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The role of T helper type 17 cells in inflammatory arthritis

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Summary

While T cells have been implicated in the pathogenesis of inflammatory arthritis for more than three decades, the focus on the T helper type 17 (Th17) subset of CD4 T cells and their secreted cytokines, such as interleukin (IL)-17, is much more recent. Proinflammatory actions of IL-17 were first identified in the 1990s, but the delineation of a distinct Th17 subset in late 2005 has sparked great interest in the role of these cells in a broad range of immune-mediated diseases. This review summarizes current understanding of the role of Th17 cells and their products in both animal models of inflammatory arthritis and human immune-driven arthritides.

Keywords: collagen arthritis, cytokines, rheumatoid arthritis, spondyloarthritis, T lymphocytes

Introduction – utility of animal models for studying the role of T helper type 17 (Th17) cells in arthritis

Animal models of arthritis are important tools for understanding the aetiology and underlying mechanisms of disease, as well as for discovering and testing new therapeutic targets. Many of these rodent models closely resemble rheumatoid arthritis (RA) pathologically, with infiltration of the joints by inflammatory cells, autoantibody production, synovial hyperplasia and erosion of cartilage and bone. Arthritis can be induced experimentally by systemic immunization with joint proteins mixed with adjuvant, local injection of microbial products or inflammatory mediators directly into the joint, or genetic mutation leading to exaggerated immune responses and spontaneous joint inflammation. There are many similarities as well as important differences in the pathogenesis of these diverse animal models of arthritis, which possibly parallel the clinical, genetic and immunological subcategories of RA and other human arthritic syndromes. Recent work suggests strongly that IL-17, an inflammatory cytokine produced primarily by $CD4^+$ Th17 cells, can play a pivotal role in the pathogenesis of many animal models of arthritis, making it a very attractive target for new therapeutics.

Collagen-induced arthritis

One of the best-characterized models of RA is collagen-induced arthritis (CIA). To induce CIA, DBA mice are immunized intradermally with type II collagen in complete Freund's adjuvant. Several weeks later, joints of the front and hind paws develop severe synovial inflammation and cellular infiltration, leading to destruction of both cartilage and bone. CIA is a T cell-dependent disease, and although Th1 cells were thought previously to be the key pathogenic subset, substantial evidence now demonstrates that Th17 cells are largely to blame. Th17 cells are characterized by production of interleukin (IL)-17A (referred to simply as IL-17), IL-17F (closely related and highly homologous to IL-17A), IL-21 and IL-22. In CIA, serum IL-17 levels increase shortly after immunization, and IL-17 mRNA is up-regulated in the synovium after the onset of arthritis [1]. Many of the $CD4^+$ T cells in the joint are IL-17-positive [2]. Several approaches have shown IL-17 to be both necessary and sufficient for joint inflammation. IL-17-deficient mice develop significantly less severe (although not completely absent) CIA, and IL-17 is important for priming collagen-specific T cells and for collagen-specific immunoglobulin $(IgG)_{2a}$ production [3]. Administration of soluble IL-17R or neutralizing antibody to IL-17, either before or after the

onset of disease, reduces significantly macroscopic joint swelling and the associated histological changes, including cellular infiltration, proteoglycan depletion, cartilage surface erosion and bone erosion [1,4]. Soluble IL-17R treatment has similar protective effects in the rat model of methylated bovine serum albumin (mBSA)-induced arthritis (also known as antigen-induced arthritis, or AIA) [5]. Conversely, local adenoviral over-expression of IL-17 in the knee of naive or immunized mice results in aggravated joint inflammation, including increased cellular infiltration, synovial hyperplasia, receptor activator of nuclear factor (NF)-B (RANK) and receptor activator of NF-B ligand (RANKL) expression, osteoclastogenesis, proteoglycan depletion, chondrocyte death and erosion of cartilage and bone [1,6]. Thus IL-17 is important both for recruitment of inflammatory cells and for joint destruction. This conclusion is supported further by a multitude of *in vitro* studies which show that IL-17 can act on synoviocytes to induce inflammatory cytokines such as tumour necrosis factor (TNF)-α, IL-1β and IL-6, chemokines such as IL-8, chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL2, and mediators of bone and cartilage loss such as RANKL and matrix metalloproteinases (MMPs) (reviewed in [7]).

Roles of Th17 pathway cytokines: transforming growth factor (TGF)-β, IL-6, IL-21 and IL-23

Further evidence for the role of Th17 cells is the contribution to CIA of cytokines responsible for inducing and maintaining Th17 differentiation. In mice, Th17 cells develop when naïve $CD4^+$ T cells are stimulated in the presence of TGF-β plus IL-6 or IL-21. The actions of TGF-β are complex, with both pro- and anti-inflammatory effects. In mice, injecting TGF-β inhibits CIA [8] and neutralizing TGF-β worsens disease [9], yet in CIA or AIA in rats, injection of TGF-β into the joint results in accelerated arthritis and enhanced neutrophil recruitment, synovial inflammation and hyperplasia, while injection of blocking antibody to TGF-β inhibits acute and chronic synovial inflammation [10–13]. Thus, the precise role of TGF-β varies greatly depending on the species, concentration, timing, target cells and cytokine microenvironment.

