CD4 T cells: fates, functions, and faults

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In 1986, Mosmann and Coffman identified 2 subsets of activated CD4 T cells, Th1 and Th2 cells, which differed from each other in their pattern of cytokine production and their functions. Our understanding of the importance of the distinct differentiated forms of CD4 T cells and of the mechanisms through which they achieve their differentiated state has greatly expanded over the past 2 decades. Today at least 4 distinct CD4 T-cell subsets have been shown to exist, Th1, Th2, Th17, and iTreg cells. Here we summarize much of what is known about the 4 subsets, including the history of their discovery, their unique cytokine products and related functions, their distinctive expression of cell surface receptors and their characteristic transcription factors, the regulation of their fate determination, and the consequences of their abnormal activation. (Blood. 2008;112:1557-1569)

Introduction

CD4 T cells play a central role in immune protection. They do so through their capacity to help B cells make antibodies, to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation, and, through their production of cytokines and chemokines, to orchestrate the full panoply of immune responses. Beginning with the groundbreaking work of Mossman and Coffman in 1986[1] showing that long-term CD4 T-cell lines could be subdivided into 2 groups, those that made IFNγ as their signature cytokine and those that produced IL-4, it has been realized that CD4 T cells are not a unitary set of cells but represent a series of distinct cell populations with different functions.

While some of these CD4 T-cell populations are actually distinct lineages of cells already distinguished from one another when they emerge from the thymus, such as "natural" regulatory T (nTreg) cells[2,3] and natural killer T cells (NKT cells),[4] several represent alternative patterns of differentiation of naive CD4 T cells. It is to the description of these cells, their functions, their patterns of differentiation, the sets of genes they express, and the consequences of abnormalities in them that this review is devoted.

Naive conventional CD4 T cells have open to them 4 (and possibly more) distinct fates that are determined by the pattern of signals they receive during their initial interaction with antigen. These 4 populations are Th1, Th2, Th17, and induced regulatory T (iTreg) cells. Mossman and Coffman recognized the Th1 and Th2 phenotypes among the set of long-term T-cell lines that they studied and the early history of this field was devoted to understanding these 2 cell populations, with Th1 cells being regarded as critical for immunity to intracellular microorganisms and Th2 cells for immunity to many extracellular pathogens, including helminths.[5,6]

Abnormal activation of Th1 cells was seen as the critical event in most organ-specific autoimmune diseases while Th2 cells were responsible for allergic inflammatory diseases and asthma. Th17 cells have been recognized much more recently but there is now a growing body of work indicating not only that these cells exist but that they play a critical function in protection against microbial challenges, particularly extracellular bacteria and fungi.[7] Further, some of the autoimmune responses formally attributed to Th1 cells, such as experimental autoimmune encephalomyelitis (EAE), collagen induced arthritis (CIA), and some forms of inflammatory bowel disease (IBD), have now been shown to be mediated, at least in part, by Th17 cells. iTreg cells are also now well established as an inducible cell population that phenotypically resembles nTreg cells, although distinguishing the function of iTreg cells from that of nTreg cells and, particularly, the relative importance of the 2 Treg populations in humans and experimental animals has been difficult. In this review, we will deal with the function of Treg cells as a group except where we explicitly speak of iTreg cells. There are also other regulatory CD4 T cells including Th3 and TR1 cells. Th3 cells are transforming growth factor β (TGF-β)–producing cells induced by oral tolerance.[8] Most of them are likely inducible regulatory T cells that express Foxp3.[9] Whether or not there are TGFβ-producing Foxp3⁻ CD4 T cells is unclear. TR1 cells are IL-10 producing cells.[10] Because all the CD4 T-cell sets including Th1, Th2, Th17 as well as Treg cells are capable of producing IL-10 under certain circumstances,[11-13] TR1 cells may not be a distinct lineage but rather may represent a certain state of each existing lineage. Finally, there may well be other sets of conventional CD4 T cells and even among the more conventional sets, important differences exist, such as the detailed pattern of cytokines that they produce.

Figure 1 summarizes much of what we know about the major sets of CD4 T cells, including their unique products, the characteristic transcription factors and cytokines critical for their fate determination and some of their functions. Each of these topics will be discussed in some depth in the subsequent sections of this review.

A little history

Initially, immunologists believed that there were fundamentally 2 types of immune responses that require the action of CD4 T cells. One was antibody-mediated and the other cell-mediated. However, there was very little progress in this area until the early 1980s, when T-cell cloning technology was developed, many cytokines were discovered and cloned, and assays for them became available.
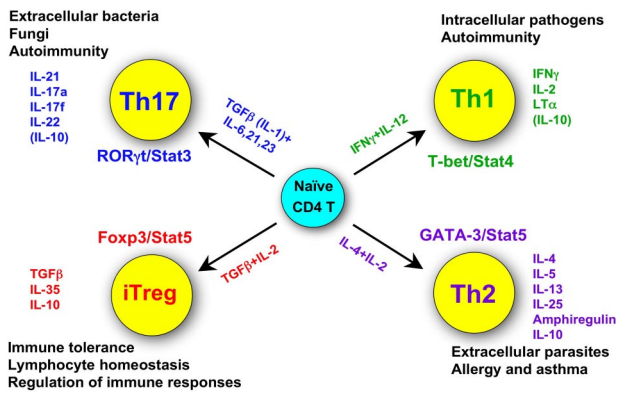
Figure 1. Summary of the 4 CD4 T helper cell fates: their functions, their unique products, their characteristic transcription factors, and cytokines critical for their fate determination.

Tim Mosmman and Bob Coffman recognized that mature CD4 T cells could be subdivided into 2 distinct populations with different sets of products and that this would endow them with unique functions.[1] Kim Bottomly was also working on this subject; she and her colleagues subdivided CD4 T-cell lines based on functional criteria, distinguishing inflammatory and helper CD4 T cells, with the latter being IL-4 producers.[14]

The translation of the differences observed in long-term CD4 T-cell lines to the behavior of normal CD4 T cells, first in vitro and then in vivo, constitutes the beginning of the Th field as a biologic subject. The earliest description of in vitro differentiation was reported in 1990 by our group and that of Susan Swain, demonstrating first that naive CD4 T cells failed to make IL-4 (or most other effector cytokines) and that these cells could be induced to develop into vigorous IL-4 producers if they were stimulated both with T-cell receptor ligands and IL-4, itself.[15,16] Within 2 to 3 days after the initiation of culture, the stimulated cells acquire the capacity to produce IL-4. It was subsequently shown that this in vitro differentiation requires a signaling pathway that includes the IL-4 receptor, the signal transducer and activator of transcription (Stat) 6 and the DNA-binding factor GATA-3.[17,18] As we will discuss later, this is far from the whole story, but "it gets us off to the races." We note in passing that in our original 1990 paper, we found that IL-2 was also necessary for cells to acquire IL-4–producing capacity, although that was largely overlooked and didn't come back for serious analysis for more than a decade.[19]

Three years later, Ken Murphy, Anne O'Garra, and their colleagues showed that naive CD4 T cells could acquire the capacity to produce IFNγ in vitro.[20] They stimulated T-cell receptor transgenic naive CD4 T cells and antigen-presenting cells with cognate antigen and heat-killed *Listeria monocytogenes* organisms; the heat-killed *Listeria* caused cells in the culture to produce IL-12, which was critical for Th1 differentiation in this system.

At first, it appeared that there was a fundamental dichotomy between the logic of differentiation process for Th1 and Th2 cells, with a CD4 T-cell endogenous product, IL-4, playing a major positive feedback role in Th2 differentiation and an exogenous product, IL-12, probably mainly from dendritic cells, playing the major inductive role for Th1 cells. However, with time and attention, the logic of the differentiation processes appears to be much closer than initially appreciated. Neutralizing IFNγ strikingly diminishes Th1 differentiation; IL-12 appears to induce some IFNγ production which then acts to up-regulate the key transcription factor T-bet[21,22] and leads to much more IFNγ production, showing a positive feedback loop for Th1 cells as well.

Immunologists attributed many autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, and their experimental models, to the action of Th1 cells. However, they were puzzled by the paradoxical finding that neutralizing or knocking out IL-12 and IFNγ had different effects on the induction of experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis. IL-12 p40 knockout mice are resistant to EAE induction whereas IFNγ knockout mice are more sensitive. The discovery of IL-23, which consisted of IL-12p40 paired with a distinctive chain (p19), led to a reassessment of the relative contributions of IL-12 and IL-23 in EAE induction.[23] Indeed, it is IL-23, not IL-12, that plays the major role in inducing EAE. Due to the linkage between IL-23 and the expression of IL-17, a new Th lineage, Th17, was soon identified.[24,25] Th17 cells are different from classical Th1/Th2 cells based on the following evidence: Th17 cells do not produce the "classical" Th1/Th2 cytokines; Th17 cells express low levels of T-bet and GATA-3; and the Th1/Th2 signature cytokines, IL-4 and IFNγ, suppress Th17 cell differentiation.[24,25]

In 2006, Stockinger, Weaver, Kuchroo, and their colleagues each showed that Th17 cells could be induced in vitro from naive mouse CD4 T cells by stimulation through their T-cell receptor (TCR) in the presence of IL-6 and TGF-β.[26-28] RORγt was identified as the master regulator gene for Th17 cells.[29] More work has revealed that the role of TGF-β in human cells may not be central to Th17 differentiation but that IL-1 has an important role.[30,31] However, very recently, 3 groups independently reported that TGF-β was also critical for human Th17 cell differentiation.[32-34] The discrepancy between these reports and previous studies may be explained by the potentially different purity of the naive T-cell population each group prepared because a small contamination with effector/memory cells may suppress de novo Th17 cell differentiation. In addition, in the earlier studies, the amount of TGF-β added to the culture and/or present in the serum is much higher than the amount required for Th17 differentiation and high levels of TGF-β inhibit Th17 cell differentiation and favor iTreg differentiation.

