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Immunity
Review

Th17 Cell Differentiation: The Long and Winding Road

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The characterization of the new lineage of IL-17-producing CD4$^+$ T helper (Th17) cells has revolutionized our current understanding of T cell-mediated immunity. Over the past five years, there have been many twists and turns as the pathways that lead to Th17 cell differentiation have been elucidated. Not least of these was the discovery that TGF-β is a crucial cytokine for Th17 cell development, suggesting that Th17 and regulatory T cell subsets share reciprocal developmental pathways during the pathogenesis or control of inflammation. This review aims to bring together the observations that have formed current opinion on factors that promote and contain Th17 cell development, in both mouse and man. Unresolved controversies in this field are also discussed: For example, IL-23 is absolutely required for disease pathogenesis in many models of Th17-cell-mediated autoimmunity, yet its role in Th17 cell development is relatively unclear.

Discovery of the Th17 Cell Subset—A Brief History

The recent advances in our understanding of the development of Interleukin (IL)-17-producing CD4$^+$ effector T cells (Th17 cells) have led to a substantial revision of the T helper subset hypothesis. In 1986, a cornerstone of modern T cell biology was laid down by Mossman and Coffman when they published two landmark papers that proposed the Th1-Th2 hypothesis (Coffman and Carty, 1986; Mosmann et al., 1986). The theory was based on the observation that distinct subsets of CD4$^+$ T helper (Th) cells expressed discrete cytokine profiles that defined their function: Th1 cells induce cell-mediated inflammatory responses, and Th2 cells provide B cell help. Importantly, Coffman predicated that the effector cytokines produced by one subset of cells would regulate the development and function of the other. This concept has been validated over the past decades by molecular and genetic studies demonstrating a complex network of cross-regulating Janus kinase (JAK) and signal transducer and activator of T cells (STAT) signaling pathways as well as transcriptional regulators such as T-bet and GATA-3 that coordinate genetic programming of the differentiating T cells.

The Th1-Th2 hypothesis has served the immunology community well, particularly in understanding infectious and allergic diseases. Nevertheless, this model does not fit all systems; there are many inconsistencies when it comes to the study of organ-specific autoimmune diseases. Th1-cell-mediated responses can certainly play an important role in the initiation of autoantigen-specific inflammation, exemplified by T cell passive-transfer studies. However, depletion of critical Th1-specific factors often gave contradictory results. For example, interferon (IFN)-γ, the hallmark Th1 mediator that is critical for antitumor and antimicrobial responses, has paradoxical roles during chronic inflammation. Injection of IL-12 or IFN-γ blocked experimental autoimmune encephalomyelitis (EAE) (Gran et al., 2004; Voorthuis et al., 1990), whereas genetic or antibody-mediated depletion of IFN-γ enhanced disease (Billiau et al., 1988; Willenborg et al., 1999), suggesting that Th1-cell-produced factors might in fact regulate chronic inflammation. Deletion of the Th1 pathway molecules IL-12(p35), IL-12Rβ2, IFN-γR, and STAT1 has further supported the role of Th1 cells in regulating rather than promoting many models of autoimmunity (Bettelli et al., 2004; Gran et al., 2002; Zhang et al., 2003). Despite these observations, immunologists (including ourselves) continued to maintain that autoimmune diseases are predominantly driven by autoreactive Th1 cells.

In 2000, IL-23 was described as the pairing of the p19 subunit with the p40 subunit, also shared with IL-12 (Oppmann et al., 2000); thus began the study of the relative contribution of IL-12 and IL-23 to chronic inflammatory diseases. On the basis of the observation that IL-12 receptor β2 is expressed in naive T cells, whereas IL-23 receptor is only found in memory-activated T cells (Parham et al., 2002), it seemed likely that because IL-12 is an important factor for Th1 cell development, subsequent exposure to IL-23 might be required for maturation of effector cells. This led to the hypothesis that these two related cytokines would initiate and then sustain Th1-cell-mediated immune responses, which were consistent with the observation that both the IL-12p40- and IL-23p19-deficient mice were resistant to Th1-associated autoimmune diseases (Cua et al., 2003). However, thorough analysis of the CNS-infiltrating cells in EAE-resistant *Il23a*$^{-/-}$ mice showed the presence of neuroantigen-specific IFN-γ secreting Th1 cells (albeit in low numbers) (Langrish et al., 2005). In addition, *Il12a*$^{-/-}$ mice, which lack IL-12 but express functional IL-23, have few IFN-γ secreting cells—yet these mice suffer from a hyperacute form of EAE (Cua et al., 2003; Gran et al., 2002). These unexpected findings suggested that IL-23 may regulate the function of a hitherto unknown population of inflammatory cells and prompted the search for an IL-23-dependent cell population capable of inducing chronic inflammation.

In 2003, Gurney and colleagues showed that IL-23 promotes the production of IL-17 by activated T cells (Aggarwal et al., 2003). Around the same time, Langrish, Murphy, and colleagues established that IL-23 is a key cytokine that induced expansion of IL-17-producing CD4$^+$ T cells and that autoimmune resistant *Il23a*$^{-/-}$ mice have very few cells capable of secreting IL-17 (Langrish et al., 2005; Murphy et al., 2003). IL-17 was known as a proinflammatory cytokine that mediates multiple chronic inflammatory responses including angiogenesis, recruitment of inflammatory cells, and induction of proinflammatory mediators by endothelial and epithelial tissues (reviewed in this issue of

Immunity by Ouyang et al. [2008]). These observations led to the proposal that IL-23 is required for the development and/or expansion of inflammatory IL-17-producing cells (Langrish et al., 2005; Murphy et al., 2003). On the basis of the characteristic cytokine profile, these cells were subsequently called ThIL-17 or Th17 (Langrish et al., 2005; McKenzie et al., 2006; Weaver et al., 2006). Gene-expression analysis of the IL-17-producing cells showed a distinct gene-expression profile compared to Th1 cells (Langrish et al., 2005). IL-23-driven cells expressed high amounts of IL-17A, IL-17F, and Tumor necrosis factor (TNF)α. In contrast, IL-12 specifically promoted expression of IFN-γ, granzyme F, granzyme G, TNF-related apoptosis-inducing ligand (TRAIL), B lymphocyte stimulator (BLYS), and Fas ligand. These differences predicted that Th1 and Th17 cells probably have distinct immune functions.

The concept that Th17 cells are responsible for driving autoimmune inflammation was finally established when it was shown that EAE was induced by passive transfer of IL-17-producing memory activated $CD4^+$ T cells (Langrish et al., 2005). In addition, both IL-12 and IFN-γ were found to suppress IL-17 production (Langrish et al., 2005; Murphy et al., 2003), and the lack of this suppression in IL-12- or IFN-γ-deficient mice may contribute to EAE exaceration. Two additional landmark studies further showed that both Th1- and Th2-cell-specific transcriptional regulators (STAT1 and T-bet, STAT6 and GATA3, respectively) inhibited Th17 cell differentiation (Harrington et al., 2005; Park et al., 2005). Thus, as predicted by Mossman and Coffman, effector cytokines produced by distinct T cell subsets governed the development and function of another. Collectively, these initial studies ushered in the concept of the Th17 hypothesis and began to change the way we viewed T cell biology and inflammatory diseases.

