Review
Cell fate decision: T-helper 1 and 2 subsets in immune responses

Chen Dong and Richard A Flavell

Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut, USA

© Current Science Ltd

Abstract

After activation CD4+ helper T cells differentiate into T-helper (Th)1 or Th2 effector cells. These two subsets are characterized by their distinct cytokine expression pattern and the immune function they mediate. Over the past years, a number of factors have been identified to affect helper T cell lineage determination, including antigen receptor, coreceptors and, most importantly, cytokine environment. In this review, we also summarize recent advancement in understanding of transcriptional and signaling regulation of the differentiation process. This knowledge will become important in the future to develop means in treating immune disorders.

Keywords: helper T cells, cytokines, immune responses
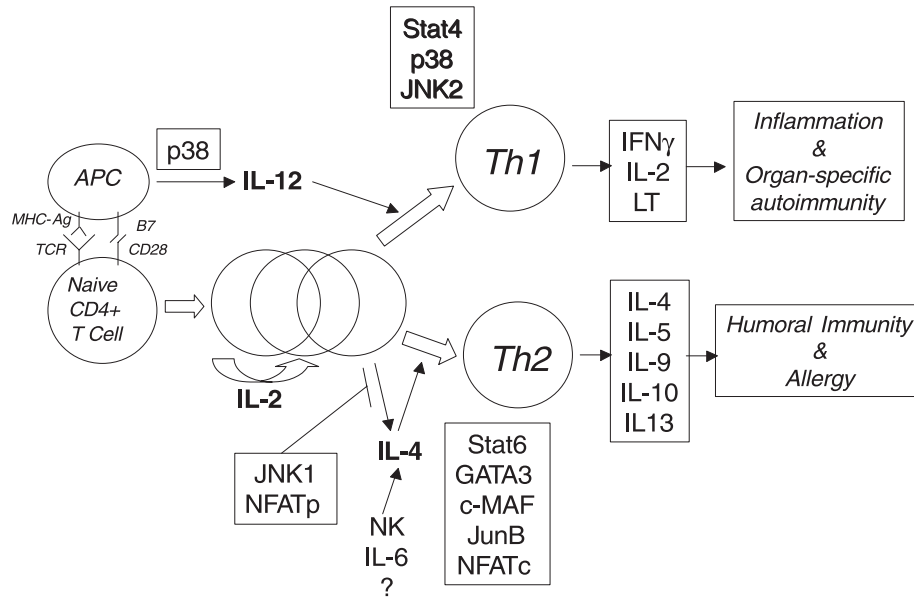
Introduction

CD4+ Th cells play a central regulatory role in immune and autoimmune responses. Like CD8+ cytotoxic T cells, Th cells develop in the thymus, with specificity to recognize specific major histocompatibility (MHC)–peptide complexes on antigen-presenting cells (APCs). After the receipt of signals through the T-cell receptor (TCR) and CD28 costimulator, these cells are triggered to produce IL-2 and enter the cell cycle. After several days of rapid cell division, these T cells differentiate into effector cells. During this process of differentiation, a new pattern of cytokine expression is established that provides the gene products that are responsible for the specific effector functions of these cells, and therefore their ability to protect the host from a variety of pathogens. Effector CD4+ T cells differ from their naïve precursors in several characteristics. In addition to their distinct cytokine expression profiles, naïve CD4+ T cells require a costimulatory signal for effective activation, whereas effector T cells are less dependent on this signal [1]. Moreover, the life-spans of naïve, effector, and memory T cells are different [2,3].

After more than a decade of research, two classes of effector CD4+ T cells have been defined on the basis of the cytokines that they secrete, and the immunomodulatory effects conferred by these cytokines [4] (Fig. 1). Effector Th1 cells produce proinflammatory cytokines such as IFN-γ and lymphotoxin-α. These cytokines organize inflammatory centers and enhance cellular immune response; moreover, intracellular pathogens such as *Mycobacteria* and *Salmonella* spp, and other intravesicular agents are killed by IFN-γ through the activation of antimicrobial defenses. Th1 cytokine production is also characteristic of many organ-specific autoimmune diseases, including rheumatoid arthritis, insulin-dependent diabetes mellitus, experimental autoimmune encephalitis,

Figure 1

Th cell differentiation.

and others. Effector Th2 cells, in contrast, produce a different profile of cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, and so on) that together instruct B cells to proliferate and differentiate into antibody-secreting plasma cells, and potentiate the function of several cell types in antiparasite responses. As such, Th2 cells play an important role in providing protection against certain extracellular pathogens, such as bacteria and a variety of parasites, and are also involved in asthmatic reactions.

Proper differentiation of naïve Th cells into Th1 or Th2 cells is critical for a T-dependent immune response. *Leishmania major* infection in mice represents one of best-studied animal models for Th differentiation and function. Most common inbred strains can mount a Th1 response and resolve the lesions; Balb/c mice, however, develop a Th2 reaction that never heals and renders them susceptible to the infection [5]. On the other hand, immunodeviation of Th1 responses to the Th2 direction has been proposed as a means to alleviate the symptoms in autoimmune diseases.

Because Th1 and Th2 cells are crucial regulators of immune responses, the mechanisms involved in the differentiation of naïve CD4+ T cells into effector Th1 or Th2 cells have been intensively studied by a number of investigators. A variety of factors have been identified to influence differentiation along these pathways, including antigen dose and costimulation (Fig. 1) [6–8]. It is generally agreed, however, that the most effective inducer of such differentiation is the cytokine environment. IL-4, in conjunc-

tion with antigenic and costimulatory signals, causes naïve T cells to differentiate into Th2 effector cells [9,10], whereas IL-12 drives naïve T cells to differentiate into Th1 cells [11,12]. IL-13, another Th2 cytokine that can bind to IL-4 receptor α-chain, plays an indispensable role in efficient Th2 cell differentiation [13]. Moreover, we showed previously [14] that IL-6 derived from APCs is able to polarize naïve CD4+ T cells into Th2 cells, by potentiating the initial production of IL-4 by recently activated CD4+ T cells. Recently, IL-18 has been shown [15] to synergize with IL-12 to potentiate further Th1 responses.

In the present review recent progress in studies of Th cell lineage commitment and cytokine production is reviewed, especially that achieved using genetically manipulated mouse models. In the space available, it was not possible to cover this entire field. Rather, a number of issues are the focus.

