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T helper 17 cell heterogeneity and pathogenicity in autoimmune disease

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Abstract

Th17 cells have been proposed to represent a new CD4⁺ T cell lineage important for host defense against fungi and extracellular bacteria, and the development of autoimmune diseases. Precisely how these cells arise has been the subject of some debate with apparent species-specific differences in mice and men. Here, we describe evolving views of Th17 specification, highlighting the contribution of TGF- β and the opposing roles of STAT3 and STAT5. Increasing evidence points to heterogeneity and inherent phenotypic instability in this subset. Ideally, better understanding of expression and action of key transcription factors and the epigenetic landscape of Th17 can help explain the flexibility and diversity of IL-17-producing cells.

Recognition of complexity of Th cell subsets

Activated CD4⁺ T cells differentiate into distinct functional subsets, characterized by heritable patterns of cytokine secretion and the expression of specific transcription factors or so-called “master regulators” [1, 2]. Along with classical T helper (Th)1 and Th2 cells [3], new subsets of T cells continue to be recognized [4–6]. Of these “new” subsets, CD4⁺ T cells that preferentially produce IL-17 have attracted tremendous attention because of the connection with autoimmune disease [7–9]. The importance of autoreactive Th17 cells in mouse models of multiple sclerosis, psoriasis, rheumatoid arthritis and inflammatory bowel disease led to the establishment of treatments affecting cytokines of the Th17 network in autoimmune disease [10–13]. In humans, therapeutics targeting IL-12/IL-23p40, IL-17 or the IL-6 receptor are already in clinical practice for some of these autoimmune diseases. Given this onslaught of studies, it is useful to review the history of this field and to consider current controversies and discuss future challenges. In this article we examine the heterogeneity and pathogenicity of Th17 cells. Therefore, we focus on the IL-17-promoting cytokines IL-6, IL-1 β , IL-23 and TGF- β and their signaling pathways causing Th17 cell diversity. We also discuss the opposite roles of STAT3, which can be activated by IL-6 and IL-23 and STAT5, which can be activated by IL-2, on Th17 differentiation.

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The criticality of IL-23 in driving autoimmunity

The heterodimeric cytokine IL-23 (p40/p19), which shares the p40 subunit with IL-12 (p40/p35), has been identified as the crucial cytokine in Th17-associated pathology. In attempts to decipher the role of IL-12 subunits in mouse models of autoimmune disease, it was noted that mice deficient in IL-12p40 were resistant to experimental autoimmune encephalomyelitis (EAE) [14, 15]. However, mice lacking the IL-12p35 subunit developed more severe disease and in this respect were similar to mice lacking interferon (IFN)- γ . In contrast, gene targeting of another partner of p40, the p19 subunit of IL-23, resulted in resistance to EAE [15]. Therefore, an immune mechanism independent from IL-12 and Th1 cells must be responsible for T cell-mediated inflammatory autoimmunity. This result was a serious challenge to a prevailing, simple dichotomous view of Th1 versus Th2 cell differentiation, and launched a new era in the understanding of autoimmune mechanisms. Strengthening the connection between IL-23 and human autoimmunity is a considerable body of genetic evidence that links *IL23R* polymorphisms with susceptibility to a range of autoimmune diseases including: Crohn's disease, spondyloarthritis, autoimmune thyroid disease, multiple sclerosis, psoriasis, psoriatic arthritis, acquired aplastic anemia, and Behcet's disease [16–22]. As will be discussed later, IL-23 activates Jak2 and Stat3, and polymorphisms of the genes encoding these signaling molecules are also linked to human autoimmunity [23–25]. Thus, in addition to mouse models, which may or may not accurately reflect immunopathogenetic mechanisms of human disease, independent genetic evidence has now linked IL-23 signaling to actual autoimmune disease in people.

Connecting the dots: IL-23 and IL-17 axis

First cloned in 1995, IL-17 was under appreciated for many years, even though there were clear data showing that it was associated with delayed-type hypersensitivity reactions and autoimmunity [8, 26]. The first work linking IL-23 to IL-17 production were *in vitro* studies using memory CD4⁺ T cells, and the authors posited that IL-23 might act during a secondary immune response to “promote an activation state with features distinct” from Th1 and Th2 cells [27]. Deficiency of IL-23 was then found to be associated with reduced production of IL-17 at sites of immune pathology [28]. Much later, the combination of IL-1 β and IL-23 was noted to potently induce IL-17 in mouse $\gamma\delta$ T cells [29, 30]. However, it proved much more challenging to drive naïve CD4⁺ T cells to differentiate to selectively produce IL-17. Initial studies using naïve T cells, argued that polarized Th1 and Th2 cells did not secrete IL-17. Stimulating cells with IL-23, while blocking IFN- γ and IL-4, resulted in small numbers of IL-17 producing T cells [31, 32]. The presence of cells that selectively produced IL-17 and not IFN- γ or IL-4 led the authors to propose that these cells represented a new helper cell lineage, Th17 cells.

