The New and Evolving Science of IL-6 in Rheumatoid Arthritis

# A Review of the Dual Signaling Mechanism of IL-6


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Dear Colleagues,

This is a very exciting time in the field of rheumatoid arthritis (RA). The more we understand from basic and clinical research about the pathogenesis of RA, the more equipped we are to understand this disease. We now know that cytokines play many key roles in the inflammation that drives RA. One such example is interleukin-6 (IL-6), a multifunctional cytokine that contributes to chronic inflammation in patients with RA.

Regeneron Pharmaceuticals and Sanofi Genzyme are excited to bring you additional educational material describing some of the fundamental immunology as well as clinical pathology we see in RA patients through a series of scientific monographs entitled *The New and Evolving Science of IL-6 in Rheumatoid Arthritis*. In this first installment, we review the signaling mechanisms of IL-6 that allow it to have widespread effects in RA.

We hope you find this series informative and engaging.

Sincerely,

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Dr Calabrese and Dr Choy were provided honoraria by Sanofi Genzyme and Regeneron Pharmaceuticals.

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# Introduction

RA is a chronic, progressive autoimmune disease characterized by debilitating articular and systemic manifestations.[1] Articular manifestations include pain, tender and swollen joints, and morning stiffness. Although the disease course varies among patients, RA leads to progressive joint destruction and loss of function in most cases, and may lead to physical disability.[1,2] Systemic manifestations include anemia, fatigue, osteoporosis, cardiovascular disease (CVD), rheumatoid nodules, and vasculitis. These manifestations may negatively impact prognosis and survival of patients with RA.[1,3]

It has been established that rheumatoid arthritis (RA) and other inflammatory diseases are driven by a complex network of cytokines, including tumor necrosis factor-α (TNF-α), interleukins (IL)-1, 4, 6, 12, 13, and 17, and interferons.[1,4] IL-6 is a multifunctional cytokine, that performs many diverse functions, including vital pro-inflammatory roles, in response to infection or injury.[1,4] Persistently elevated IL-6 signaling can play a role in disrupting homeostasis in multiple physiologic processes, which can lead to pathologic consequences in conditions of autoimmunity and chronic inflammation such as RA.[5,6] Elevated IL-6 signaling plays an important role in RA, contributing to both articular and systemic manifestations of the disease.[1,7-10] IL-6 is one of the most abundant cytokines in the serum and synovial fluid of patients with RA and correlates with both disease activity and articular destruction.[1,11,12]

The signaling features of IL-6 allow it to interact with a broad range of cells and tissues, such as: immune cells, fibroblast-like synoviocytes (FLSs), hematopoietic stem cells, hepatocytes, adipocytes, endothelial cells, and pancreatic islets.[4,7,13-16] IL-6 can signal through both a membrane-bound receptor and soluble receptor.[1] The latter differentiates IL-6 signaling from other cytokines such as TNF-α and IL-1, which are also implicated in driving inflammation in RA.[17,18] This monograph will describe the two distinct signaling mechanisms of IL-6, explaining the differences between classical (*cis*) signaling and *trans*-signaling, and the ultimate consequences of these mechanisms in inflammatory conditions such as RA.

## Role of IL-6 in the Immune Response

IL-6 signaling helps promote and coordinate the pro-inflammatory activities of cells throughout the body, contributing to increased immune cell survival and proliferation, B-cell antibody production, and a shifting of metabolic function by altering lipid and glucose utilization.[1,15,19-21] Through these functions and others, IL-6 also helps drive chronic inflammation by stimulating and facilitating interactions between both the innate and adaptive arms of the immune system **(Table 1)**.[1,4,19] At sites of infection or injury, IL-6 is first released by neutrophils and other infiltrating cells of the innate immune system as well as by adjacent endothelial cells, stimulating these cell types to carry out their respective functions.[22-24] Endothelial cells also release chemokines in response to IL-6 stimulation, which leads to further recruitment of innate immune cells that secrete IL-6, such as macrophages.[25] IL-6 facilitates generation of