T-cell receptor and costimulation regulation
T-cell receptor
Th-cell activation and differentiation require interactions of TCR with MHC class II with specific peptides. The strength of this interaction influences the outcome of Th-cell differentiation. *In vitro* studies using altered peptide ligands [8], in which activated T cells are the only source of IL-4, showed that high-affinity binding of peptide by TCR resulted in Th1 differentiation, whereas lower affinity binding resulted in Th2 differentiation. Low-affinity altered peptide ligand stimulation is characterized by failure to induce full pattern of early tyrosine phosphorylation of

TCR-associated components and a sustained calcium mobilization [16], and Th2 differentiation induced under this condition appeared to depend on IL-4 production by activated T cells [17]. Similarly, antigen dose could influence Th-cell differentiation [8]. In addition, Lezzi *et al* [18] recently showed that the duration of TCR triggering is differentially required for Th1 and Th2 development. Th1 could be achieved by a short TCR stimulation in the presence of IL-12, whereas Th2 polarization required prolonged TCR triggering.

CD4
In addition to TCR, a whole set of cell-surface receptors are also engaged by their ligands on APCs, which regulate the Th differentiation program. The CD4 coreceptor binds to class II MHC, and has been shown to signal through lck. Leitenberg *et al* [19] recently found that naïve Th cells that lack CD4 are defective in Th2 differentiation. Consistent with this, lck-deficient CD4$^+$ T cells also showed impaired Th2 development *in vitro* [20]. Furthermore, itk, which can be phosphorylated and activated by lck, has recently been shown to be essential for Th2 development [21].

CD28/cytotoxic T-lymphocyte associated antigen-4 family
CD28 is the most notable costimulatory receptor on T cells, which binds to B7.1 (CD80) or B7.2 (CD86) on activated APCs. MHC−TCR−CD4 interaction provides a first signal for T-cell activation, and B7−CD28 interaction provides the second. In Th1 clones, both signals are required for IL-2 production and cell proliferation [22]. Giving signal one in the absence of signal two rendered the Th1 clones anergized or unresponsive. In support of an essential role of B7−CD28 interaction in primary T-cell activation, studies using T cells derived from CD28-deficient mice [23] demonstrated its role in T-cell proliferation and survival. In addition, several lines of evidence indicate that the CD28 signal is crucial for Th2 differentiation. Seder *et al* [24] showed that no IL-4 was produced when the CD28−B7 interaction was blocked with hCTLA-4-Ig. We also observed that CD4$^+$ T cells required CD28 costimulation for IL-4, IL-5, and IL-10 production, whereas in its absence only IFN-γ was produced [25]. A study using an APC that was deficient for both B7.1 and B7.2 molecules [26] also demonstrated that IL-4 production and Th2 differentiation is highly dependent on B7, whereas for Th1 cells only IL-2 is sensitive to B7 regulation [27]. *In vivo*, *CD28*$^{−/−}$ mice have a reduced Th2-dependent antibody response to vesicular stomatitis virus, whereas the Th1-dependent delayed-type hypersensitivity response to lymphocytic choriomeningitis virus remains intact [28,29]. In addition, blockade of B7−CD28 interaction *in vivo* reduced the Th2 responses in both *Leishmania* and nematode infection models [30].

The molecular mechanism by which CD28 mediates Th2 differentiation is not very clear. One possibility is that CD28

amplifies TCR signals for Th2 induction. In support of this, Viola *et al* [31] observed that CD28 cosimulation resulted in reorganization of membrane lipid microdomains and sustained tyrosine phosphorylation. Consistent with this, we showed [25] that CD28 enhanced nuclear accumulation of nuclear factor of activated T cells (NFATc), a transcription factor required for Th2 differentiation. Alternatively, CD28 enhancement of cell proliferation may favor Th2 differentiation, because Bird *et al* [31] discovered that IL-4 expression requires more cell divisions than does expression of IFN-γ.

CTLA-4 is a homolog of CD28 that is strongly induced on activated T cells, which has a 10-fold higher affinity for B7 binding than does CD28 [32]. CTLA-4 plays an inhibitory costimulatory role in regulating T-cell response. Mice that are deficient in CTLA-4 display polyclonal T-cell activation and a lymphoproliferative disorder that resulted in neonatal lethality [32]. Recently, Khattri *et al* [33] found that CTLA-4-deficient T cells secrete high levels of IL-4 and IL-5, indicating that CTLA-4 functions to downregulate the CD28 signal in Th2 induction.

A third CD28 homologous molecule, inducible costimulator (ICOS), was identified just last year [34]. It is also expressed by activated T cells. It may not bind to B7 because, unlike CD28 or CTLA-4, it does not possess the MYPPPY motif in its immunoglobuln domain. ICOS costimulation enhances T-cell proliferation and cytokine production. Most notably, it is more potent in IL-10 induction than is CD28, suggesting that it can potentially regulate Th-cell development, especially at a stage where CD28 signal is downregulated by B7−CTLA-4 engagement. To add further complexity, two more B7 homologs were recently reported. One of them, B7h/B7-RP1 that is induced by the inflammatory cytokine tumor necrosis factor (TNF)-α was shown to bind to ICOS, whereas the other one, B7h1, could not [35–38]. Whether B7h/B7-RP1 is the only ligand for ICOS and what is the receptor for B7h1 requires further investigation.

Tumor necrosis factor receptor family
Several members of the TNF receptor/ligand families have been suggested to play a role in T-cell costimulation and differentiation. CD40 ligand (CD154), which is expressed by activated T cells, is crucial for T-cell-mediated immune response [39]. Its binding of CD40 on APCs results in their expression of the inflammatory cytokine IL-12 and of costimulatory factors. Recently, TNF-related activation-induced cytokine (TRANCE)/TRANCE receptor, another TNF−TNF receptor pairing, has been shown to function independently of CD40/CD40 ligand to mediate IL-12 production by dendritic cells *in vitro* and IFN-γ response during a viral infection *in vivo* [40,41]. OX40, which is also expressed by activated T cells, plays an important role in Th1-type immune responses. In mice lacking OX40, there was reduced number of IFN-γ-producing CD4$^+$ T cells in response to viral

infection [42]. On the other hand, mice deficient in OX40 ligand exhibited impaired delayed hypersensitivity [43].

IL-12 transcription in APCs and the IL-12-dependent T-cell response

IL-12, a heterodimeric cytokine that consists of p35 and p40 subunits, plays a central role in driving naïve $CD4^+$ T cells to differentiate into Th1 effector cells. IL-12 p40-deficient mice, IL-12 receptor β_1-deficient mice and mice deficient for signal transducer and activator of transcription (Stat)4, a key signaling molecule that is required in order to respond to IL-12, all exhibited defective type I cytokine immune responses [44–47].

In comparison with the IL-12 p35 promoter, the IL-12 p40 promoter and the transcription factors that bind to it are better characterized. CATA enhancer binding protein and nuclear factor-κB (NF-κB) family members, together with an ets-2-related factor, have been shown to bind to their corresponding sites in the p40 promoter and to regulate the expression of this gene [48–52]. By the use of gene disruptions in mice, interferon responsive factor (IRF)-1 and interferon consensus sequence binding protein (another member of the IRF-1 family) have been shown to be required for IL-12 p40 expression [53–56].