IL-21 produced by Th17 cells, induced in the course of Th17 differentiation,[35-37] fulfills the role of the powerful positive feedback stimulant, reinforcing the Th17 induction process and showing that Th17 development has the logic similar to that of Th1 and Th2 cells.

The Treg "revolution" has been one of the defining themes of modern immunology but reaching an understanding of how these cells differentiate has been complex. In 1995, Sakaguchi and his colleagues discovered that regulatory T cells express CD25.[38] Transfer of CD4 T cells that had been depleted of the CD25+ population into congenitally athymic mice induced autoimmune diseases while transfer of intact populations of CD4 T cells did not. In 2001, the autoimmune Scurfy mice and a human immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) patient were found to have mutations in *Foxp3*.[39-41] In 2003, Foxp3 was reported as the master transcriptional regulator for nTreg cells.[42,43]

Weiner and colleagues had reported in 1994 that oral tolerance regimens induced TGF-β–producing CD4 T regulatory cells.[8] This cell population was designated Th3 cells. In 2003, Chen et al reported that TGF-β can convert Foxp3− naive CD4 T cells into Foxp3+ CD4 T cells, that is iTreg cells.[44] It is now clear that activated naive CD4 T cells stimulated by TGF-β in the absence of proinflammatory cytokines develop into iTreg cells. The positive feedback factor here is TGF-β itself, although there is still much

uncertainty as to the relative biologic importance of nTreg and iTreg cells, particularly in humans.

Converting the Th paradigm from in vitro to in vivo situations initially met with much resistance but with time it became clear that memory and memory/effector T cells from normal priming events do display polarization in their cytokine-producing capacity, in their functions and in the range of cell surface molecules they express. Indeed, the recent description of the selective deficit in development of Th17 cells in patients with hyper-IgE syndrome (HIES or Job syndrome) strikingly validates this concept.[45] HIES patients have a genetically determined inability to signal through Stat3, due to dominant negative mutations in the SH2 domain or the DNA-binding domain of this molecule.[45-47] In humans and mice, the 3 major inducers and/or sustainers of Th17 differentiation, IL-6, IL-21 and IL-23, each use Stat3 for signal transduction. Indeed, the principal difficulties HIES patients face, recurrent staphylococcal and fungal infections, are precisely those observed in mice that cannot develop Th17 cells, strikingly validating the importance of the CD4 T-cell differentiation concept and indicating that lessons are learned, although not always perfectly, by studying experimental animals.

Th cells: cytokine produced and functions

Th cells play critical roles in orchestrating the adaptive immune responses. They exert such functions mainly through secreting cytokines and chemokines that activate and/or recruit target cells.

Th1 cells mediate immune responses against intracellular pathogens.[5,6] In humans, they play a particularly important role in resistance to mycobacterial infections. Th1 cells are also responsible for the induction of some autoimmune diseases. Their principal cytokine products are IFNγ, lymphotoxin α (LTα), and IL-2. IFNγ produced by Th1 cells is important in activating macrophages to increase their microbicidal activity.[48] LTα has been implicated as a marker for the disease progression in multiple sclerosis patients.[49] LTα-deficient mice are resistant to EAE.[50] IL-2 production is important for CD4 T-cell memory. IFNγ$^+$IL-2$^+$ cells are regarded as precursors of the Th1 memory cells.[51] IL-2 stimulation of CD8 cells during their priming phase is critical for CD8 memory formation.[52]

Th2 cells mediate host defense against extracellular parasites including helminths.[5,6] They are important in the induction and persistence of asthma and other allergic diseases. Th2 cells produce IL-4, IL-5, IL-9, IL-10, IL-13, IL-25, and amphiregulin. IL-4 is the positive feedback cytokine for Th2 cell differentiation[15,16] and is the major mediator of IgE class switching in B cells.[53] IgE binds to FcεRI on basophils and mast cells and, when interacting with a multivalent ligand, cross-links FcεRI, leading to the secretion of active mediators such as histamine and serotonin and to the production of several cytokines including IL-4, IL-13, and tumor necrosis factor α (TNF-α).

IL-5 plays a critical role in recruiting eosinophils.[54] In addition to its effect on mast cells and lymphocytes, IL-9 induces mucin production in epithelial cells during allergic reactions.[55] IL-10, produced by Th2 cells, suppresses Th1 cell proliferation.[56] IL-10 can also suppress dendritic cell function.[57] IL-13 is the effector cytokine in the expulsion of helminths and in the induction of airway hypersensitivity.[58,59] Amphiregulin is a member of the epidermal growth factor (EGF) family. It induces epithelial cell proliferation. In the absence of amphiregulin, the expulsion of the

nematode *Trichuris muris* is delayed.[60] Amphiregulin may also be important for the induction of airway hypersensitivity.

IL-25 (also known as IL-17E) is also a Th2 cytokine.[61,62] IL-25, signaling through IL-17RB, enhances the production of IL-4, IL-5, and IL-13 by a unique c-kit$^+$FcεRI$^-$ nonlymphocyte population.[63] Interestingly, IL-25 is also produced by lung epithelial cells in response to allergens.[55] Thus, IL-25 serves as an initiation factor as well as an amplification factor for Th2 responses. IL-25 can induce the production of chemokines including RANTES (CCL5) and eotaxin (CCL11) that recruit eosinophils.

Th17 cells mediate immune responses against extracellular bacteria and fungi.[7] They are responsible for, or participate in, the induction of many organ-specific autoimmune diseases. Th17 cells produce IL-17a, IL-17f, IL-21, and IL-22. IL-17a was originally cloned as CTLA-8 and is homologous to a *Herpesvirus saimiri* gene. It was renamed IL-17 when its receptor was cloned.[64] IL-17a and IL-17f are genetically linked and presumably under the control of the same locus control region (LCR). Thus, IL-17a and IL-17f are often coexpressed at the single cell level although there are also IL-17a- and IL-17f-single producing cells, suggesting the regulation of IL-17a and IL-17f expression in Th17 cells mirrors that of IL-4 and IL-13 in Th2 cells (see below). IL-17a and IL-17f both use the IL-17RA chain for their signaling, implying that they have similar functions, although IL-17a binds to IL-17RA with much higher affinity.[65] IL-17a can induce many inflammatory cytokines, IL-6 as well as chemokines such as IL-8 (also known as CXCL8), and thus has an important role in inducing inflammatory responses.[64] Both IL-17a and IL-17f recruit and activate neutrophils during immune responses against extracellular bacteria and fungi. IL-21 made by Th17 cells is a stimulatory factor for Th17 differentiation and serves as the positive feedback amplifier,[35-37] as does IFNγ for Th1 and IL-4 for Th2 cells. IL-21 also acts on CD8 T cells, B cells, natural killer (NK) cells, and dendritic cells.[66] IL-22 is produced by Th17 cells through IL-6– or IL-23–mediated Stat3 activation[67]; TGF-β inhibits IL-22 expression.[13] The aryl hydrocarbon receptor (AHR), a receptor for dioxin, is highly expressed in Th17 cells and plays an important role in the expression of IL-22.[68] IL-22 mediates IL-23–induced acanthosis and dermal inflammation.[67] IL-22 also protects hepatocytes during acute liver inflammation.[69] Strikingly, IL-22 mediates host defense against bacterial pathogens such as *Klebsiella pneumoniae*[70] and *Citrobacter rodentium*.[71] However, these functions may largely depend upon IL-23 stimulation of innate cells to produce IL-22 rather than on the action of Th17 cells.[71]

Treg cells play a critical role in maintaining self-tolerance as well as in regulating immune responses.[2] Increasing Treg numbers and/or enhancing their suppressive function may be beneficial for treating autoimmune diseases and for preventing allograft rejection. Indeed, Treg cells stimulated in vitro with alloantigen prevent both acute and chronic allograft rejection in mice.[72] On the other hand, depletion of Treg cells and/or inhibition of their function could enhance immunity against tumors and chronic infectious agents. Treg cells exert their suppressive functions through several mechanisms, some of which require cell-cell contact.[3] The molecular basis of suppression in some cases is through their production of cytokines, including TGF-β, IL-10, and IL-35. TGF-β produced by Treg cells may also result in the induction of iTreg cells from naive CD4 T cells. Although TGF-β is not absolutely required for suppression in some settings, particularly in vitro, it is very important in mediating suppression in several circumstances in vivo.[73,74] IL-10 production is critical for Treg-mediated prevention and cure of inflammatory bowel disease.[75,76] Specific deletion of

IL-10 in Treg cells by Foxp3-Cre results in the development of spontaneous colitis and enhanced lung inflammation.[77] IL-10 also plays an important role in limiting the severity of EAE at later stages. During *Leishmania* infection, Treg IL-10 production in the lesion maintains a homeostasis between the host and the pathogen, allowing a low level of pathogen persistence and a consequent continued stimulation of protective immunity.[78] IL-35, which consists of EBI3, a chain shared with IL-27, and IL-12 p35, is produced by Treg cells and contributes to suppressive activity.[79]

CD4 T cells other than Th2 and Treg cells can also produce IL-10. IL-10 production by Th1 or Th17 cells may play an important role in limiting their own effector function.[11-13] IL-10, IL-27, and TGFβ can induce IL-10 production.[10,13,80] Interestingly, *Foxp3*-deleted "Treg cells," judged by expression of GFP encoded by a *Foxp3null* locus, produce high levels of IL-10, suggesting that IL-10 production in Treg cells is independent of Foxp3.[81] The originally described TR1 cells (IL-10–producing regulatory T cells) may include many different types of cells that are capable of producing IL-10. Thus, IL-10 production by all CD4 T cells serves as a negative regulatory mechanism for limiting the immune responses to prevent host tissue damage.