IL-6, on the other hand, has robust and well-characterized inflammatory effects in multiple animal models, and a humanized blocking antibody to the IL-6 receptor is effective in RA, both as a monotherapy and in combination with methotrexate [14–16]. Injection of anti-IL-6R at the time of immunization inhibits differentiation of Th17 cells and the development of CIA, even after a second booster immunization with collagen [2]. Soluble IL-6R or anti-IL-6 can also ameliorate disease [17,18] and IL-6-deficient mice have reduced IL-17 expression and are completely resistant to CIA [19,20]. Both TGF-β and IL-6 are found in human RA synovial fluid, suggesting a mechanism by which Th17 cells may develop in the joint [21]. In addition, IL-17 augments IL-6 production by human RA synovial fibroblasts *in vitro*, thus creating the potential for a Th17-mediated, positive feedback loop central to joint inflammation [22,23].

Inhibiting IL-6 can suppress the development of Th17 cells, but studies in IL-6-deficient mice have shown that the mechanism of suppression *in vivo* may be complex. In addition to supporting Th17 differentiation, IL-6 can suppress regulatory T cells (T_{regs}). IL-6-deficient mice have increased numbers of T_{regs}, and depleting the T_{regs} with antibody to CD25 restores the Th17 population, suggesting that IL-6 is not absolutely required for Th17 development [20]. Moreover, IL-21 can substitute for IL-6 to promote Th17 differentiation both *in vivo* and *in vitro.* Furthermore, IL-21 is produced by Th17 cells and can act in an autocrine manner to enhance Th17 development [20,24–26]. A role for IL-21 in a variety of autoimmune diseases and their animal models has been proposed (for review see [27]), but relatively little is known currently about its role in arthritis. In CIA, mice treated with a soluble IL-21R-Fc fusion protein after the onset of disease demonstrate a modest but significant decrease in disease severity and down-regulation of IL-6 and IL-17 expression in spleen cell cultures [28]. Similar IL-21R-Fc treatment of rats with AIA yields more dramatic effects, suggesting that different Th17-dependent models of arthritis may be more IL-21 or IL-6 dominant.

One complicating factor is that IL-21 has a significant, non-redundant role in supporting B cell function and therefore may contribute to arthritis by up-regulation of autoantibody production, in addition to promoting Th17 differentiation. The importance of IL-21 for humoral immune responses in arthritis is evident in the K/BxN model, which is autoantibody mediated. K/BxN mice deficient in IL-21R or treated with IL-21R-Fc are resistant to disease despite increased IL-17 expression. Protection from arthritis correlates with a decrease in memory B cells, follicular helper T cells and IgG1 [29]. Much more work is needed to elucidate the role of IL-21 in arthritis, both in human RA and in animal models.

CIA was considered to be a Th1-mediated disease due to the effect of deficiency of the p40 subunit of IL-12 (a key cytokine for induction of Th1 differentiation), in conferring resistance to disease. However, the IL-12 p40 subunit is shared by IL-23, which supports the maintenance and pathogenicity of Th17 cells. A key observation concerning relative roles of Th1 and Th17 cells in CIA was made by Cua and colleagues, who demonstrated that IL-23, rather than IL-12, was critical for development of arthritis. Mice lacking the specific p19 subunit of IL-23 have significantly fewer IL-17-producing Th17 cells and no joint or bone pathology, despite normal numbers of interferon (IFN)-γ-producing Th1 cells. Mice lacking the specific p35 subunit of IL-12, on the other hand, show exacerbated arthritis pathology and increased expression of many inflammatory cytokines in the joint, including TNF-α, IL-1β, IL-6 and IL-17 [30]. Furthermore, deletion of the IFN-γ gene from the CIA-resistant B6 strain

of mice renders them susceptible to CIA and correlates with an increase in IL-6 and IL-17 expression [31]. Neutralizing IFN-γ in wild-type mice increases IL-17 expression and disease susceptibility, while neutralizing IL-17 in IFN-γ-deficient mice decreases the incidence and severity of CIA [31]. IFN-γ-deficient mice also develop augmented AIA, which correlates with increased IL-17 expression and can be treated with neutralizing antibody to IL-17 [32].

Although the precise mechanism by which IL-23 supports Th17 cell activity is not known, IL-23 is a multi-faceted inflammatory mediator, with both T cell-dependent and independent effects. In mice, IL-23 is not required for Th17 differentiation *in vitro*, but *in vivo* a lack of IL-23 severely hinders both Th17 differentiation and pathogenicity. IL-23 can induce RANKL expression on both T cells and myeloid precursor cells, leading to osteoclastogenesis *in vitro* and bone resorption *in vivo* [33,34]. Furthermore, while IL-23 up-regulates IL-17 production by Th17 cells, IL-17, in synergy with TNF-α, induces IL-23 production by fibroblast-like synoviocytes (FLS), leading to a potential positive feedback loop of inflammation and bone destruction in the joint [35]. *In vivo* treatment with neutralizing antibody to IL-23 p19 decreases inflammation and bone destruction in both the rat model of CIA and in a mouse model of Lyme arthritis, which develops following infection with *Borrelia burgdorferi*, thus showing that IL-23 plays an important role in joint inflammation in a variety of settings [36,37].

