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SERIES "SIGNALLING AND TRANSCRIPTIONAL REGULATION IN INFLAMMATORY AND IMMUNE CELLS: IMPORTANCE IN LUNG BIOLOGY AND DISEASE"
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T-helper cell type-2 regulation in allergic disease

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ABSTRACT: Substantial experimental evidence now supports the notion that allergic diseases are characterised by a skewing of the immune system towards a T-helper cell type-2 (Th2) phenotype.

Studies using both human and mouse model systems have provided key evidence for the role that Th2 cytokines play in driving many of the hallmarks of allergic inflammation. Furthermore, the signalling pathways by which Th2 cytokines exert their effects on airway target cells are rapidly being elucidated, and antagonists of the Th2 pathway are under active development.

In this review, the current knowledge of the role of T-helper cell type-2 cells in asthma is summarised, focusing on how and where T-helper cell type-2 cells differentiate from naïve precursors. The signalling molecules and transcription factors involved in T-helper cell type-2 differentiation will be reviewed in detail, in an attempt to translate studies using genetically modified mice into meaningful insights about asthma and other allergic diseases.

KEYWORDS: Asthma mechanisms, dendritic cells, gene regulation, lymphocyte subsets

Substantial evidence supports the notion that allergic asthma is an immune-mediated disease, driven in part by T-cells directed against aeroallergens and viruses [1]. CD4+ T-helper cell (Th) type-2 cells in particular, which secrete interleukin (IL)-4, IL-5, IL-9 and IL-13, are thought to contribute to many of the pathophysiological features of asthma, including airway inflammation, mucus secretion and airway hyperresponsiveness (AHR) [2]. Much of the current research is aimed at understanding how Th2 cytokines act on resident lung cells, including airway epithelium, myofibroblasts and smooth muscle, to induce the asthmatic phenotype, and the signalling pathways and transcription factors involved in this process are rapidly being elucidated [3]. The precise molecular basis for a Th2-biased immune response is complex and is likely to be multifactorial in any given individual. A genetic predisposition is suggested by the association between single nucleotide polymorphisms (SNPs) and haplotypes in the Th2 cluster, as well as Th2-signalling molecules and different allergic diseases (table 1). Poorly defined environmental exposures also contribute to the Th2 bias, in part by acting on dendritic cells and other cells that influence T-cell activation and expansion. Since a Th2 bias is characteristic of atopy in general, it seems likely that there are disease-specific susceptibility genes and environmental exposures that ultimately influence the development of a specific atopic phenotype (*e.g.* atopic dermatitis *versus* asthma); candidates in this regard are beginning to be identified [4–6]. According to this model, a Th2-biased immune system can be considered necessary but insufficient for the development of allergic asthma, which requires a ''susceptible airway'' (fig. 1). This article reviews the genetic and potential environmental factors that lead to a Th2-biased immune response. It draws heavily from studies using mouse models that identified key molecules involved in Th2 differentiation, but will emphasise areas where future research should enhance the understanding of human atopic diseases.

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TABLE 1 Association of single nucleotide polymorphisms in T-helper cell type-2 (Th2) related genes and different lung-related phenotypes

Gene	Association	Reference
Th2 cytokine cluster		
IL-4 promoter	Asthma severity	[7]
	Severe RSV disease in children	[8]
IL-13 promoter	Asthma	[9, 10]
	COPD	[11]
	IgE levels	[12]
IL-13 coding	Asthma	[13]
	IgE levels	[14]
Th2 signalling pathway		
IL-4Rα	Asthma	[15, 16]
	Lung function decline in smokers	[17]
	IgE levels	[18]
Stat6	Allergic diseases	[19]

This table is representative but not exhaustive; see references [20, 21] for recent comprehensive reviews. Reference [16] provides an example of interactions between interleukin (IL)-4R and IL-13 polymorphisms. Stat: signal transducer and activator of transcription; RSV: respiratory syncytial virus; COPD: chronic obstructive pulmonary disease; Ig: immunoglobulin.

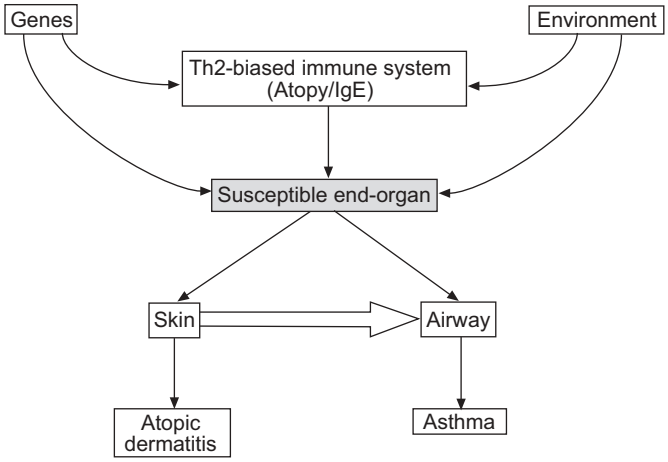

FIGURE 1. The relationship between a T-helper cell type-2 (Th2) biased immune response and organ-specific atopic diseases. This model emphasises that a Th2-bias can be considered a systemic marker of immune dysregulation, but that the clinical manifestation of different diseases is affected by organ-specific factors. The progression of atopic disease from skin to lung (*e.g.* the ''atopic march'' observed during childhood) is indicated by the large arrow. Ig: immunoglobulin.

TH2 CELLS, ASTHMA AND ATOPY

Several studies have documented increased numbers of activated CD4+ T-cells in the lungs of subjects with asthma, and, in the majority of studies to date these cells were found to express cytokines or transcription factors characteristic of Th2 cells [22–32]. In some studies this apparent Th2 bias in asthma extends outside of the airway to circulating lymphocytes [33–35]. Furthermore, research in mouse models has established the critical roles that Th2 cytokines play in the pathophysiology of asthma, including mucus hypersecretion and AHR [36–50], and a recent study emphasised the importance of IL-4 and IL-13 in airway dysfunction and remodelling in response to chronic allergen exposure [50]. In human studies, administration of recombinant IL-4 resulted in AHR in asthmatic subjects [51], whereas IL-12 suppressed eosinophils in blood and sputum (without affecting AHR [52]). Furthermore, clinically useful drugs, including inhaled corticosteroids, potently suppress Th2 cytokine gene expression [53, 54]. Taken together, these observations support the hypothesis that the expansion and activation of Th2 cells in the airway is a critical component of asthma pathophysiology [55]. A corollary of this hypothesis is that a detailed understanding of the factors responsible for the Th2 bias in asthma should identify future therapeutic targets.

Given the key role that Th2 cells and cytokines play in asthma pathophysiology, it was originally thought that inducing a Th1 response in the lung would be protective. However, it is now known that Th1 cells and their signature cytokine interferon (IFN)-γ have complex and potentially pro-inflammatory effects in the lung. Studies using mouse models have provided conflicting results about the potential role of Th1 cells and IFN-γ in airway inflammation and AHR. In some studies, Th1 cells and IFN-γ were found to dampen allergic airway inflammation [56], whereas Th1 cells and IFN-γ were found to have pro-inflammatory effects in other studies [57–59]. In a mouse model of virally induced AHR and airway remodelling, there was no attenuation of these parameters in IFN-γ-deficient mice [60]. Interestingly, there is evidence that IFN-γ expression in peripheral blood CD8+ T-cells correlated with AHR in asthmatic subjects [61], and a recent study found that IFN-γ production in cord blood CD8 T-cells predicted subsequent allergic sensitisation [62]. IFN-γ-expressing lymphocytes are present in the lungs or sputum of some subjects with asthma [63, 64], and it would seem likely that IFN-γ-secreting cytolytic CD8+ cells would be recruited to the lung during virally triggered exacerbations. Although there is some evidence for CD8+ recruitment to the lung during asthma exacerbations [65], surprisingly little is known presently about the cytokine profile of these cells. Taken together, these studies suggest that the potential role of IFN-γ in asthma is complex and depends on the location of cells expressing this cytokine and stage of the disease. Studies using specific cytokine antagonists will be needed to help clarify the role of IFN-γ and related cytokines in human subjects with asthma.

T-CELL ONTOGENY AND TH2 DIFFERENTIATION

It is currently not known exactly where Th2 cells arise in atopic individuals. Naïve T-cells exit the thymus with a productively re-arranged T-cell receptor (TCR) after having undergone positive selection and escaped negative selection. Due to their expression of homing receptors, including the peripheral lymph node addressin, naïve T-cells recirculate through central immune organs, including lymph nodes, until they encounter antigen-presenting cells (APC) bearing peptide fragments recognised by their surface TCR. If the APC is appropriately activated, then the naïve T-cell receives additional signals that initiate clonal expansion driven in part by autocrine secretion of IL-2. Interestingly, recent studies show that in addition to its effects on T-cell proliferation,

prolonged exposure to IL-2 also promotes Th2 differentiation [66]. As clonal expansion progresses, many activated T-cells undergo activation-induced cell death, which serves to limit the subsequent immune response. Some T-cells survive and differentiate into effector or memory cells, which are then recruited to peripheral mucosal sites to orchestrate cellular immunity. Based on this model, it seems reasonable to speculate that the differentiation of allergen-specific Th2 cells is initiated in regional lymph nodes. Since atopic individuals can be sensitised *via* both the skin and respiratory tract to environmental allergens, Th2 differentiation might occur in lymph nodes that drain both the skin and lung. However, recent findings in mouse models suggest that, in the absence of lymph nodes, the lung has an intrinsic capacity to initiate primary immune responses [67–69]; the precise site of naive T-cell education in these models, and how they apply to human allergic diseases, remains to be determined. Although technically challenging, it will be interesting in future research to study the route of sensitisation and fate of allergen-specific T-cells in humans with different allergic diseases. Along these lines, the study of subjects with occupational asthma may be insightful given that the route of sensitisation will vary and this disease has an onset in adulthood.

The degree of polarisation of T-cell subsets depends on the strength and duration of the polarising signals, and involves both cell-intrinsic factors, as well as exogenous signals. The relative contribution of cell-intrinsic factors in Th differentiation (*i.e.* in the ''selection'' of pre-committed precursor cells) is difficult to ascertain with certainty (for a review see [70]). The decision to express an individual allele of IL-4 or IL-13 appears to be a random event [71]. However, it is now clear that ''instruction'' by exogenous signals, including cytokines, plays a key role in driving naïve T-cell differentiation. IL-4 is the most important cytokine for Th2 differentiation, and activates the transcription factor signal transducer and activator of transcription (Stat)6, as well as other downstream effectors (see below). The primordial source of IL-4 that initiates Th2 differentiation has been debated. The observation that naïve T-cells can rapidly produce IL-4 suggested that IL-4 could come from naïve T-cells themselves [72]. In this model, if the precise signals provided during naïve T-cell activation preferentially induce IL-4 and not IFN-γ expression, then this can feed forward to amplify Th2 differentiation. Genetic polymorphisms that favour early IL-4 production would then be predicted to enhance Th2 differentiation and contribute to the Th2 bias in susceptible atopic individuals (fig. 2).

Although a non-T-cell source of IL-4 does not need to be invoked to initiate Th2 differentiation, it is worth noting that two other cell types relevant to human atopic diseases can produce IL-4, namely basophils and eosinophils. Human basophils secrete substantial amounts of IL-4 after IgE cross-linking and produce IL-4 in the allergic lung [73, 74]. In addition, experiments using transgenic mice in which IL-4-expressing cells were tagged using green fluorescent protein (GFP) found that most of the GFP-positive cells recruited to the lung during Th2 reactions were eosinophils [75]. The essential role of eosinophils in allergen-driven airway inflammation and remodelling was recently shown using two new models of eosinophil-deficient mice [76, 77]. Although eosinophils constitutively express transcription factors required for IL-4

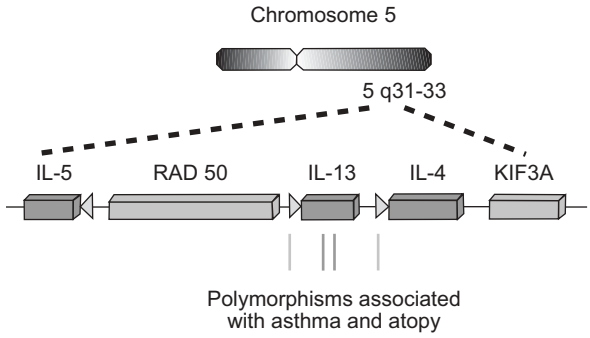

FIGURE 2. Organisation of the T-helper cell type-2 (Th2) cytokine gene cluster on chromosome 5. The relative location of the Th2 cytokine genes (rectangles) and their promoters (arrow heads) is shown. Single nucleotide polymorphisms located in promoter and coding regions associated with different asthmatic and atopic phenotypes are shown at the bottom. The figure is a schematic and is not drawn to scale. IL: interleukin; RAD: radiation sensitive 50; KIF: kinesin family member.

expression [78], the ability of these cells to secrete IL-4 protein remains controversial. Whether or not these two cell types influence naïve Th cell differentiation will depend on their ability to gain access to naïve T-cells. Along these lines, the recent demonstration that eosinophils are present in airway draining lymph nodes suggests that they might actually serve as antigen-presenting cells, but this is a controversial area [79].

