Interleukin-6 in the Pathogenesis of Rheumatoid Arthritis

Jean Y. Park, M.D., and Michael H. Pillinger, M.D.

Abstract

Cytokines such as TNF-α and IL-1β play key roles in driving the inflammation and synovial cell proliferation that characterize rheumatoid arthritis (RA) joint destruction. It is, therefore, not surprising that therapies for RA have targeted these cytokines. While blockade of TNF-α or IL-1β has been efficacious for many patients with RA, adequacy and maintenance of response are not universal, and increased risk of adverse events such as infection and malignancy remains a concern. Therefore, new targets in the treatment of RA continue to be examined. As interleukin-6 (IL-6) has been implicated in the pathogenesis of RA, blockade of its activity is of both scientific and clinical interest. The basic biology of IL-6, the in vitro animal data supporting its role in RA, and the human trials to date that test the possible efficacy of IL-6-directed therapy for RA are reviewed.

Rheumatoid arthritis (RA) is a chronic, systemic disease characterized by inflammation and cellular proliferation in the synovial lining of joints that can ultimately result in cartilage and bone destruction. Although RA has been the subject of innumerable investigations, the etiology and pathogenesis of the disease remain incompletely understood. It is clear, however, that cytokines play a key role in driving synovial cell activation leading to joint destruction. Among the most important of these cytokines in RA are TNF-α and IL-1β, whose capacity to induce inflammation has previously led to their historical designation, along with IL-6, as endogenous pyrogens. It is not surprising, therefore, that these cytokines have been targeted in the development of RA therapies. While blockade of TNF-α or IL-1β effects is efficacious for many patients with RA, not all patients respond adequately or maintain a response to these strategies. Moreover, increased risk of infection (such as tuberculosis), as well as concern about malignancy and other adverse outcomes, have raised concerns about the use of currently available biologics, particularly anti-TNF-α agents. Accordingly, the search for new targets for safe and effective therapy of RA continues.

In addition to IL-1β and TNF-α, synovial fluid and synovium from RA patients contains IL-6 activity that is significantly elevated compared to control patients with osteoarthritis. Moreover, increased IL-6 activity correlates with elevations of acute phase reactants, as well as other signs of inflammation, including fever and anemia. Additionally, IL-6 has been implicated as a trophic factor for the generation of autoantibodies (including rheumatoid factor). It is unsurprising, therefore, that blockade of IL-6 has become a focus of growing scientific and clinical interest in the treatment of RA.

In this article, we examine the role of IL-6 in RA pathogenesis. We review the basic biology of IL-6, and discuss the in vitro, animal and clinical data supporting its contribution to the RA disease process. Finally, we describe the human trials, performed to date, that test the hypothesis that IL-6-directed therapy may be of significant clinical benefit to patients with RA.

Basic Biology

IL-6 Structure, Expression, and Cell Signaling Structure

IL-6 is produced by a variety of cells including T cells, B cells, fibroblasts, endothelial cells, monocytes, keratinocytes, mesangial cells, and some tumor cells. The genes for human and murine IL-6 have been cloned and sequenced. Human IL-6 has a molecular mass of 21 to 28 kDa, and is
IL-6 Signaling and Regulation. IL-6 engagement of β1030 receptors on the cell surface results in the activation of multiple intracellular signaling pathways. This engagement occurs through the interaction of the extracellular domain of the IL-6 receptor with its ligand, IL-6. The IL-6 receptor (IL-6R) contains a 130 kDa glycoprotein (gp130), and engagement of IL-6R results in the association of the intracellular segment of IL-6R with gp130. Dimerized gp130 activates intracytoplasmic JAK tyrosine kinases, which in turn induce tyrosine phosphorylation of STAT3. STAT3 nuclear translocation results in new gene expression of acute phase proteins, which subsequently are secreted. STAT3 also induces transcription of SOCS-1, a regulatory molecule that binds to JAK to inhibit gp130 signal. Dotted arrows depict translocation events; solid lines indicate protein activation.

Figure 1  IL-6 Signaling and Regulation. IL-6 engagement of IL-6 receptors results in the activation of STAT3, which then translocates to the nucleus and up-regulates gene expression. Dotted arrows indicate translocation events; solid lines indicate protein activation.

However, human multiple myeloma cells reportedly express IL-6 receptors, suggesting that IL-6 may function as a growth factor in multiple myeloma and possibly other forms of tumor. In addition, B and T cells express IL-6 receptors, but the regulation of IL-6R expression differs between these two types of cells.

The IL-6 signaling system is regulated by negative feedback via the suppressors of cytokine signaling (SOCS) and the protein inhibitors of activated STATs (PIAS). Once STAT3 is activated, it translocates to the nucleus and up-regulates the transcription of SOCS-1, which in turn binds to the JAK tyrosine kinase and suppresses gp130 signal transduction. In contrast, PIAS are constitutively active negative regulators of STATs. PIAS-3 specifically associates with STAT3 and blocks STAT3-mediated gene transcription.

