected from myelin oligodendrocyte glycoprotein-induced EAE and failed to generate Th17 cells in the central nervous system (CNS) following disease induction (Li et al., 2007). In addition, mice transgenic for TGF-β under the control of the IL-2 promoter had enhanced Th17 differentiation and exacerbated EAE, whereas IL-6 deficiency inhibited such phenomenon (Bettelli et al., 2006), suggesting a critical role for IL-6 in Th17 differentiation *in vivo*. Considering the importance of the newly appreciated Th17 cells in pathogen clearance and autoimmunity, further study is warranted to investigate the mechanisms utilized by TGF-β and IL-6 in instructing Th17 differentiation.

IL-23 and IL-21. IL-23 and IL-21 are cytokines produced by innate and T cells respectively and are shown to be important for Th17 differentiation. The discovery of IL-23 solved a scientific dilemma that bewildered researchers for many years: the IL-12-dependent Th1 cells were thought to be essential for the induction of autoimmunity, such as EAE and collagen-induced arthritis (CIA), based on the studies performed using p40-deficient mice or antibody to neutralize p40. However, although p40 is essential for the development of CNS inflammation during EAE, mice that are deficient in IFN-γ signaling (IFN-, IFNR-, and STAT1-deficient) developed more severe pathology (Langrish et al., 2004). Similarly, during CIA, treatment with p40-specific antibodies prevented disease, but the absence of IFN-γ signaling pathway resulted in increased arthritic disease (Manoury-Schwartz et al., 1997; Vermeire et al., 1997; Chu et al., 2003). Now we know that p40 not only binds to p35 to form IL-12 but also binds to p19 to form IL-23 (Oppmann et al., 2000) and IL-23 accounts for this disparity through regulating Th17 differentiation. Indeed, IL-23-driven IL-17-producing cells are shown to be highly potent at inducing CNS immune pathology (Langrish et al., 2004), and IL-23-deficient mice are resistant to the development of CIA (Murphy et al., 2003). Unlike TGF-β and IL-6, which are critical for the induction of Th17 cells, IL-23 is currently thought to be critical for promoting the survival and proliferation of differentiated Th17 cells.

IL-23 binds to the IL-23R complex composed of IL-12Rβ1 chain and a novel receptor chain (IL-23R) related to IL-12Rβ2 and gp130. Jak2/Tyk2-STAT1, 3, 4, and 5 are involved in its intracellular signal transduction (Parham et al., 2002). In contrast to IL-12, IL-23 predominantly activates STAT3, but not STAT4.

IL-21 is an IL-2 family member that was recently found to be highly produced by Th17 cells. IL-21 can substitute for IL-6 to induce Th17 cells along with TGF-β (Korn et al., 2007; Nurieva et al., 2007; Zhou et al., 2007). IL-21-activated signaling pathways critical for Th17 differentiation and its biological function under physiological and pathological conditions remain to be established.

For Tfh differentiation

IL-21 has recently been found to be critical for Tfh differentiation. IL-21 promotes CXCR5 expression in Tfh cells through IL-21R that is highly expressed in these cells (Vogelzang et al., 2008). Mice deficient in IL-21 or IL-21R have a severe defect in numbers of Tfh cells (Nurieva et al., 2008; Vogelzang et al., 2008). Being highly produced by Tfh cells, IL-21 thus appears to promote and stabilize Tfh differentiation through an autocrine, feed-forward mechanism. Whether there are other cytokines besides IL-21 that are able to promote and maintain Tfh cells warrants further study in the future.

For Treg development and differentiation

IL-2. IL-2 is a cytokine produced mainly by activated T lymphocytes (Thornton and Shevach, 1998). The IL-2 receptor is composed of three subunits: α (CD25), β, and common γ (γ_c) chains. The γ_c chain of IL-2R is shared by the receptors for IL-4, -7, -9, -15 and -21. In T cells, binding of IL-2 leads to activation of the JAK3-STAT5 pathway (Friedmann et al., 1996; Lin and Leonard, 1997). Early studies suggest that IL-2 is specifically required for the development and maintenance of nTregs. In mice, antibody-

mediated depletion of IL-2 or genetic disruption of IL-2 signaling invariantly leads to autoimmune diseases associated with reduced numbers of peripheral Foxp3-expressing nTregs (Schorle et al., 1991; Sadlack et al., 1993; Suzuki et al., 1995; Snow et al., 2003; Setoguchi et al., 2005). However, IL-2 signaling is not absolutely required for the development of nTregs. A recent study demonstrated that substantial amounts of Foxp3$^+$ nTreg cells, albeit reduced in number, were recovered from the thymus and peripheral lymphoid organs when the IL-2 or CD25 gene was lacking (Fontenot et al., 2005). IL-2 has also been shown to activate and be required for optimal nTreg function (de la Rosa et al., 2004; Thornton et al., 2004a, b), likely through facilitating their proliferation and survival. It is therefore coming to a consensus that IL-2 safeguards the 'metabolic fitness' of nTreg to maintain their survival and homeostasis (Fontenot et al., 2005).

