IL-23-IL-17 immune axis: Discovery, Mechanistic Understanding, and Clinical Testing

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Preface

With the discovery of Th17 cells, the past decade has witnessed a major revision of the T helper subset paradigm and significant progress has been made deciphering the molecular mechanisms for T cell lineage commitment and function. In this review, we focus on the recent advances on the transcriptional control of Th17 cell plasticity and stability as well as the effector functions of Th17 cells—highlighting IL-17 signaling mechanisms in mesenchymal and barrier epithelial tissues. We also discuss the emerging clinical data showing anti-IL-17 and anti-IL-23 treatments are remarkably effective for many immune-mediated inflammatory diseases.

Introduction

The discovery of interleukin-23 (IL-23) and elucidation of the biology governed by this cytokine has led to important new insights within immunology[1–3]. IL-23 has critical roles in the pathogenesis of autoimmunity as it induces a cell population with a unique inflammatory gene signature that includes *Il17a, Il17f, Il6, Csf2, Tnf, Ccl20, Ccl22, Il1r1*, and *Il23r*[4,5]. Based on this distinct gene profile and unique Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, a novel subset of T helper (T_H) cells was coined and designated Th_{IL-17} or Th17[6,7]. The discovery of the IL-23–Th17 pathway has led to fundamental changes in our understanding of cellular immunity (Figure 1). In 1989, Mossman and Coffman proposed to divide CD4$^+$ Th cells into interferon- γ (IFNγ)-producing Th1 cells (which are dependent on STAT1) and IL-4-producing Th2 cells (which are dependent on STAT6) to explain the tendency of T cells to diverge into either a cellular or humoral immune response[8, 9]. At that time, it was predicted that additional CD4$^+$ T cell subsets may exist that *drive the full spectrum of immune responses*. Indeed, the Th1-Th2 hypothesis does not adequately explain the regulation of STAT3-dependent CD4$^+$ T cells during autoimmunity and infections[3]. In 2003, the recognition that IL-23 is indispensable for promoting autoimmunity led to rethinking of the established paradigm[1, 3, 4, 10, 11].

In addition to Th17 cells, many innate immune cells respond to IL-23 and are important in both resistance to infection and mediating autoimmune pathology. These cells are characterized by expression of the transcription factor retinoic-acid-receptor-related orphan receptor-γ (RORγt, which is encoded by *Rorc*)[12] and include subsets of γδT cells, natural killer T (NKT) cells, 'natural' Th17 cells and innate lymphoid cells (ILCs) collectively, and

are termed "Type-17" cells[13–17] (Box 1). These innate immune cells are located in non-lymphoid tissues where they are poised to respond immediately to tissue injury or pathogenic insults. Stimulation of Th17 cells and Type-17 cells with IL-1β and IL-23 induces local tissue inflammation, which is mainly mediated by Type-17 signature cytokines such as IL-17, IL-22 and granulocyte–macrophage colony-stimulating factor (GM-CSF)[4, 18–20].

The exploration of Type-17 immunity has led to the identification of previously unrecognized immune cell subsets and new paradigms for disease localization that is dependent on the distribution of cell populations that express the IL-23 receptor (IL-23R) and RORγt[+]. These concepts are at the core of novel therapeutic strategies based on neutralization of IL-17 or IL-23, which show encouraging results for treatment of psoriasis, multiple sclerosis, Crohn's disease and ankylosing spondylitis. In this Review, we highlight the roles of cytokines that promote development and stabilization of Th17 cells via complex transcriptional networks. We discuss how IL-17 signaling is unique compared to other receptor families and highlighting recent studies that reveal mechanisms by which IL-17 signaling is constrained. Finally, we discuss disease settings where anti-IL-17 and anti-IL-23 therapeutic agents worked as predicted and where they have shown unexpected effects.

Cytokines that promote Th17 cell development

The cumulative impact of RORγt^{+} Th17 cells is a function of the effector cytokines they produce and the subsequent impact on target cells and tissues bearing appropriate receptors. RORγt^{+} Th17 cells can be broadly divided into two groups: host protective cells that express both IL-17 and IL-10 [21–23] and a highly inflammatory population that express IL-17, IL-22, IFNγ and GM-CSF[4, 18]. The ultimate outcome of Th17 cell activity is determined by the balance of these effector functions. Th17 cells activated by transforming growth factor-β (TGFβ) and IL-6 promote mucosal defense, barrier tissue integrity and curtail immunopathogenic responses[21–23], whereas IL-23-activated Th17 cells promote chronic tissue inflammation during infection, granuloma formation and autoimmunity[24–26].

Initiating cytokines

Studies in 2003 and 2005 showed that IL-23 promotes the development and expansion of a pathogenic T cell population with a unique inflammatory gene signature[1, 4, 6, 7]. However, IL-23 alone cannot drive differentiation of Th17 cells from naïve CD4+ T cell precursors, indicating that additional factors are required for their lineage fate determination. Shortly thereafter, Stockinger, Weaver, and Kuchroo simultaneously showed that addition of TGFβ and IL-6 during initial T cell recognition of cognate antigen promotes Th17 cell differentiation[27–29]. IL-6 has an essential role in this process by activating signal transducer and STAT3[30], which directly drives transcription of T_H17 lineage-specific genes[31] including *Rorc*, *Il17*, and *Il23r*. STAT3 also suppresses TGFβ-induced forkhead box P3 (FOXP3) expression, and thereby inhibit the generation of regulatory T (T_{Reg}) cells[27]. Consequently, *Il6−/−* mice are unable to generate Th17 cells and are protected from developing experimental autoimmune encephalomyelitis (EAE)[32] and collagen-induced arthritis (CIA)[33]. Although IL-6 was identified as a therapeutic target long before the recognition of

Th17 cells, the recent advances have validated IL-6 as a target for the treatment of rheumatoid arthritis and other inflammatory conditions.

IL-1β signaling also has a critical role during the initial stages of Th17 cell differentiation. *Il1r1−/−* mice fail to develop antigen-specific Th17 cells and are resistant to EAE[34]. Expression of IL-1 receptor type 1 (IL-1R1) by Th17 cells is induced by IL-6, and signaling through the IL-1R promotes the transcription factor interferon-regulatory factor 4 (IRF4), which reinforces the expression of RORγt[35]. Mechanistically, IL-1β induces phosphorylation of the mammalian target of rapamycin (mTOR) in Th17 cells and thereby enhances the metabolic fitness of rapidly dividing Th17 cells during inflammation. Indeed, rapamycin treatment or mTOR-deficiency completely abolishes IL-1β-induced Th17 cell proliferation[36]. These results suggest that IL-6 functions to direct differentiation of Th17 cells, whereas IL-1β functions to enhance expansion of Th17 cells in competition with other T cell subsets in the context of a resource-limited tissue environment.

In contrast to IL-1β and IL-6, the role of TGFβ in Th17 cell differentiation is more complex. Genetic approaches that used a dominant negative TGFβ receptor II, which inhibits the formation of a functional TGFβ signaling complex, and a T cell-specific deletion of TGFβ confirmed that endogenous TGFβ induces Th17 cell development *in vivo*[37–39]. However, deletion of TGFβ also led to excessive production of IFNγ and IL-4, which inhibited Th17 cell development and expansion[37, 38]. Thus, TGFβ can either function as an obligate Th17-inducing factor or could indirectly suppress alternate cell fates. Notably, in the absence of TGFβ, IL-6 alone can promote Th17 development in T cells that lack transcription factors T-bet and STAT6[40], which suggest that TGFβ represses Th17 differentiation indirectly by repressing T-bet and transcription factor GATA-binding protein 3 (GATA-3). This complexity is further highlighted by human studies; three separate research groups show that TGFβ is required for *in vitro* Th17 cell development, whereas two other research groups found that a cocktail of IL-1β, IL-6 and IL-23 is sufficient to drive Th17 differentiation[41–45]. These discrepancies could be due to the source of the human T cells (for example peripheral blood versus umbilical cord blood), which may influence their developmental status and hence, the requirement for TGFβ. However, under *in vivo* inflammatory conditions, the presence of TGFβ is probably necessary for optimal Th17 cell differentiation.

Although the combination of TGFβ and IL-6 efficiently generates Th17 cells *in vitro*, the cells generated by this cocktail are only weakly pathogenic, and subsequent exposure to IL-23 is essential to drive pathogenicity[21, 46–50]. A recent study further supported the notion that TGFβ is not always required for *in vitro* Th17 differentiation, as Th17 cells could be generated in response to IL-1β, IL-6 and IL-23 in a TGFβ-independent manner[50]. Interestingly, these TGFβ-independent IL-23R+, RORγt+ Th17 cells are also T-bet+ and co-express IFNγ and GM-CSF[4, 19, 20, 50]; such "hybrid" T cells with diverse functional plasticity (see below) are frequently detected in lesions from patients with multiple sclerosis (MS) and mice with EAE [51, 52].

