

REVIEW

Lineage commitment in the immune system: the T helper lymphocyte grows up

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Cells of the immune system provide particularly fruitful subjects for the study of lineage commitment. Both T and B lymphocytes undergo complicated patterns of differentiation from uncommitted, nonfunctional precursor cells to highly sophisticated effector cells. The development of the helper T lymphocyte is one of the most elegant examples of this. A little over a decade ago, Mosmann and Coffman (1989) discovered that naive mouse CD4⁺ T helper lymphocytes, upon receiving an antigenic stimulus, differentiate into two distinct subsets defined both by their function and by unique cytokine profiles. These subsets, T helper 1 (Th1) and T helper 2 (Th2) (Mosmann et al. 1986; Mosmann and Coffman 1989; Paul and Seder 1994; O'Garra 1998; Rengarajan and Glimcher 2000), are responsible for cell-mediated/inflammatory immunity and humoral responses, respectively (Fig. 1). This division of labor fits nicely with previous demonstrations that an organism tends to mount either a cell-mediated or humoral response, but not both, in response to pathogens. The function of T helper cells can largely be explained by the cytokines they secrete. Cytokines (or lymphokines) are small hormone-like polypeptides that have pleiotropic biological activities in several cell types. Resting T cells do not transcribe cytokine genes, but they are rapidly induced upon coactivation through the T-cell receptor (TCR) and costimulatory receptors (Lenschow et al. 1996). Much progress has been made in identifying the signaling pathways and transcription factors that control Th1 and Th2 differentiation as shown schematically (Fig. 2a). This review will summarize what is currently known about the signals that regulate lineage commitment in T helper cells with a special focus on three subset-specific transcription factors, T-bet, GATA-3, and c-Maf, responsible for lineage commitment (Fig. 2b).

Th cell cytokines, phenotype, and function

The functional differences between the Th subsets can largely be explained by the activities of the subset-spe-

cific cytokines produced. The hallmark cytokine of Th1 cells is interferon- γ (IFN γ), and Th1 cells also produce IL-2, TNF, and LT, cytokines that mediate delayed type hypersensitivity responses and macrophage activation. The signature cytokine of Th2 cells is interleukin-4 (IL-4), and Th2 cells also secrete IL-5, IL-9, IL-10, and IL-13, cytokines that provide help to B cells and are critical in the allergic response (Arthur and Mason 1986; Paliard et al. 1988; Mosmann and Coffman 1989; Paul and Seder 1994). This paradigm extends to other species including human where clearcut human Th1 and Th2 clones have been generated (Romagnani 1992, 1994). However, simultaneous production of IL-2, IL-4, and IFN γ can be observed in human helper cells (Paliard et al. 1988).

A host of surface antigens that are unique to, or preferentially expressed on, Th1 or Th2 cells have recently been identified. Th1-preferentially expressed genes include the IFN γ receptor- β chain, IL-12 receptor β chain, IL-18 receptor, the P-selectin glycoprotein ligand-1, and the CXCR3 and CCR5 chemokine receptors (Davis et al. 1984; Bach et al. 1995; Borges et al. 1997; Szabo et al. 1997a; Bonecchi et al. 1998; Sallusto et al. 1998; Xu et al. 1998b). Markers preferentially expressed on Th2 cells include a novel IL-1-like molecule T1/ST2 and the chemokine receptors CCR3 (eotaxin receptor), CCR4, and CCR8 (Bonecchi et al. 1998; D'Ambrosio et al. 1998; Lohning et al. 1998; Sallusto et al. 1998; Xu et al. 1998a; Zingoni et al. 1998; Coyle et al. 1999; Hoshino et al. 1999; Townsend et al. 2000). T1/ST2 is a stable marker for Th2 cells in vivo that may be functionally important in the generation of Th2 responses. Antibodies against T1/ST2 attenuated eosinophilic inflammation in airways and suppressed Th2 cytokines in vivo (Lohning et al. 1998), and mice lacking T1/ST2 had impaired Th2 responses to *Schistosoma mansoni*. The preferential expression of these markers on effector Th1 and Th2 cells may be the consequence of ligand-induced events or of the cytokine milieu because surface antigens that will distinguish Th cells in the early stages of Th1 or Th2 differentiation have not yet been found. The best way to phenotypically distinguish the two subsets is by their unique cytokine profiles as determined by cellular stain-

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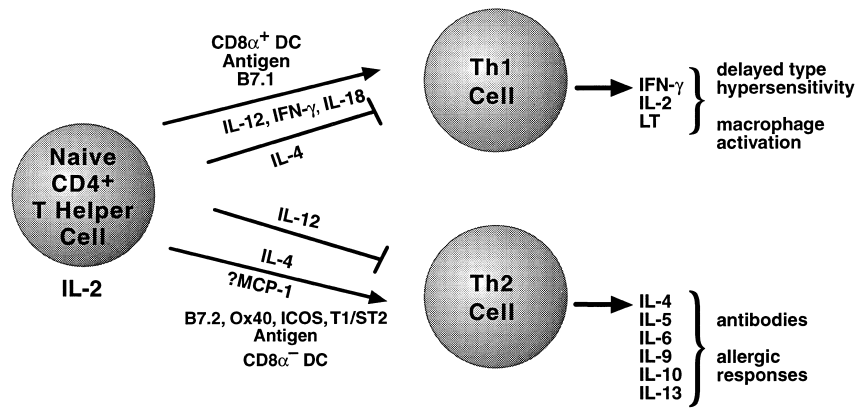


Figure 1. Signals that influence TH differentiation.

ing for IFN γ and IL-4 or by a powerful new technique that allows detection of surface IFN γ and IL-4 (Ouyang et al. 2000). We and others have also generated transgenic “knock-in” mice at the IL-4 locus (Riviere et al. 1998; Ho et al. 1999) that may prove useful in marking early Th2 cells.

Factors that influence lineage commitment

After differentiation and emigration from the thymus to the peripheral immune organs, CD4⁺ T helper cells are

termed naive T helper precursor (Thp) cells. Thp cells are functionally immature and capable of secreting only IL-2. Much has been learned about the signals that drive these naive Thp cells, which secrete only IL-2, to become Th1 or Th2 effector cells. Th1 and Th2 cells appear to derive from a common precursor that expresses the IL-4 gene (Kamogawa et al. 1993). Thp activation and differentiation in the periphery requires at least two separate signals. The first signal is delivered by the TCR/CD3, after its interaction with antigen/MHC on antigen presenting cells (APCs). The second signal is produced by a number of costimulatory or accessory molecules typified by the CD28/B7, OX40, and LFA-1/ICAM receptor–ligand pairs. Whether an immune response will be dominated by Th1 versus Th2 CD4⁺ cells is clearly influenced by the nature of these two signals (Seder and Paul 1994). However, neither of these two signals is as potent a determinant of Th cell fate as the cytokine milieu itself.

APC, antigen, costimulation Signal one is delivered by interaction of the TCR on a naive Thp cell with MHC/antigen on an APC such as a dendritic cell. The recent identification of phenotypically distinct subsets of dendritic cells has revealed unique functions for these subsets in the development of Th cells (Maldonado-Lopez et al. 1999; Pulendran et al. 1999; Rissoan et al. 1999; Smith and Fazekas de St. Groth 1999). In the mouse, CD8 α^+ lymphoid-like dendritic cells produce IL-12 and preferentially stimulate Th1 differentiation. Recently, a T-cell secreted factor called Eta1/osteopontin has been shown to drive Th1 differentiation by inducing IL-12 and inhibiting IL-10 secretion from APC (Ashkar et al. 2000). Eta1-deficient mice fail to mount DTH responses to pathogens such as herpes simplex keratitis and *Listeria monocytogenes*. A second subset of myeloid-like CD8 α^- DC have been shown to stimulate Th2 differentiation. The mechanism by which this occurs remains unclear because the APC counterpart cytokine of IL-12 for Th2 differentiation is not known, although IL-6 has been suggested as a candidate (Rincon et al. 1997). An intriguing possibility is that specific chemokines may polarize Thp cells, as evidenced by a recent report that MCP-1 deficient mice fail to mount Th2 responses (Gu et al. 2000).