Expression of cytokine and chemokine receptors by Th cells

Th1 cells

IL-12Rβ2 expression is induced by TCR activation and then maintained by IL-12 as well as by IFNγ stimulation.[82-84] IL-12Rβ1 is constitutively expressed on naive CD4 T cells and its expression is further increased in Th1 cells through an IRF1-dependent mechanism.[85] Up-regulation of the IL-12R complex conveys IL-12 hyperresponsiveness to activated cells. IL-18Rα is also up-regulated during Th1 differentiation. Although IL-18 is not involved in the differentiation of Th1 cells, it can synergize with IL-12 in inducing IFNγ, implying that IL-18 plays an important role in Th1 responses.[86,87] Although chemokine receptor expression and differentiated Th phenotype are not strictly coordinate, some receptors, such as CXCR3[88,89] and CCR5,[90] show a striking preferential expression on Th1 cells.

Th2 cells

IL-4Rα is up-regulated by IL-4 during Th2 differentiation. However, other γc cytokines may also induce IL-4Rα. CD25 (IL-2Rα) expression is higher in Th2 cells than in Th1 cells, possibly due to the action of c-Maf.[91] Such higher expression of CD25 may confer hyperresponsiveness to IL-2. The most important cell surface marker for Th2 cells is T1/ST2 (IL-33Rα).[92] T1/ST2, also known as IL-1R like 1, belongs to the IL-1R superfamily, which includes IL-1R and IL-18Rα. The function of IL-33Rα on Th2 cells may mirror the function of IL-18Rα on Th1 cells. Among the chemokine receptors, CCR3,[93] CCR4,[88,89] CCR8,[94] and CRTh2[95] tend to be expressed on Th2 cells.

Th17 cells

Th17 cells express high levels of IL-23R.[27,31,37] In addition, Th17 cells express substantial amounts of IL-1R1 and of IL-18Rα. The function of IL-18Rα on Th17 cells is unclear while IL-1R1 appears critical for IL-17 production; mice deficient in IL-1R1 are resistant to EAE, which is correlated with reduced IL-17 production.[96] This is also consistent with a requirement for IL-1 in induction of human

Th17 cells. Surprisingly, there has been little study of the expression of TGFβR on various Th cells. Among the chemokine receptors, human Th17 cells coexpress CCR6 and CCR4.[97]

Treg cells

The majority of the nTreg cells express CD25.[2] Although all activated T cells express CD25, Treg cells express the highest levels of CD25 and do so constitutively, whereas expression by conventional CD4 T cells is transient and lower. The high level of expression of CD25, IL-2Rα, on Treg cells suggests the importance of IL-2 for these cells. Treg cells also express CTLA-4, GITR, and Folr4. However, these markers are only useful for distinguishing Treg cells from naive conventional CD4 T cells because each can be induced by activation of conventional T cells. Treg cells, especially in human, express little or no IL-7Rα. The absence of IL-7Rα in combination with high levels of CD25 provides an approach to identifying Treg cells and separating them from other cells.[98] An interesting subset of Treg cells, those that express CD103,[99] also known as alpha E integrin, is mainly found in the gut or at sites of inflammation. Most iTreg cells induced in vitro express CD103.

Transcription factors critical for each T helper lineage

Transcription factors including members of the nuclear factor of activated T cell (NFAT), NF-κB, and activator protein-1 (AP-1) families are critically involved in cytokine production upon TCR and/or cytokine stimulation. Presumably, those factors are also important during the process of T helper differentiation. However, they are not the factors directly determining T helper lineage fates and are usually expressed in all lineages. Below, we will focus on the transcription factors that either are specifically expressed, or function differently, in each of the lineages.

Transcription factors for Th1 differentiation

T-bet,[21] the Th1 master regulator, is up-regulated during Th1 differentiation. Stat1, the major transducer of IFNγ signaling, plays a critical role in the IFNγ-mediated induction of T-bet.[22] Overexpression of T-bet in Th2 cells induces them to produce IFNγ and inhibits their production of IL-4. T-bet$^{-/-}$ cells have severe defects in Th1 cell differentiation. T-bet$^{-/-}$ mice spontaneously develop asthma-like diseases.[100]

However, T-bet$^{-/-}$ Th1 cells still produce some IFNγ. Eomesodermin (Eomes),[101] another T-box family member critical for IFNγ production in CD8 T cells, is up-regulated during Th1 differentiation, suggesting that it may also be involved in IFNγ production by CD4 T cells. Indeed, IL-21 treatment of Th1 cells partially inhibits IFNγ production, correlating with suppression of Eomes but not T-bet.[102]

Stat4, an IL-12 signal transducer, is important for amplifying Th1 responses.[103,104] In addition, Stat4 can directly induce IFNγ-production in activated CD4 T cells, which can initiate the positive feedback loop in which IFNγ, acting through T-bet, induces more IFNγ. IL-12/Stat4, together with an NF-κB inducer, can cause IFNγ production independent of TCR stimulation. This is best illustrated by the capacity of IL-12 and IL-18, whose receptor is expressed on Th1, but not Th2, cells to induce IFNγ production by Th1 cells in a cyclosporine A–independent matter.[86,87]

Runx3,[105,106] a transcriptional repressor important for silencing CD4 during CD8 T-cell development, is also up-regulated in Th1 cells. Overexpression of Runx3 in Th2 cells induces IFNγ production independent of T-bet (our unpublished data). Runx3-deficient cells produce less IFNγ than wild type Th1 cells.[106]

Hlx, a transcription factor induced by T-bet, interacts with T-bet and enhances T-bet-mediated IFNγ production.[107]

Transcription factors for Th2 differentiation

Stat6, activated by IL-4, is the major signal transducer in IL-4–mediated Th2 differentiation.[108-110] Stat6-deficient cells fail to develop IL-4–producing capacity in vitro; in vivo, Th2 responses independent of Stat6 activation can be obtained.[111-113] In vitro, Stat6 activation is necessary and sufficient for inducing high expression levels of the Th2 master regulator gene, GATA-3.[114,115]

Overexpression of GATA-3 in Th1 cells induces IL-4 production[116] and in the absence of GATA-3, Th2 differentiation is totally abolished in vitro and in vivo.[117,118] Even in fully differentiated Th2 cells, deleting GATA-3 completely blocks the subsequent production of IL-5 and IL-13,[117] although it has only a modest effect on IL-4 production, consistent with the presence of GATA-3-binding sites in the promoters of IL-5 and IL-13 but not in the IL-4 promoter.

There are 2 Stat5 family members, Stat5a and Stat5b.[119] They are important for cytokine-driven cell proliferation and cell survival. IL-2 potently stimulates Stat5 activation. Th2 cell differentiation requires strong Stat5 signaling.[19,120] Thus, Stat5a single knockout cells have profound defects in Th2 cell differentiation both in vitro and in vivo despite the presence and activation of Stat5b. Stat5 has been shown to directly bind to DNase I hypersensitive sites (HSII and HSIII) in the second intron of the *Il4* locus.[120]

c-Maf, which is selectively up-regulated in Th2 cells, also enhances IL-4 production but does not play a role in the production of other Th2 cytokines.[121] IRF-4 expression is required for Th2 cell differentiation.[122,123] IRF-4–deficient cells produce much less IL-4, but this defect can be rescued by overexpression of GATA-3, suggesting that IRF-4 up-regulates GATA-3.[122]

Gfi-1 is an immediate early IL-4–inducible gene.[124] TCR activation also transiently induces Gfi-1 expression. Gfi-1 selects GATA-3hi cells for growth by modulating both the upstream and the downstream IL-2 signaling events.[124,125]

Transcription factors for Th17 differentiation

RORγt is important in Th17 cell differentiation.[29] Overexpressing RORγt induces IL-17 production, whereas RORγt-deficient cells produce very little IL-17. Indeed, RORγt-deficient mice are partially resistant to EAE.

Another related nuclear receptor, RORα, is also up-regulated in Th17 cells.[126] Although RORα deletion has minimal effect on IL-17 production, deficiency in both RORγt and RORα completely abolished IL-17 production.

Stat3, the major signal transducer for IL-6, IL-21 and IL-23, is indispensable for IL-17 production and deletion of Stat3 results in the loss of IL-17 producing cells.[127-129] Stat3 is also responsible for the induction of IL-23R.

Interferon regulatory factor–4 (IRF4) has been recently reported to be critical for Th17 cell differentiation.[130] IRF4−/− T cells fail to produce any IL-17. EAE cannot be induced in IRF4−/− mice. IRF4 appears to play a role in RORγt expression but not in Foxp3 induction.
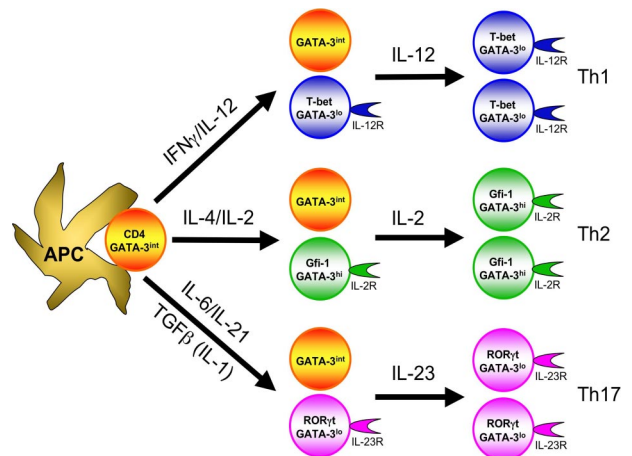

Figure 2. T-cell differentiation involves instructive differentiation as well as selective expansion of differentiated cells. The cytokines critical for the differentiation of each lineage instruct activated CD4 T cells to express their master transcription factors, T-bet for Th1, GATA-3 for Th2 and RORγt for Th17, as well as other lineage specific factors, IL-12R for Th1, Gfi-1 for Th2 and IL-23R for Th17. In many instances, only a portion of cells expresses the indicated transcription factors and adopts the differentiated phenotype. Such differentiated cells express the factors that determine responsiveness to particular cytokines, IL-12 for Th1, IL-2 for Th2 and IL-23 for Th17 cells, thus leading to selective expansion of those differentiated cells.

