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Mechanisms underlying helper T-cell plasticity: Implications for immune-mediated disease

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CD4 helper T cells are critical for proper immune cell homeostasis and host defense but are also major contributors to immune and inflammatory disease. Arising from a simple biphasic model of differentiation (ie, T_H1 and T_H2 cells). A bewildering number of fates seem possible for helper T cells. To what extent different helper cell subsets maintain their characteristic gene expression profiles or exhibit functional plasticity is a hotly debated topic. In this review we will discuss how the expression of "signature cytokines" and "master regulator" transcription factors do not neatly conform to a simple helper T-cell paradigm. Although this might seem confusing, the good news is that the newly recognized complexity fits better with our understanding of immunopathogenesis. Finally, we will discuss factors, including epigenetic regulation and metabolic alterations, that contribute to helper cell specificity and plasticity. (J Allergy Clin Immunol 2013;131:1276-87.)

Key words: *T-cell plasticity, asthma, allergic disease, epigenetics, histone modification, therapy*

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Abbreviations used

Bcl6:	B-cell lymphoma 6
Foxp3:	Forkhead box protein 3
HIF:	Hypoxia-inducible factor
H3K4me3:	Histone 3 lysine 4 trimethylation
H3K27me3:	Histone 3 lysine 27 trimethylation
IRF:	Interferon regulatory factor
mTOR:	Mammalian target of rapamycin
mTORC:	Mammalian target of rapamycin complex
PU.1:	SFFV proviral integration 1
Rorγt:	Retinoic acid receptor–related orphan receptor γt
T-bet:	T-box transcription factor
T_{FH}:	Follicular helper T
Treg:	Regulatory T

CD4 T cells are critical for host defense, but in addition to their key role as helper cells, they can also be troublemakers, driving autoimmune diseases, asthma, and allergies.[1,2] Classically, we viewed helper T cells as having 2 major fates, T_H1 and T_H2 cells (Fig 1, *A*), but we now know that the opportunities for helper diversity are far greater than just these 2 outcomes. The new diversity includes T_H17, T_H9, and T_H22 cells; follicular helper T (T_{FH}) cells; and different types of regulatory T (Treg) cells (Fig 1, *B*).[2-6] In addition, the emerging data point to the increased flexibility of these subsets. Fortunately, we are also beginning to understand the molecular basis of this complexity. This newer appreciation is not just pertinent for understanding the basic aspects of T-cell biology; on the contrary, the new insights provide a more sophisticated understanding of immune-mediated disease and new opportunities for therapy. In this review we discuss helper cell differentiation decisions and how the regulation of helper cell specificity pertains to susceptibility to immune and inflammatory disease. We will consider the intrinsic and extrinsic factors that drive specification and the mechanisms that influence flexibility. Of particular interest with respect to the issue of plasticity are advances in epigenetic technologies as they pertain to T-cell biology. The insights provided are especially relevant for immunologically mediated diseases, in which both genetic and environmental factors play key roles in susceptibility.

COMPLEXITY OF HELPER CELL FATE DETERMINATION

For more than 2 decades, it has been recognized that CD4 T cells specialize in response to microbial challenges. The first subsets recognized were denoted T_H1 and T_H2 cells based on the selective production of 2 cytokines, IFN-γ and IL-4, respectively.[7] This T_H1/T_H2 paradigm was reasonably useful for initial categorization of mechanisms involving elimination of microbial pathogens. For instance, T_H1 cells are critical for the clearance of many intracellular pathogens, such as *Leishmania major* and

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Terms in boldface and italics are defined in the glossary on page 1277.

Mycobacterium tuberculosis.[8,9] Similarly, T_H2 cells were found to be important for elimination of helminthic parasites, such as *Nippostrongylus brasiliensis* and *Schistosoma mansoni*.[10]

At first, the pathogenesis of immune-mediated disease also seemed to fit within this paradigm. Human asthma, as well as animal models of allergic airway inflammation, revealed the importance of cytokines produced by T_H2 cells, namely IL-4, IL-5, and IL-13[11-14]; the contribution of these various cytokines to the pathophysiology of airway inflammation, eosinophilia, fibrosis, and other responses is well recognized.[15-17] Moreover, ***genome-wide association studies*** of asthmatic patients have revealed the association of DNA variants in the T_H2 cytokine ***locus*** and the *IL4R* gene with susceptibility to asthma.[18-20] Equally important has been the successful use of therapeutic mAbs directed against IL-5 (mepolizumab) and IL-13 (lebrikizumab).[21-24] Such discoveries clearly point to the pathophysiologic role of these cytokines, although it is also clear that not all patients respond to these agents. Such findings clearly point to the additional complexity of these diseases.

Initially viewed as one of the products of T_H2 cells, IL-9 is an important factor that promotes mucus production; its expression is increased in the airways of asthmatic patients.[25-27] Recently, however, IL-9 has been found to be produced in a subset of cells that is distinct from classical T_H2 cells.[5,28] These cells are dubbed T_H9 cells, but precisely how they relate to other subsets and the extent to which they constitute a stable subset remain to be determined.