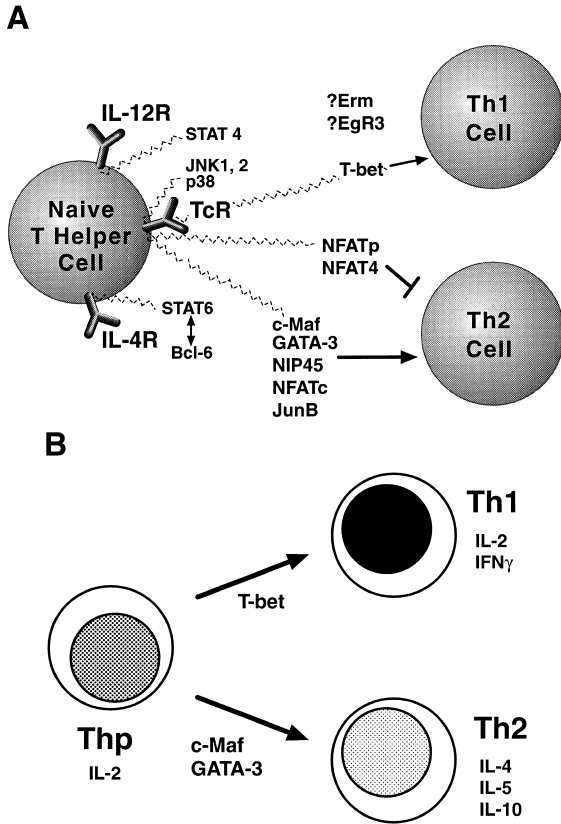


Figure 2. (A) Transcription factors that influence Th differentiation. (B) Tissue-specific factors that regulate CD4⁺ T helper cell differentiation.

The influence of antigen structure and antigen dose in Th polarization has been reviewed recently (Constant and Bottomly 1997; Tao et al. 1997; O'Garra 1998). In particular, the use of altered peptide ligands has provided convincing evidence that the strength of the signal transmitted via the TCR influences lineage commitment, perhaps by controlling the duration or magnitude of Ca^{2+} fluxes (Sloan-Lancaster et al. 1997; Grakoui et al. 1999). The role of costimulatory molecules in Th cell skewing is clear as CD28 and B7 knockout mice have significantly reduced immune responses with especially pronounced defects in generating the Th2 compartment (Freeman et al. 1993; Green et al. 1994; Schweitzer et al. 1997). What is controversial is whether the two costimulatory molecules, B7-1 and B7-2, can differentially drive Th cell development (Kuchroo et al. 1995; Brown et al. 1996; Ranger et al. 1996; Nakajima et al. 1997; Subramanian et al. 1997). A third costimulatory receptor, ICOS, functionally related to CD28 and its ligand B7-H1 (Dong et al. 1999; Hutloff et al. 1999; Yoshinaga et al. 1999; Ling et al. 2000), is thought to preferentially stimulate Th2 differentiation. Th2 cells are also preferentially expanded by CD28-dependent OX40 signaling (Akiba et al. 2000; Lane 2000; Murata et al. 2000). Recently, an opposing role for the LFA-1/ICAM-1,2 interaction in regulating Th2 development has been demonstrated because blocking this interaction results in overproduction of Th2 cytokines (Salomon and Bluestone 1998).

Cytokines The cytokines themselves play the most critical role in T helper cell polarization. The two critical cytokines that control Th1 and Th2 differentiation are IL-12 and IL-4, respectively. These two cytokines enhance the generation of their own Th subset and simultaneously inhibit the generation of the opposing subset (Scott 1991; Maggi et al. 1992; Parronchi et al. 1992; Hsieh et al. 1993; Macatonia et al. 1993; Manetti et al. 1993; Powrie and Coffman 1993; Seder et al. 1993; Trinchieri 1993; Wu et al. 1993), events that likely occur at a precursor stage (Thp) because once established, Th1 and Th2 cells cannot revert (Scott 1991; Murphy et al. 1996). The requirement for these two cytokines has been demonstrated unequivocally by the phenotype of mice that lack these cytokines, cytokine receptors, or the effector molecules downstream of the receptors. IL-12, secreted by APC, activates the Stat4 signaling pathway, and mice lacking IL-12 or Stat4 do not have Th1 cells (Kaplan et al. 1996a; Magram et al. 1996; Thierfelder et al. 1996). Two other cytokines that influence Th1 development are IL-18, whose receptor is related to the IL-1 receptor family (Cerretti et al. 1992; Okamura et al. 1995; Gu et al. 1997), and $IFN\gamma$ (Scott 1991; Meraz et al. 1996). $IFN\gamma$ activates the Stat1 pathway (Meraz et al. 1996). Mice lacking IL-18 or Stat1 have defective *in vivo* Th1 responses (Meraz et al. 1996; Takeda et al. 1998). In contrast, mice that lack IL-4, IL-4 receptor, or Stat6, the downstream signaling molecule for the IL-4 receptor, fail to develop Th2 cells in response to most stimuli (Kuhn et al. 1991; Kopf et al. 1993; Kaplan et al. 1996b; Shimoda et al. 1996; Takeda et al. 1996; Noben-Trauth et al.

1997). The biologic function of another cytokine, IL-13, partially overlaps with IL-4 because, in some instances, IL-13 drives Th2 development and IgE synthesis in an IL-4-independent fashion (Minty et al. 1993; Punnonen et al. 1993; Barner et al. 1998; Cohn et al. 1998; Emson et al. 1998; McKenzie et al. 1998). IL-13 is especially important in the asthmatic response (Wills-Karp et al. 1998).

Development of the Th1 lineage

Signaling pathways Signaling pathways emanating from both the TCR and the IL-12 receptor drive Th1 differentiation at least in part by inducing the expression of the Th1-restricted transcription factor T-bet described below (Fig. 3). The p38 MAP kinase pathway regulates $IFN\gamma$ production in $CD4^+$ T cells (Dong et al. 1998; Rincon et al. 1998; Yang et al. 1998) because p38 MAPK inhibitors and a dominant-negative p38 transgene reduced Th1 responses (Rincon et al. 1998). Targeted disruption of MKK3 (Lu et al. 1999) also diminished Th1 responses, but primarily by decreasing IL-12 production. The Jun NH_2 -terminal kinase (JNK) pathway has also been implicated in Th1 development or function. A substantial reduction in $IFN\gamma$ production was observed in JNK2-deficient T cells (Yang et al. 1998), although the decreased $IFN\gamma$ production may be secondary to impaired expression of IL-12R β_2 in JNK2-deficient T cells rather than a direct requirement for JNK2 in $IFN\gamma$ gene transcription.

IRF-1 family members also participate in Th1 development and $IFN\gamma$ production primarily, but perhaps not entirely, through controlling IL-12 production (Lohoff et al. 1997; Taki et al. 1997). Although IRF-1-deficient mice have reduced IL-12 production, there are conflicting studies on the ability of IL-12 to restore $IFN\gamma$ production in IRF-1-deficient $CD4^+$ T cells (Lohoff et al. 1997; Taki et al. 1997). Furthermore, IL-12 signaling in T cells induces IRF-1 expression, apparently through activation of Stat4 (Coccia et al. 1999). Thus, IRF-1 expression may be downstream as well as upstream of IL-12.