Later, IL-17-producing T cells were found to express the transcription factor retinoic acid orphan receptor (ROR) gamma thymus (ROR γ t), which along with ROR- α , was critical for Th17 development and was required for the development of EAE [33, 34]. ROR γ t was thus argued to be the master regulator of Th17 differentiation, a notion that supported the lineage sovereignty of Th17 cells. However, other factors are also important for Th17 differentiation and they include: Batf [35], IRF4 [36], Runx1 [37], I κ B δ [38] and IKK α [39]. Activated by IL-6 and IL-23, STAT3 was also recognized to be essential for IL-17 production in mouse and human cells, its importance being vividly illustrated in humans with hyper-IgE syndrome [40–43]. These individuals lack efficient Th17 cell development and suffer from infections with certain bacteria and fungi. In mice, deletion of STAT3 in T cells limits Th17 cell differentiation and pathology [44–49].

While an IL-23–IL-17 connection seems reasonably clear, it is also worth pointing out that IL-23 does not necessarily equal IL-17. Despite the presence of IL-17-producing T cells in EAE, absence of *Il17f* has only minor effects on EAE development and severity [50–52]. Mice deficient in *Il17a* or IL-17RA develop attenuated signs of EAE [51–53], but the severity of disease is greater than in mice lacking p40. In this regard, it is intriguing that the *Il17* locus has not emerged as a susceptibility gene for human autoimmune disease in the many genome-wide association studies that have been reported. Taken together, the data suggest that in terms of immunopathology, IL-23 should not be equated with IL-17. IL-6 signaling through STAT3 is crucial for both IL-23 receptor expression and for IL-17A and IL-17F induction, and defective IL-6 signaling is also associated with a more profound block in the development of EAE suggesting that IL-23 has effects beyond simply promoting IL-17 production [14, 15, 46, 54–58]. Thus, while it is clear that IL-23 can drive IL-17 production, it is also clear that IL-23 and IL-17 are not synonymous in terms of autoimmunity [13].

A more efficient recipe for Th17 differentiation: TGF- β and IL-6

While the importance of IL-23 in driving IL-17-mediated pathology was (and remains) clear, what was less obvious was how to efficiently generate IL-17-producing cells. Especially relevant was that naïve CD4⁺ T cells express little or no receptor for IL-23. So, what are the initiating factors that induced receptor expression and first specify Th17 fate commitment?

In vitro, culture of regulatory T (Treg) cells with naïve CD4⁺ T cells was found to efficiently drive generation of IL-17 producing cells, which was attributed to Treg cell production of TGF- β [59]; it was quickly confirmed that TGF- β and IL-6 efficiently generated populations of IL-17-producing cells from naïve precursors [60, 61]. It was notable that although TGF- β (especially with addition of exogenous IL-2) upregulated Foxp3 and generated induced Treg (iTreg) cells, IL-6 inhibited Foxp3. This suggested that Tregs and Th17 cells are developmentally related [5, 62, 63].

The next challenge was to establish whether TGF- β and IL-6 were requisite for *in vivo* Th17 generation and to discern how these factors drove Th17 specification mechanistically. Several approaches have been used including transgenic expression of dominant negative TGF- β receptor II, deletion of TGF- β in T cells using CD4-cre, and, most recently, selective deletion of TGF- β 1 in activated T cells and Treg cells [64–66]. Collectively, these models do support the idea that T cell-derived TGF- β is important for Th17 differentiation. Accordingly, genetic models in which TGF- β was absent were also resistant to EAE, leading to the view that T cell-derived TGF- β is critical for the generation of encephalitogenic Th17 cells.

Although resistance to EAE and reduced generation of Th17 cells is seen in models in which there is absence of TGF- β or disruption of TGF- β signaling in T cells, there is another consequence, namely profound autoimmunity. This pathology is characterized by overproduction of multiple cytokines especially IFN- γ and IL-4, factors known to attenuate Th17 differentiation. More importantly, IFN- γ limits the severity of EAE. This raises the question is TGF- β really important because it provides an obligate instructive signal to cells to become IL-17 producers or is TGF- β primarily limiting production of other cytokines, which in turn indirectly attenuate EAE?