It is also well appreciated that IgE is a central player in the pathophysiology of allergies and asthma.[24,29] Although the generation of IgE-producing B cells is a well-accepted action of IL-4, it is also becoming clear that a specific population of CD4 T cells are important for providing B-cell help. These cells are designated as T_{FH} cells and are identified based on their location in ***germinal centers*** and surface expression of the molecules CXCR5 and programmed cell death 1 *(PD-1)*.[4,30-32] IL-21 has been referred to as the signature cytokine for T_{FH} cells, but IL-21 is also produced by T_H1 and T_H17 cells.[33,34] In addition, T_{FH} cells can produce cytokines made by other subsets, including IFN-γ, IL-4, IL-17, and IL-10.[4,35,36] Therefore T_{FH} cells might have both overlapping and distinct contributions to disease because they can make T_H1 and T_H2 cytokines but also contribute specifically to antibody formation. Because they do not localize to tissues, the direct effects of their cytokine production are unlikely to be with regard to tissue inflammation but rather with regard to isotype-specific antibody production. Accordingly, genetic mutations in ***inducible costimulator (ICOS)*** or SLAM-associated protein *(SAP)*, genes expressed by T_{FH} cells that are necessary for interaction with B cells, result in a loss of T_{FH} cell development and thus antibody production.[37-39] In addition, patients with mutations in ***signal transducer and activator of transcription (STAT)*** 3 have reduced T_{FH} cell numbers, which might contribute to the altered antibody repertoire they display.[40]

The attempt to link common autoimmune diseases with a simple T_H1/T_H2 paradigm has been even more problematic.[41] Certainly, there is evidence that excessive activation of T_H1 cells contributes to organ-specific autoimmune diseases.[42] However, a number of lines of evidence suggest that autoimmune mechanisms cannot be reduced to the action of T_H1 cells alone. In particular, the discovery of a new cytokine, IL-23, led to the recognition of a new subset of helper T cells and their importance in autoimmunity.[43]

GLOSSARY

BLIMP1: A key transcription factor for the differentiation of B cells into antibody-secreting plasma cells within lymphoid organs.

EPIGENETICS: The term was coined by Waddington before the era of modern molecular biology. It has come to denote hereditable changes in phenotype or gene expression without changes in DNA sequence.

EPIGENOME: The term indicates the status of the genome-wide chemical changes to the DNA and histone proteins.

GENOME-WIDE ASSOCIATION STUDIES: Cohorts of patients with and without a given disease are examined across the entire genome for single nucleotide polymorphisms that are overrepresented in patients with the disease. This identifies regions of the genome that contain a variant gene or genes that confer disease susceptibility. Candidate genes are then selected based on how closely they are associated with the disease and whether their biologic function correlates with the disease under study.

GERMINAL CENTER: An area within a lymphoid follicle where affinity maturation occurs. B cells activated by antigen and helper T cells migrate into germinal centers. Somatic mutation of V region genes in these B cells generates antibodies with different affinity for antigen. Binding of B cells to antigen presented on follicular dendritic cells rescues these B cells from apoptosis. B cells with the highest affinity for antigen will have a survival advantage, which results in an average increase in the affinity of antibodies for antigen during the immune response.

INDUCIBLE COSTIMULATOR (ICOS): ICOS is a member of the CD28 family of costimulatory receptors on T cells. ICOS binds to ICOS ligand on antigen-presenting cells and promotes effector responses. Mutations in the ICOS gene have been reported in patients with common variable immunodeficiency.

IMMUNE DYSREGULATION–POLYENDOCRINOPATHY–ENTEROPATHY–X-LINKED SYNDROME: *FOXP3* mutations lead to immune system dysregulation in this disorder. Features include early-onset diabetes, diarrhea, and failure to thrive. Newborns have an eczematous rash. Serious infections can occur. Laboratory abnormalities include high IgE, normal IgG, normal IgM, and normal IgA levels. T and B subsets are also normal. Autoimmune hemolytic anemia, neutropenia, and thrombocytopenia can occur. Female carriers are usually healthy.

LOCUS: The position in a chromosome of a particular gene or allele.

MUCOCUTANEOUS CANDIDIASIS: Persistent superficial candidal infections of the mucous membranes, skin, and nails. Other defects that are associated with mucocutaneous candidiasis include CARD9/Dectin-1 deficiency and *AIRE* gene defects. Patients have selective anergy to *Candida* species on delayed-type hypersensitivity testing.

NAIVE CD4 T CELLS: T cells that have completed maturation in the thymus but have not yet encountered foreign antigen. They are characterized by no effector function, no cell cycling, and high expression of CCR7 and CD62 ligand (L-selectin and peripheral lymph node homing receptor). Their major CD45 isoform is CD45RA.

RAPAMYCIN: An immunosuppressant drug used in renal transplantation as prophylaxis against organ rejection.

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION (STAT): Transcription factors that are a part of the Janus kinase (Jak)–STAT pathway. Many cytokines use Jak-STAT pathways for signaling. There are 7 STATs (1-4, 5a, 5b, and 6). The discovery of the Jak-STAT pathway came from analyses of interferon signaling.

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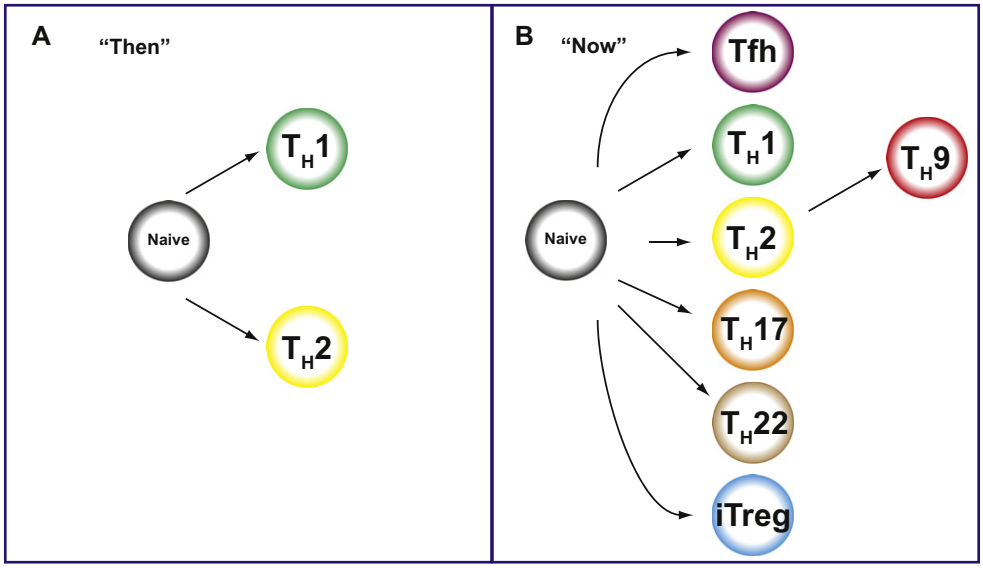

FIG 1. Helper T cells then (**A**) and now (**B**). More than 2 decades ago, we viewed helper T cells as having 2 major fates: T_H1 and T_H2 cells. However, now we recognize the new diversity of helper T cells, including T_H17, T_H9, T_H22, and T_{FH} cells and different types of Treg cells. *iTreg*, Peripheral antigen-induced Treg cells.

