Are Th17 Cells an Appropriate New Target in the Treatment of Rheumatoid Arthritis?

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Abstract

Th17 cells play crucial roles not only in host defense but also in many human autoimmune diseases and corresponding animal models. Although many of the fundamental principles regarding Th17 biology have been rapidly elucidated in mice, there remain numerous controversies regarding the differentiation, plasticity, and pathogenicity of human Th17 cells. In this review, we consider these open questions in comparison to what has already been clarified in mice, and discuss the potential impact of discoveries related to the Th17 pathway on the development of new therapeutic strategies in Th17 driven autoimmune diseases, specifically rheumatoid arthritis. Clin Trans Sci 2010; Volume 3: 319–326

Keywords: lymphocytes, rheumatology, cytokines, translational research

Introduction

Discovery of Th17 cells

Until about a decade ago, many human autoimmune diseases and the corresponding experimental animal models had been viewed as being driven by Th1 cells. However, immunologists encountered a dilemma when they realized that experimental autoimmune encephalomyelitis (EAE: a mouse model of multiple sclerosis) and collagen-induced arthritis (CIA: a mouse model of rheumatoid arthritis) both were aggravated by the absence of Th1 associated cytokines, receptors or transcription factors, such as IFN-γ, IFN-γ receptor, IL-12 p35, IL-12 receptor β 2, or Stat1.[1–5] However, mice lacking the IL-12p40 subunit, which is found not only in IL-12 but also in IL-23, were resistant to these diseases, and IL-23p19 knockout mice were shown to be protected from EAE[6] and CIA.[7] This paradox was resolved in 2005 by a discovery of new subset of CD4+ T cells that produce IL-17A and IL-17F, expand in response to IL-23, and induce EAE and CIA upon adoptive transfer in mice.[8–10] These lymphocytes, termed Th17 cells, are characterized by expression of RORγt as a master regulator gene as well as secretion of IL-17A, IL-17F, IL-21, and IL-22. Th17 cells are crucial in host defense against extracellular bacteria and some fungi, which Th1 and Th2 immunity are not fully effective against, and also play essential roles in many autoimmune or inflammatory diseases, both in mice and humans (*Table 1*).[11–13]

Discussion

What is the connection between Th17 cells and rheumatoid arthritis?

Multiple lines of evidence indicate that Th17 cells are important in rheumatoid arthritis (RA). As summarized in *Table 2*, IL-17 activates a diverse array of cell types that participate in the pathogenesis of RA, including synovial fibroblasts, monocytes, macrophages, chondrocytes, and osteoblasts. IL-17 induces production of proinflammatory cytokines, such as TNF, IL-1, IL-6, IL-23, which amplify positive feedback loops that commit naïve CD4 T cells to the Th17 lineage.[14–16] By inducing chemokine production, IL-17 indirectly attracts numerous effector T cells, B cells, monocytes, and neutrophils to the inflamed joint.[17] Of note, acting in synergy with TNF and IL-1-β, IL-17 induces CCL20,[18] which strongly attracts lymphocytes, including Th17 cells that express CCR6, a receptor for CCL20. In bone, IL-17 stimulates osteoblasts to express receptor activator of nuclear factor kappa-B ligand (RANKL).[19] Such osteoblasts then activate osteoclasts that express RANK as a membrane receptor. This interaction leads to bone resorption. Finally, induction of matrix metalloproteinases (MMPs)[20] and vascular endothelial growth factor (VEGF)[21] are crucial in tissue destruction and angiogenesis, respectively.

Brief overview of Th17 differentiation in mice

In mice, the combination of TGF-β and IL-6 or IL-21 induces Th17 cells.[22–24] IL-6 or IL-21 phosphorylates Stat3, which induces RORγt expression.[25] Stat3 and RORγt appear to cooperate with each other[26] and bind to the IL-17 promoter to induce IL-17 expression.[27,28] TGF-β induces not only RORγt but also FoxP3 (master regulator gene of regulatory T cells: Tregs),[23,29,30] which physically associates with RORγt as well as Runx1.[28,29] In the absence of proinflammatory cytokines, FoxP3, through interaction with Runx1, inhibits RORγt-directed IL-17 expression, which is crucial in maintaining homeostasis of the immune network through generation of Tregs.[28,29] However, in the presence of proinflammatory cytokines, Stat3 and IRF4 both play essential roles in IL-6/21- and IL-6-mediated down-regulation of FoxP3, respectively,[31,32] after which RORγt primarily cooperates with Runx1 to induce IL-17.[28] In this manner, IL-6 or IL-21 play a pivotal role in tipping the balance toward Th17, but not Treg differentiation by inhibiting TGF-β–mediated FoxP3 expression.[23,29]

IL-21 produced by Th17 cells in the presence of TGF-β induces Th17 cells, creating an autoamplification loop for Th17 differentiation.[26,33,34] Thus, IL-21 might play a crucial role in maintaining a precursor pool of Th17 cells when the supply of IL-6 is limited.

