

Genes that regulate interleukin-4 expression in T cells

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Interleukin-4 is an immunomodulatory cytokine which plays a central role in the regulation of allergic and atopic immune responses. Significant progress has been made in gaining a detailed understanding of the transcriptional regulation of the interleukin-4 gene. The recent identification and characterization of several key transcription factors has helped to elucidate the molecular mechanisms of T helper cell cytokine gene expression.

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Abbreviations

AP	activator protein
APC	antigen-presenting cell
bzip	basic region/leucine zipper
IFN	interferon
IL	interleukin
NF-AT	nuclear factor of activator T cells
NIP45	NF-AT interacting protein 45
RHD	Rel-homology domain
STAT	signal transducer and activator of transcription
Th	T helper
TNF	tumor necrosis factor

Introduction

CD4⁺ T helper (Th) subsets are characterized by their distinct cytokine production profiles [1*,2]. Th1 cells secrete IL-2, IFN- γ , and TNF- β , which promote cellular immune responses against intracellular pathogens and viruses. Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13, which promote humoral immunity by aiding in B cell growth and differentiation. As naïve CD4⁺ T cells have the potential to differentiate into either the Th1 or Th2 phenotype, the initial development of the appropriate Th phenotype is necessary to attain a curative immune response. A wide variety of pathological states, including autoimmune, allergic, and chronic infectious diseases, have been shown to be caused by the inappropriate development of the Th1 or Th2 subsets [3]. Thus, any manipulation that can alter the Th response may have significant therapeutic applications [4]. The cytokines IL-12 and IL-4 critically influence the differentiation of naïve CD4⁺ T cells [1*,2]. IL-12, a macrophage derived cytokine, induces Th1 development from naïve CD4⁺ T cells. IL-4 binding to the IL-4 receptor on naïve CD4⁺ T cells, leads to the production of IL-4 and other Th2

specific cytokines while suppressing the production of Th1 type cytokines.

A detailed knowledge of the regulation of Th1- and Th2-restricted cytokine gene expression is central to the understanding of Th phenotype development. The key Th2 cytokine, IL-4, has been extensively studied as a model for the phenotype specific expression of a cytokine. The IL-4 gene has been shown to be primarily regulated at the level of transcription and thus the *cis*-acting elements and *trans*-acting factors that control IL-4 gene expression have been actively sought. This review will focus on the recent identification and characterization of transcription factors that may explain the activation requirements and cell-type specificity of T cell IL-4 expression. For a comprehensive analysis of the *cis*-elements and transcription factors of the IL-4 gene, readers are referred to recent reviews [5*,6*].

Transcriptional regulation of the IL-4 gene

Any molecular mechanism proposed for IL-4 gene regulation in CD4⁺ T cells should be able to explain three important features: firstly, that IL-4 is a highly tissue-specific gene which is expressed in the Th2 cell subset and not in the Th1 cell subset; secondly, that resting T cells do not transcribe IL-4 until activated by TCR ligation or pharmacological agents, including calcium ionophores and phorbol esters; and thirdly, that expression of the IL-4 gene can be blocked by immunosuppressive drugs such as cyclosporin A. This review details recent progress in the identification of a series of transcription factors that together account for many of the above requirements for T cell IL-4 gene expression.

NF-AT/AP-1 proteins and coactivators

Transcription factors of the NF-AT (nuclear factor of activated T cells) family share some of the activation characteristics of the IL-4 gene, including activation by calcium ionophores and inhibition by cyclosporin A [7*]. Thus, the discovery of NF-AT binding sites in the IL-4 promoter prompted an investigation of the role of these factors in IL-4 expression (Figure 1). Presently, the NF-AT family contains at least four members: NF-ATp (NF-AT1 or NF-ATc2), NF-ATc (NF-AT2 or NF-ATc1), NF-AT3 (NF-ATc4), and NF-AT4 (NF-ATx or NF-ATc3) [8–13]. All members contain a Rel homology domain (RHD) distantly related in amino acid sequence to the Rel domain of NF- κ B [7*]. At least five NF-AT binding sites are present in the minimal IL-4 promoter [14,15]. Mutation or deletion of any one of these NF-AT sites results in a significant decrease in promoter activity. Of the NF-AT family members, NF-ATp and NF-ATc are the predominant NF-AT proteins expressed in human and