The discovery of an IL-17–producing population of CD4 T cells, termed T_H17 cells, helped clarify contrasting findings in experimental autoimmune encephalitis, a mouse model of multiple sclerosis. IL-23 was found to have a critical role in experimental autoimmune encephalitis pathogenicity, and selective production of IL-17 by helper T cells was linked to IL-23. Although pathogenicity of the cytokine IL-17 in patients with arthritis has been recognized since the late 1990s, the discovery of IL-23 led to the appreciation of T_H17 cells as a distinct subset.[43-47] Accordingly, mAbs that interfere with the action of IL-17, such as ixekizumab and secukinumab, appear to be useful in patients with diseases such as rheumatoid arthritis and psoriasis.[48-51] In addition to pathogenic roles in human autoimmunity and a variety of mouse models of disease, T_H17 cells contribute to host defense against extracellular bacteria, such as *Staphylococcus aureus* and *Klebsiella pneumonia*, as well as fungi.[52-54]

The importance of IL-17 applies not only to autoimmune disease but is also relevant to the pathophysiology of asthma.[55-57] One important action of IL-17 is the recruitment of neutrophils to the lung during airway inflammation and likely plays a role in steroid-resistant asthma.[55-62] IL-17 also contributes to allergen-induced airway hyperresponsiveness through direct effects on airway smooth muscle.[63] IL-17 also appears to contribute to the pathogenesis of chronic obstructive pulmonary disease and atopic dermatitis.[64-67] Conversely, IL-27 is an important negative regulator of T_H17 differentiation, which also induces T_H1 differentiation.[68-70] Interestingly, IL-27 receptor–deficient mice have exaggerated airway inflammation.[71] Although IL-27 receptor–deficient mice displayed increases in T_H2 cytokine levels, they also had slightly increased IFN-γ responses to experimental asthma challenge. This suggests that IL-27 plays a role in suppression of asthmatic responses by inhibiting the T_H2 response, and this inhibition is independent from promoting T_H1 responses.[71]

T_H17 cells can also produce IL-22, which has been shown to have important roles in protecting barrier function in the lung and gut.[72] Increased IL-22 levels are associated with severity of asthma and are present in the skin of patients with atopic dermatitis.[73,74] IL-22 neutralization in mouse models reduces eosinophil recruitment in the lung.[75] The identification of a population of CD4 T cells that make IL-22 but do not express IL-17, IL-4, or IFN-γ has led to the notion of T_H22 cells.[76] However, IL-22 is also made by non–T-cell lineages, including innate lymphoid cells.[77]

With respect to the pathogenesis of autoimmune disease, the recognition of the criticality of Treg cells was another key discovery.[6,78-82] Treg cells are essential for the maintenance of immunologic tolerance, as is vividly documented in mice or human subjects lacking the transcription factor Foxp3, which drives specification of this subset.[6,81] In human subjects this disorder is termed ***immune dysregulation–polyendocrinopathy–enteropathy–X-linked syndrome***. Treg cells can be divided into natural thymic-derived Treg cells and peripheral antigen-induced Treg cells[6,81,83,84]; however, there are a paucity of markers that distinguish these subsets.[85-87] Among the ways Treg cells suppress immune responses is through the production of the anti-inflammatory cytokines IL-10 and TGF-β.[82] Treg cells also suppress effector T-cell responses by consuming IL-2, limiting access to this important effector CD4 T-cell growth factor.[82] However, IL-2 interferes with T_H17 cell differentiation, and there are also circumstances in which Treg cells can promote T_H17 responses through the consumption of IL-2.[88-91]

In summary, although the importance of CD4 T cells in host defense and immune-mediated diseases is evident, it is also clear that they execute these functions by attaining multiple distinct fates. Advances over the last few years have led to the recognition that a simple T_H1/T_H2 view of helper T cells was a vast oversimplification. However, we face a new challenge in understanding helper T-cell function because simply adding more subsets of helper T cells to our lexicon does not provide a satisfactory understanding of immune homeostasis and immune-mediated pathology.[92]