**Table 1. Roles of IL-6 in innate and adaptive immunity.**

| Physical Barriers | Innate Immune Response | | Adaptive Immune Response | |
|---|---|---|---|---|
| • 1st line of defense<br>• Nonspecific response | • 2nd line of defense<br>• Selective, moderately specific response<br>• No immunological memory<br>• Immediate response | | • 3rd line of defense<br>• Specific response (specific antigen)<br>• Lag time from exposure to response<br>• Immunological memory after exposure | |
| | **Humoral** | **Cellular** | **Humoral** | **Cellular** |
| | **Pattern Receptors**[26,27]<br>IL-6 increase in response to LPS stimulation is dependent on toll-like receptor 4<br>**Complement**[32,35,36]<br>Complement components increase IL-6 levels in human cells<br>Loss of IL-6 function impairs de novo synthesis of C3 complement component<br>**Enzymes**[41]<br>Elevated IL-6 increases cathepsin levels and enzymatic activity<br>**Cytokines**[43]<br>IL-6–induced elevation of acute-phase proteins in response to injury or infection | **Phagocytes**[23,28,29]<br>IL-6 and IFN-γ cooperative signaling governs neutrophil trafficking and apoptosis during acute inflammation<br>IL-6 promotes monocyte differentiation into macrophages rather than dendritic cells<br>IL-6 enhances mast cell differentiation in vitro<br>**Natural Killer Cells**[42]<br>IL-6 augments the cytotoxic activity of natural killer cells | **Antibodies**[30-32]<br>Loss of IL-6 function reduces IgG production in response to antigen challenge<br>IL-6 induces immunoglobulin production in B lymphoblastoid cell lines<br>**Cytokines**[37-39]<br>IL-6 increases IL-17 production via Th17 cells | **T Cells**[33,34]<br>IL-6 inhibits $T_{reg}$ suppression of adaptive immune response to microbial infection<br>IL-6 is required for the T-cell–dependent response to viral infection<br>**B Cells**[40]<br>Inhibition of IL-6 receptor impairs somatic hypermutation in preswitch memory B cells and modulation of mutational targeting in memory B cells |

IFN-γ, interferon-gamma; IgG, immunoglobulin G; LPS, lipopolysaccharide; $T_{reg}$, regulatory T cells.

the adaptive immune response by stimulating B cells and T cells and fostering interactions between the two cell types. IL-6 stimulates antibody production—and in the case of RA, autoantibody production—by causing B cells to mature into antibody-producing plasma cells. It also increases the production of IL-21, which allows $CD4^+$ T cells to promote B-cell maturation.[30,44] In addition, the combined presence of IL-6 and transforming growth factor beta (TGF-β) stimulates naïve T cells to differentiate into T helper (Th) 17 cells.[45] Th17 cells in turn cause FLSs to release IL-6, which further promotes Th17 differentiation.[46] Th17 cells also produce IL-17, which contributes to RA pathogenesis.[4,47] B and T cells, activated in large part by IL-6, in turn release cytokines that further stimulate cells of both the innate and adaptive arms.

IL-6 released at sites of inflammation diffuses into circulation, enabling it to exert widespread effects. It is a major inducer of the acute-phase response involved in the pro-inflammatory cascade, through direct stimulation of the liver via hepatocytes.[1,43] IL-6 can also mediate fever by crossing the blood-brain barrier and initiating synthesis of prostaglandin $E_2$ ($PGE_2$) in the hypothalamus, thereby regulating body temperature.[48-50]