TH1 DIFFERENTIATION AND T-BET

In contrast to IL-4, the key cytokine driving Th1 differentiation is IL-12. A major source of IL-12 is the dendritic cell, which processes and presents soluble antigen to T-cells in the context of major histocompatibility complex. IL-12 activates the transcription factor Stat4 that enhances IFN-γ expression in part by binding to regulatory sequences near the IFN-γ gene [80]. Another key transcription factor driving Th1 polarisation and IFN-γ gene expression is T-bet. The recent discovery that T-bet is as a lineage-specific transcription factor required for IFN-γ and IL-12Rβ2 gene expression was a major advance in the understanding of Th1 differentiation [81, 82]. The signals that regulate T-bet in CD4+ cells are complex, and include the IFN receptor (*via* Stat1) and the T-cell receptor *via* poorly understood pathways [83]. T-bet appears to act in part at the level of chromatin remodelling to induce IFN-γ gene expression [84], but the precise binding sites for T-bet in and around the IFN-γ gene are currently undefined. T-bet is involved in the generation of effector CD8+ cells and natural killer cells [85, 86], and also regulates B-cell isotype switching [87]. Interestingly, there are T-bet independent pathways of IFN-γ gene expression [88].

FINOTTO *et al.* [89] reported that T-bet-deficient mice spontaneously developed an asthma-like syndrome with airway inflammation, remodelling and hyperreactivity. Furthermore, reduced expression of T-bet was found in T-cells in the lungs of subjects with allergic asthma [89]. This study suggested that allergic asthma may result from defective production of T-bet and/or Th1 cells. Given the complex role of IFN-γ in asthma (as discussed above), this result is intriguing and whether this reflects effects of T-bet on genes other than IFN-γ

warrants further investigation. The precise molecular basis for reduced T-bet expression in humans with asthma is currently unknown. Interestingly, polymorphisms in *TBX21*, the gene encoding T-bet, were recently found to be associated with a greater response to inhaled corticosteroids in asthmatic children [90]. Whether TBX21 is a susceptibility gene for asthma or other atopic disorders is currently unknown.

DENDRITIC CELLS AND TH2 DIFFERENTIATION

Dendritic cells (DCs) are the key antigen-presenting cells that activate naïve T-cells, and there has been great interest in deciphering the DC-derived signals that influence Th differentiation (excellent recent reviews have covered this topic in depth, see [91, 92]). In general, DC control of Th1 differentiation is better understood than that of Th2 differentiation and results from exposure of DCs to bacterial or other strong danger signals that initiate DC maturation. DC maturation is characterised by reduced endocytosis and enhanced antigen presentation, altered chemokine receptor expression, homing from peripheral mucosal sites to regional lymph nodes, and increased production of immunoregulatory cytokines, including IL-12. Many of these danger signals are transduced by the toll-like receptor (TLR) family of pattern recognition receptors, which are expressed on DCs and other cells of the innate immune system. Strong danger signals tend to favour Th1 immune responses by inducing the differentiation of DCs producing large amounts of IL-12 and related Th1-promoting cytokines (including IL-23, IL-27, and type-1 IFNs) (fig. 3).

Dendritic cells do not produce IL-4, the prototypic Th2-promoting cytokine, and probably initiate Th2 differentiation indirectly. One possibility is that activated DCs that produce low amounts of IL-12 (IL-12^{lo} DCs) will promote Th2 differentiation (fig. 3). Therefore, the environmental signals that lead to the maturation of IL-12^{lo} DCs can be considered susceptibility factors for the Th2 bias in atopy. Several signals have been discovered that allow DCs to mature into an IL-12^{lo}/Th2-promoting phenotype. These include low-dose endotoxin, TLR2 ligands, glucocorticoids, and many G-protein coupled receptor agonists (*e.g.* histamine, cholera toxin, prostaglandin (PG) E_2, PGD_2 and others, see table 2). Exactly how these and other signals might influence allergen-driven DC maturation in humans with asthma is not entirely clear. A note of caution is warranted regarding the potential for glucocorticoids (GCs) to induce a Th2-bias by directly acting on DCs. Some studies have shown that GCs inhibit IL-12 production in developing DCs, and that GC-exposed IL-12^{lo} DCs induce a Th2 phenotype in co-culture assays with T-cells [93, 94]. However, in these studies DCs were washed prior to co-culture with T-cells (which was done in the absence of GCs). When GCs are present during DC:T-cell co-cultures, a more physiological model of T-cell activation, IL-4 expression was potently suppressed [95]. This observation is consistent with the known ability of GCs to suppress IL-4 and Th2 gene expression *in vivo*, and is in keeping with the potent therapeutic efficacy of these drugs in asthma [54]. Therefore, the conclusion that inhaled GCs contribute to the Th2 bias in asthma is premature, and reflects an artefact of the *in vitro* culture systems used.

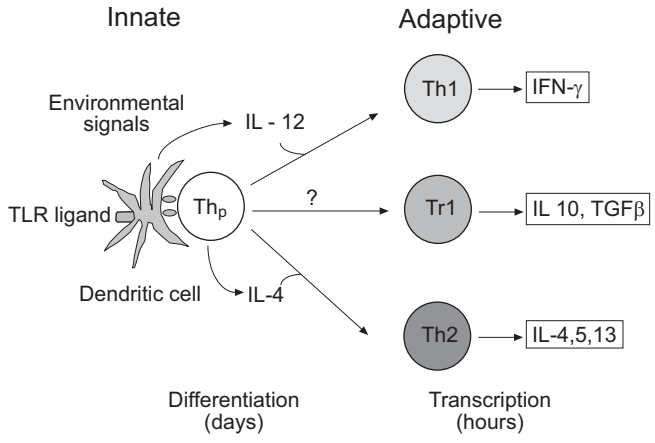

FIGURE 3. Regulation of T-cell differentiation by signals provided by dendritic cells (DCs). Strong toll-like receptor (TLR) signalling enhances IL-12 production leading to T-helper cell (Th) type-1 differentiation. Th2 differentiation, in contrast, results in the absence of IL-12 when IL-4 gene transcription in naïve T-cells is promoted. Whether there are DC-derived signals that can actively promote Th2 differentiation is not entirely clear (see text for details). Naïve T-cells can also differentiate in the periphery into regulatory phenotypes that can down regulate immunity: the signals involved in regulatory T-cell differentiation are not known but may include IL-10 (not shown). IFN: interferon; TGF-β: transforming growth factor-β.

There is some support for the idea that reduced production or activity of IL-12 is a risk factor for Th2 sensitisation in humans with allergic diseases [99], although further research is needed in this area. However, it should be noted that in the absence of IL-12, T-cells do not automatically default to the Th2 pathway (*e.g.* in IL-12-deficient mice). Thus, although reduced DC IL-12 production can be considered a risk factor for Th2 sensitisation, there are other mechanisms by which activated DCs initiate Th2 differentiation. These may include both secreted factors as well as contact-dependent signals. The distinction between IL-12^{lo} and immature DCs is not always clear, and DCs can also downregulate immunity by inducing the differentiation of regulatory T-cells (*e.g.* cells secreting IL-10, transforming growth factor-β, or other cytokines [108, 109]). The potential role of regulatory T-cells in asthma and atopy is beginning to be appreciated but is beyond the scope of this review.

TH2 RECRUITMENT AND SURVIVAL

Whether the expansion of Th2 cells in asthma is due to the ongoing recruitment of circulating T-cells into the lung or the

TABLE 2 Examples of factors that induce interleukin-12^{lo}, T-helper cell type-2 promoting dendritic cells

Examples	References
Low-dose endotoxin	[101]
TLR2 agonists	[102]
Glucocorticoids	[93, 94]
G-protein-coupled receptor ligands (*e.g.* PGE_2, cholera toxin, histamine, lipoxin)	[103–107]

TLR: toll-like receptor; PGE_2: prostaglandin E_2.

TABLE 3 Potential genetic factors involved in the T-helper cell type 2 (Th2) bias in atopy

Candidate location or gene(s)	Examples
Th2 regulatory regions that affect DNA binding of transcriptional or chromatin-based regulators	IL-4 and IL-13 promoters, LCR (?)
Th2 cytokine coding regions that affect cytokine activity	IL-13
Th2-promoting signaling molecules or transcription factors	Stat6, GATA3, many other potential candidates
Polymorphisms that inhibit the expression/function of genes that activate Th1 cytokine gene expression	IL-12Rβ2, T-bet (?)
Genes that regulate Th2 cell growth or survival	GIF-1 (?)
Genes that induce the differentiation of Th2-promoting DC	TLR2, GPCRs (?)

See table 1 for interleukin (IL)-4 and IL-13 promoter polymorphism references. References for the following SNPs are as follows: IL-13 coding region [96], signal transducer and activator of transcription (Stat)6 [97], GATA3 [98], IL-12Rβ2 [99], TLR2 [100]. Question marks indicate that no association has been shown to-date for atopic or asthmatic phenotypes. DC: dendritic cell; LCR: locus control region; GIF-1: growth-factor independent-1; TLR: toll-like receptor; GPCR: G-protein coupled receptor.

expansion and persistence of resident airway effector cells remains to be determined. Recent studies in mice suggest that ongoing T-cell recruitment is much more important than expansion of resident lymphocytes in explaining effector cell accumulation in the lung and other nonlymphoid organs [110, 111]. For example, in an allergic sensitisation and challenge model, HARRIS *et al.* [110] showed that activated T-cells migrate from the regional lymph nodes to the lung and airway, where they secrete IL-4 but are unable to further divide. The specific recruitment of IL-4 secreting Th2 cells may be mediated by Th2-attracting chemokines, such as thymus- and activation-regulated chemokine (TARC or CCL17) and macrophage-derived chemokine (MDC or CCL22) [112, 113]. The ligand for TARC and MDC is C-C chemokine receptor 4 (CCR4), which is preferentially expressed on some Th2 cells. The expression of MDC was recently shown to be potentiated by PGD_2 in a mouse model of asthma [114]. Interestingly, PGD_2 can also directly recruit Th2 cells by binding to the receptor CRTh2, which is selectively expressed on Th2 lymphocytes. Taken together, these studies show that antagonising Th2 recruitment provides a novel therapeutic target in asthma.

Although less well studied than the regulation of naïve T-cell differentiation, the persistence of memory T-cells in the periphery also plays a role on the Th2 bias in atopy. Factors that control the long-term survival of memory cells include homeostatic cytokines, such as IL-7 and IL-15 [115]. Th2 cells appear to survive longer than Th1 cells, which may be prone to apoptosis. For example, in a recent study by WU *et al.* [116], the generation of memory CD4+ Th1 cells was inhibited in cells actively secreting IFN-γ consistent with the known ability of IFN-γ to promote T-cell apoptosis [117]. These studies suggest that Th2 cells might preferentially expand in atopy regardless of the initial differentiating signals. Although there is some experimental evidence to support this idea [118, 119], further research into the factors that sustain the persistence of Th2 cells in atopy is needed. Genetic polymorphisms in these factors might also contribute to the Th2 bias in human allergic diseases (see table 3).

TH2 TRANSCRIPTIONAL REGULATION

Whether due to enhanced recruitment or persistence, T-cells that accumulate in the allergic airway tend to express Th2 cytokines. The molecular basis for selective expression of the Th2 subset of cytokine genes has come under intense scrutiny in the past decade, and several key signalling pathways and transcription factors involved in this process have been identified (fig. 4). The remainder of this review will cover the

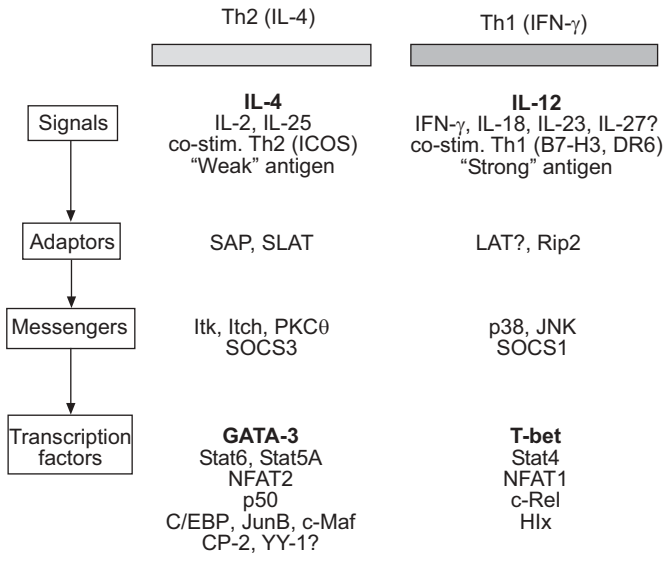

FIGURE 4. Molecules involved in T-helper cell (Th) type-1 *versus* Th2 polarisation. This figure is a compilation of studies using gene-targeted and transgenic mice and is not meant to be comprehensive. This format was suggested by a table in a review article by HO and GLIMCHER [121], which contains some of the primary references. Additional references include the following: interleukin (IL)-23 [122], IL-25 [123], IL-27 [124], B7 family [125], antigen strength ([126, 127] and references therein), signalling lymphocyte activation molecule-associated protein (SAP) [128], SWAT-70-like adapter molecule of T (SLAT) [129], linker for activation of T-cells (LAT) [130], Rip2 [131], Itk [132], Itch [133], protein kinase C (PKC)-θ [134, 135], mitogen-activated protein kinase (MAPK) review [136] and suppressor of cytokine signalling (SOCS) [137]. Additional references for transcription factors not discussed in the text include the following: nuclear factor-κB p50 [138, 139], c-Rel [140], and Hlx [141]. For some molecules it is currently unclear whether they act as primary activators of one pathway or inhibitors of the other pathway (*e.g.* IL-27 [142] and LAT [130]). IFN-γ: interferon gamma; ICOS: inducible costimulator; Stat: signal transducer and activator of transcription; NFAT: nuclear factor of activated T-cells; T-bet: T-box expressed in T-cells; c/EBP: CCAAT enhancer binding protein.

transcription factors involved in Th2 gene regulation and their potential roles in dysregulated gene expression in humans with allergic diseases.