Effects of IL-6 on immune and inflammatory Cells In Vitro

IL-6 was originally described as a T cell-derived B-cell differentiation factor. In B-cell-directed studies, stimulation of mononuclear cell preparations with pokeweed mitogen (PWM) induces production of IgM, IgG, and IgA, a process enhanced by the addition of IL-6. Moreover, in the absence of exogenous IL-6, addition of anti-IL-6 antibody to PWM-stimulated cells abrogates immunoglobulin production. However, anti-IL-6 antibody does not affect the proliferation of B cells. Thus, it is believed that IL-6 is essential for antibody production by, but not proliferation of, activated B cells.

IL-6 effects are not limited to B cells, and IL-6 exerts...
multiple effects on numerous cell types. For example, IL-6 acts upon resting T cells. Studies on mitogen-stimulated T cells and thymocytes suggest that IL-6 activates T cells by inducing both IL-2 production and IL-2 receptor expression. In contrast to B cells, IL-6 induces the proliferation of mitogen-activated T cells and thymocytes, a process that can be inhibited by addition of anti-IL-2 or anti-Tac (i.e., anti-human lymphocyte IL-2R) antibody. In addition to stimulating IL-2 synthesis and secretion, IL-6 may act synergistically with IL-2 in driving the differentiation of human T cells and thymocytes into cytotoxic T cells.

IL-6 also has effects on other marrow-cell lines. In hematopoiesis, IL-6 acts synergistically with IL-3 to induce formation of multilineage blast cell colonies and megakaryocytes. Serum amyloid A and C-reactive protein, while increased in the presence of IL-6, apparently through the activation of gp130. Given the recent interest in osteoclasts as mediators of bony erosion in RA, these observations suggest a possible direct role for IL-6 in RA joint destruction.

**IL-6 in the Pathogenesis and Treatment of RA**

**Animal Models of Arthritis and Autoimmunity**

Animal models examining the role of IL-6 initially focused either on overexpression, or inhibition or deletion of IL-6 or the IL-6 gene. In a study by Suematsu and associates, the normally unexpressed but inducible human IL-6 gene was constitutively expressed in a transgenic mouse. Polyclonal hypergammaglobulinemia, with massive plasmacytosis within the spleen and lymph nodes, was the result. In addition, these mice developed mesangiproliferative glomerulonephritis and had an increase in megakaryocytes in their bone marrow. Serum albumin levels were decreased, consistent with a role for IL-6 in the regulation of acute phase protein synthesis. These data support a direct role for IL-6 in inflammation and autoimmunity. Unfortunately, the effect of IL-6 expression on the joints of these animals was not assessed.

Antigen-induced arthritis (AIA) is an immune complex animal model employed by researchers to mimic RA. Boe and coworkers compared IL-6 knockout mice and their wild type counterpart on the ability to develop arthritis after injection with methylated bovine serum albumin (mBSA). In contrast to wild-type controls, IL-6 knockout mice failed to develop joint swelling and the histological joint lesions characteristic of AIA. Injection of IL-6 knockout mice with recombinant human IL-6 reconstituted the ability of these mice to develop arthritis.

Collagen induced arthritis (CIA) is another well-established animal model of RA, in which injection of mice with type II collagen (CII) produces an immune response directed at joint connective tissues. This model was used by Alonzi and colleagues to evaluate the role of IL-6 in arthritis in DBA/1J mice. Injection of CIA resulted in the anticipated arthritis. Moreover, serum IL-6 levels were significantly higher in those mice with higher arthritis indices. When an IL-6 null mutation was introduced, the IL-6 deficient mice produced lower titers of anti-type II collagen antibodies, and they were completely protected from arthritis. Histologic examination of knee joints from these mice confirmed an absence of inflammatory cells and tissue damage, supporting a crucial role for IL-6 in the development of CIA.

Sasai and associates also examined CIA in IL-6 deficient DBA/1J mice. Although this group observed that CIA was induced in both IL-6 deficient and IL-6, arthritis in the IL-6 mice was of delayed onset, and less severe than in their wild-type counterparts. Consistent with the studies of Alonzi and coworkers, the IL-6 mice produced less anti-CII antibodies and had decreased histological severity of arthritis; they also had less radiographic osteopenia and fewer bone erosions. While gene knockout is not at present a therapeutic option, these studies suggested the possibility that antibody therapies directed at IL-6 or designed to block IL-6 might be effective in both animal models of arthritis and human RA.