Transcription factors for Treg differentiation

As noted above, most patients with IPEX and Scurfy mice have *FOXP3/Foxp3* mutations, which result in loss of functional Treg cells. Overexpression of Foxp3 in conventional T cells converts them to a Treg phenotype and endows them with anergy and suppressive activity.[42] TGF-β induces Foxp3 expression.[44] Continuous expression of Foxp3 is critical for maintaining the suppressive activity of Treg cells.[131] Diminishing the degree of Foxp3 expression may convert Treg cells to Th2 like cells, implying a close relationship of the Th2 and Treg lineages.[132] Stat5 activation by IL-2, important for Th2 differentiation, is also required for Treg development.[133] Stat5 may contribute to Foxp3 induction through binding to its promoter.[134,135]

T helper differentiation

Th1 cell differentiation

In the initiation of Th1 responses, antigen-presenting cells (APCs), particularly activated dendritic cells, stimulate naive CD4 T cells possessing cognate T-cell receptors. APCs that produce large amounts of IL-12 as a result of their activation[136] (eg through either a combination of TLR3, TLR4, TLR7, TLR8, TLR9, and TLR11 stimulation or a single TLR activation in the presence of type I IFNs, IFNγ, or CD40L-mediated signaling) promote Th1 cell differentiation by acting on both NK cells and T cells. IL-12 activates NK cells to produce IFNγ, which in turn activates Stat1 in the responding CD4 T cells, up-regulating their T-bet expression. T-bet, in turn, induces T-cell IFNγ production and up-regulates IL-12Rβ2. Then, the IL-12Rβ2-expressing T cells, with high levels of T-bet, can be selected by IL-12, which is produced by APCs (Figure 2). IL-12, through activation of Stat4, induces IFNγ production and sustains expression of IL-12Rβ2. Thus, collaboration between IFNγ and IL-12 induces full Th1 differentiation.[137]

At later stages of Th1 differentiation, IL-18Rα is also up-regulated. IL-18Rα up-regulation requires IL-12/Stat4 signaling and is further increased by IFNγ. IL-12 and IL-18 jointly induce
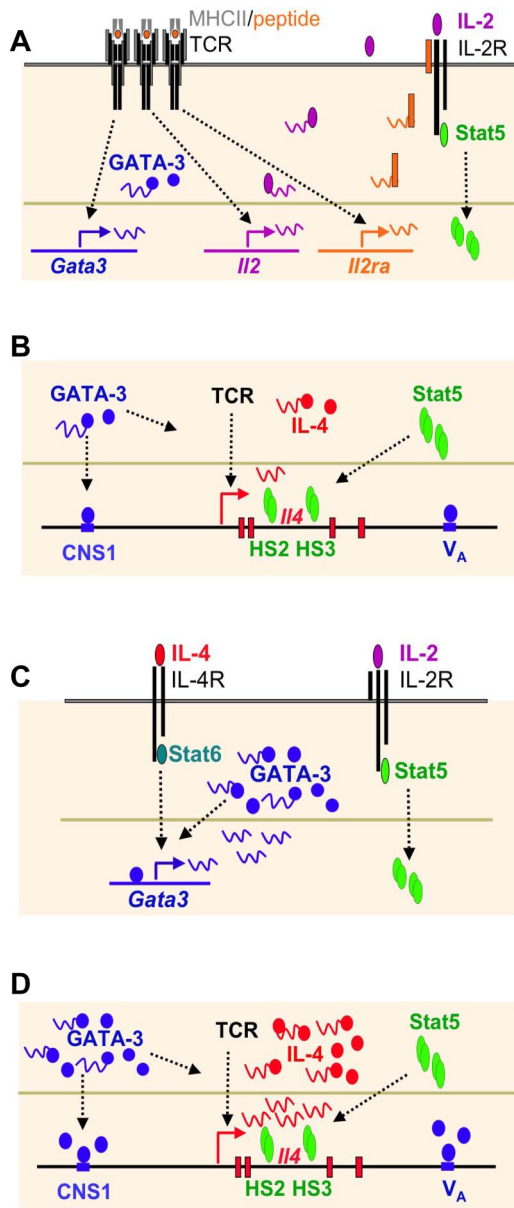
Figure 3. Th2 differentiation driven by low concentration of peptide stimulation in vitro consists of an IL-4–independent initiation phase and an IL-4–dependent amplification phase. (A) TCR stimulation by low concentration of peptide induces IL-4–independent GATA-3 expression and IL-2–mediated Stat5 activation. (B) GATA-3 binds to CNS-1 and V_A whereas activated Stat5 binds to HSII and HSIII of Il4 locus. Both are critical for TCR-mediated IL-4 production at the initial phase of Th2 cell differentiation. (C) IL-4 produced by T cells can further induce GATA-3 expression through Stat6 activation. GATA-3 also regulates itself once it reaches a certain threshold. Thus, IL-4–mediated GATA-3 expression together with IL-2–mediated Stat5 activation drives full Th2 differentiation. (D) High levels of GATA-3 and activated Stat5 play critical roles in inducing large amount of IL-4 production.

IFNγ production by Th1 cells in the absence of TCR stimulation. Such antigen-independent cytokine production is probably important for amplifying Th1 responses by recruiting other preexisting Th1 cells.

Th2 differentiation

Both IL-4 and IL-2 are required for Th2 differentiation (Figure 3) in vitro.[15,19] IL-4 can be provided exogenously, in which case IL-4–mediated Stat6 activation induces GATA-3 expression. If exogenous IL-4 is not provided, naive CD4 T cells can produce limited amounts of IL-4, as a result of TCR-mediated *Gata3*

transcription and IL-2 mediated Stat5 activation.[138] Such endogenous IL-4 production only occurs when cells receive low strength signals. The endogenous IL-4 then acts like exogenous IL-4 to up-regulate GATA-3 expression. GATA-3 has been reported to induce it own expression,[139] probably when it has reached a threshold level. The IL-4/Stat6 pathway also induces expression of Gfi-1, a transcriptional repressor, which plays an important role in selecting GATA-3high cells to grow, providing a selective component in the Th2 development pathway[124,125] (Figure 2). GATA-3 binds to regions of the *Il4/Il13* loci including DNaseI hypersensitive site Va and CNS-1 sites (see "Epigenetic changes in Th differentiation"); however, GATA-3 alone is not sufficient to induce IL-4 production. IL-2–mediated activation of Stat5 plays a critical role in inducing/maintaining accessibility at the second intron HSII and HSIII DNase I hypersensitive sites of the *Il4* locus.[120] Indeed, Stat5 is bound to these 2 sites in Th2 but not Th1 cells. The collaboration of Stat5 and GATA-3 accounts for full Th2 differentiation in vitro.[140]

Accumulating in vivo studies indicate that IL-4 is not essential for Th2 differentiation in some settings, particularly for primary Th2 responses to *Nippostrongylus brasiliensis* and *Schistosoma mansoni* infection.[111-113] The absence of IL-4 abolishes IgE switching in B cells in these infections, but Th2 cell differentiation is retained, at least partially. On the other hand, in vivo Th2 responses are completely dependent on GATA-3,[117] suggesting that there is an IL-4–independent pathway for GATA-3 induction in vivo. It has been suggested that IL-4 can be induced by Notch signaling.[141] However, Notch's role in IL-4–independent in vivo Th2 responses is still debatable. IL-4–independent Th2 responses in vivo may reflect hyperactivation of Stat5 by cytokines like IL-2, IL-7 or TSLP, because only limited amounts of GATA-3 are needed for Th2 differentiation when Stat5 is overexpressed.[120] In fact, GATA-3 expression levels in in vivo–primed Th2 cells are substantially lower than those of in vitro–primed Th2 cells.

Th17 differentiation

TGFβ is critical for Th17 cell differentiation.[26-28,32-34] TGFβ1-deficient mice are devoid of Th17 cells. More importantly, T cell–specific deletion of TGFβ1 blocks differentiation of Th17 cells during EAE induction and such mice are resistant to EAE.[74] IL-6 is produced by the cells of the innate immune system that have been activated through TLR signaling. In the presence of IL-6, TGFβ induces Th17 differentiation,[26-28] production of IL-21 and expression of IL-23R and RORγt. IL-21 can replace IL-6 in inducing RORγt and IL-17 expression.[35-37] Thus, IL-21 could serve as an amplification cytokine for Th17 differentiation. The importance of IL-21 during in vivo Th17 differentiation in different models needs to be further studied. IL-23, initially proposed as the differentiation factor for Th17 cells, fails to induce Th17 differentiation from naive mouse CD4 T cells but is critical for Th17 cell survival and/or for maintaining their function (Figure 2). Therefore, Th17 cell differentiation consists of 3 stages: a differentiation stage, based on TGFβ and IL-6; an amplification stage, mediated by IL-21; and a stabilization stage due to IL-23. Importantly, all 3 cytokines, IL-6, IL-21, and IL-23, activate Stat3.