RORγt Is the Th17-Cell-Specific Transcription Factor

After the observation that Th17 cells expressed a distinct subset of cytokines and chemokines compared to Th1 and Th2 cells, we performed an Affymetrix gene-expression analysis and found a number of Th17-cell-specific factors with potential DNA binding activities. In collaboration with Dan Littman's group, we confirmed that *Rorc*—which encodes RORγt, a retinoid orphan nuclear receptor—is specifically expressed in both human and mouse Th17 cells (Ivanov et al., 2006; Wilson et al., 2007). The Th17 cells for this analysis were initially derived by stimulation of activated T cells with IL-23. Subsequent experiments showed that stimulation of naive T cells with transforming growth factor (TGF)-β and IL-6 (see below) also enhanced RORγt mRNA expression. Expression of RORγt in naive T cells was both necessary and sufficient to induce IL-17A, IL-17F, and IL-23R expression (Ivanov et al., 2006). Furthermore, $Rorc^{-/-}$ mice are unresponsive to IL-23 stimulation, have reduced number of Th17 cells, and are resistant to autoimmune diseases. More recently, Dong and colleagues further demonstrated that RORα synergizes with RORγt to promote differentiation and function of Th17 cells (Yang et al., 2008). Thus, *Rora-Rorc* double-mutant mice harbor few Th17 cells and may be more resistant to inflammatory diseases. Upregulation of RORγt is STAT3 dependent, and maximal IL-17 production also appears to require functional STAT4 (Harris et al., 2007; Mathur et al., 2007; Yang et al., 2007)—the signaling pathways involved in Th17 cell differentiation are fully discussed in the review by O'Shea and Murray (2008) in this issue.

Cytokines that Regulate Th17 Cell Differentiation in Mice

One major issue with the Th17 hypothesis in the early days was the lack of clarity for the initial regulation of Th17 cell lineage commitment. IL-23 was initially shown to induce IL-17 production in activated T cells, and because IL-23R is not expressed on naive T cells, it is not surprising that it was difficult to demonstrate direct induction of de novo Th17 cells by IL-23. However, two papers from Dong and colleagues and Weaver and colleagues suggested that this was possible if IFN-γ and IL-4 were blocked (Harrington et al., 2005; Park et al., 2005).

In early 2006, three research groups demonstrated that TGF-β acting in the presence of proinflammatory cytokines, particularly IL-6, is sufficient to induce naive T cell differentiation into Th17 cells (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006a) (Figure 1A). Importantly, stimulation of naive T cells with TGF-β and IL-6 also enhanced RORγt mRNA expression (Ivanov et al., 2006). These studies led to the general acceptance that IL-23 does not directly enhance the effects of TGF-β and may not be required during the initial differentiation of Th17 cells in the presence of TGB-β and IL-6. The role of IL-23 will be discussed later.

Previously, TGF-β had been associated primarily with anti-inflammatory effects, particularly because it is intricately involved in the induction, maintenance, and function of $Foxp3^+$ Treg cells that are generated in the thymus (natural nTreg cells) and induced in the periphery (inducible iTregs cells) (reviewed in this issue by Li and Flavell [2008]). It was already known that IL-6 inhibited the suppressive function of nTreg cells (Pasare and Medzhitov, 2003). The surprise was that the presence of IL-6 along with TGF-β, provided either exogenously or by nTreg cells present in the culture, resulted in upregulation of IL-17 in naive T cells (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006a). In this context, IL-6 most probably serves two functions. First, IL-6 favors Th17 cell induction while inhibiting Treg cells: Korn et al. showed that IL-6-deficient mice are resistant to EAE in part because of their propensity to expand Treg cells rather than Th17 cells after immunization (Korn et al., 2007). Second, IL-6 upregulates factors in naive T cells that are probably important for further development of the Th17 cell phenotype: IL-23R and IL-21 (Ivanov et al., 2006; Zhou et al., 2007).

The in vivo source of TGF-β during Th17 cell differentiation is not clear. Several lines of evidence suggest that $Foxp3^+$ Treg cells may be involved in the differentiation of Th17 cells through the provision of TGF-β. In vitro, dendritic cells (DCs) stimulated with certain microbial components such as curdlan and lipopolysaccharide (LPS) are capable to induce Th17 cells, but this requires an exogenous source of TGF-β, which can be provided by the presence of Treg cells in the culture (LeibundGut-Landmann et al., 2007; Veldhoen et al., 2006a). In contrast, zymosan and *Mycobacterium tuberculosis* appear to induce sufficient TGF-β from DC to negate this requirement for Treg cells in vitro (Veldhoen et al., 2006b). However, Th17 cells are notably absent in mice that lack TGF-β production only in $CD4^+$ T cells (Li et al., 2007), even after immunization with adjuvant containing *Mycobacterium*, further suggesting that T cell sources of TGF-β are

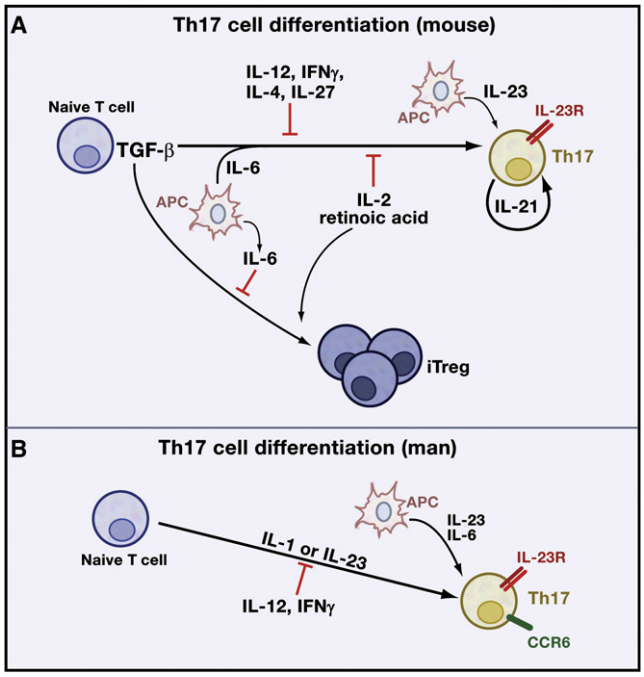

Figure 1. Th17 Cell Differentiation in Mouse and Man

(A) In mouse, naive T cells activated in the presence of TGF-β (possibly provided by Treg cells) and IL-6 begin differentiation toward the Th17 cell subset; IL-6 upregulates IL-21 and IL-23R to further their Th17 development. In the absence of IL-6, TGF-β instead induces regulatory T cells. Th17 cell development is inhibited by Th1 and Th2 cytokines, as well as IL-2 and retinoic acid.

(B) In man, IL-23 or IL-1 drives differentiation of Th17 cells that express IL23R and CCR6. IL-1's effects may be enhanced by IL-23 and/or IL-6. Th1-cell-promoting cytokines inhibit Th17 cell development.

required for induction of the Th17 cell response in vivo. It was nevertheless not clarified whether Treg cells are the important contributor of TGF-β.