Both IL-6 and IL-21, in cooperation with TGF-β, induce expression of the IL-23 receptor in a Stat3-[26] and RORγt-dependent[33] fashion. Stat4, which has been viewed to be primarily essential for IL-12 signaling for Th1 lineage commitment, was shown to also be important for IL-23-mediated expression of IL-17 in CD4 T cells *in vitro*.[35]

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DOI: 10.1111/j.1752-8062.2010.00233.x

Strong evidence:	Moderate evidence:
Multiple sclerosis	Periodontal disease
Rheumatoid arthritis	Loosening of prosthetic joint
Psoriasis	Chronic GVHD
Inflammatory bowel disease	Allograft rejection
Steroid resistant asthma	SLE

Table 1. Human autoimmune or inflammatory diseases in which contributions of Th17 cells have been suggested.

How does TGF-β contribute to human Th17 differentiation?

In humans, TGF-β induces ROR-c (human homolog of RORγt), which is essential for IL-17 expression.[36,37] However, high TGF-β suppresses ROR-c directed IL-17 expression, which is relieved either by IL-1, IL-6, or 21.[36] Thus, the basic molecular mechanism is probably conserved between the two species. Analogous to mice, it is plausible that TGF-β possesses a dual potential; induction of either Tregs or Th17 cells, depending on its concentration and on the cytokine milieu. Targeted administration of TGF-β in conjunction with neutralization of IL-1/IL-6/IL-23 might theoretically represent an effective maintenance therapy for Th17 driven autoimmune diseases including RA, provided that the disease is in remission. Dendritic cells (DCs) genetically engineered to express immunoregulatory cytokines are a potential approach to targeting T cells, and their use in inflammatory arthritis has been explored in mouse systems.[38,39]

What are the roles for proinflammatory cytokines (IL-1, IL-6, IL-21, IL-23) in human Th17 differentiation?

In humans, the combination of TGF-β and IL-6 does not induce Th17 cells. Although TGF-β was definitively shown to be indispensable, it has been controversial as to what proinflammatory cytokines in combination with TGF-β are necessary and sufficient to induce human Th17 cells. Manel reported that TGF-β + IL-1 + IL-6 or IL-21 or IL-23 was necessary and sufficient for human Th17 cell differentiation.[36] Yang and Volpe found TGF-β + IL-21 or TGF-β + IL-1 + IL-6 + IL-23 to be Th17 inducing cytokine combinations.[37,40] We will discuss potential roles of each proinflammatory cytokine in human Th17 differentiation below.

Target cell	Mediators produced	Major functions
Synovial fibroblasts	IL-6, IL-1$_\beta$, IL-23 CXCL-1, -2, -8, CCL-2, -20 G-CSF, GM-CSF COX-2, PGE$_2$ VEGF	Inflammation, Th17 differentiation Leukocyte recruitment Granulopoiesis Inflammation Angiogenesis
Monocytes/ macrophages	IL-6, IL-1$_\beta$, TNF$_\alpha$ COX-2, PGE$_2$ MMPs	Inflammation, Th17 differentiation Inflammation Tissue destruction
Chondrocytes/ osteoblasts	IL-6, IL-1$_\beta$ CXCL-1, -5, CCL-2 COX-2, PGE$_2$ Nitric Oxide, MMPs VEGF RANK, RANKL	Inflammation, Th17 differentiation Leukocyte recruitment Inflammation Tissue destruction Angiogenesis Bone resorption

Adapted from Lundy SK et al. Cells of the synovium in rheumatoid arthritis. T lymphocytes. Arthritis Res Ther. 2007; 9(1)202

Table 2. Effects of IL-17 on cells of the joint.