It is notable that despite the association between Th17 cells and inflammatory disease in both mice and humans, TGF- β and IL-6-induced Th17 cells are weakly pathogenic in mice in the setting of EAE [67–69]. One explanation for this is that TGF- β and IL-6-induced Th17 cells make IL-10, with subsequent exposure to IL-23 being required for pathogenicity

[68, 70]. Accordingly, the pathogenicity of Th17 cells generated with TGF- β can be enhanced by neutralizing IL-10 [68]. Taken together, the data suggest that the combination of IL-6 and TGF- β produces a population of cells that selectively produces IL-17 and not IFN- γ , but alone this cocktail may not be sufficient to generate cells with pathogenic potential. The anti-inflammatory phenotype of this Th17 cell population is characterized by the production of cytokines like IL-9 and IL-10, which *in vivo* can be found in ROR γ ⁺FoxP3⁺ Th17 cells [68, 71–73]. Thus, a population of Th17 cells with a phenotype similar to cells that received TGF- β signals does exist *in vivo*; however their exact function in autoimmunity and host defense has to be determined. It should also be noted that Th17 cells generated by TGF- β *in vitro* can become IFN- γ producers *in vivo* and attain diabetogenic or colitogenic potential after adoptive transfer into immunocompromised mice [74, 75].

Those pesky human cells

Shortly after the recognition of factors that promote differentiation of mouse Th17 cells, efforts were made to generate human IL-17-producing CD4⁺ T cells, which like their murine equivalent, express the master regulator ROR γ t. The cocktail of IL-6, IL-1 and IL-23 was noted to readily induce IL-17-secreting cells from naïve cells, but in some cases TGF- β was found to inhibit rather than promote Th17 development [76–81]. One argument raised was that perhaps the human cells were not quite as “naïve” as their mouse counterparts [82]. Later, when cord blood naïve T cells were used rather than adult blood along, low doses of TGF- β were found to induce Th17 development [83, 84].

Another complexity revealed in these studies was that human Th17 cells readily produced IFN- γ [78, 79]. Although pure populations of IL-17⁺IFN- γ [−] cells are easily generated in the mouse, this is harder to do in human CD4⁺ T cells. At the same time though, in lesional tissue in the setting of autoimmunity, it is quite common to see IL-17 and IFN- γ double producers; this is the case in both mouse and man [85, 86]. So it is by no means clear that in the setting of autoimmunity populations of pure IL-17⁺IFN- γ [−] cells are necessarily the relevant players.

To beta or not to beta; that is the question: Murine Th17 revisited

Given the perplexing data from human cells and the complex effects of TGF- β , we and others revisited the requirement for TGF- β in Th17 differentiation. In T cells deficient in T-bet and STAT6 expression, IL-6 alone was found to induce IL-17 production even in the absence of TGF- β signaling [87]. This was interpreted to indicate that the TGF- β acts indirectly to regulate IL-17 by suppressing factors that drive other cell fates [88], especially Th1 and Th2 cell differentiation.

As indicated above, work from both mouse and human cells indicated that STAT3 is essential for Th17 differentiation. What is the mechanism by which STAT3 participates in the development of this subset of cells? Second generation or deep sequencing technology has permitted mapping and quantitation of transcription factor binding on a genome-wide scale [89]. This technology can also be used to measure epigenetic changes and gene expression and when used in conjunction with cells from knockout mice, one can comprehensively define the genomewide actions of a given transcription factor [90]. This is precisely what was done for STAT3 in Th17 cells. STAT3 bound to and regulated multiple genes that contribute to the Th17 phenotype including: the *Il17* locus itself, *Il21*, and *Il23r* but also CCR6 [46, 49, 69]. STAT3 also bound to genes encoding transcription factors critical for Th17 differentiation including *Rorc* (which encodes ROR γ t), *Irf4*, *Batf* and *Nfibiz* [49].

Given STAT3's prominent role and its ability to bind and regulate *Il23r* expression along with key transcription factors and the contradictory information on the requirement for TGF- β in human IL-17 regulation, the question of whether TGF- β is really required for initiating Th17 differentiation has been recently revisited. By culturing cells in serum-free medium and using multiple strategies to block TGF- β signaling (including antibodies and genetic and pharmacologic approaches), the IL-23 receptor was found to be induced in the absence of TGF- β 1 and addition of IL-23 then further induced receptor expression. Consequently, the combination of IL-1, IL-6 and IL-23 was able to induce IL-17 production in a TGF- β 1-independent manner [69]. Consistent with these findings, Th17 cells are also present in the gut of mice with deficient TGF- β signaling [69, 91]. In this regard, it is worth pointing out that conditional deletion of TGF- β in T cells did not significantly alter the proportion of gut Th17 cells [66]. Moreover, deletion of SMAD2, SMAD3 or SMAD4 in T cells does not prevent Th17 development [92–94]. The expression of ROR γ t is also not reduced in SMAD2- and SMAD3-deficient T cells, and IL-17 can be induced in these cells when IL-2 is neutralized [94].