In contrast to IL-6, IL-2 is required for iTreg cell generation and nTreg cell survival (Davidson et al., 2007; Setoguchi et al., 2005; Zheng et al., 2007a) but is dispensible for, and in fact inhibits, Th17 cell differentiation (Laurence et al., 2007). Hence, IL-2-deficient mice have large numbers of Th17 cells but are deficient in peripheral Treg cells, and they suffer severe lymphoproliferative immunopathology. This is perhaps surprising, given the dependence of other helper T cell subsets on IL-2, and suggests that additional factor(s) substitute for IL-2 during Th17 cell development. One candidate is IL-21, another member of the common gamma chain cytokine family along with IL-2. IL-21 is produced by Th17 cells upon stimulation by IL-6 and by IL-21 itself, although other subsets of T cells may also produce IL-21; it is also thought to promote B cell responses (IL-21 functions are reviewed by Ouyang et al. [2008]). IL-21 acts in an autocrine manner to promote Th17 cell differentiation along with TGF-β, and may even be able to compensate for absent IL-6 for induction of RORγt and Th17 cell differentiation (Korn et al., 2007; Nurieva et al., 2007; Zhou et al., 2007). Although it is clear that IL-21 enhances the rate of Th17 cell conversion, it may not be essential for the lineage commitment of Th17 cells or their pathogenic functions.

Much like IL-2, retinoic acid has recently been shown to promote Foxp3$^+$ Treg cell induction over Th17 cell induction by TGF-β, even in the presence of IL-6. Retinoic acid binds retinoic acid receptor (RAR) to directly induce Foxp3 expression and inhibits RORγt expression and IL-17 production (Elias et al., 2008; Mucida et al., 2007; Schambach et al., 2007) (Figure 1A). In the intestine and draining mesenteric LN, CD103$^+$ dendritic cells have been shown to metabolize dietary beta carotene (vitamin A) to secrete retinoic acid (Coombes et al., 2007). These CD103$^+$ DCs also produce TGF-β and are strong inducers of Foxp3$^+$ regulatory T cells but poor inducers of Th17 cells unless retinoic acid is inhibited. In contrast, CD103$^-$ DCs produce IL-6, TNFα, and IL-23 but low amounts of TGF-β, and they are poor Treg cell inducers. Thus, in the context of TGF-β, retinoic acid and IL-6 may be key switch factors that promote the balance between Treg cell and Th17 cell development in the gut mucosa. The finding that retinoic acid is produced by DCs in this site may explain why the Th17 cell population is limited despite relatively high amounts of IL-6 in the intestinal tract. Treg cells are clearly important in maintaining tolerance in the intestine, but the role of Th17 cells in this site is less clear. Th17 cells have been isolated from the gut mucosa of patients with active Crohn's disease and are found in some mouse models of colitis (Annunziato et al., 2007; Kullberg et al., 2006; Yen et al., 2006). On the other hand, depletion of IL-17 exacerbated inflammation in the DSS model of colitis in mice (Ogawa et al., 2004), suggesting that IL-17 may have a protective and/or reparative role in the intestine after exposure to toxins or pathogens. The cellular source of this "protective" IL-17 has yet to be determined because many cell types, including myeloid cells (Kullberg et al., 2006), can produce IL-17.

Much as the Th1 and Th2 cell subsets crossregulate the differentiation of the other, they also appear to negatively regulate differentiation of Th17 cells. Addition of IL-12, IFN-γ, or IL-4 to cultures inhibits either IL-23- or TGF-β plus IL-6-stimulated differentiation of mouse and human Th17 cells (Annunziato et al., 2007; Harrington et al., 2005; Murphy et al., 2003; Park et al., 2005; Wilson et al., 2007). It is not clear for how long developing Th17 cells are susceptible to this regulation. One paradox in this regard is the observations from many investigators that a subset of Th17 cells may coproduce IFN-γ. This is particularly apparent when looking at T cells from sites of inflammation, for example, the brain of mice after EAE induction (Chen et al., 2006; McGeachy et al., 2007) or the intestine of patients with active Crohn's disease (Annunziato et al., 2007). This suggests that IFN-γ cannot always downregulate IL-17 production and may in fact contribute to the pathogenic function of Th17 cells, although this has not been thoroughly investigated. In relation to this point, it is interesting to note that mice lacking the Th1 transcription factor T-bet produce greater numbers of IL-17$^+$ cells (Park et al., 2005), presumably because of reduced IFN-γ production, yet these mice are also extremely resistant to Th17-cell-mediated models of inflammation, including EAE (Bettelli et al., 2004). It is still not clear whether the important contribution of T-bet in these models is toward the function of Th17 cells, Th1 cells, or to other cells such as DCs. T-bet may be expressed in Th17 cells stimulated with IL-23 (Chen et al., 2006), and T-bet has been suggested to positively regulate IL-23R expression (Gocke et al., 2007). However, because IL-6 also stimulates IL-23R upregulation in the presence of TGF-β, which represses T-bet, it does not seem likely that this fully explains the requirement for T-bet in Th17 cells.

Human Th17 Cell Differentiation

Although there is general agreement on the factors required for the generation of murine Th17 cells, the crucial initiating cytokines for human Th17 cell development remain less clear. Four groups have recently found that the combination of TGF-β plus IL-6 does not drive IL-17-producing T cells in vitro (Acosta-Rodriguez et al., 2007a; Chen et al., 2007; van Beelen et al., 2007; Wilson et al., 2007); in fact, TGF-β inhibited production of IL-17 (Acosta-Rodriguez et al., 2007a; Wilson et al., 2007). However, although there are similarities, these investigators did not quite reach a common consensus as to the factors that are required or sufficient to drive the Th17 cell phenotype in human T cells. Chen et al. showed that IL-23 was able to drive Th17 cell differentiation, and Wilson et al. found that culture with either IL-23 or IL-1 stimulated the Th17 cell phenotype, with little or no synergy when both cytokines were present. In contrast, Acosta-Rodriguez et al. identified IL-1 as driving human Th17 cells in vitro, with IL-23 and IL-6 able to potentiate the effects of IL-1 (Figure 1B). Thus, there are some outstanding questions remaining when it comes to the differentiation of human Th17 cells. It seems that the controversy of whether TGF-β has any role to play in their development has not yet been fully laid to rest. The description of IL-21 as an autocrine growth factor and its potential to couple TGF-β in the generation of murine Th17 cells further raises the notion that as-yet-unrecognized cytokines may have important functions in human Th17 differentiation.

Although their differentiation may not be identical, the phenotype of human Th17 cells does bear many similarities to murine Th17 cells. Both subsets produce IL-17, IL-17F, and IL-22 as hallmark cytokines. Expression of the IL-23R (Annunziato et al., 2007; Wilson et al., 2007) and the chemokine receptor CCR6 (Acosta-Rodriguez et al., 2007b; Annunziato et al., 2007; Hirota et al., 2007; Lim et al., 2008; Singh et al., 2008) additionally define this subset. It appears that IFN-γ production by Th17 cells can be even further segregated in humans on the basis of chemokine receptor expression: $CCR6^+CCR4^+$ cells produce exclusively IL-17, whereas $CCR6^+CXCR3^+$ cells coproduce IL-17 and IFN-γ (Acosta-Rodriguez et al., 2007b). Human Th17 cells may also produce IL-26 (Wilson et al., 2007), whereas murine pathogenic Th17 cells have been shown to produce TNFα and IL-6 (Langrish et al., 2005).

Stimuli that Promote Th17-Cell-Inducing Dendritic Cells

As the cytokines that promote Th17 cell differentiation have been delineated, so have the microbial factors and pathways that stimulate the Th17-cell-inducing phenotype in DC. IL-17 is important in the control or clearance of various pathogens (reviewed by Ouyang et al. [2008] and Umemura et al. [2007]). These include the extracellular bacteria *Klebsiella pneumonia* (Ye et al., 2001), *Citrobacter rodentium* (Mangan et al., 2006), and *Borrelia Burgdorferi* (Infante-Duarte et al., 2000), as well as systemic infection with the fungal pathogen *Candida albicans* (Huang et al., 2004). Candida-specific Th17 cell responses have also been observed in peripheral blood of human donors (Acosta-Rodriguez et al., 2007b). Th17 cells do not appear to be required for clearance of Mycobacterium infection but may facilitate recruitment of Th1 cells to the lungs and thus aid the response (Khader et al., 2007). An important point to bear in mind when considering effects of absence of IL-17 signaling is that IL-17 can also be produced as part of the innate immune response.

