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Expanding the effector CD4 T-cell repertoire: the Th17 lineage

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The Th1/Th2 paradigm has provided the framework for understanding CD4 T-cell biology and the interplay between innate and adaptive immunity for almost two decades. Recent studies have defined a previously unknown arm of the CD4 T-cell effector response — the Th17 lineage — that promises to change our understanding of immune regulation, immune pathogenesis and host defense. The factors that specify differentiation of IL-17-producing effector T-cells from naïve T-cell precursors are being rapidly discovered and are providing insights into mechanisms by which signals from cells of the innate immune system guide alternative pathways of Th1, Th2 or Th17 development.

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Introduction

Adaptive immune responses are vital for the efficient eradication of infectious agents, although dysregulated adaptive immune responses might also lead to autoimmune and chronic inflammatory diseases. A principal component of the adaptive immune response is the CD4 T cell, which can orchestrate the functional activity of both innate and adaptive immune systems. The directed differentiation of effector CD4 T cells by cytokines produced by pathogen-activated cells of the innate immune system provides a mechanism to coordinate the innate and adaptive immune responses for greatest host protection.

Classically, effector CD4 T cells have been divided into two distinct lineages on the basis of their cytokine production profile: cells of the T helper (Th)1 lineage, which evolved to enhance eradication of intracellular pathogens (e.g. intracellular bacteria, viruses and some protozoa), are characterized by their production of interferon (IFN) γ , a potent activator of cell-mediated immunity; and cells of the Th2 lineage, which evolved to enhance elimination of

parasitic infections (e.g. helminths), are characterized by production of interleukin (IL)-4, IL-5, and IL-13, which are potent activators of B-cell immunoglobulin (Ig)E production, eosinophil recruitment and mucosal expulsion mechanisms (mucous production and hypermotility). Immune pathogenesis that results from dysregulated Th1 responses to self or commensal floral antigens can promote tissue destruction and chronic inflammation, whereas dysregulated Th2 responses can cause allergy and asthma.

Recent studies have suggested a greater diversification of the CD4 T-cell effector repertoire than that encompassed by the Th1/Th2 paradigm. This knowledge has forced a reassessment of the Th1 lineage in autoimmunity. New studies that link the cytokines IL-23 and IL-17 to immune pathogenesis previously attributed to the Th1 lineage have led to the delineation of a new effector CD4 T-cell arm — referred to as Th17.

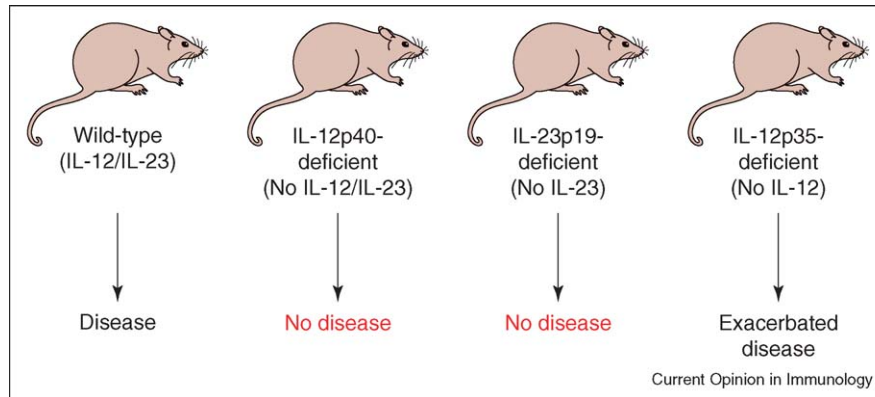
In this review, we highlight recent advances that have resulted in the characterization of the Th17 cells as the product of a developmental lineage distinct from that of Th1 and Th2, and discuss implications for immune regulation, host defense and autoimmunity mediated by this lineage.