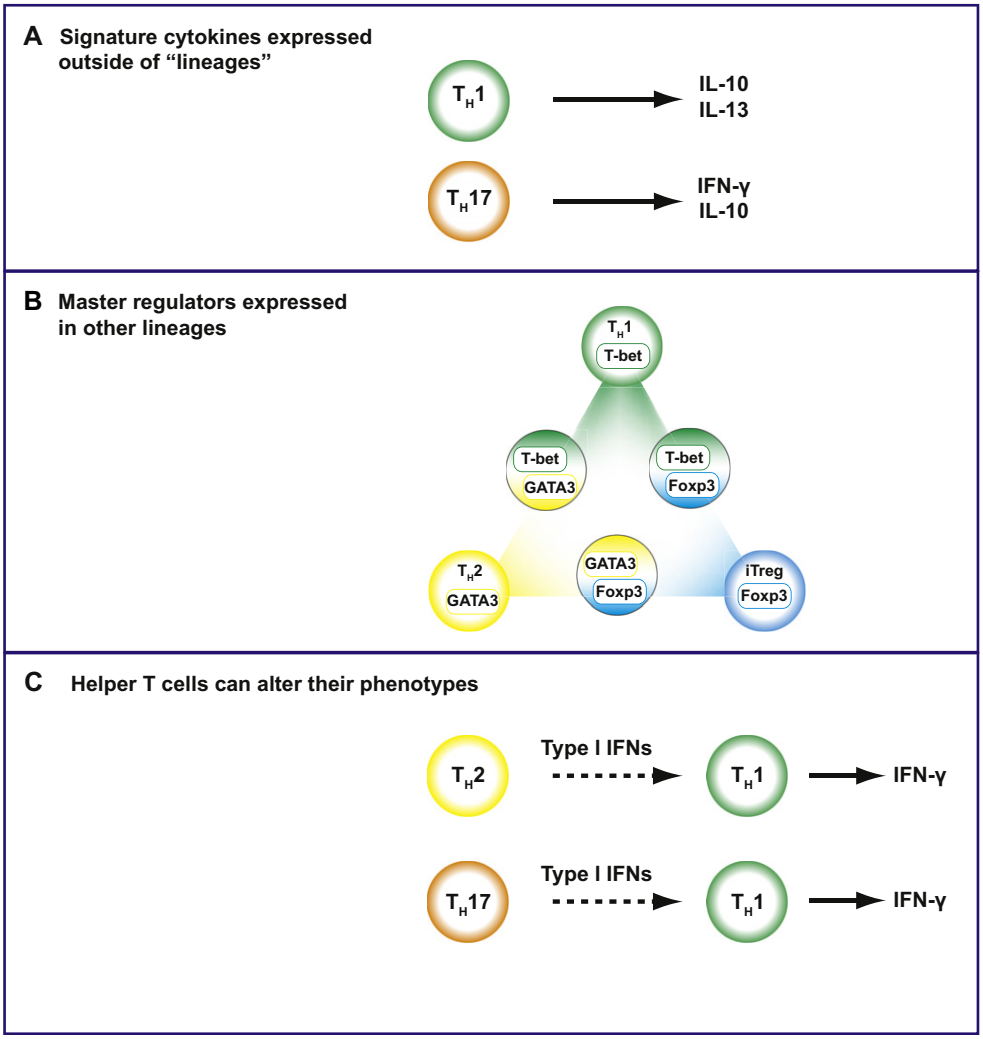

FIG 2. Plasticity and promiscuity in helper T cells. Recent advances have allowed us to point out several pieces of evidence of the plasticity of helper T cells. Helper T cells can express the signature cytokines (**A**) and the master regulators (**B**) outside of lineages. Moreover, helper T cells can alter their phenotypes by the environmental stimulations (**C**). *iTreg,* Peripheral antigen-induced Treg cells.

FLEXIBILITY OF HELPER CELL RESPONSES

The diversity of outcomes available to ***naive CD4 T cells*** provided new ways of conceptualizing the pathogenesis of immune-mediated disease and new opportunities for therapy. However, it is often tacitly assumed that these subsets are stable or behave as lineages, with defined signature cytokines, distinct transcriptional profiles, and unique master regulator transcription factors. Initial experiments argued that T_H1 and T_H2 cells did conform to this view. Recently, however, the exceptions to these notions have also become evident. What was also less clear is whether the more recently described other helper T cells behave as lineages.

Indeed, it is now very clear that CD4 T cells can be remarkably flexible in their responses. It is not infrequent for CD4 T cells to coexpress more than 1 signature cytokine, particularly *in vivo* (Fig 2, *A*). T_H17 cells, for example, readily become IFN-γ producers.[93-97] In fact, the conversion of IL-17 producers to IFN-γ producers is an important aspect of immunopathogenesis in disease models and likely in human autoimmune disease.[95] Even polarized T_H2 cells can acquire the ability to produce IFN-γ.[98] Similarly, in asthmatic patients memory/effector cells can be identified that produce both T_H17 and T_H2 cytokines.[99] These hybrid cells appear to be important drivers of pathology. This fits with the immunopathologic features of diseases, such as atopic dermatitis, which exhibit T_H2 characteristics early on but later show T_H1-like disease.[100]

Although they are clearly important in promoting B-cell responses, T_{FH} cells do not produce a single signature cytokine; on the contrary, they can express a range of different cytokines in different circumstances. Ordinarily, Treg cells do not produce effector cytokines; however, there are data arguing that Treg cells can be unstable and can acquire the ability to produce such cytokines. However, this remains a controversial topic.[101-107]

Initially, the so-called T_H2 cytokines included IL-4, IL-5, IL-13, and IL-10. However, we now appreciate that IL-10 is broadly produced by multiple types of helper T cells, including T_H1 and T_H17 cells.[108-110] In addition, we also now recognize that IL-13 can be made without IL-4.[111,112] T_H2 cells might, in fact, be heterogeneous,[113-115] and cells that produce IL-5 and

not IL-4 might be more differentiated cells. Similarly, T_H17 cells are also heterogeneous; some are pathogenic in autoimmune disease, and other are not.[116]

FACTORS THAT REGULATE PLASTICITY VERSUS PHENOTYPIC STABILITY

Given that CD4 T cells can exhibit features of stability but also apparently retain the potential for flexible responses, the question arises as to how immediately phenotypic conversion occurs and what mechanisms promote flexibility in differentiating helper T cells.