murine T cells [16–18]. *In vitro* analysis demonstrated that the NF-AT proteins present in activated Th1 and Th2 clones equivalently bind the IL-4 promoter NF-AT sites and can transactivate a reporter construct containing multimerized IL-4 NF-AT sites [17,19]. Surprisingly, analysis of NF-ATp deficient mice demonstrated increased IgE levels and increased IL-4 production upon stimulation of T cells derived from these mice [20•,21•]. These findings suggest a negative role for NF-ATp in regulating IL-4 gene expression but do not identify whether this role is mediated directly through active suppression of IL-4 gene transcription or indirectly through non-transcriptional mechanisms. Together, the above studies suggest that NF-ATc is the dominant transactivator of the IL-4 promoter. The generation of mice deficient in NF-ATc should clarify its role in IL-4 expression.

Despite their activity in the regulation of IL-4 gene expression, it is unlikely that the NF-AT proteins can explain the Th2 specific expression of the IL-4 gene. The NF-ATp and NF-ATc proteins are expressed in both Th1 and Th2 cells and bind with a similar affinity to the IL-4 promoter NF-AT sites [11,12,18,22]. Furthermore, studies using mice transgenic for a reporter construct containing multimerized IL-4 promoter NF-AT sites showed comparable reporter activity in the Th1 and Th2 cells derived from these mice [23].

Recently the gene encoding NIP45 (NF-AT interacting protein 45) was cloned, using the yeast two-hybrid method, on the basis of its interaction with the Rel homology domain of NF-ATp [24•]. Inspection of the amino acid sequence of NIP45 revealed a highly basic amino-terminal domain that demonstrated no homology to any known functional protein domain. Transcripts encoding this factor are enriched in lymphoid tissue and testis. NIP45 mRNA is present in naïve Thp cells as well as Th1 and Th2 cells, and these NIP45 mRNA

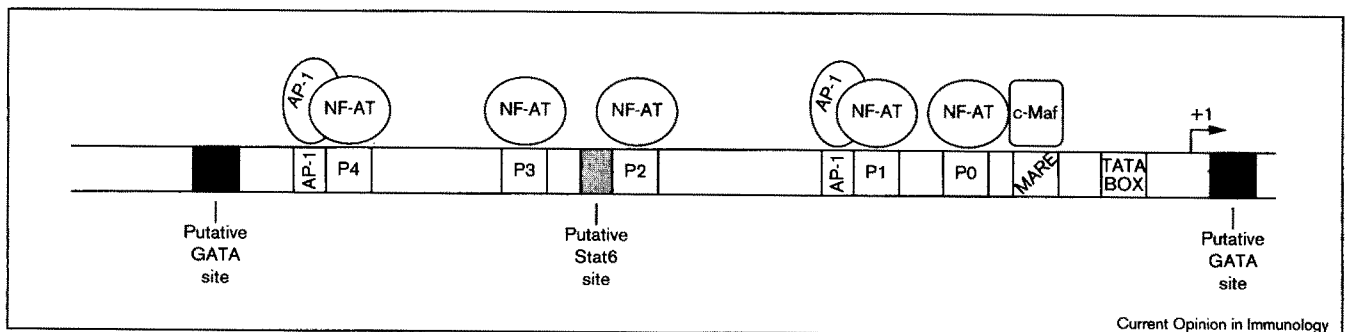
levels are not altered by TCR stimulation (B Lieberman, unpublished data). NIP45 cannot bind DNA and alone it has virtually no transcriptional activity. NIP45 was shown, however, to synergize with NF-ATp and c-Maf (see below) to transactivate the IL-4 promoter. In this study transient expression of NIP45 with NFATp and c-Maf in the M12 B lymphoma cell line induced high level endogenous IL-4 protein production [24•]. Thus, NIP45 appears to function as a potent coactivator of IL-4 gene transcription. The role of NIP45 in the expression of other NF-AT dependent cytokine genes remains to be elucidated.