Thymic stromal lymphopoietin. Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine that binds to IL-7Rα. TSLP was shown to induce the generation of thymic nTreg specifically via DC *in vitro* (Watanabe et al., 2005). TSLP-expressing Hassall's corpuscles interact with and activate lysosomal membrane glycoprotein$^+$ DCs, which display upregulated CD80 and CD86 but not proinflammatory cytokines. More importantly, TSLP-activated DC potently induced CD4$^+$ thymic nTreg in a TCR- and co-stimulation-dependent manner. This study demonstrates that interactions among epithelial cells, DC, and T cells bridged by cytokines are important for the generation of thymic nTreg (Watanabe et al., 2005). It nevertheless remains to be addressed whether such *in vitro* systems recapitulate what happens *in vivo*.

TGF-β. TGF-β is a family of pleiotropic cytokines consisting of TGF-βs, bone morphogenetic proteins, activins, and growth differentiation factors (Chang et al., 2002). TGF-β1 is the isoform predominantly expressed in the immune system (Li et al., 2006). TGF-β1 binds to a hetero-dimeric receptor that initiates intracellular signaling through Smad and mitogen-activated protein kinase (MAPK) (Massague, 1998). Early studies revealed that TGF-β1 promotes the *de novo* generation of iTreg accompanied by increased Foxp3 expression following TCR stimulation in human and mouse cells (Chen et al., 2003; Fantini et al., 2004; Wan and Flavell, 2005). These studies prompted later investigation on TGF-β function in nTreg development and revealed that TGF-β is required for both the development and the peripheral maintenance of nTreg cells in mice (Marie et al., 2005; Liu et al., 2008). TGF-β1 is obligatory for the generation of both Treg and Th17 cells, with additional cytokines such as IL-6 or IL-21 for the latter. In addition, it is also involved in the differentiation of newly identified Th9 cells (Dardalhon et al., 2008; Veldhoen et al., 2008). The mechanism through which TGF-β achieves such diverse functions needs to be addressed in the future.

IL-10. IL-10 is a cytokine first described as cytokine synthesis inhibitory factor (Fiorentino et al., 1989). The binding of IL-10 to its receptor activates JAK1/Tyk2-STAT1α and 3 in target T cells. IL-10 is a cytokine highly expressed in Tr1 cells and is critical for the function of this Treg subset. IL-10 promotes the generation of Tr1 cells *in vitro*. When human or mouse CD4$^+$ T cells are chronically activated in the presence of IL-10, they differentiate into Tr1 cells (Groux et al., 1997; Bacchetta et al., 2002). However, whether IL-10 signaling is crucial for the generation or function of Tr1 cell *in vivo* remains to be tested.

MAP kinase pathways

Following TCR and cytokine receptor activation, the MAPK signaling cascade is engaged. Three key components of MAPK pathways; ERK, JNK and p38 are constitutively expressed in T cells. Their activation relies on being phosphorylated by upstream kinases. JNK and p38 MAPK are both reportedly involved in Th1 and Th2 differentiation. JNK1-deficient CD4 T cells preferentially expressed IL-4 without a defect in IFN-γ production (Constant et al., 2000; Dong et al., 2000). JNK2, however, is selectively activated in Th1 cells following TCR stimulation and required for IFN-γ production (Yang et al., 1998). p38 MAPK was shown to preferentially participate in IFN-γ production, because inhibition of p38 by a pharmacological drug SB203580 or a dominant negative form of p38 resulted in diminished IFN-γ expression with no effect on IL-4 secretion. In addition, a constitutively active form of MKK6, an MAPK that activates p38 MAPK, augmented IFN-γ production (Rincon et al., 1998). GADD45γ, a factor able to activate p38 and JNK independent of MAPK, is rapidly and preferentially induced in Th1 cells (Lu et al., 2001). CD4 T cells lacking GADD45γ displayed reduced IFN-γ and Th1 responses, correlating with diminished p38 MAPK and JNK activity (Lu et al., 2001). MAPKs play an important role of relaying upstream cell surface signal to the downstream nuclear factors. Because of the vast numbers of family members and extensive crosstalk among them, pinpointing the specific function of each MAPK in Th differentiation is difficult. Nevertheless, the importance of MAPK in these biological processes has been demonstrated and further investigation is warranted. Because many pharmacological drugs targeting MAPKs have been and are being developed, information gained from studying MAPK in T cell differentiation may readily be translated into clinical benefits of treating immune diseases in humans.