IL-12 is secreted mainly by APCs upon innate immune recognition of pathogen-associated molecular patterns, including lipopolysaccharide. Lipopolysaccharide has recently been determined to function through Toll-like receptors [57]. In addition, as stated above, IL-12 expression can also be regulated by signals from members of the TNF receptor family, including CD40 and TRANCE receptor. How signals from these receptors activate IL-12 transcription machinery is not yet completely understood. Our laboratory has recently found that p38 mitogen-activated protein kinase (MAPK) pathway, activated by lipopolysaccharide or CD40, plays a crucial role in IL-12 regulation [58]. Macrophages isolated from mice deficient in the p38 kinase, MAPK kinase (MKK)3, have a profound defect in IL-12 p35 and p40 transcription when stimulated by lipopolysaccharide. Furthermore, $Mkk3^{-/-}$ dendritic cells failed to express IL-12 in response to CD40 activation. In a mouse macrophage cell line, activation of the p38 pathway activates IL-12 p40 promoter activity. These results demonstrate that p38 MAPK is necessary and sufficient to mediate IL-12 expression.

IL-12 receptor expression by T cells is regulated during Th differentiation, which may impact on their responsiveness to IL-12 and resultant lineage determination. Szabo et al [59] showed that IL-12 receptor β_2 subunit, which is not expressed in naïve T cells, is induced by TCR ligation. IL-4 inhibits its expression, which may lead the cells to become unresponsive to IL-12 and differentiate into the Th2 subsets. On the other hand, IFN-γ overcomes this inhibition.

Interleukin-4 transcription regulation

IL-4 is a cytokine that is not only produced by differentiating and differentiated Th2 cells, but also is the key factor driving Th2 differentiation. The source of the initial IL-4 that primed the Th2-type response *in vivo* has been debated for some years. Natural killer T cells produce IL-4 very rapidly upon TCR engagement and were therefore thought to be the regulatory cell for Th2 reaction. Mice that are deficient for CD1, and thus for natural killer T cells, have normal Th2 responses, however, which suggests that IL-4 may come from other sources [60,61]. Balb/c mice, during *Leishmania major* challenge, developed a Th2-polarized response [62]. In these mice, during the early phase of infection a subset of T cells bearing $V\beta4^+V\alpha8^+$ TCR that recognizes *Leishmania* antigen secrete IL-4 and may be responsible for the Th2 skewing *in vivo* [62]. Whether this is a regulatory cell or simply an antigen-specific $CD4^+$ T cell remains to be determined.

Recently, two reports [63,64] demonstrated that IL-4 is expressed in a monoallelic manner. Bix and Locksley [64] established Th2 cell clones from Balb/c X CaST/Ei F1 mice and analyzed the allelic expression of the IL-4 gene. They found that most clones were expressed in a monoallelic manner and that the allelic pattern was transmitted as a stable epigenetic trait. In anther study, Riviere *et al* [63] generated a mouse strain in which one allele of the IL-4 gene was replaced by human CD2 by gene targeting. Using this model, they convincingly showed that most Th2 cells expressed only the functional IL-4 allele or the targeted allele. Estimation of the frequency of monoallelic versus biallelic expression in this model also suggests a stochastic process in the activation of each individual allele, in which each cytokine gene can be turned on probabilistically to generate diverse profiles of cytokine expression and effector population.

The IL-4 gene is located on chromosome 11 in a locus containing the gene for the other Th2 cytokines IL-5 and IL-13, which suggests a mechanism to coordinate the expression of these genes. Two groups have identified several deoxyribonuclease I-hypersensitivity sites in IL-4 and IL-13 gene loci that are associated with Th2 differentiation [65,66], which may indicate a chromatin remodeling process during Th2 commitment that makes the loci more accessible to the transcription machinery. In addition, IL-4 locus remodeling is accompanied by demethylation and was shown to require both antigen receptor and IL-4 cytokine signaling [66]. However, the functional significance of these hypersensitive sites, and whether they are enhancers for transcription factor binding or one or more of them constitute a locus-control region, as reported for β-globin [67] and CD2 loci [68], are still unclear.

The 800-bp proximal promoter of the IL-4 gene has been extensively studied, and it confers Th2 specificity in

transgenic mice despite a substantially lower expression level than the endogenous gene [69]. Furthermore, the NFAT–activating protein 1 (AP1) composite element in this region, when placed in an IL-4 or IL-2 minimal promoter, is expressed with Th2 specificity, indicating a role for the NFAT and AP1 transcription factor families in regulation of IL-4 gene expression [69–71].

Nuclear factor of activated T cells
Thus far five NFAT members have been identified – NFATp (NFAT1 or c2), NFATc (NFAT2 or c1), NFAT3 (NFATc4), NFAT4 (NFATc3 or x), and NFAT5 – all of which contain a rel homology domain that is responsible for DNA binding [72]. The first four NFATs are cytoplasmic and translocated into the nucleus only after dephosphorylation in their serine-proline rich and serine rich regions by calcineurin phosphatase [72]; NFAT5 is constitutively nuclear [73]. Unlike NF-κB proteins, NFATs bind to DNA in a complex with other factors, including AP-1 molecules [72,74]. In fact, NFAT–AP1 composite sequences exist in promoters of numerous cytokine and immune effector genes, and have been shown to be critical for regulation of these genes. Deletion or mutation of NFAT sites in the IL-4 promoter abolished its transcription activity [75]. NFATc and NFATp have been shown [74,76] to bind to the NFAT–AP1 site in the IL-4 promoter and to activate IL-4 transcription in cell lines. Analysis of knockout mice for these two genes distinguished their potential roles in IL-4 regulation, however. In mice that are deficient for NFATp or for both NFATp and NFAT4, there is an exaggerated Th2 response [77–79]. In contrast, NFATc knockout mice displayed defective Th2 differentiation [80,81]. Thus, NFATp and NFATc seem to play opposite regulatory roles in IL-4 regulation. Precisely why this is so is still a puzzle.