Adjacent to two of the five IL-4 promoter NF-AT sites there are non-canonical activator protein (AP)-1 sites [14,19]. These sites are similar to the well defined NF-AT-AP-1 sites of the IL-2 promoter [25,26]. The AP-1 family of transcription factors are bzip (basic region/leucine zipper) proteins which include the Jun, Fos, activating transcription factor (ATF)/cAMP response element binding protein (CREB) and Maf subfamilies [27]. AP-1 family members such as Fra-1, Fra-2, JunB and JunD are capable of binding to the AP-1 sites of the IL-4 promoter [19]. A functional role for AP-1 family members is suggested by studies demonstrating that mutation of the IL-4 promoter AP-1 sites resulted in a 50% decrease in promoter activity [19]. Despite several studies demonstrating the differential recruitment of AP-1 members to the NF-AT sites in Th1 and Th2 cells [19,28,29], none of the Fos or Jun AP-1 members have been shown to be exclusively expressed or activated in a Th phenotype specific manner.

Role of Stat6 in IL-4 transcription and Th2 development

The STAT (signal transducer and activator of transcription) proteins are a family of tyrosine phosphorylated, cytokine-activated transcription factors [30]. One of the STAT family members, Stat6, has been shown to be

Figure 1



Transcription factor binding sites of the proximal IL-4 promoter. This schematic diagram shows the *cis*-acting sites (AP-1, P0-4, and MARE, as indicated by the labelled boxes) of, and the *trans*-acting factors (AP-1, NF-AT and c-Maf, as indicated by the labelled structures) that bind to, the proximal 300 base pairs of the murine IL-4 promoter. This model is simplified for clarity and contains only those transcription factors referred to in this review. The relative positions of the potential Stat6 and GATA-3 binding sites are also noted. The TATA box binds the general transcription factor TFIID. The start of transcription is indicated by +1.

rapidly activated by IL-4 receptor ligation [31]. As IL-4 is a potent inducer of Th2 development, transcription factors activated by IL-4 are potential candidates for inducing T cell IL-4 gene expression and eventual Th2 phenotype development. The critical role of Stat6 in Th2 development was demonstrated by the targeted disruption of the Stat6 gene in mice. Three groups independently showed that mice deficient in Stat6 were unable to generate Th2 cells [32–34]. Although these studies clearly define a requirement for Stat6 in Th2 development, the relationship between Stat6 activation and Th2 development is uncertain. Lederer *et al.* [35] demonstrated that activated Stat6 isolated from Th2 cells could bind a region of the IL-4 promoter that contained a putative Stat6 binding site (Figure 1). This region, when multimerized in a reporter construct and transfected into the TA3 B cell line, was transactivated in response to IL-4, thus suggesting a functional consequence of Stat6 binding. It has been postulated that Stat6 may mediate the Th2 specific expression of IL-4 through an autocrine mechanism by binding and transactivating the IL-4 promoter. There is yet, however, no direct evidence that Stat6 transactivates the IL-4 promoter in T cells or that the Stat6 site of the IL-4 promoter is required for promoter activity. Moreover, exogenous IL-4 does not enhance the expression of IL-4 in mature Th2 cells. Thus, while Stat6 plays an essential role in Th2 differentiation, its direct role in IL-4 transcription remains unclear.

