Interleukin-6
A Key Mediator of Systemic and Local Symptoms in Rheumatoid Arthritis

Bruce N. Cronstein, M.D.

Abstract
Interleukin-6 (IL-6) is a pleiotropic cytokine, present at elevated levels in patients with rheumatoid arthritis (RA). IL-6 signaling involves both a specific IL-6 receptor (IL-6R) and a ubiquitous signal-transducing protein, gp130 that is also utilized by other members of the IL-6 family. IL-6 signaling occurs by two mechanisms. Conventional signaling involves the binding of IL-6 to transmembrane IL-6R on cells expressing this receptor. In contrast, trans-signaling involves binding between the complex of soluble IL-6R/IL-6 and membrane-bound gp130. Trans-signaling allows IL-6 to affect cells that do not express IL-6R, including many synovial cells. The biological activities of IL-6 contribute to both systemic and local RA symptoms. IL-6 is a strong inducer of the acute-phase response, which can result in fever, secondary amyloidosis, anemia, and elevations in acute-phase proteins, such as C-reactive protein (CRP). The ability of IL-6 to induce B-cell differentiation may lead to the formation of rheumatoid factor and other autoantibodies. In joints, IL-6 promotes osteoclast activation and induces the release of matrix metalloproteinases, thus contributing to joint damage. In patients with RA, IL-6 levels correlate with markers of disease activity and clinical symptoms, and animal studies support the concept that this cytokine plays a role in the development of inflammatory arthritis. Clinical trials with tocilizumab, a humanized monoclonal antibody to soluble IL-6R, have shown that blocking IL-6 signaling reduces RA symptoms and markers of disease activity. Current evidence thus strongly supports the association between IL-6 and RA symptoms and suggests that IL-6 blockade will be a useful therapeutic strategy for patients with this disease.

Interleukin-6 (IL-6) is a multifunctional cytokine that was originally identified as a B-cell differentiation factor involved in the maturation of antibody-producing cells. Since then, IL-6 has been found to have a wide array of additional activities, including effects on T cells, blood vessels, and neurons. In addition, IL-6 is closely related to other cytokines with diverse and, in some cases, redundant, activities, such as leukemia-inhibitory factor and ciliary neurotrophic factor.

IL-6 is expressed at high levels in several inflammatory diseases, including systemic juvenile arthritis, systemic lupus erythematosus, Crohn’s disease, and rheumatoid arthritis (RA). The evidence that this cytokine serves as a key mediator of systemic and local manifestations of RA is compelling. Blocking IL-6 may thus have the potential to control symptoms and slow disease progression in patients with RA.

IL-6 Mediated Signaling
IL-6 is a single-chain protein that is produced by T cells, B cells, monocytes, fibroblasts and certain other cell types. IL-6 and other members of the IL-6 family are arranged in four long α-helical structures. IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin-1 are some of the most notable cytokines that belong to the IL-6 family.

IL-6 can transmit signals to cells in two ways: conventional receptor-bound signaling and through trans-signaling in conjunction with soluble IL-6 receptor (IL-6R). Both forms of signaling require the IL-6R and a signal-transducing transmembrane protein, known as gp130, reviewed by Rose-John. In conventional signaling, IL-6 binds to IL-6R on the cell surface. This complex then associates with gp130,
resulting in dimerization of gp130 and signal transduction. Other members of the IL-6 family also utilize gp130 for signal transduction, but they require cytokine-specific receptors in order to exert activity. Interactions with gp130 are thought to explain part of the functional redundancy exhibited by the IL-6 family.\(^8\)

IL-6R also exists in a soluble form, produced either by proteolytic cleavage or by translation from an alternatively spliced mRNA. This soluble form is the molecule that is involved in trans-signaling. IL-6 binds to the soluble IL-6R, and the IL-6/soluble IL-6R complex then associates with gp130 on the surface of cells, resulting in gp130 dimerization and signal transduction.\(^7\)