Autoimmune pathogenesis revisited: the IL-12-IFN γ versus IL-23-IL-17 cytokine networks

A number of chronic inflammatory disorders develop as the result of uncontrolled self-reactive effector CD4 T cells. Experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA), two prototypical autoimmune mouse models, have been traditionally associated with dysregulated Th1 responses. An important basis for this association has been a number of studies that have described ablation of disease development in gene-targeted mice deficient in the p40 subunit of IL-12 (*Il12p40* or *Il12b*) or mice treated with neutralizing antibodies specific for IL-12p40 [1–3]. With the description of a new IL-12 family member, IL-23 [4], which also has the p40 subunit but is paired with a distinct second chain (IL-23p19 instead of IL-12p35), it became apparent that this association should be revisited. This was especially important in view of data that are inconsistent with a dominant role for the Th1 lineage in immune pathogenesis: mice deficient in IFN γ , the principal cytokine produced by Th1 cells, are susceptible to EAE and CIA, as are mice deficient in IFN γ R signaling (*Ifngr*^{-/-} and *Stat1*^{-/-}) [5–10].

Resolution of this paradox came from studies that examined the development of EAE and CIA in mice

Figure 1



This schematic shows the pathogenic role for IL-23, not IL-12, in mouse models of autoimmunity. Studies by Cua and co-workers [11,12] have demonstrated that disease development requires IL-23, but not IL-12, in EAE and CIA. Compared with wild-type susceptible mice, mice deficient for IL-23 (*Il23p19^{-/-}*) and both IL-23 and IL-12 (*Il2p40^{-/-}*) failed to develop disease after antigenic challenge, whereas mice deficient for IL-12 (*Il12p35^{-/-}*) developed more severe disease.

specifically deficient in either IL-12 or IL-23 (Figure 1). In two landmark studies, Cua and co-workers [11,12] found that mice deficient in the IL-23p19 subunit (*Il23p19^{-/-}*; lacking IL-23 only) or the IL-12p40 subunit (*Il12p40^{-/-}*; lacking IL-12 and IL-23) were resistant to EAE and CIA, whereas IL-12p35-deficient mice (*Il12p35^{-/-}*; lacking IL-12 only) remained susceptible. Moreover, mice that lack the IL-12 receptor complex (*Il12rb2^{-/-}*) also succumbed to these diseases, which demonstrates a requirement for IL-23, but not IL-12, in pathogenesis [13].

Additional insights emerged from analysis of the cytokine phenotypes of effector CD4 T cells primed with type II collagen in the CIA studies [12]. Although immunization of *Il12p19*-deficient mice did not result in autoimmunity, IFN γ -positive Th1 effector cells were generated at levels comparable to that of wild-type mice, which did develop arthritis. Conversely, induction of disease in *Il12p35*-deficient mice was associated with diminished frequencies of IFN γ -positive CD4 T cells. Together, these data establish an inverse correlation between Th1 induction and disease development. In contrast, there was a positive correlation between the availability of IL-23 and the development of effector CD4 T cells that produced IL-17 — a potent T-cell-derived pro-inflammatory cytokine linked to IL-23-induced inflammation [14–16]. In accordance with these findings, IL-17-deficient mice demonstrate impaired joint inflammation following type II collagen immunization [17]. Furthermore, neutralization of IL-17 decreases disease severity [18–20], and overexpression of IL-17 in the joints exacerbates disease [18,21]; this links the development of self antigen-reactive IL-17-producing effector CD4 T cells, but not IFN γ -producing effectors, to autoimmune inflammation. Thus, in at least some

forms of autoimmunity, it is the IL-23–IL-17 cytokine axis, and not the IL-12–IFN γ axis, that is crucial for disease pathogenesis.

