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Role of TH-17 Cells in Rheumatic and Other Autoimmune Diseases

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Abstract

In humans multiple pathways can induce TH-17 cell differentiation, whereas in mice this process is mostly modulated by IL-6 and TGF-β. IL-17 produced by TH-17 cells has been associated with a number of inflammatory autoimmune diseases including psoriasis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, and rheumatoid arthritis. In this review, we have primarily focused on the role of TH-17 cells/IL-17 in the pathogenesis of rheumatoid arthritis and experimental arthritis. The potential role of TH-17 cells in rheumatoid arthritis progression has been demonstrated by correlating the percent TH-17 cells or levels of IL-17 with rheumatoid arthritis disease activity score and C-reactive protein levels. Further, previous studies suggest that IL-17 mediated vascularization may lay the foundation for rheumatoid arthritis joint neutrophil and monocyte recruitment as well as cartilage and bone destruction. The profound role of IL-17 in the pathogenesis of experimental arthritis may be due to its synergistic effect with TNF-α and IL-1β. Although the initial clinical trial employing anti-IL-17 antibody has been promising for rheumatoid arthritis, future studies in humans will shed more light on how anti-IL-17 therapy affects rheumatoid arthritis and other autoimmune disease pathogenesis.

Keywords: TH-17; IL-17; Rheumatoid arthritis; Autoimmune diseases; Experimental arthritis; Inflammation; Angiogenesis

Abbreviations: BFGF: Basic Fibroblast Growth Factor; CIA: Collagen Induced Arthritis; CRP: C-Reactive Protein; DAS 28: Disease Activity Score; HGF: Hepatocyte Growth Factor; LTi: Lymphoid Tissue Inducer; MMP: Matrix Metalloproteinases; OA: Osteoarthritis; OPG: Osteoprotegerin; PGIA: Proteoglycan-Induced Arthritis Model; RA: Rheumatoid Arthritis; RORC: RAR-Related Orphan Receptor C; SLE: Systemic Lupus Erythematosus; SEFIR: SEF/IL17R Domain; VEGF: Vascular Endothelial Growth Factor; C/EBP: CCAAT/enhancer binding proteins; UTR: 3' Untranslated Region

Introduction

TH-17 cells are a subgroup of CD4+ lymphocytes that function predominantly at mucosal surfaces to protect against extracellular pathogens and are involved in the inflammatory process through the recruitment of neutrophils and in autoimmune diseases [1,2]. Human TH-17 cell development is regulated by transcription factor, RAR-related orphan receptor C (RORC) and these cells express IL-17A, IL-17F, IL-21, IL-22 and IL-26 [3]. Originally TH-17 cells were characterized by expression of IL-17A, commonly referred to as IL-17, though more recently, the lectin receptor, CD161, has been identified as a specific marker for human TH-17 cells [4].

IL-17A was the first member identified of the IL-17 cytokine family, which consists of 6 members IL-17A through IL-17F (Table 1). The IL-17 family members function as either homodimers or heterodimers [5,6]. IL-17A and IL-17F are both produced by TH-17 cells, however IL-17A is significantly more potent in initiating signaling and causing autoimmune responses. IL-17B is produced by cells of the gastrointestinal tract, pancreas and neurons, IL-17C is produced by cells of the prostate and fetal kidney, and IL-17D is secreted by cells of the muscles, brain, heart, lung, pancreas and adipose tissues [7,8]. IL-17E also known as IL-25 is produced by lymphocytes, lung epithelial cells, alveolar macrophages, eosinophils, basophils, NKT cells, TH-2 cells, and mast cells [9,10]. IL-17E is capable of initiating a TH-2 response which suppresses TH-17 cell differentiation. IL-17 was initially believed to be solely expressed by TH-17 cells, however it is now known that IL-17 can be produced by γδ T cells [11] , lymphoid tissue inducer (LTi) cells [12], mast cells [13], and neutrophils [14].

