T-cell subsets: **Transcriptional control in the Th1/Th2 decision**

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Transcriptional mechanisms in CD4+ T cells and antigen-presenting cells determine the activation or differentiation of Th1 and Th2 helper cell subsets, and also form attractive targets for therapeutic intervention in the balance of Th1/Th2 responses.

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Naive (precursor) CD4+ helper T cells recognize specific major histocompatibility complex (MHC)–peptide combinations on antigen-presenting cells (APCs) via interactions with the T-cell receptor (TCR), thereby providing the first signal required for activation. The second, costimulatory signal, is provided by accessory molecules expressed on APCs, such as the widely studied B7 family of proteins. B7 proteins are the ligands for the CD28 and CTLA-4 costimulatory molecules that are expressed on T cells. The combination of these two signals induces interleukin-2 (IL-2) synthesis and secretion, IL-2 receptor expression, clonal expansion and differentiation of precursor CD4+ T cells into effector T helper (Th) cells.

Two subsets of effector Th cells have been defined on the basis of their distinct cytokine secretion patterns and their immunomodulatory effects: Th1 cells produce inflammatory cytokines, such as tumor necrosis factor β (TNF-β) and interferon-γ (IFN-γ), and enhance cellular immunity; Th2 cells produce a different group of cytokines — IL-4, IL-5, IL-6, IL-10 and IL-13 — and help B cells secrete antibodies. The differentiation of naive T cells into the Th1 or Th2 phenotype has important biological implications in terms of susceptibility or resistance to a particular disease.

The Th1/Th2 differentiation step requires new protein synthesis and gene expression. An understanding of the transcriptional mechanisms that are involved in the differentiation and/or activation of Th1 and Th2 cells might therefore provide potential targets for therapeutic intervention. In this commentary, we will summarize the transcription factors that have been implicated in this process.

Th1 or Th2 – a multifactorial decision

The selective differentiation of precursor CD4+ T cells into Th1 and Th2 cells is established during the initial priming of these cells and is influenced by a variety of extracellular factors, one of which is the dose of antigen during priming [1]. Several lines of evidence indicate that the source of costimulation is another critical factor in the Th1/Th2 differentiation process. The two members of the B7 family, B7.1 and B7.2, can be differentially expressed on dendritic cells, Langerhans cells, activated monocytes, T cells and B cells. Recently, B7.1 and B7.2 have been described to play distinct roles in the differentiation of Th1 and Th2 cells, although their specific functions remain unclear [2].

The most effective inducer of differentiation is the cytokine environment present during the priming of the precursor cells. Thus, the presence of IL-4 promotes differentiation into Th2 cells, whereas IL-12 and IFN-γ drive precursor Th cells to differentiate into Th1 cells [3]. IL-12 is secreted by APCs — mainly macrophages and dendritic cells — but the source of the initial production of IL-4 that triggers differentiation remains unclear. Recently, APC-derived IL-6 was found to polarize naive CD4+ T cells into Th2 cells by inducing the initial production of IL-4 in CD4+ T cells [4]. Moreover, an increased Th1 response and a reduced Th2 response to *Borrelia burgdorferi* infection was observed in IL-6-deficient mice (J. Anguita, M.R., S. Santana. S.W. Berthold, R.A.F. and E. Fikrig, unpublished observations). The balance between IL-6 and IL-12 production by APC therefore plays a critical role in the control of Th1 and Th2 responses. Additional mechanisms are important for the Th2 responses: IL-7 also primes human naive CD4+ T cells for IL-4 production [5].

The overall relevance and relative contribution of each of these components in the differentiation process remains controversial; however, as all components are derived from APCs, their activation events are central to the differentiation of Th1 and Th2 cells. Thus, a simple model of differentiation might include all these elements if the Th1/Th2 decision is the result of a bidirectional communication between the CD4+ T cells and APCs. The expression of specific cytokine genes in APCs could be determined by the intracellular signals provided by MHC class II (upon interaction with antigen), B7.1 or B7.2 (upon ligation with CD28 or CTLA-4), CD40 (upon interaction with the CD40 ligand) and other potential costimulatory molecules. This cytokine environment would then modulate the polarization of the T-cell component. It is therefore important to determine not only the signaling pathways and transcriptional mechanisms that control the expression of IL-4 and IFN-γ during the differentiation of Th1 and Th2 cells, but

also the signaling pathways and transcriptional mechanisms that lead to the expression of specific cytokines in the APCs.