The studies mentioned above specifically examined TGFβ1. In a recent report, it was proposed that TGFβ3 promotes pathogenic T_H17 cells by upregulating IL-23R expression[53]. Interestingly, TGFβ3 was induced by IL-23 and acted endogenously in a feed forward loop

to enhance IL-23R expression, thereby amplifying the IL-23 signal[53]. Taken together, these studies indicate that TGFβ together with IL-6 can drive development of IL-17- and IL-10-producing cells that have mucosal defensive functions; however, subsequent exposure to IL-23 is required for development of autoimmune-associated Th17 cells.

Differentiating and stabilizing cytokines

TGFβ- and IL-6-driven Th17 cells have limited inherent pathogenicity, and exposure to IL-23 is essential for the maturation of inflammatory Th17 cells[1]. This is supported by genetic studies that link *IL23R* polymorphisms with susceptibility to autoimmune diseases such as psoriasis, psoriatic arthritis, ankylosing spondylitis, multiple sclerosis and Crohn's disease[54–58]. Accumulating data clearly indicates that IL-23 promotes pathogenicity of mature Th17 cells by several mechanisms, including maintenance of Th17 signature genes (*Rorc* and *Il17*), induction of effector genes (*Il22, Csf2* and *Ifng*), downregulation of repressive factors (IL-2, IL-27 and IL-12) and amplification of its own signal by upregulation of *Il23r* expression.

By using an approach that restricts IL-23R deficiency to T cells, we have shown that without IL-23 signaling, Th17 cells are arrested at an early activation stage and are unable to mediate encephalitogenicity in EAE [48]. Subsequent studies revealed that IL-23R is needed for co-expression of GM-CSF; accordingly, GM-CSF-deficient mice are resistant to EAE and GM-CSF-deficient T_H17 cells cannot transfer EAE to naive recipients [19, 20]. IL-23 also induces IFNγ expression in Th17 cells, and IFNγ+IL-17+ cells are highly pathogenic[52, 59]. To gain inflammatory function, Th17 cells must mitigate the impact of inhibitory cytokines such as IL-27 and IL-2 [60, 61]. Indeed, committed IL-23R+ Th17 cells are highly resistant to IL-27- and IL-2-mediated suppression[62]. This phenomenon could be due to downregulation of IL-27R in mature Th17 cells, and IL-23R signaling may also induce transcription factors that partner with RORγt to inhibit repressive signals, and thereby stabilize the Th17 lineage. Pathogenic Th17 cells induced by IL-23 in the absence of TGFβ also express reduced levels of aryl hydrocarbon receptor (AHR), C-MAF and IL-10 compared with TGFβ driven Th17 cells[53, 59]. AHR and C-MAF are induced under TGFβ- and IL-6-driven Th17 differentiation conditions and transactivate IL-10 expression[63–65]. However, it is unclear whether IL-23 directly suppresses these factors to drive pathogenicity. The ability of IL-23 to drive differentiation and stabilization of pathogenic Th17 cells is upregulation of IL-23R expression, which is STAT3-dependent [10, 59]. Taken together, these studies establish that TGFβ, IL-6 and IL-1β are essential elements that initiate Th17 cell development, whereas IL-23 serves as a pivotal factor that drives both the differentiation and inflammatory functions of pathogenic Th17 cells.

Transcriptional control of Th17 cells

CD4+ T_H cell populations are considered unique lineages based on their characteristic expression of transcriptional regulators and signature cytokines. RORγt serves as the master regulator for T_H17 cells, and *Rorc*−/− mice lack T_H17 cells and exhibit reduced autoimmune disease severity[12]. In contrast, enhanced expression of RORγt in naïve CD4+ T cells induces key genes essential for T_H17 cell differentiation[12]. RORγt cooperates with a network of transcription factors — most notably, STAT3 IRF4 and basic leucine zipper transcription

factor ATF-like (BATF) — to initiate the complete differentiation program. The activation of STAT3 by IL-6 and IL-23 is a key event in Th17 lineage specification. **ChIP-Seq analysis** has demonstrated that STAT3 directly regulates the Th17 cytokine and cytokine receptor genes *Il17*, *Il17f* and *Il23r*, and it also controls expression of the Th17-specific transcription factors *Rorc, Batf, and Irf4* [31]. In addition, STAT3 binds both the promoter regions of *Il17a* and *Il17f* and their intergenic regions with enhancer elements that undergo histone modifications during differentiation[31]. These findings are also relevant to humans as patients with *STAT3* mutations suffer from autosomal dominant **hyper-IgE syndrome** (AD-HIES; also known as Job's syndrome), and are susceptible to recurrent pulmonary infections and chronic mucocutaneous candidiasis due to reduced IL-17-producing T cells [66, 67] (see below).

BATF is a member of the activator protein 1 (AP-1) family of transcription factors, which is upregulated in T_H cells following T cell receptor (TCR) activation[68, 69]. A study from 2009 showed that BATF is essential for T_H17 differentiation, and mice lacking BATF are resistant to EAE[69]. *Batf*$^{-/-}$ mice have normal TGFβ and IL-6 signaling and can upregulate RORγt early on during T cell development, but fail to maintain RORγt expression. However, overexpression of RORγt could only partially rescue Th17 defects in *Batf*$^{-/-}$ cells and expression of both BATF and RORγt is required to drive IL-17 expression, which suggests that BATF and RORγt cooperatively control Th17 differentiation. Furthermore, BATF forms a dimer with JUNB that binds directly to the promoters of *Il17*, *Il21* and *Il22* in Th17 cells. IRF4 was originally reported to be essential for T_H2 differentiation[70] and has now also been implicated in T_H17 differentiation[71]. IRF4-deficient mice are protected from EAE and exhibit a defect in T_H17 differentiation that stems from impaired RORγt expression. However, similar to BATF, overexpression of RORγt in *Irf4*$^{-/-}$ T cells could only partially restore IL-17 production, which indicates that both RORγt and IRF4 are required to establish the T_H17 lineage.

Two recent studies show that BATF and IRF4 are "pioneer factors" that cooperatively bind and govern chromatin accessibility, which enables RORγt recruitment and binding to Th17 signature genes[72, 73]. A comprehensive ChIP-Seq analysis revealed a striking overlap between BATF and IRF4 promoter occupancy in Th17 cells[73]. Furthermore, these transcription factors form a complex and regulate each other's DNA binding activities. Since BATF and IRF4 binding promotes strong association and co-occupancy by Th17-defining transcription factors (STAT3 and RORγt), BATF and IRF4 seem to serve as initiating factors that regulate chromatin accessibility in T_H cells. Surprisingly, co-occupancy by these transcription factors was also observed in TCR activated but non-polarized Th0 cells. Thus, BATF and IRF4 are bona fide pioneer factors that remodel chromatin to enable access of lineage-specifying transcription factors induced following environment signals.

Transcription of a gene is regulated by sequence-specific binding of proteins to the promoter region and/or to distant regulatory enhancer sites. Enhancer activity is measured by histone modification marks such as the histone acetyltransferase p300, which are recruited by lineage specifying signals, and the presence of these marks indicates a transcriptionally active regulatory domain. Strikingly, BATF, IRF4 and STAT3 deficiency substantially reduces p300 binding, but a lack of RORγt has almost no effect on the enhancer landscape.

Although RORγt is referred to as a master transcription factor, in fact only a few Th17 genes —*Il17*, *Il17f*, and *Il23r*—are exclusively dependent on RORγt [73]. Instead, RORγt acts as a modulator that fine-tunes a pre-established Th17 lineage program. RORγt positively regulates the Th17-specific genes while simultaneously repressing alternate lineage fates, by exploiting the altered enhancer landscape initially created by BATF, IRF4 (induced by TCR) and STAT3 (induced by cytokines) (Figure 2). These new insights argue against the notion of a single factor governing lineage specificity, and instead indicates that a transcription factor complex regulates fate commitment [74]. Furthermore, these results are consistent with studies that have identified other transcription regulators, such as T-bet (Th1) and Foxp3 (Treg) as factors that take advantage of an enhancer DNA landscape already established by STAT proteins[75, 76].

Another key transcription factor in Th17 fate determination is hypoxia-inducible factor 1α (HIF-1α), which is a key sensor of hypoxia (Figure 2). HIF-1α is induced upon TCR activation, and its expression is further enhanced by hypoxic conditions associated with tissue inflammation as well as IL-6-and STAT3-dependent differentiation signals[77]. Mechanistically, HIF-1α directly binds and drives transcription of RORγt and forms a complex with RORγt and p300 to drive IL-17 expression. HIF-1α can also form a complex with FOXP3, and targets it for proteasomal degradation. In this way, proinflammatory environmental cues such as hypoxia stabilize the Th17 lineage.