The Th2 genes are located in a cluster on chromosome 5q (fig. 2). IL-4 and IL-13 share similar intron/exon structure and sequence homology, and are thought to have arisen from a primitive gene duplication event. Although the 5′-flanking regions of the IL-4, IL-5 and IL-13 genes all contain TATA sequences (required for the assembly of the RNA polymerase II-containing basal initiation complex), overall promoter sequence homology is otherwise limited. This suggests that each promoter will allow the assembly of unique transcription factor complexes, and that the expression of a given Th2 cytokine can be fine-tuned at the level of promoter-driven transcription initiation. In support of this notion, individual Th2 cytokines and their alleles can be selectively expressed when cell populations are examined on an individual cell basis [71, 120].

Since the original description of a binding site for the transcription factor nuclear factor of activated T-cells (NFAT) in the IL-4 promoter [143], discrete binding sites for many other factors have been identified therein (for recent review see [144]). Based on sequence conservation between species, the 5′ boundary of the IL-4 promoter is generally considered to contain several hundred base pairs upstream from the transcription start site but this is inevitably an arbitrary distinction. In transient transfection assays using reporter constructs, the IL-4 promoter is inducible in response to cell stimulation, implying that some of the factors responsible for enhanced IL-4 gene expression act at the promoter level [138]. Using combinations of *in vitro* DNA-binding assays (*e.g.* gel shift assays) and functional studies using wild-type and mutated promoter reporter constructs, several factors have been shown to bind to and enhance IL-4 promoter activity, including members of the NFAT, activation protein 1 (AP-1), and C/enhancer binding protein (EBP) families [145–147]. Other nuclear factors that bind to the IL-4 promoter include signal transducer and activator of transcription (Stat6), CCAAT-box binding protein 2 (CP-2), and YY-1 [148–150], and a current challenge in the field is to determine how different factors interact in a combinatorial way to lead to specific patterns of gene expression. Notably absent from this list is GATA3, the signature transcription factor of Th2 cells [151]. There is currently little evidence that GATA3 binds directly to high affinity sites in the IL-4 promoter, and overexpressed GATA3 does not transactivate the IL-4 promoter (for example see [152]). The exact mechanism by which GATA3 enhances IL-4 gene expression in Th2 cells is complex and is considered further below.

Nuclear factor of activated T-cells

NFAT cells comprise of a family a calcium-sensitive transcription factors that is critically important for the expression of many T-cell cytokines, including IL-2 and IL-4, as well as for the induction of tolerance [153, 154]. NFAT proteins are constitutively cytoplasmic, but translocate to the nucleus after dephosphorylation by the calcium-activated phosphatase calcineurin (fig. 5). The IL-4 promoter contains at least six purine-rich NFAT sites (termed the P elements P0–P5), and current thinking is that DNA-bound NFAT proteins interact with other transcription factors to enhance the rate of IL-4 transcription in activated T-cells [145, 155]. At some P elements, NFAT interacts with AP-1 family members to form a heterotrimeric transcription factor complex. As AP-1 is activated by a distinct signalling pathway (involving of protein kinase C (PKC)/ras activation), these so-called composite NFAT sites represent a point of signal integration in the nucleus [156]. The structural basis for NFAT AP-1 interactions has been solved and involves an extended surface of interaction between NFAT and AP-1 that also covers ~15 base pairs of the DNA template [157]. This intimate interaction between NFAT and AP-1 helps explain the fact that AP-1 consensus sites are not readily detectable at the IL-4 promoter P elements that support combinatorial interactions [155]. NFAT activation without AP-1 (*e.g.* after cell stimulation with

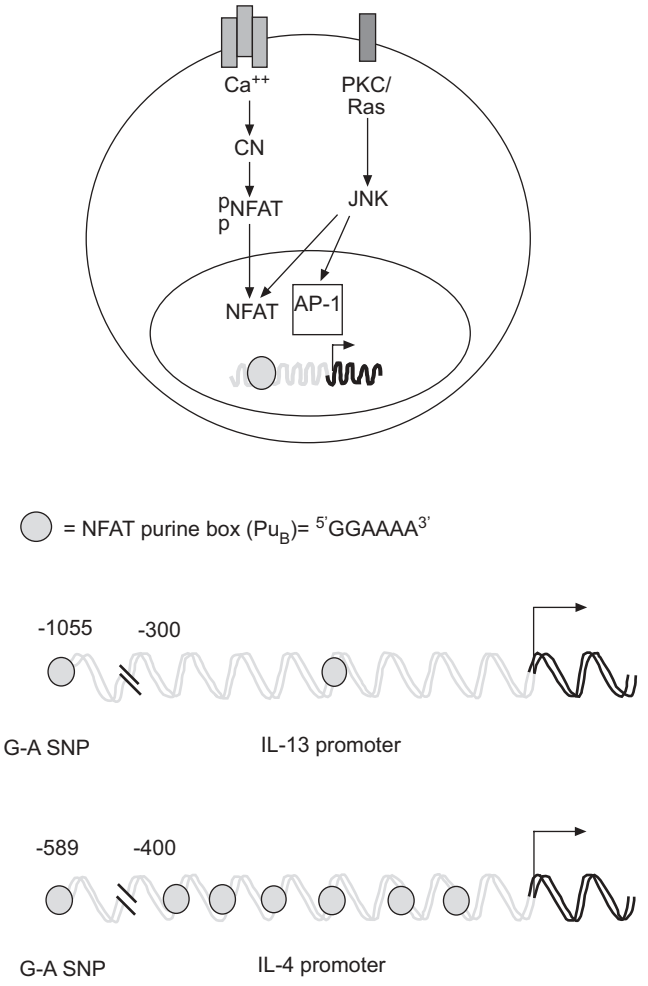

FIGURE 5. Nuclear factor of activated T-cells (NFAT) comprise a family of calcium-activated transcription factors that bind to the interleukin (IL)-4 and IL-13 promoters. Cytoplasmic NFAT is dephosphorylated by the phosphatase calcineurin (CN) that promotes its nuclear entry. Nuclear NFAT can interact with members of the activation protein 1 (AP-1) family to form a transcriptionally active complex. AP-1 proteins are activated by phosphorylation by c-Jun N-terminal kinases (JNK), which can also re-phosphorylate NFAT and promote its nuclear export (not shown). Single nucleotide polmorphisms (SNPs) are located 5′ of the IL-13 and IL-4 promoter regions that affect the binding of NFAT and are associated with asthma and/or chronic obstructive pulmonary disease phenotypes in different kindreds. PKC: protein kinase.

calcium ionophore alone) induces a state of T-cell unresponsiveness referred to as anergy (or tolerance) [158]. This is due to the induction of different molecules that inhibit proximal TCR-dependent signalling events, such as tyrosine phosphatases and ubiquitin ligases [159]. Interestingly, the requirements for NFAT:AP-1 complex formation differ between the Th2 cytokines. For example, NFAT-dependent IL-4 promoter transactivation requires an intact AP-1-interacting domain, whereas an NFAT mutant incapable of binding AP-1 can still transactivate the IL-13 promoter [160]. The physiological consequences of this in relation to T-cell anergy are unclear at present, but one implication is that the signalling requirements for IL-4 and IL-13 gene expression will be different.

Both the IL-4 and IL-13 promoter regions contain SNPs that are associated with asthma or asthma severity in different populations (table 1). Interestingly, both of these polymorphisms affect the binding of NFAT. The IL-4 promoter polymorphism is located at position -589 relative to the transcription start site, and has an allele frequency of ~0.5 and 0.2 in African-American and Caucasian subjects, respectively [7]. The C-T SNP, which recreated a higher affinity NFAT site, is associated with asthma severity (*i.e.* reduction in forced expiratory volume in one second by 4.5% compared with wild-type subjects), especially in white subjects [7]. The IL-13 promoter SNP (at position -1055) also recreates a higher affinity NFAT site, and is thought to function in a complex manner by reducing poorly understood inhibitory effects of CD2 and other signals on IL-13 gene expression [9]. Therefore, one way NFAT might contribute to the Th2 bias in asthma is due to polymorphic binding sites in Th2 cytokine promoters.

NFAT binds to additional purine boxes located within the IL-5 and IL-13 promoters, as well as to distal enhancer elements [161, 162]. One interesting NFAT site was discovered 3′- of the IL-4 gene using DNase hypersensitivity analysis [163]. This site, termed V_A, possesses enhancer activity in reporter constructs and can support the binding of NFAT and GATA3 in a Th2-dependent manner as determined by chromatin immunoprecipitation (ChIP) assay (fig. 6) [164]. Thus, even though NFAT proteins are not differentially expressed in Th1 *versus* Th2 cells (see below), occupancy of the V_A regulatory element was restricted to the Th2 lineage. In a follow-up series of experiments, SOLYMAR *et al.* [164] used gene targeting to delete the V_A region in mice, and observed a striking reduction in IL-4 expression in both bone-marrow derived mast cells (BMMCs) as well as *in vitro* differentiated Th2 cells. Interestingly, IL-13 expression was also partially reduced in V_A-null mice, especially in BMMCs. These results confirmed that V_A is a critical enhancer of IL-4 and Th2 cytokine gene expression, and underscore the notion that many transcription factor binding sites are located 3′ of coding regions [165]. AVNI *et al.* [166] showed that NFAT and GATA3 functionally cooperate at V_A to enhance IL-4 promoter activity in transient transfection assays. Together with the recent observation that NFAT also cooperates with T-bet to regulate IFN-γ gene expression [167], these data suggest that NFAT can interact with lineage-restricted factors to induce cytokine gene expression in both Th1 and Th2 cells.

There are five currently known NFAT family members that differ in their tissue distribution and regulation. NFAT1

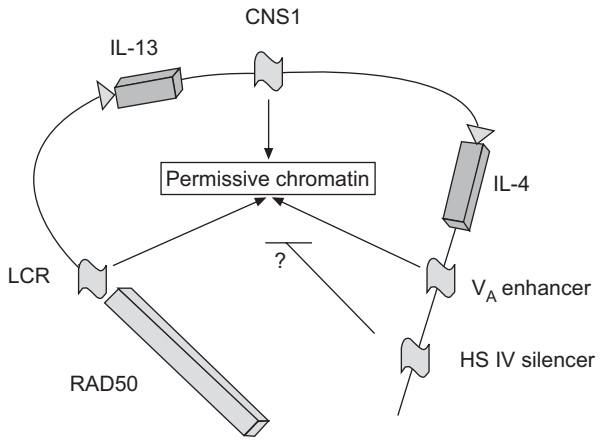

FIGURE 6. Conserved regulatory elements in the T-helper cell (Th) type-2 gene cluster identified using DNase hypersensitivity analyses and/or cross-species sequence alignment. A locus control region (LCR) in the 3′-region of the RAD50 gene was recently identified using transgenic mice. Conserved nucleotide sequence-1 (CNS-1) is located in between the interleukin (IL)-4 and IL-13 genes and has been implicated in Th2 lineage commitment. Two nearby elements 3′ of the IL-4 gene have opposing functions. The V_A enhancer is required for maximal IL-4 gene expression and Th2 differentiation, whereas the hypersensitive site IV silencer suppresses IL-4 in naïve, Th1 and Th2 cells. CNS: conserved nucleotide sequence; HS: hypersensitive.

(NFATp or NFATc2), NFAT2 (NFATc or NFATc2) and NFAT4 (NFTAx or NFATc3) predominate in immune cells [153]. Despite its name, the expression of NFAT is not restricted to T-cells, but can also be detected in B-cells, muscle cells, mast cells, basophils and eosinophils [78, 168]. The evidence available todate would argue that NFAT proteins should not have a dominant role in Th differentiation. This conclusion is based on the following three observations. First, the consensus binding sites of different NFAT proteins are almost identical (reflecting conserved DNA-binding domains), implying that target genes of different NFAT proteins should be very similar. Secondly, there are no major differences in NFAT expression when comparing highly polarised Th1 and Th2 cells. Thirdly, NFAT can interact with lineage restricted factors (*e.g.* GATA-3 and T-bet) to activate both IL-4 and IFN-γ gene expression. However, experiments using gene-targeted and chimeric mice uncovered a surprisingly important role for NFAT2, but not NFAT1 or NFAT4, in Th2 polarisation [169, 170]. In fact, deletion of NFAT1 (especially in combination with NFAT4) resulted in a phenotype of hyperactive immunity, characterised by heightened IL-4 gene expression and eosinophil recruitment to the lung [169, 171, 172]. As both NFAT1 and NFAT2 can activate the IL-4 or IL-13 promoters in transient transfection assays [173], enhanced IL-4 gene expression in NFAT1-null mice probably does not reflect a direct repressive effect of NFAT1 on Th2 gene expression. Instead, the Th2-bias of these mice may be the result of immune hyperproliferation due to the inhibited regulation of NFAT1-dependent genes that keep Th2 lymphocyte proliferation and/or cellcycle progression in check (*e.g.* CDK4 and ROG [174, 175]). NFAT proteins can therefore influence patterns of Th cytokine gene expression both directly and indirectly and the effect of a given NFAT family member will depend on

differences in strength and duration of activation, and possibly interaction with unique co-factors.

