Mechanisms of allergic diseases

T-cell effector pathways in allergic diseases: Transcriptional mechanisms and therapeutic targets

Talal A. Chatila, MD,a Ning Li, PhD,b Maria Garcia-Lloret, MD,a Hyon-Jeen Kim, PhD,b and Andre E. Nel, MD, PhDb

Los Angeles, Calif

Originally interpreted within the framework of a binary TH1/TH2 paradigm, our knowledge of the pathogenesis of atopic diseases has broadened to incorporate the contribution of regulatory cells and the newly described proinflammatory TH17 cell lineage. The commitment of peripheral T-cell clones to undergo differentiation into one of those lineages is shaped by self-reinforcing transcriptional circuits that center on key transcriptional regulators: T-box expressed in T cells (T-box1), GATA-3 (TH2), forkhead box p3 (T regulatory cells), and retinoid-related orphan receptor γ/retinoid-related orphan receptor α (TH17). These circuits function both to establish the respective lineage phenotype and to enable epigenetic changes that maintain those phenotypes long-term. This evolving view of how signaling and transcriptional networks generate effector T-cell responses suggests novel therapeutic approaches to reprogram effector T-cell lineage commitment in allergic diseases in favor of tolerance induction. (J Allergy Clin Immunol 2008;121:812-23.)

Key words: Transcriptional regulation, T-cell differentiation, TH1, TH2, Treg, TH17, T-bet, GATA-3, Foxp3, RORγt, atopic disease, asthma, therapeutic strategies, oxidative stress, Nrf2

Transcriptional regulation of immune response is dependent on a number of coordinated events involving a network of transcription factors (TFs). Sequential activation and combinatorial interactions of TFs are the most important determining factors that govern the fate and function of immune cells. Similar to other organs and tissues, numerous TFs are involved in regulating immune function.

Originally interpreted within the framework of a binary TH1/TH2 paradigm, our knowledge of the pathogenesis of atopic diseases has broadened to incorporate the contribution of T regulatory (Treg) cells and the newly described
Abbreviations used

ARE: Antioxidant response element
αTreg: Adaptive T regulatory
c-MAF: Musculoaponeurotic fibrosarcoma oncogene homolog
Foxp3: Forkhead box p3
GR: Glucocorticoid receptor
IL-12Rβ1: IL-12 receptor β1
IPLEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked
NFAT: Nuclear factor of activated T cells
NR: Nuclear receptor
Nrt2: Nuclear factor-erythroid 2-related factor 2
nTreg: Natural T regulatory
ROR: Retinoid-related orphan receptor
RUNX: Runt-related transcription factor
STAT: Signal transducer and activator of transcription
T-bet: T-box expressed in T cells
TCR: T-cell receptor
TF: Transcription factor
Tr-1: T regulatory type 1
Treg: T regulatory

GLOSSARY

3′ ENHANCER: Enhancer DNA sequences increase gene transcription. The 3 enhancer sequencer can be located at long distances in upstream, downstream, or intronic locations in DNA relative to the transcription initiation site.

CHROMATIN REMODELING: A process whereby DNA that is tightly bound around histones and inaccessible to proteins becomes “opened” by the action of histone acetylases allowing gene transcription. Some coactivators of TFs have the ability to acetylate histones, with hypo-acetylation associated with gene silencing. Acetylation removes positive charges on lysine residues of histones and loosens the electrostatic interaction with negatively charged DNA.

Cytotoxic T-Lymphocyte-Associated Antigen 4 (CTLA-4), Glucocorticoid-Induced TNF Receptor-Related Protein (GITR): CTLA-4 limits activation through the costimulatory molecules CD80/CD86. GITR interacts with its receptor (GITRL) on antigen-presenting cells to coactivate effector T cells. The exact role of GITR in activating Treg cell suppressive activity is unclear, but the expression of GITR in Treg cells depends on the TF Foxp3.

Dominant-Negative, Antisense DNA, Small Interfering RNA (siRNA): These terms all describe techniques that reduce gene expression. A dominant-negative TF binds DNA but does not allow transcriptional activation to occur; antisense DNA binds to target mRNA and recruits ribonuclease H (degrades RNA) or inhibits translation to protein; siRNA silences RNA by targeting it for degradation.

EPIGENETIC: Genetic changes affecting cellular phenotype (as defined by gene expression) through DNA methylation, chromatin remodeling, and histone modifications are called epigenetic changes. These changes are transmissible to progeny but are also reversible, because the genetic code remains unchanged.

GATA-3: GATA-3 is a member of the guanosine, adenosine, thymidine, adenosine (GATA) family of zinc-finger DNA binding proteins. GATAs 1-3 are hematopoietic, and GATAs 4-6 are nonhematopoietic. GATA-3 is upregulated in T1 cells, which is important for IL-4, IL-5, and IL-13 gene expression. Blunting of GATA-3 expression decreases T1-mediated asthma.

Granzymes: Granzymes are serine proteases from cytotoxic T cells required for effective target cell killing; granzymes can act in concert with perforin.

Histone Acetylase: See Chromatin Remodeling.

Hypomethylated: Methylation normally occurs at CpG dinucleotides and prevents TFs from binding to DNA. Methylation also recruits methyl-CpG binding proteins that repress chromatin remodeling. Hypomethylated DNA is thus more readily transcribed.

IL-12, IFN-γ: IL-12 is composed of biologically active p40/p35 heterodimers stimulates production of IFN-γ and produces a shift to a Th1 cell phenotype. IL-12p40, IL-12Rβ1, STAT1, and IFN-γ receptor deficiency all lead to severe nontuberculous mycobacterial infections.

IL-17: IL-17 induces histone acetylation via nuclear factor-κB activation of gene transcription. Glucocorticoids can interfere with IL-17–induced histone acetylation.