IL-1

Unlike IL-6, IL-1 is not absolutely indispensable for Th17 differentiation in mice; however, deficiency of IL-1 signaling significantly impairs Th17 differentiation both *in vivo* and *in vitro*.[41] Furthermore, lack of IL-1 signaling delays the onset and reduces the incidence and severity of EAE.[41] At a low concentration of TGF-β, IL-1 synergizes with IL-6 + IL-23 to induce Th17 cells without down-regulating FoxP3.[41] IL-1 signaling is essential for expression of RORγt and IRF-4, whereas over-expression of RORγt and IRF-4 restores IL-17 expression independent of IL-1.[41]

Given such significant contributions of IL-1 to Th17 differentiation in mice, it would be surprising if IL-1 were not involved in development of human Th17 cells. Expression of the IL-1 receptor in naïve and memory CD4 T cells from peripheral blood is associated with higher expression of IL-17, ROR-c, IRF4, and IL-23 receptor, even before T cell receptor triggering.[42] Whereas it is not entirely clear how IL-1 promotes human Th17 differentiation, IL-1 appears to synergize either with IL-6, IL-21, or IL-23 to induce Th17 cells in the presence of TGF-β.[36] This is consistent with what was defined in mice, that the IL-1 receptor is induced by IL-6 and is dependent on Stat3 and RORγt expression.[41] How the signaling cascade triggered by the IL-1R is involved in human Th17 differentiation remains to be investigated.

In clinical practice, the IL-1 receptor antagonist: anakinra, has been used to treat RA. Several RCTs and metaanalyses indicated that treatment with anakinra in patients with RA refractory to methotrexate not only improved the clinical outcomes, including ACR 20, 50, and 70 responses and functional status, but also delayed the radiographic progression.[43–46] However, the potency of anakinra in RA is typically inferior to other biologics such as TNF antagonists.[43] In general, anakinra is well tolerated. A study involving 1,399 patients revealed a slightly higher incidence of serious infections in anakinra group, none of which were attributed to opportunistic organisms or resulted in death.[47] The risk of infections correlates with either concomitant use of steroid[48] or a higher dose (>100mg/day) of anakinra.[49]

IL-6

In humans, the role for IL-6 in Th17 differentiation has not been as rigorously studied as in mice. Whereas IL-6 + TGF-β with/without IL-23 do not induce human Th17 cells, IL-6 appears to synergize with IL-1 in the presence of TGF-β.[36] Although Yang indicated that the combination of IL-6 + TGF-β induced a level of ROR-c comparable to the effect of IL-21 + TGF-β, only the latter combination induced Th17 cells.[40] Volpe demonstrated that lack of IL-6 did not completely abrogate, but substantially impaired, Th17 differentiation.[37] In view of these conflicting data, whether IL-6 is absolutely indispensable for human Th17 differentiation awaits further investigations.

The efficacy and safety of an anti-IL-6 receptor monoclonal antibody (tocilizumab) have been proven in RA. Several RCTs indicated that tocilizumab is effective in patients with RA refractory to conventional DMARDs or TNF antagonists, with greater efficacy at higher doses.[50] One of these studies showed that tocilizumab in combination with methotrexate reduced markers of systemic bone resorption as well as cartilage turnover.[51]

IL-21

As noted above, IL-21 might be an autoamplification factor for Th17 cells in mice. Moreover, the IL-21 receptor Fc fusion protein was shown to improve clinical and histologic signs of CIA.[52]

In humans, the combination of TGF-β + IL-21 induced Th17 cells *in vitro*.[40] Although the mechanism of how IL-21 is involved in human Th17 differentiation is unclear, IL-21 appears to synergize with IL-1 in the presence of TGF-β in analogous fashion to IL-6.[36] In considering the role of IL-21 in RA, it is important to keep in mind that IL-21 is produced not only by Th17 cells themselves, but also by other T cell subsets, such as T follicular-helper cells, and that IL-21 has important effects on B cells that are likely significant in RA.

Further clarification of roles for IL-21 in human Th17 development will provide a rationale for blockade of IL-21 or the IL-21 receptor in RA. Besides IL-21 itself or the IL-21 receptor, downstream molecules of the IL-21 signaling cascade, such as Jak3, might represent an effective target in suppressing human Th17 differentiation. CP-690,550 is an orally active Jak inhibitor that was initially developed as a "selective Jak3 inhibitor" for treatment of organ allograft rejection,[53] but has now turned out to be a pan-Jak inhibitor. A randomized controlled trial of 264 patients with active RA showed that all tested doses of CP-690,550 were superior to placebo in ACR 20-response rate (70%–81% vs. 29%) by week 6 as well as ACR 50 and 70 responses by week 4.[54] Therapeutic efficacy was noted as early as week 1. The incidence of infection in the treatment group was around 30% as compared to 26% in placebo group, none of which were attributed to opportunistic organisms or resulted in death.[54]

IL-23

In mice, IL-23 is dispensable for Th17 differentiation, but important for proliferation and maturation of the Th17 lineage.[10,55] The IL-23 receptor is induced in a Stat3-[26] and RORγt-dependent[33] fashion, but not in naïve CD4 T cells.