The expression of the transmembrane form of IL-6R is confined mainly to hepatocytes, monocytes, and macrophages, as well as some lymphocytes.\(^7\) Accordingly, conventional IL-6 signaling is mostly limited to these cell types. In contrast, gp130 is ubiquitously expressed, and soluble IL-6R is found in most body fluids, including serum and synovial fluids.\(^6,7,9\) The trans-signaling process thus allows IL-6 signaling in tissues that do not express the transmembrane form of IL-6R. In particular, trans-signaling probably mediates most of the effects of IL-6 in the joint, as many synovial cells do not express transmembrane IL-6R. Furthermore, IL-6 alone was not sufficient to trigger inflammation in a mouse model of RA, whereas a fusion protein of IL-6 and IL-6R resulted in arthritis.\(^4,10\)

For both conventional and trans-signaling, signal transduction occurs through the Janus-activated kinase (JAK)/signal transducers and activators of transcription (STAT) pathway and the mitogen-activated protein kinase (MAPK) cascade.\(^6\) The IL-6/IL-6R/gp130 complex associates with JAK, an intracytoplasmic tyrosine kinase, resulting in autophosphorylation and activation of JAK. The activated JAK molecule mediates tyrosine phosphorylation of gp130, leading to the recruitment and phosphorylation of STAT signaling proteins. Once activated by phosphorylation, the STAT molecules dimerize and enter the nucleus, where they influence gene expression, reviewed by Scheller and colleagues.\(^11\) Stimulated gp130 also recruits tyrosine phosphatases involved in the MAPK signaling pathway, leading to the activation of molecules involved in cell survival and stress responses.\(^6,11\)

In contrast to soluble IL-6R, soluble gp130 acts as a natural inhibitor of IL-6 signaling. The complex of soluble gp130/soluble IL-6R also inhibits IL-6 activity and may act to regulate systemic responses to IL-6.\(^6\)

### Biological Activities of IL-6

IL-6 is a pleiotropic cytokine with diverse activities.\(^8,12\) These activities contribute to both systemic and local symptoms associated with RA (Table 1). The role that IL-6 plays in the shift from acute to chronic inflammation may help explain its involvement in the development of RA.

#### Activities Related to Systemic Symptoms

One of the most important systemic actions of IL-6 is induction of the acute phase response. Acute phase proteins are produced primarily by the liver and include proteins that promote the immune response through activation of complement, induction of proinflammatory cytokines, and stimulation of neutrophil chemotaxis. Other acute-phase proteins, such as proteinase antagonists, opsonins, and procoagulants, help limit tissue destruction.\(^13\) In humans, two of the most prominent acute phase proteins are CRP and serum amyloid A.\(^5\) Serum amyloid A is the protein involved in secondary amyloidosis, which is frequently observed in patients with chronic diseases, particularly RA.\(^14\)

Other aspects of the acute phase response, regulated at least in part by IL-6, include the induction of fever and the secretion of hepcidin, an iron regulatory peptide synthesized in the liver. Overproduction of hepcidin interferes with iron absorption.

### Table 1  Biological Activities of IL-6 That May Be Related to RA Symptoms\(^1,2,4,5,12\)

<table>
<thead>
<tr>
<th>IL-6 Activity</th>
<th>Symptoms</th>
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<tbody>
<tr>
<td>• Activities Related to Systemic Symptoms</td>
<td>Elevated levels of C-reactive protein, serum amyloid, and other acute-phase proteins</td>
</tr>
<tr>
<td>Induction of acute-phase response</td>
<td>Fever</td>
</tr>
<tr>
<td>Induction of B-cell differentiation and antibody formation</td>
<td>Secondary amyloidosis</td>
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<tr>
<td>Stimulation of T cell activation and differentiation and macrophage differentiation</td>
<td>Anemia of chronic disease</td>
</tr>
<tr>
<td>• Activities Related to Local Symptoms</td>
<td>Rheumatoid factor production</td>
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<tr>
<td>Activation of endothelial cell production</td>
<td>Inflammatory immune response</td>
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<tr>
<td>Stimulation of synoviocyte proliferation and osteoclast activation</td>
<td>Recruitment of leukocytes to inflammatory sites, local inflammation</td>
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<tr>
<td>Induction of matrix metalloproteinases</td>
<td>Synovial pannus formation</td>
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<td>Joint and cartilage damage</td>
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release from macrophages and with iron absorption; this dysregulation is believed to cause the development of anemia in chronic disease.  