Palindrome: DNA sequences that read identically 5′ to 3′ on each strand of DNA are termed palindromic—for example, the STAT consensus binding site: 5′-TTCC(Xn)GAA-3′, 3′-AAG(Xn)CTT-5′.

Retinoic Acid Receptor (RAR), Peroxisome Proliferator-Activated Receptor (PPAR): RAR and PPARβ/δ both bind retinoic acid, both dimerize with retinoid X receptor; ligand binding protein ratios will tip the balance to retinoic acid binding to RAR, causing cell survival, or PPARβ, causing apoptosis. PPARγ can have anti-inflammatory effects in asthma.

Signal Transducer and Activator of Transcription (STAT): The signal transducer and activator of transcription family of TFs become phosphorylated, dimerize, and bind to palindromic DNA elements in response to Janus-activated kinase pathways. STAT1,2 is involved in IFN signaling. STAT3 is involved in IL-2, IL-6, IL-10 signaling and in IL-21, IL-1, IL-23 signaling for RORγT+ T17 cells. STAT4 is involved in IL-12 signaling and activates T-beta transduction. STAT5 is required for IL-2–stimulated T cell development. STAT6 is important for IL-4, IL-13 signals and activates GATA-3 gene expression.

T17, IL-17: T17 cells are CD4+ T cells that produce IL-17A, IL-17F, IL-6, IL-21, IL-22, and TNF-α and are involved in autoimmunity. T17 CD4+ T cells’ production of IL-17 is increased by IL-23 secreted by dendritic cells. IL-23 activation of the TF STAT3 maintains the T17 phenotype of the CD4+ T cells. IL-17 induces IL-1β, IL-6, and chemokine CXCL1,2,8 production, causing increased neutrophil recruitment and possible fibrosis. IL-17 is thought to reduce tissue pathology in autoimmune diseases.

Transcription factor (TF): TFs are proteins that bind DNA to activate or repress gene expression. TFs are often modular in composition with the DNA-binding, dimerization, transactivation, and/or transrepression domains. TFs often belong to families defined by conserved modules—for example, homeobox, leucine zipper, and forked head.

The Editors wish to acknowledge Seema Aceves, MD, PhD, for preparing this glossary.
TRANSCRIPTIONAL CIRCUITS AND TH CELL PHENOTYPE MAINTENANCE

The capacity of master regulatory factors to orchestrate TH cell differentiation is critically dependent on the induction by those factors of transcriptional circuits that establish the TH cell phenotype and maintain its stability. They also benefit from epigenetic changes that are brought about by the action of those circuits, and from the modular nature of TF domains, which allow 1 factor to simultaneously mediate interactions with DNA, other TFs, histones, and histone-modifying enzymes. Expression of alternatively spliced forms of these factors presents an additional layer of regulation that may enable selective interactions with other transcriptional regulators that, ultimately, affect cell fate decisions. Once their expression is established by cell-extrinsic signals, many lineage-determining TFs maintain their own expression by autoinduction. Examples of how such attributes govern T-cell differentiation are described here.

T-bet and TH1 differentiation

The development of the TH1 response requires T-cell receptor (TCR) stimulation and TH1-promoting cytokines from the antigen presenting cells such as IL-12 and IFN-γ. Although a major function of this axis of immunity is to eliminate intracellular pathogens such as viruses and bacteria, unhindered TH1 responses also play a role in the pathogenesis of autoimmune diseases. The signaling pathway that leads to the development of TH1 lineage is initiated by the pathogens that stimulate IFN-γ and IL-12 production. Under TH1-inducing conditions, expression of native TH1 cells to IFN-γ during TCR engagement activates signal transducer and activator of transcription (STAT)–1, which in turn, activates its downstream TF, T-box expressed in T cells (T-bet). T-bet has been considered a specific master regulator for TH1 differentiation, and there is a strong correlation between T-bet expression and IFN-γ production.

T-bet, also known as TBX21, is a member of the T-box family of TFs. Members of this family share a conserved DNA binding domain known as a palindromic 20-bp DNA sequence. T-bet itself is a 530–amino acid long protein in which the T-box DNA binding domain is flanked by 2 transcriptional activation domains. T-bet is essential for the genetic program of CD4+ TH1 cell lineage differentiation. Another T-box factor, eomesodermin, is a paralog of T-bet that promotes IFN-γ production and cytolytic activity of CD8+ T cells.

T-bet displays the attributes of a TH1 lineage commitment factor, including the capacity to initiate TH1 cell differentiation independently, to activate its own expression, and to promote chromatin remodeling at target loci. Ectopic expression of T-bet leads to strong transactivation of the IFN-γ gene and, reciprocally, represses TH2 gene expression. Importantly, forced expression of T-bet in established TH2 cells reprograms the cytokine profile into that of TH1 cells. These attributes of T-bet are enabled both by the direct action of T-bet at target promoters, including those of IFN-γ, IL-12 receptor β1 (IL-12Rβ1), and IL-4 genes, and by the activation of secondary transcriptional circuitries that work in synergy with T-bet to enforce the TH1 genetic program (Fig 2). One such circuit involves the TF runt-related transcription factor (RUNX)–3, which is induced in differentiating TH1 cells in a T-bet–dependent manner. Unlike T-bet, RUNX3 is unable to initiate TH1 differentiation independently. However, the binding of both factors to the IFN-γ promoter enables maximal gene expression. The 2 factors also synergize to repress IL-4 gene expression by cooperatively binding to a composite response element in a silencer region at the 3’ end of the IL-4 gene. A second transcriptional circuit involving a T-bet–induced homeobox factor, H2.0-like homeobox (HLX), also acts to reinforce IFN-γ gene activation positively. These interactions enable the
establishment of stable epigenetic changes at the IFN-γ and IL-4 gene loci that sustain a terminally differentiated Th1 phenotype. T-bet induces stable DNase I hypersensitivity sites in the IFN-γ locus, reflecting active chromatin remodeling that involves histone acetylation and methylation. These changes are ultimately transmitted into daughter cells of terminally differentiated Th1 cells in a T-bet–independent manner.