Role of Stat4 in Th1 development Th1 development involves signaling through Stat4, cloned by homology to other STATs (Zhong et al. 1994) but later found to be

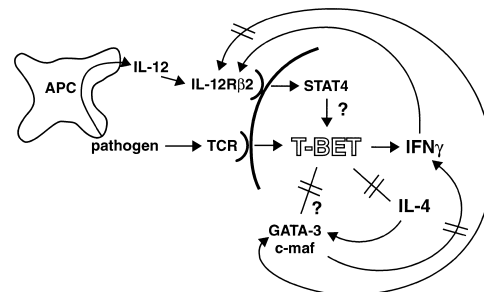


Figure 3. Transcription factors that control Th1 differentiation.

dedicated to IL-12 signaling in the mouse (Jacobson et al. 1995). Stat4 activation was first correlated with IFN γ expression by recognizing the loss of IL-12-induced Stat4 activation in Th2 cells (Szabo et al. 1995), and its role in Th1 development later proven using Stat4-deficient mice (Kaplan et al. 1996a; Thierfelder et al. 1996). Clearly not all IFN γ production by T cells is Stat4-dependent (Kaplan et al. 1998; Carter and Murphy 1999). Stat6/Stat4 double-deficient T cells produce some IFN γ (Kaplan et al. 1998), and CD8 $^+$ T cells are largely Stat4-independent for TCR-induced IFN γ production, in contrast to CD4 $^+$ T cells (Carter and Murphy 1999). Although Stat4 is critical in the generation of Th1 cells, its role in directly controlling IFN γ gene transcription is unclear. Stat4 may act on the IFN γ gene via cooperative binding to nonconsensus low-affinity STAT sites in the IFN γ gene promoter and first intron (Xu et al. 1996), but TCR-activated Th1 cells produce IFN γ without Stat4 activation (Ouyang et al. 1999; Yang et al. 1999). In addition, IFN α -induced Stat4 activation in human T cells is not sufficient to activate IFN γ transcription (Rogge et al. 1998). Although IL-12 activates Stat1 and Stat3 as well as Stat4, only Stat4 is required for Th1 differentiation. Explanations for this selectivity might include induction of unique Stat4-activated targets or involve selective interactions with Stat4-interacting coactivators. Several STAT factors have been shown to interact with the general transcriptional coactivators p300/CBP (Bhattacharya et al. 1996; Zhang et al. 1996; Kozus et al. 1998).

Type I interferons in Th1 responses Although the control of human and mouse Th differentiation is quite similar, one important difference has emerged. In human T cells, the cytokine IFN α , not IL-12, controls Th1 development and IFN γ production (Parronchi et al. 1992; Brinkmann et al. 1993; Rogge et al. 1997). This is in contrast to the inability of IFN α to induce Th1 development in the mouse (Wenner et al. 1996; Rogge et al. 1998). Both IL-12 and IFN α activate Stat4 and induce Th1 development in human T cells, whereas only IL-12 exerts these effects in murine T cells (Rogge et al. 1998). Although IL-12 receptor expression appears to be modulated in the mouse, being lost in Th2 cells (Xu et al. 1998b), the constitutive expression of IFN α receptors on human T cells provides an attractive explanation for the observation that human T cells show less exclusive polarization of IFN γ and IL-4 than mouse T cells.

IL-1 family cytokines and signaling in Th1 development IL-12 and IL-4 are considered the dominant factors in Th1 and Th2 development, but other factors, especially the cytokine IL-18, also contribute. Differential actions of IL-1 on T-cell subsets were recognized early on (Lichtman et al. 1988; Taylor-Robinson and Phillips 1994; Shibuya et al. 1998), and IL-1R-deficient mice display enhanced Th2 responses in vivo (Satoskar et al. 1998). More recently, IL-18, an IL-1-related factor (Ghayur et al. 1997; Gu et al. 1997; Fantuzzi et al. 1998), was shown to be a selective activator of IFN γ in Th1, but

not Th2, cells. IL-1 and IL-18 signaling pathways share similar components. Both IL-1 and IL-18 activate IRAK (Robinson et al. 1997), NF- κ B in Th1 cells (Matsumoto et al. 1997; Robinson et al. 1997), and TRAF6 (Kojima et al. 1998). MyD88-deficient mice not only lose IL-1-induced function but also IL-18-mediated functions (Adachi et al. 1998). IRAK-deficient mice have defective IL-18-mediated natural killer (NK) and Th1-type responses to pathogens in vivo (Kanakaraj et al. 1999). In addition, potential IL-18 activation of the MAP kinase pathway has been reported (Tsuji-Takayama et al. 1997).

Powerful synergy between IL-12 and IL-18 for IFN γ production was reported (Micallef et al. 1996; Kohno et al. 1997). IL-18-deficient mice (Takeda et al. 1998) show reduced LPS-induced IFN γ production after priming with *Propionibacterium acnes*, and mice deficient in both IL-18 and IL-12 have a more severe defect in IFN γ production than either strain alone. Although IL-18 is clearly important for IFN γ production in vivo, its mechanism of action in T-cell development remained unclear. O'Garra and colleagues clarified this issue by showing that IL-18 does not induce development of Th1 cells but acts after IL-12-induced Th1 development to increase IFN γ produced by differentiated Th1 cells (Robinson et al. 1997). IL-12 and IL-18 together induced full IFN γ production by Th1 cells, independently of stimulus through the TCR. The synergistic actions of IL-12 and IL-18 on the IFN γ promoter were proposed to be mediated by a Stat4 binding site and an adjacent AP-1 binding site (Barbulescu et al. 1998).

Transcriptional regulation of Th1-specific cytokines

IL-2 IL-2 is expressed exclusively in T lymphocytes and is the cytokine produced earliest during Th differentiation. It is the major cytokine produced by the naive Thp cell. Differentiated effector Th1, but not Th2, cells also produce IL-2 albeit at lower levels than primary, stimulated Thp cells. IL-2 expression is primarily controlled at the transcriptional level, although costimulation via CD28 regulates IL-2 expression post-transcriptionally, by increasing IL-2 mRNA stability (Umlauf et al. 1995). TCR engagement activates MAP kinase and Ca $^{2+}$ -dependent signaling pathways, both essential for IL-2 expression, and leads to the activation of several transcription factors including members of the nuclear factor of activated T cells (NFAT) and activator protein 1 (AP-1) families (Durand et al. 1988; Shaw et al. 1988; Marx 1993; Northrop et al. 1994; Jain et al. 1995). In resting T cells, NFAT proteins reside in the cytoplasm in a phosphorylated state. Upon stimulation they are rapidly dephosphorylated by the phosphatase calcineurin and translocate into the nucleus where, in combination with AP-1 proteins, they activate the transcription of multiple cytokine genes, including IL-2 (Jain et al. 1995). The repression of cytokine gene transcription by the immunosuppressants cyclosporin A (CsA) and FK506 is explained by their blockade of calcineurin leading to inhibition of Ca $^{2+}$ -mediated, and hence NFAT-dependent, pathways

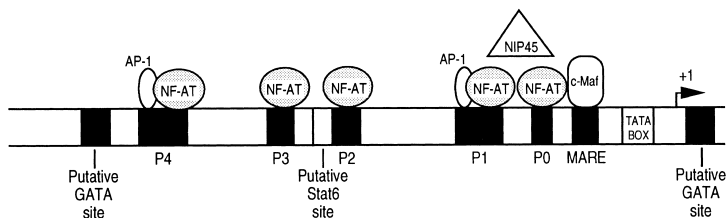
(Emmel et al. 1989), as well as their blockade of JNK activation (Shibasaki et al. 1996).