The IL-17 receptor family consists of 5 members, IL-17RA through IL-17RE, whose structures are not homologus to other cytokine receptor families [15,16]. IL-17 receptors are type I transmembrane proteins that have conserved structural elements including a cytoplasmic SEF/IL17R domain (SEFIR) and two extracellular fibronectin III-like domains [5]. IL-17RA can bind to either IL-17A, IL-17E, or IL-17F, however it binds to IL-17A with the highest affinity [17]. IL-17RB can bind to either IL-17B or IL-17E and is also known as IL-25 receptor [16]. IL-17RC can bind either IL-17A or IL-17F, while IL-17RE binds only to IL-17C [18,19]. IL-17RD does not have a known ligand. IL-17 receptor family members function as receptor complexes with multiple members binding to ligands, possibly in a 2 step binding process where one IL-17 ligand must bind to a receptor before the second IL-17 ligand can bind a second receptor in the complex [17]. It is thought that these complexes can be either homomeric or heteromeric, for example, IL-17RA can pair with IL-17RC to bind and transmit signals from ligands such as IL-17A and IL-17F [20]. Of the IL-17 receptor family members, IL-17RA is expressed ubiquitously while IL-17RC is expressed mostly on non-hemopoietic cells [17-21].

Differentiation of mice and human Th-17 cells

Origin of and factors that modulate human TH-17 cells have been discussed intensely over the past few years. Three independent studies demonstrated that in mice TGF-β and IL-6 are responsible for polarizing naïve CD4+ T cells to TH-17 and the transcription factor RORγt is an essential component in this process [22-24]. However, the role of TGF-β in human TH-17 differentiation is less clear. It was shown that TGF-β can inhibit human TH-17 differentiation while IL-6 and IL-1β were the driving factors for this development [25]. Consistently, others have shown that while IL-1β or IL-23 is necessary

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Family member	Receptor	Source	Pathological role
IL-17(A)	IL-17RA and RC	TH-17 cells, CD8+ T cells, γδ T cells, NK cells, NKT cells	Involved in autoimmune pathology, neutrophil and monocyte trafficking and angiogenesis
IL-17B	IL-17RB	Cells of the gastrointestinal tract, pancreas and neurons	Not identified
IL-17C	IL-17RE	Cells of the prostate and fetal kidney	Not identified
IL-17D	unknown	Cells of the muscles, brain, heart, lung, pancreas and adipose tissue	Not identified
IL-17E (IL-25)	IL-17RA and RB	lymphocytes, lung epithelial cells, alveolar macrophages, eosinophils, basophils, NKT cells, TH-2 cells, mast cells	Induces TH-2 cell responses and therefore suppresses TH-17 cell polarization
IL-17F	IL-17RA and RC	TH-17 cells, CD8+ T cells, γδ T cells, NK cells, NKT cells	Neutrophil recruitment

Table 1: The human IL-17 cytokine family members source of expression and pathological role.

for human TH-17 differentiation from naïve human CD4+ T cells, the presence of TGF-β was not required [26]. Interestingly, direct contact of LPS or peptidoglycan activated monocytes with naïve CD4+ T cells was able to polarize naïve CD4+ T cells to TH-17 cells [27]. In contrast to these findings, others have shown that TGF-β together with other proinflammatory factors such as IL-23, IL-1β and IL-6, was critical for human TH-17 cell development [28,29] and lack of TGF-β induces a shift from TH-17 to a TH-1 profile [29]. It was also demonstrated that IL-21 and TGF-β could uniquely promote human naïve CD4+ T cells into TH-17 cells by activating RORC2 which is the human homologue of mouse RORγt [30]. It was thought that the presence of TGF-β in serum containing media, and therefore purity and/or naivety of cells, may have lead to the discrepancy in these results [31]. Further, while both human and mouse TH-17 cells secrete IL-17, IL-17F, IL-21, IL-22 and CCL20 production of IL-26 is only induced by human TH-17 cells as there is no known murine homolog for this cytokine [32-34]. Collectively, previous studies indicate that while in mice IL-6 and TGF-β can induce robust levels of TH-17 cells (40-80%) this phenomen on may not be as straight forward in humans where multiple factors can differentially modulate TH-17 cell development to modest levels (10%).