The current authors demonstrated in T-cell lines that glucocorticoids interfere with NFAT2-driven IL-4 promoter activity *via* a novel protein-protein interaction between the glucocorticoid receptor and NFAT2 [176], which may help explain some of the inhibitory effects of GC on IL-4 gene expression. New calcineurin antagonists are under development that may also prove to be useful agents in allergic asthma, especially if used in conjunction with glucocorticoids. Interestingly, there is some evidence that activation of NFAT proteins correlates with predisposition to atopy and asthma. For example, CHAN *et al.* [177] showed that NFAT was preferentially activated in peripheral blood mononuclear cells of subjects with atopic dermatitis using nonspecific pharmacologic agonists. In addition, KEEN *et al.* [178] showed that NFAT2 activation correlated with preferential IL-4 gene expression in ''asthma susceptible'' A/J mice (compared with resistant C3H/HeJ mice). The biochemical basis for these differences is unclear at present but deserves further study.

The activator protein-1 family (including junB) and c-Maf

Ap-1 is made up of members of the c-Fos and c-Jun families of transcription factors that dimerise *via* their leucine zippers and regulate the expression of diverse groups of genes in multiple lung cell types [179]. As discussed above, AP-1 interacts with NFAT in IL-4 gene regulation, and a similar interaction is involved in regulation of the IL-5 promoter [161]. It is currently unknown whether AP-1 proteins are involved in IL-13 gene regulation: preliminary evidence suggests that NFAT:AP-1 interactions are dispensable for IL-13 promoter activity [161]. Several reports show that distinct AP-1 family members are important for IL-4 gene expression in Th2 cells. For example, LI *et al.* [180] showed that junB was preferentially expressed in Th2 cells, activated the IL-4 promoter in transient transfection assays and enhanced IL-4 gene expression in transgenic animals. This result was surprising because junB was originally discovered as a kinase-inactive mutant that could repress the transacting functions of c-Jun [181]. In a recent paper, HARTENSTEIN *et al.* [186] generated transgenic mice expressing junB under the control of ubiquitin promoter in order to rescue the embryonic lethality of junB-null mice. Lymphocytes isolated from these chimeric animals expressed little or no junB and showed significantly reduced levels of IL-4. Furthermore, there was a concomitant decrease in ovalbumin-induced airways inflammation in junB-null chimeric mice. Clinical studies linking junB activation and/or expression in humans with asthma are currently lacking.

Another leucine-zipper containing transcription factor important for IL-4 transcriptional activation in Th2 cells is c-Maf. Since the original description by HO *et al.* [183] that c-Maf was preferentially expressed in Th2 clones and could induce IL-4 gene expression when overexpressed in B-cell lines, several studies have refined the understanding of the role of this factor in Th2 gene regulation. Current thinking is that c-Maf directly activates IL-4 but not IL-13 gene expression, and can do so in concert with other factors, including NIP-45, NFAT, and junB [180, 184, 185]. Furthermore, c-Maf can directly suppress IFN-γ production, independently of IL-4 [186]. Thus, these studies suggest that c-Maf plays an important role in Th2 polarisation. Studies using c-Maf gene-targeted mice should prove interesting in this regard. Interestingly, LI-WEBER *et al.* [187] found that the Maf response element was dispensable for full activation of the human IL-4 promoter, possibly reflecting subtle sequence divergence downstream of this site. Thus, the function of c-Maf in transcriptional regulation of the human IL-4 gene requires further study. Interestingly, two studies found increased expression of c-Maf in bronchial biopsies or induced sputum leukocytes in asthmatics compared with controls [31, 188]. The function of c-Maf in these cells, and whether this increased expression reflects Th2 skewing of cells in asthmatics or other factors, remains to be determined.

Other AP-1 proteins besides JunB and cMaf have been shown to bind the IL-4 promoter in gel shift assays, but to date none has demonstrated selective expression in Th subsets. However, the c-Jun N-terminal kinases JNK1 and JNK2, which regulate c-Jun activity, play complex roles during Th2 differentiation. Experiments using gene-targeted or transgenic mice found that JNK1 surprisingly antagonised Th2 differentiation, especially during early stages of naïve T-cell activation. This was attributed to phosphorylation of other targets besides AP-1, such as NFAT2. In contrast, JNK activity was required for full IL-4 expression in effector T-cells. These studies underscored the notion that Th differentiation in naïve T-cells and subsequent cytokine gene expression in re-stimulated effector T-cells are distinct biochemical events with distinct intermediaries. This notion is also useful for considering the roles of other transcription factors in Th2 gene regulation, including Stat6 and GATA3 (see below).

CCAAT-enhancer binding protein

Members of the CCAAT-EBP family are also potent transactivators of the IL-4 gene. C/EBP-β in particular binds to multiple sites in the IL-4 promoter, some of these in a Th2-speficic manner, and mutational analysis indicates that these sites are essential for IL-4 transcriptional regulation [147, 187]. C/EBP also activates the IL-5 promoter [189]. The signalling cascades that activate C/EBP in T-cells are complex, and appear to be distinct from those that activate NFAT. For example, C/EBP is activated in T-cells in a PKC-dependent, calcineurin-independent manner. One possibility is that signals downstream of the IL-6 receptor (known to activate C/EBP in other cell types) are involved in this process. However, although IL-6 can enhance IL-4 gene expression and Th2 differentiation [190], this was recently attributed to a novel enhancing effect of IL-6 on NFAT2 expression [191]. Additional studies of the effects of IL-6 and related cytokines on C/EBP activation in T-cells should help address this issue. Studies using retrovirally-mediated overexpression of C/EBP-β in mouse lymphocytes provided strong confirmation that this factor enhances IL-4 (and represses IFN-γ) expression in primary cells [192]. Future studies in which the levels of C/EBP-β can be genetically manipulated in mice should help address its role in allergen-driven Th2 pulmonary immune responses. Interestingly, defective expression of C/EBPα was recently found in bronchial smooth muscle cells (but not circulating lymphocytes) in subjects with asthma [193]. This defect in C/EBPα conferred resistance to the inhibition of smooth muscle cell proliferation by glucocorticoids, which was restored by overexpressing C/EBPα. This study showed

that while C/EBP-β can contribute to Th2-mediated inflammation, defective expression of C/EBP-α also leads to smooth muscle dysfunction in asthma [194]. This has important consequences for understanding how to position C/EBP as a potential therapeutic target. The ideal strategy would be to antagonise C/EBP-β in lymphocytes while restoring C/EBPα in bronchial smooth muscle cells, which will be a challenging task.

Signal transducer and activator of transcription-6

Ligation of the IL-4 and IL-13 receptors leads to the phosphorylation and activation of latent cytoplasmic Stat6, and current thinking is that Stat-6 mediates most of the transcriptional programmes that are downstream of IL-4/IL-13 signalling. The essential role of IL-4 in Th2 polarisation was discussed above, and studies using gene-targeted mice demonstrated that this requires Stat6 [195]. The molecular mechanisms by which Stat6 activates Th2 cytokine gene transcription are indirect. The current authors and others detected Stat6 binding sites in the IL-4 promoter, suggesting an initial model in which IL-4-induced Stat6 simply enhanced transcriptional activation of the IL-4 gene. However, in transient transfection assays Stat6 paradoxically repressed IL-4 promoter activity by displacing NFAT [148]. In support of this model, a recent study by DORADO *et al.* [196] showed that Stat6 downregulates IL-4 expression at later stages of Th2 cell activation. That Stat6 can downregulate gene expression (including chemokines) in the allergic lung was recently demonstrated by FULKERSON *et al.* [197]. Whether or not Stat6 directly regulates the transcription of other Th2 cytokines will require further research. One way that Stat6 might indirectly promote Th2 expression is at the level of chromatin remodelling; recent data suggest that Stat6 binds to a locus control region required for opening chromatin structure in the Th2 gene cluster on chromosome 5 (see below). A third way Stat6 might indirectly regulate Th2 responses is by enhancing the expression of other Th2 transcription factors, such as GATA3 and c-Maf (fig. 7) [198]. Although two GATA3 promoter regions have been identified [199], direct evidence that Stat6 activates the GATA3 promoter is currently lacking. Another indirect effect of Stat6 is to promote the survival of Th2 cells. In two key studies, ZHU and co-workers [200, 201] showed that Stat6 is necessary and sufficient for the Th2 expansion in part by inducing the expression of growth factors (*e.g.* Gfi-1) that promote the proliferation of Th2 cells and prevent their apoptosis. Recent studies have shown that the requirement for Stat6 in Th2 differentiation is not absolute. For example, in gene-targeted mice lacking the inhibitory molecule CTLA-4, Th2 differentiation can proceed in the absence of Stat6 [202]. This study showed that unopposed TCR signalling can substitute for Stat6 in some settings (possibly due to the recently described ability of IL-2 and Stat5 to drive Th2 differentiation).

Studies using gene-targeted mice have also demonstrated an essential role for Stat6 in allergic airway inflammation [203]. Molecules induced by Stat6 in the lung are numerous and include Th2-induced chemokines, such as eotaxin (CCL11) and possibly mucus genes. Stat6 is ubiquitously expressed, and regulates IL-4- and IL-13-induced gene expression in multiple lung cell types. KUPERMAN *et al.* [46] generated transgenic mice

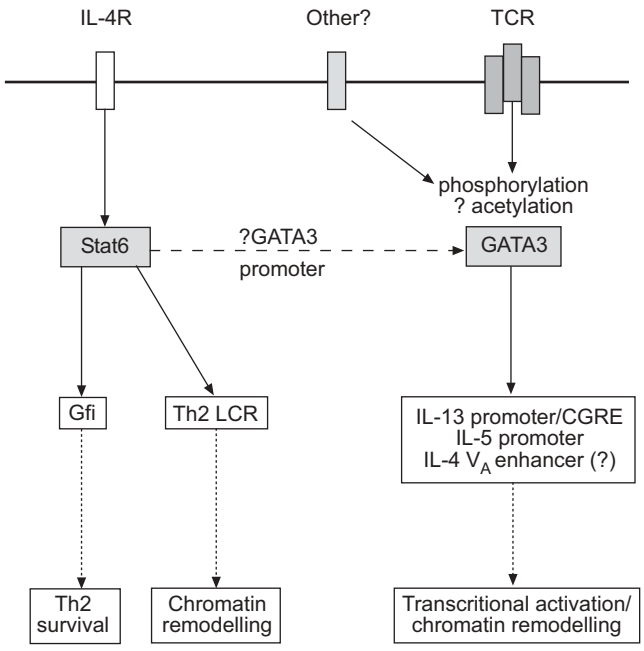

FIGURE 7. Regulation of T-helper cell type-2 (Th2) differentiation by signal transducer and activator of transcription (Stat)6 and GATA3. Interleukin (IL)-4-activated Stat6 can enhance Th2 responses by inducing the expression of genes that promote Th2 survival (*e.g.* Gfi), and binding to a locus control region (LCR) located in the Th2 gene cluster. The potential ability of Stat6 to enhance GATA3 expression is indicated by the dashed line. The signalling pathways that activate GATA3 in re-stimulated Th2 cells require further study. GATA3 can bind to several sites in the Th2 gene cluster to induce chromatin remodelling in naïve Th cells, and activate IL-13 and IL-5 transcription in re-stimulated Th2 cells. TCR: T-cell receptor; CGRE: conserved GATA response element; Gfi: growth factor independent-1.

in which Stat6 expression was restored in airway epithelial cells in an otherwise Stat6-null background. Mucus production and AHR, two cardinal features of asthma, were induced in these Stat6-expressing mice, but not controls, in an IL-13-dependent manner [46]. This was associated with the expression of a remarkably small subset of genes [204]. These studies suggested that the key IL-13/Stat6 target in asthma is a structural lung cell. However, adoptive transfer experiments using wild-type and Stat6-deficient T-cells found that the expression of Stat6 in lymphocytes (and not the recipient lung) was essential for AHR [205]. In a model of helminth-induced lung eosinophilia, it was recently shown that Stat6 expression in a bone-marrow derived cell was essential for eosinophil recruitment to the lung [75]. Future studies in which Stat6 expression can be genetically manipulated in discrete cell lineages should help identify the key Th2 target cell(s) in models of asthma. Interestingly, Stat6 was found to be overexpressed in bronchial mucosal biopsies from subjects with atopic asthma compared with nonatopic asthmatics or controls [31]. In addition, allergen challenge induced the expression of Stat6 in the nasal mucosa that could be blocked by topical steroids [206]. Since most studies using purified cells *ex vivo* show that Stat6 is equally expressed in Th1 and Th2 cells, these studies suggest that the airway microenvironment promotes Stat6 expression: the biochemical basis of this remains to be determined. Single nucleotide polymorphisms

in Stat6 have been associated with asthma, but their functional significance is currently unknown [97].