Treg cell differentiation

TGFβ also plays a major role in iTreg differentiation[44] and is important for nTreg development.[142] Deleting TGFβ from Treg cells results in diminished suppressive function and poor survival in vivo.[74,143] In the absence of proinflammatory cytokines, TGFβ
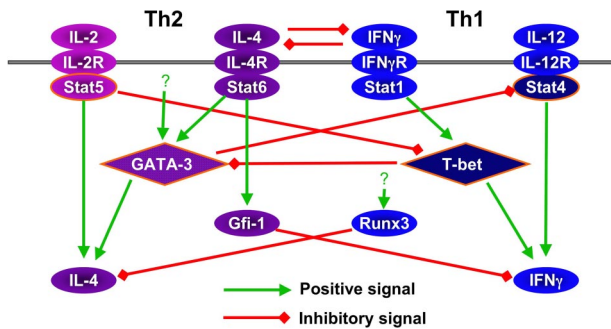
Figure 4. Cross regulation among the factors that are involved in Th1 and Th2 differentiation.

induces iTreg differentiation from naive mouse CD4 T cells.[26] TGFβ activates Smad3 while TCR stimulation induces NFAT activation. Smad3 and NFAT collaborate in remodeling the *Foxp3* enhancer region and promote Foxp3 expression.[144] IL-2–mediated Stat5 activation is also required for the induction of Foxp3 expression.[133,135,145] Both TGFβ and IL-2 are required for the survival and function of Treg cells even after they have differentiated.

Cross-regulation of T-helper differentiation

As described, Th differentiation involves positive feedback by cytokines. The differentiation process also actively involves cross-inhibition of other lineage fates. Mutual suppression between IFNγ and IL-4 signaling was the take-off point for studies of cross-regulation.[5,6] TGFβ was also found to suppress both Th1 and Th2 differentiation,[146] and both IL-4 and IFNγ inhibit Th17 differentiation.[24,25]

The cross-regulation of Th cell differentiation by cytokines may be partly explained by interaction of master genes. T-bet suppresses GATA-3 function by direct binding of the factors.[147] Although it has not been studied carefully, such interactions may also be important for IL-4–mediated suppression of Th1 development. TGFβ induces RORγt expression in both Th17 and Treg cells, whereas Foxp3 is only found in Treg cells.[148] Despite RORγt expression, Treg cells do not produce IL-17. The suppression of RORγt function in Treg cells is explained by the direct protein-protein binding between it and Foxp3. In addition, a low concentration of TGFβ can induce RORγt expression, whereas Foxp3 induction requires high concentrations of TGFβ. Thus, the amount of TGFβ as well as the presence or absence of proinflammatory cytokines determines the balance of RORγt and Foxp3 expression and thus whether the Th17 or the Treg fate is adopted. Besides direct interaction between lineage-specific transcription factors, competition for DNA binding has also been reported. Stat5 may compete with Stat3 for binding to the promoter of *Il17*, with the consequence that IL-17 production is suppressed.[129]

Another level of cross-regulation is through transcriptional regulation of critical factors. GATA-3 has been reported to down-regulate Stat4.[149] Strong Stat5 activation inhibits T-bet expression.[120] On the other hand, T-bet can suppress GATA-3 expression.[84]

Finally, cross-regulation occurs at levels of cytokine transcription. Foxp3 suppresses IL-2 through its binding to NFAT[150] as well as to Runx1.[151] Runx3 inhibits IL-4 production through binding to the HSIV region of the *Il4* locus.[105] GATA-3 deficiency results in spontaneous IFNγ production, independent of IL-12 and IFNγ.[117] Gfi-1, which acts to favor Th2 cell growth, suppresses both IFNγ[125] and IL-17 production (our unpublished data). The factors expressed in Th17 cells that are responsible for suppressing cytokine production of other lineages are unknown. Interestingly, interchromosomal interaction occurs between *Ifng* and *Il4* in naive T cells[152]; this may prove of importance in cross-regulation. The cross-regulation between Th1 and Th2 factors are shown in Figure 4.

Epigenetic changes in Th differentiation

As with all processes of differentiation, whole sets of genes are activated or repressed during the transition of naive CD4 T cells to Th1, Th2, Th17, and iTreg cells, and these differentiated states are associated with heritable changes in the conformation of key genes. Indeed, new technologies now being brought to bear will give a fuller assessment of the degree of genome-wide epigenetic modification than could previously be achieved. Zhao and his colleagues[153] are pioneers in the analysis of genome-wide patterns of histone modification that are critical for regulation of gene expression in the 4 major types of Th cells.

Much work has been done on how the accessibility of signature cytokine genes for each of the differentiated cell types is modified in the course of differentiation. Of these, most is known about *Il4* and its congener *Il13* and it is on these that we will concentrate (see Figure 5 for detailed regulatory elements and their binding to transcription factors). The *Il4* and *Il13* genes are closely linked on human chromosome 5q31 and the syntenic region on mouse chromosome 11 as part of a larger genetic assemblage that includes *Il3*, *Csf2*, *Irf1*, *Il5*, *Rad50*, and *Kif3a*.

An LCR for *Il4-Il13* has been identified that lies in a 25 Kb region at the 3′ end of *Rad50*, approximately 20 Kb and 40 Kb 5′ of *Il13* and *Il4*, respectively.[154] The LCR was defined by using a bacterial artificial chromosome (BAC) containing *Il4* and *Il13* and showing that transgenic mice expressing this BAC displayed copy number–dependent, position-independent expression of the cytokine genes. By carrying out a set of deletions, Flavell and his colleagues showed that the region in *Rad50* described above contained the LCR. This Th2 LCR is both necessary and sufficient for locus control activity directed toward the neighboring *Il4* and *Il13* genes. In cells such as fibroblasts, which do not transcribe Th2 cytokines, the *Il4*, *Il13*, and *Il5* genes form a minimal core
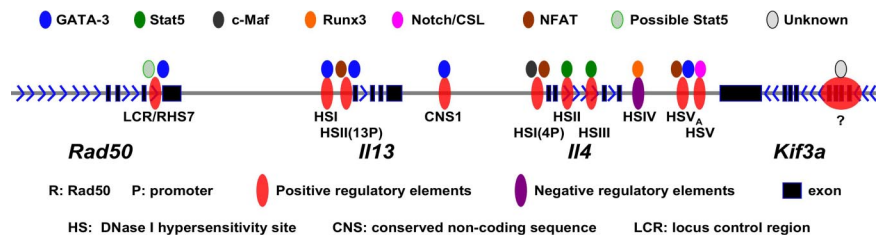

Figure 5. Positive and negative regulatory elements within *Il4/Il13* loci and their binding to transcription factors.

interacting structure. In naive T cells as well as in Th1 and Th2 cells, the LCR is recruited into this interacting structure. In contrast to naive and Th1 cells, one particular site within the LCR (RHS7)[155] becomes DNase I hypersensitive and is demethylated[154,156] within 48 hours of the initiation of Th2 differentiation. It is known that deleting this portion of the LCR diminishes but does not abolish IL-4 production in Th2 cells. Precisely how the LCR regulates the accessibility and transcription of *Il4* and *Il13* is not certain. Although GATA-3 binds to RHS7, it is not sufficient to induce the activation of LCR. The demethylation of RHS7 during Th2 differentiation seems to be partially dependent on IL-2/Stat5 signaling.[156] It is possible that GATA-3 and Stat5 besides directly regulating *Il4* gene also collaborate in regulating the LCR.

Analysis of *Il4* in Th1 and Th2 cells revealed a series of notable differences in DNase I hypersensitivity. Among a series of sites, a set within an *Il4-Il13* intergenic region (conserved noncoding sequence 1 or CNS1),[157,158] 2 in the second intron of *Il4*[159] and 2 3′ of the *Il4* coding region (HSV and HSVa, associated with CNS2)[160] appear particularly important. The CNS-1 and HSVa regions were shown by chromatin immunoprecipitation studies to have bound GATA-3 in Th2 but not Th1 cells and 2 DNase I hypersensitivity sites (those within the *Il4* second intron, designated HSII and HSIII) to have bound Stat5a. It has been shown that overexpressing either GATA-3 or constitutively active Stat5a in cells stimulated under Th1-inducing conditions allows the cells to produce IL-4.[120] The Stat5a effect does not occur in cells that are genetically deficient in GATA-3[117] and anti–IL-2 diminishes the capacity of GATA-3 overexpression to allow IL-4 production.[19] Thus, it would appear that GATA-3 and Stat5, the former induced by TCR and/or IL-4/Stat6 stimulation and the latter by IL-2, bind to sequences in the *Il4* locus and lead to accessibility, as measured either by patterns of histone modification or restriction enzyme accessibility.

In addition to genetic regions that enhance IL-4 expression, there is a region in the 3′ portion of *Il4*, HSIV, acted on by Runx3, that represses IL-4 transcription.[105] Runx3 is expressed at substantially higher levels in Th1 than Th2 cells.[106] This is one of several examples of cross-inhibition between the differentiated Th cells as discussed above.

Much still remains to be established as to how the distinctive patterns of gene accessibility are initially induced and how they are maintained but the detailed analysis of the *Il4* region and the ease of achieving alternative patterns of Th differentiation indicate that *Il4* and the other key cytokine genes can provide insight into mechanisms of gene regulation in immune cells.

One very striking property of some of the cytokine genes, most notably *Il4* and *Il13*, is that they are often expressed monoallelically. This monoallelic expression can be explained by probabilistic determination of transcription such that each *Il4* (or *Il13*) allele has a given probability of expression in Th2 cells that is determined by its pattern of gene accessibility.[161] Because these probabilities are often relatively low, many (but not all) cells express only one of the 2 alleles during any one stimulation period. We have suggested that probabilistic regulation of transcription may provide a selective advantage because of the biology of cytokine-producing cells and the functions they mediate. A particular example is IL-4's control of immunoglobulin class switching to IgE. Switching requires a direct interaction between antigen-specific T cells and B cells, with the formation of an immunologic synapse. IL-4 mainly acts across short distances so the IL-4–producing T cells can only stimulate their interacting B cells to switch. We argue that regulating the proportion of Th2 cells that make IL-4 through probabilistic transcription (with monoallelism as the consequence)

would provide finer control over the switching process than trying to regulate the amount of IL-4 each CD4 T cell makes.