Transcription factors

Cytokine expression profile defines and determines effector T cell lineages. As always seen in developmental biology where a specific lineage correlates with the activity of one or more transcription factors, studies were set forth to investigate whether different Th lineages are controlled by specific transcription factors. Great progress has been made in the endeavor; the transcription factors controlling Th1, Th2, Th17 and Treg differentiation have been identified.

For Th1 differentiation

T-bet

T-bet, also known as Tbx21 (Szabo et al., 2000), belongs to the T-box family of transcription factors and is the only known T-box gene specifically expressed in the lymphoid system. T-bet is

rapidly and specifically induced in developing Th1 but not Th2 cells, and its expression appears to be controlled by both TCR and IFN-γR-STAT1 signals (Lighvani et al., 2001; Afkarian et al., 2002), but not by the IL-12-STAT4 pathway (Lighvani et al., 2001; Mullen et al., 2001; Afkarian et al., 2002). T-bet expression seems to be self-regulated; retrovirally expressed T-bet induced T-bet in Th2 cells (Mullen et al., 2002), which is dependent on STAT1 (Afkarian et al., 2002), likely through IFN-γ signaling. Indeed, IFN-γR-STAT1 signaling maintains high-level of T-bet expression in developing Th1 cells (Lighvani et al., 2001; Afkarian et al., 2002). Thus, in a feed-forward manner, IFN-γ promotes Th1 commitment through T-bet (Mullen et al., 2001; Afkarian et al., 2002).

Gain- and loss-of-function studies of T-bet highlight T-bet's role in Th1 development. *In vitro*, retrovirus-mediated over-expression of T-bet in differentiating or fully differentiated Th2 cells resulted in an induction of IFN-γ expression, accompanied by a reduction of IL-5 and, to a lesser extent, of IL-4 (Szabo et al., 2000). Such an effect of T-bet is cell-intrinsic because IL-4 and IL-5 expression were unchanged in control Th2 cells that co-existed in the same culture. Under similar experimental settings, the strength of TCR signaling appeared to be important in quantitatively affecting T-bet expression levels and thus Th1 differentiation (Afkarian et al., 2002). In addition, T-bet-deficient CD4 T cells failed to produce IFN-γ in response to TCR stimulation, even under Th1-polarizing conditions. These mice failed to mount a Th1 response to either protein antigen immunization or to *L. major* infection; instead an increase of Th2 cytokines was observed (Szabo et al., 2002) contributing to the spontaneous asthmatic condition found in these mice (Finotto et al., 2002). Therefore, T-bet is critically involved in initiating Th1 development through both inducing the Th1 program and repressing the opposing Th2 program.

Hlx

Hlx is a Th1-specific homeobox gene that interacts specifically with T-bet. Its expression becomes detectable 3 days after Th1 differentiation and is restricted to Th1 but not Th2 T cell clones (Mullen et al., 2002). Hlx appears to be a target gene for T-bet because retrovirus-mediated expression of T-bet and a dominant negative form of T-bet induced and inhibited Hlx expression, respectively. Hlx and T-bet synergistically promote IFN-γ expression when co-expressed (Mullen et al., 2002). Thus, as a cofactor for T-bet, Hlx appears to enhance the activities of T-bet and thus Th1 differentiation. Further study is needed to investigate whether Hlx promotes or is required for optimal Th1 responses under physiological conditions.