In spite of advances in deciphering the Th17 transcriptional network, we still lack a clear understanding of IL-23-dependent mechanisms that orchestrate the pathogenic Th17 differentiation process. STAT3 activation alone cannot explain the unique requirement for IL-23 in pathogenic T_H17 cell commitment since IL-6 is an even more potent activator of STAT3. Additional transcriptional regulators or signaling pathways induced by IL-23 must operate to promote T_H17 inflammatory effector cell function. Also, it will be important to assess the Th17 enhancer landscape created by IL-6 compared with IL-23 to determine whether divergence at an epigenetic level impacts functional outcomes.

Th17 cell plasticity versus stability

Plasticity is defined as the ability of Th17 cells to acquire divergent functional capabilities while maintaining the fundamental Th17 program as defined by RORγt and IL-17 expression. Recent published data indicates that Th17 cells retain a high degree of plasticity that enables them to co-express alternate lineage-specifying transcription factors and effector cytokines [23, 50, 52, 53, 78]. Such functional heterogeneity endows Th17 cells with an enhanced capacity to traffic to a broad range of anatomical sites and efficiently promote diverse functions including host defense, barrier tissue integrity and wound healing responses.

Factors that define regulation of Th17 plasticity is an area of intensive investigation. Although RORγt is essential for the development of Th17 cells, it is not sufficient to promote all functional capabilities. As mentioned above, IL-23 might be a pivotal factor dictating Th17 functional diversity through induction of alternate lineage specific transcription factors (such as T-bet) and effector genes (such as *Il22, Csf2* and *Ifng*), while

still maintaining the core Th17 program (which entails expression of *Rorc* and *Il17*)[4, 25]. We have recently identified that transcription factor B-lymphocyte-induced maturation protein 1 (BLIMP1, which is encoded by *Prdm1*) is induced by IL-23 and acts both as a gene activator and repressor to induce Th17 maturation (Unpublished data, R. Jain and D. J. Cua). BLIMP1 coordinates with RORγt to enhance expression of IL-23R and GM-CSF while simultaneously repressing IL-2 and alternate fate determining factors (such as *Bcl2*, *Foxp3*, *Gata3*). Interestingly, BLIMP1 directly engages the *Il23r, Il17f* and *csf2* loci in close proximity to RORγt, p300 and STAT3, which suggests that BLIMP1 is part of a complex that regulates Th17 pathogenic function (Unpublished data, R. Jain and D. J. Cua).

Th17 cell plasticity could be explained by epigenetic modifications. Recent advances in genome-wide analysis of histone modifications enables examination of permissive and repressive histone marks, H3K4me3 and H3K27me3, respectively, on lineage specifying genes. Interestingly, Th17 cells display both active and repressive histone marks on *Tbx21* and *Gata3*, whereas Th1 and Th2 cells show only repressive marks on *Rorc* and *Il17* loci. In other words, Th17 cells appear to be highly poised for subsequent activation under appropriate environmental stimuli[79]. Interestingly, T_{Reg} cells are the only other cell type with such dual modifications on master transcription factors, which gives them the unique advantage of functional diversity and plasticity.

The concept of Th17 stability is more than a question of functional malleability. Stability is defined by whether RORγt-expressing Th17 cells can lose their core transcriptional and functional characteristics and become RORγt-"ex-Th17" cells[52]. The concept of Th17 stability has been explored by employing both *in vitro* and *in vivo* approaches. The *in vitro* polarization approach, in which the inducing signals are constantly present, has been unable to demonstrate that Th17 cells are unstable. However, *in vivo* systems that use mouse strains with a expressing a *Cre* recombinase from IL-17A promoter have greatly enhanced our understanding of this important question [22, 52, 80]. Crossing the *Il17a-Cre* allele to *Rosa26*-enhanced yellow fluorescence protein (YFP) reporter mice generated a fate mapping system in which *Il17*-expressing cells are permanently marked with YFP. This cell tagging system has demonstrated that "bona fide" Th17 cells can be destabilized and converted to Th1 cells under proper environmental stimuli. Histone modification analysis of cytokine bead captured IL-17$^+$ cells from the inflamed intestine confirmed these results[81]. However, an epigenomic analysis of ex-Th17 cells at a single cell level is required to gain a deeper molecular understanding of Th17 stability and to avoid any variations caused by heterogeneity of the population. For instance, it would be revealing to analyze epigenetic changes in Th17 cells that acquire Th1 functions. Do they undergo complete epigenetic remodeling of Th17 associated genes, or do they retain epigenetic evidence of their Th17 origins? Are former Th17 cells capable of reacquiring the original Th17 program upon TCR stimulation because they retain a core epigenetic status? Understanding the plasticity of Th17 cells at the epigenetic level may have important advantages. First, it could predict the developmental stages of Th17 cells that would be susceptible to reprogramming towards the Treg cell phenotype. This cellular reprogramming could have added anti-inflammatory benefits, in addition to 'depleting' Th17 cells. Second, it is vital to understand the durability of the Th17 effector cell suppression and/or reprogramming. It is critical to recognize

whether "ex Th17 cells" can revert back to their original pathogenic phenotype once the effects of drugs have subsided.

IL-17 signal transduction

As outlined above, much effort has been devoted to defining cellular sources of IL-17 and understanding the subsequent regulation of its expression. However, the biological impact of IL-17 is mediated through its receptor in downstream target cells, which is outlined in this section. Cells that respond to IL-17 are mainly, though not exclusively, non-hematopoietic and the effects of IL-17 are most similar to other innate inflammatory effectors, especially those that belong to the IL-1 family of cytokines and TLR ligands[82]. In particular, IL-17 is a potent regulator of neutrophils, which is mediated by IL-17-induced downstream genes such as those that encode the granulocyte colony-stimulating factor (G-CSF) and CXC chemokines. The IL-17 receptor (IL-17R) has many unique and/or unexpected properties in terms of structure and signaling, and ultimately a detailed understanding of these events may reveal potential novel drug targets or avenues for therapeutic intervention.

Positive activation of signaling

When IL-17R was cloned in 1995 it was noteworthy for its striking lack of similarity to known receptor families[83]. Subsequently, it became clear that the IL-17R (now known as IL-17RA) was the founding member of a new subclass of cytokine receptors, which comprises five receptor subunits, IL-17RA–IL-17RE, that have homology to one another but not to other receptor families[82] (Figure 3). All subunits of the IL-17R family exhibit a broad expression pattern; IL-17RA is most highly expressed in hematopoietic cell types and IL-17RC in non-hematopoietic cells[84]. Most of the homology within the IL-17R family is located in the conserved cytoplasmic motif SEF/IL-17R (SEFIR), which suggests a common mode of signaling that is probably distinct from other cytokine receptors. A landmark bioinformatics study demonstrated that this domain was related to the Toll/IL-1R (TIR) domain. Recent studies have revealed that the IL-17RA subunit is shared among several members of this family (Figure 3). IL-17RA is essential for signaling through IL-17E (also known as IL-25) when partnered with IL-17RB, and also for signaling through IL-17C when partnered with IL-17RE[85, 86]. The only other protein identified to have a SEFIR domain is the adaptor ACT1 (also known as CIKS)[87]; indeed, ACT1 is essential for signal transduction by all analyzed IL-17R family members[82, 88, 89].

Despite the similarity between IL-17R and TLR/IL-1Rs, studies of IL-17 have revealed several differences in how IL-17R operates. For example, ACT1 is not a positive activator of signaling in any system outside the IL-17R family[90]. ACT1 serves both as a signaling adaptor, to recruit tumour necrosis factor receptor (TNFR)-associated factor (TRAF) proteins to direct activation of several downstream signaling cascades[88], and as an E3 ligase to mediate ubiquitination of TRAF6[91]. Recruitment and ubiquitination of TRAF6 leads to activation of the canonical NF-κB pathway and the mitogen-activated protein kinase (MAPK) pathways, which include extracellular signal-regulated kinase (ERK), p38 and JUN N-terminal kinase (JNK). Consequently, activation of these pathways by IL-17 is impaired in the absence of either ACT1 or TRAF6[88, 89, 92]. Alternatively, ACT1 can recruit TRAF2 and TRAF5, which promote activation of an mRNA stability pathway via the SF2/ASF or

HuR complexes[93, 94]. Regulation of these alternative pathways occurs via inducible ACT1 phosphorylation, which is mediated by Ikki (Ikkε) and TBK1 (Figure 4)[95, 96]. ACT1 function and stability is in turn regulated by the chaperone protein hsp90. Inhibition of hsp90 results in proteasomal degradation of ACT1, which in turn leads to defective IL-17 signaling[97]. ACT1 appears to be required for most IL-17-dependent signaling events[98] and the majority of phenotypes $Act1^{-/-}$ mice are similar to $Il17ra^{-/-}$ mice. Notably, a single-nucleotide polymorphism (SNP) in ACT1 has been associated with psoriasis[99]. Thus, in the IL-17R family all known signaling emanates from ACT1, which leads to an activation of pro-inflammatory signaling cascades.