Several adjuvants including LPS and CpG promote some Th17-cell-promoting cytokines such as IL-23 and IL-6 production from DCs, but these components also induce strong IL-12 responses and therefore promote Th1 cell responses as well as low amounts of IL-17 (LeibundGut-Landmann et al., 2007). However, the *Saccharomyces cerevisiae* cell-wall component zymosan, as well as *Mycobacteria*, which both contain ligands for TLR2 but do not signal exclusively through this pathway, promote strong Th17 cell responses in murine cell culture and in vivo (Veldhoen et al., 2006b). *Mycobacterium tuberculosis* is the key ingredient of complete Freund's adjuvant (CFA), the widely used adjuvant for murine immunizations; although CFA was long thought to induce Th1 cell responses, it is now clear that it also very effectively induces Th17 cell responses. This is associated with the capacity to strongly induce IL-6 and IL-23 as well as TGF-β, allowing the differentiation of naive T cells into Th17 cells without the requirement for addition of exogenous TGF-β as was required for LPS-induced Th17 cells (Veldhoen et al., 2006a). The source of antigen presenting cell (APC) may also influence the response elicited: Acosta-Rodriguez and colleagues found that monocytes from human PBMC produced large amounts of IL-1 and IL-6 but not IL-12 in response to various TLR stimulants including LPS and peptidoglycan and were therefore good inducers of Th17 cells. However, generation of dendritic cells by culture of monocytes with IL-4 resulted in cells that produced greater amounts of IL-12 in response to these stimulants and were therefore poor inducers of the Th17 cell phenotype (Acosta-Rodriguez et al., 2007a).

β-glucan components in zymosan also signal through TLR-independent pathways to promote Th17 cell induction (Gantner et al., 2003). Fungal β-glucans, including Curdlan, bind to the C type lectin Dectin-1 on dendritic cells and trigger IL-23 production (LeibundGut-Landmann et al., 2007). These Dectin-1-activated DCs also produce proinflammatory cytokines including TNFα and IL-6 and upregulate costimulatory molecules such as CD80 and CD40. However, in contrast to TLR-stimulated DCs, Dectin-1 stimulated DCs do not produce substantial amounts of IL-12 and appear to preferentially induce Th17 over Th1 cell responses. An additional TLR-independent pathway for generation of Th17-promoting DC is the ligation of the intracellular receptor NOD2 by muramyldipeptide (MDP)—a breakdown product of internalized peptidoglycan (van Beelen et al., 2007). The signaling pathways for NOD2 and Dectin-1 may converge in their shared use of caspase recruitment domain (CARD) 9.

Interestingly, peptidoglycan joins zymosan as one of the few microbial components that can be substituted for Mycobacteria during the induction of autoimmune inflammation in the mouse model of EAE (Visser et al., 2005). It is likely that the common factor is the triggering of Th17-cell-promoting pathways in DCs by these pathogen components, although this has not been investigated for peptidoglycan. In humans, the link between infection and autoimmune disease remains intriguing yet elusive. However, the discovery of pathogens that preferentially promote Th17 cell responses and their corresponding ability to promote Th17-cell-mediated autoimmune inflammation in murine models is very suggestive. Further compelling examples

of this link are found in murine arthritis models. Infection with *Borrelia burgdorferi*, the causative agent of Lyme's disease, can lead to subsequent development of arthritis, and in mice, this is dependent on IL-17 (Burchill et al., 2003). Fungal β-glucans have also been found to trigger onset of arthritis via dectin-1 receptor activation in genetically susceptible SKG mice (bearing a mutation in the SH2 domain of ZAP70) (Yoshitomi et al., 2005).

Th17 Cells in Immunopathology

It is now clear that IL-23 and IL-17 are associated with a number of human autoimmune disorders, validating the findings in mouse models (IL-17 functions are more extensively reviewed by Ouyang et al. [2008]). One of the earliest human disease associations for IL-17 was demonstrated in rheumatoid arthritis (RA). Lebecque and coworkers showed that IL-17 induced human synovial cells to produce IL-6, IL-8, and TNF, suggesting that IL-17 may directly act on stromal cells to promote inflammation (Fossiez et al., 1996; Kotake et al., 1999). In addition, Th17 cells found in human arthritic synovium expressed receptor activator of nuclear factor kappa B ligand (RANKL), which is a potent cytokine that promotes formation of bone resorptive osteoclasts (Kim et al., 2007). These observations suggest that Th17 cells are likely to be highly pathogenic in RA. Likewise, IL-17$^+$ CD4$^+$ and CD8$^+$ T cells have been identified in active lesions in the brain from multiple sclerosis (MS) patients (Tzartos et al., 2008). Prat and colleagues elegantly demonstrated that receptors for IL-17 and IL-22 are present on inflamed endothelium in MS lesions and that these cytokines may potentiate migration of inflammatory T cells across the blood-brain barrier (Kebir et al., 2007). Psoriasis is another immune-mediated inflammatory disease that has been linked to inappropriate Th17 cell responses. The Th17 factors RORγt, IL-17, IL-22, and IL-23 mRNA are all elevated in psoriatic lesional skin (Lee et al., 2004; Teunissen et al., 1998; Wilson et al., 2007; Wolk et al., 2004). Anti-p40 treatment—which inhibits both IL-12 and IL-23—was highly efficacious for treatment of psoriasis (Krueger et al., 2007). Future clinical studies using anti-p19 will determine whether IL-23 is the target of choice for treatment of this skin inflammatory disease.

Currently, one of the most powerful tools for studying human diseases is genome-wide disease-association analysis. Using this strategy, Cho and colleagues screened 547 patients and case-control cohorts and identified IL-23R as an inflammatory-bowel-disease gene (Duerr et al., 2006). A number of IL-23R single-nucleotide polymorphisms (SNPs) including Arg381Gln were found to be highly associated with IBD. This study underscores the critical importance of IL-23 signaling in regulation of chronic tissue inflammation. At this point, it is not clear whether it is gain of function or loss of function that lead to human IBD. Additional genetic studies have since shown that IL-23R polymorphism is also linked to psoriasis, psoriatic arthritis, ankylosing spondylitis, and multiple-sclerosis-disease susceptibility (Burton et al., 2007; Capon et al., 2007; Cargill et al., 2007; Illes et al., 2008; Smith et al., 2007). Together, these studies demonstrate that IL-23 and/or IL-23R are potential targets for treatment of chronic inflammatory disorders such as psoriasis and Crohn's disease. In addition, because IL-17 is clearly involved in joint inflammation and bone erosion, it is probably an effective therapeutic target for treatment of RA.

TGF-β-IL-6 Promotes IL-10: A Self-Regulating Mechanism for Th17 Cells?