T-bet expression is induced on signaling via the IFN-γ receptor and the downstream TF STAT1, and both IFN-γ receptor deficiency and STAT1 deficiency result in profound defects in T-bet expression and Th1 differentiation. IFN-γ activates T-bet expression by a STAT1-dependent mechanism and, in turn, T-bet induces the transcription of the IFN-γ gene to establish a positive feedback loop. T-bet can also promote IFN-γ production indirectly by upregulating IL-12 receptor β2 expression, which combines with the constitutively expressed IL-12Rβ1 to form a functional IL-12 receptor. IL-12, acting via STAT4, can then induce IFN-γ production independent of T-bet. An alternative model of Th1 differentiation has also been proposed that lays emphasis on the IL-12/STAT4 circuit as the initiator of Th1 cells in a T-bet–independent manner.

True T-bet deficiency has not been reported in human beings, but specific T-bet haplotypes have been associated with asthma and airway hyperresponsiveness. Loss of function mutations in components of the Th1 circuits in human beings, including STAT1 and IFN-γ and IL-12 receptors, give rise to susceptibility to mycobacterial infections. In the case of STAT1, which links with the IFN-α and IFN-β receptors, there is also susceptibility to some viral infections as well. These mutations have not been linked to profound Th2 skewing, consistent with the capacity of more than 1 pathway (STAT1, STAT4) to mediate T-bet activation. On the other hand, mutations in Tyk2, a Janus kinase family member that participates in several cytokine signaling pathways involved in innate and adaptive immunity, including type I (IFN-α/β) and type II (IFN-γ IFNs, IL6, IL-10, IL-12, and IL-23), gives rise to impaired Th1 cell differentiation, overt Th2 skewing, and a hyper-IgE–like syndrome with susceptibility to bacterial, mycobacterial, viral, and fungal infections.

**GATA-3 and Th2 differentiation**

Th2 cells are involved in humoral immune responses and are essential in host defense against parasitic infection. On the other hand, Th2-driven responses are central to the development of atopic diseases. Differentiation of naive CD4+ cells into effector Th2 cells requires IL-4 receptor and TCR stimulation and is characterized by the production of IL-4, IL-5, and IL-13. Th2 lineage commitment is typically initiated by signaling via the IL-4/IL-4 receptor/STAT6 axis, which upregulates expression of GATA-3. In turn, GATA-3 autoinduces its own expression in a classic positive feedback loop. GATA-3, a member of the GATA family of zinc finger–type TFs, is both necessary and sufficient for Th2 cell differentiation.

**FIG 2.** Transcriptional circuitries that enforce Th1 and Th2 lineage commitment. Each circuit is composed of 1 primary (T-bet or GATA-3) and a number of secondary regulators that are induced and mobilized by the primary transcription factors. In addition to enforcing commitment to the appropriate lineage, these circuits simultaneously repress commitment to alternative lineages. HLX, H2.O-like homeobox.
Expression of GATA-3 in naïve T cells is sufficient to drive their differentiation into Th2 cells, whereas its forced expression in developing Th1 cells drives de novo Th12 cytokine expression and induces chromatin remodeling at target cytokine gene loci. 28,30,31 Antagonism of GATA-3 expression in T cells inhibits Th2 cell differentiation both in vitro and in vivo. 29,32 The primacy of GATA-3 in the establishment of the Th2 cell lineage is further evidenced from experiments with STAT6-deficient T cells, which differentiate into full-fledged Th2 cells on their transduction with GATA-3. 30

The requirement of GATA-3 for IL-4 production early in Th2 differentiation is intimately tied to chromatin remodeling at the Il4 locus. However, this requirement becomes less acute when the Th12 phenotype has been established. Disruption of the GATA-3 gene in established Th2 cells is associated with a mild decrease in the number of IL-4–producing Th2 cells and a 2- to 3-fold decrease in the magnitude of IL-4 production. 33,34 Chromatin remodeling at the IL-4 locus in established Th2 cells is largely maintained, but the accessibility of the IL-4 gene at its 3' enhancer remains dependent on the presence of GATA-3. The effect of sudden GATA-3 deficiency on the production by established Th2 cells of other Th2 cytokines is more severe, with complete loss of IL-5 and IL-13 expression. Thus, overall, the continued expression of GATA-3 is required for the effective maintenance of Th2 cell differentiation.

In addition to its central function in enabling Th12 cytokine production, GATA-3 promotes Th2 cell differentiation by 2 other complementary mechanisms. First, it makes possible the expansion of Th2 cells by the action of growth factor independent 1 (GFI), an IL-4/STAT6–inducible gene that promotes Th2 cell proliferation. Second, GATA-3 inhibits T-bet expression and Th1 cytokine production in Th2 cells. De novo deficiency of GATA-3 in established Th2 cells is rapidly followed by the upregulation of T-bet expression and IFN-γ production. 33,35

Consistent with the Th2 bias encountered in asthma, GATA-3 mRNA is elevated in the airways of patients with asthma compared with controls. 36 Antagonism of GATA-3 by the expression in T cells of a dominant-negative GATA-3 transgene or by the delivery into the airways of antisense DNA or small inhibitory RNA (siRNA) ameliorates allergic inflammation in experimental asthma models and decreases airway eosinophilia and Th2 cytokine production. 32,37,38

Downstream of GATA-3 is the TF musculoaponeurotic fibrosarcoma oncogene homolog (c-MAF), which is expressed in Th12 cells and binds to the proximal IL-4 promoter (Fig 2). 30,39,40 c-MAF is a basic leucine zipper TF that selectively induces IL-4 expression in synergy with other factors such as the nuclear factor of activated T-cells (NFAT) interacting protein 45 and NFAT at the c-MAF response element within the IL-4 proximal promoter. 41 Overexpression of c-MAF enhances IL-4 production and, secondarily, Th2 responses. By contrast, c-MAF–deficient T cells differentiated under Th12 conditions show a selective decrease in IL-4 production, but not other Th12-specific cytokines. Although c-MAF functions as a specific transactivator of the IL-4 gene, it appears to be dispensable for chromatin remodeling at the IL-4 locus.