GATA-3
Using the representation difference analysis approach, we found GATA-3 to be selectively expressed in the Th2 pathway [82]. Existing in naïve CD4+ T cells and shortly after activation, GATA-3 mRNA and IL-4 gene expression are extinguished in Th1 cells, whereas GATA-3 levels are elevated in Th2 cells. Interestingly, through analysis of IL-5 promoter, Zhang *et al* [83] also identified GATA-3 as a Th2-specific factor. In addition, Ouyang *et al* [84] found that diminished GATA-3 expression in Th1 differentiation is dependent on IL-12. During the past 2 years, GATA-3 has been shown by several studies to be the critical regulatory transcription factor involved in Th2 differentiation. We showed [82] that T cells isolated from transgenic mice in which GATA-3 was forced to be overexpressed produced IL-4, IL5, IL-6, and IL-10 mRNA, even when they developed under Th1 conditions in the complete absence of exogenous IL-4. Zhang *et al* [83] also found that GATA-3 could activate the IL-5 promoter *in vitro*. Moreover, GATA-3 was also reported to inhibit T-cell responsiveness to IL-12 in IFN-γ [84]. On the other hand, reduction of

GATA-3 function in cloned Th2 cells by antisense led to the extinction of IL-4, IL-5, IL-6, IL-10, and IL-13 Th2 cytokine gene mRNA expression and protein secretion [82]. This result was confirmed by a more recent study [85], in which expression of a dominant-negative mutant of GATA-3 in mice in a T-cell-specific manner reduced the expression of all of the Th2 cytokines IL-4, IL-5, and IL-13, and diminished airway hypersensitivity *in vivo*. Finally, Arai *et al* [86] recently showed that retrovirally expressed GATA-3 could cause fully committed Th1 cloned T cells to produce IL-4 and IL-5. In another study, Ouyang *et al* [87], using a similar approach, found that GATA-3 expression could rescue Th2 development in Stat6-deficient cells, resulting in Th2 cytokine expression and the establishment of Th2-specific deoxyribonuclease I hypersensitive sites in the IL-4 locus. These experiments show that GATA-3 is the master Th2 regulatory factor, and is both necessary and sufficient to generate Th2 responses. How GATA-3 works is still not understood, however. The proximal promoter of IL-4 gene lacks a strongly functional GATA-3-binding site, so we believe that its major role is likely to be as a Th2-specific enhancer(s) or perhaps as a locus control region (eg in the IL-4, IL-5, and IL-13 regions).

c-*Maf*
c-*Maf* is a basic region/leucine zipper transcription factor that is not expressed by naïve cells, and is induced when cells differentiate into the Th2 lineage [88]. It binds to a site in the proximal IL-4 promoter. Overexpression of c-*maf* strongly induced IL-4 expression in non-T cells. Transgenic mice in which c-*maf* overexpression was driven by the CD4 promoter exhibited enhanced Th2 responses that were dependent on IL-4 [89]. Decreased IFN-γ production seen in these mice is not dependent on IL-4. Unlike, GATA-3, however, c-*maf* does not appear to act by itself, as c-*maf*-overexpressing Th cells failed to express IL-4 under Th1-skewing conditions [89]. The importance of c-*maf* in IL-4 expression is further strengthened by a report that c-*maf*-/- Th and natural killer T cells are defective in IL-4 production [90]. Th2 differentiation and cytokine production appear normal in the absence of c-*maf*, however, which is probably due to a compensatory role by IL-13 that is normally expressed. Thus, c-*maf* is a transcription factor that plays an important role in IL-4, but not of other cytokine gene expression.

JunB
JunB, a member of the c-*jun* family of transcription factors, was found to be selectively expressed in Th2 cells [70,91]. JunB binds specifically to the AP-1 site of the P1 element, and when junB was overexpressed in transgenic mice it caused developing Th1 cells to overproduce IL-4 and IL-5, and to a lesser extent IL-6 and IL-10, which are all Th2 products [91]. The mechanism whereby JunB achieved this overexpression was through synergy with c-*maf* [91]. JunB and c-*maf* synergized in transient transfection studies

using reporters for the promoter proximal element of the IL-4 promoter. This synergy was specific for JunB, because neither c-*jun* or JunD is capable of synergizing. Interestingly, to perform this function JunB required phosphorylation at two threonine residues (102 and 104) by c-*jun* amino-terminal kinase (JNK), and mediated c-*maf* binding to DNA in a phosphorylation-dependent manner. Paradoxically, although it has previously been considered to be a transcriptional repressor, JunB activates transcription in the context of IL-4 promoter.

Signaling pathways that mediate T-helper-cell differentiation

Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathways

First identified as downstream of IFN signaling, the JAK–STAT pathway has been demonstrated to be essential for cytokine regulation of cell growth and differentiation. In general, cytokine-induced receptor dimerization activates JAK kinases, which then phosphorylate and initiate the nuclear transport of STAT transcription factors. STAT4 is activated by IL-12, and STAT4-deficient mice exhibited a profound Th1 defect [45,46]. On the other hand, STAT6, which acts downstream of IL-4, is required for Th2 differentiation [92–94]. Bcl-6 was reported to compete with STAT6 for the same binding site, and thus was considered as a negative regulator of Th2 responses [95]. In support of this, Bcl-6 knockout mice exhibited autoimmune diseases that were characterized as Th2 type [95]. Although STAT function in Th-cell differentiation has been well established, their immediate downstream target gene(s) remain unknown. For instance, the proximal IL-4 promoter lacks a STAT site. There is a report [96] that STAT6 may bind to a site in an intronic repressor and released the inhibition. Whether this is the exact physiological function for STAT6 remains to be determined. Alternatively, STAT6 may function to regulate expression of other transcription factors that are involved in Th2 differentiation, and attractive candidate genes are GATA-3 and c-*maf*. Indeed, Kurata *et al* [97] found that retroviral expression of STAT6 can result in GATA-3 and c-*maf* expression and Th2 cytokine production in cells differentiated into the Th1 lineage.

Extracellular-signal-regulated kinase (ERK)–mitogen-activated protein kinase pathway

There are three major MAPK pathway in mammals: ERK, p38 and JNK [98]. Each pathway consists of three cascades of activation events (i.e. MAPK kinase kinases phosphorylate MAPK kinases, which then activate MAPKs). The ERK pathway was first identified downstream of oncogenic Ras, and is often involved in the regulation of cell growth and differentiation. There are two isoforms of ERK, ERK1 and ERK2, which are sometimes referred to as p44/p42 MAPKs. They can be activated by MAPK/ERK kinase (MEK)1 and 2 upstream kinases. The RAS–ERK

pathway plays an important role in early thymocyte differentiation and selection, and its role in peripheral Th-cell differentiation was not studied until recently. Yamashita *et al* [99], using dominant H-RAS transgenic mice in which ERK activation by TCR was severely compromised, showed that this pathway is required for Th2-cell differentiation [99]. Similar results were found using wild-type cells treated with inhibitors against MEKs. Those investigators went further to show that the ERK pathway functions to enhance IL-4-induced STAT6 and IL-4 receptor phosphorylation, which suggests a mechanism of cross-regulation among different signaling pathways.

P38–mitogen-activated protein kinase pathway

The p38 and JNK protein kinases are also members of the MAPK family that are activated in response to stress and inflammatory cytokines. These enzymes are also activated by MKKs, specifically MKK3 and MKK6 in the case of p38 [99]. The p38 MAPKs comprise members of a parallel stress-activated MAPK pathway. Here, too, there are multiple isoforms and p38 is found as four enzymes: p38-α, p38-β, p38-γ, and p38-δ.