Transcriptional regulation mechanisms in Th1 and Th2 CD4+ T cells

It remains unclear how the interplay between the TCR complex, costimulatory molecules and cytokine-mediated signals induces or represses the expression of specific cytokine genes in CD4+ T cells during Th1/Th2 differentiation. However, numerous studies have focused on the identification and characterization of specific transcription factors that are expressed exclusively in either of these T-cell subsets.

STAT4 and STAT6 transcription factors are induced upon tyrosine phosphorylation triggered by IL-12 and IL-4 stimulation, respectively. To date, no other cytokine has been able to induce STAT4, and only IL-13 in addition to IL-4 can trigger the phosphorylation and translocation of STAT6. The development of Th1 cells in response to either IL-12 or *Listeria monocytogenes* infection is impaired in STAT4-deficient mice [6,7]. Similarly, as expected, an impairment of IL-4-mediated Th2 responses was observed in STAT6-deficient mice [6,8,9]. Nevertheless, direct regulation of IFN-γ and IL-4 gene expression by STAT4 or STAT6, respectively, has not been demonstrated. No functional STAT6-binding sites have yet been identified in the promoter region of the IL-4 gene. It is, however, intriguing that IL-4 receptor expression is not upregulated in response to IL-4 in STAT6-deficient mice [6], suggesting that STAT6 may also be involved in controlling the expression of the IL-4 receptor gene. Recently, a STAT6 binding site has been found to negatively regulate a Th1-specific IL-4 silencer [10]. No functional STAT4-binding site has yet been found in the promoter of IFN-γ gene.

The proto-oncogene c-*maf* has also been identified as a potent transactivator of the IL-4 promoter [11], and is expressed in Th2 clones, but not in Th1 clones. Low levels of c-*maf* are expressed in naive T cells, suggesting that it may play a role in the initial activation of IL-4 gene expression. Levels of c-*maf* expression are then greatly upregulated upon Th2 differentiation. These kinetics of expression suggest that c-*maf* might be responsible for the high levels of IL-4 produced by fully differentiated Th2 cells upon restimulation (as in Th2 clones). Additional factors might be involved in the regulation of the initial expression of the IL-4 gene during the differentiation of Th2 cells.

More recently, it has been shown that GATA-3, a transcription factor required for T-cell development, is expressed at a high level in naive CD4+ T cells and Th2 cells, but at a low level in Th1 cells [12]. As developing Th1 effector cells commit to the prototypic lineage, they suppress GATA-3 expression. In Th2 clones, antisense GATA-3 inhibits the expression of IL-4, IL-6, IL-10 and IL-13 and, to a lesser extent, IL-5. Furthermore, overexpression of GATA-3 in transgenic mice results in sustained expression of IL-4, IL-6 and IL-10 genes in Th1 cells and GATA-3 directly transactivates the IL-4 [12] and IL-5 [13] promoters. Together, these studies suggest that GATA-3 plays a key role in promoting Th2 differentiation, and, as a result, GATA-3 expression is suppressed in developing Th1 cells [12].