The regulatory regions of the IL-2 gene have been well delineated *in vitro* and have begun to be characterized *in vivo* as well. A 300-bp minimal promoter/enhancer region has been mapped that is sufficient for tissue-specific expression of the IL-2 gene in Th1 cells *in vitro* (Lederer et al. 1994). This region contains binding sites for multiple factors including inducible proteins like NFAT, AP-1 (Jun and Fos), and NF- κ B (p50, p65, c-Rel) family members and constitutive factors like Oct proteins (Durand et al. 1988; Jain et al. 1995; Rooney et al. 1995b; Maggirwar et al. 1997; Shapiro et al. 1997; Ullman et al. 1991) (Fig. 4). Although the CD28 response element has been mapped and shown to bind NFAT proteins, NF- κ B proteins such as c-Rel, ATF-1, CREB2, and HMG I(Y), it remains unclear which if any of these factors binds *in vivo* (Himes et al. 1996; Shapiro et al. 1998). Although the identity of the proteins bound to sites in this 300-bp promoter *in vivo* is not yet clear, the functional relevance of each of these sites *in vitro* has been established by point mutations that substantially impair transcriptional activity (Rooney et al. 1995b). *In vivo* footprinting of the IL-2 locus in T cells revealed a combinatorial interaction of multiple proteins on the IL-2 promoter/enhancer rather than autonomous binding of single factors (Rothenberg and Ward 1996). Although the 300-bp promoter/enhancer region is sufficient for high-level Th1-

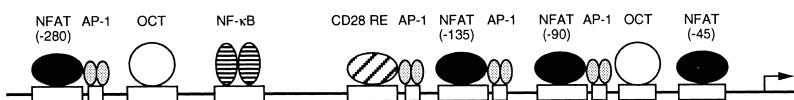
specific expression *in vitro*, this has not been borne out by studies in transgenic mice where at least 600 bp of the IL-2 promoter has been required (Brombacher et al. 1994). This is consistent with earlier studies that identified DNase I hypersensitivity (HS) sites outside of this region (Siebenlist et al. 1986). Footprinting and DNase I HS studies revealed TCR-inducible T-cell-specific HS sites between -600 and -300 bp upstream of the transcriptional start site (Rothenberg and Ward 1996; Ward et al. 1998) and activation-inducible sites between -400 and -300 bp upon activation (Ward et al. 1998). Identification of the proteins binding to these regions *in vivo* may yield factors that influence chromatin accessibility and remodeling.

Nevertheless, it is important to note that, in contrast to IFN γ and IL-4 as described below, the transcription factors that direct the tissue-specific expression of IL-2 have not yet been defined. None of the transcription factors described above is Th1 specific, and none, by itself, can account for the Th1-specific expression of IL-2. Thus, it is not possible to reconstitute the expression of IL-2 in a nonproducer cell as can be done for IFN γ with T-bet and for IL-4 with c-Maf (Ho et al. 1996; Hodge et al. 1996a; Szabo et al. 2000). It is possible that Th1-specific expression of IL-2 may be accomplished by the precise temporal and spatial combination of proteins at the transcription complex or, more likely, by a single factor that has not yet been identified.

The IL-4 Promoter



The IL-2 Promoter



IFN- γ Regulatory Regions

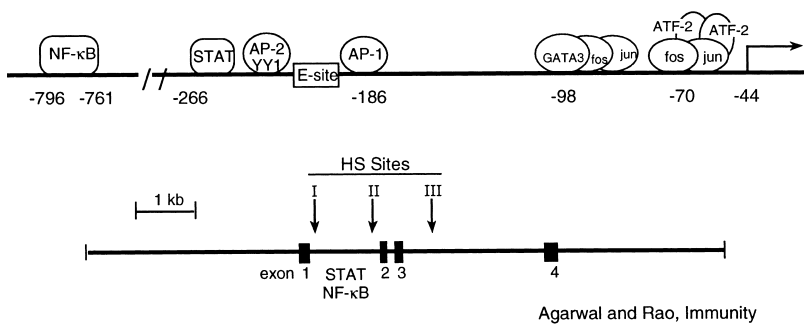


Figure 4. Regulating regions of cytokine genes.

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IFN- γ Th1 cells, CD8⁺ cells, and NK cells are the major secretors of *IFN- γ* that is induced within 24 hr after stimulation of naive cells (Lederer et al. 1996). Very little is known about the regulatory regions of the *IFN- γ* gene. For example, the location of elements that direct its tissue-specific expression has not been established in vitro or in vivo although early studies designated 8.6 kb as conferring T cell-specific expression (Agarwal and Rao 1998a). The Th1-specific regions within this sequence have not yet been identified. Reporter constructs containing 500 bp or 3 kb of upstream sequence are active but are expressed in both Th1 and Th2 cells (Young et al. 1994).

The 500-bp region is induced by phorbol ester and ionomycin and inhibited by CsA in vitro. A fairly detailed analysis of sites within the proximal promoter has been performed using both mouse and human T cells as expression systems. Two essential regulatory elements that mirror the expression of the endogenous gene were identified between -108 and -40 bp, the distal element (-96 to -80 bp) being a consensus GATA motif that binds GATA-3 in vitro (Penix et al. 1993) and the proximal regulatory element (-73 to -48 bp) capable of binding CREB, ATF-1, ATF-2, c-Jun, and Oct-1 proteins (Penix et al. 1996). The proximal element, when dimerized, recapitulates the inducibility and CsA sensitivity of *IFN- γ* in Jurkat T cells and in transgenic mice although additional NFAT sites exist outside of the proximal regulatory element, and NFAT proteins transactivate and interact with these sites in vitro (Campbell et al. 1996; Aune et al. 1997; Sweetser et al. 1998). NF- κ B proteins (c-Rel, p50, and p65) and possibly NFAT proteins bind to and transactivate regions in the first intron of the *IFN- γ* gene (Sica et al. 1997), and mice that express a dominant-negative I κ B α transgene that inhibits NF- κ B have substantially impaired *IFN- γ* production (Aronica et al. 1999). Ying-Yang 1 (YY1) interacts with a negative regulatory element and was initially thought to inhibit *IFN- γ* expression (Ye et al. 1996) but was subsequently shown to also transactivate *IFN- γ* through binding to an element between the two NFAT sites (Sweetser et al. 1998).

Although a functional role for ATF-2, NF- κ B, AP-1, GATA-3, NFAT, and Stat4 sites in the *IFN- γ* promoter or introns has been demonstrated, these factors cannot account for tissue-specific expression (Penix et al. 1993, 1996; Young et al. 1994; Xu et al. 1996; Ye et al. 1996; Barbulescu et al. 1997; Sica et al. 1997; Zhang et al. 1998b). Several reports have suggested that tissue-specific regulatory elements are located in the first and third introns as demonstrated by Th1-preferential DNase I hypersensitive sites as discussed below, but the actual *cis* elements responsible have not yet been identified (Young et al. 1994; Agarwal and Rao 1998b).

T-bet, a transcription factor that controls IFN- γ transcription and Th1 lineage commitment The approach that several laboratories took to unravel the genetic programs that controlled Th lineage commitment was to isolate the factors that accounted for the Th-subset selective expression of *IFN- γ* and IL-4, reasoning that such

factors should be important in specifying lineage fate. This search resulted in the isolation of one factor, T-bet, that is Th1 specific and controls *IFN- γ* gene transcription, and two Th2-specific factors, GATA-3 and c-Maf that control IL-4 gene transcription.

Very little was known about the control of *IFN- γ* . The two transcription factors whose absence results in a failure to generate the Th1 compartment and hence *IFN- γ* are Stat4 (Kaplan et al. 1996a; Thierfelder et al. 1996) and IRF-1 (Lohoff et al. 1997; Taki et al. 1997), neither of which is Th1 specific. Although Stat4, the downstream signaling molecule for IL-12, is clearly important in driving Th1 differentiation from naive Thp cells, it does not appear to be required in primed Th1 cells (Carter and Murphy 1999). The absence of Th1 cells in IRF-1-deficient mice can be attributed to IL-12 deficiency because IRF-1 directly regulates the IL-12 gene (Lohoff et al. 1997; Taki et al. 1997). A new member of the Ets family called ERM is both Th1 specific and is induced by IL-12 in a Stat4-dependent manner, but its function is as yet unknown (Ouyang et al. 1999).