CP-2 and Yin-Yang-1

During a detailed deletion analysis, the current authors discovered binding sites for additional factors in the IL-4 promoter, including CP-2 and Yin-Yang-1 (YY-1) [149, 150]. Emerging data suggest that these factors play a previously underappreciated role in IL-4 gene regulation. CP-2 (also known as LBP1) was originally identified as a transcription factor required for α-globin expression. It is now known that CP-2 is expressed in multiple cells types and can regulate the expression of many genes. Using transient transfection assays, the current authors found that the integrity of the IL-4 promoter CP-2 site was essential for both constitutive and inducible promoter activity. Furthermore, the expression of a dominant negative CP-2 construct (LBP-1d) inhibited IL-4 gene expression in the D10 Th2 line [149]. The signalling pathways that lead to CP-2 activation in T-cells are currently under study, and involves members of the MAPK family. Interestingly, in ongoing studies the current authors have found that expression of CP-2 is also upregulated in differentiating human Th2 cells. Thus, CP-2 represents a novel transcriptional regulator of IL-4.

Using similar approaches, the current authors uncovered four binding sites for the pleiotropic factor YY-1 in the proximal IL-4 promoter. These sites (termed the Y elements, Y0–Y4) are located adjacent to or overlapping with the purine-rich NFAT sites described above. Using a careful mutagenesis strategy to preserve the integrity of the adjacent NFAT site, the authors found that mutation of Y0 had a particularly drastic effect on IL-4 promoter activity in transient transfection assays [150]. Surprisingly, in co-transfection assays the authors did not observe any positive interactions between YY-1 and NFAT proteins in driving the IL-4 promoter. Thus, despite their close association on the DNA-template, YY-1 and NFAT appear to enhance IL-4 transcriptional activity by distinct mechanisms. YY-1 can interact with a diverse array of other co-factors including histone-modifying enzymes [207], and one possibility is that YY-1 recruits these enzymes to the IL-4 locus. YY-1 has been shown to repress the IL-5 promoter in T-cell lines [208], and can also bind to the IFN-γ promoter [209]. The ability of YY-1 to regulate IFN-γ promoter activity is controversial, with both enhancing and repressing effects shown in different experiments [209, 210]. Whether or not YY-1 plays a role in T-cell polarisation is currently unknown. YY-1 was recently shown to regulate MCP-4 expression *via* a polymorphic site in the MCP-4 promoter [211]. Since deletion of YY1 results in early embryonic lethality, it will be necessary to manipulate the expression of YY1 at different stages of lymphocyte development in order to address its role in T-cell differentiation and allergic airway responses. Although it is known to be a constitutively nuclear phospho-protein, emerging data suggest that the expression and/or function of YY-1 can be altered by post-translational modifications. For example, BOVOLENTA *et al.* [212] showed that YY-1 was proteolytically degraded in IL-2-treated peripheral blood mononuclear cells, and YAO *et al.* [213] showed that the transacting function of YY-1 was regulated by lysine acetylation. Studies aimed at dissecting the signalling cascades that regulate the expression and function of YY-1 in the lung are ongoing.

GATA3

Since the original description by ZHANG *et al.* [214] that GATA3 expression was upregulated in Th2 cells but downregulated during Th1 differentiation, research by several laboratories has documented the key role this factor plays in the coordinate induction of Th2 cytokine gene expression and differentiation [151]. The preferential expression of GATA3 in Th2 cells involves signals provided by Stat6 and nuclear factor-κB [41, 72, 198], as well as an auto-activation positive feedback loop that stabilises GATA3 expression [215]. The signals that extinguish GATA3 expression following T-cell activation under neutral or Th1 conditions are less clear. Another important property of GATA3 is its ability to repress IFN-γ gene expression, thus serving to reinforce the Th2 phenotype [216]. Interestingly, distinct domains of GATA3 appear to be required for activation of Th2 cytokines compared with repression of IFN-γ [217]. Definitive evidence of the essential role for GATA3 in Th2 differentiation was recently provided by ZHU *et al.* [218]. Using conditional deletion of GATA3 in lymphocytes, ZHU *et al.* demonstrated that GATA3 was essential for both initiating Th2 responses and maintaining Th2 survival *in vivo*. Furthermore, deletion of GATA3 resulted in Th1 differentiation in the absence of Th1-inducing factors (*e.g.* IL-12), showing that GATA3 is a principal switch for Th1/Th2 responses [218]. The precise binding sites of GATA3 in the Th2 locus are under study. High-affinity GATA3 binding sites are located upstream of the IL-5 and IL-13 genes, but interestingly not in the IL-4 promoter [163, 214, 219]. In the report by ZHU *et al.* [218] conditional deletion of GATA3 in already committed Th2 cells inhibited the expression of IL-5 and IL-13, but only slightly blocked IL-4 production (little change in the number of IL-4 positive cells but ~50% reduction in their mean fluorescence intensity by intracellular staining and flow cytometry). This is compatible with a model in committed Th2 cells, in which GATA3 acts at the transcriptional level to primarily enhance promoter-driven expression of IL-5 and IL-13, with less effects on IL-4, although this a controversial area where more research is needed. The ability of GATA3 to initiate Th2 differentiation may be independent of its ability to promote transcriptional activation; the potential role of GATA3 in chromatin remodelling at the Th2 gene cluster is discussed in the concluding section of this review.

Several studies have directly linked GATA3 with allergic airway inflammation in mouse models and in man. For example, FINOTTO *et al.* [220] showed that inhaled GATA3 antisense oligonucleotides suppressed Th2 cytokine expression and AHR in mice. ZHANG *et al.* [40] created transgenic mice expressing an inducible GATA3 dominant negative in T-cells, and provided definitive evidence of the role of GATA3 in allergen-driven Th2 cytokine production and airway inflammation. Studies analysing GATA3 expression by immunohistochemistry found that GATA3 expressing cells were more numerous in airway biopsies from asthmatic compared with control subjects, and that GATA3-positive cells were strikingly increased following allergen challenge [29, 221]. If GATA3 expression is used as a surrogate marker of Th2 commitment, then these studies show that there is a striking enrichment of

Th2 cells following allergen challenge. A recent study found that three haplotypes within the GATA3 gene were associated with asthma and atopy-related phenotypes [98]. This study lends support to the idea that Th2 skewing in allergic individuals has a genetic basis.

Th2 chromatin-based gene regulation

In addition to the acute transcriptional events outlined above, which occur after TCR and co-stimulatory molecule-dependent activation of NFAT and other factors, there is now substantial evidence the transition of a naïve Th cell into a Th2 effector is also regulated at the level of chromatin remodelling. In this model, chromatin structure at the Th2 locus in resting naïve T-cells is generally repressive for transcription because of tight interactions between histone and other proteins and the DNA template. Under Th2-biasing conditions (*e.g.* strong IL-4 receptor), chromatin is remodelled into a more permissive form that allows easier access of NFAT and other transcription factors required for sustained, high-level IL-4 gene expression. Remodelled states of chromatin are passed on to daughter cells after mitosis and represent an epigenetic trait [222]. The precise biochemical basis of chromatin remodelling in Th2 cells is under investigation, and involves post-translational acetylation and methylation of histones as well as passive demethylation of DNA [166, 223, 224]. Equally important in Th2 cells is silencing of the IFN-γ gene which also happens in part at the epigenetic level.

The DNA elements that regulate chromatin remodelling are under active study. Definitive evidence that an element acts at the chromatin level requires experiments using gene-targeted or transgenic mice in which the element of interest is used to drive expression of a reporter gene. Elements that confer copy-number dependent and integration site-independent patterns of gene expression when to linked reporter genes are operationally defined to as locus control regions (LCRs) [225]. LCRs have been characterised at a few genetic loci and are thought to spread open chromatin domains over thousands of base pairs of DNA, thus facilitating the expression of gene clusters. LCRs may or may not act as classical enhancers in standard transfection assays. A candidate LCR was recently identified in the 3′ region of the RAD50 gene (fig. 6) [224]. The DNA binding factors that regulate the LCR are under investigation by several groups, and appear to include Stat6 but interestingly not GATA3 [226]. In addition to the LCR, several elements around the IL-4 gene act in concert at the chromatin level to restrict IL-4 gene expression to Th2 cells [227].

The LCR and other regulatory elements were discovered on the basis of DNase hypersensitivity analyses. Another means of identifying important noncoding elements involved in gene regulation involves cross-species sequence comparison. If an intronic or noncoding element is conserved in multiple species, this argues that it serves a functional role presumably in gene regulation. In a ground-breaking paper, LOOTS *et al.* [228] identified several regions throughout the Th2 locus that were highly conserved, located mostly in noncoding regions, and several hundred base pairs in length. One of these was ~400 base pairs in length and located between the IL-4 and IL-13 genes (conserved nucleotide sequence-1 (CNS-1), fig. 6) [228]. Deletion of CNS-1 in mice resulted in a reduced frequency of IL-4-positive cells and a modest impairment in IL-4 production per cell, reflecting reduced lineage commitment consistent with effects on chromatin remodelling [229]. The response of CNS-1-null mice to inhaled helminth challenge was blunted (*e.g.* reduced AHR and immunoglobulin E), showing that despite small changes in overall IL-4 expression-reduced lineage commitment can have substantial effects on pulmonary Th2 immune responses [229]. The combination of DNase hypersensitivity analysis and cross-species sequence alignment was used to identify other regulatory elements important for IL-4 gene expression, the V_A enhancer and hypersensitive (HS) IV silencer. Both of these are located 3′ of the IL-4 gene, and have been deleted in the germ line in mice with interesting phenotypes (fig. 6). The V_A enhancer, which binds NFAT and GATA3, was discussed above and is required for full IL-4 expression in T-cells and mast cells. The phenotype of HS IV gene targeted mice was recently reported [230]. IL-4 and IL-13 expression were enhanced in naïve HS IV-null T-cells with concomitant Th2 skewing. Furthermore, IL-4 was aberrantly expressed in Th1 cells and impaired the Th1 immune response to *Leishmania major*. At present, contributions of the V_A enhancer and HS IV silencer to chromatin remodelling are unclear. It will be interesting to discover the identity of transacting factors that bind to the putative LCR, CNS-1 and other noncoding regulatory regions in the Th2 gene cluster. Studying the expression and regulation of these factors in Th2 immune responses in asthma should prove worthwhile. LOOTS *et al.* [231] recently developed a computational tool for high-throughput discovery of conserved DNA sequences termed rVista. rVista combines clustering of predicted transcription factor binding sites and the analysis of interspecies sequence conservation to maximise the identification of functional elements. This web-based tool should be helpful to many investigators interested in gene regulation.

DIFFERENCES BETWEEN MOUSE AND MAN?

Polarisation of the cytokine repertoire is a feature of both CD4+ and CD8+ T-cells. Although originally defined in mouse T-helper clones [232], Th2 polarisation has been observed in many species including cats, dogs, cows, horses, monkeys and humans. Due to the ease with which they can be grown and genetically manipulated, the vast majority of studies todate have been performed using mouse cells. Human T-cells behave similarly and can be polarised *in vitro* by stimulation *via* the TCR, co-stimulatory receptors and exogenous IL-4 [233, 234]. Thus, Th2 differentiation represents a programme of coordinated gene expression that is conserved through evolution. ROGAN *et al.* [235] recently showed, using a novel transgenic mouse model, that human Th2 cytokine genes are coordinately induced under Th2 conditions similar to their mouse counterparts. Thus, even though they are not genetically identical, the human and mouse Th2 gene clusters are both subject to coordinate regulation *in vivo*. However, there are some apparent species-specific differences between man and mouse in the Th differentiation programme. For example, there is inter-subject variability in Th2 polarisation, and it is often necessary to use prolonged polarising conditions to uncover distinct Th subsets using human T-cells (*e.g.* up to several weeks [234]). This probably reflects both genetic variability in the outbred human population, as well as difficulties in obtaining truly naïve precursor T-cells to study. One way

around the latter problem is to use cord-blood derived T-cells [233]. In addition, differences in the expression and role of polarising transcription factors in human *versus* mouse Th2 cells are beginning to emerge. For example, despite polarisation of the cytokine repertoire, the extinction of GATA3 and T-bet under Th1 and Th2 conditions, respectively, is often not as dramatic using human Th cells (De Fanis and Casolaro, John Hopkins Asthma and Allergy Centre, Baltimore, USA; personal communication). It was also recently found that T-bet does not extinguish IL-2 or IL-4 in human Th2 cells as effectively as in mouse Th2 cells [236]. In addition, c-Maf is not so clearly a Th2-restricted factor in human Th cells [234], and the human IL-4 promoter c-Maf response element is dispensable for full promoter activity [187]. The physiological significance of these observations is currently uncertain, but one possibility is that there are alternative pathways to Th2 cytokine gene expression in human T-cells (*i.e.* c-Maf- and/or GATA3-independent?); this should be an interesting area for future research.