Th17 differentiation and the balance of STAT3 vs STAT5 activation

As already mentioned, inclusion of Treg cells in naïve CD4 T cells cultures promotes Th17 differentiation [59]. However, more recent work argues that Treg cell production of TGF- β is dispensable for Th17 differentiation [66, 95] and there is an alternative explanation for the original finding regarding Treg cells promoting Th17 cells. IL-2 was recently found to potently inhibit Th17 differentiation [45] whereby blocking IL-2 or IL-2 signaling *in vitro* and *in vivo* results in marked expansion of IL-17-producing cells. Two very recent papers conclude that the mechanism by which Treg cells promote Th17 differentiation is via their ability to act as IL-2 “sinks”, offering a re-interpretation of the original Treg cell finding [95, 96]. In this scenario, consumption of IL-2 by CD25⁺ Tregs promotes the differentiation of Th17 cells *in vitro* and *in vivo*, and in this manner Tregs can help clear *C. albicans* infection in mice by enhancing IL-17 production [96].

In aggregate, these results indicate that IL-2 is an important regulator of IL-17 production. It then becomes an important question to define the mechanisms through which it inhibits IL-17 expression. STAT5 is responsible downstream of IL-2. Even though STAT5 directly regulates the *Foxp3* gene, this is not the mechanism underlying the inhibition of IL-17 and nor is IL-2–STAT5 acting primarily to downregulate Ror γ t. Rather, STAT5 acts directly on the *Il17* gene. As STAT3 directly binds the promoter and enhancer elements in *Il17*, STAT5 binding to this region was also assessed [46]. Surprisingly, STAT5 binds the same elements as STAT3, and furthermore, displaces STAT3. By varying the levels of cytokines that activate STAT3 versus STAT5, it was found that the balance of activation of these two transcription factors determines the outcome fate of the T cells. That is, under circumstances in which there is minimal activation of STAT5, the threshold for Th17 differentiation is greatly reduced. In this context, it should be borne in mind that multiple factors can activate both STAT3 and STAT5, so a number of factors might influence the proclivity of cells to make IL-17. Recall for instance that different preparations of media tend to promote or inhibit Th17 differentiation [97]. Specifically, unknown factors in media complemented with fetal bovine serum for instance seem to activate STAT5 and thereby inhibit Th17 differentiation. Presumably other factors *in vivo* can also tip the balance.

Instability and heterogeneity of Th17 cells

After the identification of Th17 cells as a putative new subset linked to autoimmunity, several groups generated Th17 cells *in vitro* and adoptively transferred the cells to provoke autoimmune disease. In addition to the aforementioned problem that not all Th17 cells are

pathogenic, another issue emerged. Specifically, after transfer *in vivo*, Th17 cells quickly acquired the ability to produce IFN- γ and lost their ability to produce IL-17 [74, 98]. This was recapitulated *in vitro* in that, with multiple rounds of culture, Th17 cells begin to produce IFN- γ . TGF- β inhibited this tendency, but Th17 cells express IL-12 receptors and readily produce IFN- γ in response to exposure to IL-12 [75]. The tendency to produce IFN- γ was explained by the accessibility of the *Ifng* locus, as measured by DNase hypersensitivity mapping, which was surprisingly similar in Th17 and Th1 cells [99]. Moreover, upon stimulation with IL-12 the *Ifng* locus in Th17 cells undergoes rapid epigenetic remodeling with increased H3K4me3 and decreased H3K27me3. Moreover, the *Tbx21* gene (which encodes T-bet, a Th1 “master regulator”) shows bivalent (H3K4me3 and H3K27me3) epigenetic marks in Th17 cells, indicating that this transcription factor gene is poised for expression [100]. Further evidence for plasticity and instability of Th17 cells was provided by IL-17A reporter mice [86]. That is, a substantial proportion of IFN- γ ⁺ T cells during EAE were previous IL-17 producers and the appearance of IL-17⁺IFN- γ ⁺ T cells required IL-23 signaling.

Another issue that arises when generating Th17 cells in the absence of TGF- β (i.e. with IL-1, IL-6 and IL-23) was that their transcription profile is quite different from conventional, TGF- β -induced Th17 cells. Importantly, in the absence of TGF- β , the cells express transcription factors expressed by Th1 cells, T-bet and Hlx1 [69]. IL-6, IL-1 and IL-23-generated Th17 cells also express IL-33 [69], which is associated with inflammatory immune responses. Adoptive transfer experiments showed that Th17 cells made in this manner are much more pathogenic than TGF- β -induced cells, which express higher levels of c-Maf and Ahr, CCL20, IL-9, and IL-10 [68, 69, 71, 101]. This is notable in that human Th17 cells and clones can co-express ROR γ t and T-bet [102], as do lesional T cells from patients with multiple sclerosis also express T-bet along with ROR γ t [85].