IL-1Ra$^{-/-}$ mice – the IL-1/IL-17 connection

Further evidence for the pathogenic role of IL-23 and Th17 cells comes from several models of spontaneous arthritis which develop as a result of genetic manipulations affecting immune regulation. Mice deficient in the naturally occurring IL-1 receptor antagonist display exaggerated IL-1R signalling, resulting ultimately in a T cell-dependent, RA-like disease, characterized by synovial inflammation, autoantibody production and erosion of cartilage and bone. Importantly, T cells from IL-1Ra-deficient mice produce increased amounts of IL-17, and IL-17/IL-1Ra double-deficient mice are completely resistant to disease [38]. In addition, treating arthritic mice with neutralizing antibody to IL-17 stops the progression of disease and reduces cellular infiltration, proteoglycan depletion and bone erosion (although to a lesser extent than does neutralizing antibody to IL-1) [39].

IL-17 clearly plays a role in the arthritis caused by excessive IL-1 activity, yet IL-1 does not up-regulate IL-17 production directly by *in vitro*-stimulated T cells, implying that over-expression of IL-17 *in vivo* is mediated by an indirect mechanism. In addition, IL-1 appears to play a more important role in Th17 development *in vivo* than *in vitro*. Kim and colleagues discovered that IL-23 is up-regulated in response to IL-1 and over-expressed in IL-1Ra-deficient spleens and joints. Furthermore, neutralizing antibody to IL-23 blocks the up-regulation of IL-17 in spleen cell cultures stimulated with IL-1, suggesting that IL-1-induced IL-17 production in IL-1Ra-deficient mice is mediated by IL-23 [40]. Immunohistochemistry of joints from IL-1Ra-deficient mice shows a high level of IL-23 p19, which co-localizes with CD4 and IL-17 [33]. Moreover, intra-articular injection of adenoviral IL-23 in IL-1Ra-deficient mice accelerates arthritis, with increased RANK and RANKL expression and more severe bone destruction [33]. These and many other *in vitro* studies strongly implicate both IL-23 and IL-17 as mediators of osteoclastogenesis and bone destruction.

SKG mice implicate autoreactive Th17 cells that express CCR6

SKG mice have a mutation of ZAP-70, a key signalling intermediate downstream of the T cell receptor, which results in defective negative selection of self-reactive T cells. The mice develop spontaneous, T cell-mediated arthritis and demonstrate many of the extra-articular comorbidities found in RA, including interstitial pneumonitis, subcutaneous nodules and vasculitis [41]. As in IL-1Ra-deficient mice, arthritis in SKG mice is Th17-dependent. SKG mice deficient in IL-17 or IL-6 are completely resistant to disease, while deficiency of IFN-γ exacerbates disease [42]. However, spontaneous development of autoreactive Th17 cells is not sufficient to induce disease. Onset of arthritis requires further Th17 expansion and activation via homeostatic proliferation or stimulation of innate immunity by microbial products, such as zymosan [42].

Th17 cells in both humans and mice (including SKG mice) express the chemokine receptor CCR6, and both produce and respond to the CCR6 ligand CCL20 [43,44]. Inflamed synoviocytes, in particular FLS, express CCL20, which is up-regulated by IL-1β, IL-17 and TNF-α. In addition, *in vivo* treatment with anti-CCR6 antibody suppresses SKG arthritis, suggesting that arthritogenic, CCR6-expressing Th17 cells migrate to inflamed joints in response to CCL20, which is both directly produced and up-regulated by Th17 cells [43].

Streptococcal cell wall-induced arthritis illuminates downstream actions of IL-17

Streptococcal cell wall (SCW)-induced arthritis is a chronic, erosive polyarthritis that can be induced in euthymic, susceptible Lewis rats by a single intraperitoneal (i.p.) injection of SCW. The van den Berg group recently pioneered a version of SCW-induced arthritis in mice. A single intra-articular injection of SCW induces localized, acute joint inflammation, which evolves into a chronic, destructive arthritis after repeated injections. The mouse model of SCW-induced arthritis is particularly interesting, because distinct cytokines appear to mediate the different phases and aspects of disease.

In this new chronic mouse model of SCW-induced arthritis, IL-17R expression is required for full progression of

chronic, destructive arthritis, but not for acute joint swelling [45,46]. Mice lacking IL-17R, specifically in the radiation-resistant cells of the joint, show a significant decrease in synovial infiltration, bone and cartilage erosion, and joint expression of many inflammatory cytokines, chemokines and selectins. In addition, local IL-17 gene transfer is sufficient to turn acute, macrophage-driven joint inflammation into severe chronic arthritis with aggravated cartilage damage [47]. Interestingly, both IL-17R and IL-1β are required for chronic arthritis and cartilage erosion but not for acute joint swelling. TNF-α, on the other hand, is required for acute joint swelling but not for chronic erosive disease [48,49]. Over-expression of IL-17 can, however, overcome the requirement for IL-1β during cartilage erosion in both SCW-induced arthritis and CIA, suggesting that IL-17 can induce joint damage independent of IL-1 [47]. Similarly, over-expression of IL-17 enhances inflammation and cartilage destruction during SCW-induced arthritis, even in the absence of TNF-α, and in the combined absence of TNF-α and IL-1β [50].