Regulation of robust adaptive T cell responses is crucial for preventing host tissue damage during clearance of infectious pathogens and most probably also during periods of remission from autoimmune disease. In this regard, IL-10 has long been known to be important for regulation of Th1 cell responses to pathogens. However, it was only recently shown that the Th1 effector cells are themselves important coproducers of IL-10 with IFN-γ, and thus "self-regulate" (Anderson et al., 2007; Jankovic et al., 2007). IL-27 production by activated DCs promotes IL-10 production in Th1 cells and may therefore be an important mediator of this type of regulation (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007).

Our lab and others have recently demonstrated that Th17 cells generated in the presence of TGF-β plus IL-6 include a subset of cells that produce IL-10 along with IL-17 (McGeachy et al., 2007; Stumhofer et al., 2007). Unlike Th1 cells, IL-27 was found to inhibit the generation of Th17 cells but promote IL-10 (Stumhofer et al., 2007). Hence, although IL-27 is known to regulate the severity of Th17 cell responses, the mechanisms may be different to Th1 cell regulation.

While investigating the regulation of effector Th17 cells, we found that restimulation of activated Th17 cells in the presence of TGF-β and IL-6 also resulted in IL-10 production concomitant with increased IL-17 (McGeachy et al., 2007). Analogous to the Th1 cell situation, this T cell-produced IL-10 was able to regulate Th17 cell immunopathology, which was demonstrated by reduced disease severity after cotransfer of TGF-β-plus-IL-6-stimulated T cells with IL-23-stimulated (pathogenic) T cells. Hence, we proposed that continued exposure of differentiating Th17 cells to TGF-β and IL-6 results in regulation through production of IL-10, as well as other mechanisms (Figure 2). Just as Th1 cells that produce IL-10 are not considered regulatory T cells, we do not consider these cells "regulatory Th17 cells" but rather that this IL-10 production may be a mechanism of self-regulation of the otherwise potentially dangerous Th17 cell response. Importantly, IL-23 does not maintain the IL-10 induced by TGF-β plus IL-6 but also does not directly inhibit IL-10 production. Hence, stimulation with IL-23 results in gain of pathogenic function, whereas continued exposure to TGF-β may self-limit the response even in the presence of IL-23, and the balance of signals will determine the outcome of the response.

There is an unresolved question over whether Treg cells can suppress Th17 cells, but there are suggestions that in contrast to IFN-γ, IL-17 production and/or Th17 cell development may not be downregulated by Treg cells in vitro (Annunziato et al., 2007; O'Connor et al., 2007). Defective Treg cell function or numbers has been associated with several autoimmune diseases that are now also Th17-cell-associated (Baecher-Allan and Hafler, 2004). Also, there is some debate over whether DCs or T cells are more pivotal targets for Treg cells. Our studies with TGF-β and IL-6 activation of effector cells suggested that although IL-17 may not be downregulated, the outcome of interactions between Treg cells and effector Th17 cells could in fact be regulation of the inflammatory response. However, the issue of whether Treg cells can effectively inhibit Th17 cell responses has yet to be fully clarified. In any case, it appears that continued strong proinflammatory signals in the presence of TGF-β plus

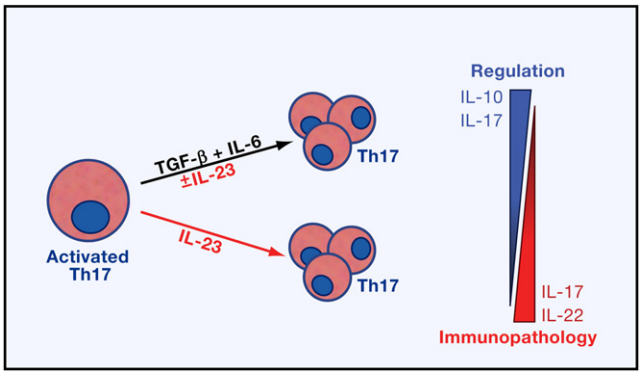

Figure 2. Regulation of Th17 Cell Responses by TGF-β- and IL-6-Induced IL-10

Continued stimulation of activated murine Th17 cells with TGF-β and IL-6 results in enhancement of IL-17 and IL-10. In contrast, IL-23 only enhances IL-17. The balance of cells stimulated under each condition will ultimately determine whether the Th17 cell response is regulated or leads to severe tissue damage, as depicted by the sliding scale on the right.

IL-6 will trigger mechanisms of self-regulation in Th17 cells to limit bystander immunopathology.

What Is the Role of IL-23 in Th17 Cell Biology?

The findings that TGF-β plus IL-6 alone may not be sufficient to induce the full pathogenic Th17 cell phenotype coupled with the observations in IL-23-deficient mice raise again the question: What is the bona fide function of IL-23 in Th17 cell biology? Because IL-23 is not required for initial IL-17 induction in vitro, and the IL-23R is not expressed on naive T cells, the early proposals that IL-23 directly drives differentiation of the Th17 cell lineage (Harrington et al., 2005; Langrish et al., 2005; Park et al., 2005) have been predominantly discounted. Several alternative suggestions for the function of IL-23 have been put forth, and even started to gain acceptance, but we feel the evidence is still inconclusive.

The most commonly presumed function of IL-23 is to expand differentiated Th17 cells or maintain IL-17 production, two overlapping ideas based on similar data. These conclusions were reached after in vitro observations that the presence of IL-23 during culture of activated cells results in increased proliferation of activated T cells (Oppmann et al., 2000) and increased frequencies of IL-17$^+$ cells (Langrish et al., 2005) or that IL-23 was required during restimulation of TGF-β plus IL-6-stimulated cells to maintain their IL-17 production (Veldhoen et al., 2006a). In a similar vein, it has also been suggested that IL-23 may stabilize the phenotype of Th17 cells through STAT3-dependent mechanisms (Yang et al., 2007; Zhou et al., 2007), although IL-6 and IL-21 also share the STAT3 signaling pathway with IL-23. Finally, an alternative hypothesis for the current data is that IL-23 is a survival factor for Th17 cells (Elson et al., 2007).

Although all of these suggestions broadly concur with the reduced frequencies of IL-17$^+$ cells generated in IL-23-deficient mice, they have yet to be convincingly supported in vivo, and these functions of IL-23 may not provide a full picture. In addition, they do not incorporate the findings that IL-23 can promote expression of additional pathogenic factors in Th17 cells, including IL-22, that are required for disease pathogenesis in certain models including psoriasis (Liang et al., 2006; Zheng et al., 2007b).

In the *Citrobacter rodentium* model described by Mangan et al., IL-17$^+$ cells were observed in *Il23a*$^{-/-}$ mice but were unable to clear the infection (Mangan et al., 2006). The mechanisms for this observation were not investigated, but one explanation could be a requirement for IL-23 to promote the effector function of Th17 cells. However, it is also clear that IL-23 can promote inflammatory functions in cells of the innate immune system (Cua et al., 2003; Uhlig et al., 2006) (Michel et al., 2007); therefore, the results obtained in IL-23-deficient animals need to be interpreted with care.

Rather than expanding or stabilizing pre-existing Th17 cells, could IL-23 in fact have an earlier role during their differentiation in vivo? IL-23R expression is upregulated remarkably early in response to IL-6 (within 1 to 2 days [Zhou et al., 2007], making it possible that IL-23 begins to act quite early during Th17 cell differentiation. These findings suggest that IL-23 may be more central to differentiation of effector Th17 cells than is currently appreciated, and it might not merely expand or enhance survival of pathogenic Th17 cells.