In humans, Volpe showed that lack of IL-23 did not completely abrogate, but substantially impaired Th17 differentiation *in vitro*.[37] In the presence of TGF-β, IL-23 appears to synergize with IL-1 in inducing the IL-23 receptor, ROR-c, and IL-17A/F.[36] This synergism with IL-1 is more robust than IL-6 or IL-21.[36] Given these data, IL-23 might play more important roles in human Th17 differentiation than in mice. Whereas no clinical study targeting IL-23 in RA has been reported yet, a phase II randomized clinical trial involving 320 patients with moderate-to-severe psoriasis showed that treatment with a monoclonal antibody against the IL-12/23 p40 subunit led to a dose-response improvement of the psoriasis area and severity index.[56] The incidence of serious adverse events was comparable between treatment and placebo group. However, in the treatment of RA, blockade of IL-23p19, an IL-23-specific component not shared with IL-12, would likely be more efficacious with less toxicity, in view of the regulatory effects of IL-12 on IL-17 production.

Besides IL-23 itself and its receptor, molecules downstream in the IL-23 signaling cascade, namely Jak2 or Tyk2, could be appealing targets for therapy. INCB028050 is a selective orally available Jak1/Jak2 inhibitor. Treatment with INCB028050 led to improvements in clinical, histologic, and radiographic aspects of rat adjuvant arthritis.[57] In this study, INCB028050 inhibited IL-6- and IL-23-mediated phosphorylation of Stat3 in a dose-dependent fashion along with selective inhibition of Jak1/Jak2. Expression of IFN-γ, IL-12, IL-17, IL-21, and IL-22 were suppressed by 50%–80%. Taking all observations summarized above into account, we propose a model of human Th17 differentiation as described in *Figure 1*.

What transcription factors drive human Th17 differentiation?

Our knowledge as to what transcription factors drive human Th17 cells is very limited. ROR-c was proven to be essential for IL-17 expression *in vitro*.[36] T cells from patients with dominant negative mutations in Stat3 (autosomal-dominant hyper-IgE syndrome: primary immunodeficiency characterized by recurrent lung and skin infections, elevated serum IgE, pathologic fractures, characteristic face, and high palate) have impaired IL-17 production *ex vivo*.[58] While these data suggest probable important involvement of these transcription factors in human Th17 differentiation, there remain numerous open questions, such as i) what proinflammatory cytokines phosphorylate Stat3; ii) whether Stat3 induces ROR-c by binding to the ROR-c promoter; iii) whether Stat3 and ROR-c bind to the IL-17 promoter to induce IL-17 expression, and iv) which other transcription factors (Stat4, IRF4, Runx1 etc.) are involved in IL-17 expression.

No clinical studies targeting ROR-c have been conducted to date, which is not surprising given the relatively limited data as to how ROR-c is involved in human Th17 development.

When it comes to Stat inhibition, one must block either their recruitment to cytokine receptors, dimerization, or DNA binding, since Stats do not have enzymatic activity. Over-expression of dominant negative alleles, mutants that cannot be phosphorylated, will not be very practical in the clinical setting in the near future. Instead, phosphopeptides corresponding either to motifs in cytokine receptors that prevent Stat recruitment or to conserved motifs in Stats themselves that prevent Stat dimerization might be a feasible strategy in disrupting Stat activation.[59–61] Decoy oligonucleotides may also deserve consideration given their potential for preventing Stat DNA binding.[62]

A few lines of indirect evidence support the rationale for Stat3 inhibition in treating Th17 driven autoimmune diseases, specifically RA. Socs3 is a negative feedback regulator of Stat3 that is induced by IL-6 and is abundantly expressed in synovial tissues in patients with RA.[63] Adenovirus carrying Socs3 cDNA was shown to be an effective tool in reducing phosphorylation of Stat3 and ameliorating CIA, and was more potent than a dominant negative form of Stat3.[63] CD4 T cells co-cultured with DCs transduced with Socs3, (which inhibit IL-23-mediated phosphorylation of Stat3 and have high secretion of IL-10 but low IL-23p19 expression), produce less IL-17 than those co-cultured with nontransduced DCs.[64] Auranofin: a sulphur-containing gold compound, which has previously been used in the treatment of RA, was shown to inhibit IL-6-mediated phosphorylation of Stat3 in fibroblast-like synoviocytes and impair its translocation to the nucleus.[65]

In light of the probable contribution of Stat4 to Th17 development in mice[35] as well as the observation that Stat4 deficient mice were protected from CIA,[66] EAE,[67] and inflammatory bowel disease,[68] Stat4 inhibition also might deserve consideration in treating Th17 driven autoimmune diseases, including RA.