FLEXIBLE EXPRESSION OF MULTIPLE MASTER REGULATORS

In the conventional view a given helper T-cell subset is defined based on its ability to produce a single signature cytokine, as well as corresponding to a single master transcription factor. T_H1, T_H2, T_H9, T_H17, T_{FH}, and Treg cells express T-box transcription factor (T-bet; encoded by *Tbx21*),[117,118] GATA3,[119] SFFV proviral integration 1 (PU.1),[120] retinoic acid receptor–related orphan receptor γt (Rorγt),[47] B-cell lymphoma 6 (Bcl6),[30,32] and forkhead box protein 3 (Foxp3),[79,121] respectively.

However, recent data show that, like the signature cytokines, the expression of T-cell master regulators is far more complicated than originally appreciated. For example, T-bet and GATA3 are transiently coexpressed in recently activated CD4 T cells and can functionally interact, limiting the action of GATA3.[122] In addition, after viral infection, T-bet and GATA3 can also be stably coexpressed in previously committed T_H2 cells to create a population termed T_H2+1 cells that has features of both T_H1 and T_H2 cells.[98] This provides a means by which previously committed CD4 T cells can maintain flexibility in functional responses.

T-bet and Bcl6 can also be expressed simultaneously.[33,123-125] In fact, the same cytokines that induce Bcl6 can also induce T-bet.[33] Because T-bet is induced, it can bind Bcl6 and interfere with the ability of Bcl6 to act on its target genes. In this case master regulators can fine tune function for each other. Thus the balance between T-bet and Bcl6 expression could be important for the decision between a T_H1 and a T_{FH} cell.

Simultaneous expression of Foxp3 with T-bet, GATA3, or Bcl6 has also been documented in Treg cells. This has been argued to be functionally relevant for control of T_H1, T_H2, and T_{FH} cell responses, respectively.[104,126-131] In this way the expression of multiple master regulators can be viewed as a means of specialization of regulatory responses. In other cases master regulators are important for more than 1 lineage. T_H9 cells express the transcription factors interferon regulatory factor (IRF) 4 and PU.1.[120,132-134] Furthermore, IRF4 is proved to be important for T_H2, T_H9, and T_H17 cells.[135]

These examples of coexpression make it appropriate to revisit our views of the master regulator. There are now clear examples in which helper T cells express more than 1 master regulator (Fig 2, *B*). In fact, the complex modes of expression can modulate function, specialize responses, or preserve flexibility.

SENSING THE ENVIRONMENT

The dynamic expression of master regulators is controlled both positively and negatively by other factors. Among the more relevant factors are STAT family DNA-binding proteins. Typically, we associated STAT4, STAT6, STAT3, and STAT5 with T_H1, T_H2, T_H17, and Treg cells, respectively. However, unlike the master regulators, STATs are not necessarily differentially expressed among subsets. Cytokine receptor expression can be downmodulated, but the action of STATs can be redundant. For instance, IL-12 acting through STAT4 is an important driver of T-bet expression. In T_H2 cells IL-12 receptor β2 is downregulated, thus making T_H2 cells resistant to the effect of IL-12. However, type I interferons acting through STAT1 can also induce T-bet expression. In this way interferons can reprogram T_H2 cells in the setting of viral infection (Fig 2, *C*).[98]

Similarly, STAT3, being activated by IL-6 and IL-21, promotes T_H17 differentiation. However, STAT5, in response to IL-2, can bind the same sites in the *IL17* locus and inhibit IL-17 expression.[89] IL-2 acting on STAT5 also inhibits Bcl6 expression.[136]

STAT5 is a critical positive regulator of Foxp3 expression; in fact, the phenotypic stability of Treg cells requires the expression of the high-affinity IL-2 receptor.[107] Conversely, activation of STAT3 can limit Foxp3 expression; helper T cells that lack STAT3 exhibit a more stable Foxp3 expression.[137]

Another example of cytokines mediating an antagonism between STAT molecules, which alters helper T-cell fate, can be found in patients with gain-of function *STAT1* mutations who have ***mucocutaneous candidiasis***. These patients have a deficiency in T_H17 cells, which are also key to anti-*Candida* species defense.[138] It has been suggested that because IL-27 can suppress T_H17 differentiation and because IL-27 signals through STAT1, these patients have a failure of differentiation into T_H17 cells because of overexuberant STAT1 signaling triggered at least in part by IL-27.[139]

THE HELPER T-CELL TRANSCRIPTION FACTOR NETWORK

Although STATs and master regulators are critical for helper cell differentiation, it is overly simplistic to try to explain the diverse functionalities of helper T cells based on these 2 classes of factors. In reality, specification requires a cohort of critical transcription factors working in concert. During T-cell development, CD4 T cells express an array of transcription factors that dynamically change over the course of commitment in the thymus, allowing them to diverge from CD8 T cells.[140] Factors such as Thpok, Runt-related transcription factor (RUNX) 3, Runx1, Ets1, Tox, and the E proteins E2A and HEB are all induced at discrete steps to drive commitment to either the CD4 or CD8 lineage. It is in the context of these other transcription factors that master regulators and STATs exert their effects. Even GATA3 has critical roles in thymic development, aside from its function in T_H2 cells.[141] Thus any given transcription factor can have stage-specific functions.

In addition to factors required for development, several other transcription factors have been described that are critical for CD4 T cells but are not specifically required for only 1 subset. IRF4 is important for the differentiation of T_H2, Treg, T_H17, T_H9, and T_{FH} cells.[134,142-145] To function, IRF4 complexes with members of the activator protein 1 family, making family members like basic leucine zipper transcription factor (BATF) and c-Maf necessary for several CD4 T-cell subsets.[146-150]

Additional transcription factors that are important for T_H1 cells include Hlx, Runx3, and the Ets family members.[151-153] In addition to c-Maf, the activator protein 1 family member JunB is required for T_H2 cells, as well as the transcription factor Gfi-1.[154,155] The transcription factor NFIL3 (E4BP4) is a key

regulator of IL-13 production.[111,156] Recently, hypoxia-inducible factor (HIF) 1, Runx1, Aiolos, and Fosl2 have all been demonstrated to be important for T_H17 cells.[148,157-159] Coupled to this, we appreciate that factors such as Klf2, Bcl6, and ***Blimp1*** (encoded by PRDM1) influence the extent to which cells exhibit features of effector cells.[160,161] Despite the importance of Foxp3, other factors are important contributors to the phenotype of various regulatory cells.[162-167]

Given that helper T cells express a panoply of key transcription factors, it is naive to interrogate expression of 1 transcription factor, master regulator or otherwise, and make inferences about functionality. There is not a simple correlation between one helper cell lineage and expression of a single master regulator of transcription.[168] Multiple transcription factors work in concert to effect complex cellular decisions; fortunately, the technological advances in imaging and sequencing facilitate measuring numerous factors simultaneously.