IL-17 induces signaling mainly in cells of non-hematopoietic origin, including epithelial and mesenchymal cell types. The genes that are induced by IL-17 largely explain its pro-inflammatory activities; for example, IL-17 induces expression of chemokines such as CXC-chemokine ligand 1 (CXCL1), CXCL2 and CXCL5 that promote neutrophil chemotaxis[100]. IL-17 also induces expression of CC-chemokine ligand 20 (CCL20), which promotes trafficking of mucosal-associated cells expressing CC-chemokine receptor 6 (CCR6); notably, CCR6 is a characteristic receptor on IL-17-expressing cells such as Th17 cells and ILC3s [101]. Other cytokines induced by IL-17, such as IL-6 and G-CSF, regulate myeloid lineages and especially neutrophils. Additionally, IL-17 is a strong inducer of **antimicrobial peptides** (AMPs), particularly β-defensins, which can prevent infection at mucosal surfaces and skin and that may also exert chemotactic activity[102]. Lipocalin-2 (LCN2; also known as 24p3) is another IL-17-induced antimicrobial protein that controls bacterial growth by restricting access to dietary iron[103].

The CCAAT/enhancer-binding protein (C/EBP) family of transcription factors is also activated by IL-17 signal transduction, and is often just as important as NF-κB in the regulation of target genes. For example, the *Il6* and *Lcn2* promoters require both NF-κB and C/EBP elements, as deletion of either element renders their promoters insensitive to IL-17-mediated signals[104, 105]. IL-17 induces expression of C/EBPδ mRNA and protein, and to a lesser extent C/EBPβ[104, 106]. The latter C/EBPδ protein is regulated extensively by posttranscriptional or posttranslational modifications, including alternative translation and phosphorylation[107]. Phosphorylation of C/EBPβ depends on a GSK3β- and ERK-dependent pathway, which functions to dampen IL-17 dependent signaling (see below)[108].

On their own, IL-17 (and IL-17F) are modest activators of signaling, but they function cooperatively with other pro-inflammatory molecules, particularly TNFα but also IFNγ, IL-22, lymphotoxin, IL-1β and lipopolysaccharide[109]. The molecular basis for this synergy is not completely understood, and probably involves multiple mechanisms. In synovial tissue, IL-17 upregulates TNFRII expression, and thereby enhances responsiveness to TNFα signaling[110]. For some genes, cooperativity between IL-17 and TNFα occurs at the level of the promoter (for example *Il6* and *Lcn2*) and/or mRNA stability[111]. IL-17 also upregulates expression of IκBζ — a positive regulator of the NF-κB family — which in turn promotes expression of at least some target genes[112]. The ability of IL-17 to signal cooperatively with other cytokines is probably an important aspect of its biology, since inflammatory environments contain multiple cytokines that can act in concert.

Negative regulation of IL-17 signaling

Dysregulated IL-17 production and signaling is associated with the development of several autoimmune diseases, thus it is not surprising that there are multiple layers of restriction that control IL-17-mediated inflammatory signaling. As outlined below, a variety of inhibitory mechanisms have been identified (Figure 4).

Deubiquitylating enzymes (DUBs) can temper IL-17-mediated signal transduction by targeting TRAFs; ubiquitin C-terminal hydrolase 25 (USP25) removes K63-linked ubiquitin chains on both TRAF6 and TRAF5, which inhibits transcription of IL-17 target genes as well as IL-17-induced mRNA stability USP25 deficiency is associated with enhanced IL-17-dependent pulmonary inflammation and increased susceptibility to EAE[113].

We recently identified **A20** (*Tnfaip3*) as a vital feedback inhibitor of IL-17 signaling[114]. SNPs in *TNFAIP3* are associated with numerous autoimmune and lymphoproliferative diseases, including psoriasis, rheumatoid arthritis (RA) and certain B cell malignancies[115]. IL-17 induces expression of *Tnfaip3* mRNA and A20 protein levels, which triggers deubiquitination of TRAF6 and downregulation of NF-κB and MAPK activation (particularly JNK). Notably, A20 associates directly with IL-17RA through the receptor's C-terminal "C/EBPβ activation domain" (CBAD)[114]. The CBAD is also an interaction site for TRAF3, another negative regulator of IL-17 signaling[116], and deletion of the CBAD was shown several years ago in a structure-function analysis of IL-17RA to enhance IL-17 signaling[117]. Intriguingly, A20 appears to inhibit IL-17 in a noncanonical fashion. In other systems, A20 needs to interact with accessory factors, such as TAX1-binding protein 1 (TAX1BP1) and E3 ubiquitin protein ligase ITCH, in order to hydrolyze K63-linked substrates efficiently[118] and deletion of TAXBP1 or ITCH results in a phenotype similar to the lack of A20, albeit less severe[119, 120]. However, knockdown of either TAX1BP1 or ITCH do not inhibit IL-17 signaling, which suggests that A20 might use alternate adaptors. Indeed, we found that A20 associates with anaphase-promoting complex subunit 5 (APC5)[121, 122], which is a subunit of a multi-protein E3 ubiquitin ligase complex. Together, these data show that reversal of K63 ubiquitination on TRAF6 via A20 is an important downregulatory strategy for restricting IL-17-mediated inflammation.

K48-linked ubiquitination and proteasomal degradation of adaptor proteins is another means of regulating inflammatory signaling. IL-17R signaling is inhibited by the Ub ligase complex β-TrCP, which induces K48 ubiquitination on phosphorylated forms of ACT1 that in turn leads to its degradation through the proteasome and consequent dampening of IL-17-dependent signaling[123]. TRAF proteins also play negative roles in regulating inflammatory signaling pathways by IL-17 [124]. For example, TRAF3 associates with IL-17RA through a consensus TRAF binding site in the CBAD of the receptor. TRAF3 competes with ACT1 for binding to IL-17RA and thereby dampens IL-17-dependent signals[116]. Similarly, TRAF4 directly binds Act1 upon IL-17 stimulation and competes for its association with TRAF6. Consistently, TRAF3 and TRAF4 limit IL-17-mediated development of EAE[116, 126].

Some data implicate **microRNAs** in regulating both Th17 development and IL-17 signaling. The microRNA MiR-155 expressed in CD4+ T cells positively regulates Th17 differentiation[127], which may be due to blockade of suppressor of cytokine signalling 1

(SOCS1), a negative regulator of JAK/STAT signaling[128]. Consequently, MiR155−/− mice or mice in which this miRNA is silenced are resistant to EAE[127, 129]. In terms of signaling, MiR-23b keeps IL-17-induced NF-κB activation in check by targeting the degradation of TAB2/3 and IKKα. Consistently, patients with RA and systemic lupus erythematosus (SLE) show reduced expression of miR-23b [130].

As noted above, C/EBPs regulate IL-17-induced gene expression. Interestingly, C/EBPβ is both a positive and negative regulator of the IL-17 pathway. Although C/EBPβ can positively transactivate IL-17 target genes[104], C/EBP phosphorylation of C/EBPβ by the kinases Erk and GSK3β correlates with a decrease in IL-17-induced gene expression. Notably, phosphorylation through GSK3β is dependent on the CBAD, another negative regulatory event mediated through the IL-17RA distal domain [108].

Effector cytokines of Th17 cells

Th17 cells in steady state

Naturally, Th17 cells did not evolve to cause autoimmunity, but to provide effective host defense against pathogens. Experiments using IL-17- and IL-17R-knockout mouse models have demonstrated increased susceptibility to a wide variety of infectious organisms, including bacteria, parasites, fungi and viruses. However, examination of families or individuals with specific mutations in the Th17 pathway indicated a surprisingly limited role for Th17 cells and IL-17 in immunity to infections, with susceptibility to commensal fungi (mainly the commensal *Candida albicans*) by far the dominant disease seen in these settings (Summarized in Table 1). Although the reasons for the dichotomy between mice and humans are still unclear, this is similar to findings in MyD88-deficient humans, who show a relative restricted susceptibility to pyogenic bacteria, in contrast to the broadly susceptible MyD88-knockout mice[131].