Concluding remarks

Although IL-17 was discovered in 1995 and IL-23 was discovered in 2000, we still have much to learn about what it means for a T cell to make IL-17. An obvious question is whether these cells really represent a “lineage”. Clearly differentiated Th cells seem to be more flexible with respect to lineage commitment than we initially thought and there is accumulating evidence that subsets can express more than one “master regulator” [2]. For instance, we now know that a subset of FoxP3⁺ Tregs can co-express T-bet, which is required for appropriate trafficking [103]. Moreover, GATA3⁺ Th2 cells have been reported to convert into a phenotype that co-expresses IL-4, IFN- γ , GATA3 and T-bet, allowing them to serve a protective role in viral infection [104]. Th17 cells are no exception to this emerging complexity; on the contrary, they appear to be particularly unstable and readily become Th1 cells. IL-17 producing cells may be a heterogeneous collection and it is possible that this heterogeneity may be influenced by the site and circumstances of induction. For instance, perhaps in environments with high levels of TGF- β , populations that express IL-17, and possibly IL-10, but little IFN- γ , might be generated. One could imagine that such cells would be useful in terms of host defense but would have limited pathological potential (perhaps at mucosal sites). On the contrary, T-bet⁺ROR γ t⁺ T cells might be more relevant in terms of autoimmune mechanisms, which in many circumstances represent a mixed picture of Th1 and Th17-mediated pathology. Certainly, in human disease this seems to be the case. Evidence that allergic asthma is promoted by IL-17-producing IL-4⁺ Th cells that co-express GATA3 and ROR γ t is particularly intriguing in this respect [105]. It would not be surprising to learn in the future that “Th17” cells represent a spectrum of cells with subtly different functionalities.

Another unresolved question is whether TGF- β “promotes” Th17 differentiation or if it primarily acts by limiting expression of T-bet, GATA3 and other transcription factors associated with other fates. Since TGF- β is ubiquitous, there are probably no circumstances in which Th17 cells arise in the absence of this cytokine. The question is more of a mechanistic problem. Does deletion of TGF- β impair Th17 differentiation because Th1 differentiation is exacerbated? Is the protective effect of eliminating TGF- β in EAE simply a reflection of the fact that IFN- γ is protective in such models? Or does TGF- β have more direct roles in actively instructing cells to become IL-17 producers? The finding that STAT3 and STAT5 compete to regulate IL-17 production adds a new level of complexity to regulation of this gene; the finding raises the possibility that multiple factors might fine tune expression.

The understanding of cytokine signaling and its role in controlling T cell responses are the basis for the development of effective, safe and more specific therapies. IL-23 is clearly functionally and genetically linked to a number of autoimmune diseases. The insights about the pathogenic potential of IL-23 in experimental mice led to the establishment of therapies targeting IL-23. Targeting IL-12 and IL-23 with an antibody against the shared subunit, p40, is highly effective in autoimmune diseases as psoriasis and Crohn’s disease, but it remains to be determined whether selectively targeting IL-23 will be as effective. Surprisingly, in patients with relapsing-remitting multiple sclerosis the effect of anti-p40 therapy was disappointing. This may reflect the complexity of the pathogenesis of human autoimmune disease that may be different from experimental mouse models [106]. Antagonizing the action of IL-6 is effective in the treatment of some autoimmune diseases, but whether this is related to effects on Th17 cells has not been established. The efficacy and safety of neutralizing IL-17 itself is under clinical investigation in autoimmune settings, but initial reports suggest different responses in psoriasis and rheumatoid arthritis [107]. Clearly more work is required to fully understand the relevance of the IL-23/IL-17 connection in the spectrum of autoimmune disease. However, one thing’s for sure - we will continue to learn a great deal about Th17 cells in human autoimmunity. With any luck, we will soon be able to better sort out when and where to use drugs that target these cells and improve the treatment of these diseases.

References

1. Zhu J, et al. Differentiation of effector CD4 T cell populations. *Annu Rev Immunol.* 2010; 28:445–489. [PubMed: 20192806]
2. O’Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science.* 2010; 327:1098–1102. [PubMed: 20185720]
3. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol.* 1989; 7:145–173. [PubMed: 2523712]
4. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol.* 2002; 2:389–400. [PubMed: 12093005]
5. Korn T, et al. IL-17 and Th17 Cells. *Annu Rev Immunol.* 2009; 27:485–517. [PubMed: 19132915]
6. Crotty S. Follicular Helper CD4 T Cells (T(FH)). *Annu Rev Immunol.* 2011; 29:621–663. [PubMed: 21314428]
7. Weaver CT, et al. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol.* 2007; 25:821–852. [PubMed: 17201677]
8. Miossec P, et al. Interleukin-17 and type 17 helper T cells. *N Engl J Med.* 2009; 361:888–898. [PubMed: 19710487]
9. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. *Curr Opin Immunol.* 2007; 19:281–286. [PubMed: 17433650]
10. Goverman J. Autoimmune T cell responses in the central nervous system. *Nat Rev Immunol.* 2009; 9:393–407. [PubMed: 19444307]