Th-17 cells in human autoimmune inflammatory disease

A number of inflammatory autoimmune diseases including psoriasis, SLE, MS, inflammatory bowel disease and RA have been associated with TH-17 cells. In psoriasis, which is a chronic skin disease characterized by keratinocyte hyperplasia, inflammation, T cell invasion, and angiogenesis, TH-17 cells are predominantly located in the dermis of skin lesions [35]. Further, IL-17 and other TH-17 cytokines such as IL-22 and IL-23 are involved in the pathogenesis of psoriasis [36-38]. In psoriasis skin lesions, IL-17 mRNA levels increase with disease activity [35]. Disease resolution correlated with decreased IL-17 and IL-23 expression levels in psoriasis patients who responded to treatment with the soluble TNFα receptor fusion protein, Etanercept [39]. In vitro experiments with human keratinocytes showed that IL-17 treatment resulted in increased production of ICAM-1, IL-6 and IL-8 [36]. Also IL-17F treatment of kerotinocytes showed greater increase in IL-6 expression compared to IL-17 or TNFα- activated cells suggesting that IL-17F produced by TH-17 cells causes the inflammation in psoriasis partly through induction of IL-6 by keratinocytes [40]. Studies demonstrate that kerotinocytes treated with IL-17 and IL-22 had increased CCL20/MIP-3α expression which may drive CCR6+ TH-17 cell recruitment into the skin lesions perpetuating the disease process [41]. These results suggest that several TH-17 associated cytokines such as IL-17, IL-17F and IL-22 play an important role in pathogenesis of psoriasis by inducing the production of similar downstream targets. Therefore, response to therapy can suppress these common inflammatory pathways.

Another chronic autoimmune disease, SLE, also features elevated levels of IL-17. SLE is a systemic disease in which autoantibodies initiate immune complex formation resulting in chronic inflammation in locations including the skin and kidneys. In patients with SLE, IL-17 is produced by both TH-17 cells and TCRαβ CD4- CD8- T cells [42,43]. Additionally, in these patients IL-17 levels are elevated in the sera [44] and in sites of inflammation such as the skin [45] and kidneys [46,47] . The over production of IL-17 is thought to potentiate the systemic inflammation observed in patients with SLE in a couple of ways. First, IL-17 stimulation of B cells can increase B cell activation and antibody production resulting in greater amounts of autoantibody production and immune complex formation [43-48]. Secondly, IL-17 released at inflammation locations such as the skin can amplify the immune response by increasing the influx of effector cells [49].

MS is an autoimmune central nervous system disease that involves the destruction of myelin sheets resulting in impairment of nerve signal transduction. IL-17 production was elevated in MS cerebral spinal fluid and blood [50] however, in comparison, greater IL-17 levels were detected in the cerebral spinal fluid which correlated with clinical exacerbations and neutrophil infiltration [51]. More recently, histological studies demonstrated that IL-17 expressing cells were more likely found in active rather than inactive areas of MS lesions [52]. It is hypothesized that IL-17 and TH-17 cytokine IL-22 may be involved in the pathogenesis of MS by weakening the blood brain barrier. Endothelial cells of the blood brain barrier express receptors for IL-17 and IL-22 and it was shown that IL-17 and IL-22 could disrupt the blood brain barrier possibly through the production of reactive oxygen species [53,54]. Hence, disruption of the blood brain barrier would allow autoantibodies and inflammatory mediator's access to the myelin sheets allowing the pathology to occur.

Inflammatory bowel disease includes both Crohn's disease and ulcerative colitis and is a chronic autoimmune inflammatory condition of the gastrointestinal tract. IL-17 has been found to be elevated in the intestinal mucosa and within the sera of patients with inflammatory bowel disease compared to normal controls [55,56]. IL-17 was also produced from gut TH-17 cells in patients with Crohn's disease [57]. Further IL-17F mRNA has been found in greater levels in inflamed lesions of Crohn's disease patients compared to non-inflamed areas [58]. These human studies suggest a role for TH-17 cells and IL-17 in the pathogenesis of inflammatory bowel disease. However, the exact function of IL-17 in inflammatory bowel disease is somewhat controversial as animal studies have shown both protective and pathogenic roles for IL-17 [59,60].