**Foxp3 and Treg cell differentiation**

T regulatory cells play a key role in tolerance to self-antigen and prevention of autoimmune diseases, as well as in inappropriate immune responses involved in allergic diseases. 42-44 A majority of peripheral Treg cells are programmed in the thymus and are known as natural Treg (nTreg) cells. 44 Other Treg cells, also known as induced or adaptive (aTreg) cells, are derived de novo from a naïve CD4+ precursor pool in peripheral lymphoid tissues after encountering exogenous antigen under the influence of TGF-β.

T regulatory cells are characterized by the expression of a distinctive combination of surface antigens including the IL-2 receptor α-chain (CD25), cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), and glucocorticoid-induced TNF receptor–related protein (GITR). Unlike conventional T cells, they do not produce IL-2, but are dependent of IL-2 and TGF-β for peripheral expansion and function. 35,45 A cardinal feature of Treg cells is their high level expression of the forkhead-family TF forkhead box p3 (Foxp3), which is indispensable to their suppressive activity, phenotype stability, and survival in the periphery. 46 In human beings, loss of function Foxp3 mutations result in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. 42,46 The hallmark of IPEX is immune dysregulation caused by the lack of functional Treg cells. It typically presents during infancy with enteropathy, autoimmune endocrinopathy, immune-mediated cytopenias, dermatitis, and intense allergic dysregulation manifesting as food allergy and elevated IgE levels. Foxp3 deficiency in mice, whether a result of natural or induced mutations, gives rise to a fatal autoimmune and inflammatory disorder called Scurfy that has many of the features of IPEX. IPEX-like syndromes in human beings and in mice also arise from mutations along the IL-2 signaling pathway, including loss of function mutations in IL-2 receptor α-chain (CD25) and the IL-2–responsive TF STAT5b, the latter with an associated phenotype of resistance to growth hormone. 49,51

The essential role of Foxp3 in Treg cell biology is supported by several lines of evidence. Forced expression of Foxp3 in effector T cells endows them with regulatory properties and some, but not all, of the phenotypic markers of Treg cells. 52,53 Foxp3 deficiency is permissive to the development in the thymus of Treg cell precursors that share many of the phenotypic and genetic attributes of Treg cells. However, unlike Foxp3-sufficient Treg cells, Foxp3-deficient Treg cell precursors fail to mediate suppression. 54,55 Once in the periphery, they acquire attributes of an activated cytotoxic cell phenotype. They express high levels of mRNA-encoding granzymes, some killer cell markers, and a mixed Th1 and Th2 cytokine profile. In particular, they secrete large amounts of IL-4 and other Th12 cytokines, which accounts for much of the allergic dysregulation associated with Foxp3 deficiency. 54-56 Circulating Foxp3-deficient Treg cell precursors also exhibit a high rate of apoptotic death, possibly as a result of suboptimal response to growth factors such as IL-2. The continued requirement for Foxp3 expression to maintain the phenotype of mature Treg cells in the periphery was demonstrated in experiments in which the acute inactivation of the Foxp3 locus rapidly led to the loss of Treg cell regulatory function. Acute Foxp3 deficiency also alters the transcriptional program of Treg cells in a manner reminiscent of that of Foxp3-deficient Treg cell precursors. 57

Foxp3 was initially thought to function as a transcriptional repressor. 58 However, it has become clear that Foxp3 may function as either a transcriptional activator or repressor depending on the context. 59,60 The transcriptional functions of Foxp3 are enabled by its capacity of its different domains to interact with distinct
sets of regulatory proteins to form large macromolecular transcriptional complexes. An N-terminal domain that mediates transcriptional activation and repression associates with the histone acetyltransferase Tat-interactive protein, 60 kd, and class II histone deacetylases HDAC7 and HDAC9. A zinc finger domain and leucine zipper domains mediate a second set of interactions, with the leucine zipper domain especially important for homologimerization and heteroligimerization of Foxp3 with related members of the Foxp family. The distal part of the protein includes a carboxyl-terminal forkhead domain that mediates binding to specific DNA response elements. It also includes residues that contribute to the physical association of Foxp3 with other TFs, including NFAT and RUNX1/acute myelogenous leukemia-1, both of which contribute to the transcriptional program and suppressive functions of Treg cells, including the TCR/NFAT, IL-2/STAT5, and TGF-β pathways converges on the Foxp3 promoter/enhancer to activate Foxp3 gene expression efficiently.

Foxp3 cooperates with other TFs, including NFAT and RUNX1/AML1, to activate transcription of a number of genes and suppress that of others (such as IL2). A target of Foxp3 is the Foxp3 gene itself, which is endowed with a number of forkhead factor response elements. Foxp3 deficiency is associated with decreased Foxp3 expression in the Treg cell precursors that do develop, consistent with autoinduction of Foxp3 by a positive feedback loop.