IL-6 mediates diverse effects on immune cells. With respect to RA, one of its most important activities is the induction of B-cell differentiation and antibody formation, which may stimulate the production of autoantibodies such as rheumatoid factor. Other effects on immune cells include induction of T-cell activation and differentiation and macrophage differentiation. These activities may contribute to the inflammatory immune response that occurs in patients with RA.

**Activities Related to Local Symptoms**

IL-6 activates endothelial cell production, leading to the release of IL-8 and monocyte chemotactant protein, expression of adhesion molecules, and recruitment of leukocytes to inflammatory sites. In addition, IL-6 can stimulate synovioocyte proliferation and osteoclast activation, leading to synovial pannus formation. IL-6 acts with IL-1 to increase production of matrix metalloproteinases, which may contribute to joint and cartilage destruction. However, IL-6 may also have protective effects in the joint, as suggested by the finding that this cytokine induces the expression of the tissue inhibitor of metalloproteinase and stimulates proteoglycan synthesis when injected into the joints of mice with antigen-induced arthritis.

**IL-6 and Chronic Inflammation**

IL-6 exerts a significant influence on the course of inflammation in humans. There is evidence that IL-6 is capable of mediating both proinflammatory effects, including the induction of intercellular adhesion molecules and the recruitment of leukocytes, and anti-inflammatory effects, such as suppression of the proinflammatory cytokines, tumor necrosis factor and IL-1. The balance between the proinflammatory and anti-inflammatory effects of IL-6 may influence the development of chronic inflammation and disease. In particular, trans-signaling mediated by the IL-6/soluble IL-6R complex is believed to promote the recruitment of monocytes, a key step in the shift from acute to chronic inflammation (Fig. 1).

**Evidence Supporting the Role of IL-6 in RA**

**IL-6 Levels in Humans**

High IL-6 levels are frequently observed in patients with chronic inflammatory diseases. In patients with RA, elevated levels of IL-6 and soluble IL-6R are found in serum and in the synovial fluid of affected joints. IL-6 levels in patients with RA are higher than those in patients with osteoarthritis. Other chronic inflammatory diseases, including juvenile RA, are also associated with elevated IL-6 levels.

The possibility that increased IL-6 levels have clinical importance in RA is supported by correlations between IL-6 concentrations and disease activity. In several studies, IL-6 levels have been found to correlate with surrogate markers of disease activity, including rheumatoid factor, erythrocyte sedimentation rate, and CRP. Correlations between IL-6 levels and clinical manifestations, including morning stiffness, number of inflamed joints, and Ritchie’s disease activity index, have also been reported. Treatment with disease-modifying antirheumatic drugs is associated with decreases in IL-6 serum levels, and these declines correlate

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**Figure 1** IL-6 mediated shift from acute to chronic inflammation. (1) Following acute inflammatory response, IL-6 is secreted by endothelium, monocytes, macrophages, and other cell types. It then binds to soluble IL-6R (sIL-6R). (2) Trans-signaling through gp130 leads to monocyte recruitment. (3) Prolonged expression of IL-6 leads to neutrophil apoptosis, phagocytosis, and mononuclear accumulation at the site of injury. Open arrows indicate processes related to polymorphonuclear leukocyte activation in acute inflammation. Solid arrows relate to mononuclear shift in chronic inflammation. IL-6 indicates interleukin-6; JAK, Janus-activated kinases; MCP, monocyte chemotactant protein; and PMN, polymorphonuclear leukocyte. (Adapted from Gabay, 2006.)
with improvements in the number of inflamed joints and morning stiffness.24

The mechanisms responsible for high IL-6 levels in patients with chronic inflammatory diseases remain unclear. It has been suggested that dysregulated expression may involve promoter polymorphisms in the IL-6 gene. Although different genotypes at two sites in the IL-6 promoter, -622 and -174, did not appear to increase susceptibility to RA, certain genotypes were associated with increased disease activity and lower age of onset,25,26 suggesting that promoter polymorphisms may influence disease severity.