IL-12 and IL-23 induce distinct effector CD4 T-cell subsets

In light of the positive link of the IL-23–IL-17 cytokine axis with EAE and CIA, efforts to understand possible lineage relationships between IFN γ - and IL-17-producing T-cell effectors intensified. It had been found independently that IL-23 elicited IL-17 production from CD4 T cells of the effector or memory phenotype, but not from naïve CD4 T cells [14]. These data, along with the common features shared by IL-12 and IL-23, suggested that IL-23 might be acting on a common Th1 precursor, or even on mature Th1 cells, to stimulate IL-17 production. It was thus suggested that IFN γ - and IL-17-producing CD4 T cells might represent a common lineage [12,22]. However, a striking feature of the phenotypic characterization of effector cells from the IL-12- and IL-23-deficient mice was the segregation of cytokine expression by IFN γ and IL-17 producers, such that individual T cells showed a strong tendency towards mutually exclusive production of either IFN γ or IL-17. In a subsequent study, Langrish *et al.* [23^{••}] extended these findings by demonstrating that these distinct subpopulations of effector T cells also develop during EAE. Importantly, they also showed that IL-17-enriched CD4 T cells produced by *ex vivo* culture with IL-23 following *in vivo* priming were ~10-fold more potent on a per-cell basis than IL-12-derived IFN γ -positive Th1 cells at transferring disease upon adoptive transfer into unimmunized recipients. These data indicated that the IFN γ and IL-17 cells are not only distinct in their cytokine profile but also in their biological functions.

Although these data indicated that IFN γ - and IL-17-producing effector CD4 T cells were largely divergent and developed under differential stimulation with IL-12 and IL-23, the extent of this divergence was not well-defined. Langrish *et al.* [23^{••}] therefore performed real-time polymerase chain reaction analysis to quantitate the relative expression of more than 200 genes in CD4 T cells primed *in vivo* and then cultured *ex vivo* with antigen and IL-12 or IL-23. Interestingly, many genes were differentially expressed between the two subsets. The IL-12-polarized cells (i.e. prototypic Th1 cells) preferentially expressed genes associated with cytotoxicity (such as IFN γ , FasL and granzymes), whereas the IL-23-cultured cells expressed genes associated with chronic inflammation (such as IL-17A, IL-17F, IL-6, TNF α and pro-inflammatory chemokines). These results further highlighted the phenotypic and functional differences between the IFN γ - and IL-17-producing CD4 T cells, which suggests that these are distinct populations of effector cells, each having a unique role in the adaptive immune system. However, the lineage relationships between the two phenotypes remained unclear.

Pathways to effector CD4 T-cell differentiation

Since the initial studies of T-cell receptor (TCR) transgenic mice when it was established that Th1 and Th2 cells can both develop from the same pool of naïve precursors [24,25], there has been extensive investigation of the factors and signaling pathways that distinguish these lineages [26]. There is now general consensus regarding many of the broad features of these developmental programs, although certain details remain contentious [27].

Th1 differentiation is initiated by coordinate signaling through the TCR and signal transducer and activator of transcription (STAT)1-associated cytokine receptors. Both type I and type II IFNs can activate STAT1 by way of their respective receptors, as can the IL-12 family member IL-27 [28–30]. Each of these receptors are expressed on naïve T-cell precursors and are activated depending on the availability of their respective ligands, which are thought to be produced primarily by cells of the innate immune system. STAT1 signaling in antigen-activated naïve precursors upregulates the transcription factor T-bet (also known as Tbx-21), which is thought to be a master regulator of Th1 differentiation [31,32]. T-bet potentiates expression of the *Ifng* gene and upregulates the inducible chain of the IL-12 receptor (IL-12R β 2), but suppresses Th2-associated factors. Induction of a competent IL-12 receptor on developing Th1 cells enables IL-12 signaling through STAT4, which further potentiates IFN γ production and induces expression of IL-18R α , thereby conferring responsiveness to IL-18 by mature Th1 cells. The IL-12-driven component of Th1 development results in mature effector cells that can produce IFN γ through either TCR-dependent or TCR-

independent (IL-12 plus IL-18) pathways [33,34]. Thus, the later stage of Th1 differentiation induced by IL-12 enables mature Th1 cells to produce IFN γ in an antigen-independent manner, not unlike cells of the innate immune system such as natural killer cells.

Th2 differentiation is initiated by TCR signaling in concert with IL-4 receptor signaling via STAT6. Signals that emanate from the TCR and IL-4 receptors act cooperatively to upregulate low-level expression of GATA3, a master regulator of Th2 differentiation [35–37]. GATA3 autoactivates its own expression and drives epigenetic changes in the Th2 cytokine cluster (*I/4*, *I/5* and *I/13* genes), while suppressing factors crucial to the Th1 pathway, such as STAT4 and the IL-12R β 2 chain. Thus, early IL-4 signaling rapidly initiates positive and negative feedback loops that serve to reinforce early commitment to Th2 development while blocking Th1 development. An important facet of the Th1/Th2 developmental paradigm, as in many developmental strategies, is therefore the presence of reiterative feedback mechanisms that propagate early lineage decisions once initiated.