Several lines of evidence have shown that HMG-CoA reductase inhibitors, statins, ameliorated Th17 driven autoimmune disease, partly through inhibition of Stat3 or Stat4. In patients with multiple sclerosis, simvastatin-induced Socs3, and suppressed Stat3 phosphorylation as well as IL-6 and IL-23 expression in monocytes.[69] In this study, simvastatin suppressed expression

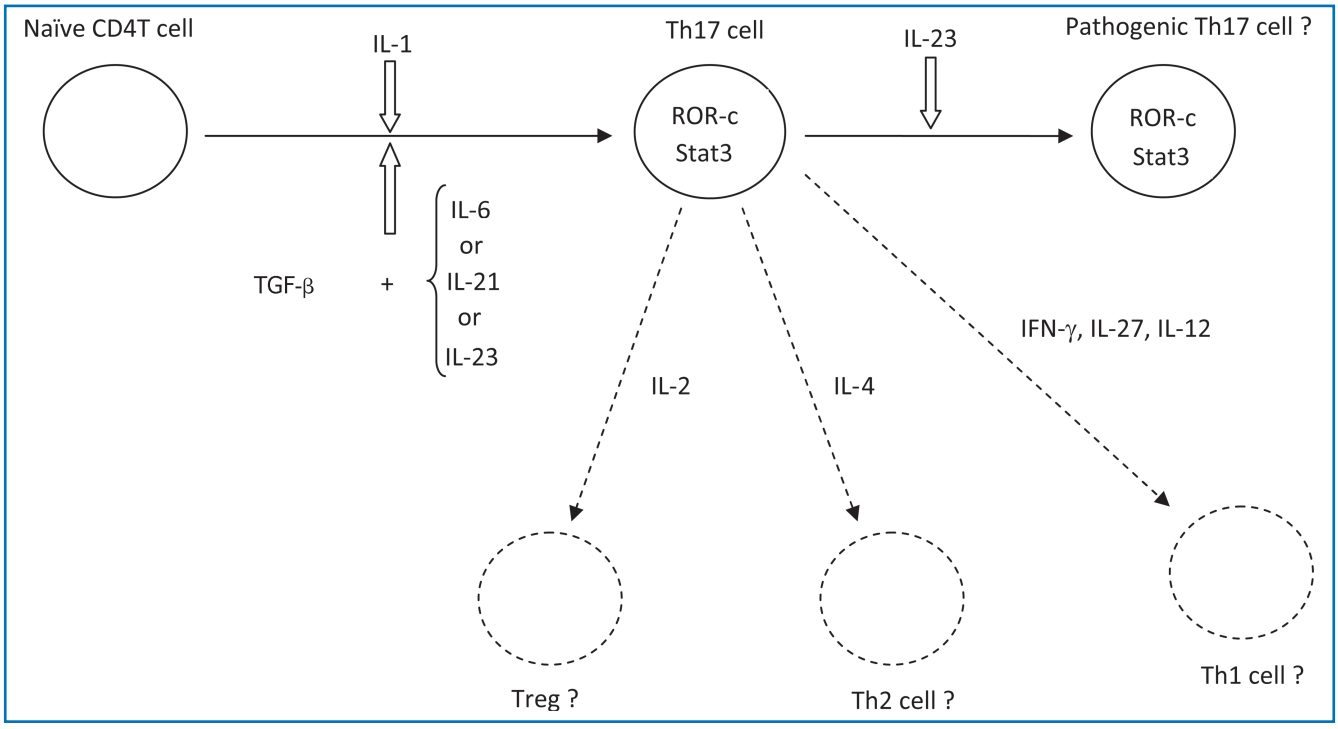

Figure 1. Model of human Th17 differentiation: IL-1 signaling appears to cooperate with a pathway driven by TGF-β+ IL-6/IL-21/IL-23 to induce human Th17 cells, associated with expression of ROR-c and Stat3. IL-23 might promote differentiation of human Th17 cells to a pathogenic phenotype, which contribute to autoimmune pathology. Human Th17 cells, depending on the stage of development, might lose Th17 phenotype or be redirected to Th1 cells by IFN-γ/IL-27/IL-12, Th2 cells by IL-4, or Tregs by IL-2.

of ROR-c and IL-17 in CD4 T cells cultured without antigen presenting cells.[69] Orally administered statins are effective in preventing and treating EAE[70] and CIA.[71] Statins inhibit Stat4 and enhance Stat6 phosphorylation, both in T cells from mice with EAE and *in vitro*, associated with reduced production of IL-12 and IFN-γ, but increased secretion of IL-4 and IL-5.[70] Upon adoptive transfer, these Th2 cells conferred resistance to EAE.