TRANSCRIPTOMIC VIEWS OF HELPER T-CELL SPECIFICATION

Although helper T-cell subsets were initially defined based on their selective cytokine production, the advent of microarray technology provided the opportunity to define the global patterns of gene expression or "transcriptomes" of the helper T-cell subset. What became obvious is that different types of helper T cells exhibit a large cassette of genes that contribute to their functionality. For instance, the regulation of chemokine and chemokine receptor expression is an important feature of different subsets of helper T cells. T_H1 cells preferentially express CCR5 and CXCR3, whereas T_H2 cells are characterized by the expression of CCR4 and CCR8.[169] CCR6 and CXCR5 are important for T_H17 and T_{FH} cells, respectively.[169] In addition, deep sequencing technology coupled to chromatin immunoprecipitation techniques has allowed the first views of how various helper cell–expressed transcription factors act on a genome-wide scale to contribute to helper cell transcriptomes. This is important because we can begin to determine direct versus indirect effects. If a transcription factor is important, we can more precisely dissect why this is the case.

What we have learned is that many of the key genes associated with particular fates are direct targets of STATs and master regulators. These transcription factors bind at thousands of sites in the genome in a sequence-specific manner and regulate the transcription of their target genes. STATs and master regulators are known to activate many genes, although they are also responsible for silencing of genes expressed in other cell fates. Disrupted binding of transcription factors by disease-associated single nucleotide polymorphisms are now being linked to changes in the transcriptional profile in relevant cell types.[170] However, it must be emphasized that when we consider stability versus flexibility of helper T cells, more often than not, only a few genes are interrogated and not entire transcriptomes. However, as the technology for measuring global gene expression becomes available, it will be important to factor in global information on all genes as we consider to what extent different populations of cells appear to be stable.

EPIGENOMIC VIEWS OF HELPER T-CELL STABILITY AND FLEXIBILITY

Although the specific functions of different helper T cells are obviously a reflection of distinctive patterns of global gene expression and the action of transcription factors, this is not the whole story. Transcription factors do not act in isolation; for them to exert their effect, the region of the genome on which they are acting must be accessible. We are now beginning to understand what this means on a genome-wide scale and to identify the biochemical and cell biology underpinnings of what it means for a gene and its regulatory elements to be accessible. Classically, the term epigenetic has been used to refer to heritable changes in gene expression that are not due to changes in the DNA sequence. It is now clear that many factors contribute to how and when different portions of the DNA code can be read. From this perspective, a modern view of ***epigenetics*** can be viewed as encompassing the combined action of the many factors that will be further discussed below. On a global scale, this can be referred to as epigenomics.

DNA is bound to histone molecules to form nucleosomes, which can exist in accessible (euchromatin) or inaccessible (heterochromatin) states. The histone components of the nucleosome are associated with an array of posttranslational covalent modifications. For example, histone 3 lysine 4 trimethylation (H3K4me3) is associated with active promoters, and histone 3 lysine 36 trimethylation (H3K36me3) is indicative of active transcription of genes. In contrast, histone 3 lysine 27 trimethylation (H3K27me3) and histone 3 lysine 9 trimethylation (H3K9me3) are marks of silenced genes. For example, the H3K4me3 and H3K27me3 status of the *PU.1* promoter acts as a unique regulator of T_H9 memory acquisition and T_H9 immunity.[171]

A variety of enzyme complexes (Trithorax and Polycomb complexes) deposit these marks, and other enzymes (eg, Jmjd3) can remove these marks; however, these are just a few of the many modifications that have been described. The movement of nucleosomes is also regulated by ATP-dependent enzymes (Brg1, the Swi-Snf complex, and other factors). DNA methylation is another important factor that dictates whether genes can be read. All these factors working in concert influence the accessibility of genes to the action of transcription factors.

However, it needs to be emphasized that genes represent only 2% of the genome. Equally impressive are recent discoveries provided by the Encode project, which has shown that 80% of the so-called junk DNA is active. Among the vast numbers of switches and regulatory hubs that reside in the junk DNA are enhancers. We have known for many years that the regulation of a limited number of genes is carefully regulated by a complex architecture of distal enhancers. For instance, many studies focused on the distal cis-regulatory elements of lineage-specific cytokine genes. The *IFNG/Ifng* locus encompasses approximately 200 kb with multiple distal enhancers.[172] The *IL4/Il4* locus also comprises multiple enhancers, as well as a silencer element.[173] Enormous stretches of DNA are required for proper regulation of these key genes. Similarly, the *Foxp3* gene is also regulated by distinct enhancer elements.[174] Current technology now allows enumeration of active and poised enhancers on a genome-wide scale. What is becoming clear is that the enhancer landscape is cell specific, and in this way, cell identity is a reflection not only of genes expressed at any given time but also of what genes might be expressed based on accessibility of key regulatory elements. Finally, gene expression is also regulated by the actions of long noncoding RNAs and microRNAs.