c-Maf, a Th2 specific transcription factor

None of the above described transcription factors have been demonstrated to be expressed in a Th phenotype specific manner that could account for the differential gene expression in Th1 and Th2 cells. Recently, we have shown that the proto-oncogene c-Maf is selectively expressed in Th2 clones, but not in Th1 clones, B cells or monocytes [36•]. Importantly, expression of c-Maf is induced during *in vitro* Th2 differentiation, but not during Th1 differentiation. The c-Maf protein is a bzip transcription factor belonging to the Maf subfamily of the AP-1 protein family. All Maf family members recognize a 12 or 13 base pair sequence designated TPA maf response element (T-MARE) or CRE maf response element (C-MARE) [37]. An MARE site is present in the proximal IL-4 promoter and homodimers of c-Maf are capable of binding this site. A reporter construct containing a multimerized IL-4 MARE site was found to be active in the murine Th2 clone D10 but not the Th1 clone AE7, thus demonstrating Th2 specificity [38]. Moreover, c-Maf, in synergy with NF-ATp, was shown to initiate endogenous IL-4 production in the M12 B lymphoma cell line which does not normally secrete IL-4. In this B cell line further addition of NIP45 caused a dramatic increase in endogenous IL-4 production to levels similar to that seen in mature Th2 cells ([24•], LH Glimcher, unpublished data).

c-Maf's transcriptional activity appears to be specific to the IL-4 promoter. c-Maf, although capable of robust transactivation of the IL-4 promoter, is unable to transactivate the promoters of other Th2 cytokine genes such as IL-5 or IL-10 (I-C Ho, unpublished data). It is possible that c-Maf is the first of a series of yet to be discovered cytokine-specific transcription factors which interact with more widely expressed factors such as NF-AT and NIP45 to confer the exquisite specificity of cytokine gene expression.

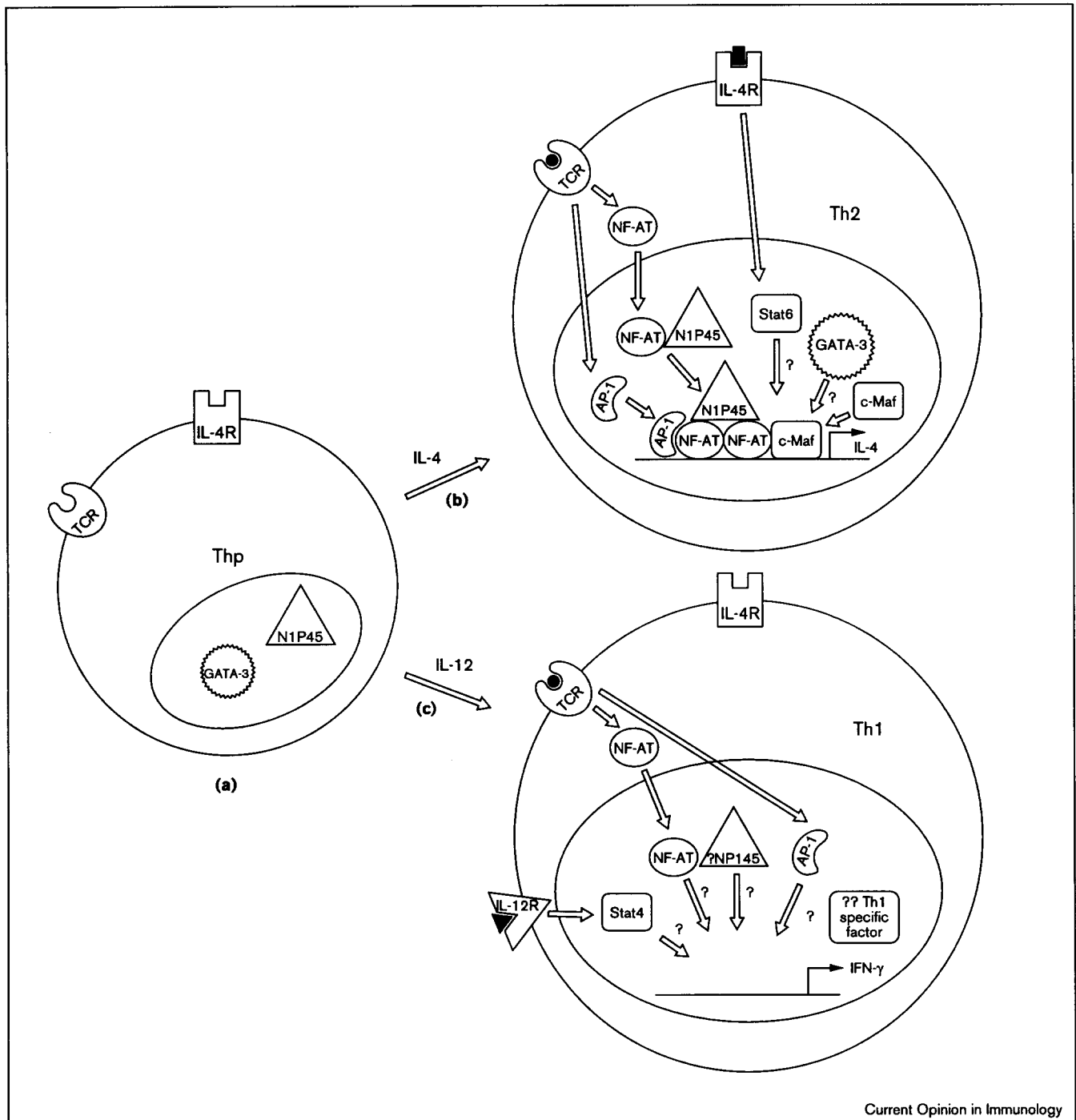
GATA-3, a regulator of Th2 type cytokines