Summary and Future Perspectives

Over the past few years, there have been remarkable advances in our understanding of the regulation of T cell responses during immune homeostasis and disease pathogenesis. The key areas of discovery have been identification of the cytokines and transcriptional regulators that control the function of distinct T cell subsets. We now recognize that specific cytokines can induce activation of transcriptional factors such as T-bet, GATA3, RORγt, and Foxp3, which control the development and function of Th1, Th2, Th17, and Treg cells, respectively. However, there are still many unknowns. Although in vitro studies suggest that T cells follow a strict developmental program when activated with discrete cytokines, in vivo T cells are actually exposed to a complex cytokine milieu and display considerable plasticity. For example, many of the CD4$^+$ T cells isolated from an inflamed central nervous system during EAE do not exclusively belong to either the Th1 or Th17 cell subsets as currently defined because they coproduce IL-17 and IFN-γ. For many years, the presence of Th1 cell signature cytokines implicated Th1 cells in many of the diseases that are now associated with Th17 cells, raising the possibility that inflammatory autoimmune diseases are not necessarily restricted to just Th1 or Th17 cell type responses. The possible synergistic or antagonistic interactions between these subsets in vivo need to be more fully clarified.

The study of human Th17 cell differentiation and function is only in its infancy, and there remains much to know about how the cells that drive inflammation in human diseases are generated. Although human genetic evidence suggests that targeting IL-23 or IL-23R may benefit IBD patents, more work is also needed to understand the precise function of this potent immune regulator. Whether IL-23 is important in the generation or expansion of pathogenic Th17 cells and the relative contribution of IL-23 to activation of inflammatory myeloid cells are still unclear. Careful analysis of the in vivo expression of IL-23R and the fate of IL-23R-deficient cells should help to finally resolve these issues.

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REFERENCES

Acosta-Rodriguez, E.V., Napolitani, G., Lanzavecchia, A., and Sallusto, F. (2007a). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat. Immunol. *8*, 942–949.

Acosta-Rodriguez, E.V., Rivino, L., Geginat, J., Jarrossay, D., Gattorno, M., Lanzavecchia, A., Sallusto, F., and Napolitani, G. (2007b). Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat. Immunol. *8*, 639–646.

Aggarwal, S., Ghilardi, N., Xie, M.H., de Sauvage, F.J., and Gurney, A.L. (2003). Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J. Biol. Chem. *278*, 1910–1914.

Anderson, C.F., Oukka, M., Kuchroo, V.J., and Sacks, D. (2007). CD4(+)CD25(-) Foxp3(-) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J. Exp. Med. *204*, 285–297.

Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., Parente, E., Fili, L., Ferri, S., Frosali, F., et al. (2007). Phenotypic and functional features of human Th17 cells. J. Exp. Med. *204*, 1849–1861.

Awasthi, A., Carrier, Y., Peron, J.P., Bettelli, E., Kamanaka, M., Flavell, R.A., Kuchroo, V.K., Oukka, M., and Weiner, H.L. (2007). A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat. Immunol. *8*, 1380–1389.

Baecher-Allan, C., and Hafler, D.A. (2004). Suppressor T cells in human diseases. J. Exp. Med. *200*, 273–276.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235–238.

Bettelli, E., Sullivan, B., Szabo, S.J., Sobel, R.A., Glimcher, L.H., and Kuchroo, V.K. (2004). Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. J. Exp. Med. *200*, 79–87.

Billiau, A., Heremans, H., Vandekerckhove, F., Dijkmans, R., Sobis, H., Meulepas, E., and Carton, H. (1988). Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-gamma. J. Immunol. *140*, 1506–1510.

Burchill, M.A., Nardelli, D.T., England, D.M., DeCoster, D.J., Christopherson, J.A., Callister, S.M., and Schell, R.F. (2003). Inhibition of interleukin-17 prevents the development of arthritis in vaccinated mice challenged with Borrelia burgdorferi. Infect. Immun. *71*, 3437–3442.

Burton, P.R., Clayton, D.G., Cardon, L.R., Craddock, N., Deloukas, P., Duncanson, A., Kwiatkowski, D.P., McCarthy, M.I., Ouwehand, W.H., Samani, N.J., et al. (2007). Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat. Genet. *39*, 1329–1337.

Capon, F., Di Meglio, P., Szaub, J., Prescott, N.J., Dunster, C., Baumber, L., Timms, K., Gutin, A., Abkevic, V., Burden, A.D., et al. (2007). Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. Hum. Genet. *122*, 201–206.

Cargill, M., Schrodi, S.J., Chang, M., Garcia, V.E., Brandon, R., Callis, K.P., Matsunami, N., Ardlie, K.G., Civello, D., Catanese, J.J., et al. (2007). A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am. J. Hum. Genet. *80*, 273–290.

Chen, Y., Langrish, C.L., McKenzie, B., Joyce-Shaikh, B., Stumhofer, J.S., McClanahan, T., Blumenschein, W., Churakovsa, T., Low, J., Presta, L., et al. (2006). Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J. Clin. Invest. *116*, 1317–1326.

Chen, Z., Tato, C.M., Muul, L., Laurence, A., and O'Shea, J.J. (2007). Distinct regulation of interleukin-17 in human T helper lymphocytes. Arthritis Rheum. *56*, 2936–2946.

Coffman, R.L., and Carty, J. (1986). A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. J. Immunol. *136*, 949–954.

Coombes, J.L., Siddiqui, K.R., Arancibia-Carcamo, C.V., Hall, J., Sun, C.M., Belkaid, Y., and Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J. Exp. Med. *204*, 1757–1764.

Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., et al. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature *421*, 744–748.

Davidson, T.S., DiPaolo, R.J., Andersson, J., and Shevach, E.M. (2007). Cutting edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells. J. Immunol. *178*, 4022–4026.

Duerr, R.H., Taylor, K.D., Brant, S.R., Rioux, J.D., Silverberg, M.S., Daly, M.J., Steinhart, A.H., Abraham, C., Regueiro, M., Griffiths, A., et al. (2006). A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science *314*, 1461–1463.

Elias, K.M., Laurence, A., Davidson, T.S., Stephens, G., Kanno, Y., Shevach, E.M., and O'Shea, J.J. (2008). Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. Blood *111*, 1013–1020.

Elson, C.O., Cong, Y., Weaver, C.T., Schoeb, T.R., McClanahan, T.K., Fick, R.B., and Kastelein, R.A. (2007). Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. Gastroenterology *132*, 2359–2370.

Fitzgerald, D.C., Zhang, G.X., El-Behi, M., Fonseca-Kelly, Z., Li, H., Yu, S., Saris, C.J., Gran, B., Ciric, B., and Rostami, A. (2007). Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat. Immunol. *8*, 1372–1379.

Fossiez, F., Djossou, O., Chomarat, P., Flores-Romo, L., Ait-Yahia, S., Maat, C., Pin, J.J., Garrone, P., Garcia, E., Saeland, S., et al. (1996). T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J. Exp. Med. *183*, 2593–2603.

Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S., and Underhill, D.M. (2003). Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J. Exp. Med. *197*, 1107–1117.

Gocke, A.R., Cravens, P.D., Ben, L.H., Hussain, R.Z., Northrop, S.C., Racke, M.K., and Lovett-Racke, A.E. (2007). T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. J. Immunol. *178*, 1341–1348.

Gran, B., Chu, N., Zhang, G.X., Yu, S., Li, Y., Chen, X.H., Kamoun, M., and Rostami, A. (2004). Early administration of IL-12 suppresses EAE through induction of interferon-gamma. J. Neuroimmunol. *156*, 123–131.