Circulating levels of IL-6 drastically increase in response to physiologic cues but are otherwise maintained at low levels. Based on several reports, serum levels of circulating IL-6 in healthy subjects range from 1 pg/mL to 16 pg/mL.[51-56] In response to severe infections, serum IL-6 levels may reach 10,000 pg/mL, with significant, albeit less dramatic, increases reported in other inflammatory and infectious diseases.[54,57,58] In RA, reports of serum IL-6 levels have varied, ranging from 5 pg/mL to 200 pg/mL,[52,53,55,59,60] with 100-to-1000-fold higher concentrations found in synovial fluid.[12,52,53,55,60,61]

# The Molecular Mechanisms of IL-6 Signaling

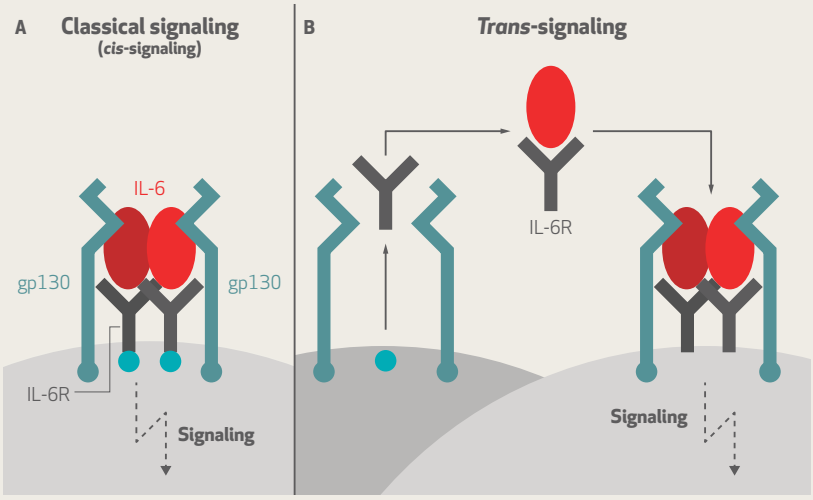


**Figure 1A and 1B. IL-6 molecular signaling.** IL-6 signals through two distinct mechanisms. **(A)** IL-6 binds to its membrane receptor. **(B)** IL-6 binds to its soluble receptor. In each case, the IL-6 receptor binds to ubiquitously expressed gp130, to activate signaling. Figure 2A and 2B adapted from Dayer JM and Choy E. *Rheumatology (Oxford)*. 2010;49:15-24.