A transcription factor that may be more widely involved in the induction and maintenance of the Th2 pattern of cytokine secretion is GATA-3. GATA-3, a zinc finger DNA binding protein, was first identified as a T cell specific transcription factor that binds to the enhancers of the TCR α and δ genes [39,40]. It is expressed in both immature and mature T cells. Targeted disruption of the GATA-3 gene completely blocks early T cell development, suggesting a crucial role for GATA-3 in T cell lineage formation [41]. Recently, two groups demonstrated that expression of GATA-3 is selectively suppressed during Th1 differentiation, and is induced during Th2 differentiation [42•,43]. In addition, GATA-3 overexpression was shown to transactivate an IL-4 promoter construct in the M12 B lymphoma cell line upon phorbol myristate acetate and ionomycin stimulation [42•]. Two potential canonical GATA binding sites are present in the IL-4 promoter (Figure 1) but whether GATA-3 can directly bind and transactivate the IL-4 gene remains to be determined. Th1 cells derived from GATA-3 transgenic mice continued to express Th1 type cytokines but they also expressed the normally Th2-specific genes IL-4, IL-6, and IL-10 (low level IL-5 expression was also detected) as assayed by reverse transcriptase polymerase chain reaction. Thus, in contrast to c-Maf, which appears to be IL-4 specific, GATA-3 may function as a more general regulator of Th2 cytokine expression.

Environmental influences on IL-4 production

The identification of the transcription factors described above has provided insights into the regulation of the IL-4 gene and also suggests potential targets for the manipulation of IL-4 expression and consequently the developing immune response. These discoveries, however, fail to demonstrate a link between the local environment and subsequent Th cell development. Pathogens themselves clearly influence the type of Th response that develops in the host. Intracellular bacteria such as *Listeria monocytogenes* result in antigen-presenting cell (APC) IL-12 production, thus initiating the Th1 differentiation process of naïve T cells [44]. In this manner, innate immunity directs the outcome of specific immunity leading to the successful elimination of the pathogen. In Th2 development, the initial stimulus for IL-4 production, and thus for Th2 differentiation, has not been clearly defined. Recently,