STAT4

STAT4 is critical for IL-12 signaling and thus the full commitment of Th1 cells (Ouyang et al., 1999; Yang et al., 1999). However, it is dispensable for initial IFN-γ expression (Magram et al., 1996b; Kaplan et al., 1998; Schijns et al., 1998; Brombacher et al., 1999; Oxenius et al., 1999; Mullen et al., 2001; Jankovic et al., 2002). The mechanism of STAT4-promoted IFN-γ expression is largely unknown. STAT4 may act through binding to regulatory genomic regions of the IFN-γ gene (Xu et al., 1996) or through interacting with general transcriptional co-activators p300/CBP (Bhattacharya et al., 1996; Zhang et al., 1996; Korzus et al., 1998). Thus, with need-to-be-defined mechanisms, STAT4 is critical for amplifying IFN-γ production, but not the initial IFN-γ expression.

For Th2 differentiation

GATA-3

GATA-3 is a member of the GATA family of transcription factors. While it was previously shown to be critical for embryo development, GATA-3 dictates Th2 differentiation by regulating Th2 cytokine production (Zheng and Flavell, 1997) through binding to the IL-4 locus that encompasses the IL-4, IL-5 and IL-13 genes. Forced expression of GATA-3 in Th1 cells led to the induction of IL-4 production (Zheng and Flavell, 1997). Th2 differentiation and responses are virtually abolished *in vitro* and *in vivo* when GATA-3 is deficient in T cells (Zheng and Flavell, 1997). In addition, even in fully differentiated Th2 cells, abrogation of GATA-3 blocked IL-5 and IL-13 production (Zhu et al., 2004), although IL-4 production was only modestly affected, likely due to the fact that GATA-3-binding sites were found in the promoters of IL-5 and IL-13 but not in IL-4.

STAT6

STAT6, activated by IL-4 stimulation, is the major signal transducer in IL-4-mediated Th2 differentiation *in vitro* (Shimoda et al., 1996; Takeda et al., 1996; Kaplan et al., 1996a). However, *in vivo*, Th2 responses can be obtained independently of STAT6 (Finkelman et al., 2000; Jankovic et al., 2000; Min et al., 2004). *In vitro*, one of the mechanisms of STAT6 is to promote Th2 differentiation through inducing high levels of the transcription factor GATA-3. STAT6 activities were found necessary and sufficient for inducing GATA-3 (Kurata et al., 1999; Zhu et al., 2001).

c-Maf, IRF-4 and Gfi-1

c-Maf is an AP-1 family transcription factor selectively up-regulated in Th2 cells. It is required for the production of IL-4 but not other Th2 cytokines (Kim et al., 1999). IRF-4 is also shown to be required for Th2 cell differentiation (Lohoff et al., 2002; Rengarajan et al., 2002). IRF-4-deficient cells produce much less IL-4, but this defect can be rescued by over-expression of GATA-3, suggesting that IRF-4 promotes Th2 differentiation through up-regulating GATA-3 (Lohoff et al., 2002). Gfi-1 is a transcription factor immediately induced following IL-4-stimulation (Zhu et al., 2004) and TCR stimulation. Gfi-1 appears to function by selecting GATA-3^{hi} cells for growth by modulating both upstream and downstream events of IL-2 signaling (Zhu et al., 2004, 2006).

For Th17 differentiation

Retinoic acid-related orphan receptors (ROR) are the key transcription factors in Th17 differentiation. ROR-γt mRNA is upregulated in T cells in response to IL-23 and its expression highly correlates with IL-17 expression (Ivanov et al., 2006). Forced expression of ROR-γt in naïve CD4^{+} T cells induced Th17. IL-17 expression was greatly reduced if the CD4 T cells were deficient

in ROR-γt. *In vivo*, ROR-γt deficiency led to drastically reduced Th17 cells, although residual IL-17-producing cells were found. Therefore, ROR-γt appears to be necessary for Th17 differentiation. The target genes directly regulated by ROR-γt have not yet been identified, although IL-17 is a good candidate because conserved ROR responsive elements were found in its promoter (Jetten et al., 2001). ROR-γt deficiency did not result in total abrogation of Th17 differentiation. This could be owing to partial compensation from another ROR family member, ROR-α. ROR-α is highly expressed in Th17 cells and can be induced by TGF-β and IL-6. Through ectopic expression, ROR-α promoted Th17 differentiation by itself or synergistically with ROR-γt. ROR-α deficiency led to Th17 reduction (Yang et al., 2008). Therefore, ROR-α and ROR-γt are both critical and somewhat redundant in promoting Th17 differentiation. Further studies are needed to investigate the mechanisms through which ROR controls Th17 differentiation and the roles of other factors, besides ROR, in regulating Th17 differentiation.