Treg cells are unique among the effector T-cell subsets in being composed of 2 developmentally distinct populations: natural Treg (nTreg) cells, which develop in the thymus, and adaptive Treg (aTreg) cells that are induced de novo in the periphery from conventional T cells. The 2 populations display a close affinity in their regulatory function and phenotype but are not identical. The phenotypic and genetic attributes of nTreg cells are “hard-wired,” with most of them persisting even in the absence of Foxp3. This is a reflection of an irreversible commitment to the Treg cell lineage that occurs in the course of thymic selection and maturation of nTreg cells. In contrast, aTreg cells are “plastic,” developing on antigenic stimulation of conventional T cells in the presence of TGF-β and IL-2, The de novo activation of Foxp3 transcription in aTreg cells proceeds through the agency of several TFs that include NFAT (TCR), Smad3 (TGF-β), and STAT5 (IL-2). Foxp3, whose expression in aTreg cells is induced by the action of TGF-β and T-cell receptor signaling, is absolutely required for the suppressive functions of aTreg cells, similar to the situation of their nTreg cell counterparts (D. Haribhai, T. A. Chatila, C. B. Williams, unpublished data, December 2007). aTreg cells have been demonstrated to develop during induction of oral tolerance to an allergen and may play an important role in tolerance induction in immunotherapy. Their phenotype, however, maybe less stable than that of nTreg cells. Whereas the Foxp3 locus is stably hypomethylated in natural Treg cells, it is weakly so in adaptive Treg cells. The suppressive function and Foxp3 expression levels of the latter may accordingly decline over time.

In addition to the Foxp3+ aTreg cells, another class of Treg cells is the Foxp3+IL-10+ T regulatory type 1 (Tr-1) cells, derived by the ex vivo activation of naive CD4+ T cells in the presence of IL-10 or by IL-10–conditioned dendritic cells. Previous studies attempting to track these cells in vivo have been impeded by the lack of clear markers that could distinguish Tr-1 cells from Foxp3+ nTreg and aTreg cells, which also express IL-10, especially after activation. Recent studies
have overcome this limitation by using IL-10 locus-tagged mice, revealing Foxp3+ Tr-1–like cells to be particularly abundant in the small and large intestine, where they play an essential role in downregulating the inflammatory response triggered by the commensal flora.\textsuperscript{75-77} Foxp3+ Tr-1–like cells share with Foxp3+ aTreg cells a requirement for TGF-β for their in vivo differentiation, but it remains unclear whether Tr-1 cells branch off from a common differentiation pathway with Foxp3+ aTreg cells or arise by a separate pathway. Although earlier studies have implicated Tr-1 in tolerance to allergens, especially after immunotherapy, the precise contributions of Foxp3+ Treg cells and Foxp3+ Tr-1 cells to induced tolerance to allergens remain to be carefully analyzed with the newer tools now available.\textsuperscript{8,}\textsuperscript{8}

**RORγT/RORα and Th17 differentiation**

In addition to the Th1, Th2, and Treg cell lineages, another effector T-cell lineage has been recently introduced, namely Th17 cells.\textsuperscript{79,80} Th17 cells are characterized by the production of their distinct cytokines including IL-17A, IL-17F, and IL-22. The IL-23/IL-17 axis has been reported to be involved in immunity to bacterial and fungal pathogens.\textsuperscript{81-84} The proinflammatory role of Th17 was first identified in autoimmune disorders such as experimental autoimmune encephalitis and collagen-induced arthritis.\textsuperscript{85,86} Emerging evidence indicates that Th17 may also be involved in the pathogenesis of various chronic immune-inflammatory diseases in human beings.\textsuperscript{85,86} Although the exact role of Th17 in atopy and asthma is still unclear, available data suggest that this T-cell lineage may contribute to the pathogenesis of atopic diseases such as asthma, at least in part, by inducing neutrophilic inflammation. Data from experimental models suggest both a proinflammatory and a regulatory role for IL-17 in experimental asthma, depending on the degree of sensitization and the phase of the allergic inflammatory response.\textsuperscript{87,88}

The development of Th17 is independent of the STAT signaling pathway required for Th1 and Th2 differentiation, but it shares a common requirement with Treg cells for TGF-β, at least in the murine system. Although TGF-β alone induces Treg cell differentiation, a combination of TGF-β and IL-6 leads to Th17 commitment.\textsuperscript{89,90} Th17 cells do not express the specific TFs of Th1, Th2, and Treg, such as T-bet, GATA-3, and Foxp3.\textsuperscript{6} Instead, retinoid-related orphan receptor (ROR)–γt has been identified as the distinct TF that is necessary and sufficient for the signaling pathway that leads to Th17 differentiation.\textsuperscript{22,23}

Nuclear receptors (NRs) function as ligand-dependent DNA-binding proteins that translate physiological signals into gene regulation. Classic examples of this family include the receptors for steroid hormones, the retinoic acid receptors, and the peroxisome proliferator-activated receptor, all of which have been clearly shown to have important immunoregulatory functions. The NR superfamily includes “orphan” receptors for which no ligands have been identified. The RORs (α, β, and γ) make up a distinct subfamily of NR. Members of this subgroup share a similar structure with a highly conserved DNA binding domain and a less conserved putative ligand binding domain. Multiple isoforms generated by alternative splicing or differential promoter use have been identified for each member of the RORS (RORα1-4, RORβ1 and 2, and RORγ1and 2, the latter also known as RORγt).\textsuperscript{89}

The 495–amino acid long RORγt is an isoform of the orphan nuclear receptor RORγ that lacks the latter’s N-terminal 24 amino acids. RORγt is transcribed from an alternative promoter within the second exon of the RORγ gene (resulting in a 495 amino acid protein lacking the N-terminal 24 residues of RORγ).\textsuperscript{90,91} RORγ and RORγt appear to be functionally equivalent. However, whereas RORγ is widely expressed in several tissues, RORγt is almost exclusively expressed in the thymus.\textsuperscript{92} It is most abundant in thymocytes but is not expressed in B cells or in most T cells except the Th17 subset.