11. Nestle FO, et al. Psoriasis. *N Engl J Med.* 2009; 361:496–509. [PubMed: 19641206]
12. van den Berg WB, Miossec P. IL-17 as a future therapeutic target for rheumatoid arthritis. *Nat Rev Rheumatol.* 2009; 5:549–553. [PubMed: 19798029]
13. Ahern PP, et al. The interleukin-23 axis in intestinal inflammation. *Immunol Rev.* 2008; 226:147–159. [PubMed: 19161422]
14. Becher B, et al. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J Clin Invest.* 2002; 110:493–497. [PubMed: 12189243]
15. Cua DJ, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature.* 2003; 421:744–748. [PubMed: 12610626]
16. Lock C, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med.* 2002; 8:500–508. [PubMed: 11984595]
17. Nair RP, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet.* 2009; 41:199–204. [PubMed: 19169254]
18. Takaku T, et al. Interleukin-23 receptor (IL-23R) gene polymorphisms in acquired aplastic anemia. *Ann Hematol.* 2009; 88:653–657. [PubMed: 19165485]
19. Remmers EF, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behcet's disease. *Nat Genet.* 2010; 42:698–702. [PubMed: 20622878]
20. Liu Y, et al. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet.* 2008; 4:e1000041. [PubMed: 18369459]
21. Burton PR, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet.* 2007; 39:1329–1337. [PubMed: 17952073]
22. Reveille JD, et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet.* 2010; 42:123–127. [PubMed: 20062062]
23. Danoy P, et al. Association of variants at 1q32 and STAT3 with ankylosing spondylitis suggests genetic overlap with Crohn's disease. *PLoS Genet.* 2010; 6:e1001195. [PubMed: 21152001]
24. Anderson CA, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet.* 2011; 43:246–252. [PubMed: 21297633]
25. Barrett JC, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet.* 2008; 40:955–962. [PubMed: 18587394]
26. Nakae S, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity.* 2002; 17:375–387. [PubMed: 12354389]
27. Aggarwal S, et al. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J Biol Chem.* 2003; 278:1910–1914. [PubMed: 12417590]
28. Langrish CL, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med.* 2005; 201:233–240. [PubMed: 15657292]
29. Sutton CE, et al. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity.* 2009; 31:331–341. [PubMed: 19682929]
30. Martin B, et al. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity.* 2009; 31:321–330. [PubMed: 19682928]
31. Harrington LE, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol.* 2005; 6:1123–1132. [PubMed: 16200070]
32. Park H, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol.* 2005; 6:1133–1141. [PubMed: 16200068]
33. Ivanov II, et al. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell.* 2006; 126:1121–1133. [PubMed: 16990136]
34. Yang XO, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR α and ROR γ . *Immunity.* 2008; 28:29–39. [PubMed: 18164222]
35. Schraml BU, et al. The AP-1 transcription factor Batf controls T(H)17 differentiation. *Nature.* 2009; 460:405–409. [PubMed: 19578362]

36. Brüstle A, et al. The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. *Nat Immunol.* 2007; 8:958–966. [PubMed: 17676043]
37. Zhang F, et al. Interactions among the transcription factors Runx1, ROR γ and Foxp3 regulate the differentiation of interleukin 17-producing T cells. *Nat Immunol.* 2008; 9:1297–1306. [PubMed: 18849990]
38. Okamoto K, et al. IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors. *Nature.* 2010; 464:1381–1385. [PubMed: 20383124]
39. Li L, et al. Transcriptional regulation of the Th17 immune response by IKK α . *J Exp Med.* 2011
40. Chen Z, et al. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. *Proc Natl Acad Sci U S A.* 2006; 103:8137–8142. [PubMed: 16698929]
41. Mathur AN, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. *J Immunol.* 2007; 178:4901–4907. [PubMed: 17404271]
42. Minegishi Y, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature.* 2007; 448:1058–1062. [PubMed: 17676033]
43. Milner JD, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature.* 2008; 452:773–776. [PubMed: 18337720]
44. Yang XO, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem.* 2007; 282:9358–9363. [PubMed: 17277312]
45. Laurence A, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity.* 2007; 26:371–381. [PubMed: 17363300]
46. Yang XP, et al. Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. *Nat Immunol.* 2011; 12:247–254. [PubMed: 21278738]
47. Liu X, et al. Loss of STAT3 in CD4⁺ T cells prevents development of experimental autoimmune diseases. *J Immunol.* 2008; 180:6070–6076. [PubMed: 18424728]
48. Harris TJ, et al. Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. *J Immunol.* 2007; 179:4313–4317. [PubMed: 17878325]
49. Durant L, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity.* 2010; 32:605–615. [PubMed: 20493732]
50. Komiyama Y, et al. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol.* 2006; 177:566–573. [PubMed: 16785554]
51. Haak S, et al. IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. *J Clin Invest.* 2009; 119:61–69. [PubMed: 19075395]
52. Ishigame H, et al. Differential roles of interleukin-17A and -17F in host defense against mucocutaneous bacterial infection and allergic responses. *Immunity.* 2009; 30:108–119. [PubMed: 19144317]
53. Gonzalez-Garcia I, et al. IL-17 signaling-independent central nervous system autoimmunity is negatively regulated by TGF- β . *J Immunol.* 2009; 182:2665–2671. [PubMed: 19234160]
54. Chen Y, et al. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest.* 2006; 116:1317–1326. [PubMed: 16670771]
55. Samoilova EB, et al. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: roles of IL-6 in the activation and differentiation of autoreactive T cells. *J Immunol.* 1998; 161:6480–6486. [PubMed: 9862671]
56. Dong C. Regulation and pro-inflammatory function of interleukin-17 family cytokines. *Immunol Rev.* 2008; 226:80–86. [PubMed: 19161417]
57. Yang XO, et al. Regulation of inflammatory responses by IL-17F. *J Exp Med.* 2008; 205:1063–1075. [PubMed: 18411338]
58. Puel A, et al. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. *Science.* 2011; 332:65–68. [PubMed: 21350122]
59. Veldhoen M, et al. TGF β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity.* 2006; 24:179–189. [PubMed: 16473830]
60. Bettelli E, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature.* 2006; 441:235–238. [PubMed: 16648838]