For Treg differentiation

Foxp3 is an X-linked transcription factor belonging to the Fork-head protein family. It is specifically highly expressed in nTreg cells and can be induced by TGF-β following antigenic stimulation in iTreg cells. Since its discovery in 2002, Foxp3 is now recognized as the master regulator for functional Treg. Over-expression of Foxp3 in conventional T cells converts them to a Treg phenotype endowed with anergy and suppressive activity (Fontenot et al., 2003). Mutation of Foxp3 in human and mice invariably results in systemic lymphoproliferative autoimmune syndrome in Immunodeficiency, Polyendocrinopathy, and Enteropathy, X-Linked Syndrome patients and Scurfy mice because of the lack of functional Treg (Chen et al., 2003). Continuous expression of Foxp3 is critical for maintaining the suppressive activity of Treg cells (Williams and Rudensky, 2007), and reduced Foxp3 expression led to abrogated suppressive function and a conversion of Treg cells into Th2 like cells (Wan and Flavell, 2007). Substantial efforts have been invested to elucidate how Foxp3 functions in Treg. It appears that Foxp3 is able to affect hundreds of target genes but the ones specific for Treg function have been elusive. In addition, Foxp3-expressing cells were shown to be generated even in the absence of Foxp3 protein (Gavin et al., 2007). Therefore, Foxp3 is not absolutely required for the development of Treg but rather is critical for the establishment and stabilization of the suppressive function of these cells (Gavin et al., 2007). Obviously, much work is needed to address how Foxp3 functions and is regulated in T cells.

Mutual-exclusion, plasticity and interconvertibility of effector T cells: when the boundary blurs

While we appreciate the seemingly clear delineation of different classes of effector T cells, we start to realize that nature works its wonders in an efficient and yet not-so-simple way—different Th cells not only functionally interact but also possess plasticity and inter-convertibility (Figure 1).

Mutual exclusion

Th1 and Th2 programs were found to be mutually exclusive since the day they were discovered. Immediately following TCR activation, IFN-γ and IL-4 are both upregulated in a cytokine-independent manner. Within hours of activation, the cytokine-dependent phase of Th development ensues. At this stage, the induction and maintenance of high-levels of T-bet or GATA-3 expression appears necessary for Th1 or Th2 commitment, respectively (Grogan et al., 2001). T-bet expression is promoted by the IFN-γR-STAT1 pathway (Lighvani et al., 2001; Afkarian et al., 2002) and by itself (Mullen et al., 2002). T-bet may potentiate IFN-γ production by stably remodeling the chromatin surrounding the IFN-γ gene to achieve permanent lineage commitment. While IFN-γ production is maintained, IL-4 expression is extinguished in developing Th1 cells. The IFN-γ and IL-12 signaling pathways have been implicated in the suppression of GATA-3 expression (Ouyang et al., 2000) and in the silencing of the Th2 loci (Manetti et al., 1993; Seder et al., 1993; Hsieh et al., 1993b; Mountford et al., 1999). Developing Th1 cells are further sustained through T-bet-induced expression of IL-12Rβ2 (Mullen et al., 2001; Afkarian et al., 2002), facilitating their response to the Th1-driving cytokine IL-12. Thus, early T-bet expression appears to be a master switch to turn on the Th1 program while turning off the Th2 program. In a similar fashion, the Th2 lineage commitment factor GATA-3 switches on the Th2 program but turns off the Th1 program (Zheng and Flavell, 1997). GATA-3 is predominantly induced by the IL-4-STAT6 signaling pathway (Ouyang et al., 1998; Kurata et al., 1999) to both increase IL-4 production and suppress IFN-γ and IL-12Rβ2 expression (Ouyang et al., 1998; Ferber et al., 1999), potentially through remodeling chromatin to make Th2 loci more accessible (Lee et al., 2000; Ouyang et al., 2000). Thus, T-bet or GATA-3 decides a common precursor to follow the Th1 or Th2 path. After a decision is made, the differentiation program of the other lineage will be shut off. The exact mechanism of how T-bet and GATA3 reciprocally regulate the expression of Th1 and Th2 cytokines at the epigenetic level is an important question. Interestingly, it has been found that the loci encoding IFN-γ and IL-4 are physically close during Th1–Th2 differentiation and become separated after full differentiation (Spilianakis et al., 2005). Thus, gene loci on different chromosomes can juxtapose, allowing a common regulatory complex to be used for Th1–Th2 differentiation.