Functionally, we already know that epigenetic regulation of helper T cells is important.[175,176] Deletion of BRG1 interferes with IFN-γ production.[177] Similarly, the cohesin protein complex

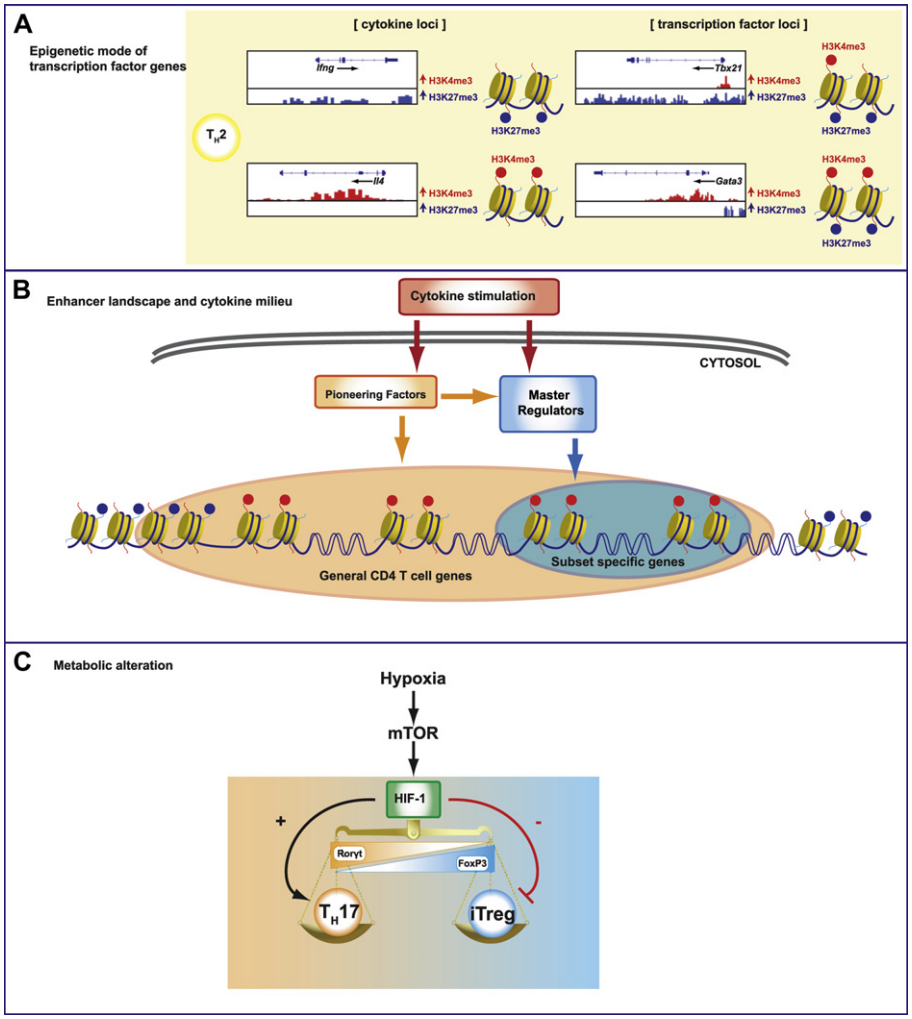

FIG 3. Mechanisms underlying plasticity of helper T cells. **A**, Specific modifications of the histone molecules that make up the nucleosome are associated with accessibility. **B**, Cytokine milieu can induce pioneering factors and activate different STATs, which are indispensable for establishing the genomic epigenetic landscapes of developing helper T cells and dictating the accessibility of key target genes. Master regulators can be better viewed as critical modulators but not drivers of the chromatin architecture underlying helper cell identity. **C**, Alterations in T-cell metabolism also have critical roles in the plasticity of helper T-cell lineages. *iTreg*, Peripheral antigen-induced Treg cells.

is also important for the maintenance of gene architecture; deletion of a component of this complex also blocks IFN-γ.[178,179] Disruption of the Trithorax complex causes aberrant T_H2 differentiation,[180,181] and deletion of the H3K9 methylase results in inappropriate expression of T_H1 genes in T_H2 cells.[182] Deletion of the DNA methyltransferase DNMT1 in T cells leads to loss of silencing of T_H1 and T_H2 genes in the opposing subset.[183-185]

Thanks to technological advances, genome-wide mapping of histone modifications in helper T cells has been accomplished.[103] Consistent with the standard lineage commitment view of helper cell differentiation, signature cytokine genes exhibit unopposed permissive marks (H3K4me3) in the appropriate subsets (eg, *Ifng* in T_H1 cells) and repressive marks (H3K27me3) in other subsets that do not express these cytokines (Fig 3, *A*). Contrary to expectation, genes encoding master regulators, including *Tbx21*, *Gata3*, *Bcl6*, *Runx3*, and *Prdm1*, have complex marks: the genes exhibit both accessible and repressive marks (Fig 3, *A*).[103] This helps explain the flexible expression of master regulator genes. The action of master regulators and STAT proteins on global histone modifications has also been elucidated. STATs can affect target genes by affecting transcription and epigenetic modifications or by only affecting transcription (approximately 11%). For a sizable number of genes, STATs only affect epigenetic modifications (approximately 20%). It is clear that deletion of some master regulators has an effect on the ***epigenome***. However, not all lineage-specific factors are essential for the epigenetic status: T-bet and GATA3 clearly have global epigenetic effects, but other factors, such as Rorγt and Foxp3, have little effect on the epigenetic landscapes of their respective subsets. Overall, it appears that target genes of master regulators, such as Foxp3 and Rorγt, are prepared by other transcription factors, and these master regulators are mostly modulators of expression and have focused effects on promoters.[148,163,186]

The global enhancer landscapes of helper T-cell subsets have begun to be elucidated. This has already provided a number of surprises. First is the extent to which the active enhancer

landscapes are distinct. T_H1 and T_H2 cells exhibit roughly 10,000 of these elements, of which only half are shared. If macrophages are also analyzed, the number of elements decreases to roughly 1,000 active elements. This is very consistent with evolving notions of the cell-specific nature of enhancer landscapes.