Role of Th-17 cells in RA pathogenesis

Synovial tissue explants from RA but not osteoarthritis (OA) spontaneously produce biologically active IL-17 [61]. Increased levels of IL-17 are also detected in RA synovial fluid and in T cell rich areas of RA synovial tissues compared to OA synovial tissue and fluid [61-

63]. We found that TH-17 cells were significantly elevated in RA synovial fluid compared to RA and normal peripheral blood cells [63]. Others have shown that expression of CCR4 and CCR6 on RA TH-17 cells demonstrates selective migration of these cells to the site of inflammation [64]. This group of investigators shows that increased TH-17 presence in peripheral blood of early stage RA patients is suggestive of the contribution of TH-17 cells to disease onset. Further, percentage of TH-17 cells and levels of IL-17 strongly correlate with disease activity score (DAS 28) and C-reactive protein (CRP) suggesting the importance of these cells in disease progression [64]. The potential importance of TH-17 cells/IL-17 in RA is supported by a randomized, placebo-controlled and double blind phase I study where RA patients that received DMARD plus neutralizing monoclonal antibody against IL-17 achieved an ACR 20 more rapidly compared to those receiving DMARD alone [65]. Consistently a two year prospective study analyzing RA synovial tissues demonstrated that IL-17 and TNF-αmRNA levels are synergistic prognostic factors for worse out come [66]. These studies clearly demonstrate that TH-17 cells play an important role in RA pathogenesis.

Synergistic effect of Il-17 with other proinflammatory cytokines

The direct proinflammatory effects of IL-17 may be small when compared to those of IL-1β and TNF-α. However, IL-17 enhances many of the effects of IL-1β and TNF-α. IL-17 stimulates the production of IL-1 and TNF-α from human macrophages [67]. IL-17 also enhances IL-1-mediated IL-6 production by RA synovial tissue fibroblasts [68], as well as TNF-α induced synthesis of IL-1, IL-6 and IL-8 [69]. Many of the IL-17 activated genes contain CCAAT/enhancer binding proteins (C/EBP) in their promoter which cooperates with NF-κB in inducing the transcription of these proinflammatory factors [70]. In RA synovial tissue fibroblasts, IL-17 interacts with IL-1 and TNF- α to amplify the secretion of CCL20 [71]. IL-17, in combination with TNF- α, induces significantly higher levels of nitric oxide and osteoclastic resorption compared to each cytokine alone [72,73]. The mechanism by which IL-17 mediates synergistic effect is through enhancing mRNA stability of AU-rich elements in the 3' untranslated region (UTR) of many cytokines and chemokines [74,75]. In short, a major role of IL-17 may be amplifying the effects of macrophage derived proinflammatory cytokines and hence be the missing link between T cells in RA joint and the effector phase of RA.

Role of Th-17 in granulopoiesis and neutrophil migration

It is shown that neutrophils are critical in the early stage of arthritis and are abundantly present in the RA joint and synovial fluid of patients with active disease [76]. In mice, overexpression of IL-17 can expand both neutrophil progenitors in bone marrow and spleen [77]. This effect is due to IL-17-induced G-CSF production since neutralization of G-CSF, but not deletion of IL-17RA, markedly attenuates this effect [77-79] suggesting that IL-17 plays an indirect role in granulopoiesis. Local expression of IL-17 enhances neutrophil migration in mouse ankle joints as well as the peritoneal cavity [80,81] . In the rat airway, IL-17 mediates neutrophil recruitment via induction of macrophage inflammatory protein protein-2 (rMIP-2) [82]. Like granulopoiesis, neutrophil chemotaxis caused by conditioned media from IL-17-stimulated gastric epithelial cells was inhibited by a neutralizing antibody to IL-8 but not to IL-17, suggesting that IL-17 is unable to directly induce neutrophil chemotaxis [83]. Further, results from our laboratory demonstrates that neutralizing antibody to CXCL5, but not CXCL1, significantly suppresses neutrophil trafficking to IL-17-induced arthritis ankle joints indicating that IL-17 mediated CXCL5 plays a role in this process [84]. Therefore if these studies in rodents translate into human, IL-17 may contribute to RA disease onset by inducing IL-8 and/or CXCL5 production that is involved in recruitment of neutrophils to the RA joint.