These results may have important implications for the possible clinical value of IL-17 neutralizing therapies. Due to the fact that IL-17 often synergizes with TNF-α and IL-1β, one might worry that targeting IL-17, in addition to TNF-α or IL-1β, would be redundant and offer no additional benefit. The data discussed here, however, suggest that IL-17 can mediate disease, especially cartilage erosion, independently of TNF-α and IL-1β, thus making it a potentially valuable therapeutic target. The differential timing of the dependence on TNF-α *versus* IL-1β and IL-17 also suggests that different cytokine-blocking treatments may be most efficacious at different stages of disease. While the therapeutic value of neutralizing TNF-α in arthritis is well established, recent studies in CIA showed that, paradoxically, TNF blockade increased numbers of pathogenic Th1 and Th17 cells in the lymph node, but reduced their accumulation in the joint, thus raising many questions about the effect of TNF blockade on IL-17 expression in humans [51]. These hypotheses clearly need to be explored clinically. Given the rapidly increasing number of biological therapies available, deciding which cytokine to target at what stage of disease in each patient represents a major hurdle to providing the most successful and personalized patient care.

Proteoglycan-induced arthritis shows that Th1 cells can still be arthritogenic

Many animal models of arthritis are Th17-mediated, but there are exceptions to every rule. Finnegan and colleagues have shown that the mouse model of proteoglycan-induced arthritis (PGIA) is not dependent on IL-17, but rather is mediated primarily by IFN-γ [52]. Mice deficient in IFN-γ develop more severe CIA but significantly milder PGIA. Loss of IL-17, on the other hand, has no effect on the onset or severity of PGIA, including cellular infiltration, synovial hyperplasia and anti-proteoglycan antibodies [52]. Interestingly, the IL-17-deficient mice with PGIA had reduced joint expression of IL-6 and RANKL, but normal levels of TNF-α and bone erosion – due possibly to increased joint expression of IL-1β. These results suggest that IL-17 is important for RANKL and IL-6 expression, but that IL-1β can mediate bone loss through alternative mechanisms.

CIA and PGIA also differ in their dependence on IL-12 and IL-27, both of which support Th1 differentiation but inhibit Th17. Mice deficient in IL-12 p35 or IL-27R develop PGIA with reduced incidence and severity, which correlates with a significant loss of IFN-γ expression [52,53]. In CIA, on the other hand, loss of IL-12 p35 enhances disease, and treatment with IL-27 reduces disease [30,54]. Despite these differences, however, arthritic joints from mice with both PGIA and CIA express IFN-γ, IL-17, TNF-α, IL-1β and IL-6 [52]. The important lesson from these contrasting models is that one cell type, such as Th1 cells, can be either protective (as in CIA) or pathogenic (as in PGIA), and what may appear to be opposing cytokine networks *in vitro* can trigger highly similar clinical manifestations *in vivo*. It will be interesting to learn whether RA patients fall into different subclasses, depending on the relative abundance, and thus the potential pathogenicity, of Th1 *versus* Th17 cells.

Is rheumatoid arthritis a Th17-driven disease?

Rheumatoid arthritis (RA) is a chronic autoimmune disease, with symmetrical involvement of small joints of the hands and feet, characterized by synovitis as well as bone and cartilage destruction. Several studies have evaluated the tissue distribution of IL-17 in RA. While there is some discrepancy regarding the serum levels of IL-17 and the frequency of Th17 cells in the systemic circulation in RA, most reports agree that IL-17 is increased in the synovial fluid and synovial tissue in RA (Tables 1 and 2). IL-17 is expressed in the T cell rich areas of the synovium and is secreted primarily by $CD4^+CD45RO^+$ memory T cells in the synovium and peripheral blood [55–63]. Th17 cells from RA peripheral blood express the receptor for IL-23, as well as chemokine receptors CCR6 and CCR4. Approximately a third of the Th17 cells in RA also express IL-22 or IFN-γ [63]. The frequency of Th17 cells correlates with markers of disease activity in RA, such as CRP and tender joint count. Interestingly, in a prospective study in patients with RA, increased expression of IL-17 and TNF-α mRNA in synovium were associated independently with more severe joint damage, but expression of IFN-γ was associated with protection from joint damage [64].

TNF-α, IL-1β, IL-6, and now IL-17, have been identified as pathogenic cytokines in RA. TNF neutralizing therapy is being used with significant success and an IL-6 receptor blocking antibody is also effective. Furthermore, trials are currently under way to evaluate the safety and efficacy of anti-IL-1β and anti-IL-17 monoclonal antibodies in patients with RA. Thus the regulation of TNF-α, IL-1β, IL-6 and

Table 1. Distribution of interleukin (IL)-17 and T helper type 17 (Th17) cells in the peripheral blood, synovial fluid and synovium of patients with rheumatoid arthritis.