In a randomized controlled trial of 116 patients with RA treated with either 40mg of atorvastatin or placebo in conjunction with disease modifying therapy, treatment with atorvastatin was associated with significant, albeit modest, improvement DAS28 as well as better DAS28-response rate (31%) than placebo(10%).[72]

Can human Th17 cells be redirected to other lineages as is the case in mice?

A growing body of evidence suggests that Th17 cells represent a heterogeneous population of T cells with dynamic phenotypic and functional properties, both in mice and humans. *In vivo*, IL-17(+)IFN-γ(+) cells and RORγt(+)FoxP3(+) cells[26,73] were identified in both mice and humans. *In vitro*, the combination of high TGF-β and IL-6 induces IL-17(+)IL-22(–) cells in mice and humans,[37,74] which may be either more or less pathogenic, depending on the specific diseases or affected organs. Whereas the combination of TGF-β and IL-6 induces IL-17(+)IL-10(+) cells, which are less pathogenic Th17 cells in mice, restimulation of these cells by IL-23-induced IL-17(+)IL-10(–) cells, which are more pathogenic.[75]

It was previously speculated that each subset of helper T lymphocytes represented a fixed phenotype tightly linked to expression of a lineage-specific transcription factor in a mutually exclusive pattern. However, it is now clear that helper T lymphocytes can be redirected to other lineages in mice depending on the cytokine milieu. Th1 and Th2 cells, which have been viewed as relatively fixed lineages, can be inter-converted to each other through exposure to IL-4 and IFN-γ, respectively.[76] *In vitro*-generated Th17 cells lose IL-17A and IL-17F expression without constant exposure to TGF-β and IL-6, and can be redirected to Th1 cells through exposure to IFN-γ or IL-12.[77] Whether Th17 to Th2 conversion can be induced by IL-4 is controversial. Our laboratory has shown that *in vitro*-generated Th17 cells lose IL-17 expression when exposed to IL-4 in a Stat6-dependent fashion, but do not convert to Th2 cells. Th17 cells became resistant to regulation by IL-4 after 3 rounds (3 weeks) of restimulation in Th17 inducing conditions, associated with loss of Stat6 phosphorylation but upregulation of Socs5 and Socs1 (L Cooney and D Fox, submitted), analogous to IL-27 inhibition of *de novo* differentiation of Th17 cells in a Stat1-dependent fashion, but inability of IL-27 to suppress already committed Th17 cells.[78] In contrast, Lexberg found that *in vitro*-generated Th17 cells readily lost Th17 phenotype and converted to Th2 cells when exposed to IL-4, even after 18 days of polarization towards Th17 cells while *in vivo*-generated Th17 cells maintained the Th17 phenotype even under Th1 or Th2 inducing condition.[79] The discrepancies may be attributed to differences in the type of antigen presenting cells, protocol for Th17 polarization, purity of Th17 cells used for restimulation, and method of restimulation by IL-4.

In the setting of Treg-specific deletion of the RNaseIII enzyme Dicer that is required for microRNA biogenesis, Tregs can be reprogramed to Th1 and Th2 cells.[80] Tregs can be redirected to Th17[30,81] and follicular helper T cells (Tfh)[82] through exposure to IL-6 or IL-21 and interaction with B cells through CD40 respectively. In humans, Tregs can be reprogramed to Th17 cells through exposure to IL-1 and IL-6 *in vitro*.[73]

In light of such complex phenomena concerning the heterogeneity and plasticity of helper T cells, O'Shea proposed a more sophisticated model, that views helper T cells as expressing various ratios of master regulator genes (T-bet, Gata3, and RORγt), which could dynamically change during the course of differentiation and development depending on the cytokine milieu.[83]

Besides master regulator genes, it would be paramount to consider Signal Transducer and Activator of Transcription proteins (STATs) in discussing the differentiation and plasticity of helper T cells. In mice, Th17 differentiation is inhibited by Stat1 phosphorylation by IFN-γ or IL-27, Stat4 phosphorylation by IL-12, Stat5 phosphorylation by IL-2, or Stat6 phosphorylation by IL-4, all of which, except for Stat6, were shown to directly bind to the IL-17 promoter.[8,35,84–86]

An important question is whether human Th17 cells can be regulated or redirected to other lineages through exposure to IFN-γ, IL-27, IL-12, IL-2, or IL-4. This question is related to the issues of whether immune tolerance can be restored by cytokines with opposing phenotypes after generation of pathogenic Th17 cells in human autoimmune diseases. *Ex vivo* expanded human Th17 cells have reduced IL-17, but increased IFN-γ expression after treatment with IL-12, correlated with decreased expression of RORγt, but increased expression of T-bet.[87]