To test the possible efficacy of IL-6R blockade, Takagi and colleagues administered a rat anti-murine IL-6R monoclonal antibody (MR16-1) to DBA/1J mice immunized with CII. MR16-1 delayed the onset and reduced the severity of CIA in these mice. These effects were only seen when the monoclonal antibody was administered early in the induction of arthritis, either on experimental Day 0 (day of the first collagen injection) or Day 3. Thus, IL-6 expressed immediately...
in response to CII injection may play an important role in the development of CIA, and MR16-1 may suppress CIA by inhibiting IL-6 signal transduction in this early stage.

Tocilizumab (formerly MRA) is a humanized murine anti-human IL-6R antibody that has been shown to cross-react with the IL-6R of the cynomolgus monkey, but not with the murine receptor. As CIA can be induced in nonhuman primates like cynomolgus monkeys, the effect of tocilizumab on the development of CIA was studied in these animals. Control monkeys treated with CII developed clinical signs of arthritis 4 weeks after the first injection with CII. Treatment with tocilizumab (10 mg/kg intravenous) significantly inhibited the onset of joint inflammation and reduced the increases in serum CRP, fibrinogen, and ESR seen in the controls. Histological examination at 14 weeks revealed no pathological changes in the tocilizumab group, while the control group demonstrated synovial proliferation, pannus formation, infiltration of neutrophils, angiogenesis, and cartilage and bone destruction in their joints. These results indicated that targeting IL-6R may be a useful antiarthritic strategy in multiple mammalian species, and suggested the potential applicability of this approach to the treatment of RA in humans.46

**Human Rheumatoid Arthritis: Clinical Trials of IL-6 Signal Blockade**

To date, the only anti-IL-6 strategy to be tested in humans has been the use of tocilizumab. Using recombinant DNA technology, tocilizumab was humanized by grafting the complementarity-determining regions from a murine antibody into a human IgG
t. The result was a functional IL-6R antigen-binding site in a reshaped human antibody.47 In vitro, tocilizumab has been confirmed to bind both the membrane-bound and soluble forms of IL-6R and to inhibit the proinflammatory activity of IL-6 (Fig. 2).

Choy and associates48 reported the first study of tocilizumab efficacy in RA patients in 2002. Forty-five patients with active RA were randomized to receive a single intravenous administration of tocilizumab at doses of 0.1, 1, 5, or 10 mg/kg. After two weeks, primary efficacy analysis revealed a statistically significant improvement in American College of Rheumatology (ACR) 20% response criteria in the 5 mg/kg tocilizumab group versus placebo. Patients receiving either 5 mg/kg or 10 mg/kg of tocilizumab experienced a significant decrease in disease activity score (DAS) and serum inflammatory markers (ESR and CRP) after two weeks, compared with those who received 0.1 or 1 mg/kg of tocilizumab or placebo. Thirty-four of the 45 patients reported adverse events, the most common being diarrhea (8 patients, 17.8%). However, this symptom occurred at comparable rates in all treatment groups, including placebo. No serious adverse events were attributed to the medication itself.

The safety, pharmacokinetics, and efficacy of repetitive dosing of tocilizumab in the treatment of RA were studied by Nishimoto and coworkers49 in 2003. In an open-label, Phase I/II clinical study, 15 patients with active RA were administered tocilizumab (2, 4 or 8 mg/kg IV) twice weekly for 6 weeks. Tocilizumab was generally well-tolerated by all participants. Though a total of 70 adverse events were reported, none was deemed severe. However, laboratory evaluation during the study revealed increases in total blood cholesterol, low-density lipoprotein (LDL), and triglyceride levels. While the precise implication of this finding is not yet established, it has been postulated that IL-6 may function as a regulator of lipid metabolism. Serologic evaluation revealed no new appearance of antinuclear or anti-dsDNA in these patients; antibodies to tocilizumab itself also were not observed. With each subsequent administration, the serum concentration and half-life of tocilizumab increased in all three doses studied. That the half-life was prolonged at all doses suggested that it might be possible to further extend the dosing interval of tocilizumab in the treatment of RA. Ultimately, 80% of patients achieved an ACR20 at 6 weeks, and 33% achieved an ACR50. Improvement in ESR and CRP were also noted in these study patients.