The p38–MAPK pathway is selectively activated in mouse Th1 effector cells [100]. Imidazole inhibitors of the p38 kinases block IFN-γ production by Th1 cells in a dose-dependent manner, but have no effect on IL-4 production by Th2 cells. Furthermore, transgenic mice in which a dominant-negative p38-α transgene was directed by the LCK distal promoter showed reduced IFN-γ cytokine secretion and mRNA production [100]. T cells from mice deficient in the p38 upstream kinase MKK3 have an intrinsic defect to produce normal levels of IFN-γ, even when provided with APCs from a wild-type B6 mouse [58]. The effect of the p38 pathway on IFN-γ is probably transcriptional, because IFN-γ reporters are also inhibited by dominant-negative p38-α construct in Jurkat cells, indicating that p38 regulation is necessary for IFN-γ expression. On the other hand, transgene-encoded constitutively active MKK6, one of the upstream MKKs that activates p38 kinase, led to the predicted opposite result, namely the activation of p38 MAPK and the consequent activation of IFN-γ transcription to higher levels than those seen in control transgene negative cells [100].

Candidate downstream targets of p38 regulation are likely to include transcription factors of the activating transcription factor (ATF) family. Studies of the IFN-γ promoter, the prototype Th1-specific cytokine, led to the identification of c-*jun*/ATF2 sites and a series of other ATF-binding sites within two functionally active elements called the proximal and distal IFN-γ elements [101,102]. Transgenic mice in which these two elements were linked to luciferase reporter constructs [103,104] showed that the proximal element that carries a c-*jun*/ATF2 site exhibited Th1 specificity, whereas the distal element did not.

C-*jun* amino-terminal kinase–mitogen-activated kinase pathway

The JNK group of MAPKs are also known as stress-activated protein kinases, which are activated by exposure of cells to cytokines and a variety of environmental stresses. JNK activation is mediated by dual phosphorylation on threonine and tyrosine residues in a Thr-Pro-Tyr motif by two MKKs known as MKK4 and MKK7 [105]. MKK4, also known as SEK1, was initially identified as a component of the JNK signaling pathway, but it can phosphorylate p38 as well.

JNK phosphorylates c-Jun and increases AP1 transcriptional activity. JNK activation in Jurkat cells has been shown to require both TCR and CD28 ligation, and JNK is suggested to integrate signals from both sets of receptors to induce IL-2 expression [106]. However, induction of JNK activity in primary mouse T cells is slow and peaks at approximately 2–3 days after T-cell activation, during which time there is substantial *de novo* synthesis of JNK protein [107]. Thus, there is an apparent paradox with regard to the timing of IL-2 gene expression, which occurs within several hours of T-cell activation, at a time when there is little JNK activity. JNK activity does, however, correlate well with the expression of effector cytokines in both the Th1 and Th2 pathways. Indeed, knockout mice for either *Jnk1* or *Jnk2* genes exhibited defective phenotypes associated not with Th-cell activation, but with Th-cell differentiation. Mice that are deficient in JNK2 show deficient Th1 immune responses [108]. Reduced levels of IFN-γ are found and this appears to derive from a reduced production of the IL-12 receptor β_2-chain. This deficiency itself is a consequence of the failure to produce optimal levels of IFN-γ early during the response to T-cell receptor and costimulator ligation. Addition of IFN-γ to JNK2-deficient T cells restores the deficit and confers normal Th1 function on these cells *in vitro*. In contrast, the Th2 responses of JNK2-deficient mice appear relatively normal.

JNK1-deficient mice, in contrast, show an exaggerated Th2 response [25]. Even when cultured under Th1 conditions, the Th2 cytokines IL-4, IL-5, and IL-10 are produced in significant amounts. Consistent with this exaggerated Th2 response, infection of JNK1 mice with *Leishmania* leads to greatly exacerbated disease with failure to heal skin lesions; the disease advances to ulceration in a manner similar to that in the BALBc mouse, which also has a profoundly strong Th2 response [Constant *et al*, unpublished data]. Th2 responses themselves are exaggerated and JNK1 mice that are immunized with protein antigens develop enhanced Th2-cell responses in recall assays *in vitro* [25]. Most notably, *Jnk1*$^{-/-}$ T cells produced Th2 cytokines in the absence of CD28 costimulation and differentiate preferentially into Th2 cells *in vitro* when stimulated with anti-CD3, irradiated APCs, and IL-2, whereas the wild-type became mostly Th1 cells [25]. This is probably caused by enhanced IL-4 production at the early phase (ie 24-h and 48-h time point) of Th-cell activation. Examination of Th2 transcription factors expression at this stage shows that JNK1-deficient mice have elevated NFATc in the nucleus [25]. Because NFATc-deficient mice have reduced IL-4 production and NFAT itself can efficiently transactivate the IL-4 promoter, we believe that this elevated NFAT leads to exaggerated production of Th2 cytokines. It is likely, therefore, that JNK1 plays a role in negatively regulating the level of NFATc in the nucleus. The mechanisms that underlie this process are under further investigation.

I-κB kinase-nuclear factor-κB pathway

NF-κB proteins normally exist in the cytoplasm associated with inhibitor of NF-κB (I-κB) molecules. Upon activation by upstream kinases, NF-κB-inducing kinase or MEK kinase 1 for instance, I-κB kinases are activated and phosphorylate I-κB proteins, which leads to their dissociation from NF-κB and degradation by ubiquination-dependent proteosomes, and NF-κB proteins translocate into the nucleus to trans-activate target genes. NF-κB factors are known for their essential roles in innate immune responses (ie production of inflammation cytokines and B7 molecules). Their roles in Th cell differentiation have just begun to receive attention and appreciation. Using transgenic mice expressing a I-κB mutant that can not be phosphorylated, thereby inhibiting NF-κB nuclear transport, Aronica *et al* [110] recently demonstrated that the NF-κB pathway is required for Th1-dependent delayed-type hypersensitivity responses, but not for a Th2-characterized airway hypersensitivity response. The molecular mechanism for this observation and the downstream gene(s) mediated by NF-κB certainly require further investigation.

Conclusion

Recent years have seen rapid progress in study of Th-cell differentiation. Th1/Th2 lineage determination is mediated by TCR, coreceptors and, most importantly, the cytokine environment (Fig. 1). A number of signaling pathways positively or negatively regulate the transcription factors that are responsible for polarized cytokine production (Fig. 1). An in-depth understanding of these events is likely to be achieved in the coming years, and the complexity of the differentiation program will be further appreciated with realization of exquisite regulation and cross-regulation of each pathway and factor. We believe that improvement of our knowledge in the field will greatly benefit development of efficient pharmacologic means for treating immune disorders that are suffered by millions of people today.