Soon after the discovery of Th17 cell, the studies of its relationship with other known Th cells ensued. Based mostly on the studies *in vitro*, Th17 and iTreg were found to be mutually exclusive (Korn et al., 2007). Following activation in the presence of TGF-β, CD4 T cells differentiate into Foxp3-expressing iTreg without IL-17 expression. However, if IL-6 is also present, activated CD4 T cells deviate to Th-17 cells without Foxp3 expression. The mechanism of such a mutual exclusion could be owing to Foxp3 expression in iTreg and ROR-γt expression in Th17 cells. It appears that high-level Foxp3 expression leads to the

repression of ROR-γt and thus Th17 differentiation, and ROR-γt expression inhibits Foxp3 and prompts IL-17 production (Zhou et al., 2008). What is worth mentioning is that iTreg seem to be in a particular situation in that the high level of Foxp3 expression induced by TGF-β results in the shut-down of all known lineage development. It thus appears that Foxp3 function supersedes all transcription factors for T cell differentiation. With the increasing interest in Th17 and Treg, more efforts will be invested in addressing how these two cell fates are determined in relation with other known effector T cells.

Plasticity and inter-convertibility

We have become accustomed to the idea that different Th cells are 'committed' to their path. Certainly, most *in vitro* differentiation models suggest this is the case and such 'commitments' provide simplified experimental models to allow us understand Th cell functions and gene regulation. T cells in our body often have to mount different responses towards different insults on a short notice. The time required to let recently activated Th cells die, to replenish the apoptotic T cells and then to launch another effective immune response might be too long to accommodate such a task. One may question: are Th cells really fully committed? And is it possible that Th cells possess certain plasticity so that they can convert into other types of effectors under the right conditions? Thus, the body can fight insults more quickly and more energy-efficiently.

Recently, emerging evidence suggests that under certain conditions, seemingly committed T cells indeed possess plasticity and may convert into other types of effector cells (Figure 1). Long-lived Th1 effector/memory cells are able to turn off IFN-γ expression *in vivo*, appearing to be ready to 're-differentiate' (Harrington et al., 2008). nTreg may downregulate Foxp3 expression and generate Th2 cytokines during autoimmune syndrome and lung allergic responses (Wan and Flavell, 2007; Joetham et al., 2008). In addition, when the Treg lineage CD4 T cells lose Foxp3 expression, they generate IL-4, IFN-γ as well as IL-17 (Gavin et al., 2007). Moreover, nTreg can become Th17 cells in the presence of activated inflammatory DC, likely through the action of IL-6 (Radhakrishnan et al., 2008). Seemingly fully differentiated Th17 cells were also shown to convert into Th1 cells in the absence of TGF-β (Lee et al., 2009). How Th cells achieve such plasticity is a question not fully addressed. One contributing mechanism could be that the permissive epigenetic states of the entire set of genes associated with different lineages in a particular Th subset allow its alternative paths (Wei et al., 2009). Under physiological conditions, how such plasticity is achieved and what decides the inter-conversion amongst different Th cells are important questions that need further investigation.

Closing remarks

We appreciate the functional importance of Th cells in fighting pathogens, inducing inflammation and autoimmunity as well as maintaining self-tolerance and immune homeostasis. Th cells accomplish such tasks by deploying specialized lineages—Th1, Th2, Th17, Tfh, Th9 and Treg, with certain overlapping functions. Current knowledge gained on the function and regulation of Th cells derives mostly from a reductionist approach using well-controlled, simplified *in vitro* differentiation systems. With the development of reporter mice that allow us to track different Th subsets *in vivo*, in the future we will be able to address how Th cells function and are induced under physiological settings. Recent discovery of several new Th lineages on one hand further energizes the research on Th cells; on the other hand, it raises the question of how many 'Th lineages' truly exist. More importantly, as we start to realize that Th cells are plastic and can be converted into other Th cells, especially under physiological conditions, it becomes possible that the traditionally defined 'Th lineage' might not be terminally differentiated but rather transiently specialized effector T cells that are ready to perform different tasks to efficiently counter various immune insults. In the future, study will be needed to elucidate the mechanisms through which T cells acquire and re-acquire particular cytokine profiles to regulate immunity in an ever-changing microenvironment. Nevertheless, one consistent theme in Th biology is that the cytokine milieu dictates what specialized effector T cells are induced. Much more work is needed in the years to come to reveal what effects different cytokines and their combinations have on Th function and through what mechanisms.

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