T-bet was isolated using an IL-2 promoter-reporter and a cDNA library prepared from activated Th1 cells in a yeast one-hybrid screen. Several independent clones obtained all proved to encode the same gene, a novel member of the T-box family of transcription factors. The gene was named *T-bet* for T-box expressed in T cells. The T-box family, whose founding member is *Brachury* or *T* for short-tailed mutation in mice (Herrmann et al. 1990), is defined by the presence of a highly conserved T-box DNA-binding domain (for review, see Meisler 1997; Papaioannou 1997; Smith 1997). There have now been isolated multiple members of this family in species ranging from nematodes to humans, and the T-box domain of T-bet is most similar to the mouse gene *T-brain* (Bulfone et al. 1995) and the *Xenopus* gene *eomesodermin* (Rao 1994). Several of the T-box proteins play important roles in mesoderm development. Mutations in one allele of the human *Tbx5* and *Tbx3* genes are responsible for the human congenital diseases Holt-Oram syndrome and ulnar-mammary syndrome, respectively (Bamshad et al. 1997; Basson et al. 1997; Li et al. 1997; Spranger et al. 1997), whereas the chicken *Tbx4* and *Tbx5* genes govern limb bud identity (Rodriguez-Esteban et al. 1999; Takeuchi et al. 1999). There are several consensus T-box binding sites in the IL-2 promoter and in both the promoter and third intron of the *IFN- γ* gene, where the Th1-specific DNase I hypersensitive site is located.

Expression and function analyses of T-bet revealed the following: T-bet is expressed only in Th1 cells and in NK cells, the known producers of *IFN- γ* . In these cells, T-bet transcripts and protein are rapidly induced upon signaling through both the TCR and the IL-12 receptor (S. Szabo and L.H. Glimcher, unpubl.). The expression of T-bet completely correlates with the expression of *IFN- γ* in all cells examined including B cells that can be induced to produce T-bet and *IFN- γ* upon stimulation via the CD40 receptor in the presence of IL-12 and IL-18 (Yoshimoto et al. 1997). Transient transfection assays in a Th0 non-*IFN- γ* -producer lymphoma revealed that T-bet

was a potent transactivator of the IFN γ gene and a repressor of the IL-2 gene. Provision of T-bet to these cells induced endogenous IFN γ production. To assess the effect of T-bet on primary T cells, CD4 T cells were transduced with a bicistronic T-bet/GFP retroviral construct. The function of T-bet was determined in unskewed CD4 T cells, developing Th2 cells, and in fully polarized CD4 and CD8 T cells. Remarkably, in all cases, provision of T-bet resulted in the generation of large numbers of IFN γ -producing Th1 cells that expressed Th1 lineage markers. Most exciting, the effect of T-bet expression was twofold. It not only induced the production of IFN γ but also simultaneously shut off the production of the Th2 cytokines IL-4 and IL-5. The expression of IL-2 was also reduced, consistent with a role for T-bet in driving differentiation. These effects were observed in early developing Th2 cells and also in fully committed Th2 and Tc2 cells. T-bet is therefore a master regulator of Th1 lineage commitment and accomplishes this function both by activating Th1-inducing genetic programs and by terminating the opposing Th2-inducing genetic programs. Much more remains to be learned about the function and mechanism of the action of T-bet. The analysis of T-bet-deficient and overexpressor transgenic mice should prove informative.

Development of the Th2 lineage

Signaling pathways Two major signaling pathways drive Th2 differentiation: TCR and IL-4 receptor (Fig. 5). As discussed below, signals emanating from the TCR activate the transcription of two Th2-specific factors, c-Maf and GATA-3, and also regulate the expression and the activation of the NFAT and AP-1 transcription factors. Upon signaling through the IL-4 receptor, Stat6 is recruited to bind to the cytoplasmic domain of the IL-4 receptor where it is then phosphorylated by Jak1 and Jak3 kinases leading to Stat6 dimerization and translo-

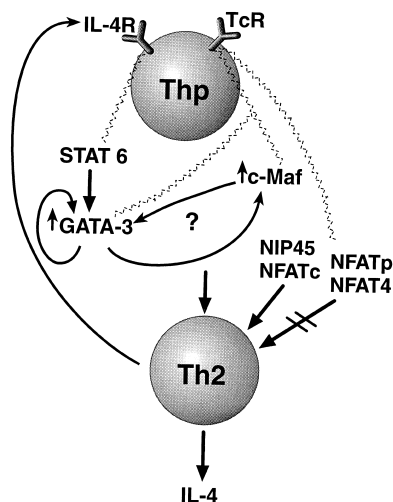


Figure 5. Transcription factors that control TH2 differentiation.

cation to the nucleus (Hoey and Grusby 1999). Although it is unlikely that Stat6 itself directly controls IL-4 gene transcription to any significant degree (Lederer et al. 1996), it does activate a number of IL-4 target genes such as germ-line IgE gene transcription and switch recombination (Linehan et al. 1998), class II MHC, IL-4R, CD23 (Kaplan et al. 1996b; Shimoda et al. 1996; Takeda et al. 1996), and GATA-3 (Ouyang et al. 1998). Ectopic expression of an inducible, activated Stat6 into developing Th1 cells induces c-Maf and GATA-3 with subsequent expression of IL-4 (Kurata et al. 1999). There is controversy about whether the JNK kinase pathway, another signaling pathway, regulates Th differentiation. Mice that lack JNK1 or JNK2 have been reported by one group to have increased Th2 and defective Th1 differentiation. Enhanced Th2 and normal Th1 responses were found in JNK1-deficient T cells (Dong et al. 1998), when T cells were activated under neutral in vitro conditions (Dong et al. 1998; Yang et al. 1998). However, the Th2 skewing observed in JNK2^{-/-} mice was not observed in an independently generated strain of JNK2-deficient mice (Sabapathy et al. 1999).

Stat6-independent pathways of Th2 differentiation do exist (Kaplan et al. 1999; Ouyang et al. 2000). BCL-6, a transcriptional repressor, inhibits Stat6 activity by competing for the Stat6 binding sites (Dent et al. 1997). Mice lacking BCL-6 display a profound Th2 phenotype (Dent et al. 1997). BCL-6 may control Th cell generation in both IL-4- and Stat6-independent manners as evidenced by the continuing Th2 phenotype of mice that lack both IL-4 and BCL-6 or both Stat6 and BCL-6 (Dent et al. 1997, 1998). c-Maf and NFAT proteins are also attractive candidates for factors responsible for Stat6-independent IL-4 production because they themselves are not Stat6 responsive. Nevertheless, although Stat6-independent pathways for IL-4 production exist, there can be little doubt of the critical role of this factor in generating the Th2 compartment in vivo as evidenced by the phenotype of mice lacking Stat6 (Kaplan et al. 1996b; Shimoda et al. 1996; Takeda et al. 1996).

Transcriptional regulation of Th2-specific cytokines

IL-4 The expression of IL-4 is restricted to a subset of $\alpha\beta$ CD4 (Th2) and CD8 (Tc2) T cells, NK T cells, $\gamma\delta$ T cells, mast cells, basophils, and eosinophils (Abbas et al. 1996). Naive Thp cells express IL-4 mRNA by 24 hr after stimulation via the TCR, and levels peak at 48 hr (Lederer et al. 1996). Studies in transgenic mice established that 3 kb of the IL-4 promoter conferred expression that mirrored endogenous IL-4 expression (Todd et al. 1993). Later studies that generated a series of IL-4 promoter transgenics demonstrated that the -741- to +60-bp region was preferentially expressed in Th2 cells. A trimer of the region spanning an NFAT-AP-1 site (-88 to -61) was moderately Th2 specific. As for the IL-2 promoter, however, expression was low relative to endogenous IL-4 suggesting the presence of enhancer elements outside of the promoter (Wenner et al. 1997).