As noted above, patients with AD-HIES have autosomal dominant-negative mutations in *STAT3* that are associated with reduced frequency of Th17 cells and susceptibility to chronic mucocutaneous candidiasis (CMC), *Staphylococcus aureus* infections and pulmonary infections[66, 132]. The reduced frequency of Th17 cells in CMC is associated with mutations that impair fungal sensing (*CARD9*), IL-23 signaling (*IL12RB1, IL12B*) or enhance negative regulation of Th17 differentiation (*STAT1*) [133–135]. Similarly, CMC but not other infections occurs patients with **APS-1/APECED, who** have naturally-occurring neutralizing antibodies against Th17 cytokines[136, 137]. Null mutations in *IL17RA* or *ACT1* and dominant-negative mutations in *IL17F* also promote CMC and some staphylococcal infections[138, 139]. Therefore, deficiencies in Th17 and/or IL-17 are strongly linked to defects in fungal immunity and these association studies support data from mouse studies while revealing the apparent resilience of the human immune system in the face of immunodeficiency[140].

Th17 cells in disease states

While Th17 cells did not evolve to cause disease, they are programed to provide formidable host protective responses—often associated with rapid recruitment and activation of granulocytes and macrophages capable of producing tissue damaging reactive oxygen species (ROS) [141]. During prolonged and dysregulated exposure to IL-1 and IL-23, Th17

cells recruit inflammatory myeloid cells to cause severe local tissue injury[35, 142]. Experiments using *Il17-, Il22-, Il23a- or Il23ra*-deficient mice as well as antibody-mediated inhibition have demonstrated a requirement for these cytokines in EAE, CAIA, CIA, IBD, AS, and psoriasis disease models[4, 5, 25, 142, 143]. Based on these preclinical models and human GWAS studies and pharmacogenomic disease association analyses, psoriasis, IBD, and AS have emerged as the leading disease indications for anti-IL-17 and anti-IL-23 treatments[54–58, 144].

Psoriasis is an immune-mediated inflammatory disorder characterized by the activation of both adaptive and innate Type-17 responses[145]. The pathogenic involvement of IL-23 in psoriasis is supported by the increased expression of IL-23 in psoriatic lesional skin and its ability to induce **psoriasiform** characteristics in a preclinical model of intradermal IL-23 administration[143]. Injection of IL-23 promoted dermal acanthosis, neutrophil recruitment, and infiltration of IL-17- and IL-22-producing T cells. In addition, inhibition of IL-23 led to significant reduction of IL-17 and IL-22 in psoriatic lesional skin, which supports that these cytokines are key regulatory factors for this disease. IL-17-producing $CD4^+$ T cells, $CD8^+$ T cells, γδT cells and ILCs can all be found in the psoriatic skin[146]. Additional skin resident cells such as keratinocytes, fibroblasts and endothelial cells respond to IL-17 and produce autocrine factors that promote rapid cell division and neo-angiogenesis, which strongly contribute to the disease process. Inappropriate secretion of anti-microbial peptides (such as β-defensins, lipocalin, LL-37, S100A7) and chemokines (CXCL 1,2,3,5, IL-18, CCL20) by keratinocytes further recruit and activate mast cells, neutrophils, and inflammatory macrophages[147]. This interaction between Type-17 lymphoid cells and tissue resident keratinocytes/fibroblasts creates amplification loops that drive epidermal hyperplasia and pro-inflammatory conditions—all of which are hallmarks of psoriasis pathogenesis.

The seronegative spondyloarthropathies (SPA) are a heterogeneous group of immunological diseases that follows specific types of viral and bacterial infections and include ankylosing spondylitis, psoriatic arthritis and the 'reactive' arthritis[148]. In contrast to rheumatoid arthritis where inflammation is accompanied by bony erosion and destruction, spondyloarthropathy is characterized by new bone formation where ligaments attach onto bone—the entheseal organ. We have recently uncovered the presence of RORγt+ IL-23R +CD3+CD4-CD8-Sca1+ entheseal resident cells and that their production of IL-17 and IL-22 are the major cause of STAT3-dependent osteoblast-mediated bone remodeling[142]. The new bone appears as inappropriate bony outgrowths from spinal vertebrae, which can fuse and immobilize the spine causing the ultimate morbidity for this disease[149]. This condition is commonly associated with uveitis, aortic valve inflammation, psoriasis and inflammatory bowel disease. Indeed, uveitis accounts for significant comorbidity in ankylosing spondylitis, affecting 40% of patients. In addition, up to 70% of patients with ankylosing spondylitis have microscopically evident bowel inflammation[150]. These observations suggest that there is a fundamental underlying pathology linking these immune disorders. Recently GWAS have identified multiple SNPs in IL-23R, which are associated with either protection from ankylosing spondylitis, psoriasis, IBD or increased susceptibility to these autoimmune conditions[54–58].

The same IL-23R SNPs found in individuals with ankylosing spondylitis and psoriasis were present in patients with Crohn's disease along with *Nod2 / Card15* (sensor for bacterial cell wall)[54, 151, 152]. This is consistent with the body's immune system having an elevated Th17 response attacking the intestinal mucosa, possibly against commensal bacterial antigens. Preclinical animal studies have also identified IL-23 as a key inflammatory mediator that promotes chronic tissue injury responses. In Crohn's disease, increased expression of IL-17A mRNA and intracellular protein in the intestinal mucosa has been reported[153]. Elevated fecal IL-17A levels were also described in active Crohn's disease[154]. Taken together, these observations implicate the IL-23-IL-17 immune axis in the pathogenesis for Crohn's disease and the related immune disorders such as psoriasis and spondyloarthropathies and provide a rationale for targeted-antibody therapy.

Targeting the Th17 pathway in disease

IL-17 and IL-23 are emerging as essential factors in the disease process of many autoimmune disorders. Currently, a number of pharmaceutical companies are testing antibody drugs targeting IL-17A, IL-17RA, IL-17A/IL-17F (an antibody recognizing common motifs of the 2 related cytokines), IL-17A/TNF (bi-specific antibody with one CDR recognizing IL-17A and the other recognizing TNF), p40 subunit of IL-23 and IL-12, as well as p19 subunit of IL-23—in psoriasis, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, autoimmune uveitis, asthma, and multiple sclerosis (Table 2). To date, the antibody drugs that specifically neutralize IL-23 or IL-17A have shown remarkable effectiveness for the treatment of psoriasis. These agents are also showing initial promising results in AS and MS.

Currently, four companies are in the final stages of clinical testing of anti-IL-23 and anti-IL-17 for psoriasis (Table 3). The phase 2 proof of concept studies for Ixekizumab (anti-IL-17A), Secukinumab (anti-IL-17A), Brodalumab (anti-IL-17RA), and Tildrakizumab (anti-IL-23p19)[155–158] showed that inhibition of IL-17, IL-17RA, or IL-23p19 had similar safety profiles and therapeutic efficacy (Table 3), comparable or better than current standard of care drugs such as ustekinumab (anti-p40 of IL-12 and IL-23) and etanercept (anti-TNF)[159]. All agents showed impressive efficacy with 70–80% of the patients achieving PASI-75, which is defined as having >75% disease clearance, and nearly half reaching PASI-100, a remarkable 100% disease clearance (Table 3). Currently, all four compounds have advanced to Phase 3 trials. These clinical data support the concept that IL-17 and IL-23 are key "check points" for the psoriatic inflammatory skin conditions.

Conventional treatment for Crohn's disease includes immunosuppression with corticosteroids, methotrexate and anti-TNF agents. Many patients became secondary failures due to intolerance or loss of initial response (for example development of anti-drug antibody). Ustekinumab, which targets the p40 unit of both IL-12 and IL-23, was tested in patients with moderate to severe Crohn's disease[160]. The study showed that patients with elevated C reactive protein (CRP), colonic inflammation, and primaly anti-TNF 'failures' had better overall treatment response rates. These results are consistent with the concept of targeting the IL-23-IL-17 immune axis may reduce disease burden in patients with Crohn's and suggest that targeting additional check points along this pathway may be effective.

Based on preclinical studies of anti-IL-17 blockade in IBD and the encouraging ustekinumab study, two groups evaluated anti-IL-17 in IBD. Unexpectedly, trials of Brodalumab (anti-IL-17RA) and Secukinumab (anti-IL-17A) for Crohn's Disease were terminated due to lack of efficacy and/or disease exacerbation[161, 162]. None of the anti-IL-17RA treatment groups showed improvement in Crohn's Disease Activity Index response compared to placebo. Moreover, results showed a disproportionate worsening of disease in anti-IL-17RA-treated patients. A higher frequency of fungal infections was observed in patients on Secukinumab (9%) compared with placebo (0%), consistent with the possibility that inhibition of IL-17 may increase susceptibility to CMC (see above). However, it is unclear why this condition appeared in Crohn's disease but not psoriasis patients. To compound the situation, post hoc analysis revealed that the subgroup of anti-IL-17A-treated patients with disease exacerbation had elevated inflammatory markers including CRP and fecal calprotectin (Reg3g), which are known targets of IL-23 and IL-22. It is intriguing that blockade of IL-17 results in mucosal tissue damage associated with up-regulation of IL-23 and RORγt-dependent inflammatory signals. One plausible explanation is that the basal levels of IL-17 *may be* gut protective. Indeed, *Il17-/-*mice have increased gut mucosal permeability, predisposing to higher incidence and severity of IBD [163] perhaps due to impaired tight junction integrity (Tato and Cua, unpublished observation). These findings may explain differences between targeting IL-23 p40/p19—which leaves tonic levels of IL-17 intact—versus complete ablation of IL-17.