Effects of Il-17 on cartilage degradation and bone erosion

Local inflammation is involved in cartilage degradation by suppressing proteoglycan and collagen synthesis as well as causing extracellular matrix breakdown. Earlier studies have demonstrated that IL-17 alone and in synergy with IL-1β inhibits cartilage proteoglycan synthesis in murine explants by inducing production of nitric oxide [85]. IL-17 treated bovine chondrocytes demonstrated increased expression of matrix metalloproteinases (MMP)1, 3 and 13 which are factors involved in degradation of extracellur matrix [86]. IL-17 can also promote cartilage breakdown by synergizing with TNF- α, IL-1β and IL-6 to increase collagen degradation and MMP release [86,87]

IL-17 induces the expression of RANKL in osteoblasts [88] and can further synergize with TNF- α in osteoclastic resorption [73-89]. A naturally occurring decoy protein osteoprotegerin (OPG) can inhibit the interaction of RANK and RANKL, however high dose of OPG could only partially inhibit IL-17/TNF- α mediated bone resorption [89]. In collagen induced arthritis (CIA) local expression of IL-17 in ankle joints enhances bone erosion by mediating an imbalance in RANKL/OPG in favor of RANKL levels [90]. Taken together these observations suggest that IL-17 can directly and in combination with IL-1β and TNF- α result in RA bone and cartilage destruction.

Unique characteristics of Il-17 in RA

Angiogenesis is an early and a critical event in the pathogenesis of RA. Hence, neovascularization is involved in leukocyte ingress into the synovium during the development and progression of RA [91,92]. Our recent data demonstrates that IL-17 can intensify inflammation by promoting angiogenesis and subsequently recruiting inflammatory cells to the RA joint [93]. We show that IL-17 is capable of endothelial chemotaxis at concentrations present in RA synovial fluid. Further, ligation to IL-17RC on endothelial cells and activation of PI3K/AKT pathway is responsible for IL-17-induced endothelial migration and tube formation [93]. Expression of IL-17 in RA synovial fluid and IL-17RC on endothelial cells plays an important role in RA synovial fluid mediated migration [93]. *In vivo*, IL-17 enhances vascularity in experimental arthritis and induces blood vessel development in matrigel plugs [93] (Figure 1). However, there are also data to suggest that IL-17 can indirectly induce angiogenesis, by promoting production of proangiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) [94,95] from cells in the RA joint. We demonstrate that in addition to the direct effect of IL-17/IL-17R on angiogenesis, joint IL-17-mediated CXCL5, but not CXCL1, plays a key role in IL-17-induced arthritis and vascularization [84]. Angiogenesis mediated by IL-17 may lay the foundation for recruitment of leukocytes and therefore studies were performed to better understand the mechanism by which IL-17 may promote inflammation and monocyte trafficking in RA. It was shown that IL-17 induces monocyte migration at concentrations available in RA joint by ligation to IL-17RA and IL-17RC through activation of p38 MAPK pathway [96]. However, the direct effects of IL-17 does not account for all its chemotactic ability *in vivo* since neutralization of CCL2 significantly reduces IL-17 induced monocyte migration into the peritoneal cavity [81] (Figure 1). Production of CCL2 was detected in IL-17 activated macrophages and RA synovial tissue fibroblasts, two cells types important for RA pathogenesis [81]. The potential role of IL-17 in RA angiogenesis and monocyte extravasation

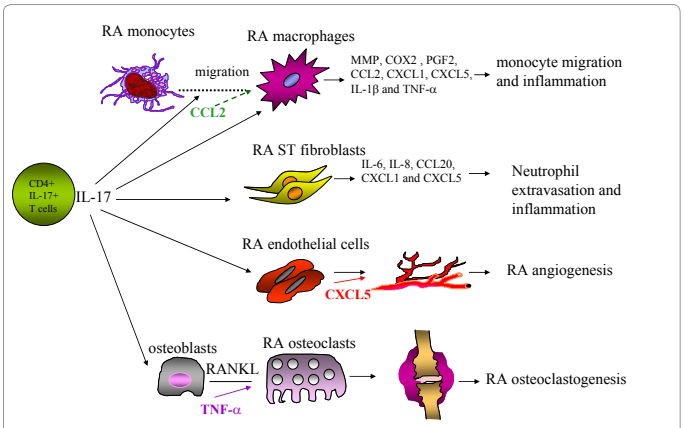

Figure 1: Schematic overview of the pathological role of IL-17 in RA. Several cell types are affected by IL-17 in RA joints. IL-17 can induce migration of RA peripheral blood monocytes to the inflamed joints where they differentiate to macrophages. RA synovial tissue macrophages and fibroblasts activated with IL-17 can produce a number of proinflammatory factors that can mediate inflammation and neutrophil recruitment. Angiogenesis mediated by IL-17 can provide the inflamed joint with nutrients and oxygen and thereby perpetuate the vicious inflammatory cycle. IL-17 can also play a role in RA bone destruction by enhancing RANKL production alone or in synergy with TNF-α.