Although short term, repetitive administration of tocilizumab appeared to be safe and effective in the Nishimoto study, the optimal dosing schedule for the medication still was not established.49 Moreover, the small size of the study precluded the identification of potential but uncommon complications. Accordingly, a multicenter, double-blind, placebo-controlled trial of tocilizumab in patients with refractory RA was conducted by the same investigators. One hundred sixty-four patients with active RA were recruited and randomized to receive tocilizumab (4 mg/kg or 8 mg/kg) or placebo every 4 weeks for 3 months. Compared with placebo, tocilizumab significantly improved disease activity in a dose-dependent manner. After 3 months, 78% of patients in the 8 mg/kg group achieved an ACR20 response, compared to 57% in the 4 mg/kg group and 11%
in the placebo group (p < 0.001). As in the prior studies, serum markers of inflammation improved (76% patients who received 8 mg/kg tocilizumab normalized their CRP). Incidence of adverse events was equivalent in the placebo and treatment groups (56% in the placebo group, 59% in the 4 mg/kg group, and 51% in the 8 mg/kg group). Most of the adverse events reported were mild, but one patient died of reactivation of chronic active Epstein-Barr virus (EBV) and subsequent hemophagocytic syndrome approximately 2 months after receiving a single dose of tocilizumab (8 mg/kg). The mechanism of reactivation of this EBV infection was not known. Consistent with the previous studies, an increase in blood cholesterol levels was observed in 44% of the tocilizumab patients. There was no increase in cardiovascular complications associated with this increase in lipid profiles during the study period. Mild increases in liver transaminases and decreases in white blood cell counts also were detected but were transient. Tocilizumab again did not induce antinuclear or anti-dsDNA antibodies in this study, but two patients developed anti-tocilizumab antibodies and were withdrawn. Overall, this study showed that tocilizumab therapy for active RA significantly reduces disease activity and is generally well-tolerated.50

A second Phase II trial, conducted in Europe [Chugai Humanized Antirheumatic Interleukin Six Monoclonal Antibody (CHARISMA) trial], included 359 patients with active RA who had previously experienced incomplete responses to methotrexate (greater than 10 mg/week for 6 months). Patients were randomly assigned to receive tocilizumab or placebo every 4 weeks for 12 weeks, together with either 10 mg to 25 mg methotrexate or placebo weekly. Treatment with 8 mg/kg of tocilizumab, alone (p < 0.05) or with methotrexate (p < 0.001), resulted in significant increases in ACR20 responses compared to methotrexate alone. No significant difference in ACR20 responses was observed between the tocilizumab only, and tocilizumab plus methotrexate groups. However, ACR50 and ACR70 responses in this trial were increased only in the tocilizumab plus methotrexate arm, compared to either tocilizumab or methotrexate therapy alone. DAS28 was reduced in a dose-dependent manner in all tocilizumab-treated patients beginning at 4 weeks, except in a cohort of patients receiving 2 mg/kg of tocilizumab alone. As in the previous trials, the drug was generally well-tolerated, with the majority of adverse events considered mild to moderate in severity.51

Most recently, a clinical trial (SAMURAI; Study of Active Controlled Monotherapy Used for Rheumatoid Arthritis, an IL-6 Inhibitor) has examined the ability of tocilizumab monotherapy to inhibit radiographic progression of structural joint damage in RA. This multicenter, randomized controlled trial enrolled 306 patients with active RA of less than 5 years’ duration. The patients were randomized to receive either tocilizumab monotherapy at 8 mg/kg intravenously every 4 weeks or conventional disease modifying antirheumatic drugs (DMARDs) for 52 weeks. Radiographs of hands and feet were scored by the van der Heijde-modified Sharp method. Overall, the tocilizumab group showed less radiographic change in Total Sharp Score (TSS) compared to the conventional DMARD group. In addition, the tocilizumab group experienced significant increases in ACR20, 50, and 70 responses, as compared with conventional DMARD therapy. Overall, tocilizumab was shown again to be generally well tolerated although cholesterol levels (as well as LDL, triglyceride and HDL levels) were again observed to rise. Three cases of malignancy (two breast cancer, one colon cancer) were observed in patients in the tocilizumab arm; the significance of this observation remains to be determined.52

While the above studies indicate that treatment of RA patients with tocilizumab (alone or in concert with methotrexate) can decrease disease activity and erosions in the short term, the long-term durability and safety of the tocilizumab response still need to be determined. Currently, a collaborative phase III clinical trial, enrolling over 4000 patients in 41 countries, is underway to validate the conclusions of the prior studies and to further assess the prevention of RA radiographic progression through the use of tocilizumab.

Conclusion

IL-6 is a pleiotropic cytokine with multiple roles in the regulation of inflammation and hematopoiesis. Clinical trials to date suggest that tocilizumab, a monoclonal antibody directed at the IL-6R, may be both effective and generally well-tolerated in the treatment of RA. Larger phase III studies are currently under way to confirm the findings of the initial studies and to delineate more clearly the long-term outcomes of therapy targeted at the IL-6 signaling system. As IL-6 has been implicated in the pathogenesis of other inflammatory conditions, such as systemic juvenile idiopathic arthritis, Castleman’s disease, and Crohn’s disease, the capacity to block IL-6 activity could also have implications for the treatment of these and other inflammatory and autoimmune disorders.

References


38. Mor A, Abramson SB, Pillinger MH. The fibroblast-like synovial cell in rheumatoid arthritis: A key player in inflammation


Disclosure Statement
Jean Y. Park, M.D., and Michael H. Pillinger, M.D., have no potential financial conflicts of interest to disclose.