Conclusions and perspectives

Since the discovery of the IL-23-Th17 immune pathway a decade ago, immunologists and clinicians have worked diligently to bring this novel therapeutic strategy to the clinic, which is now showing encouraging results for psoriasis, Crohn's disease, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis[160, 164]. However, this treatment strategy is complex. It was initially assumed that IL-23 controls the production of pathogenic IL-17 and that these cytokines are 'duplicate' targets. Recent clinical results suggest that is not the case at all. We are now beginning to appreciate that anti-IL-23p19 versus anti-IL-17 treatments each has its own beneficial effects as well as unique challenges in different disease settings. For example, anti-IL-17 showed good therapeutic efficacy for the treatment of psoriasis—even surpassing anti-TNF therapy[159165], but failed in Crohn's disease. The search for better clinical efficacy biomarkers is critically needed to improve patient stratification and disease indication selection. In addition, better understanding of Th17 biology and cellular mechanisms would allow discovery of additional targets for inflammatory diseases.

Progress in deciphering IL-17R-mediated signal transduction cascades has advanced considerably in recent years. Data indicate that, although these signaling pathways in many respects overlap with IL-1R and TLR-mediated pathways, there are also distinct processes that seem to be IL-17 specific. Understanding these events, particularly those that are unique to the IL-17 pathway, may reveal novel treatment strategies. Similarly, new insights into the epigenetics and transcriptional control of Th17 cells have revealed new treatment paradigms. A novel approach could be through destabilizing the Th17 lineage to induce reprogramming or functional suppression that could have immense therapeutic potential. Indeed, suppression of JAK and TYK2-dependent STAT3 activation[166, 167] as well as direct

inhibition of RORγt—using small molecule pharmacologic agents—have demonstrated impressive efficacy in preclinical disease models[168–171]. It is only a matter of time before these new therapeutic approaches will begin to make a difference in the clinic. The discovery IL-23-IL-17 immune axis has thus brought about fundamental changes in cellular immunology and more importantly improved the quality of life for many patients.

Acknowledgments

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Glossary terms

ChIP-Seq

Hyper-IgE syndrome	Inherited immune deficiency usually due to mutations in STAT3 and associated with reduced Th17 cell frequency. Disease is characterized by elevated IgE and eosinophilia, "cold" staphylococcal abscesses, eczema, pulmonary infections and CMC.
APS-1/APECED	Autoimmune polyendocrine syndrome/autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. Inherited autoimmune disorder due to mutations in the *AIRE* gene targeting multiple endocrine tissues. Disease is associated with a high incidence of mucocutaneous candidiasis, thought to arise due to neutralizing auto-antibodies against IL-17A, IL-17F and/or IL-22.

Antimicrobial peptides	short peptides (typically 12–50 amino acids) with bactericidal and fungicidal activities. Some may also exhibit chemotactic activities.
A20	Gene product of the Tumor necrosis factor alpha induced protein 3 (TNFAIP3) gene. Zinc finger protein with E3 ligase and deubiquitinase activity that downregulates signaling by multiple inflammatory effectors.
microRNAs	Single-stranded RNA molecules of approximately 21–23 nucleotides in length that regulate the expression of other genes.
psoriasiform	
Crohn's disease	

Box 1

"Type 17" subsets of cells ubiquitously express RORγt and IL-23R. Their development is Thymic dependent with the exception of Group 3 ILCs. Adaptive CD4+ IL-17-producing cells require IL-6 signaling during initial TCR-mediated activation. All other subsets do not require IL-6 activation and are capable of responding to IL-1 and IL-23 signaling upon emigrating from the thymus. These "innate" immune cells are poised to produce IL-17 upon sensing inflammatory cytokines as well as stress and injury signals. While the adaptive Th17 cells reside primarily in secondary lymphoid organs, the "innate" Type 17 cells are situated in a broad range of peripheral tissues, where they directly survey the interface between the host and the environment.