Th17 cells develop by way of a lineage distinct from Th1 and Th2 cells

Given the conserved structural and functional features of IL-12 and IL-23 and their receptors, and the observed induction of IFN γ along with IL-17 by IL-23-stimulated memory CD4 T cells, it has been tempting to assume overlap in the differentiation programs that give rise to Th1 cells and cells that produce IL-17. Especially in view of the limited effect of IL-23 to promote the development of IL-17 effectors from naïve CD4 precursors [14,23^{••}], it is not surprising that models of IL-17 effector differentiation have been linked to the Th1 pathway. However, two recent reports — one from our group [38[•]] and the other from Park *et al.* [39[•]] — have provided compelling evidence that the pathway that leads to generation of IL-17-producing effectors, which has been termed the Th17 lineage, is distinct from that of the Th1 lineage, and therefore represents the third arm of the CD4 T-cell effector repertoire: Th1, Th2 and Th17.

In our own studies [38[•]], two key observations stimulated further experiments that established a dichotomy between Th1 and Th17. The first was that developing Th1 cells generated *in vitro* were not amenable to IL-17 production or proliferation by stimulation with IL-23. This indicated that Th1 cells were not IL-23 responsive, as had been previously proposed. Th2 cells behaved in a similar manner: they were resistant to IL-23 effects. Second, and perhaps more importantly, attempts to generate IL-17 producers from naïve precursors by addition of IL-23 uniformly failed, until it was found that the presence of either IFN γ or IL-4 in primary cultures potently inhibited Th17 development. Thus, TCR stimulation of naïve CD4 T cells in the presence of IL-23

alone did not result in a significant fraction of IL-17-producing cells. However, activation of CD4 T cells under these same conditions with concurrent IFN γ and IL-4 neutralization induced development of a discrete population of IL-17-positive CD4 T cells, which were negative for IFN γ and IL-4. Additionally, the ability of IFN γ -deficient CD4 T cells to secrete IL-17 was impeded when cultures were supplemented with exogenous IFN γ or IL-4. Thus, Th1 and Th2 cells were both resistant to IL-23-induced IL-17 expression and were potentially inhibitory of Th17 development.

These results strongly suggested that the development of IL-17-producing effectors was by way of a lineage distinct from Th1 and Th2. To further explore this, the key signaling pathways for Th1 and Th2 differentiation were examined. CD4 T cells deficient in either STAT1 or STAT6 — the principal signaling molecules downstream of IFN γ and IL-4, respectively — exhibited augmented IL-17 production. This provided a potential explanation for the observed segregation of IFN γ - and IL-17-producing CD4 T cells in CIA and EAE: IFN γ acts autonomously to enhance IFN γ expression and to repress IL-17 production. Also, the inhibition of Th17 development by IFN γ provides a mechanism that might explain the enhanced susceptibility of *Ifng*^{-/-}, *Ifngr*^{-/-}, *I112p35*^{-/-} and *Stat1*^{-/-} mice to these murine models of autoimmunity, in which the IL-23–IL-17 cytokine axis drives disease pathogenesis. Interestingly, the addition of exogenous IL-17 did not similarly suppress Th1 or Th2 polarization (LEH, unpublished). This suggests that IL-17 cannot directly potentiate its own differentiation by extinguishing Th1 or Th2 differentiation by way of IL-17, although it is possible that other factors that promote the development of, and/or are secreted by, IL-17-positive CD4 T cells are capable of suppressing Th1 and Th2 development in favor of Th17 development (see below).