It is now well established that RORγt has a crucial role in T-cell homeostasis and lymphoid organ development. Early studies showed that RORγ-deficient mice are devoid of lymph nodes and Peyer patches, but in contrast with lymphoantigen-null mice, have normal splenic follicles.\textsuperscript{92,93} In the thymus, there is a depletion of CD4+ CD8+ double-positive thymocytes, which undergo accelerated apoptosis caused in part by a downregulation of the expression of the antiapoptotic gene Bcl-XL. More recently, RORγt has been shown to be necessary and sufficient for the in vivo and in vitro generation of the Th17 cells, a distinct lineage of Th cells fundamental in host defense and autoimmunity.\textsuperscript{92} RORγt-null mice have markedly diminished tissue-infiltrating Th17 cells and display increased resistance to experimental autoimmune encephalomyelitis. The residual Th17 activity could be ascribed to the action of a second retinoid-related orphan receptor, RORα.\textsuperscript{95} Similar to RORγt, RORα is highly expressed in Th17 cells, and its expression is induced in naive T cells in an IL-6, TGF-β, and STAT3-dependent manner. Forced expression of either RORγt or RORα is sufficient to confer a Th17 phenotype in naive CD4+ cells, whereas their combined expression synergistically includes IL-17 expression and Th17 cell differentiation. Like RORγt, RORα deficiency diminishes Th17 differentiation, whereas their compound deficiency globally impairs Th17 differentiation and completely protects against experimental autoimmune encephalomyelitis.\textsuperscript{95}

In human T cells, induction of Th17 differentiation is also associated with upregulation of RORγt expression. However, human Th17 differentiation appears to be dependent on IL-1β, rather than TGF-β, acting in synergy with either IL-23 or IL-6.\textsuperscript{82,96} It has been pointed out that this apparent species-specific difference in cytokine requirement for Th17 differentiation may reflect the less naive nature of the human T cells under study.\textsuperscript{97} However, whether it reflects a more fundamental difference in the mechanisms of Th17 differentiation between murine and human T cells remains to be established.

Biochemical studies on RORγt/RORγ have been limited, and it remains unclear whether it functions as a monomer or as a homodimer or heterodimer or whether its functional activity requires binding of a ligand. However, it appears RORγt can function as a context-dependent transcriptional activator or repressor.\textsuperscript{88} In the context of Th17 differentiation, RORγt could directly transactivate IL-17A and IL-17F genes or act as a chromatin-remodeling factor that opening the II17 locus for other factors to bind directly to II17 promoters.

The series of steps leading to Th17 differentiation has recently been elucidated (Fig 4, A). Differentiation is initiated by the concerted action of TGF-β and IL-6 and is reinforced by other cytokines such as IL-21 and IL-23.\textsuperscript{99,101} The molecular mechanisms by which these cytokines establish Th17 lineage commitment and expand Th17 cells are beginning to emerge. RORγt mRNA is upregulated by IL-6–induced activation of the STAT3 TF, and
STAT3-deficient mice have markedly decreased T\textsubscript{H}17 cells. IL-6 also induces IL-21 expression in activated T cells in a STAT3-dependent but ROR\textgamma-negative manner. IL-21 mediates an autocrine positive feedback loop that is important for T\textsubscript{H}17 differentiation: it acts in an IL-6-independent manner to induce ROR\textgamma expression and T\textsubscript{H}17 differentiation. It also upregulates the expression of the IL-23R subunit, which combines with the IL-12R\beta1 to form a functional IL-23 receptor. The latter cytokine acts in synergy with TGF-\beta to promote T\textsubscript{H}17 differentiation further. Downstream of IL-23, the TF STAT4 is dispensable for the initial generation of T\textsubscript{H}17 cells but appears important for IL-23-driven expansion.

In addition to STAT3 and ROR\textgamma/ROR\alpha, IFN regulatory factor 4 (IRF-4) has emerged as a third factor important to T\textsubscript{H}17 differentiation.\textsuperscript{102} Compared with wild-type cells, IRF-4-deficient T\textsubscript{H} cells have less expression of ROR\textgamma after stimulation with IL-6, and IL-6 also fails to downregulate Foxp3 expression in response to TGF-\beta. This abnormal regulation of both Foxp3 and ROR\textgamma leads to compromised T\textsubscript{H}17 cell differentiation in favor of Treg cell induction. Collectively, these studies reveal an interlocked series of cytokine-activated and self-reinforcing transcriptional circuits that guide T\textsubscript{H}17 differentiation.

It can be anticipated that defects involving the IL-6 receptor pathway would impair Th-17 cell differentiation. Two heritable primary immunodeficiency disorders that affect IL-6 receptor signaling have been identified recently. The first is the hyper-IgE syndrome, which is frequently associated with hypomorphous loss of function mutations in the DNA-binding and Src homology 2 domains of STAT3.\textsuperscript{103,104} The spectrum of infections associated with this disorder, especially fungal infections, would be consistent with defective T\textsubscript{H}17 differentiation, and recent evidence suggests that patients with hyper-IgE syndrome exhibit such a defect.\textsuperscript{84,139} Mutations in Tyk2, which give rise to a hyper-IgE-like syndrome, may also act in part by impairing signaling via the IL-6 and IL-23 receptors.\textsuperscript{26} Both cases point to a hitherto unappreciated yet prominent role for STAT3 in the regulation of IgE levels whose molecular basis remains to be established.