Our laboratory has shown that DCs genetically engineered to express IL-4 suppress IL-17 production from T cells and ameliorate CIA even in the presence of strong Th17 inducing stimuli, such as IL23, in mice.[38,39] Whether similar phenomena will be observed in humans and with other cytokines; IFN-γ, IL-27, IL-12, and IL-2, including alteration of sensitivity to regulation depending on the stage of Th17 development, would be an area for vigorous research. Such information would bear on whether DCs genetically engineered to express IFN-γ, IL-27, IL-12, IL-2, or IL-4 could be a potential therapeutic modality in RA.

What is the implication of IL-17(+) IFN-γ(+)cells in RA?

IL-17(+)IFN-γ(+)cells are identified in several human autoimmune diseases, including RA. Whether these cells represent a distinct population or cells in the process of differentiation to Th1 or Th17 lineage remains unclear. Since the discovery of Th17 cells, it has also been a matter of debate whether pathogenesis of RA is mainly driven by Th1 cells, Th17 cells, or both, and whether this depends on the stage of the disease.

In proteoglycan-induced arthritis (PGIA), although IFN-γ deficient mice develop less severe arthritis with delayed onset, they eventually succumb to arthritis.[88] Although IL-17 deficiency does not protect mice from PGIA, IFN-γ(–/–) or T-bet(–/–) mice developed more severe PGIA than IFN-γ/IL-17 or T-bet/IL-17 double deficient mice.[88] The data can be interpreted to imply that IL-17-mediated pathology is regulated by IFN-γ in PGIA. In CIA, lack of IFN-γ signaling leads to severe disease.[4,5] Neutralization of IFN-γ was beneficial at an earlier stage of CIA, but aggravated the disease at a later stage.[89] These data implicate the possibility that roles for Th1 and Th17 immunity in mouse model of arthritis vary depending on the stage of the disease.

Studies in other animal models of autoimmune diseases might provide important clues in dissecting the link between Th1 and Th17 immunity in the development of RA. Th17 cells are able to induce colitis upon transfer into immunodeficient mice, but many of them convert to Th1 cells.[77] Diabetogenic BDC2.5 CD4 T cells polarized *in vitro* to the Th17 phenotype lose IL-17 expression and express IFN-γ after adoptive transfer into NOD-SCID mice, ultimately causing beta-cell destruction and diabetes.[90] Finally, data showing that Th1 cells can be reprogramed to Th17 cells in mice and humans is lacking. In light of these observations, it might be plausible to speculate that an earlier stage of RA is primarily driven by Th17 cells, which gradually convert to IL-17(+)IFN-γ(+) cells and are finally replaced by Th1 cells during the progression of disease. If this were the case, the presence of IL-17(+)IFN-γ(+)cells might indicate that the disease is in the stage of conversion from Th17 to Th1 driven disease. In this regard, it would be quite intriguing to know whether there is any consistent link between the ratio of IL-17(+)cells versus IL-17(+)IFN-γ(+) cells versus IFN-γ(+) cells in peripheral blood or synovial fluid and clinical and radiologic stage of disease in patients with RA. This might define a therapeutic window when IL-17 blockade will be beneficial in treating RA.

Which phenotypic features define the pathogenicity of human Th17 cells?

Understanding the diverse array of phenotypes and functions of Th17 cells, some of which may be more pathogenic and others more tolerogenic, it has been a matter of debate as to which subset of Th17 cells drives human autoimmune diseases. Furthermore, IL-17-mediated immunopathology appears to be regulated by IFN-γ in mouse models of arthritis as above. From these perspectives, it is not entirely clear whether IL-17 blockade itself would be beneficial throughout all stages of RA. Regardless, there are some promising studies supporting the rationale for IL-17 blockade in both mice and humans. IL-17 receptor Fc fusion protein attenuated clinical, radiologic, and histologic manifestations of rat adjuvant arthritis in a dose-dependent fashion.[91] In a double bind randomized controlled trial of 77 patients, treatment with humanized anti-IL-17 monoclonal antibody (LY2439821) led to greater changes in DAS28 score as well as ACR 20, 50, and 70 responses than placebo.[92] There was no apparent dose-response relationship in treatment-related adverse events.