61. Mangan PR, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*. 2006; 441:231–234. [PubMed: 16648837]
62. Weaver CT, et al. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity*. 2006; 24:677–688. [PubMed: 16782025]
63. Cua DJ, Kastelein RA. TGF-beta, a 'double agent' in the immune pathology war. *Nat Immunol*. 2006; 7:557–559. [PubMed: 16715066]
64. Veldhoen M, et al. Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat Immunol*. 2006; 7:1151–1156. [PubMed: 16998492]
65. Li MO, et al. T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity*. 2007; 26:579–591. [PubMed: 17481928]
66. Gutcher I, et al. Autocrine Transforming Growth Factor-beta1 Promotes In Vivo Th17 Cell Differentiation. *Immunity*. 2011; 34:396–408. [PubMed: 21435587]
67. Yang Y, et al. T-bet is essential for encephalitogenicity of both Th1 and Th17 cells. *J Exp Med*. 2009; 206:1549–1564. [PubMed: 19546248]
68. McGeachy MJ, et al. 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*. 2007; 8:1390–1397. [PubMed: 17994024]
69. Ghoreschi K, et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature*. 2010; 467:967–971. [PubMed: 20962846]
70. Jager A, et al. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J Immunol*. 2009; 183:7169–7177. [PubMed: 19890056]
71. Elyaman W, et al. IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. *Proc Natl Acad Sci U S A*. 2009; 106:12885–12890. [PubMed: 19433802]
72. Nowak EC, et al. IL-9 as a mediator of Th17-driven inflammatory disease. *J Exp Med*. 2009; 206:1653–1660. [PubMed: 19596803]
73. Lochner M, et al. In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. *J Exp Med*. 2008; 205:1381–1393. [PubMed: 18504307]
74. Bending D, et al. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J Clin Invest*. 2009
75. Lee YK, et al. Late developmental plasticity in the T helper 17 lineage. *Immunity*. 2009; 30:92–107. [PubMed: 19119024]
76. Acosta-Rodriguez EV, et al. 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*. 2007; 8:942–949. [PubMed: 17676045]
77. Wilson NJ, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol*. 2007; 8:950–957. [PubMed: 17676044]
78. Chen Z, et al. Distinct regulation of interleukin-17 in human T helper lymphocytes. *Arthritis Rheum*. 2007; 56:2936–2946. [PubMed: 17763419]
79. Volpe E, et al. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat Immunol*. 2008; 9:650–657. [PubMed: 18454150]
80. Cosmi L, et al. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. *J Exp Med*. 2008; 205:1903–1916. [PubMed: 18663128]
81. Santarlasci V, et al. TGF-beta indirectly favors the development of human Th17 cells by inhibiting Th1 cells. *Eur J Immunol*. 2009; 39:207–215. [PubMed: 19130583]
82. Laurence A, O'Shea JJ. T(H)-17 differentiation: of mice and men. *Nat Immunol*. 2007; 8:903–905. [PubMed: 17712339]
83. Yang L, et al. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature*. 2008; 454:350–352. [PubMed: 18469800]