**THERAPEUTIC STRATEGIES OF TARGETING TFs**

Transcription factors are the key regulators of immune responses and inflammation. Therefore, pharmacologic approaches of targeting TFs may be used as a strategy to treat diseases in the immune system, such as asthma. The strategies that modify the actions of TFs include direct blocking of their activation, interfering with their expression using nucleic acid–based technologies, and altering their interactions with other TFs.\textsuperscript{105} Here we present select examples of such approaches.

**Glucocorticoid receptor**

Glucocorticoids have been used as an effective long-term treatment for asthma. Their action is mediated through the glucocorticoid receptor (GR), which is widely distributed in the lung and regulates the expression of many proinflammatory genes.\textsuperscript{30} GR is a Cys4 zinc finger TF containing hormone binding, DNA binding, and transactivation domains. GR can have either positive (transactivation) or negative (transrepression) effects on transcription.\textsuperscript{106,107} In the absence of the ligand, the GR is predominantly located in the cytoplasm in an inactive multiprotein complex, which includes 2 molecules of heat shock protein of 90 kd. Binding of glucocorticoids to GR leads to the dissociation of the multiprotein complex and nuclear translocation of GR. Once in the nucleus, GR binds to a DNA binding sequence known as the glucocorticoid response elements as a homodimer that induces the transcriptional activation of a number of genes, many of which have anti-inflammatory effects.\textsuperscript{106,107} In contrast, transrepression can proceed with or without GR binding to DNA. Although the former activity is performed by GR binding to a negative glucocorticoid response elements site, repression can also proceed through the ability of the GR to bind to other TFs, such as activator protein-1, nuclear factor-\kappaB, NFAT, and STAT5.\textsuperscript{87} Transrepression is responsible for the inhibitory effects of glucocorticoids on many proinflammatory cytokines, chemokines, adhesion molecules, proinflammatory receptors, and enzymes. An important mechanism by which transrepression proceeds is through the recruitment by the GR of histone deacetylases to the activated transcription complex, which results in the reversal of histone acetylation of activated inflammatory genes and suppression of transcription at those loci.\textsuperscript{108-110} Taken together, the extensive use of glucocorticoids to treat allergic airway inflammation is a well-established therapeutic strategy that forms the bases of anti-inflammatory interaction in asthma.

**Foxp3**

The suppressive characteristics of Treg cells have made these cells attractive candidates for immunotherapy, as suggested by experimental attempts to affect autoimmune processes in animals through adoptive transfer of Treg cells.\textsuperscript{111,112} Examples of disease processes that can be controlled by this approach include gastritis, thyroiditis, oophoritis, inflammatory bowel disease, graft-versus-host disease, arthritis, and systemic lupus erythematous.\textsuperscript{113-115} Similar treatment strategies are now being developed in human beings, in whom it is envisaged that the cellular therapy with antigen-specific Treg cells may allow the development of long-term immune modulation strategies without the problem of general
Current clinical trials with antigen-specific IL-10–producing Tr-1 cells in haploidentical patients receiving bone marrow transplant are underway. Other approaches include the derivation for therapeutic use of human cell lines that stably express ectopic Foxp3, which converts both naive and antigen-specific memory CD4+ T cells into cells with Treg cell–like properties. Treg cell therapy may also be rendered more effective by combining it with immunomodulatory drugs that spare Treg cells while targeting conventional T cells. One such drug is rapamycin, an inhibitor of the mTor pathway. The latter, which branches off the IL-2/phosphoinositide-3-kinase pathway, plays an important role in T-cell proliferation and survival. The mTor pathway is relatively inactive in Treg cells, which use an alternative IL-2 receptor–coupled STAT5 pathway to mediate cell growth and proliferation. A second approach is to use histone deacetylase inhibitors, which promote the development of Foxp3+ Treg cells.

A pharmacologic approach of particular therapeutic relevance to allergic diseases is to bolster aTreg cell–dependent oral tolerance by manipulating the retinoic acid pathway. It is well established that the induction of oral tolerance is associated with the in situ production of Foxp3+ aTreg cells. The in situ conversion of naive T cells into aTreg cells, which takes place on antigen presentation by CD103+ dendritic cells in gut-associated lymphoid tissue and is strictly TGF-β–dependent, is greatly augmented by retinoic acid and reciprocally antagonized by inhibitors of retinal dehydrogenase. Importantly, retinoic acid inhibits the IL-6–driven programming of TGF-β–treated T cells into proinflammatory T\textsubscript{H}17 cells in favor of Treg cell differentiation. These findings, if proven applicable to the human mucosal immune system, open up the possibility of pharmacologically enhancing oral tolerogenic therapies such as sublingual immunotherapy with retinoic acid analogs or concurrent vitamin A supplementation. They also usher in the potential to shift effector T-cell differentiation in inflammatory diseases away from T\textsubscript{H}17 and in favor of the Treg cell lineage (Fig 4, B).

### Nuclear factor-erythroid 2-related factor 2

Oxidative stress has been implicated in the pathogenesis of a variety of diseases including asthma, rheumatoid arthritis, and other inflammatory diseases. The last several years, evidence has begun to emerge demonstrating that the antioxidative nuclear factor-erythroid 2-related factor 2 (Nrf2) pathway plays a critical role in modulation of redox signaling involved in the inflammatory process, suggesting that the Nrf2 pathway may be used to suppress airway inflammation and other oxidative stress–mediated inflammatory processes.