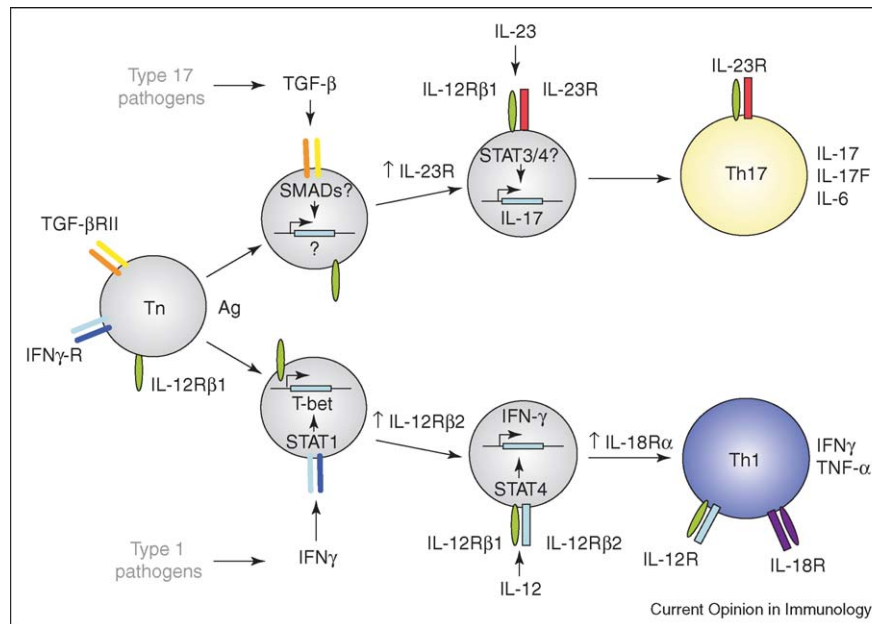
A key regulator of Th1 lineage commitment is T-bet (encoded by the *Tbx21* gene), and it is likely that a common Th1/Th17 precursor cell would differentiate via a T-bet-dependent pathway. T-bet is a member of the extensive T-box family of transcription factors that is upregulated in developing Th1 cells, but not Th2 cells, and it is crucial for optimal expression of IFN γ and IL-12R β 2 by Th1 cells. T-bet expression can be stimulated in CD4 T cells by IFN γ or IL-27 signaling through STAT1. However, it is possible that other pathways are utilized for T-bet upregulation, because *Stat1*^{-/-} and *Tbx21*^{-/-} mice display distinct phenotypes following EAE induction [10,40]. Notably, T-bet-deficient mice do not succumb to EAE; this has been attributed to the inability of these mice to generate Th1 effector cells. However, given the finding that Th17 cells, not Th1 cells, are crucial for EAE induction, it was conceivable that T-bet might be an important transcription factor in Th17

differentiation and that Th1 and Th17 cells might indeed develop from a common progenitor cell.

Nevertheless, it was found that Th17 development was unimpaired in T-bet-deficient CD4 T cells [38[•]]. Furthermore, immunization of T-bet-deficient mice with myelin oligodendrocyte glycoprotein (a protein antigen used to induce EAE) resulted in the development of a discrete population of IL-17-producing CD4 T cells [39[•]], suggesting that the resistance of T-bet-deficient mice to development of EAE is not attributable to a defect in Th17 development. Thus, although a role for T-bet in Th17 biology cannot be excluded at this time, it is unlikely to have intrinsic effects on Th17 development. By contrast, it is worth noting that molecules known to induce T-bet (e.g. IFN γ and STAT1) potentially suppress Th17 differentiation *in vitro*, which suggests that T-bet might, in fact, have a role in inhibiting IL-17 secretion from CD4 T cells. In this regard, Park *et al.* [39[•]] examined the development of IL-17-producing cells in immunized mice deficient in IFN γ and T-bet. Irrespective of the targeted deficiency examined, IL-17-positive cells developed normally, which supports the *in vitro* data of our group that show that these factors are dispensable for Th17 differentiation. Therefore, although the precise role of T-bet in autoimmune inflammation remains elusive, these data show clearly that T-bet is not required for IL-17 production from CD4 T cells, reiterating the notion that Th17 effector cells develop independently of Th1 and Th2 cells.