Study	Patient characteristics	Samples	Findings
Kohno *et al.* [144]	52 RA (25 on infliximab, 11 on etanercept)	• Synovial tissue from patients with RA or OA • PBMC from RA and HC	• Synovial expression of IL-17 mRNA unchanged • IL-17 gene expression in PBMCs higher in RA than HC
Yamada *et al.* [145]	123 RA 28 HC (patients with RA were on steroids, methotrexate, gold salts or biologicals)	• PBMCs from RA and HC • Mononuclear cells from synovial fluid	• Frequency of Th1, Th17 cells remained unchanged between RA and HC • Th1 cells >Th17 cells in the synovial fluid of patients with RA
Ziolkowska *et al.* [58]	15 RA 8 OA 20 HC (patients with RA were on low-dose steroids and DMARDS)	• Serum • Synovial fluid	• Increased amounts of IL-17 in serum and synovial fluid in RA
Kotake *et al.* [57]	43 RA 9 OA 4 trauma 7 gout	• Synovial fluid • Synovial tissue	• Increased IL-17 in synovial fluid in RA • IL-17$^+$ cells present in RA, not OA synovium
Shahrara *et al.* [60]	14 RA (patients with RA were on DMARDS and biologicals) OA HC	• Synovial fluid • Synovial tissue • Peripheral blood	• Increased IL-17 mRNA in synovial tissue in RA in comparison to OA and HC • Similar numbers of Th17 cells in HC and RA PB • Increased frequency of Th17 cells in RA synovial fluid compared to RA peripheral blood
Chabaud *et al.* [56]	21 RA 12 OA 3 trauma	• Synovial tissue • Synovial tissue explant cultures	• IL-17 increased in synovial tissue explant cultures in RA compared to OA and trauma patients • Increased expression of IL-17 mRNA in RA *versus* OA synovial tissue
Jandus *et al.* [61]	10 PsA 10 RA 10 AS 5 vitiligo 25 controls (patients were on various doses of prednisone, DMARDS and biologicals)	• Peripheral blood	• Increased frequency of peripheral blood Th17 cells in PsA and AS but not in RA
Shen *et al.* [63]	20 AS 12 RA 16 healthy controls	• Peripheral blood	• Increased frequency of peripheral blood Th17 cells in AS and RA • Increased frequency of IL-22 expressing cells in RA and AS • IL-17 is increased in *ex vivo* culture of PBMCs in RA as well as AS, but reached significance in AS

RA: rheumatoid arthritis; OA: osteoarthritis; PBMC: peripheral blood mononuclear cells; HC: healthy controls: DMARDS: disease modifying anti-rheumatic drugs; PB: peripheral blood: AS: ankylosing spondylitis; PsA: psoriatic arthritis.

Table 2. Distribution of interleukin (IL)-17 and T helper type 17 (Th17) cells in the peripheral blood, synovial fluid and synovium of patients with inflammatory arthritis.

	Serum IL-17	IL-17 mRNA in PBMCs	Th17 cells in PBMCs	Synovial fluid IL-17	IL-17 mRNA in synovium	Th17 cells in synovial fluid	Th17 cells in synovium
RA	++ [57,58]	+ [144]	+/– [60,61,145]	++ [57,58]	+/– [144] ++ [56,60]	+/– [145] ++ [60]	+ [57]
AS	++ [128,129]		++ [61,63]				
PsA			++ [61]				
ReA				++ [136]			

PBMC: peripheral blood mononuclear cells; RA: rheumatoid arthritis; AS: ankylosing spondylitis; ReA: reactive arthritis; PsA: psoriatic arthritis.

IL-17 is quite pertinent in the context of RA and provides insight into the perpetual activation of the inflammatory cascade in the synovium. The receptor for IL-17 is expressed ubiquitously and binding of IL-17 to its receptor on monocytes, macrophages, chondrocytes, osteoblasts and fibroblasts induces the secretion of TNF-α, IL-1β, IL-6 and IL-23. All these cytokines, either in combination with each other or with TGF-β, can contribute to the differentiation of Th17 cells from human memory or naive T cells [24,65–71]. Furthermore, IL-1β has also been shown to induce the generation of Th17 cells from T_{regs} [72]. This is particularly important, as TNF-α, IL-1β and IL-6 are abundant in the joint and provide the ideal cytokine mileu for the generation and maintenance of Th17 cells. Additionally, there is synergy between IL-17, TNF-α and IL-1β in mediating downstream effector functions, and neutralization of TNF-α in combination with IL-1β and IL-17 is most effective in suppressing IL-6 production and collagen degradation in *ex vivo* cultures of RA synoviocytes [73].

The interaction of T cells and fibroblast-like synoviocytes (FLS) contributes to sustained inflammation in the joints. In addition to resting T cells and antigen activated T cells, T cells which have been activated exclusively by cytokines, such as IL-6, IL-2 and TNF-α, referred to as cytokine activated T cells, may represent an important component of the synovial T cell population [74]. Resting as well as cytokine activated T cells induce production of IL-6, IL-8 and prostaglandin E_2 (PGE_2) by FLS, which is augmented by IL-17 [22,75]. FLS can, in turn, induce a proliferative response of resting T cells to superantigens [76]. Moreover, the interaction between cytokine activated T cells and FLS is dependent on membrane anchored TNF-α on the T cell surface [22]. In addition to FLS, osteoblasts can also induce superantigen-dependent proliferation of T cells, and T cells can induce IL-6 production in osteoblasts, which is augmented by IL-17 [77]. These findings indicate a pathogenic role of IL-17 in RA and provide insight into the possible mechanisms underlying the perpetual activation of IL-17 producing cells in the joints, leading to sustained inflammation and consequent tissue damage.