84. Manel N, et al. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgamma. *Nat Immunol.* 2008; 9:641–649. [PubMed: 18454151]
85. Kebir H, et al. Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. *Ann Neurol.* 2009; 66:390–402. [PubMed: 19810097]
86. Hirota K, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol.* 2011; 12:255–263. [PubMed: 21278737]
87. Das J, et al. Transforming growth factor beta is dispensable for the molecular orchestration of Th17 cell differentiation. *J Exp Med.* 2009; 206:2407–2416. [PubMed: 19808254]
88. Lazarevic V, et al. T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORgamma. *Nat Immunol.* 2011; 12:96–104. [PubMed: 21151104]
89. Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet.* 2008; 9:387–402. [PubMed: 18576944]
90. O'Shea JJ, et al. Genomic views of STAT function in CD4(+) T helper cell differentiation. *Nat Rev Immunol.* 2011; 11:239–250. [PubMed: 21436836]
91. Qin H, et al. TGF-beta promotes Th17 cell development through inhibition of SOCS3. *J Immunol.* 2009; 183:97–105. [PubMed: 19535626]
92. Yang XO, et al. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity.* 2008; 29:44–56. [PubMed: 18585065]
93. Martinez GJ, et al. SMAD2 positively regulates the generation of Th17 cells. *J Biol Chem.* 2010; 285:29039–29043. [PubMed: 20667820]
94. Takimoto T, et al. Smad2 and Smad3 are redundantly essential for the TGF-beta-mediated regulation of regulatory T plasticity and Th1 development. *J Immunol.* 2010; 185:842–855. [PubMed: 20548029]
95. Chen Y, et al. Foxp3(+) Regulatory T Cells Promote T Helper 17 Cell Development In Vivo through Regulation of Interleukin-2. *Immunity.* 2011; 34:409–421. [PubMed: 21435588]
96. Pandiyan P, et al. CD4(+)CD25(+)Foxp3(+) Regulatory T Cells Promote Th17 Cells In Vitro and Enhance Host Resistance in Mouse Candida albicans Th17 Cell Infection Model. *Immunity.* 2011; 34:422–434. [PubMed: 21435589]
97. Veldhoen M, et al. Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J Exp Med.* 2009; 206:43–49. [PubMed: 19114668]
98. Shi G, et al. Phenotype switching by inflammation-inducing polarized Th17 cells, but not by Th1 cells. *J Immunol.* 2008; 181:7205–7213. [PubMed: 18981142]
99. Mukasa R, et al. Epigenetic instability of cytokine and transcription factor gene loci underlies plasticity of the T helper 17 cell lineage. *Immunity.* 2010; 32:616–627. [PubMed: 20471290]
100. Wei G, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity.* 2009; 30:155–167. [PubMed: 19144320]
101. Beriou G, et al. TGF-beta induces IL-9 production from human Th17 cells. *J Immunol.* 2010; 185:46–54. [PubMed: 20498357]
102. Annunziato F, et al. Phenotypic and functional features of human Th17 cells. *J Exp Med.* 2007; 204:1849–1861. [PubMed: 17635957]
103. Koch MA, et al. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat Immunol.* 2009; 10:595–602. [PubMed: 19412181]
104. Hegazy AN, et al. Interferons direct Th2 cell reprogramming to generate a stable GATA-3(+)T-bet(+) cell subset with combined Th2 and Th1 cell functions. *Immunity.* 2010; 32:116–128. [PubMed: 20079668]
105. Wang YH, et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med.* 2010; 207:2479–2491. [PubMed: 20921287]
106. Steinman L. Mixed results with modulation of TH-17 cells in human autoimmune diseases. *Nat Immunol.* 2010; 11:41–44. [PubMed: 20016509]

107. Hueber W, et al. Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci Transl Med.* 2010; 2:52ra72.

Figure 1. Heterogeneity of Th17 cells

The conventional mode of inducing Th17 differentiation is to culture cells with IL-6, IL-1β and TGF-β. IL-6 acts to upregulate IL-23R and IL-21 expression. Subsequently, IL-6, IL-21 and IL-23, acting through STAT3, directly regulate many of the phenotype-defining Th17 genes. Though low levels of TGF-β clearly promote IL-17 production (a), a major unresolved question is its mechanism of action with respect to this cytokine. One can view TGF-β as providing an important positive instructive signal. However, TGF-β is not required to induce IL-23R expression. In addition, it is possible to generate Th17 cells with little or no TGF-β (b). Thus, an alternative explanation is that the major action of TGF-β is to inhibit IL-2, IFN-γ, T-bet and other factors that antagonize IL-17 production (c). Of note is that Th17 cells generated with little or no TGF-β are phenotypically and functionally distinct from conventional Th17 cells in terms of the transcription factors they express and the cytokines they produce. Also, Th17 cells are inherently unstable and can readily express IFN-γ, which further inhibits IL-17 production. Precisely, what the *in vivo* correlates of these different populations of Th17 cells are has not been defined exactly. However, in sites of autoimmune-mediated damage, cells that express RORγt and T-bet are present (d). IL-2 is key factor that negatively regulates IL-17 production. The inhibition of IL-17 by IL-2 is mediated through STAT5, which can directly compete with STAT3 to limit *Il17* transcription. Tregs can provide TGF-β for IL-17 production, but more importantly promote Th17 cell differentiation by consuming IL-2 (e). Therefore, Treg participate in host defense and pathogen clearance by inducing IL-17.