The promoter region of the IL-4 gene has been studied *in vitro* by several groups (Abe et al. 1992; Bruhn et al. 1993; Chuvpilo et al. 1993; Szabo et al. 1993; Todd et al. 1993; Kubo et al. 1994b; Rooney et al. 1994, 1995a; Tara et al. 1995; Weber et al. 1998). Similar to IL-2, IL-4 promoter activity is anti-CD3, phorbol ester and Ca^{2+} ionophore inducible, and CsA inhibitable (Todd et al. 1993; Kubo et al. 1994a,b). Deletions of the murine IL-4 5' sequence established that 87 bp of the proximal promoter conferred cell-specific and activation-dependent transcription *in vitro* (Bruhn et al. 1993; Szabo et al. 1993; Lederer et al. 1994). Extensive mutational analyses identified five NFAT sites, two of which are composite NFAT/AP-1 sites, within the proximal promoter that are critical for inducible IL-4 expression (Chuvpilo et al. 1993; Brown and Hural 1997; Szabo et al. 1997b). Mutations in each of these sites severely impaired promoter activity *in vitro* (Chuvpilo et al. 1993; Szabo et al. 1993; Todd et al. 1993; Hodge et al. 1995; Rooney et al. 1995b). Each of these sites bind NFATc1 and NFATc2 (Timmerman et al. 1997), and two of them also bind AP-1 proteins such as Fra-1, Fra-2, JunB, and JunD in an NFAT-dependent manner (Hodge et al. 1995; Rooney et al. 1995a). Although NFAT and AP-1 family proteins cannot explain Th2-specific IL-4 gene expression (Rooney et al. 1994), the phenotype of mice that lack one or more of the NFAT proteins has revealed an extremely important role, as detailed below, for this family in controlling cytokine gene expression, especially in Th2 cells. There may also be an especially important role for the AP-1 family member JunB in driving Th2 differentiation (Li et al. 1999).

NFAT NFAT was first identified as a TCR-inducible factor that regulated the IL-2 gene (Durand et al. 1988; Shaw et al. 1988; Crabtree 1989; Rao et al. 1997). NFAT proteins also regulate the promoters of multiple other cytokine genes expressed in T cells, including IL-4, GM-CSF, IL-3 and TNF α (Miyatake et al. 1991; Chuvpilo et al. 1993; Goldfeld et al. 1993; Masuda et al. 1993; Rooney et al. 1994, 1995a; Cockerill et al. 1995). Isolation of the genes encoding these proteins has yielded four NFAT family members called NFATc1 (also called NFATc, NFAT2), NFATc2 (also called NFATp, NFAT1), NFATc3 (NFAT4 and NFATx), and the nonlymphoid NFATc4 (NFAT3) that are highly homologous within a region distantly related to the Rel domain (McCaffrey et al. 1993; Northrop et al. 1994; Hoey et al. 1995; Masuda et al. 1995). NFAT family members differ markedly in amino- and carboxy-terminal regions (Luo et al. 1996), and this, together with their differing tissue distribution and response to stimuli (Hoey et al. 1995; Ranger et al. 1998b), suggested that their functions might differ, as indeed proved to be the case.

Although the three lymphoid NFAT proteins are expressed in both Th1 and Th2 cells (Ranger et al. 1998b), the phenotype of single NFATc1- and NFATc2-deficient mice suggests that NFAT proteins might reciprocally regulate Th cytokines and consequently the Th1/Th2 balance. This turned out to be the case for NFAT-regu-

lated Th2 cytokine production. T cells from mice lacking NFATc1 in the lymphoid system have reduced IL-4 production (Ranger et al. 1998a; Yoshida et al. 1998), consistent with a function of NFATc1 as a direct transcriptional activator of the IL-4 gene. Conversely, NFATc2 and NFATc3 negatively control proliferative responses, Th2 cell formation, and lymphocyte activation as revealed by the phenotype of mice lacking these genes (Hodge et al. 1996b; Xanthoudakis et al. 1996; Kiani et al. 1997; Oukka et al. 1998). Mice lacking NFATc2 display a moderate increase in Th2-type cytokines (Hodge et al. 1996b; Xanthoudakis et al. 1996). Mice lacking NFATc2 display a moderate increase in Th2-type cytokines (Hodge et al. 1996b; Xanthoudakis et al. 1996). Although mice lacking NFATc3 have normal cytokine production (Oukka et al. 1998), the very dramatic phenotype of mice lacking both NFATc2 and NFATc3 (DKO mice) proved that these two members had redundant function (Ranger et al. 1998b). These DKO mice had massive overproduction of Th2 cytokines with concomitant extreme elevations in levels of IgG1 and IgE, immunoglobulins whose production is IL-4 dependent. The clinical consequences of this Th1/Th2 imbalance were severe allergic blepharitis and interstitial pneumonitis characterized by influx of eosinophils and mast cells.

The constitutive localization of NFATc1 to the nucleus in the DKO T cells is consistent with unopposed transactivation of IL-4 by NFATc1 (Ranger et al. 1998a; Yoshida et al. 1998), which likely acts in concert with the Th2-specific transcription factors c-Maf and GATA-3 (Ho et al. 1996; Zheng and Flavell 1997) to drive IL-4 gene transcription. Although it is possible that the Th2 phenotype of the NFAT DKO is explained by a repressor function of NFATc2, it more likely reflects the dysregulation of additional, as yet unknown, NFAT target genes, critical in regulating the response of Thp and Th2 cells to TCR and cytokine receptor signaling.

GATA-3 and c-Maf: Th2-specific transcription factors

Substantial progress has been made in identifying the transcription factors responsible for the tissue-specific expression of the IL-4 gene in Th2 cells (Szabo et al. 1997b; Glimcher and Singh 1999; Glimcher et al. 1999) (Figs. 4 and 5). Two Th2-specific factors, the c-Maf proto-oncogene and the GATA-3 zinc finger factor, have been identified (Ho et al. 1991, 1996; Ting et al. 1996; Zhang et al. 1997; Zheng and Flavell 1997). Non-Th2-specific factors such as Stat6, NFAT proteins (Durand et al. 1988; Shaw et al. 1988; Crabtree 1989; McCaffrey et al. 1993; Northrop et al. 1994; Hoey et al. 1995; Masuda et al. 1995; Rao et al. 1997), and a novel nuclear antigen, NFAT-interacting protein 45 kD (NIP45) (Hodge et al. 1996a), also help to regulate IL-4 production.

GATA-3 The zinc finger protein GATA-3 was initially cloned as a T-cell-specific transcription factor that bound to the Ta3 element of the TCR α gene enhancer (Ho et al. 1991). Deletion of the GATA-3 gene produces lethal abnormalities of the central nervous and hemato-

poietic systems (Pandolfi et al. 1995), whereas mice lacking GATA-3 in the lymphoid system do not generate T cells because GATA-3-deficient thymocytes arrest at the immature double-negative stage (Ting et al. 1996; Hendriks et al. 1999). A key discovery by two groups was the selective expression of GATA-3 in Th2 cells (Zhang et al. 1997; Zheng and Flavell 1997). Ectopic expression of a GATA-3 transgene led to increased levels of Th2 cytokines, whereas a dominant-negative GATA-3 transgene inhibited Th2 differentiation (Zheng and Flavell 1997; Zhang et al. 1999). GATA-3 directly controls IL-5 gene expression through binding to and transactivating elements in the region -70 to -59 (Siegel et al. 1995; Zhang et al. 1997, 1998a; Lee et al. 1998). GATA-3 probably does not directly bind to and transactivate the IL-4 promoter (Zhang et al. 1997, 1998a). Rather, several genomic regions within the IL-4/IL-13 locus have GATA-3-dependent enhancer activity (Ouyang et al. 1998) suggesting GATA-3 may augment expression of IL-4 or IL-13 via interactions at sites distant from the proximal-promoter.