Gran, B., Zhang, G.X., Yu, S., Li, J., Chen, X.H., Ventura, E.S., Kamoun, M., and Rostami, A. (2002). IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: Evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. J. Immunol. *169*, 7104–7110.

Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat. Immunol. *6*, 1123–1132.

Harris, T.J., Grosso, J.F., Yen, H.R., Xin, H., Kortylewski, M., Albesiano, E., Hipkiss, E.L., Getnet, D., Goldberg, M.V., Maris, C.H., et al. (2007). Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. J. Immunol. *179*, 4313–4317.

Hirota, K., Yoshitomi, H., Hashimoto, M., Maeda, S., Teradaira, S., Sugimoto, N., Yamaguchi, T., Nomura, T., Ito, H., Nakamura, T., et al. (2007). Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. J. Exp. Med. *204*, 2803–2812.

Huang, W., Na, L., Fidel, P.L., and Schwarzenberger, P. (2004). Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J. Infect. Dis. *190*, 624–631.

Illes, Z., Safrany, E., Peterfalvi, A., Magyari, L., Farago, B., Pozsonyi, E., Rozsa, C., Komoly, S., and Melegh, B. (2008). 3′UTR C2370A allele of the IL-23 receptor gene is associated with relapsing-remitting multiple sclerosis. Neurosci. Lett. *431*, 36–38.

Infante-Duarte, C., Horton, H.F., Byrne, M.C., and Kamradt, T. (2000). Microbial lipopeptides induce the production of IL-17 in Th cells. J. Immunol. *165*, 6107–6115.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell *126*, 1121–1133.

Jankovic, D., Kullberg, M.C., Feng, C.G., Goldszmid, R.S., Collazo, C.M., Wilson, M., Wynn, T.A., Kamanaka, M., Flavell, R.A., and Sher, A. (2007). Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J. Exp. Med. *204*, 273–283.

Kebir, H., Kreymborg, K., Ifergan, I., Dodelet-Devillers, A., Cayrol, R., Bernard, M., Giuliani, F., Arbour, N., Becher, B., and Prat, A. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat. Med. *13*, 1173–1175.

Khader, S.A., Bell, G.K., Pearl, J.E., Fountain, J.J., Rangel-Moreno, J., Cilley, G.E., Shen, F., Eaton, S.M., Gaffen, S.L., Swain, S.L., et al. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4(+) T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nat. Immunol. *8*, 369–377.

Kim, K.W., Cho, M.L., Lee, S.H., Oh, H.J., Kang, C.M., Ju, J.H., Min, S.Y., Cho, Y.G., Park, S.H., and Kim, H.Y. (2007). Human rheumatoid synovial fibroblasts promote osteoclastogenic activity by activating RANKL via TLR-2 and TLR-4 activation. Immunol. Lett. *110*, 54–64.

Korn, T., Bettelli, E., Gao, W., Awasthi, A., Jager, A., Strom, T.B., Oukka, M., and Kuchroo, V.K. (2007). IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature *448*, 484–487.

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M.T., et al. (1999). IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J. Clin. Invest. *103*, 1345–1352.

Krueger, G.G., Langley, R.G., Leonardi, C., Yeilding, N., Guzzo, C., Wang, Y., Dooley, L.T., and Lebwohl, M. (2007). A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. N. Engl. J. Med. *356*, 580–592.

Kullberg, M.C., Jankovic, D., Feng, C.G., Hue, S., Gorelick, P.L., McKenzie, B.S., Cua, D.J., Powrie, F., Cheever, A.W., Maloy, K.J., and Sher, A. (2006). IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. J. Exp. Med. *203*, 2485–2494.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., and Cua, D.J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J. Exp. Med. *201*, 233–240.

Laurence, A., Tato, C.M., Davidson, T.S., Kanno, Y., Chen, Z., Yao, Z., Blank, R.B., Meylan, F., Siegel, R., Hennighausen, L., et al. (2007). Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity *26*, 371–381.

Lee, E., Trepicchio, W.L., Oestreicher, J.L., Pittman, D., Wang, F., Chamian, F., Dhodapkar, M., and Krueger, J.G. (2004). Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. J. Exp. Med. *199*, 125–130.

LeibundGut-Landmann, S., Gross, O., Robinson, M.J., Osorio, F., Slack, E.C., Tsoni, S.V., Schweighoffer, E., Tybulewicz, V., Brown, G.D., Ruland, J., and Reis e Sousa, C. (2007). Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat. Immunol. *8*, 630–638.

Li, M.O., and Flavell, R.A. (2008). Contextual regulation of inflammation: A duet by transforming growth factor-β gand Interleukin-10. Immunity *28*, this issue, 468–476.

Li, M.O., Wan, Y.Y., and Flavell, R.A. (2007). T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity *26*, 579–591.

Liang, S.C., Tan, X.Y., Luxenberg, D.P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., and Fouser, L.A. (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J. Exp. Med. *203*, 2271–2279.

Lim, H.W., Lee, J., Hillsamer, P., and Kim, C.H. (2008). Human Th17 Cells Share Major Trafficking Receptors with Both Polarized Effector T Cells and FOXP3+ Regulatory T Cells. J. Immunol. *180*, 122–129.

Mangan, P.R., Harrington, L.E., O'Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., Hatton, R.D., Wahl, S.M., Schoeb, T.R., and Weaver, C.T. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. Nature *441*, 231–234.

Mathur, A.N., Chang, H.C., Zisoulis, D.G., Stritesky, G.L., Yu, Q., O'Malley, J.T., Kapur, R., Levy, D.E., Kansas, G.S., and Kaplan, M.H. (2007). Stat3 and Stat4 direct development of IL-17-secreting Th cells. J. Immunol. *178*, 4901–4907.

McGeachy, M.J., Bak-Jensen, K.S., Chen, Y., Tato, C.M., Blumenschein, W., McClanahan, T., and Cua, D.J. (2007). TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat. Immunol. *8*, 1390–1397.

McKenzie, B.S., Kastelein, R.A., and Cua, D.J. (2006). Understanding the IL-23-IL-17 immune pathway. Trends Immunol. *27*, 17–23.

Michel, M.L., Keller, A.C., Paget, C., Fujio, M., Trottein, F., Savage, P.B., Wong, C.H., Schneider, E., Dy, M., and Leite-de-Moraes, M.C. (2007). Identification of an IL-17-producing NK1.1(neg) iNKT cell population involved in airway neutrophilia. J. Exp. Med. *204*, 995–1001.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., and Coffman, R.L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. *136*, 2348–2357.

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., and Cheroutre, H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science *317*, 256–260.

Murphy, C.A., Langrish, C.L., Chen, Y., Blumenschein, W., McClanahan, T., Kastelein, R.A., Sedgwick, J.D., and Cua, D.J. (2003). Divergent pro- and anti-inflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J. Exp. Med. *198*, 1951–1957.

Nurieva, R., Yang, X.O., Martinez, G., Zhang, Y., Panopoulos, A.D., Ma, L., Schluns, K., Tian, Q., Watowich, S.S., Jetten, A.M., and Dong, C. (2007). Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature *448*, 480–483.

O'Connor, R.A., Malpass, K.H., and Anderton, S.M. (2007). The inflamed central nervous system drives the activation and rapid proliferation of Foxp3+ regulatory T cells. J. Immunol. *179*, 958–966.

Ogawa, A., Andoh, A., Araki, Y., Bamba, T., and Fujiyama, Y. (2004). Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice. Clin. Immunol. *110*, 55–62.