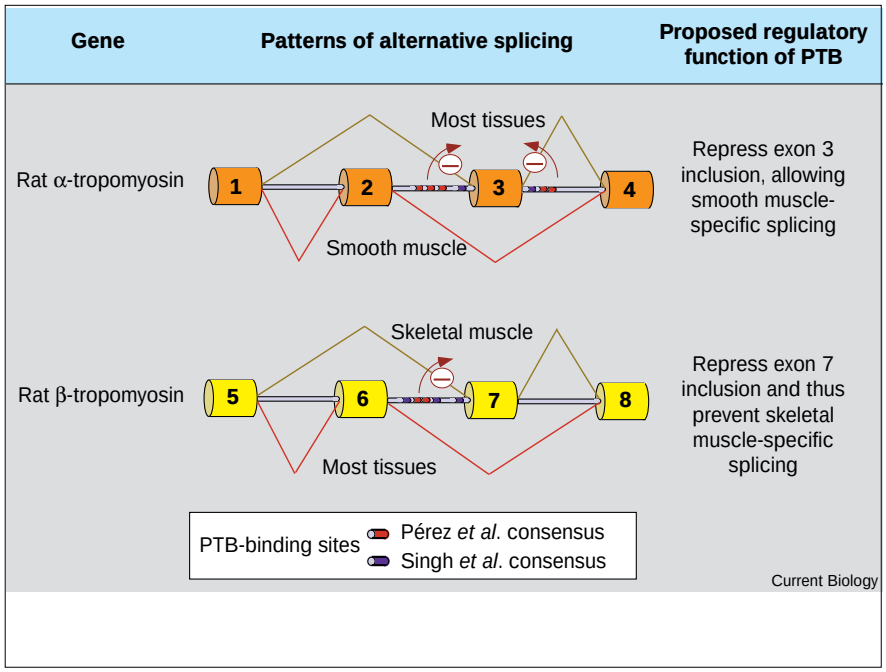
The activator protein-1 (AP-1) and nuclear factor of activated T cells (NFAT) families of transcription factors are involved in the expression of IL-2, IL-4 and other cytokine genes. We have shown that AP-1 and NFAT are highly transcriptionally active in Th2 cells, whereas only low levels of AP-1 and NFAT transcriptional activity are detectable in Th1 cells [14,15]. These results correlate with the overexpression of JunB in the Th2 subset, suggesting that JunB might play a role in the activation of the Th2 cells. However, hyperproliferation and increased Th2 responses *in vivo* to *Leishmania major* infection have been reported in mice deficient for NFAT1 (NFATp), one of the members of the NFAT family [16,17]. In contrast, a reduction in the initial production of IL-4 and other Th2 cytokines has also been observed after *in vitro* TCR ligation on T cells from these mice [18], supporting the role of NFAT in the regulation of Th2-specific cytokine genes. As NFAT now comprises a large family of related transcription factors, it is possible that other members of the NFAT family compensate for the lack of NFAT1. Although we could not demonstrate differences in the NFAT complexes that might have explained the selective activation of NFAT in Th2 cells [15], E. Serfling and S. Chuvipilo (personal communication) have recently observed a distinct longer nuclear persistence of NFAT1 in Th2 cells. As the differences in NFAT and AP-1 are found in Th1 and Th2 effector cells, and not during the priming/differentiation phase, it is likely that the key role of these factors is in the production of effector cytokines, not the determination of the T-helper phenotype [14].

Another factor, nuclear factor (NF)-IL6, is expressed in mouse Th2 clones, but not in Th1 clones, and enhances IL-4 promoter activity [19]. NF-IL6 was originally described to be induced by IL-6, suggesting an attractive hypothesis to explain its role. Further studies are needed, however, to test the role of NF-IL6 as a mediator of the IL-6-induced differentiation of Th2 cells.

The above data indicate that the control of the Th1/Th2 polarization is achieved by multiple transcription factors with several checkpoints during differentiation and activation of the effector cells (Figure 1). Nevertheless, most of these transcription factors are involved in late stages of

Figure 1

Transcriptional checkpoints in the differentiation of Th1 and Th2 cells. The transcriptional control of Th1/Th2 differentiation occurs at both sides of the mirror: T cells and antigen-presenting cells (APC). The relative expression of the transcription factors is represented by the size of the font; question marks indicate the results to be tested; asterisks indicate high transcriptional activity.



differentiation or during initial activation. An understanding of the transcriptional mechanisms that control the earliest events in the differentiation of naive CD4+ T cells as well as the expression of the polarizing factors remains incomplete.

Transcriptional regulation in the APC: the other side of the mirror

It is essential to dissect not only the molecular bases for the regulation of cytokine genes in the CD4+ T cells, but also the regulation of the genes expressed in the APCs (Figure 1). Recent studies from two different groups have revealed a role for another transcription factor, the interferon regulatory factor 1 (IRF-1), in the control of the development of Th1 responses by affecting the cytokine production in APCs [20,21].