Acknowledgments

References

1. Croft M, Bradley LM, Swain SL: **Naive versus memory CD4 T cell response to antigen.** *J Immunol* 1994, **152**:2675–2685.
2. Tough DF, Sprent J: **Lifespan of lymphocytes.** *Immunol Res* 1995, **14**:1–12.
3. Sprent J: **Lifespans of naive, memory and effector lymphocytes.** *Curr Opin Immunol* 1993, **5**:433–438.
4. Paul WE, Seder RA: **Lymphocyte response and cytokines.** *Cell* 1994, **76**:241–251.
5. Louis J, Himmelrich H, Parra-Lopez C, *et al*: **Regulation of protective immunity against** *Leishmania major* **in mice.** *Curr Opin Immunol* 1998, **10**:459–464.
6. Pfeiffer C, Stein J, Southwood S, *et al*: **Altered peptide ligands can control CD4 T lymphocyte differentiation** *in vivo*. *J Exp Med* 1995, **181**:1569–1574.
7. Bretscher PA, Wei G, Menon JN, *et al*: **Establishment of stable, cell-mediated immunity that makes 'susceptible' mice resistant to** *Leishmania major*. *Science* 1992, **257**:539–542.
8. Constant S, Pfeiffer C, Woodard A, *et al:* **Extent of T cell receptor ligation can determine the functional differentiation of naïve CD4+ T cells.** *J Exp Med* 1995, **182**:1591–1596.
9. Le Gros G, Ben-Sasson SZ, Seder R, *et al*: **Generation of interleukin 4 (IL-4)-producing cells** *in vivo* **and** *in vitro*: **IL-2 and IL-4 are required for** *in vitro* **generation of IL-4 producing cells.** *J Exp Med* 1990, **172**:921–929.
10. Swain SL, Weinberg AD, English M, *et al*: **IL-4 directs the development of Th2-like helper effectors.** *J Immunol* 1990, **145**:3796–3806.
11. Hsieh CS, Macatonia SE, Tripp CS, *et al:* **Development of Th1 CD4+ T cells through IL-12 produced by** *Listeria*-**induced macrophages.** *Science* 1993, **260**:547–549.
12. Seder RA, Gazzinelli R, Sher A, *et al*: **IL-12 acts directly on CD4+ T cells to enhance priming for IFNg production and diminish IL-4 inhibition of such priming.** *Proc Natl Acad Sci USA* 1993, **90**:10188–10192.
13. McKenzie GJ, Emson CL, Bell SE, *et al*: **Impaired development of Th2 cells in IL-13-deficient mice.** *Immunity* 1998, **9**:423–432.
14. Rincón M, Anguita J, Nakamura T, *et al*: **IL-6 directs the differentiation of IL-4-producing CD4+ T cells.** *J Exp Med* 1997, **185**:461–469.
15. Robinson D, Shibuya K, Mui A, *et al*: **IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkB.** *Immunity* 1997, **7**:571–581.
16. Sloan-Lancaster J, Allen PM: **Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology.** *Annu Rev Immunol* 1996, **14**:1–27.
17. Tao X, Grant C, Constant S, *et al*: **Induction of IL-4-producing CD4+ T cells by antigenic peptides altered for TCR binding.** *J Immunol* 1997, **158**:4237–4244.
18. Iezzi G, Scotet E, Scheidegger D, *et al*: **T cell polarization contingent on the duration of TCR and cytokine signaling.** *Eur J Immunol* 1999, **29**:4092–4101.
19. Leitenberg D, Boutin Y, Constant S, *et al*: **CD4 regulation of TCR signaling and T cell differentiation following stimulation with peptides of different affinities for the TCR.** *J Immunol* 1998, **161**:1194–1203.
20. Yamashita M, Hashimoto K, Kimura M, *et al*: **Requirement for p56lck tyrosine kinase activation in Th subset differentiation.** *Int Immunol* 1998, **10**:577–591.
21. Fowell DJ, Shinkai K, Liao XC, *et al*: **Impaired NFATc translocation and failure of Th2 development in itk-deficient CD4+ Tcells.** *Immunity* 1999, **11**:399–409.
22. Schwartz RH: **T cell clonal anergy.** *Curr Opin Immunol* 1997, **9**:351–357.
23. Shahinian A, Pfeffer K, Lee KP, *et al*: **Differential T cell costimulatory requirements in CD28-deficient mice.** *Science 1993,* **261**:609–612.
24. Seder RA, Germain RN, Linsley PS, *et al*: **CD28-mediated costimulation of interleukin 2 (IL-2) production plays a critical role in T cell priming for IL-4 and interferon gamma production.** *J Exp Med* 1994, **179**:299–304.
25. Dong C, Yang DD, Wysk M, *et al*: **Defective T cell differentiation in the absence of** *Jnk1*. *Science* 1998, **282**:2092–2095.
26. Schweitzer AN, Sharpe AH: **Studies using antigen-presenting cells lacking expression of both B7-1 (CD80) and B7-2 (CD86) show distinct requirements for B7 molecules during priming versus restimulation of Th2 but not Th1 cytokine production.** *J Immunol* 1998, **161**:2762–2771.
27. Guerder S, Carding SR, Flavell RA: **B7 costimulation is necessary for the activation of the lytic function in cytotoxic T lymphocyte precursors.** *J Immunol 1995,* **155**:5167–5174.
28. Shahinian A, Pfeffer K, Lee KP, *et al*: **Differential T cell costimulatory requirements in CD28-deficient mice.** *Science* 1993, **261**:609–612.
29. Greenwald RJ, Lu P, Halvorson MJ, *et al*: **Effects of blocking B7-1 and B7-2 interactions during a type 2 in vivo immune response.** *J Immunol* 1997, **158**:4088–4096.
30. Murphy ML, Engwerda CR, Gorak PM, *et al*: **B7-2 blockade enhances T cell responses to** *Leishmania donovani*. *J Immunol* 1997, **159**:4460–4466.
31. Viola A, Schroeder S, Sakakibara Y, *et al*: **T lymphocyte costimulation mediated by reorganization of membrane microdomains.** *Science* 1999, **283**:680–682.
32. Bird JJ, Brown DR, Mullen AC, *et al:* **Helper T cell differentiation is controlled by the cell cycle.** *Immunity* 1998, **9**:229–237.
33. Waterhouse P, Penninger JM, Timms E, *et al*: **Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4.** *Science* 1995, **270**:985–988.
34. Khattri R, Auger JA, Griffin MD, *et al*: **Lymphoproliferative disorder in CTLA-4 knockout mice is characterized by CD28-regulated activation of Th2 responses.** *J Immunol* 1999, **162**:5784–5791.
35. Hutloff A, Dittrich AM, Beier KC, *et al*: **ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28.** *Nature* 1999, **397**:263–266.
36. Swallow MM, Wallin JJ, Sha WC: **B7h, a novel costimulatory homolog of B7.1 and B7.2, is induced by TNFα.** *Immunity* 1999, **11**:423–432.
37. Yoshinaga SK, Whoriskey JS, Khare SD, *et al*: **T-cell co-stimulation through B7RP-1 and ICOS.** *Nature* 1999, **402**:827–832.
38. Dong H, Zhu G, Tamada K, *et al*: **B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion.** *Nature Med* 1999, **5**:1365–1369.
39. Grewal IS, Flavell RA: **CD40 and CD154 in cell-mediated immunity.** *Annu Rev Immunol* 1998, **16**:111–135.
40. Green EA, Flavell RA: **TRANCE-RANK, a new signal pathway involved in lymphocyte development and T cell activation.** *J Exp Med* 1999, **189**:1017–1020.
41. Bachmann MF, Wong BR, Josien R, *et al*: **TRANCE, a tumor necrosis factor family member critical for CD40 ligand-indenpendent T helper cell activation.** *J Exp Med* 1999, **189**:1025–1031.
42. Kopf M, Ruedl C, Schimitz N, *et al*: **OX40-deficient mice are defective in Th cell proliferation but are competent in generating B cell and CTL responses after virus infection.** *Immunity* 1999, **11**:699–708.
43. Chen AI, McAdam AJ, Buhlmann JE, *et al*: **Ox40-ligand has a critical costimulatory role in dendritic cell:T cell interactions.** *Immunity* 1999, **11**:689–698.
44. Magram J, Connaughton SE, Warrier RR, *et al*: **IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses.** *Immunity* 1996, **4**:471–481.
45. Kaplan MH, Sun Y-L, Hoey T, *et al*: **Impaired IL-12 responses and enhanced development of Th2 cells in Stat-4 deficient mice.** *Nature* 1996, **382**:174–177.
46. Thierfelder WE, van Deursen JM, Yamamoto K, *et al*: **Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells.** *Nature* 1996, **382**:171–174.
47. Wu C, Ferrante J, Gately M K, *et al*: **Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor.** *J Immunol* 1997, **159**:1658–1665.
48. Plevy SE, Gemberling JHM, Hsu S, *et al*: **Multiple control elements mediate activation of the murine and human interleukin 12 p40 promoters: evidence of functional syngerny between C/EBP and rel proteins.** *Mol Cell Biol* 1997, **17**:4572–4588.
49. Ma X, Chow JM, Gri G, *et al*: **The interleukin 12 p40 gene promoter is primed by interferon-gamma in monocytic cells.** *J Exp Med* 1996, **183**:147–157.
50. Ma X, Neurath M, Gri G, *et al*: **Identification and characterization of a novel ets-2-related nuclear complex implicated in the activation of the human interleukin-12 p40 gene promoter.** *J Biol Chem* 1997, **272**:10389–10395.
51. Murphy TL, Cleveland MG, Kulesza P, *et al*: **Regulation of interleukin 12 p40 expression through an NF-kappa B half-site.** *Mol Cell Biol* 1995, **15**:5258–5267.
52. Gri G, Savio D, Trinchieri G, *et al*: **Synergistic regulation of the human Interleukin-12 p40 promoter by NF-kB and Ets transcription factors in Epstein-Barr virus-transformed B cells and macrophages.** *J Biol Chem* 1998, **273**:6471–6438.