IL-17, the IL-17 receptor and signalling pathways

There are six isoforms of IL-17: IL-17A, -B, -C, -D, -E and -F. IL-17A and F have been implicated in autoimmunity and share a 50% homology. These two isoforms exist either as a homodimer or an A/F heterodimer. To date five distinct proteins have been identified that function as components of receptors for IL-17: IL-17RA, IL-17Rh1, IL-17RC, IL-17RD and IL-17RE. IL-17RA associates physically with IL-17RC [78,79], and both are over-expressed by peripheral blood mononuclear cells (PBMCs) of patients with RA and by RA synoviocytes. Both IL-17A and F, each of which has been implicated in RA, signal through IL-17RA and IL-17RC to induce similar, but not identical, patterns of expression of proinflammatory genes. Studies by Zrioual *et al.* [59] have shown that IL-17A regulates more genes than IL-17F, but both could induce expression of important proinflammatory genes, including CCL20, IL-23, IL-6, IL-8, E-selectin, CXCR4 and granulocyte colony-stimulating factor (G-CSF). Studies utilizing siRNA to suppress the expression of IL-17RA and IL-17RC in RA synoviocytes have shown that induction of IL-6 and IL-8 in response to IL-17 is dependent on full expression of IL-17RA and IL-17RC [80]. Furthermore, the synergistic effects of IL-17A, IL-17F and TNF-α to induce IL-6, CCL-20 and TNF receptor II were dependent on both IL-17RA and IL-17RC. These studies did not find any synergistic effects of IL-17 and TNF-α on IL-17RA, IL-17RC or TNF receptor I expression. Such findings point to the importance of blocking both subunits of IL-17 receptor, IL-17RA as well as IL-17RC, in order to achieve maximal therapeutic benefit.

IL-17 binding to its receptor initiates several discrete signalling pathways, all of which are important in the pathogenesis of joint inflammation. For example, IL-17-induced expression of IL-23 p19 in RA FLS is dependent on the phosphatidylinositol 3 (PI3)-kinase/AKT (protein kinase B), natural killer (NK)-kB, and p38 mitogen activated protein kinase (MAPK) pathways [81]. In contrast, IL-17-induced secretion of IL-6 and IL-8 from FLS is dependent on NK-kB

and PI3-kinase/AKT, but independent of p38 MAPK signalling [23]. Thus IL-17 utilizes multiple signalling pathways to induce various proinflammatory cytokines important in the pathogenesis of RA.

Th17-related cytokines in RA

TGF-β plays a significant role in the differentiation of Th17 cells in both mice and humans. TGF-β is present in synovial fluid and is expressed by RA FLS. Immunohistochemical studies have shown that TGF-β is located predominantly in the cartilage–pannus junction in the RA joints [82–87]. Current evidence suggests that TGF-β plays a pathogenic role in bone and cartilage destruction in RA by synergizing with TNF-α and RANKL to induce osteoclast differentiation [88–90]. TGF-β can also induce osteoclastogenesis by inhibition of osteoprotegrin production by FLS [91]. In addition, exogenous TGF-β induces matrix metalloproteinases in RA FLS [82,92]. The role of TGF-β in initiating or sustaining a pathogenic Th17 response in RA remains to be evaluated.

Elevated levels of IL-23 are found in the sera and synovial fluid of patients with RA, and expression of the IL-23 p19 is increased in FLS and synovial macrophages [60,81,93]. Expression of IL-23 by RA synovial macrophages increases further upon stimulation with peptidoglycan, and *ex vivo* treatment of synovial fluid mononuclear cells with IL-23 augments the frequency of Th17 cells [60]. *In vitro* studies of RA FLS show that IL-17 can synergize with TNF-α to induce expression of the IL-23 p19 subunit. On the other hand, the induction of IL-23 p19 expression by IL-1β is independent of IL-17. Both IL-12 and IL-23 receptors signal via signal transducer and activator of transcription-4 (STAT-4), and STAT-4 is important in the generation and maintenance of Th17 cells [94]. STAT-4 has been identified as one of the susceptibility genes for RA [95,96] and it is plausible that STAT-4 risk alleles could augment the sensitivity of Th17 cells to stimulation by IL-23, and thus maintain an activated Th17 response.