Nrf2, a basic leucine zipper redox-sensitive TF, interacts with the antioxidant response element (ARE), which is found on a large set of genes involved in the oxidative stress response, such as γ-glutamylcysteine ligase, heme oxygenase-1, and thioredoxin. Products of these genes have been shown to play an important role in a variety of pathological processes including protection against oxidative damage to tissues, cancer prevention, and inhibition of apoptosis. Nrf2 activation is induced in response to oxidative stress stimuli and electrophilic chemicals, including a number of food chemicals such as the isothiocyanates, sulforaphane, and α-lipoic acid. The activation process is governed by a series of specific chemical reactants that involve oxidative cross-linking of the critical SH group in a chaperone molecule, Keap I, which, under normal conditions, sequesters Nrf2 in the cytosol and targets it for proteasomal degradation. The cross-linking of these thiol groups on Keap I releases Nrf2 from its sequestrating complex and allows it to translocate to the nucleus, where it binds to the ARE, forms a complex with TF small Maf and other proteins, and induces gene expression (Fig 5).
Emerging evidence indicates that oxidative stress has profound effects on both innate and adaptive immunity.\textsuperscript{132,133} Oxidative stress downregulates T\textsubscript{H}1 and promotes T\textsubscript{H}2 immunity through perturbation of dendritic cell maturation and IL-12 production.\textsuperscript{132,133} An even more profound immune perturbation is observed in Nrf2-null mice, which, along with reduced antioxidant capacity, develop a female-preponderant multiorgan lupuslike inflammatory disease.\textsuperscript{134} Nrf2 deficiency enhances susceptibility to lung injury and inflammation and exaggerates allergic airway inflammation in experimental asthma models.\textsuperscript{135,136} Cumulatively, these data suggest that Nrf2 exerts important immunoregulatory and anti-inflammatory effects that may be of therapeutic value in allergic diseases, including asthma. Nrf2 agonists such as sulforaphane may be useful in suppressing airway inflammation, especially in people whose asthma could be promoted by exposure to pro-oxidative stimuli such as ambient air pollution, or a putative subset of patients with asthma who could be more prone to the development of asthma because of a weakened antioxidant defense.\textsuperscript{105,137}

Conclusion

A more complex, and gratifying, view of effector T-cell differentiation in the periphery has recently emerged, propelled by the elucidation of cytokine programs and transcriptional circuits governing peripheral T-cell differentiation into the Treg and T\textsubscript{H}17 cell lineages. A more refined view of T\textsubscript{H}1/T\textsubscript{H}2 lineage commitment has also emerged, aided by the elucidation of secondary, reinforcing transcriptional circuits and epigenetic changes that consolidate differentiation into the respective cell lineage. Several gaps remain in our knowledge of the differentiation pathways and long-term fate of the newer cell lineages. The differentiation programs of other cell lineages such as Tr-1 cells, although beginning to yield to investigation, remain largely obscure.\textsuperscript{77} The position in this new scheme of intermediate phenotypes such as IL-10–producing T\textsubscript{H}1 cells is also uncertain, as are the precise mechanisms by which environmental cues that are prevalent in affluent societies bias effector cell differentiation in favor of atopic diseases.\textsuperscript{5,138} These uncertainties hint at further complexities along the way, but they also point to opportunities for novel and effective interventions in the treatment and prevention of allergic diseases.

REFERENCES

3. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 paradigm. Nat Rev Immunol 2006;6:679-86.
89. Dzhagali O, Zhang N, He YY. The roles of orphan nuclear receptors in the develop-
90. Jetten AM, Kurebayashi S, Ueda E. The ROR nuclear orphan receptor subfamily:
91. He YY, Deflos ML, Ojala EW, Bevan MJ. RORgamma t, a novel isoform of an
orphan receptor, negatively regulates Fas ligand expression and IL-2 production in
T cells. Immunology 1998;9:797-806.
92. Sun Z, Unutmaz D, Zou YR, Sunshine MJ, Unutmaz D, Zou YR, Sunshine MJ,
inoid-related orphan receptor gamma (RORgamma) is essential for lymphoid
94. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgamma directs the differentiation program of pro-
helper 17 lineage differentiation is programmed by orphan nuclear receptors
96. Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Matsson
JD, et al. Development, cytokine profile and function of human interleukin 17-
2007;8:903-5.
98. Ivanov II, Zhou L, Littman DR. Transcriptional regulation of TH17 cell differen-
TH(17) cell differentiation by promoting sequential engagement of the IL-21 and
autocrine regulation by IL-21 in the generation of inflammatory T cells. Na-
ture 2007;448:480-3.
of inflammatory TH(17) cells requires interferon-regulatory factor 4. Nat Immuno-
104. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-
negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syn-
105. Roth M, Black JL. Transcription factors in asthma: are transcription factors a new
107. Ziegler RF, Bluestone JA. Regulatory T-cell physiology and application to treat
109. Guida MA, Stabilini A, Romagnolo ME. Rapamycin selectively expands CD4(+)
111. Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, et al. Deacute-
ylation inhibition promotes the generation and function of regulatory T cells. Nat
112. Benson MJ, Pino-Lagos K, Rosenblatt M, Noelie RJ. All-trans retinoic acid me-
113. Chatila TA. Extra-intestinal manifestations of gastro-intestinal allergy: effector
114. Chen XL, Kunsch C. Induction of cytotoxic protective genes through Nr2f2/antioxidant
response element pathway: a new therapeutic approach for the treatment of in-
115. Li N, Alam J, Venkatesan M, Eiguren-Fernandez A, Schmitz D, Di Stefano E,
et al. Nrzf2 is a key transcription factor that regulates antioxidant defense in mac-
rophages and epithelial cells: protecting against the proinflammatory and oxidiz-
118. He YW, Deftos ML, Ojala EW, Bevan MJ. RORgamma t, a novel isoform of an
120. Allan SE, Alstad AN, Merinol N, Crenlien NK, Amendola M, Bacchetta R, et al. Generation of potent and stable human CD4(+) T regulatory cells by activation-