IRF-1 was originally described as a protein that binds to DNA sequences in the promoters of the IFN-α and IFN-β genes, although it was also reported that IRF-1 is involved in the regulation of several genes that are inducible by IFN-α/β and IFN-γ [22]. Compared with normal mice, IRF-1-deficient mice are less resistant to infection with encephalomyocarditis virus, but not to infection by other viruses [23,24]. In addition, development of CD8+ T cells and expression of the nitric oxide synthase gene are also impaired in these mice. Taki *et al.* [21] and Lohoff *et al.* [20] found that the absence of IRF-1 expression strongly blocks the development of a Th1 response. *In vivo* studies of IRF-1-deficient mice showed a decrease in IFN-γ production and an enhanced disease susceptibility in response to *L. major* infection [20]. IRF-1-deficient mice were also more susceptible to *L. monocytogenes* infection, but showed highly efficient expulsion of *Nippostrongylus brasiliensis*, a phenomenon associated with an increase in the Th2 response [21].

As is the case with STAT4, however, no binding site for IRF-1 has been reported in the promoter of the IFN-γ gene, suggesting that IRF-1 does not directly upregulate the expression of the IFN-γ gene. In fact, enhanced IFN-γ production has been previously observed in CD8+ T cells from IRF-1-deficient mice. Interestingly, Lohoff *et al.* [20] have identified a profound defect in IL-12 production by macrophages in infected IRF-1-deficient mice upon re-stimulation with lipopolysaccharide (LPS), indicating that a lack of secretion of IL-12 by APCs could be the primary factor that promotes Th2 development. Similar results were obtained in the studies of Taki *et al.* [21]: IL-12p40 mRNA was highly induced in wild-type peritoneal macrophages upon LPS treatment, whereas no IL-12p40 message could be detected in activated macrophages from IRF-1-deficient mice. It was previously shown that IL-12p40 is a target for IRF-1 and that IRF-1 in combination with NFκB can co-operate to mediate transcription of the IL-12p40 gene [25]. Together, these results highlight the importance of studying the cytokines or other molecules expressed in APCs that are involved in the differentiation process as well as the cytokine genes expressed during the activation of Th1 or Th2 cells.

These two reports differ in their conclusions regarding the relevance of IRF-1 in CD4+ T cells. By transferring IRF-1-deficient CD4+ T cells into RAG-deficient mice, Lohoff *et al.* [20] showed that IRF-1-deficient CD4+ T cells can mount a normal Th1 response and clear *L. major* infections, suggesting that APCs are responsible for the Th2 phenotype in IRF-1-deficient mice. In contrast, Taki *et al.* [21] revealed that IRF-1-deficient CD4+ T cells were hyporesponsive to IL-12 *in vitro*, although no significant differences in the expression of IL-12 receptor β1 and β2 genes between wild-type and IRF-1-deficient mice were observed. It is possible that this discrepancy between the two studies is due to the *in vivo* versus *in vitro* systems used.

Genetic background is another important factor that affects the resistance and susceptibility to a particular disease. Mice with a resistant genetic background — such as C57BL/6 — develop a Th1 response during infection with *L. major* that can clear the parasite rapidly. In contrast, susceptible mice — BALB/c — develop a *L. major*-specific Th2 response that results in the visceralization of the disease and rapid death. The identification of the differences between the two genetic backgrounds has been the goal of numerous studies. Recently, a locus on murine chromosome 11 has been identified that controls the maintenance of IL-12 responsiveness and, therefore, the subsequent Th1/Th2 response [26]. Several genes related to the immune response have been found in that locus including, interestingly, the murine IRF-1 gene.

Not surprisingly, perhaps, the intense study of transcriptional regulation during Th1/Th2 differentiation has led to the elucidation of multiple transcription factors that are involved in regulating this process. This regulation occurs in response to numerous environmental factors that directly or indirectly influence the decision of a naive CD4+ T cell to become a Th1 or Th2 effector cell. Transcriptional events within the CD4+ T cells themselves, as well as in the APCs, are critical to this decision, and both cell types form attractive targets for therapeutic intervention in the balance between Th1 and Th2 immune responses.

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