53. Giese NA, Gabriele L, Doherty TM, *et al*: **Interferon (IFN) consensus sequence-binding protein, a transcription factor of the IFN regulatory factor family, regulates immune responses in vivo through control of interleukin 12 expression.** *J Exp Med* 1997, **186**:1535–1546.

54. Lohoff M, Ferrick D, Mittrucker H-W, *et al*: **Interferon response factor-1 is required for a T helper 1 immune response in vivo.** *Immunity* 1997, **6**:681–689.

55. Scharton-Kersten T, Contursi C, Matsumi A, *et al*: **Interferon consensus sequence binding protein-deficient mice display resistance to intracellular infection due to a primary defect in interleukin 12 p40 induction.** *J Exp Med* 1997, **186**:1523–1534.

56. Taki S, Sato T, Ogasawara K, *et al*: **Mutistage regulation of Th1-type immune responses by the transcription factor IRF-1.** *Immunity* 1997, **6**:673–679.

57. Kopp EB, Medzhitov R: **The Toll-receptor family and control of innate immunity.** *Curr Opin Immunol* 1999, **11**:13–18.

58. Lu H-T, Yang DD, Wysk M, *et al:* **Defective IL-12 production in mitogen-activated protein (MAP) kinase kinase 3 (*Mkk3*)-deficient mice.** *EMBO J* 1999, **18**:1845–1857.

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

60. Chen YH, Chiu NM, Mandal M, *et al*: **Impaired NK1+ T cell development and early IL-4 production in CD1-deficient mice.** *Immunity* 1997, **6**:459–467.

61. Mendiratta SK, Martin WD, Hong S, *et al*: **CD1d1 mutant mice are deficient in natural T cells that promptly produce IL-4.** *Immunity* 1997, **6**:469–477.

62. Launois P, Maillard I, Pingel S, *et al*: **IL-4 rapidly produced by V-beta-4 V-alpha-8 CD4(+) T cells instructs the development and susceptibility to *Leishmania major* in Balb/C mice.** *Immunity* 1997, **6**:541–549.

63. Riviere I, Sunshine MJ, Littman DR: **Regulation of IL-4 expression by activation of individual alleles.** *Immunity* 1998, **9**:217–228.

64. Bix M, Locksley RM: **Independent and epigenetic regulation of the interleukin-4 alleles in CD4+ T cells.** *Science* 1998, **281**:1352–1354.

65. Agarwal S, Rao A: **Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation.** *Immunity* 1998, **9**:765–775.

66. Takemoto N, Koyano-Nakagawa N, Yokota T, *et al*: **Th2-specific DNase I-hypersensitive sites in the murine IL-13 and IL-4 intergenic region.** *Int Immunol* 1998, **10**:1981–1985.

67. Blom van Assendelft G, Hanscombe O, Grosveld F, *et al*: **The beta-globin dominant control region activates homologous and heterologous promoters in a tissue-specific manner.** *Cell* 1989, **56**:969–977.

68. Greaves DR, Wilson FD, Lang G, *et al*: **Human CD2 3'-flanking sequences confer high-level, T cell-specific, position-independent gene expression in transgenic mice.** *Cell* 1989, **56**:979–986.