Given that the IL-12 and IL-23 receptors both use IL-12R β 1 as one of their two subunits and that IL-12 preferentially signals by way of STAT4–STAT4 homodimers [41], it is tempting to speculate a role for STAT4 in IL-23 signaling. Consistent with this, STAT4-deficient mice are resistant to the chronic inflammatory disorders in which the IL-23–IL-17 pathway drives disease, including CIA and EAE, suggesting that STAT4 is required for development of pathogenic Th17 cells [42]. Indeed, signaling through the IL-23R complex has been shown to phosphorylate STAT1, STAT3 and STAT4, as well as to induce the formation of STAT3–STAT4 heterodimers, suggesting that both STAT3 and STAT4 might be important for IL-17 production from CD4 T cells [4,43]. However, STAT4-deficient CD4 T cells were unimpaired in their ability to produce IL-17 *in vitro* and *in vivo* [38[•],39[•]], indicating that this transcription factor is dispensable for commitment to the Th17 lineage and IL-17 production. Whether STAT4 is required for IL-23-dependent effects on developing Th17 cells that are unrelated to IL-17 production remains to be determined (see below), as does a possible role for STAT3. Collectively, however, these data support a divergent model for Th1 and Th17 effector differentiation (Figure 2), and emphasize the counter-regulatory features of these two lineages.

Figure 2



Model of Th1 versus Th17 lineage development from naïve CD4 T cell precursors (Tn). This model emphasizes the distinct lineages leading to mature Th1 and Th17 effector cells (see main body of text for details). Question marks denote speculative or unknown aspects of Th17 differentiation that are yet to be defined.

Other factors that contribute to Th17 differentiation

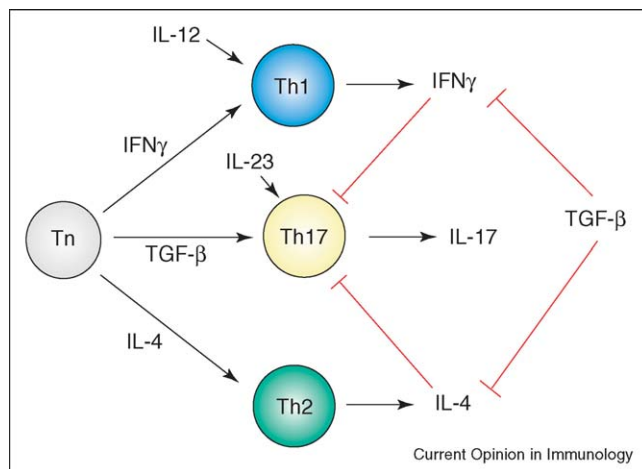
A key feature of Th1 differentiation is the requirement for induced expression of the IL-12R β 2 subunit to confer IL-12 responsiveness. This is most efficiently mediated through STAT1- and T-bet-dependent signals, and therefore occurs downstream of IFN actions early in Th1 commitment. Because the IL-23-specific component of the IL-23 receptor complex, IL-23R, must also be induced on developing Th17 cells, IL-23 actions are likely to occur downstream of factors that induce IL-23R (Figure 2), and IL-23 might not be required for Th17 commitment or IL-17 expression.

To address this, we recently re-examined the requirement for IL-23 in the development of IL-17-producing effectors (PRM *et al.*, unpublished; see Update). We used IL-12p40-deficient (*Il12b*^{-/-}) mice as a source of IL-23- (and IL-12-)deficient antigen-presenting cells (APCs) with which to examine Th17 development under defined conditions of IL-23 availability. Under Th17-polarizing conditions (neutralization or deficiency of IFN γ and IL-4), comparable frequencies of IL-17-producing effectors were generated from naïve CD4 T-cell precursors, irrespective of the availability of exogenous IL-23. Thus, it appears that IL-23 is not required for development of IL-17-competent effectors. In view of the established role for IL-23 in the development of Th17-dependent autoimmunity, this implies that IL-23 signaling might not be

required for Th17 commitment and IL-17 production, but instead might be important for amplifying and/or stabilizing the Th17 phenotype in chronic inflammation. If so, this could explain apparent inconsistencies in the STAT4-dependency of Th17 functions. It is clear that additional studies will be necessary to address this.