**IL-6 in Animal Models of RA**

Some of the strongest evidence in support of the association between IL-6 dysregulation and RA has come from studies in animal models of RA. Mice bearing a gp130 mutation that results in excess IL-6 signaling spontaneously develop an RA-like joint disease; this process is dependent on lymphocytes and is accompanied by autoantibody formation.27 On the other hand, mice genetically engineered to be IL-6 deficient are resistant to the development of collagen-induced or chronic autoimmune arthritis.28-30 Intra-articular administration of IL-6 into the joints of IL-6-deficient mice did not result in arthritis, but a soluble IL-6/IL-6R fusion protein caused joint swelling and other symptoms of arthritis.10 Together, these findings suggest that reduced levels of IL-6 protect against development of inflammatory arthritis, while excess IL-6 signaling contributes to disease development. The IL-6/soluble IL-6R complex appears to be the primary mediator of IL-6 signaling in the joint.

**Testing the Hypothesis: IL-6 Blockade in Humans**

The strong association between IL-6 and RA symptoms and the role of IL-6 in the development of inflammatory arthritis in murine models of RA suggested that neutralization of IL-6 could be beneficial to patients with this disease. Initial attempts at IL-6 blockade utilized a monoclonal antibody to human IL-6, but this agent resulted in the formation of immune complexes that prolonged the half-life of IL-6; the monoclonal antibody was, therefore, unsuccessful in blocking IL-6 activity. Subsequent studies have suggested that polyclonal antibodies may be better suited to neutralization of IL-6, reviewed by Kishimoto.8 A humanized monoclonal antibody to IL-6R (tocilizumab, previously known as MRA) provided a more successful option for IL-6 blockade in patients with RA. The findings of trials with tocilizumab are detailed elsewhere in this supplement. Briefly, tocilizumab has been found to block both conventional IL-6 signaling and trans-signaling.8 In patients with RA, tocilizumab infusions reduced disease activity and normalized acute-phase reactants.31-33 These studies appear to confirm the important role of IL-6 in RA manifestations and suggest that IL-6 blockade may be a successful strategy for controlling RA.

**Conclusions**

IL-6 has multiple biologic activities that could contribute to the systemic and local symptoms observed in patients with RA. The association between IL-6 and RA disease processes that was suspected from initial observational studies has garnered strong support from correlative analyses and studies in genetically engineered mice. More recently, the blockade of IL-6 conventional and trans-signaling by tocilizumab, a monoclonal antibody to soluble IL-6R, has been shown to mediate improvements in RA symptoms and disease activity. Dysregulation of IL-6 thus appears to contribute to both systemic and local RA symptoms. In addition to providing a promising therapeutic strategy for patients with RA, tocilizumab and other IL-6 neutralizing agents may allow scientists to unravel the relative contributions of IL-6 conventional versus trans-signaling in RA, as well as provide insights into the mechanisms by which IL-6 promotes the pathogenesis of this disease.

**References**

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Disclosure Statement
Bruce N. Cronstein, M.D., holds patents on use of adenosine A2A receptor agonists to promote wound healing and use of A2A receptor antagonists to inhibit fibrosis as well as a patent on use of adenosine A1 receptor antagonists to treat osteoporosis and other diseases of bone. He has served as a consultant for King Pharmaceutical (licensee of the abovementioned patents); CanFite Biopharmaceuticals; Bristol-Myers Squibb; Cellzome; Tap Pharmaceuticals; Prometheus Laboratories; Regeneron (Westat, DSMB); Sepracor; Amgen; Endocyte; and Protalex. He has received honoraria from speakers bureaus of Tap Pharmaceuticals, Amgen, and Roche. He owns stock in CanFite Biopharmaceuticals which was received for membership on its Scientific Advisory Board. He has also received grants from King Pharmaceuticals and the National Institutes of Health.