In mice, IL-22 is produced by Th17 cells in an IL-23-dependent fashion, but not by Th17 cells induced by TGF-β and IL-6 alone, implicating that IL-22 might be a marker of maturation of Th17 cells.[75] However, it is controversial whether IL-17(+)IL-22(+) cells are pathogenic or not. In fact, IL-22 deficient mice are not protected from EAE.[93] IL-22 is protective in liver,[94] gut,[95] and myocardial inflammation.[96] On the other hand, IL-22 induces keratosis in mouse models of psoriasis and plays a role in human psoriasis as well.[97] IL-22 may promote the breach of the blood–brain barrier in T cell-mediated CNS autoimmunity.[98] In RA, IL-22 induces proliferation of synovial fibroblasts through the IL-22 receptor expressed on these cells.[99] In bleomycin-induced airway inflammation in mice, IL-22 was tissue protective in the absence of IL-17, but became pathogenic in cooperation with IL-17.[100] Based on these data, it might be plausible to speculate that whether IL-22 is pathogenic or tolerogenic is entirely context dependent. Therefore, before considering this molecule as a potential target of therapy, the role for IL-22 in Th17 driven autoimmune disease needs to be rigorously determined depending on the type of disease, affected organs, and the presence of concomitant IL-17.

What factors drive the pathogenic features of Th17 cells?

Given the lack of a convincing answer to the last question, this also remains an open question. Our hypothesis is that IL-23,

Drugs	Conceptual Th17 target	Molecular target	Stage of drug development
Anakinra	Differentiation	IL-1 receptor	Approved for RA
Tocilizumab	Differentiation	IL-6 receptor	Approved for RA
Ustekinumab	Differentiation and pathogenicity	IL-23p40	Approved for severe plaque psoriasis
Statins	Differentiation and pathogenicity	Multiple	Efficacy proven in a RCT
Auranofin	Differentiation and pathogenicity	Stat3	Previously used
CP-690,550 (pan-Jak inhibitor)	Differentiation and pathogenicity	IL-6, 21, 23 signaling	Phase II trials suggest efficacy
LY2439821 (Anti-IL17A monoclonal antibody)	Pathogenicity	IL-17A	Phase I trial completed
IL-21 receptor Fc fision protein	Differentiation	IL-21 receptor	Animal study
INCB028050 (Jak1/Jak2 inhibitor)	Differentiation and pathogenicity	IL-6, 23 signaling	Animal study
Monoclonal Ab againt IL-23p19	Differentiation and pathogenicity	IL-23p19	Not studied yet
ROR-c inhibitor	Differentiation	ROR-c	Not studied yet
Stat3 inhibitor	Differentiation and pathogenicity	Stat3	Not studied yet
Stat4 inhibitor	Differentiation and pathogenicity	Stat4	Not studied yet
DCs expressing TGF-β	Differentiation	multiple	Not studied yet
DCs expressing IFN-γ/IL-27/IL-12/IL-2/IL-4	Plasticity	Stats?	Not studied yet
IL-22 blockade	Pathogenicity?	IL-22	Awaits further studies

Table 3. Novel therapeutic strategies in RA targeting multiple aspects of Th17 biology.

which appears to cooperate with IL-1 to induce human Th17 cells in the context of low TGF-β, also plays a crucial role in conferring pathogenicity to human Th17 cells. First, the IL-23 receptor is induced in a Stat3[26] as well as RORγt-dependent[33] fashion, but not in naïve CD4T cells in mice. Second, IL-21, which is produced by Th17 cells and promotes Th17 differentiation in an autocrine fashion,[26,33,34] requires IL-23 for its expression in mice. Third, only IL-23 receptor positive Th17 cells migrated to the site of inflammation in EAE, producing more IL-17A than IL-23 receptor negative cells.[55] Finally, a crucial role for IL-23 in CIA was suggested given development of severe CIA in mice deficient for IL-12p35, but not in mice deficient for IL-12/23p40 or IL-23p19, implicating a pathogenic role of IL-23, but not IL-12.[7]

One potential approach to determine the role of IL-23 in conferring pathogenicity to Th17 cells in RA would be to analyze the ability of naïve CD4 T cells cultured in TGF-β + IL-1 + IL-6 + various concentrations of IL-23 to interact with and activate fibroblast-like synoviocytes *in vitro*. If this approach defines a role for IL-23 in pathogenesis of RA, it would further support the rationale for use of a monoclonal antibody against IL-23p19 or the IL-23 receptor, as discussed previously.

Conclusion

Although recent studies have implicated numerous novel therapeutic strategies in RA as targeting multiple distinct aspects of development and function of Th17 cells (*Table 3*), whether these new modalities are feasible options in the actual clinical setting relies on further research to answer many open questions concerning the differentiation, plasticity, and pathogenicity of human Th17 cells.

Acknowledgments

This work was supported NIH/5 RO1 AR38477 and NIH/1 P30 AR48310-01.

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