SUMMARY

The understanding of the cellular and molecular regulation of immune responses has increased at a dramatic pace. There is now have an unprecedented understanding of the molecular factors and biochemical processes involved in Th2 gene regulation. Many key insights have been obtained using gene-targeted or genetically modified mouse strains, and the challenge for the future will be to translate this understanding into clinically meaningful insights into asthma pathophysiology and therapy.

REFERENCES

1 Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med* 2001; 344: 350–362.
2 Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004; 22: 789–815.
3 Kelly-Welch AE, Melo ME, Smith E, *et al.* Complex role of the IL-4 receptor alpha in a murine model of airway inflammation: expression of the IL-4 receptor alpha on nonlymphoid cells of bone marrow origin contributes to severity of inflammation. *J Immunol* 2004; 172: 4545–4555.
4 Allen M, Heinzmann A, Noguchi E, *et al.* Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat Genet* 2003; 35: 258–263.
5 Zhang Y, Leaves NI, Anderson GG, *et al.* Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat Genet* 2003; 34: 181–186.
6 Laitinen T, Polvi A, Rydman P, *et al.* Characterization of a common susceptibility locus for asthma-related traits. *Science* 2004; 304: 300–304.
7 Burchard EG, Silverman EK, Rosenwasser LJ, *et al.* Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. *Am J Respir Crit Care Med* 1999; 160: 919–922.
8 Choi EH, Lee HJ, Yoo T, Chanock SJ. A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in Korean children. *J Infect Dis* 2002; 186: 1207–1211.
9 Van der Pouw Kraan T, Van Veen A, Boeije LC, *et al.* An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes and Immunity* 1999; 1: 61–65.
10 Howard TD, Whittaker PA, Zaiman AL, *et al.* Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001; 25: 377–384.
11 van der Pouw Kraan TC, Kucukaycan M, Bakker AM, *et al.* Chronic obstructive pulmonary disease is associated with the -1055 IL-13 promoter polymorphism. *Genes Immun* 2002; 3: 436–439.
12 Liu X, Beaty TH, Deindl P, *et al.* Associations between specific serum IgE response and 6 variants within the genes IL4, IL13, and IL4RA in German children: the german multicenter atopy study. *J Allergy Clin Immunol* 2004; 113: 489–495.
13 Heinzmann A, Mao X, Akaiwa M, *et al.* Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet* 2000; 9: 549–559.
14 Graves PE, Kabesch M, Halonen M, *et al.* A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000; 105: 506–513.
15 Mitsuyasu H, Izuhara K, Mao XQ, *et al.* Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 1998; 19: 119–120.
16 Howard TD, Koppelman GH, Xu J, *et al.* Gene–gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *Am J Hum Genet* 2002; 70: 230–236.
17 He JQ, Connett JE, Anthonisen NR, Sandford AJ. Polymorphisms in the IL13, IL13RA1, and IL4RA genes and rate of decline in lung function in smokers. *Am J Respir Cell Mol Biol* 2003; 28: 379–385.
18 Hershey G, Friedrich M, Esswein L, Thomas M, Chatila T. The association of atopy with a gain-of-function mutation in the alpha-subunit of the IL-4 receptor. *N Eng J Med* 1997; 337: 1720–1725.
19 Tamura K, Arakawa H, Suzuki M, *et al.* Novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene is associated with allergic diseases. *Clin Exp Allergy* 2001; 31: 1509–1514.
20 Shirakawa I, Deichmann KA, Izuhara I, Mao I, Adra CN, Hopkin JM. Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. *Immunol Today* 2000; 21: 60–64.
21 Vercelli D. Genetic polymorphism in allergy and asthma. *Curr Opin Immunol* 2003; 15: 609–613.
22 Robinson DS, Hamid Q, Ying S, *et al.* Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326: 298–304.
23 Robinson DS, Bentley AM, Hartnell A, *et al.* Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness. *Thorax* 1993; 48: 26–32.
24 Robinson D, Hamid Q, Bentley A, *et al.* Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993; 92: 313–324.

25 Corrigan CJ, Hamid Q, North J, *et al.* Peripheral blood CD4 but not CD8 T-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2- type pattern: effect of glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1995; 12: 567–578.

26 Leung DY, Martin RJ, Szefler SJ, *et al.* Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. *J Exp Med* 1995; 181: 33–40.

27 Ying S, Durham S, Corrigan C, *et al.* Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am Rev Respir Cell Mol Biol* 1995; 12: 477–487.

28 Humbert M, Durham S, Ying A, *et al.* IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic amd nonatopic asthma. *Am Rev Respir Crit Care Med* 1996; 154: 1497–1504.

29 Nakamura Y, Ghaffar O, Olivenstein R, *et al.* Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999; 103: 215–222.

30 Tamaoki J, Kondo M, Sakai N, *et al.* Effect of suplatast tosilate, a Th2 cytokine inhibitor, on steroid-dependent asthma: a double-blind randomised study. *Lancet* 2000; 356: 273–278.

31 Christodoulopoulos P, Cameron L, Nakamura Y, *et al.* TH2 cytokine-associated transcription factors in atopic and nonatopic asthma: evidence for differential signal transducer and activator of transcription 6 expression. *J Allergy Clin Immunol* 2001; 107: 586–591.

32 Brightling CE, Symon FA, Birring SS, *et al.* TH2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. *J Allergy Clin Immunol* 2002; 110: 899–905.

33 van der Pouw Kraan TC, Boeije LC, de Groot ER, *et al.* Reduced production of IL-12 and IL-12-dependent IFN-gamma release in patients with allergic asthma. *J Immunol* 1997; 158: 5560–5565.

34 Caramori G, Lim S, Ito K, *et al.* Expression of GATA family of transcription factors in T-cells, monocytes and bronchial biopsies. *Eur Respir J* 2001; 18: 466–473.

35 Gemou-Engesaeth V, Fagerhol MK, Toda M, *et al.* Expression of activation markers and cytokine mRNA by peripheral blood CD4 and CD8 T cells in atopic and nonatopic childhood asthma: effect of inhaled glucocorticoid therapy. *Pediatrics* 2002; 109: E24.

36 Wills-Karp M, Luyimbazi J, Xu X, *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282: 2258–2261.

37 Grunig G, Warnock M, Wakil AE, *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma [see comments]. *Science* 1998; 282: 2261–2263.

38 Cohn L, Homer RJ, MacLeod H, *et al.* Th2-induced airway mucus production is dependent on IL-4Ralpha, but not on eosinophils. *J Immunol* 1999; 162: 6178–6183.

39 Zhu Z, Homer RJ, Wang Z, *et al.* Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999; 103: 779–788.

40 Zhang DH, Yang L, Cohn L, *et al.* Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* 1999; 11: 473–482.

41 Das J, Chen CH, Yang L, *et al.* A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat Immunol* 2001; 2: 45–50.

42 Cohn L, Herrick C, Niu N, *et al.* IL-4 promotes airway eosinophilia by suppressing IFN-gamma production: defining a novel role for IFN-gamma in the regulation of allergic airway inflammation. *J Immunol* 2001; 166: 2760–2767.

43 Temann UA, Ray P, Flavell RA. Pulmonary overexpression of IL-9 induces Th2 cytokine expression, leading to immune pathology. *J Clin Invest* 2002; 109: 29–39.

44 Venkayya R, Lam M, Willkom M, *et al.* The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am J Respir Cell Mol Biol* 2002; 26: 202–208.

45 Mattes J, Yang M, Mahalingam S, *et al.* Intrinsic defect in T cell production of interleukin (IL)-13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J Exp Med* 2002; 195: 1433–1444.

46 Kuperman DA, Huang X, Koth LL, *et al.* Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 2002; 8: 885–889.

47 Zimmermann N, King NE, Laporte J, *et al.* Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. *J Clin Invest* 2003; 111: 1863–1874.

48 Kamata T, Yamashita M, Kimura M, *et al.* SRC homology 2 domain-containing tyrosine phosphatase SHP-1 controls the development of allergic airway inflammation. *J Clin Invest* 2003; 111: 109–119.

49 Aronica MA, McCarthy S, Swaidani S, *et al.* Recall helper T cell response: T helper 1 cell-resistant allergic susceptibility without biasing uncommitted CD4 T cells. *Am J Respir Crit Care Med* 2004; 169: 587–595.

50 Leigh R, Ellis R, Wattie JN, *et al.* Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. *Am J Respir Crit Care Med* 2004; 169: 860–867.

51 Shi HZ, Deng JM, Xu H, *et al.* Effect of inhaled interleukin-4 on airway hyperreactivity in asthmatics. *Am J Respir Crit Care Med* 1998; 157: 1818–1821.

52 Bryan SA, O'Connor BJ, Matti S, *et al.* Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356: 2149–2153.

53 Umland SP, Nahrebne DK, Razac S, *et al.* The inhibitory effects of topically active glucocorticoids on IL-4, IL-5, and interferon-gamma production by cultured primary CD4+ T cells. *J Allergy Clin Immunol* 1997; 100: 511–519.

54 Georas S. Inhaled glucocorticoids, lymphocytes, and dendritic cells in asthma and obstructive lung diseases. *Proc Am Thorac Soc* 2004; 1: 215–221.

55 Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003; 111: 450–463.

56 Kumar RK, Herbert C, Webb DC, *et al.* Effects of anticytokine therapy in a mouse model of chronic asthma. *Am J Respir Crit Care Med* 2004; 170: 1043–1048.

57 Randolph DA, Stephens R, Carruthers CJ, *et al.* Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 1999; 104: 1021–1029.

58 Ford JG, Rennick D, Donaldson DD, *et al.* Il-13 and IFN-gamma: interactions in lung inflammation. *J Immunol* 2001; 167: 1769–1777.

59 Cui J, Pazdziorko S, Miyashiro JS, *et al.* TH1-mediated airway hyperresponsiveness independent of neutrophilic inflammation. *J Allergy Clin Immunol* 2005; 115: 309–315.

60 Walter MJ, Morton JD, Kajiwara N, *et al.* Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J Clin Invest* 2002; 110: 165–175.

61 Magnan AO, Mely LG, Camilla CA, *et al.* Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. Increased IFN-gamma-producing CD8(+) T cells in asthma. *Am J Respir Crit Care Med* 2000; 161: 1790–1796.

62 Rowe J, Heaton T, Kusel M, *et al.* High IFN-gamma production by CD8+ T cells and early sensitization among infants at high risk of atopy. *J Allergy Clin Immunol* 2004; 113: 710–716.

63 Krug N, Madden J, Redington AE, *et al.* T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996; 14: 319–326.

64 Cho SH, Stanciu LA, Holgate ST, *et al.* Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4+ and CD8+ T cells in atopic asthma. *Am J Respir Crit Care Med* 2005; 171: 224–230.

65 Castro M, Bloch SR, Jenkerson MV, *et al.* Asthma exacerbations after glucocorticoid withdrawal reflects T cell recruitment to the airway. *Am J Respir Crit Care Med* 2004; 169: 842–849.

66 Cote-Sierra J, Foucras G, Guo L, *et al.* Interleukin 2 plays a central role in Th2 differentiation. *Proc Natl Acad Sci USA* 2004; 101: 3880–3885.

67 Constant SL, Brogdon JL, Piggott DA, *et al.* Resident lung antigen-presenting cells have the capacity to promote Th2 T cell differentiation *in situ*. *J Clin Invest* 2002; 110: 1441–1448.

68 Gajewska BU, Alvarez D, Vidric M, *et al.* Generation of experimental allergic airways inflammation in the absence of draining lymph nodes. *J Clin Invest* 2001; 108: 577–583.

69 Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, *et al.* Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat Med* 2004; 10: 927–934.

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

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

72 Grogan JL, Mohrs M, Harmon B, *et al.* Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. *Immunity* 2001; 14: 205–215.

73 Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, *et al.* IgE-dependent IL-4 secretion by human basophils. The relationship between cytokine production and histamine release in mixed leukocyte cultures. *J Immunol* 1994; 153: 1808–1817.

74 Nouri-Aria KT, Irani AM, Jacobson MR, *et al.* Basophil recruitment and IL-4 production during human allergen-induced late asthma. *J Allergy Clin Immunol* 2001; 108: 205–211.

75 Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 2004; 20: 267–277.

76 Lee JJ, Dimina D, Macias MP, *et al.* Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004; 305: 1773–1776.

77 Humbles AA, Lloyd CM, McMillan SJ, *et al.* A critical role for eosinophils in allergic airways remodeling. *Science* 2004; 305: 1776–1779.

78 Seminario M-C, Guo J, Bochner B, *et al.* Human eosinophils constitutively express NFATp and NFATc. *J All Clin Immunol* 2001; 107: 143–152.

79 van Rijt LS, Vos N, Hijdra D, *et al.* Airway eosinophils accumulate in the mediastinal lymph nodes but lack antigen-presenting potential for naive T cells. *J Immunol* 2003; 171: 3372–3378.

80 Usui T, Nishikomori R, Kitani A, *et al.* GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet. *Immunity* 2003; 18: 415–428.

81 Szabo SJ, Kim ST, Costa GL, *et al.* A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; 100: 655–669.

82 Szabo SJ, Sullivan BM, Stemmann C, *et al.* Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. *Science* 2002; 295: 338–342.