69. Wenner CA, Szabo SJ, Murphy KM: **Identification of IL-4 promoter elements conferring Th2-restricted expression during T helper cell subset development.** *J Immunol* 1997, **158**:765–773.

70. Rincón M, Dérijard B, Chow C-W, *et al*: **Reprogramming the signaling requirement for AP-1 (activator protein-1) activation during differentiation of precursor CD4+ T cells to effector Th1 and Th2 cells.** *Genes Function* 1997, **1**:51–68.

71. Rincón M, Flavell RA: **Transcription mediated by NFAT is highly inducible in effector CD4+ Th2 cells but not in Th1 cells.** *Mol Cell Biol* 1997, **17**:1522–1534.

72. Rao A, Luo C, Hogan PG: **Transcription factors of the NFAT family: regulation and function.** *Annu Rev Immunol* 1997, **15**:707–747.

73. Lopez-Rodriguez C, Aramburu J, Rakeman AS, *et al*: **NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun.** *Proc Natl Acad Sci USA* 1999, **96**:7214–7219.

74. Boise LH, Petryniak B, Mao X, *et al*: **The NFAT-1 DNA binding complex in activated T cells contains Fra-1 and JunB.** *Mol Cell Biol* 1993, **13**:1911–1919.

75. Rooney JW, Hoey T, Glimcher LH: **Coordinate and cooperative roles for NF-AT and AP-1 in the regulation of the murine IL-4 gene.** *Immunity* 1995, **2**:473–483.

76. Timmerman LA, Healy JI, Ho SN, *et al*: **Redundant expression but selective utilization of nuclear factor of activated T cells family members.** *J Immunol* 1997, **159**:2735–2740.

77. 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.

78. Xanthoudakis S, Viola JP, Shaw KT, *et al*: **An enhanced immune response in mice lacking the transcription factor.** *Science* 1996, **272**:892–895.

79. 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.

80. Yoshida H, Nishina H, Takimoto H, *et al*: **The transcription factor NF-ATc1 regulates lymphocyte proliferation and Th2 cytokine production.** *Immunity* 1998, **8**:115–124.

81. Ranger AM, Hodge MR, Gravallese EM, *et al*: **Delayed lymphoid repopulation with defects in IL-4-driven responses produced by inactivation of NF-ATc.** *Immunity* 1998, **8**:125–134.

82. Zheng W-P, Flavell RA: **The transcription factor GATA-3 is necessary and sufficient for Th2 gene expression in CD4+ T cells.** *Cell* 1997, **89**:587–596.

83. Zhang DH, Cohn L, Ray P, *et al*: **Transcriptional 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.

84. Ouyang W, Ranganath SH, Weindel K, *et al*: **Inhibition of Th1 development mediated by GATA-3 through an IL-4- independent mechanism.** *Immunity* 1998, **9**:745–755.

85. Zhang D-H, 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.

86. Arai N, Lee HJ, Ferber I, *et al*: **Multiple levels of regulation of Th2 cytokine gene expression.** *Cold Spring Harbor Lab Symp Quant Biol* 2000 (in press).

87. 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.

88. 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.

89. 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.

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

91. Li B, Tournier C, Davis RJ, *et al*: **Regulation of T helper cell differentiation by the transcription factor JunB.** *EMBO J* 1999, **19**:420–432.

92. Takeda K, Tanaka T, Shi W, *et al*: **Essential role of Stat6 in IL-4 signaling.** *Nature* 1996, **380**:627–630.

93. Shimoda K, van Deursen J, Sangster MY, *et al*: **Lack of IL4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene.** *Nature* 1996, **380**:630–633.

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

95. Dent AL, Shaffer AL, Yu X, *et al*: **Control of inflammation, cytokine expression, and germinal center formation by BCL-6.** *Science* 1997, **276**:589–592.

96. Kubo M, Ransom J, Webb D, *et al*: **T-cell subset-specific expression of the IL-4 gene is regulated by a silencer element and STAT6.** *EMBO J* 1997, **16**:4007–4020.

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

98. Whitmarsh AJ, Davis RJ: **Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways.** *J Mol Med* 1996, **74**:589–607.

99. Yamashita M, Kimura M, Kubo M, *et al*: **T cell antigen receptor-mediated activation of the Ras/mitogen-activated protein kinase pathway controls interleukin 4 receptor function and type-2 helper T cell differentiation.** *Proc Natl Acad Sci USA* 1999, **96**:1024–1029.

100. Rincón M, Enslen H, Raingeaud J, *et al*: **Interferon-γ expression by Th1 effector T cells mediated by the p38 MAP kinase signaling pathway.** *EMBO J* 1998, **17**:2817–2829.

101. Penix L, Weaver WM, Pang Y, *et al*: **Two essential regulatory elements in the human interferon gamma promoter confer activation specific expression in T cells.** *J Exp Med* 1993, **178**:1483–1496.

102. Penix LA, Sweetser MT, Weaver WM, *et al*: **The proximal regulatory element of the interferon-γ promoter mediates selective expression in T cells.** *J Biol Chem* 1996, **271**:31964–31972.

103. Zhang F, Wang DZ, Boothby M, *et al*: **Regulation of the activity of IFN-γ promoter elements during Th cell differentiation.** *J Immunol* 1998, **161**:6105–6112.
104. Aune TM, Penix LA, Rincón M, *et al*: **Memory and effector, but not naive T cells, express transcriptional activity under the control of two IFNγ promoter elements.** *Mol Cell Biol* 1997, **17**:199–204.
105. Ip YT, Davis RJ: **Signal transduction by the c-Jun NH$_2$-terminal kinase (JNK/SAPK) – from inflammation to *Drosophila* development.** *Curr Opin Cell Biol* 1998, **10**:205–219.
106. Su B, Jacinto E, Hibi M, *et al*: **JNK is involved in signal integration during costimulation of T lymphocytes.** *Cell* 1994, **77**:727–736.
107. Weiss L, Whitmarsh AJ, Yang DD, *et al*: **MAP kinase signal transduction regulated by gene expression and phosphorylation.** *J Exp Med* 2000, **191**:139–146.
108. Yang D, Conze D, Whitmarsh AJ, *et al*: **Differentiation of CD4+ T cells to Th1 cells requires MAP kinase JNK2.** *Immunity* 1998, **9**:575–585.
109. Aronica MA, Mora AL, Mitchell DB, *et al*: **Preferential role of NF-kB/Rel signaling in the type1 but not type 2 T cell-dependent immune responses *in vivo*.** *J Immunol* 1999, **163**:5116–5124.

Authors' affiliation: Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut, USA

Correspondence: Richard A Flavell, Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, FMB 412, 310 Cedar St, New Haven, CT 06520, USA.
Tel: +1 203 785 7024; fax: +1 203 737 1764;
e-mail: fran.manzo@yale.edu