was also documented when local expression of IL-27 in CIA ankle joints suppressed TH-17 differentiation as well as IL-17-mediated vascularization and macrophage staining [97]. These studies suggest that IL-17 can perpetuate inflammation by driving angiogenesis which can result in subsequent recruitment of neutrophils (acute phase) and monocytes (chronic phase) thereby amplifying chronic inflammation in RA through multiple pathways.

Role of Il-17 in experimental arthritis

It has been shown that IL-17 plays a profound role in experimental arthritis. CIA was markedly reduced in IL-17-/- mice [98]. Early neutralization of IL-17 using an IL-17R IgG Fc fusion protein in CIA suppresses the onset of the disease [99]. Consistently, treatment of CIA after disease onset using anti-IL-17 antibody decreases the severity of inflammation and bone destruction in CIA [100]. Blocking of IL-17 in antigen induced arthritis suppresses both IL-1β and TNF-α indicating that IL-17 is an important upstream inflammatory mediator [101]. Local overexpression of IL-17 using an adenoviral vector results in joint inflammation and cartilage proteoglycan depletion in the knees of naive mice [81,84,102]. Further, IL-17-induced joint inflammation and cartilage erosion was markedly reduced in TNF-α-/- mice but not in IL-1 deficient mice [102]. These data suggest a requirement for TNF-α and not IL-1 for the induction of IL-17-induced arthritis. However, IL-17 may also act independently of TNF-α after disease onset [102]. Interestingly a very recent paper shows that neutralizing both IL-17 and TNF-α ameliorates CIA joint damage to a greater extent compared to each factor alone implicating the synergistic inflammatory effect of IL-17 and TNF-α [87]. IL-23-/- mice were resistant to CIA and this correlated with an absence of IL-17-producing CD4+ T cells, despite normal IFN-γ production by TH1 cells [103]. In contrast to the significant role of TH-17 cells in CIA pathogenesis, proteoglycan-induced arthritis model (PGIA) is dependent on IFN-γ production and hence suppression of TH-17 differentiation by IL-27 or deletion of IL-17 does not affect disease pathogenesis [104,105]. Ectopic expression of IL-27 in CIA mice reduces TH-17 mediated angiogenesis and monocyte trafficking as well as TH-17 differentiation by downregulating IL-1β and IL-6, two important TH-17 cell polarizing factors [97]. Mounting evidence from experimental arthritis models and RA demonstrates that IL-17 is involved in the initial and progression phase of disease which supports IL-17 as a therapeutic target in RA.

Conclusion

Identification of TH-17 cells has been a paradigm shifting event and has therefore questioned the importance of TH-1 cell involvement in autoimmune disorders. Strong evidence supports that TH-17 cells are pathologically important in chronic inflammatory and autoimmune disorders, namely psoriasis, SLE, inflammatory bowel disease, MS, and RA. In this review we have specifically focused on the implication of TH-17 cells/IL-17 in RA. Interestingly, IL-17 is one of the few T cell derived cytokines found in RA joints where the majority of inflammatory factors are produced from synovial tissue fibroblasts and macrophages. The role of IL-17 in neutrophil recruitment as well as cartilage and bone erosion is well established and more recently a novel function of IL-17 in RA angiogenesis and monocyte extravasation has been identified. The direct proinflammatory effect of IL-17 is often smaller than TNF-α and IL-1β, however IL-17 synergizes with these cytokines by enhancing their production and action in experimental arthritis and RA joint. Inhibition of TH-17 differentiation or IL-17 function ameliorates pathogenesis of experimental arthritis models and neutralizing anti-IL-17 was able to improve RA symptoms. Since blockade of IL-6 function (an important downstream target of IL-17) has yielded to some success in RA patients, it is tempting to speculate that anti-IL-17 therapy can be employed in anti-TNF-α non responders or in adjunct to anti-TNF-α therapy. Therefore, more human studies are required to respond to these inquiries.

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