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

84 Mullen AC, High FA, Hutchins AS, *et al.* Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. *Science* 2001; 292: 1907–1910.

85 Sullivan BM, Juedes A, Szabo SJ, *et al.* Antigen-driven effector CD8 T cell function regulated by T-bet. *Proc Natl Acad Sci USA* 2003; 100: 15818–15823.

86 Townsend MJ, Weinmann AS, Matsuda JL, *et al.* T-bet regulates the terminal maturation and homeostasis of NK and Valpha14i NKT cells. *Immunity* 2004; 20: 477–494.

87 Peng SL, Szabo SJ, Glimcher LH. T-bet regulates IgG class switching and pathogenic autoantibody production. *Proc Natl Acad Sci USA* 2002; 99: 5545–5550.

88 Way SS, Wilson CB. Cutting edge: immunity and IFN-gamma production during Listeria monocytogenes infection in the absence of T-bet. *J Immunol* 2004; 173: 5918–5922.

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

90 Tantisira KG, Hwang ES, Raby BA, *et al.* TBX21: A functional variant predicts improvement in asthma with the use of inhaled corticosteroids. *Proc Natl Acad Sci USA* 2004; 101: 18099–18104.

91 Kuipers H, Lambrecht BN. The interplay of dendritic cells, Th2 cells and regulatory T cells in asthma. *Curr Opin Immunol* 2004; 16: 702–708.

92 Lambrecht BN, Hammad H. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat Rev Immunol* 2003; 3: 994–1003.

93 Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. *J Immunol* 1997; 158: 5589–5595.

94 DeKruyff RH, Fang Y, Umetsu DT. Corticosteroids enhance the capacity of macrophages to induce Th2 cytokine synthesis in CD4+ lymphocytes by inhibiting IL-12 production. *J Immunol* 1998; 160: 2231–2237.

95 Matsue H, Yang C, Matsue K, *et al.* Contrasting impacts of immunosuppressive agents (rapamycin, FK506, cyclosporin A, and dexamethasone) on bidirectional dendritic cell-T cell interaction during antigen presentation. *J Immunol* 2002; 169: 3555–3564.

96 Arima K, Umeshita-Suyama R, Sakata Y, *et al.* Upregulation of IL-13 concentration *in vivo* by the IL13 variant associated with bronchial asthma. *J Allergy Clin Immunol* 2002; 109: 980–987.

97 Duetsch G, Illig T, Loesgen S, *et al.* STAT6 as an asthma candidate gene: polymorphism-screening, association and haplotype analysis in a caucasian sib-pair study. *Hum Mol Genet* 2002; 11: 613–621.

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

99 Kondo N, Matsui E, Kaneko H, *et al.* Atopy and mutations of IL-12 receptor beta 2 chain gene. *Clin Exp Allergy* 2001; 31: 1189–1193.

100 Eder W, Klimecki W, Yu L, *et al.* Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; 113: 482–488.

101 Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; 196: 1645–1651.

102 Redecke V, Hacker H, Datta SK, *et al.* Cutting edge: activation of toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* 2004; 172: 2739–2743.

103 Kalinski P, Hilkens CM, Snijders A, Snijdewint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* 1997; 159: 28–35.

104 Braun MC, He J, Wu CY, Kelsall BL. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor beta1 and beta2 chain expression. *J Exp Med* 1999; 189: 541–552.

105 Caron G, Delneste Y, Roelandts E, *et al.* Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol* 2001; 167: 3682–366.

106 Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest* 2001; 108: 1865–1873.

107 Aliberti J, Hieny S, Reis ESC, Serhan CN, Sher A. Lipoxin-mediated inhibition of IL-12 production by DCs: a mechanism for regulation of microbial immunity. *Nat Immunol* 2002; 3: 76–82.

108 O'Garra A, Vieira PL, Vieira P, *et al.* IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. *J Clin Invest* 2004; 114: 1372–1378.

109 Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2002; 2: 46–53.

110 Harris NL, Watt V, Ronchese F, *et al.* Differential T cell function and fate in lymph node and nonlymphoid tissues. *J Exp Med* 2002; 195: 317–326.

111 Reinhardt RL, Bullard DC, Weaver CT, *et al.* Preferential accumulation of antigen-specific effector CD4 T cells at an antigen injection site involves CD62E-dependent migration but not local proliferation. *J Exp Med* 2003; 197: 751–762.

112 Sekiya T, Miyamasu M, Imanishi M, *et al.* Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. *J Immunol* 2000; 165: 2205–2213.

113 Vulcano M, Albanesi C, Stoppacciaro A, *et al.* Dendritic cells as a major source of macrophage-derived chemokine/CCL22 *in vitro* and *in vivo*. *Eur J Immunol* 2001; 31: 812–822.

114 Honda K, Arima M, Cheng G, *et al.* Prostaglandin D2 reinforces Th2 type inflammatory responses of airways to low-dose antigen through bronchial expression of macrophage-derived chemokine. *J Exp Med* 2003; 198: 533–543.

115 Marrack P, Kappler J. Control of T cell viability. *Annu Rev Immunol* 2004; 22: 765–787.

116 Wu CY, Kirman JR, Rotte MJ, *et al.* Distinct lineages of T(H)1 cells have differential capacities for memory cell generation *in vivo*. *Nat Immunol* 2002; 3: 852–858.

117 Refaeli Y, Van Parijs L, Alexander SI, *et al.* Interferon gamma is required for activation-induced death of T lymphocytes. *J Exp Med* 2002; 196: 999–1005.

118 Jayaraman S, Castro M, O'Sullivan M, *et al.* Resistance to Fas-mediated T cell apoptosis in asthma. *J Immunol* 1999; 162: 1717–1722.

119 Akdis M, Trautmann A, Klunker S, *et al.* T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells. *FASEB J* 2003; 17: 1026–1035.

120 de Boer BA, Fillie YE, Kruize YC, *et al.* Antigen-stimulated IL-4, IL-13 and IFN-gamma production by human T cells at a single-cell level. *Eur J Immunol* 1998; 28: 3154–3160.

121 Ho IC, Glimcher LH. Transcription: tantalizing times for T cells. *Cell* 2002; 109 Suppl: S109–S120.

122 Bettelli E, Kuchroo VK. IL-12- and IL-23-induced T helper cell subsets: birds of the same feather flock together. *J Exp Med* 2005; 201: 169–171.

123 Hurst SD, Muchamuel T, Gorman DM, *et al.* New IL-17 family members promote Th1 or Th2 responses in the

lung: *in vivo* function of the novel cytokine IL-25. *J Immunol* 2002; 169: 443–453.
124 Villarino AV, Huang E, Hunter CA. Understanding the pro- and anti-inflammatory properties of IL-27. *J Immunol* 2004; 173: 715–720.
125 Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002; 2: 116–126.
126 Brogdon JL, Leitenberg D, Bottomly K. The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells. *J Immunol* 2002; 168: 3825–3832.
127 Jorritsma PJ, Brogdon JL, Bottomly K. Role of TCR-induced extracellular signal-regulated kinase activation in the regulation of early IL-4 expression in naive CD4+ T cells. *J Immunol* 2003; 170: 2427–2434.
128 Cannons JL, Yu LJ, Hill B, *et al.* SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity* 2004; 21: 693–706.
129 Tanaka Y, Bi K, Kitamura R, *et al.* SWAP-70-like adapter of T cells, an adapter protein that regulates early TCR-initiated signaling in Th2 lineage cells. *Immunity* 2003; 18: 403–414.
130 Aguado E, Richelme S, Nunez-Cruz S, *et al.* Induction of T helper type 2 immunity by a point mutation in the LAT adaptor. *Science* 2002; 296: 2036–2040.
131 Kobayashi K, Inohara N, Hernandez LD, *et al.* RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. *Nature* 2002; 416: 194–199.
132 Miller AT, Wilcox HM, Lai Z, *et al.* Signaling through Itk promotes T helper 2 differentiation via negative regulation of T-bet. *Immunity* 2004; 21: 67–80.
133 Fang D, Elly C, Gao B, *et al.* Dysregulation of T lymphocyte function in itchy mice: a role for Itch in TH2 differentiation. *Nat Immunol* 2002; 3: 281–287.
134 Marsland BJ, Soos TJ, Spath G, Littman DR, Kopf M. Protein kinase C theta is critical for the development of *in vivo* T helper (Th)2 cell but not Th1 cell responses. *J Exp Med* 2004; 200: 181–189.
135 Salek-Ardakani S, So T, Halteman BS, Altman A, Croft M. Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C theta. *J Immunol* 2004; 173: 6440–6447.
136 Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 2002; 20: 55–72.
137 Egwuagu CE, Yu CR, Zhang M, Mahdi RM, Kim SJ, Gery I. Suppressors of cytokine signaling proteins are differentially expressed in Th1 and Th2 cells: implications for Th cell lineage commitment and maintenance. *J Immunol* 2002; 168: 3181–3187.
138 Casolaro V, Georas S, Song Z, *et al.* Inhibition of NF-AT-dependent transcription by NF-κB: implications for differential cytokine gene expression. *Proc Natl Acad Sci USA* 1995; 92: 11623–11627.
139 Valanciute A, le Gouvello S, Solhonne B, *et al.* NF-kappa B p65 antagonizes IL-4 induction by c-maf in minimal change nephrotic syndrome. *J Immunol* 2004; 172: 688–698.
140 Hilliard BA, Mason N, Xu L, *et al.* Critical roles of c-Rel in autoimmune inflammation and helper T cell differentiation. *J Clin Invest* 2002; 110: 843–850.
141 Mullen AC, Hutchins AS, High FA, *et al.* Hlx is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. *Nat Immunol* 2002; 3: 652–658.
142 Artis D, Villarino A, Silverman M, *et al.* The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. *J Immunol* 2004; 173: 5626–5634.
143 Abe E, De Waal Malefyt R, Matsuda I, *et al.* An 11 base-pair DNA sequence motif apparently unique to human interleukin 4 gene confers responsiveness to T-cell activation signals. *Proc Natl Acad Sci USA* 1992; 89: 2864–2870.
144 Li-Weber M, Krammer PH. Regulation of IL4 gene expression by T cells and therapeutic perspectives. *Nat Rev Immunol* 2003; 3: 534–543.
145 Szabo S, Gold J, Murphy T, Murphy K. Identification of cis-acting regulatory elements controlling interleukin-4 gene expression in T cells: roles for NF-Y and NF-AT. *Mol Cell Biol* 1993; 13: 4793–4805.
146 Hodge MR, Rooney JW, Glimcher LH. The proximal promoter of the IL-4 gene is composed of multiple essential regulatory sites that bind at least two distinct factors. *J Immunol* 1995; 154: 6397–6405.
147 Li-Weber M, Salgame P, Hu C, Davydov I, Krammer P. Differential interaction of nuclear factors with the PRE-I enhancer element of the human IL-4 promoter in different T cell subsets. *J Immunol* 1997; 158: 1194–1200.
148 Georas S, Cumberland J, Burke T, Chen R. Stat6 inhibits interleukin 4 promoter activity in T cells. *Blood* 1998; 92: 4529–4538.
149 Casolaro V, Keane-Myers AM, Swendeman SL, *et al.* Identification and characterization of a critical CP2-binding element in the human interleukin-4 promoter. *J Biol Chem* 2000; 275: 36605–36611.
150 Guo J, Casolaro V, Seto E, *et al.* Yin-yang 1 activates interleukin-4 gene expression in T cells. *J Biol Chem* 2001; 276: 48871–48878.
151 Ray A, Cohn L. Th2 cells and GATA-3 in asthma: new insights into the regulation of airway inflammation. *J Clin Invest* 1999; 104: 985–993.
152 Zhang D-H, Yang L, Ray A. Differential responsiveness of the IL-5 and IL-4 genes to transcription factor GATA-3. *J Immunol* 1998; 161: 3817–3821.
153 Rao A, Luo C, Hogan P. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 1997; 15: 707–747.
154 Crabtree GR. Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. *Cell* 1999; 96: 611–614.
155 Burke TF, Casolaro V, Georas SN. Characterization of P5, a novel NFAT/AP-1 site in the human IL-4 promoter. *Biochem Biophys Res Commun* 2000; 270: 1016–1023.
156 Nolan G. NF-AT-AP-1 and Rel-bZIP: hybrid vigour and binding under the influence. *Cell* 1994; 77: 795–798.
157 Chen L, Glover JN, Hogan PG, *et al.* Structure of the DNA-binding domains from NFAT, Fos and Jun bound specifically to DNA. *Nature* 1998; 392: 42–48.
158 Macian F, Garcia-Cozar F, Im SH, *et al.* Transcriptional mechanisms underlying lymphocyte tolerance. *Cell* 2002; 109: 719–731.

159 Heissmeyer V, Macian F, Im SH, *et al.* Calcineurin imposes T cell unresponsiveness through targeted proteolysis of signaling proteins. *Nat Immunol* 2004; 5: 255–265.

160 Macian F, Garcia-Rodriguez C, Rao A. Gene expression elicited by NFAT in the presence or absence of cooperative recruitment of Fos and Jun. *Embo J* 2000; 19: 4783–4795.