IL-21 has been shown recently to be important in Th17 biology. IL-21, in combination with TGF-β and IL-1β, induces the differentiation of naive human CD4 T cells into Th17 cells expressing IL-17, retinoic acid-related orphan receptor (RORγT), IL-23 receptor and CCR6 [66,97]. IL-21 is increased in the peripheral blood and synovial fluid in RA. Lymphocytes from RA peripheral blood and synovial fluid, as well as FLS and synovial macrophages, have increased expression of the IL-21 receptor [98,99]. Stimulation of peripheral blood and synovial fluid T cells by IL-21 increases production of TNF-α and IFN-γ [99], while blocking IL-21 with an IL-21 receptor fusion protein decreases secretion of TNF-α, IL-6 and IL-1β from RA synoviocytes *in vitro* [100]. In addition to its effects on T cell cytokines, IL-21 induces B cell activation and expansion and differentiation of plasma cells [101]. Both T cells and B cells play important and indispensable roles in RA, and IL-21/IL-21 receptor may a critical interaction linking the T cell response with the B cell activation and autoantibody production.

The IL-21 receptor belongs to the common γ-chain cytokine receptor family, which also includes the IL-15 receptor. IL-15 is yet to be explored fully in the context of IL-17 biology, but this cytokine may be involved in the regulation of IL-17 in RA. IL-15 is expressed strongly by peripheral blood and synovial fluid T cells in RA, and exogenous IL-15 augments IL-17 secretion by PBMCs [58]. IL-15 has also been shown to be produced by FLS and mediate up-regulation of both IL-17 and TNF-α in T cells. IL-17 and TNF-α can, in turn, induce FLS to produce IL-15 and IL-6, creating yet another positive feedback loop leading to exaggerated Th17 responses [102]. Consistent with these observations, anti-IL-15 antibody reduced TNF-α, IL-1β and IL-6 production by RA synoviocytes *ex vivo* [100]. In a phase I/II study in RA, anti-IL-15 was well tolerated and led to significant clinical improvement. In this study 63% of patients achieved American College of Rheumatology criteria (ACR) 20, 38% ACR 50 responses and 25% ACR 70 responses [103]. These results are comparable to the effect of widely used TNF neutralizing therapies given in the absence of methotrexate.

In addition to IL-17, Th17 cells also produce IL-22. IL-22 is involved in mucosal immunity, and the role of this cytokine in RA remains to be understood. There is increased expression of both IL-22 and the IL-22 receptor in the synovium in RA, [104], and IL-23 can induce IL-22 in human T cells [105]. The source of IL-22, its pathogenicity and its functional significance in RA remain to be evaluated.

Th17/IL-17 effector functions in RA

One of the important functions of IL-17 is the recruitment of monocytes, macrophages, neutrophils and lymphocytes into the inflamed joint. This is achieved indirectly by the interaction of chemokines with their respective receptors. Chemokines are classified into four groups: C, CC, CXC, and CX3C chemokines. The CXC chemokines are subgrouped further into ELR^+ and ELR^-, based on the presence or absence of an N terminus Glu-Leu-Arg. ELR^+ chemokines attract neutrophils and induce angiogenesis, both of which are hallmark features of the inflamed RA synovium, and ELR^- chemokines have angiostatic properties [106–108]. Angiogenesis plays an important role in RA pathogenesis, as the newly formed blood vessels sustain inflammation and augment the migration of inflammatory cells into the joint. IL-17 can augment both angiogenesis and chemotaxis via up-regulation of ELR^+ CXC chemokines such as CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 and CXCL8 [80]. IFN-γ, on the other hand, inhibits the expression of ELR^+ CXC chemokines and down-regulates angiogenesis. IL-17 can aid angiogenesis further by inducing expression of vascular endothelial growth factor (VEGF) [109].

IL-17 induces CCL20 expression, which attracts immature dendritic cells, naive B cells and memory T cells, including

Th17 cells [73,110,111]. IL-17 can also induce stromal derived factor (SDF-1) which mediates the chemotaxis of T cells, B cells and monocytes to the RA synovium [112]. Furthermore, IL-17 stimulates the migration of monocytes [113], which are involved in the differentiation of Th17 cells *in vitro*. Leucocyte recruitment is enhanced further by up-regulation of G-CSF and granulocyte–macrophage-colony stimulating factor (GM-CSF), two mediators of granulopoiesis which are both induced by IL-17 [114,115].

Clearly, IL-17 can enhance inflammation, cellular infiltration, and angiogenesis in arthritis, but it can also mediate the bone and cartilage damage which is characteristic of RA. Periarticular bone erosion requires RANKL-expressing osteoclasts, which are abundant in inflamed joints [116–118]. RANKL is expressed on osteoclasts and binding of RANKL to RANK plays a critical role in the maturation, activation, migration and survival of osteoclasts [119–123]. Th17 cells play an important role in regulating this pathway. Th17 cells themselves can express RANKL and IL-17 is also a potent inducer of RANKL expression on osteoclasts [7,57,124,125]. The activity of the osteoclasts can be potentiated further by other cytokines, which are either induced by IL-17 or synergize with IL-17, such as IL-1β, IL-6 and TNF-α [57,126]. IL-17 can also induce MMPs and nitric oxide in chondrocytes and thus initiate and maintain cartilage destruction [127,128].

Spondyloarthropathies and IL-17

Spondyloarthritis (SpA), a chronic inflammatory disease of unknown aetiology which affects 0·5% of the population, includes psoriatic arthritis (PsA), ankylosing spondylitis (AS), reactive arthritis (ReA), inflammatory bowel disease-associated arthritis and undifferentiated spondyloarthropathy (uSpA). SpA is manifested as inflammation of the spine, peripheral joints, entheses, eyes, intestines and/or skin.