The actions of STATs and master regulators are also quite different. STATs have a major effect on the acquisition of active elements. In contrast, the effect of the master regulators is much more restricted, although master regulators vary in their capacity to affect global landscapes. Some master regulators, such as T-bet, Rorγt, and Foxp3, have very small effects on creating active enhancer elements; rather, these factors appear to highjack existing sites that are already created by other factors.[148,163,186] Studies on these 3 transcription factors support the idea that these so-called master regulators have focused roles, particularly on the promoter of their target genes and are not capable of globally changing the chromatin signature of enhancers.

In summary, cytokines change the behavior of cells and work in conjunction with other transcription factors to effect changes in chromatin and gene expression (Fig 3, *B*). The extent to which cytokines acting through STATs influence the global chromatin organization was unexpectedly broad. Alterations in gene expression have long been understood to be the result of signaling events because transcription factors can be activated or induced by exogenous factors. However, the epigenome is increasingly understood to be the outcome of signal transduction events as well. If we consider cell identity as a reflection of what functionalities are apparent and those that are possible based on accessibility to alternative gene programs, it is clear that alterations in the cytokine milieu can have substantial effects on cell identity.

BACK TO THE FUTURE: METABOLIC REGULATION OF HELPER T CELLS

A surprising new development is the appreciation that alterations in T-cell metabolism have critical roles in selective regulation of cytokine production. Alterations in the availability of nutrients has long been known to affect immune responses, such as in patients with type II diabetes or after profound starvation. The transition from a resting naive T cell into an activated, rapidly proliferating effector T cell requires a substantial change in the metabolic machinery within the cell. However, it was not recognized that these were 2 distinct effects. We now appreciate that the metabolism of a proliferating lymphoblast resembles that of a cancer cell with an ability to rapidly consume both glucose and amino acids, chiefly for the purpose of creating new protein and nucleotides for cell growth.[187] Consequently, little of the ATP generated by glucose metabolism is provided by aerobic metabolism. The majority is generated by anaerobic glycolysis.[187]

The increased uptake of glucose and glutamine in activated lymphocytes is to be mediated by the Ras/mitogen-activated protein kinase and phosphoinositide 3-kinase pathways, which are activated by T cell receptor– and IL-2–dependent signals. Activation of either receptor is capable of sustaining T-cell proliferation *in vitro*, and both are able to activate the kinase mammalian target of rapamycin (mTOR), which comprises 2 functionally distinct signaling complexes: mTOR complex (MTORC) 1 and 2 (mTORC2).[188] mTORC1 is activated by the anabolic phosphoinositide 3-kinase/AKT signaling pathway and is inactivated in the absence of available nutrients. It is a checkpoint regulator of protein synthesis through the phosphorylation of the p70 S6 kinase and 4E-BP1.[188] In contrast, mTORC2 is required for the phosphorylation of several AGC family kinases, including AKT, serum- and glucocorticoid-induced protein kinase 1, and some protein kinase C family members.[189,190]

Genetic deletion of mTOR or inhibition by ***rapamycin*** in mouse T cells inhibits effector cell differentiation and promotes generation of Treg cells.[191] It is of note that Treg cells do not depend on anaerobic glycolysis to the same degree as effector cell lineages.[192] T cells that lack RAPTOR, a key component of mTORC1, demonstrate impaired T_H1 and T_H17 differentiation,[193] whereas T cells that lack RICTOR, a key component of mTORC2, have impaired T_H2 differentiation.[194] HIF1 is a key metabolic sensor. HIF promotes T_H17 differentiation and attenuates Treg cell development (Fig 3, *C*).[157,195] The extent to which metabolic perturbations might affect polarized subsets of helpers is only just beginning to be studied. However, the idea that acute metabolic changes can selectively affect helper T cell genetic programs has profound implications.

CONCLUSIONS

Ultimately, why should these plasticity and epigenomic studies matter to clinical allergists? Understanding the complex programs of differentiation of helper T cells in response to intrinsic and extrinsic clues is a fascinating basic science problem; however, there are also several benefits. From the point of view of someone interested in the genetics of allergy, a better view of the landscape of critical transcription binding sites might open a new paradigm for finding genetic causes of common allergic diseases. Instead of overt syndromes, polymorphisms in individual transcription factor binding sites might alter the risk for disease and provide a very specific cause for certain symptom constellations, as well as ultimately hinting toward better therapeutic interventions. Finding polymorphisms in junk DNA might initially seem unrevealing; however, because we understand the functional relevance of the junk, some surprises might emerge. Such examples have been found in nonallergic settings already.[196]

Although genetics plays a large part in the susceptibility to autoimmune and allergic disease, environmental factors are also major contributors.[197-199] Better understanding of the factors that influences the epigenomes of CD4 T cells could allow us to develop interventions that keep the good phenotypes of helper T cells while skewing the bad ones away from their pathogenic effector capacities, even after they have already differentiated from the naive state. This is exciting because we have targeted therapies, both biologic and oral agents, that can influence responsiveness to cytokines[200,201]; precisely how these therapies influence epigenomes and transcriptomes of immune cells and how this relates to cellular plasticity will be exciting to ascertain. Will we be able to thoughtfully reprogram immune cells? Perhaps immunotherapy or some of the cytokine or anticytokine therapies in place now do this. However, a more sophisticated understanding will help us develop cellular assays, which could more accurately predict their efficacy in a perspective patient and/or follow the treatment to determine whether it is working before clinical improvement is noted. In an era of personalized medicine, such insight and tools will become more and more important. The exciting part is that we have the tools and are now able to get started.

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