161 Ogawa K, Kaminuma O, Okudaira H, *et al.* Transcriptional regulation of the IL-5 gene in peripheral T cells of asthmatic patients. *Clin Exp Immunol* 2002; 130: 475–483.

162 Dolganov G, Bort S, Lovett M, *et al.* Coexpression of the interleukin-13 and interleukin-4 genes correlates with their physical linkage in the cytokine gene cluster on human chromosome 5q23–31. *Blood* 1996; 87: 3316–3326.

163 Agarwal S, Avni O, Rao A. Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer *in vivo*. *Immunity* 2000; 12: 643–652.

164 Solymar DC, Agarwal S, Bassing CH, *et al.* A 3′ enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells. *Immunity* 2002; 17: 41–50.

165 Cawley S, Bekiranov S, Ng HH, *et al.* Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell* 2004; 116: 499–509.

166 Avni O, Lee D, Macian F, *et al.* T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 2002; 3: 643–651.

167 Lee DU, Avni O, Chen L, *et al.* A distal enhancer in the interferon-gamma (IFN-gamma) locus revealed by genome sequence comparison. *J Biol Chem* 2004; 279: 4802–4810.

168 Schroeder JT, Miura K, Kim HH, *et al.* Selective expression of nuclear factor of activated T cells 2/c1 in human basophils: evidence for involvement in IgE-mediated IL-4 generation. *J Allergy Clin Immunol* 2002; 109: 507–513.

169 Ranger AM, Oukka M, Rengarajan J, *et al.* Inhibitory function of two NFAT family members in lymphoid homeostasis and Th2 development. *Immunity* 1998; 9: 627–635.

170 Peng SL, Gerth AJ, Ranger AM, *et al.* NFATc1 and NFATc2 together control both T and B cell activation and differentiation. *Immunity* 2001; 14: 13–20.

171 Hodge MR, Ranger AM, Charles de la Brousse F, *et al.* Hyperproliferation and dysregulation of IL-4 expression in NF-ATp-deficient mice. *Immunity* 1996; 4: 397–405.

172 Viola JP, Kiani A, Bozza PT, *et al.* Regulation of allergic inflammation and eosinophil recruitment in mice lacking the transcription factor NFAT1: role of interleukin-4 (IL-4) and IL-5. *Blood* 1998; 91: 2223–2230.

173 Monticelli S, Rao A. NFAT1 and NFAT2 are positive regulators of IL-4 gene transcription. *Eur J Immunol* 2002; 32: 2971–2978.

174 Baksh S, Widlund HR, Frazer-Abel AA, *et al.* NFATc2-mediated repression of cyclin-dependent kinase 4 expression. *Mol Cell* 2002; 10: 1071–1081.

175 Miaw SC, Kang BY, White IA, *et al.* A repressor of GATA-mediated negative feedback mechanism of T cell activation. *J Immunol* 2004; 172: 170–177.

176 Chen R, Burke T, Cumberland J, *et al.* Glucocorticoids inhibit calcium- and calcineurin-dependent activation of the human IL-4 promoter. *J Immunol* 2000; 164: 825–832.

177 Chan SC, Brown MA, Willcox TM, *et al.* Abnormal IL-4 gene expression by atopic dermatitis T lymphocytes is reflected in altered nuclear protein interactions with IL-4 transcriptional regulatory element. *J Invest Dermatol* 1996; 106: 1131–1136.

178 Keen JC, Sholl L, Wills-Karp M, *et al.* Preferential activation of nuclear factor of activated T cells c correlates with mouse strain susceptibility to allergic responses and interleukin-4 gene expression. *Am J Respir Cell Mol Biol* 2001; 24: 58–65.

179 Reddy SP, Mossman BT. Role and regulation of activator protein-1 in toxicant-induced responses of the lung. *Am J Physiol Lung Cell Mol Physiol* 2002; 283: L1161–L1178.

180 Li B, Tournier C, Davis RJ, *et al.* Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *EMBO J* 1999; 18: 420–432.

181 Deng T, Karin M. JunB differs from c-Jun in its DNA-binding and dimerization domains, and represses c-Jun by formation of inactive heterodimers. *Genes Dev* 1993; 7: 479–490.

182 Hartenstein B, Teurich S, Hess J, *et al.* Th2 cell-specific cytokine expression and allergen-induced airway inflammation depend on JunB. *EMBO J* 2002; 21: 6321–6329.

183 Ho IC, Hodge MR, Rooney JW, *et al.* The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* 1996; 85: 973–983.

184 Hodge MR, Chun HJ, Rengarajan J, *et al.* NF-AT-Driven interleukin-4 transcription potentiated by NIP45. *Science* 1996; 274: 1903–1905.

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

186 Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J Exp Med* 1998; 188: 1859–1866.

187 Li-Weber M, Salgame P, Hu C, *et al.* Th2-specific protein/DNA interactions at the proximal nuclear factor-AT site contribute to the functional activity of the human IL-4 promoter. *J Immunol* 1998; 161: 1380–1389.

188 Taha R, Hamid Q, Cameron L, Olivenstein R. T helper type 2 cytokine receptors and associated transcription factors GATA-3, c-MAF, and signal transducer and activator of transcription factor-6 in induced sputum of atopic asthmatic patients. *Chest* 2003; 123: 2074–2082.

189 Li-Weber M, Giaisi M, Krammer PH. Roles of CCAAT/enhancer-binding protein in transcriptional regulation of the human IL-5 gene. *Eur J Immunol* 2001; 31: 3694–3703.

190 Rincon M, Aunguita J, Nakamura T. Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4+ T cells. *J Exp Med* 1997; 185: 461–469.

191 Diehl S, Chow CW, Weiss L, *et al.* Induction of NFATc2 expression by interleukin 6 promotes T helper type 2 differentiation. *J Exp Med* 2002; 196: 39–49.

192 Berberich-Siebelt F, Klein-Hessling S, Hepping N, *et al.* C/EBPbeta enhances IL-4 but impairs IL-2 and IFN-gamma induction in T cells. *Eur J Immunol* 2000; 30: 2576–2585.

193 Roth M, Johnson PR, Borger P, *et al.* Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N Engl J Med* 2004; 351: 560–574.

194 Borger P, Black JL, Roth M. Asthma and the CCAAT-enhancer binding proteins: a holistic view on airway inflammation and remodeling. *J Allergy Clin Immunol* 2002; 110: 841–846.

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

196 Dorado B, Jerez MJ, Flores N, *et al.* Autocrine IL-4 gene regulation at late phases of TCR activation in differentiated Th2 cells. *J Immunol* 2002; 169: 3030–3037.

197 Fulkerson PC, Zimmermann N, Hassman LM, Finkelman FD, Rothenberg ME. Pulmonary chemokine expression is coordinately regulated by STAT1, STAT6, and IFN-gamma. *J Immunol* 2004; 173: 7565–7574.

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

199 Asnagli H, Afkarian M, Murphy KM. Cutting edge: identification of an alternative GATA-3 promoter directing tissue-specific gene expression in mouse and human. *J Immunol* 2002; 168: 4268–4271.

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

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

202 Bour-Jordan H, Grogan JL, Tang Q, *et al.* CTLA-4 regulates the requirement for cytokine-induced signals in T(H)2 lineage commitment. *Nat Immunol* 2003; 4: 182–188.

203 Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *J Exp Med* 1998; 187: 939–948.

204 Kuperman DA, *et al.* Dissecting asthma using focused transgenic modelling and functional genomics. *J Allergy Clin Immunol* 2005; 116: 305–311.

205 Tomkinson A, Duez C, Lahn M, *et al.* Adoptive transfer of T cells induces airway hyperresponsiveness independently of airway eosinophilia but in a signal transducer and activator of transcription 6-dependent manner. *J Allergy Clin Immunol* 2002; 109: 810–816.

206 Ghaffar O, Christodoulopoulos P, Lamkhioued B, *et al. In vivo* expression of signal transducer and activator of transcription factor 6 (STAT6) in nasal mucosa from atopic allergic rhinitis: effect of topical corticosteroids. *Clin Exp Allergy* 2000; 30: 86–93.

207 Thomas MJ, Seto E. Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? *Gene* 1999; 236: 197–208.

208 Schwenger GT, Fournier R, Hall LM, *et al.* Nuclear factor of activated T cells and YY1 combine to repress IL-5 expression in a human T-cell line. *J Allergy Clin Immunol* 1999; 104: 820–827.

209 Ye J, Cippitelli M, Dorman L, Ortaldo J, Young H. The nuclear factor YY1 suppresses the human gamma interferon promoter through two mechanisms: inhibition of AP1 binding and activation of a silencer element. *Mol Cell Biol* 1996; 16: 4744–4753.

210 Sweetser MT, Hoey T, Sun YL, Weaver WM, Price GA, Wilson CB. The roles of nuclear factor of activated T cells and ying-yang 1 in activation-induced expression of the interferon-gamma promoter in T cells. *J Biol Chem* 1998; 273: 34775–34783.

211 Kalayci O, Birben E, Wu L, *et al.* Monocyte chemoattractant protein-4 core promoter genetic variants: influence on YY-1 affinity and plasma levels. *Am J Respir Cell Mol Biol* 2003; 29: 750–756.

212 Bovolenta C, Camorali L, Lorini AL, *et al. In vivo* administration of recombinant IL-2 to individuals infected by HIV down-modulates the binding and expression of the transcription factors ying-yang-1 and leader binding protein-1/late simian virus 40 factor. *J Immunol* 1999; 163: 6892–6897.

213 Yao YL, Yang WM, Seto E. Regulation of transcription factor YY1 by acetylation and deacetylation. *Mol Cell Biol* 2001; 21: 5979–5991.

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

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

216 Sundrud MS, Grill SM, Ni D, *et al.* Genetic reprogramming of primary human T cells reveals functional plasticity in Th cell differentiation. *J Immunol* 2003; 171: 3542–3549.

217 Takemoto N, Arai K, Miyatake S. Cutting edge: the differential involvement of the N-finger of GATA-3 in chromatin remodeling and transactivation during Th2 development. *J Immunol* 2002; 169: 4103–4107.

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

219 Yamashita M, Ukai-Tadenuma M, Kimura M, *et al.* Identification of a conserved GATA3 response element upstream proximal of the IL-13 gene locus. *J Biol Chem* 2002; 29: 29.

220 Finotto S, De Sanctis GT, Lehr HA, *et al.* Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J Exp Med* 2001; 193: 1247–1260.

221 Nakamura Y, Christodoulopoulos P, Cameron L, *et al.* Upregulation of the transcription factor GATA-3 in upper airway mucosa after *in vivo* and *in vitro* allergen challenge. *J Allergy Clin Immunol* 2000; 105: 1146–1152.

222 Ansel KM, Lee DU, Rao A. An epigenetic view of helper T cell differentiation. *Nat Immunol* 2003; 4: 616–623.

223 Lee DU, Agarwal S, Rao A. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. *Immunity* 2002; 16: 649–660.

224 Fields PE, Lee GR, Kim ST, Bartsevich VV, Flavell RA. Th2-specific chromatin remodeling and enhancer activity in the th2 cytokine locus control region. *Immunity* 2004; 21: 865–876.

225 Li Q, Peterson KR, Fang X, Stamatoyannopoulos G. Locus control regions. *Blood* 2002; 100: 3077–3086.
226 Lee DU, Rao A. Molecular analysis of a locus control region in the T helper 2 cytokine gene cluster: a target for STAT6 but not GATA3. *Proc Natl Acad Sci USA* 2004; 101: 16010–16015.
227 Lee R L, Fields P E, Flavell R A. Regulation of IL-4 gene expression by distal regulatory elements and GATA-3 at the chromatin level. *Immunity* 2001; 14: 447–459.
228 Loots GG, Locksley RM, Blankespoor CM, *et al.* Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. *Science* 2000; 288: 136–140.
229 Mohrs M, Blankespoor CM, Wang ZE, *et al.* Deletion of a coordinate regulator of type 2 cytokine expression in mice. *Nat Immunol* 2001; 2: 842–847.
230 Ansel KM, Greenwald RJ, Agarwal S, *et al.* Deletion of a conserved Il4 silencer impairs T helper type 1-mediated immunity. *Nat Immunol* 2004; 5: 1251–1259.
231 Loots GG, Ovcharenko I, Pachter L, Dubchak I, Rubin EM. rVista for comparative sequence-based discovery of functional transcription factor binding sites. *Genome Res* 2002; 12: 832–839.
232 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136: 2348–2357.
233 Webb LM, Feldmann M. Critical role of CD28/B7 costimulation in the development of human Th2 cytokine-producing cells. *Blood* 1995; 86: 3479–3486.
234 Cousins DJ, Lee TH, Staynov DZ. Cytokine coexpression during human TH1/TH2 cell differentiation: direct evidence for coordinated expression of th2 cytokines. *J Immunol* 2002; 169: 2498–2506.
235 Rogan DF, Cousins DJ, Santangelo S, *et al.* Analysis of intergenic transcription in the human IL-4/IL-13 gene cluster. *Proc Natl Acad Sci USA* 2004; 101: 2446–2451.
236 Lametschwandtner G, Biedermann T, Schwarzler C, *et al.* Sustained T-bet expression confers polarized human TH2 cells with TH1-like cytokine production and migratory capacities. *J Allergy Clin Immunol* 2004; 113: 987–994.