Immunologic abnormalities resulting from mutations or polymorphisms in the pathways of Th differentiation

One of the most telling pieces of evidence regarding the importance of the various differentiated cell types is the consequence of their absence or abnormalities in their development in humans. We presented in "A little history" the consequences of dominant negative mutations in *STAT3*, which were the failure of human CD4 T cells to develop into Th17 cells.[45] This failure can explain a principal abnormality suffered by individuals with HIES, susceptibility to staphylococcal and fungal infections. This established both the key role of "Stat3 users" in human Th17 differentiation and the central role of Th17 cells in protection against certain types of infections.

A second striking example of a human mutation causing an impact on one of the key T-cell subsets is the effect of disabling mutations in *FOXP3*,[41] which lead to the human IPEX syndrome. IPEX is the acronym for immunodysregulation, polyendocrinopathy, and enteropathy, X-linked. The key elements of IPEX are the appearance early in life of intractable diarrhea, eczema, hemolytic anemia, diabetes mellitus, or thyroid autoimmunity. In the initial description, there were exaggerated responses to viral infections. Remarkably, affected infants often display type I diabetes within the first days after birth. This constellation of events appears to be accounted for by the inability of affected individuals to develop nTreg or iTreg cells. The mouse genetic equivalent, the *Scurfy* mouse, also demonstrates a serious autoimmune disease resulting in death between 16 and 25 days of age. The immunopathology of Scurfy mice has a substantial Th2 component. Chatila and colleagues have proposed designating the human disorder X-linked autoimmunity-allergic dysregulation syndrome (XLAAD) because of a Th2 bias in the response of affected humans.[162] Here again, the impact of the human mutation illustrates the critical role Treg cells play in controlling autoimmune/immunopathologic responses by conventional T cells and validates the importance of Foxp3 in the induction and/or function of these cells. It further argues that in the absence of Treg cells there is a greater likelihood of Th2 differentiation. Interestingly, mutations in *IL2R*A (encoding CD25, IL-2Rα), which is constitutively expressed on most Treg cells, results in an IPEX-like syndrome.[163]

Individuals with haploinsufficiency of *GATA3* develop the hypoparathyroidism, sensorineural deafness, and renal dysplasia (HDR) syndrome.[164] An analysis of these patients revealed that their levels of Th2 cells and the capacity of their naive CD4 T cells to develop into Th2 cells in vitro is diminished as was their serum concentration of IgG4, switching to which is dependent upon IL-4.[165] Pykäläinen and colleagues have reported that polymorphisms in *GATA3* in Finnish populations are associated with elevated IgE levels and greater susceptibility to asthma.[166] Polymorphisms have also been shown to exist in *TBX21* (the gene that encodes T-bet); some are associated with enhanced incidence of asthma and airway hyperresponsiveness.[167] The former results imply that hyperactivity of GATA-3 favors Th2 differentiation and the latter that diminished activity of T-bet relieves the restraint on Th2 differentiation normally exerted by T-bet or other proteins in the Th1 differentiation pathway.

A mutation from glutamine to arginine at position 576 in the cytoplasmic domain of the IL-4Rα is common among the patients with elevated IgE and severe atopic dermatitis.[168] However, this single mutation by itself does not affect IL-4–mediated CD23 induction.[169] Another IL-4Rα variant Ile50Val is also associated with atopic asthma and has a dominant effect on Stat6 activation and IgE production.[170,171] Mutations in *IL12RB1* (the gene that encodes IL-12Rβ1) and *IL12B* (encoding IL-12 p40) are associated with increased susceptibility to mycobacterial and salmonella infection[172,173] and, in one instance, to infection with Nocardia.[174] IL-12 and IL-23 both use p40 as a constituent and their receptors both use IL-12Rβ1. Because IL-12 plays an important role in inducing Th1 differentiation and IL-23 is important in sustaining the Th17 phenotype, such mutations could diminish levels of either or both Th1 and Th17 cells. Mutations in *IFNG* or *IFNGR1* in humans are associated with increased susceptibility to intracellular infections.[175-177] This suggests that the major abnormality in individuals with mutations in *IL12RB1* or *IL12B* is in the development of Th1 cells rather than Th17 cells. Furthermore, *IL23R* mutation is associated with inflammatory bowel diseases including Crohn disease.[178]

Minegishi and colleagues have reported an unusual form of HIES that is associated with mutations in *TYK2*, encoding Tyk2, a member of the Jak family of protein tyrosine kinases.[179] Tyk2 plays a role in signaling by type I IFN, IL-6, IL-10, IL-12, and IL-23. While the cellular defects in this individual are not completely clear, the results are consistent with diminished development of Th1 and Th17 cells and enhanced development of Th2 cells.

Closing remarks

CD4 T cells represent a remarkable cell population. They are central to protection against a wide range of pathogens and do so through the adoption of a series of distinct differentiated states, each evolved under the pressure of a particular set of pathogens. The process through which the naive cells differentiate into these distinct states shows several similar features. TCR engagement is essential. A major product of the differentiated cells is a principal stimulant, providing a potent positive feedback that can enforce the development of a high degree of polarization. The Jak/Stat pathways and a specific Stat in association with one of 4 master regulators, T-bet, GATA-3, RORγt, and Foxp3, are essential for the differentiation process. In a real sense, the study of this process has illuminated how central cytokines are to the mounting of effective immune responses and, through the commonalties in their pathway of differentiation, support the assertion that cytokine biology is more than a collection of isolated facts but rather involves a set of principles in which knowledge about any of the pathways points the way to a deeper understanding of the others. The analysis of the effects of mutations in key players in the differentiation process has also provided a much deeper understanding of the true biologic function of this set of cells that are so central to the mounting of effective and regulated immune responses.

Authorship

Contribution: J.Z. and W.E.P. wrote the paper.

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References

1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986;136:2348-2357.

2. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol. 2004;22:531-562.

3. Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity. 2006;25:195-201.

4. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. Annu Rev Immunol. 2007;25:297-336.

5. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989;7:145-173.

6. Paul WE, Seder RA. Lymphocyte responses and cytokines. Cell. 1994;76:241-251.

7. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity. 2006;24:677-688.

8. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science. 1994;265:1237-1240.

9. Carrier Y, Yuan J, Kuchroo VK, Weiner HL. Th3 cells in peripheral tolerance. I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF-beta T cell-transgenic mice. J Immunol. 2007;178:179-185.

10. Groux H, O'Garra A, Bigler M, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature. 1997;389:737-742.

11. Jankovic D, Kullberg MC, Feng CG, et al. Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J Exp Med. 2007;204:273-283.

12. Anderson CF, Oukka M, Kuchroo VJ, Sacks D. CD4(+)CD25(-)Foxp3(-) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J Exp Med. 2007;204:285-297.

13. McGeachy MJ, Bak-Jensen KS, Chen Y, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol. 2007;8:1390-1397.

14. Killar L, MacDonald G, West J, Woods A, Bottomly K. Cloned, Ia-restricted T cells that do not produce interleukin 4(IL 4)/B cell stimulatory factor 1(BSF-1) fail to help antigen-specific B cells. J Immunol. 1987;138:1674-1679.

15. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. J Exp Med. 1990;172:921-929.

16. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. J Immunol. 1990;145:3796-3806.

17. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89:587-596.

18. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem. 1997;272:21597-21603.

19. Cote-Sierra J, Foucras G, Guo L, et al. Interleukin 2 plays a central role in Th2 differentiation. Proc Natl Acad Sci U S A. 2004;101:3880-3885.

20. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1

CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science. 1993;260:547-549.

21. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100:655-669.

22. Lighvani AA, Frucht DM, Jankovic D, et al. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc Natl Acad Sci U S A. 2001;98:15137-15142.

23. Cua DJ, Sherlock J, Chen Y, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature. 2003;421:744-748.

24. Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6:1133-1141.

25. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6:1123-1132.

26. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441:235-238.

27. Mangan PR, Harrington LE, O'Quinn DB, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature. 2006;441:231-234.

28. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 2006;24:179-189.

29. Ivanov II, McKenzie BS, Zhou L, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006;126:1121-1133.

30. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol. 2007;8:942-949.

31. Wilson NJ, Boniface K, Chan JR, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol. 2007;8:950-957.

32. Yang L, Anderson DE, Baecher-Allan C, et al. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. Nature. Prepublished May 11, 2008 as DOI 10.1038/nature07021.

33. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol. 2008;9:641-649.

34. Volpe E, Servant N, Zollinger R, et al. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. Nat Immunol. 2008;9:650-657.

35. Korn T, Bettelli E, Gao W, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature. 2007;448:484-487.

36. Nurieva R, Yang XO, Martinez G, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature. 2007;448:480-483.

37. Zhou L, Ivanov II, Spolski R, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol. 2007;8:967-974.

38. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155:1151-1164.

39. Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001;27:68-73.

40. Patel DD. Escape from tolerance in the human X-linked autoimmunity-allergic disregulation syndrome and the Scurfy mouse. J Clin Invest. 2001;107:155-157.

41. Wildin RS, Ramsdell F, Peake J, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001;27:18-20.

42. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003;4:330-336.

43. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299:1057-1061.

44. Chen W, Jin W, Hardegen N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med. 2003;198:1875-1886.

45. Milner JD, Brenchley JM, Laurence A, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature. 2008;452:773-776.

46. Holland SM, DeLeo FR, Elloumi HZ, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med. 2007;357:1608-1619.

47. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature. 2007;448:1058-1062.

48. Suzuki Y, Orellana MA, Schreiber RD, Remington JS. Interferon-gamma: the major mediator of resistance against Toxoplasma gondii. Science. 1988;240:516-518.

49. Selmaj K, Raine CS, Cannella B, Brosnan CF. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. J Clin Invest. 1991;87:949-954.