Both serum IL-17 levels and circulating Th17 frequency are elevated in patients with AS, although there is no correlation with disease activity or biomarkers of bone turnover, such as bone-specific alkaline phosphatase and tartarate-resistant acid phosphatase [129]. In patients with AS, IL-17 and IL-23 are elevated in serum and are augmented after *in vitro* culture of PBMCs with baterial superantigen. Moreover, PBMCs from patients with AS produce more IL-17 after *in vitro* stimulation with IL-23 compared to healthy controls [130]. Th17 cells from patients with AS also express cytokines such as IL-22, IFN-γ, IL-2 and TNF-α and chemokine receptors CCR6 and CCR4 [61,63]. In contrast, the IL-12/23 p40 subunit is not elevated in serum of patients with SpA (including AS and PsA) when compared to patients with OA, although the synovial fluid level of p40 is higher [131]. Although administration of anti-p40 in psoriasis has led to significant improvement of skin inflammation, the significance of the p40 subunit in inflammatory arthritis remains to be understood and clinical trials are currently under way evaluating anti-p40 in PsA [132]. Th17 cells are increased in the peripheral blood of patients with PsA compared to patients with RA. In addition, the Th17 cells from PsA co-express other inflammatory cytokines such as IL-2, IFN-γ and TNF-α, suggesting that Th17 cells may play a role in the pathogenesis of PsA [61].

In a recent study, patients with AS and Crohn's disease had increased expression of the IL-23 p19 subunit in intestinal biopsy samples in comparison to normal controls. Surprisingly, the increased expression of IL-23 was associated with increased expression of IL-17 only in Crohn's disease but not AS [133]. This is particularly interesting, as several polymorphisms of the IL-23 receptor gene have been associated with AS, inflammatory bowel disease and psoriasis [134,135]. It is possible, but not yet proven, that these polymorphisms confer resistance or sensitivity to IL-23-mediated stimulation of Th17 cells. These findings support further the possible role of a dysfunctional IL-23/IL-17 axis in AS and PsA.

The role of IL-17 and Th17 cells in reactive arthritis and inflammatory bowel disease associated arthritis remains to be evaluated, although synovial fluid levels of IL-17 are increased in patients with reactive arthritis or undifferentiated arthritis in comparison to RA and OA [136].

IL-17 in juvenile arthritis

Children with juvenile idiopathic arthritis (JIA) have elevated synovial fluid levels of IL-17, and exogenous IL-17 induces FLS to produce proinflammatory cytokines and MMPs in *ex vivo* cultures [137]. Children with JIA and healthy controls have a similar frequency of Th17 cells in the peripheral circulation, but there are increased numbers of $CD4^+$ Th17 cells in the synovial fluid and synovium in JIA. The Th17 cells in the inflamed joints express CCR6 as well as CCR4 and some also express IL-22 or IFN-γ [138]. Although much remains to be known regarding the generation and regulation of Th17 cells associated with JIA, the phenotype of the Th17 cells in JIA and RA have several similarities, suggesting that Th17 cells may play a similar inflammatory role in both diseases.

Regulation of IL-17 in inflammatory arthritis

Current evidence provides substantial support for the role of IL-17 and Th17-related cytokines in the pathogenesis of inflammatory arthritis. While IL-23, IL-21, IL-15, IL-1β, TNF-α and IL-6 contribute to the development and maintenance of Th17 cells, IL-4, IFN-γ and IL-12 suppress differentiation of Th17 cells and secretion of IL-17. Dendritic cells are genetically modified to secrete IL-4 suppress IL-17 responses and reduce arthritis in CIA [139,140]. In this same model injection of an adenoviral vector expressing IL-4 reduces IL-17 and mitigates the severity of arthritis [141].

The value of regulating IL-17 or Th17 pathway cytokines is being tested in clinical studies of patients with inflamma-

tory arthritis. Phase I/II clinical trials of anti-IL-17 in RA were completed recently and preliminary data suggest a therapeutic effect in at least one of these trials [142,143]. In addition, two phase II trials of anti-IL-17 neutralizing antibody, one in PsA and the other in AS, are currently under way (NCT00809614 and NCT00809159). A phase II clinical trial in PsA using anti-p40 was completed recently and results are pending (NCT00267956), and a trial with an oral IL-12/IL-23 inhibitor is ongoing in patients with RA (NCT00642629). IL-6 and IL-1 are also among the molecular targets of biological agents that are pertinent to Th17 cells.

In view of the existence of multiple IL-17 isoforms, the complexity of the IL-17 receptor(s), the various ways of inducing Th17 cells and the production of proinflammatory cytokines other than IL-17 by these cells, the best way to target the Th17 axis in human disease is far from obvious, and may differ among the various forms of human inflammatory arthritis. It will probably require many years of careful clinical studies to determine this. Such studies are also likely to offer further insights into the pathogenesis of human arthritis and the role of the Th17 pathway.

Disclosure

None of the authors have conflicts of interest, or any relevant financial interest, in any company or institution that might benefit from this publication.

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