| Cell Type	TCR usage	Developmental Timing	Activation requirements	Primary Tissue location (steady state)	Refs
Adaptive Th17	Diverse TCR	Thymic, adolescent to adult	Antigen-specific TCR activation IL-6, TGFb, IL-1, IL-23	Lymphoid organs	[12]
Natural Th17	Diverse TCR Self reactivity?	Thymic, adolescent to adult	IL-1, IL-23	Skin and mucosal tissues	[17]
IL-17-producing gd T cells	Invariant TCR Vg6, Vg4, Vg1 Variable TCR	Fetal and neonatal thymic development Adolescent to adult	Dectins, TLRs, IL-1, IL-23 TCR + IL-1 and IL-23	Mucosal and peripheral tissues Lymphoid organs	[13] [172]
iNKT	Oligoclonal	Thymic, adolescent to adult	CD1, glycolipids	Liver	[173]
Group 3 ILC	None	Independent of thymic development; throughout life	Dectins, TLRs, IL-1, IL-23	Gut, skin	[174]
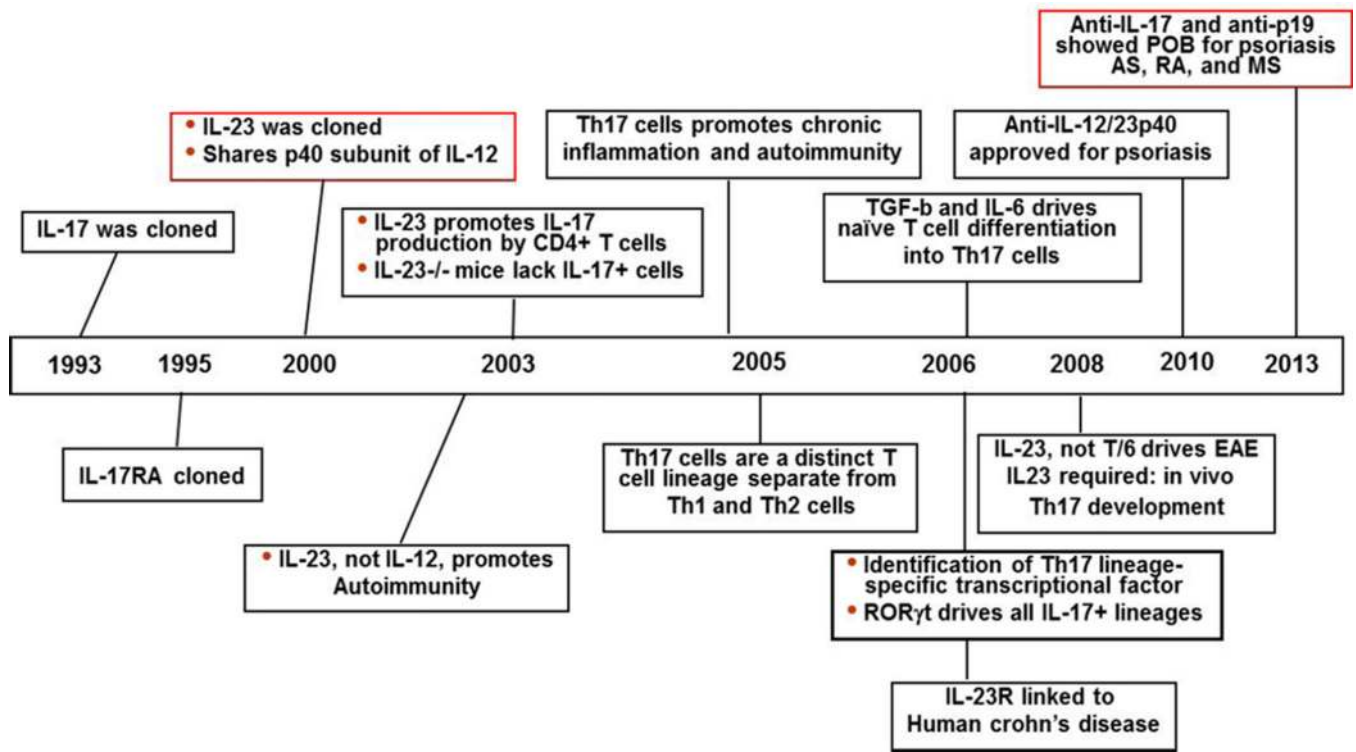
Figure 1.
Discovery of the IL-23/Th17 immune pathway
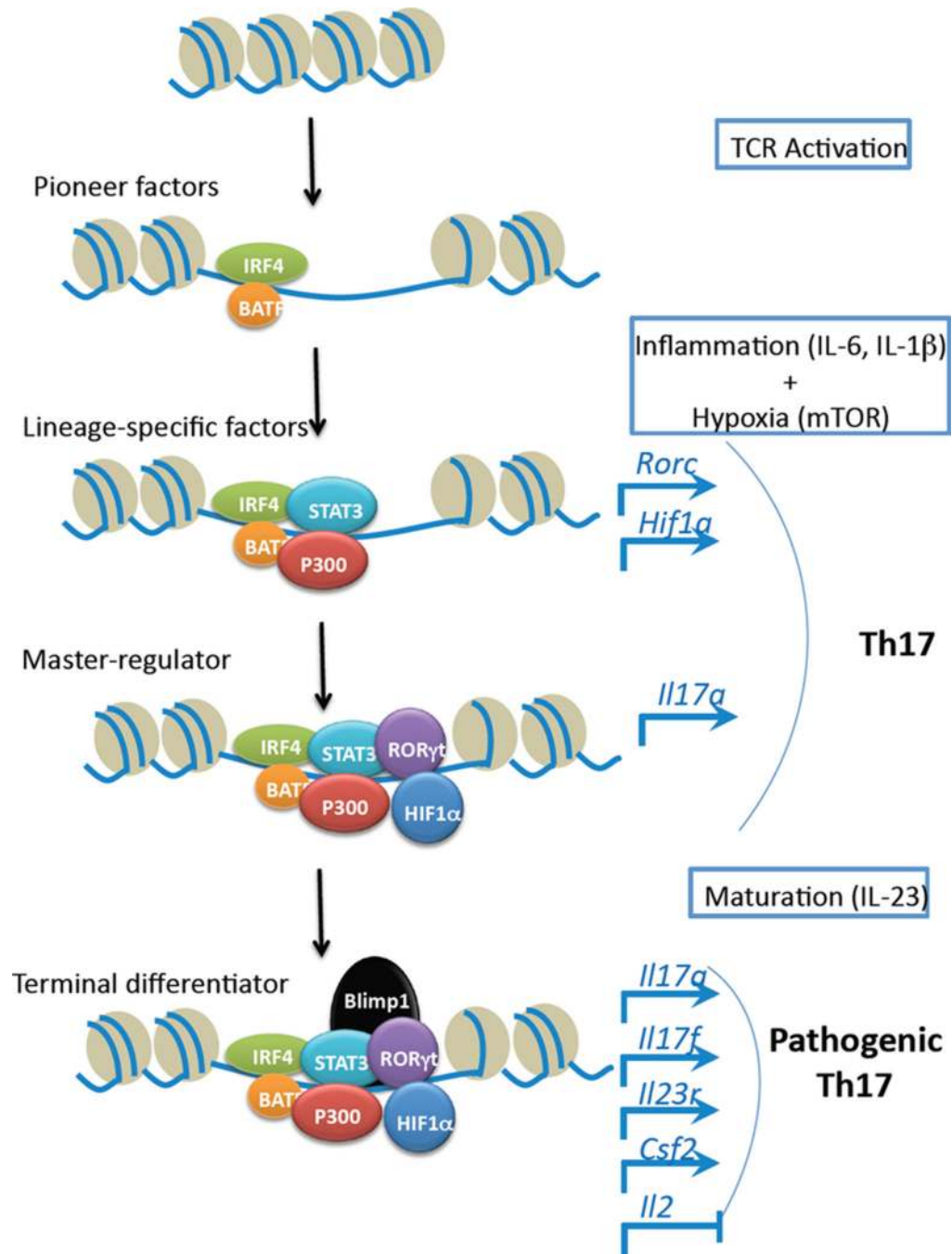
Figure 2.
Transcriptional control of Th17 cells
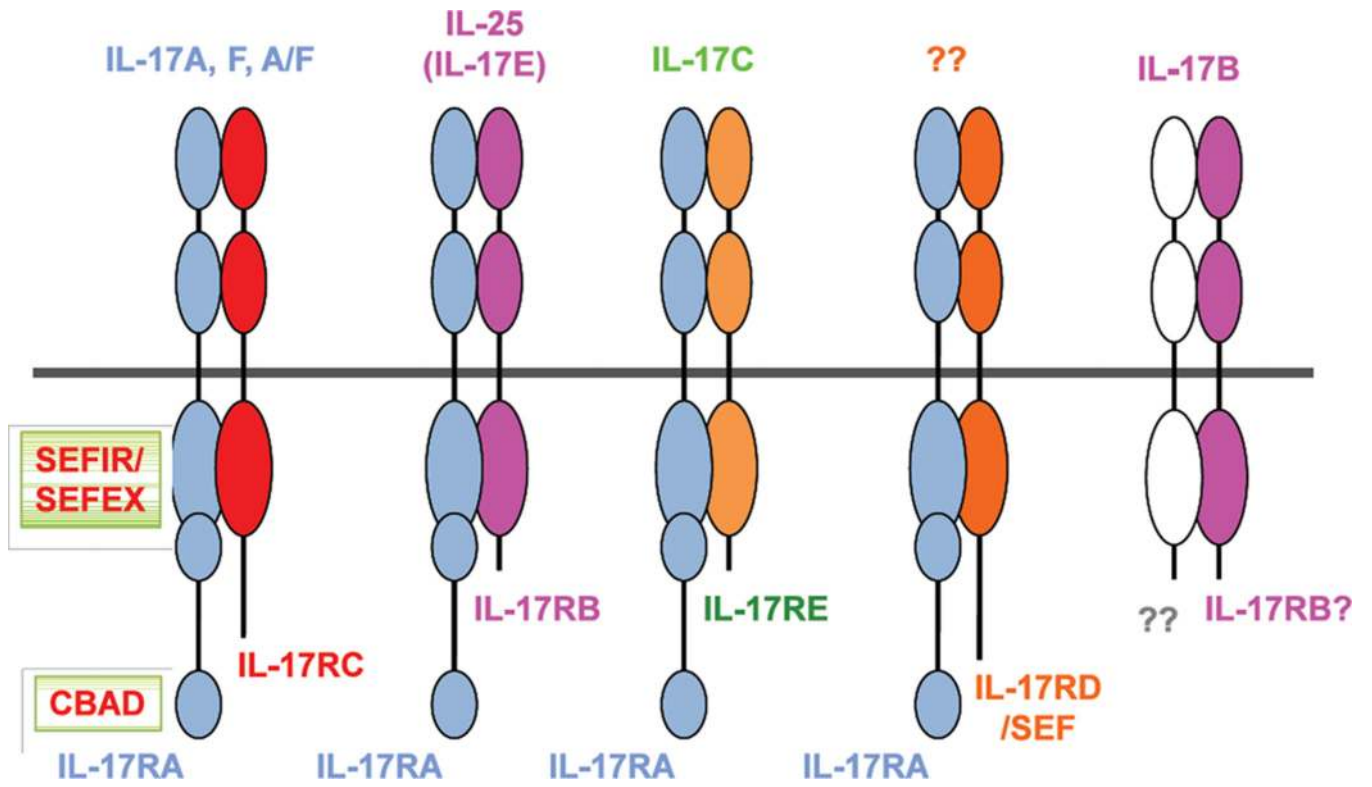
Figure 3.
IL-17 receptor family members
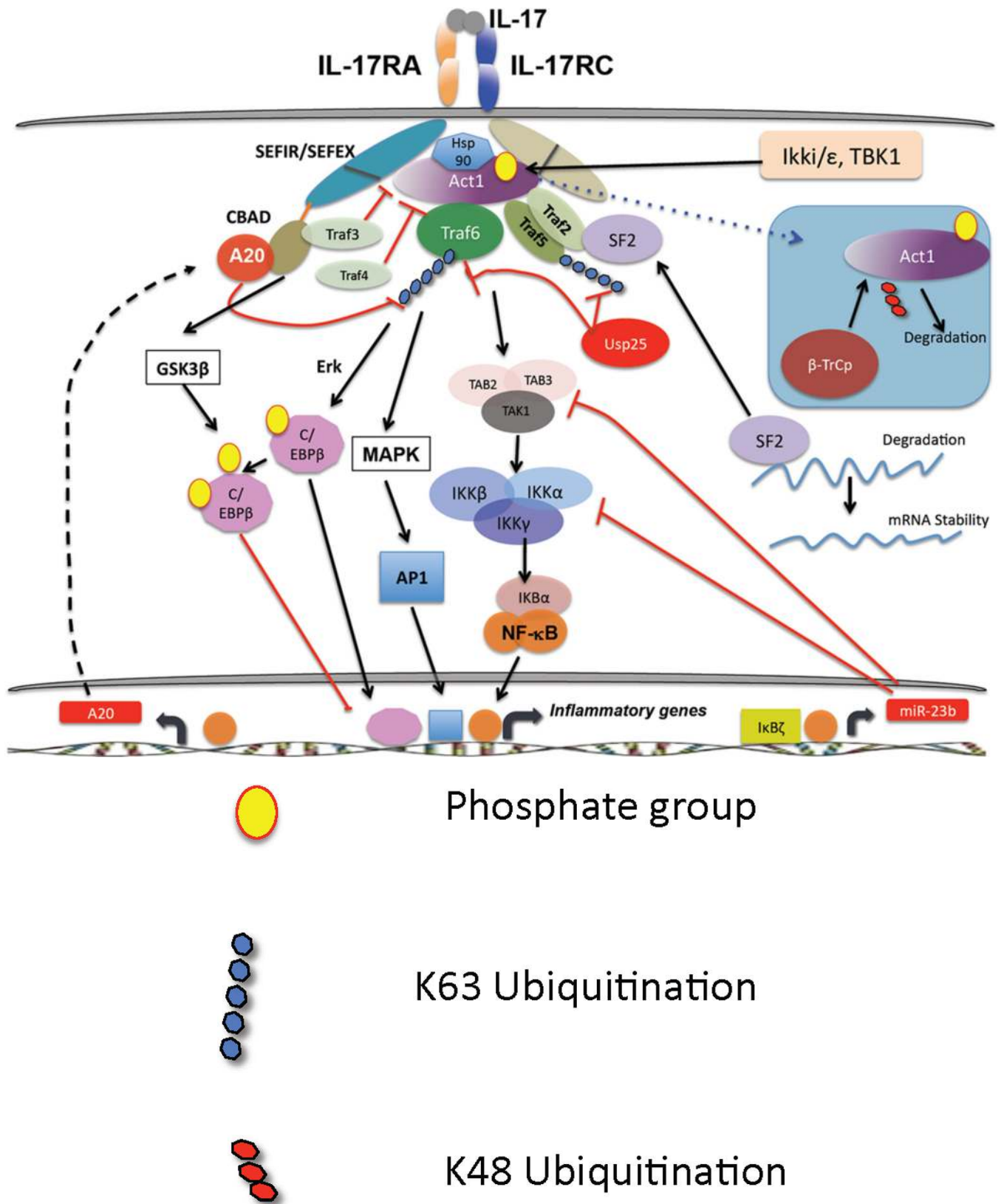
Figure 4.