The absence of a requirement for IL-23 in early Th17 development begs the question of what factors are necessary for Th17 commitment and IL-17 expression. Important clues have come from the demonstrated inhibitory actions of IFN γ and IL-4 on Th17 development (Figure 3). In further recent studies, we have examined factors that might directly or indirectly block the inhibitory actions of IFN γ to determine their possible actions on Th17 development. It has been reported that TGF- β inhibits Th1 and Th2 development, at least in part through blockade of effector cytokine production [44], although it also inhibits the Th1 developmental program by way of the T-cell intrinsic effects [45,46]. We have now examined the effects of TGF- β on Th17 development, and, remarkably, have found that this cytokine induces IL-17 expression in the absence of IL-23 (PRM *et al.*, unpublished; see Update). Importantly, the effect of TGF- β on Th17 induction is independent of simple inhibition of IFN γ , as it induces much stronger IL-17 responses than deficiency of IFN γ alone. Furthermore, under the conditions used for Th17 induction, TGF- β did not induce strong Treg development. Thus, these

Figure 3



Antagonistic cytokine networks control CD4 effector T-cell differentiation. Recent studies have established that Th1 and Th2 effector cytokines, IFN γ and IL-4, respectively, potentially inhibit Th17 development. Furthermore, TGF- β , a cytokine previously implicated in Treg development and function, appears to be required for Th17 development, both through indirect effects (blockade of IFN γ and IL-4 production by cells of the innate immune system) and through direct effects on naïve CD4 T-cell precursors (Tn).

results support a model in which competing effects of antagonistic cytokines act early to establish Th17 lineage commitment, similar to the competitive effects of IFN γ and IL-4 for Th1 and Th2 (Figure 3).

APCs can direct effector T-cell development not only by their production of cytokines but also through the expression of certain surface molecules such as costimulators. Inducible costimulatory molecule (ICOS)–ICOSL interactions are important for IL-4 secretion and Th2 development, but have little effect on IFN γ production and Th1 development [47,48]. It has been shown that this interaction might also facilitate Th17 development and the induction of CIA [39*,49], suggesting an additional mechanism for divergence of the Th1 and Th17 lineages. However, ICOS-deficient mice develop augmented EAE [47], suggesting that a simple correlation between ICOS signaling and Th17 development might be flawed. Also, it has recently been reported that APC expression of different Notch ligands can direct effector differentiation: Delta promotes Th1 differentiation, whereas Jagged promotes Th2 differentiation [50]. It remains to be determined whether these molecules (or others) have an active role in regulation of Th17 development.

Conclusions

The rapid emergence of the IL-23–IL-17 cytokine network as a central player in immune pathogenesis has been followed by studies that have linked these cytokines to a new CD4 T-effector lineage — Th17 — that has strong pathogenic potential. Although not addressed in this brief

review, it is certain that the Th17 lineage evolved to control certain classes of pathogens, analogous to the specialized functions of Th1 and Th2 for handling intracellular pathogens and parasitic infections, respectively. Given the prominent association of IL-23 and IL-17 with host protection in a growing number of bacterial infection models (e.g. *Klebsiella pneumoniae* [51]), it is not unlikely that the Th17 lineage evolved to cope with a range of extracellular bacterial pathogens, although more studies will be needed to define the range of pathogens linked to Th17.

Irrespective of the focus on autoimmunity or host defense, a number of crucial questions remain to be answered. How are IL-23 and IL-12 differentially regulated by pathogens and their products, and how might inappropriate Th17 responses be induced and lead to immune pathogenesis? Is there a master regulator of the Th17 differentiation program, analogous to T-bet and GATA3 for Th1 and Th2 lineages, respectively? How might TGF- β initiate Th17 development while playing a role in regulatory T-cell function? What role does IL-23 play in amplifying or maintaining Th17 responses *in vivo*? Are there complementary or antagonistic roles for Th17 and Th1 responses in chronic inflammatory disease? Clearly, a great deal is yet to be learned in this field of research, but with the discovery of this novel pathway in immune regulation, the foundation for a new era in treatment of diseases of immunity has been laid.

Update

The study cited in the text as PRM *et al.*, unpublished, has now been accepted for publication [52*]. A complementary study by Veldhoen *et al.* [53*] has also been published while this article was in revision. Each of these studies identifies TGF- β as an important factor in driving the development of Th17 cells.

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- of special interest
- of outstanding interest

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