Oppmann, B., Lesley, R., Blom, B., Timans, J.C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., et al. (2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity *13*, 715–725.

O'Shea, J.J., and Murray, P.J. (2008). Cytokine signaling modules in inflammatory responses. Immunity *28*, this issue, 477–487.

Ouyang, W., Kolls, J.K., and Zheng, Y. (2008). The biological functions of T helper 17 effector cytokines in inflammation. Immunity *28*, this issue, 454–467.

Parham, C., Chirica, M., Timans, J., Vaisberg, E., Travis, M., Cheung, J., Pflanz, S., Zhang, R., Singh, K.P., Vega, F., et al. (2002). A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J. Immunol. *168*, 5699–5708.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. *6*, 1133–1141.

Pasare, C., and Medzhitov, R. (2003). Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science *299*, 1033–1036.

Schambach, F., Schupp, M., Lazar, M.A., and Reiner, S.L. (2007). Activation of retinoic acid receptor-alpha favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation. Eur. J. Immunol. *37*, 2396–2399.

Setoguchi, R., Hori, S., Takahashi, T., and Sakaguchi, S. (2005). Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. J. Exp. Med. *201*, 723–735.

Singh, S.P., Zhang, H.H., Foley, J.F., Hedrick, M.N., and Farber, J.M. (2008). Human T Cells That Are Able to Produce IL-17 Express the Chemokine Receptor CCR6. J. Immunol. *180*, 214–221.

Smith, R.L., Warren, R.B., Eyre, S., Ho, P., Ke, X., Young, H.S., Griffiths, C.E., and Worthington, J. (2007). Polymorphisms in the IL-12beta and IL-23R Genes Are Associated with Psoriasis of Early Onset in a UK Cohort. J. Invest. Dermatol. Published online November 22, 2007. 10.1038/sj.jid.5701140.

Stumhofer, J.S., Silver, J.S., Laurence, A., Porrett, P.M., Harris, T.H., Turka, L.A., Ernst, M., Saris, C.J., O'Shea, J.J., and Hunter, C.A. (2007). Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat. Immunol. *8*, 1363–1371.

Teunissen, M.B., Koomen, C.W., de Waal Malefyt, R., Wierenga, E.A., and Bos, J.D. (1998). Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J. Invest. Dermatol. *111*, 645–649.

Tzartos, J.S., Friese, M.A., Craner, M.J., Palace, J., Newcombe, J., Esiri, M.M., and Fugger, L. (2008). Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am. J. Pathol. *172*, 146–155.

Uhlig, H.H., McKenzie, B.S., Hue, S., Thompson, C., Joyce-Shaikh, B., Stepankova, R., Robinson, N., Buonocore, S., Tlaskalova-Hogenova, H., Cua, D.J., and Powrie, F. (2006). Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. Immunity *25*, 309–318.

Umemura, M., Yahagi, A., Hamada, S., Begum, M.D., Watanabe, H., Kawakami, K., Suda, T., Sudo, K., Nakae, S., Iwakura, Y., and Matsuzaki, G. (2007). IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. J. Immunol. *178*, 3786–3796.

van Beelen, A.J., Zelinkova, Z., Taanman-Kueter, E.W., Muller, F.J., Hommes, D.W., Zaat, S.A., Kapsenberg, M.L., and de Jong, E.C. (2007). Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. Immunity *27*, 660–669.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006a). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179–189.

Veldhoen, M., Hocking, R.J., Flavell, R.A., and Stockinger, B. (2006b). Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. Nat. Immunol. *7*, 1151–1156.

Visser, L., Jan de Heer, H., Boven, L.A., van Riel, D., van Meurs, M., Melief, M.J., Zahringer, U., van Strijp, J., Lambrecht, B.N., Nieuwenhuis, E.E., and Laman, J.D. (2005). Proinflammatory bacterial peptidoglycan as a cofactor for the development of central nervous system autoimmune disease. J. Immunol. *174*, 808–816.

Voorthuis, J.A., Uitdehaag, B.M., De Groot, C.J., Goede, P.H., van der Meide, P.H., and Dijkstra, C.D. (1990). Suppression of experimental allergic encephalomyelitis by intraventricular administration of interferon-gamma in Lewis rats. Clin. Exp. Immunol. *81*, 183–188.

Weaver, C.T., Harrington, L.E., Mangan, P.R., Gavrieli, M., and Murphy, K.M. (2006). Th17: An effector CD4 T cell lineage with regulatory T cell ties. Immunity *24*, 677–688.

Willenborg, D.O., Fordham, S.A., Staykova, M.A., Ramshaw, I.A., and Cowden, W.B. (1999). IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: A possible role for nitric oxide. J. Immunol. *163*, 5278–5286.

Wilson, N.J., Boniface, K., Chan, J.R., McKenzie, B.S., Blumenschein, W.M., Mattson, J.D., Basham, B., Smith, K., Chen, T., Morel, F., et al. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat. Immunol. *8*, 950–957.

Wolk, K., Kunz, S., Witte, E., Friedrich, M., Asadullah, K., and Sabat, R. (2004). IL-22 increases the innate immunity of tissues. Immunity *21*, 241–254.

Yang, X.O., Panopoulos, A.D., Nurieva, R., Chang, S.H., Wang, D., Watowich, S.S., and Dong, C. (2007). STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J. Biol. Chem. *282*, 9358–9363.

Yang, X.O., Pappu, B.P., Nurieva, R., Akimzhanov, A., Kang, H.S., Chung, Y., Ma, L., Shah, B., Panopoulos, A.D., Schluns, K.S., et al. (2008). T Helper 17 Lineage Differentiation Is Programmed by Orphan Nuclear Receptors RORalpha and RORgamma. Immunity *28*, 29–39.

Ye, P., Rodriguez, F.H., Kanaly, S., Stocking, K.L., Schurr, J., Schwarzenberger, P., Oliver, P., Huang, W., Zhang, P., Zhang, J., et al. (2001). Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J. Exp. Med. *194*, 519–527.

Yen, D., Cheung, J., Scheerens, H., Poulet, F., McClanahan, T., McKenzie, B., Kleinschek, M.A., Owyang, A., Mattson, J., Blumenschein, W., et al. (2006). IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. J. Clin. Invest. *116*, 1310–1316.

Yoshitomi, H., Sakaguchi, N., Kobayashi, K., Brown, G.D., Tagami, T., Sakihama, T., Hirota, K., Tanaka, S., Nomura, T., Miki, I., et al. (2005). A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. J. Exp. Med. *201*, 949–960.

Zhang, G.X., Yu, S., Gran, B., Li, J., Siglienti, I., Chen, X., Calida, D., Ventura, E., Kamoun, M., and Rostami, A. (2003). Role of IL-12 receptor beta 1 in regulation of T cell response by APC in experimental autoimmune encephalomyelitis. J. Immunol. *171*, 4485–4492.

Zheng, S.G., Wang, J., Wang, P., Gray, J.D., and Horwitz, D.A. (2007a). IL-2 is essential for TGF-beta to convert naive CD4+CD25- cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. J. Immunol. *178*, 2018–2027.

Zheng, Y., Danilenko, D.M., Valdez, P., Kasman, I., Eastham-Anderson, J., Wu, J., and Ouyang, W. (2007b). Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature *445*, 648–651.

Zhou, L., Ivanov, I.I., Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D.E., Leonard, W.J., and Littman, D.R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat. Immunol. *8*, 967–974.