Table 1

Human mutations that impact the Th17 pathway

Gene	Syndrome	Disease susceptibility	Connection to Th17 pathway	Refs
STAT3	Autosomal dominant-Hyper-IgE Syndrome (Job's Syndrome)	Mucocutaneous candidiasis Staph aureus Cold abcesses Viral susceptibility Etc.	Reduced Th17 frequency, defects in IL-6, IL-23, IL22 signal transduction	67, 175
TYK2	Autosomal recessive-Hyper-IgE Syndrome (Job's Syndrome)	Mucocutaneous candidiasis Staph aureus Cold abcesses Other?	Loss of IL-23 signaling (presumed)	176
IL-12p40			Reduced Th17 (and Th1) frequency	177
IL12RB1			Reduced Th17 (and Th1) frequency	178, 179
IL17F		Mucocutaneous candidiasis Staph aureus?	Dominant negative mutation in IL-17F that blocks IL-17F and IL-17A/F signaling	138
IL17RA		Mucocutaneous candidiasis Staph aureus	Loss of function in IL-17RA	138
Dectin-1		Mucocutaneous candidiasis	Induces Th17 differentiation in response to β-glucans	175
CARD9		Mucocutaneous and disseminated candidiasis	Induces Th17 differentation in response to fungal PAMPs via CLRs	133
STAT1		Mucocutaneous candidiasis Viral susceptibility?	Reduced Th17 frequency, increased IL-27 and Type I IFN signaling	134, 135
AIRE	APS-1	Endocrinopathy, mucosal candidiasis	Neutralizing Abs against Th17 cytokines (IL-17A, IL-17F and/or IL-22)	136, 137
ACT1		Mucocutaneous candidiasis		139
IL17RC		Mucocutaneous candidiasis	Loss of function in IL-17RC	J-L Casanova and A Puel, personal communication

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Table 2

human diseases being treated with anti-p40, anti-p19, anti-IL–17, and anti-IL–17RA

Company	Agent	Target	Indications	Stage	Clin Trial ID
Eli Lilly	Ixekizumab (Ly2439821)	IL–17A	Psoriasis Rheumatoid arthritis	Phase 3 Ph 2 complete	NCT01597245 NCT00966875
Novartis	Secukinumab (AIN457)	IL–17A	Psoriasis Rheumatoid arthritis Ankylosing spondylitis Psoriatic arthritis Asthma Multiple sclerosis Type 1 Diabetes Crohn's disease	Phase 3 Ph 3 Ph 3 Phase 3 Ph 2 Ph 2 Ph 2 Ph 2terminated	NCT01544595 NCT01770379 NCT01358175 NCT01892436 NCT01478360 NCT01874340 NCT02044848 NCT01009281
Amgen/ MedImmune	Brodalumab (AMG 827)	IL–17 Receptor A	Psoriasis Psoriatic arthritis Asthma Crohn's disease	Phase 3 Ph 3 Ph 2 Ph 2suspended	NCT01708590 NCT02024646 NCT01902290 NCT01150890
Abbott AbbVie	ABT-122	IL–17A/ TNFa	Rheumatoid arthritis	Phase 1	NCT01853033
Johnson & Johnson Janssen Biotech	Stelara (Ustekinumab) (CNTO 1275)	p40 subunit of IL–12 and IL–23	Psoriasis Crohn's disease Ankylosing spondylitis Rheumatoid arthritis Psoriatic arthritis Multiple sclerosis GvHD Atopic dermatitis	Approved 2009 Phase 3 Phase 2 Phase 2 Phase 2 Phase 2 Phase 2 Phase 2	approved NCT01369329 NCT01330901 NCT01645280 NCT01009086 NCT00207727 NCT01713400 NCT01945086
Abbott	Briakinumab ABT-874	p40 subunit of IL–12 and IL–23	Crohn's disease Psoriasis Multiple Sclerosis	Ph 2terminated Phase 3 Phase 2	NCT00562887 NCT00626002 NCT00086671
Merck	Tildrakizumab (MK 3222) (SCH 900222)	IL–23p19	Psoriasis	Phase 3	NCT01729754
Johnson & Johnson Janssen Biotech	Guselkumab CNTO 1959	IL–23p19	Psoriasis Rheumatoid arthritis	Phase 2 Phase 2	NCT01483599 NCT01645280
Amgen/ MedImmune	AMG 139	IL–23p19	Psoriasis Crohn's disease	Phase 1 Phase 1	NCT01094093 NCT01258205

Company	Agent	Target	Indications	Stage	Clin Trial ID
Eli Lilly	LY3074828	IL-23p19	Psoriasis	Phase 1	NCT01947933
Boehringer Ingelheim	BI 655066	IL-23p19	Ankylosing spondylitis Crohn's disease Psoriasis (single rising dose)	Phase 2 Phase 2 Phase 2	NCT02047110 NCT02031276 NCT02054481

Table 3

Primary Psoriasis clinical end point in 4 separate Phase II dose-ranging studies

Phase II Studies	Dosage	Primary endopoint: PASI 75 (% patients)	Secondary endopoint: PASI 100 (% patients)	Refs
Ixekizumab (anti-IL-17A) n=142	5 × Placebo 5 × 10 mg 5 × 25 mg 5 × 75 mg 5 × 150 mg	8 29 77 83 82	0 0 17 38 39	155
Secukinmab (anti-IL-17A) n=404	3 × Placebo 3 × 25 mg 3 × 75 mg 3 × 150 mg	9 18 57 81		155
Brodalumab (anti-IL-17RA) n=198	3 × Placebo 6 × 70 mg 6 × 140 mg 6 × 210 mg 3 × 280 mg	0 33 77 82 67	0 10 38 62 29	155
Tilrakizumab (anti-IL-23p19) n=350	6 × Placebo 6 × 5 mg 6 × 25 mg 6 × 100 mg 6 × 200 mg	2 33 58 62 74		158

The primary endpoint of the studies is percent of patients with >75% disease clearance as defined by the Psoriasis Area and Severity Index (PASI-75). All four therapeutic agents showed treatment response rate of 74% to 83% for PASI-75 at doses ranging from 25 to 100 mg/kg. The Placebo groups showed 0% to 9% response rates for PASI-75. Two studies also reported a secondary endpoint of PASI-100 (defined as 100% disease clearance). Patients treated with Ixekizumab (150 mg/kg) and Brodalumab (210 mg/kg) showed 38% and 62% PASI-100, respectively.