In addition to increasing expression of Th2-selective cytokines, GATA-3 inhibits Th1 development independently of IL-4, perhaps in part by inhibition of IL-12R β 2 expression (Ouyang et al. 1998). Although GATA-3 is a Stat6-inducible gene, the developmental program initiated by GATA-3 can operate independently of Stat6 (Ouyang et al. 2000). When GATA-3 was expressed by retrovirus in Stat6-deficient T cells, all components of Th2 development were observed, including induction of Th2 cytokines, inhibition of Th1 cytokines, induction of c-Maf, and the formation of DNase I hypersensitive sites in the IL-4 locus. In addition, the endogenous GATA-3 gene was observed to be induced in these cells, despite the addition of IL-12 to the culture, normally a signal that inhibits GATA-3 expression (Ouyang et al. 1998). These results suggest a pathway of GATA-3 autoactivation, which could provide a mechanism for acquisition of the IL-4 independence that develops in Th2 cells described by Paul and colleagues (Huang et al. 1997).

The potential importance of this Stat6-independent pathway has been brought out by two recent studies. First, Jankovic et al. (2000) reported that Th2 cells can develop both in vivo in response to *S. mansoni* infection even in T cells lacking Stat6 or IL-4 receptor, suggesting the existence of an unrecognized pathway stabilizing Th2 development once these cells have emerged. Second, Finkelman and colleagues (2000) showed that in the primary response to pathogens, Stat6-deficient mice generated wild-type IL-4 responses, delineating a requirement for Stat6 in only the secondary response. These findings are all consistent with a model in which the initial expression of GATA-3 can be independent of IL-4 and Stat6 activation but is repressible by IL-12; in the absence of such repression, GATA-3 can be induced through Stat6-independent autoactivation to induce the development of the Th2 phenotype.

c-Maf c-Maf was isolated using a yeast two hybrid screen with NFATc1 as bait and cDNAs from activated

Th2 cells as library (Ho et al. 1996). c-Maf, the cellular homolog of the avian viral oncogene v-maf, is a member of the AP-1 family of basic region/leucine zipper factors and binds to a consensus site (MARE) in the proximal IL-4 promoter. The expression of c-Maf is limited to Th2 cells, and its own expression is induced by signals transmitted via the TCR. Ectopic expression of c-Maf in all cells tested including yeast potentially activates the IL-4 promoter. In CD4⁺ T cell lines prepared from patients with atopic disease, c-Maf is expressed at very high levels in Th2 clones but minimally in Th0 clones that produce IFN γ and very little IL-4. Extremely strong evidence for the critical role of c-Maf in controlling IL-4 production has been gathered in vivo with the production of c-Maf overexpressor transgenics (Ho et al. 1998) and most convincingly with mice that lack c-Maf (Kim et al. 1999). c-Maf transgenic mice have an increased Th2 immune response in vivo and in vitro that can be ablated by backcrossing onto an IL-4-deficient background (Ho et al. 1998). Targeting of the c-Maf locus revealed severely impaired IL-4 production in the absence of c-Maf (Kim et al. 1999). These data provide very strong evidence that c-Maf is required for the production of IL-4 in vivo. However, the provision of c-Maf to mature effector Th1 cells cannot allow them to produce IL-4, suggesting that other factors such as Stat6 or GATA-3 may also be required.

An attractive overall schema (Fig. 5) suggests that signals through the TCR activate both c-Maf and GATA-3 as well as NFATc1 transcription factors, whereas IL-4 signaling induces GATA-3 and Stat6 activity. GATA-3 is autoregulatory (Ouyang et al. 2000), and it is possible that GATA-3 and c-Maf regulate each other in an auto-crine loop, resulting in IL-4 production. GATA-3 down-regulates expression of the IL-12R β 2 chain, whereas both GATA-3 and c-Maf repress IFN γ production in Thp cells thus inhibiting the Th1 pathway (Ho et al. 1998; Ouyang et al. 2000).

DNA replication and monoallelic cytokine gene expression

The above discussion has emphasized signaling and transcriptional control of cytokine expression underlying a predominantly instructional model of T helper development. However, certain recent results have raised the issue of whether T helper commitment might not rely on stochastic or selective processes. Cytokine-driven responses in bulk populations do not necessarily distinguish between instructive or selective models of T cell differentiation. Recently, Coffman and Reiner (1999) have outlined three competing models of T helper development: instructive differentiation, selective differentiation, and a hybrid instructive/selective model. In the first model, all daughter cells of uncommitted progenitors respond to specific differentiating signals to acquire one particular developmental state. In the second, daughter cells of a progenitor adopt multiple development states in a random or stochastic manner, which later appears to polarize only by selective outgrowth of one cell type in responses to "polarizing" cytokines.

Their hybrid model adds an effect of cytokines on altering the ratio in the initial developmental fates but retains the component of selective outgrowth in response to cytokines for achieving polarization.

Examining the stability or reversibility in bulk cultures reveals the potential for early reversibility and of heterogeneity but does not address issues of single cell fate determination (Murphy et al. 1996; Coffman et al. 1999). Experiments using a lineage ablation approach, in which the IL-4 promoter drove expression of thymidine kinase (Kamogawa et al. 1993; Minasi et al. 1993; Nakamura et al. 1997), suggested that precursors to both Th1 and Th2 cells could proceed through a stage where the IL-4 promoter was active. Favoring the early commitment to the Th2 pathway is the rapid loss of a functional IL-12 receptor (Szabo et al. 1997a). Early Th1 populations, in contrast, exhibit greater reversibility (Mocci and Coffman 1995, 1997; Szabo et al. 1997a) due perhaps to uncommitted precursors within the early Th1 population (Mocci and Coffman 1997).

The possibility of epigenetic regulation of cytokine expression has recently sparked a renewed interest in non-instructional models of T helper development. Monoallelic expression using polymorphic IL-2 alleles from F₁ mouse strains was revealed in both T cell clones and primary T cells to occur in a substantial fraction of T cells (Hollander et al. 1998), suggesting that alleles of IL-2 were not activated uniformly in all T cells. Similar findings for IL-4 alleles in Th2 cells were later reported (Bix and Locksley 1998), and these observations extended using reporter genes or knock-ins targeted to both the IL-2 (Naramura et al. 1998) and IL-4 loci (Riviere et al. 1998), allowing individual alleles to be examined in early populations of developing TCR transgenic T cells. In contrast to earlier findings, however (Hollander et al. 1998), biallelic expression of both IL-2 and IL-4 was observed as the predominant pattern in T-cell activation (Naramura et al. 1998; Riviere et al. 1998). The level of T-cell activation could also alter the distribution between monoallelic and biallelic expression (Riviere et al. 1998). These data favor a model in which activation of alleles is a stochastic process whose probability depends on TCR signal strength.

Reiner and colleagues have proposed that cell cycle is a more important regulator of T helper development than instructive cytokine signals based on distinct requirements for numbers of cell division in initiating cytokine expression (Bird et al. 1998). Naive T cells were immediately capable of producing IL-2, but IFN γ and IL-4 production correlated with the number of cell divisions after activation, IFN γ requiring only one to two cell divisions, but IL-4, four divisions. Similar results reported for cytokines that are not differentially expressed among Th1 and Th2 cells, such as IL-3 (Gett and Hodgkin 1998), implied that these differences may not control Th1/Th2 commitment. Furthermore, a very recent analysis (Richter et al. 1999) provided evidence against a strict cell-division model and in favor of an instructional model in which the epigenetic changes induced by TCR and IL-4R signaling are required for cyto-

kine gene expression. This study found that naive T cells were capable of initiating IL-4 production after priming even when cell division was blocked as long as entry into the S phase of the cell cycle was allowed. Once again, the required epigenetic changes appeared to be induced by cytokines, consistent with an instructional model of development.