Figure 2



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A model for Th cell specific cytokine gene expression. (a) Resting, naïve Th precursor (Thp) cells are unable to secrete cytokines but express the transcription factor GATA-3, and N1P45. Upon antigen stimulation in the presence of either (b) IL-4 or (c) IL-12, these Thp cells differentiate along the Th2 and Th1 pathways, respectively. (b) Antigen stimulation under Th2-inducing conditions results in Th cell IL-4 gene expression through the induction of a series of transcription factors: IL-4 binds to the IL-4 receptor on naïve T cells resulting in Stat6 activation; TCR stimulation results in the activation and nuclear translocation of NF-AT proteins, permitting their interaction with N1P45; AP-1 proteins are synthesized; GATA-3 expression increases and the production of c-Maf protein begins. Some of the transcription factors bound to the IL-4 promoter are depicted. (c) In the case of naïve Thp cells activated under Th1-inducing conditions, c-Maf expression is not induced and GATA-3 expression is extinguished. The lack of these two signals contributes to Th1 commitment and the absence of IL-4 expression. Expression of other transcription factors such as Stat4, AP-1, NF-AT, N1P45 and, as yet, unidentified Th1-specific factors leads to the induction of IFN- γ expression, a Th1 cytokine. Question marks indicate uncertainties.

Rincon *et al.* [45] suggested that IL-6, a component of the inflammatory and acute phase immune response, induces IL-4 production from naïve T cells themselves. This IL-4 could then act in an autocrine fashion to direct Th2 development. Thus, in a manner that parallels IL-12 induced Th1 differentiation, pathogen induced APC IL-6 production provides a possible link between innate immunity and Th2 differentiation. Although this is a satisfying model, IL-6 may not be the primary inducer of Th2 development, as mice deficient in the IL-6 gene are capable of developing Th2 type responses upon infection with pathogenic organisms [46,47].

A model for IL-4 regulated Th differentiation

Recent progress on the transcriptional regulation of the IL-4 gene allows the proposal of a molecular model for the regulation of IL-4 gene expression during Th cell differentiation (Figure 2). In their resting state, naïve Th precursor cells are unable to secrete IL-4. Following antigen stimulation under Th2 inducing conditions (for the purposes of the model, in the presence of IL-4) the following events occur in the precursor cells: firstly, IL-4 binds to the IL-4 receptor on the naïve T cells resulting in Stat6 activation; secondly, TCR stimulation results in the activation and translocation of NF-AT proteins to the nucleus where they can interact with NIP45; thirdly, *de novo* synthesis of AP-1 proteins begins; fourthly, GATA-3 expression increases and finally the induction of c-Maf expression begins. The latter events, increased GATA-3 expression and c-Maf upregulation, appear to be critical for Th cell expression of IL-4 and subsequent Th cell commitment to the Th2 pathway. The signals that lead to the upregulation of c-Maf and GATA-3 have yet to be defined. A potential role for Stat6 in this upregulation is suggested by its rapid activation upon IL-4 receptor ligation and the demonstrated obligate role of Stat6 in Th2 development [32–35]. At this time, however, no direct evidence for such a role of Stat6 exists. At later time points in activation, further upregulation of c-Maf allows the developing Th2 cells to secrete large amounts of IL-4. In the case of naïve Th precursor cells activated under Th1-inducing conditions (i.e. in the presence of IL-12 and the absence of IL-4), c-Maf expression is not upregulated and GATA-3 expression is extinguished. Based on the current model, the lack of these two signals contributes to Th1 commitment and the absence of IL-4 expression.

Conclusions

The proposed Th cell differentiation mode; may be generalizable to the regulation of other Th2 type cytokines. Expression of GATA-3 alone is insufficient for high level Th2 cytokine expression but it maintains the ability of the developing Th cells to express Th2 cytokines [42•]. High level expression of a given Th2 cytokine may further require the expression of a cytokine-specific and possibly cell type specific factor. In the case of the IL-4 gene, c-Maf may serve as the cytokine-specific transcription factor. Other Th1 and Th2 genes may require similar

cytokine-specific transcription factors for optimal cytokine production. The elucidation of cytokine-specific and phenotype-specific transcription factors should provide us with targets for the precise manipulation of ongoing immune responses.

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