50. Suen WE, Bergman CM, Hjelmstrom P, Ruddle NH. A critical role for lymphotoxin in experimental allergic encephalomyelitis. J Exp Med. 1997;186:1233-1242.

51. Darrah PA, Patel DT, De Luca PM, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. Nat Med. 2007;13:843-850.

52. Williams MA, Tyznik AJ, Bevan MJ. Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. Nature. 2006;441:890-893.

53. Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature. 1993;362:245-248.

54. Coffman RL, Seymour BW, Hudak S, Jackson J, Rennick D. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. Science. 1989;245:308-310.

55. Longphre M, Li D, Gallup M, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest. 1999;104:1375-1382.

56. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med. 1989;170:2081-2095.

57. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683-765.

58. Wynn TA. IL-13 effector functions. Annu Rev Immunol. 2003;21:425-456.

59. Urban JF Jr, Noben-Trauth N, Donaldson DD, et al. IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. Immunity. 1998;8:255-264.

60. Zaiss DM, Yang L, Shah PR, Kobie JJ, Urban JF, Mosmann TR. Amphiregulin, a TH2 cytokine enhancing resistance to nematodes. Science. 2006;314:1746.

61. Fort MM, Cheung J, Yen D, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity. 2001;15:985-995.

62. Angkasekwinai P, Park H, Wang YH, et al. Interleukin 25 promotes the initiation of proallergic type 2 responses. J Exp Med. 2007;204:1509-1517.

63. Fallon PG, Ballantyne SJ, Mangan NE, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J Exp Med. 2006;203:1105-1116.

64. Yao Z, Fanslow WC, Seldin MF, et al. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity. 1995;3:811-821.

65. Hymowitz SG, Filvaroff EH, Yin JP, et al. IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. EMBO J. 2001;20:5332-5341.

66. Leonard WJ, Spolski R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. Nat Rev Immunol. 2005;5:688-698.

67. Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature. 2007;445:648-651.

68. Veldhoen M, Hirota K, Westendorf AM, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature. 2008;453:106-109.

69. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity. 2007;27:647-659.

70. Aujla SJ, Chan YR, Zheng M, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med. 2008;14:275-281.

71. Zheng Y, Valdez PA, Danilenko DM, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med. 2008;14:282-289.

72. Joffre O, Santolaria T, Calise D, et al. Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. Nat Med. 2008;14:88-92.

73. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. Immunity. 2006;25:455-471.

74. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity. 2007;26:579-591.

75. Asseman C, Read S, Powrie F. Colitogenic Th1 cells are present in the antigen-experienced T cell pool in normal mice: control by CD4+ regulatory T cells and IL-10. J Immunol. 2003;171:971-978.

76. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med. 1999;190:995-1004.

77. Rubtsov YP, Rasmussen JP, Chi EY, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008;28:546-558.

78. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM,

Sacks DL. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature. 2002;420:502-507.

79. Collison LW, Workman CJ, Kuo TT, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature. 2007;450:566-569.

80. Stumhofer JS, Silver JS, Laurence A, et al. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol. 2007; 8:1363-1371.

81. Gavin MA, Rasmussen JP, Fontenot JD, et al. Foxp3-dependent programme of regulatory T-cell differentiation. Nature. 2007;445:771-775.

82. Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J Exp Med. 1997;185:817-824.

83. Afkarian M, Sedy JR, Yang J, et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. Nat Immunol. 2002;3:549-557.

84. Usui T, Preiss JC, Kanno Y, et al. T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. J Exp Med. 2006;203: 755-766.

85. Kano S, Sato K, Morishita Y, et al. The contribution of transcription factor IRF1 to the interferon-gamma-interleukin 12 signaling axis and TH1 versus TH-17 differentiation of CD4+ T cells. Nat Immunol. 2008;9:34-41.

86. Robinson D, Shibuya K, Mui A, et al. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity. 1997;7: 571-581.

87. Yang J, Zhu H, Murphy TL, Ouyang W, Murphy KM. IL-18-stimulated GADD45 beta required in cytokine-induced, but not TCR-induced, IFN-gamma production. Nat Immunol. 2001;2:157-164.

88. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med. 1998;187:875-883.

89. Bonecchi R, Bianchi G, Bordignon PP, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med. 1998;187:129-134.

90. Loetscher P, Uguccioni M, Bordoli L, et al. CCR5 is characteristic of Th1 lymphocytes. Nature. 1998;391:344-345.

91. Hwang ES, White IA, Ho IC. An IL-4-independent and CD25-mediated function of c-maf in promoting the production of Th2 cytokines. Proc Natl Acad Sci U S A. 2002;99:13026-13030.

92. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23:479-490.

93. Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science. 1997;277:2005-2007.

94. D'Ambrosio D, Iellem A, Bonecchi R, et al. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. J Immunol. 1998;161:5111-5115.

95. Nagata K, Tanaka K, Ogawa K, et al. Selective expression of a novel surface molecule by human Th2 cells in vivo. J Immunol. 1999;162:1278-1286.

96. Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J Exp Med. 2006;203:1685-1691.

97. Acosta-Rodriguez EV, Rivino L, Geginat J, et al.

Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol. 2007;8:639-646.

98. Liu W, Putnam AL, Xu-Yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med. 2006;203:1701-1711.

99. Lehmann J, Huehn J, de la Rosa M, et al. Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25-regulatory T cells. Proc Natl Acad Sci U S A. 2002;99:13031-13036.

100. Finotto S, Neurath MF, Glickman JN, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. Science. 2002;295:336-338.

101. Pearce EL, Mullen AC, Martins GA, et al. Control of effector CD8+ T cell function by the transcription factor Eomesodermin. Science. 2003;302: 1041-1043.

102. Suto A, Wurster AL, Reiner SL, Grusby MJ. IL-21 inhibits IFN-gamma production in developing Th1 cells through the repression of Eomesodermin expression. J Immunol. 2006;177:3721-3727.

103. Kaplan MH, Sun YL, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature. 1996;382:174-177.

104. Thierfelder WE, van Deursen JM, Yamamoto K, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature. 1996;382:171-174.

105. Djuretic IM, Levanon D, Negreanu V, Groner Y, Rao A, Ansel KM. Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. Nat Immunol. 2007;8:145-153.

106. Naoe Y, Setoguchi R, Akiyama K, et al. Repression of interleukin-4 in T helper type 1 cells by Runx/Cbf beta binding to the Il4 silencer. J Exp Med. 2007;204:1749-1755.

107. Mullen AC, Hutchins AS, High FA, et al. Hlx is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. Nat Immunol. 2002;3:652-658.

108. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity. 1996;4:313-319.

109. Shimoda K, van Deursen J, Sangster MY, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature. 1996;380:630-633.

110. Takeda K, Tanaka T, Shi W, et al. Essential role of Stat6 in IL-4 signalling. Nature. 1996;380:627-630.

111. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the in vivo or in vitro development of CD4+ lymphocytes with a Th2 cytokine profile. J Immunol. 2000;164:3047-3055.

112. Min B, Prout M, Hu-Li J, et al. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. J Exp Med. 2004;200: 507-517.

113. Finkelman FD, Morris SC, Orekhova T, et al. Stat6 regulation of in vivo IL-4 responses. J Immunol. 2000;164:2303-2310.

114. Kurata H, Lee HJ, O'Garra A, Arai N. Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity. 1999; 11:677-688.

115. Zhu J, Guo L, Watson CJ, Hu-Li J, Paul WE. Stat6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. J Immunol. 2001;166:7276-7281.

116. Ouyang W, Ranganath SH, Weindel K, et al. Inhibition of Th1 development mediated by GATA-3

through an IL-4-independent mechanism. Immunity. 1998;9:745-755.

117. Zhu J, Min B, Hu-Li J, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004;5:1157-1165.

118. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc Natl Acad Sci U S A. 2004;101:1993-1998.

119. Lin JX, Leonard WJ. The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene. 2000;19:2566-2576.

120. Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. Immunity. 2003;19:739-748.

121. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity. 1999;10:745-751.

122. Lohoff M, Mittrucker HW, Prechtl S, et al. Dysregulated T helper cell differentiation in the absence of interferon regulatory factor 4. Proc Natl Acad Sci U S A. 2002;99:11808-11812.

123. Rengarajan J, Mowen KA, McBride KD, Smith ED, Singh H, Glimcher LH. Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. J Exp Med. 2002; 195:1003-1012.

124. Zhu J, Guo L, Min B, et al. Growth factor independent-1 induced by IL-4 regulates Th2 cell proliferation. Immunity. 2002;16:733-744.

125. Zhu J, Jankovic D, Grinberg A, Guo L, Paul WE. Gfi-1 plays an important role in IL-2-mediated Th2 cell expansion. Proc Natl Acad Sci U S A. 2006; 103:18214-18219.

126. Yang XO, Pappu BP, Nurieva R, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity. 2008;28:29-39.

127. Harris TJ, Grosso JF, Yen HR, et al. Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. J Immunol. 2007;179:4313-4317.

128. Mathur AN, Chang HC, Zisoulis DG, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol. 2007;178:4901-4907.

129. Laurence A, Tato CM, Davidson TS, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity. 2007;26:371-381.

130. Brüstle A, Heink S, Huber M, et al. The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. Nat Immunol. 2007; 8:958-966.

131. Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol. 2007;8:277-284.

132. Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature. 2007;445:766-770.

133. Davidson TS, DiPaolo RJ, Andersson J, Shevach EM. Cutting Edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells. J Immunol. 2007;178:4022-4026.

134. Yao Z, Kanno Y, Kerenyi M, et al. Nonredundant roles for Stat5a/b in directly regulating Foxp3. Blood. 2007;109:4368-4375.

135. Burchill MA, Yang J, Vogtenhuber C, Blazar BR, Farrar MA. IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells. J Immunol. 2007;178: 280-290.

136. Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol. 2007;7:179-190.

137. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol. 2002;2:933-944.

138. Yamane H, Zhu J, Paul WE. Independent roles

for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. J Exp Med. 2005;202:793-804.

139. Ouyang W, Lohning M, Gao Z, et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity. 2000;12:27-37.

140. Zhu J, Yamane H, Cote-Sierra J, Guo L, Paul WE. GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. Cell Res. 2006;16:3-10.

141. Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. Cell. 2004;117:515-526.

142. Liu Y, Zhang P, Li J, Kulkarni AB, Perruche S, Chen W. A critical function for TGF-beta signaling in the development of natural CD4+CD25+Foxp3+ regulatory T cells. Nat Immunol. 2008;9:632-640.

143. Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. J Exp Med. 2005;201:1061-1067.

144. Tone Y, Furuuchi K, Kojima Y, Tykocinski ML, Greene MI, Tone M. Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol. 2008;9:194-202.

145. Burchill MA, Yang J, Vang KB, et al. Linked T cell receptor and cytokine signaling govern the development of the regulatory T cell repertoire. Immunity. 2008;28:112-121.

146. Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. J Immunol. 2000;165:4773-4777.

147. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science. 2005;307:430-433.

148. Zhou L, Lopes JE, Chong MM, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008;453:236-240.

149. Usui T, Nishikomori R, Kitani A, Strober W. GATA-3 suppresses Th1 development by down-regulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet. Immunity. 2003;18:415-428.

150. Wu Y, Borde M, Heissmeyer V, et al. FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell. 2006;126:375-387.

151. Ono M, Yaguchi H, Ohkura N, et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature. 2007;446:685-689.

152. Spilianakis CG, Lalioti MD, Town T, Lee GR, Flavell RA. Interchromosomal associations between alternatively expressed loci. Nature. 2005; 435:637-645.

153. Barski A, Cuddapah S, Cui K, et al. High-resolution profiling of histone methylations in the human genome. Cell. 2007;129:823-837.

154. Fields PE, Lee GR, Kim ST, Bartsevich VV, Flavell RA. Th2-specific chromatin remodeling and enhancer activity in the Th2 cytokine locus control region. Immunity. 2004;21:865-876.

155. Lee GR, Spilianakis CG, Flavell RA. Hypersensitive site 7 of the TH2 locus control region is essential for expressing TH2 cytokine genes and for long-range intrachromosomal interactions. Nat Immunol. 2005;6:42-48.

156. Kim ST, Fields PE, Flavell RA. Demethylation of a specific hypersensitive site in the Th2 locus control region. Proc Natl Acad Sci U S A. 2007;104: 17052-17057.

157. Loots GG, Locksley RM, Blankespoor CM, et al. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. Science. 2000;288:136-140.

158. Mohrs M, Blankespoor CM, Wang ZE, et al. Deletion of a coordinate regulator of type 2 cytokine expression in mice. Nat Immunol. 2001;2:842-847.

159. Henkel G, Weiss DL, McCoy R, Deloughery T, Tara D, Brown MA. A DNase I-hypersensitive site in the second intron of the murine IL-4 gene defines a mast cell-specific enhancer. J Immunol. 1992;149:3239-3246.

160. Solymar DC, Agarwal S, Bassing CH, Alt FW, Rao A. A 3′ enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells. Immunity. 2002;17:41-50.

161. Guo L, Hu-Li J, Paul WE. Probabilistic regulation in TH2 cells accounts for monoallelic expression of IL-4 and IL-13. Immunity. 2005;23:89-99.

162. Chatila TA, Blaeser F, Ho N, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest. 2000;106:R75-81.

163. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. J Allergy Clin Immunol. 2007;119:482-487.

164. Van Esch H, Groenen P, Nesbit MA, et al. GATA3 haplo-insufficiency causes human HDR syndrome. Nature. 2000;406:419-422.

165. Skapenko A, Leipe J, Niesner U, et al. GATA-3 in human T cell helper type 2 development. J Exp Med. 2004;199:423-428.

166. Pykalainen M, Kinos R, Valkonen S, et al. Association analysis of common variants of STAT6, GATA3, and STAT4 to asthma and high serum IgE phenotypes. J Allergy Clin Immunol. 2005; 115:80-87.

167. Raby BA, Hwang ES, Van Steen K, et al. T-bet polymorphisms are associated with asthma and airway hyperresponsiveness. Am J Respir Crit Care Med. 2006;173:64-70.

168. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med. 1997; 337:1720-1725.

169. Risma KA, Wang N, Andrews RP, et al. V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function. J Immunol. 2002;169:1604-1610.

170. Mitsuyasu H, Izuhara K, Mao XQ, et al. Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma. Nat Genet. 1998;19:119-120.

171. Mitsuyasu H, Yanagihara Y, Mao XQ, et al. Cutting edge: dominant effect of Ile50Val variant of the human IL-4 receptor alpha-chain in IgE synthesis. J Immunol. 1999;162:1227-1231.

172. Fieschi C, Dupuis S, Catherinot E, et al. Low penetrance, broad resistance, and favorable outcome of interleukin 12 receptor beta1 deficiency: medical and immunological implications. J Exp Med. 2003;197:527-535.

173. de Jong R, Altare F, Haagen IA, et al. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. Science. 1998;280:1435-1438.

174. Picard C, Fieschi C, Altare F, et al. Inherited interleukin-12 deficiency: IL12B genotype and clinical phenotype of 13 patients from six kindreds. Am J Hum Genet. 2002;70:336-348.

175. Jouanguy E, Dupuis S, Pallier A, et al. In a novel form of IFN-gamma receptor 1 deficiency, cell surface receptors fail to bind IFN-gamma. J Clin Invest. 2000;105:1429-1436.

176. Jouanguy E, Altare F, Lamhamedi S, et al. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. N Engl J Med. 1996;335:1956-1961.

177. Newport MJ, Huxley CM, Huston S, et al. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med. 1996;335:1941-1949.

178. Duerr RH, Taylor KD, Brant SR, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science. 2006; 314:1461-1463.

179. Minegishi Y, Saito M, Morio T, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity. 2006;25: 745-755.

Jinfang Zhu

I have always had a great passion for studying natural sciences, possibly because of a gene, which was inherited by my 6-year-old son who is devoting his time to his "Challenge Math" and "Aha" books. Being good at math, my family expected me to be a mathematician, but I wanted to be a robot designer when I was in middle school in China. My biology teacher, an old friend of my father, convinced me to list biochemistry as one of the choices for my major while applying for college. My scientific path was completely diverted from my original plan when I was indeed enrolled in the Department of Biology, NanKai University, to study biochemistry. Within less than a year, I came to the conclusion that designing robots would not be as fun as studying living creatures. I have been very fortunate to meet many wonderful mentors at several critical points of my career. After I obtained my Bachelor's degree with highest honors, I entered the PhD program at the Shanghai Institute of Biochemistry (now known as Shanghai Institute of Biochemistry and Cell Biology), Chinese Academy of Sciences. I began to work on interleukin (IL)-2–mediated signal transduction and gene regulation under the supervision of Prof Xinyuan Liu, a member of the Chinese Academy of Sciences, and Prof ZhongCheng Zheng. I was amazed by the elegant regulations of the signaling pathways in the immune cells with positive and negative feedbacks. After receiving my PhD degree, I decided to visit one of the top immunology labs in the United States. Dr William E. Paul, who discovered IL-4, offered me a great opportunity to work in the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Since then, I have been working on the collaboration and cross-regulation between cytokines and transcription factors during the activation, differentiation, and expansion of CD4 T cells. Throughout all these years, I find that the results of experiments often give disappointment as well as satisfaction, but the knowledge derived always gives joy and excitement, particularly when I focus on what is learned not only from my own work but also that of others.The building of my own research career, as with CD4 T-cell differentiation, has been deeply influenced by many outstanding mentors, especially Dr William Paul, who constantly stimulates me with his "IL-2" and "IL-4." Although my "fate" is not yet fully determined and my "functions" need to be revealed, I believe my deep passion in immunology should compensate my "faults," if any.

William E. Paul

I have always been motivated by the wish to contribute something to the store of human knowledge. I recall reading, perhaps when I was in college, a slender volume of lectures by Michael Heidelberger, the father of quantitative immunochemistry, outlining the remarkable specificity of antibodies and how these molecules interact with their cognate antigens. The elegance of this work and of the ideas that were developed from it entranced me. Although in medical school I made a foray into endocrinology, upon coming to the National Institutes of Health as a clinical associate I edged toward immunology through my work with Bill Odell and Jack Wilber on the development of the TSH radioimmunoassay. The NIH experience committed me to research and I went on to spend 6 remarkable years with Baruj Benacerraf, first at New York University (NYU) and then back at NIH, which made that commitment virtually irrevocable. While at NYU, I had the delight of working in the adjoining laboratory to Michael Heidelberger, who had come to NYU in his second postretirement job at the age of 75. Michael continued to work at NYU until well after his 100th birthday, proving how durable careers in immunology can be. As I mentioned, I had returned to NIH with Benacerraf but still thought of myself as a physician-scientist. However, when Baruj left NIH to become the chairman of pathology at Harvard Medical School, I was appointed as his successor to lead the NIAID Laboratory of Immunology. Perhaps with too little reluctance, I embarked on a laboratory research career that has continued to this day, with a 4-year interlude in which I accepted Harold Varmus's summons to lead the Office of AIDS Research. I have had the good fortune to work with a remarkable set of postdoctoral fellows and other colleagues. Indeed, my coauthor in preparing this article, Jeff Zhu, ranks as among the best of these marvelous scientists. I close by pointing out how valuable it is to be blessed with "students" of exceptional ability and cite the Talmudic quotation, "[F]rom my teachers I have learned much, from my colleagues still more, but from my students most of all."