Role of chromatin structure

Genomic DNA is packaged into nucleosomes, which then form higher order chromatin structures. Chromatin remodeling is accompanied by changes in nucleosomal positioning, an event that is ATP dependent. It has been suggested that cytokine gene loci, including IL-2, IL-4, IL-12, IL-13, IFN γ , and GM-CSF, undergo changes in chromatin structure that allow access to gene-specific transcription factors, likely mediated in part through acetylation of histones. Distal control regions such as enhancers and LCRs have been shown to regulate accessibility to gene loci by regulating chromatin structure (Ernst and Smale 1995). The location of regions that control these changes have been identified in cytokine genes using both assessment of CpG methylation status and DNase I hypersensitivity assays since transcriptionally active chromatin is hypomethylated and is more accessible to nucleases (Agarwal and Rao 1998a; Kadonaga 1998; Takemoto et al. 1998; Agarwal et al. 1999). The most complete information on the role of chromatin remodeling in the regulation of cytokine genes is available for two cytokines, IL-4 and IFN- γ , and is summarized below and reviewed more thoroughly by Rengarajan and Glimcher (2000).

Regulation of the IL-4 and IFN- γ loci Several observations suggested that the IL-4 gene was regulated at the level of chromatin structure. First, in vitro analyses had indicated that only 157 bp of the proximal IL-4 promoter was required for tissue-specific expression in Th2 cells. Nevertheless, in vivo, the proximal 800 bp of the promoter was required to confer significant Th2-selective expression of an IL-4 promoter-reporter transgene (Wenner et al. 1997). However, neither 800 bp nor up to 3 kb of sequence were sufficient to achieve expression equivalent to the endogenous IL-4 gene, suggesting the presence of additional elements for optimal expression (Todd et al. 1993). Second, differentiated, effector Th2 cells produce IL-4 more rapidly and at higher levels than naive Thp cells, implying that the IL-4 regulatory regions are more accessible or "poised" in effector, but not in naive, T cells (Reiner and Seder 1999).

Evidence for chromatin remodeling In both mouse and human chromosomes, the Th2 cytokine genes IL-4, IL-5, and IL-13 are clustered together within 150 kb, consistent with the notion that these genes compose a single chromosomal locus that may be controlled by long-range modulation of chromatin structure. Rao and colleagues (Agarwal and Rao 1998b) provided evidence in favor of this model by using both methylation and DNase I hy-

Lineage commitment in the immune system

persensitivity assays to identify regions in the IL-4/IL-13 locus that responded to stimulation through the TCR. Differentiated Th2 cells and established Th2 clones had an accessible chromatin structure as evidenced by the presence of five clusters of HS sites over the IL-4 locus. Naive T helper cells, in contrast, like Th1 cells, possessed only one of the five HS sites. Within 48 hr of antigen activation, naive Thp rapidly acquired the chromatin phenotype of differentiated Th2 cells, implicating these regions in important regulatory functions (Agarwal and Rao 1998b). The acquisition of these sites was dependent on both IL-4 and Stat6 (Agarwal and Rao 1998b). The Th2-specific transcription factor GATA-3 induced the same changes when transduced into differentiated Th1 cells or Stat6-deficient cells, indicating that GATA-3 acts to remodel this locus downstream of Stat6 (Ouyang et al. 2000). Furthermore, naive Thp cells possessed a hypermethylated IL-4 locus, whereas differentiated Th2 cells display decreased CpG methylation at the IL-4 locus. This latter result is consistent with the observation that treatment of naive Thp cells with trichostatin A and 5-azacytidine (histone deacetylase and methylase inhibitors, respectively) accelerates the kinetics of IL-4 production (Bird et al. 1998). Factors like GATA-3 and Stat6 may directly remodel chromatin structure to allow TCR-induced factors like c-Maf and NFAT to access their specific binding sites in the IL-4 locus and promote rapid transcription of IL-4 (Agarwal and Rao 1998b); NFAT proteins may also directly alter chromatin configuration. Mice that lack both NFATc2 and NFATc3 vastly overproduce Th2 cytokines and display a highly allergic phenotype (Ranger et al. 1998b) suggesting that NFAT proteins may help regulate the balance between the active/inactive state of the IL-4/IL-5/IL-13 locus during the initiation of Th2 differentiation.

Although chromatin remodeling may also be important for the IFN γ gene, less is known about this locus than the IL-4 locus. Tissue (Th1)-specific DNase I HS regions have been identified in both the first and third introns of the IFN γ gene (Young et al. 1994; Agarwal and Rao 1998b), and these assays also demonstrate alterations of the IFN γ locus in naive Th1 cells compared with Th2 cells (Agarwal and Rao 1998b). The methylation patterns of the IFN γ locus have also been analyzed during T-cell differentiation. IFN γ -producer cells like Th1 and CD8 $^+$ T cells display hypomethylation that appears to be a stable, long term, inheritable trait (Young et al. 1994; Fitzpatrick et al. 1999), whereas the same locus is methylated in Th2 cells. The assembly of Th1-specific transcription factors may remodel the chromatin in a configuration that facilitates access to other factors required to transcribe the IFN γ gene. It will be of particular interest to determine whether T-bet is involved in the remodeling of the IFN γ locus during Th1 differentiation.

Summary

The genetic programs that specify lineage commitment in the T helper cell are beginning to be elucidated (Fig. 6).

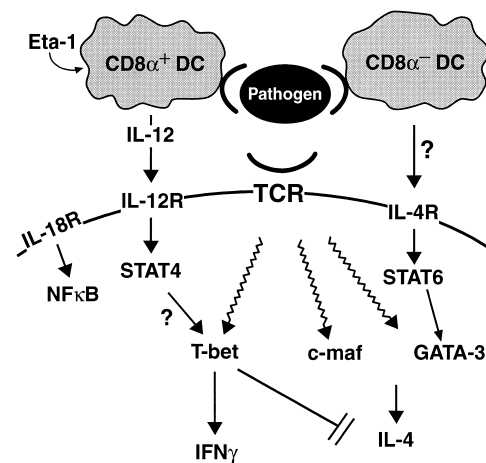


Figure 6. Overall schema of molecular basis of Th lineage commitment.

An attractive model to posit considers T-bet and GATA-3 as the master regulators of Th1 and Th2 differentiation, respectively, with c-Maf as the downstream factor that directly and selectively controls IL-4 gene transcription. The expression of T-bet and GATA-3 during Th differentiation are mirror images. T-bet and GATA-3 are strikingly similar in their ability to induce one lineage while simultaneously repressing the opposing lineage. T-bet may lie downstream of IL-12/Stat4 and IFN γ (Stat1), the major inducers of Th1 differentiation, whereas GATA-3 is activated by IL-4/Stat6, the critical Th2-inducing cytokine. It is tempting to postulate that T-bet and GATA-3 regulate each other with T-bet suppressing GATA-3 expression during Th1 differentiation and GATA-3 inhibiting T-bet expression during Th2 development. Cytokine gene expression may more broadly reflect a balance between repressors and activators. Transcription factors such as T-bet that activate Th1 genetic programs repress Th2 programs (Szabo et al. 2000), whereas GATA-3 and c-Maf are activators in Th2 cells but play an opposing function as repressors in Th1 cells (Ho et al. 1998; Ouyang et al. 1998). Another example is the Stats. Although Stat4-deficient mice lack Th1 responses, mice lacking both Stat4 and Stat6 mount a partial Th1 response suggesting that Stat6 may act as a repressor in Th1 cells (Kaplan et al. 1998). We would propose that the fate of a developing Th cell may be determined by the relative predominance of T-bet and GATA-3 elicited by external signals such as pathogens, autoantigens, or allergens. Analysis of the regulatory regions of the T-bet, c-Maf, and GATA-3 genes themselves should yield prove informative in revealing the signaling pathways and molecules upstream of these three critically important transcription factors.

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