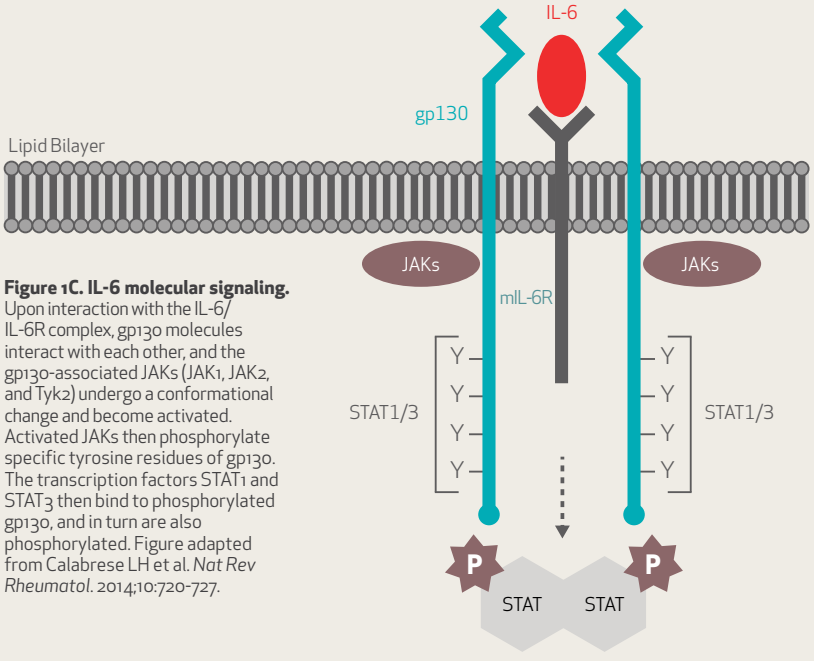


**Figure 1C. IL-6 molecular signaling.** Upon interaction with the IL-6/IL-6R complex, gp130 molecules interact with each other, and the gp130-associated JAKs (JAK1, JAK2, and Tyk2) undergo a conformational change and become activated. Activated JAKs then phosphorylate specific tyrosine residues of gp130. The transcription factors STAT1 and STAT3 then bind to phosphorylated gp130, and in turn are also phosphorylated. Figure adapted from Calabrese LH et al. *Nat Rev Rheumatol*. 2014;10:720-727.

## The membrane-bound IL-6 receptor mediates *cis*-signaling

In classical signaling, otherwise known as *cis*-signaling, IL-6 binds to its membrane-bound receptor (mIL-6R), which is mainly expressed in hepatocytes and hematopoietic cells—such as some types of T cells, monocytes/macrophages, activated B cells, and neutrophils.[25,62] Binding of IL-6 to mIL-6R is not sufficient to activate signaling, as the complex must first associate with the signal-transducing glycoprotein 130 (gp130)—a membrane protein expressed in all tissues—which is described further below **(Figure 1A and B).**[1,63]

The IL-6 activation signal is relayed from the cell membrane, through the cell's interior, and into the nucleus through a cascade of phosphorylation events.

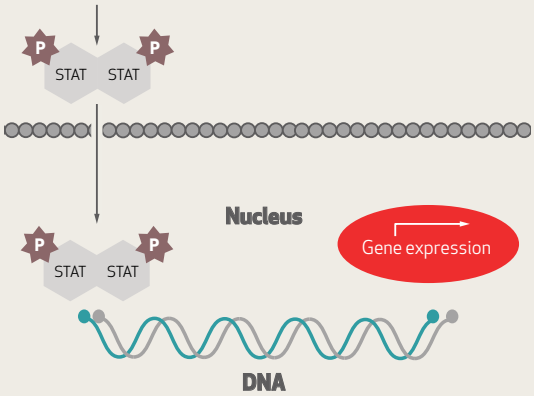


**Figure 1D. IL-6 molecular signaling** Phosphorylated STATs then bind to each other, allowing for their translocation into the nucleus, where they bind to DNA and directly activate gene expression. Figure adapted from MacFarlane LA and Todd DJ. *Int J Rheum Dis.* 2014;17:359-368.

Upon interaction with the IL-6/IL-6R complex, gp130 molecules interact with each other, and the gp130-associated Janus family tyrosine kinases (JAK1, JAK2, and Tyk2) undergo a conformational change and become activated **(Figure 1C)**.[64] Once activated, the JAK kinases can then phosphorylate specific tyrosine residues of gp130, which are required for activation of downstream signal transduction pathways.[63]

Activation of JAKs by IL-6 signaling ultimately affects gene expression through Signal Transducer and Activator of Transcription 3 (STAT3) and STAT1—transcription factors that are recruited to the phosphorylated residues of gp130, and then are also phosphorylated (pSTAT) by JAK kinases at critical tyrosine residues **(Figure 1C)**.[63] Complexes of pSTAT proteins enter the nucleus and bind to the specific DNA sequences in the regulatory regions of their target genes, where they can induce gene expression **(Figure 1D)**.[63,65] Elevated STAT3 activity has been detected in the synovial tissue from mice induced with arthritis, and a recent study demonstrated that circulating constitutive, pSTAT3 levels in $CD4^+$ T cells correlated with serum IL-6 levels in a cohort of patients with early RA.[66,67]
In addition to the activation of the canonical JAK/STAT pathway, IL-6 signaling activates the Ras/Raf/Mitogen-activated Map Kinases (MAPK) signaling pathway through effects on the phosphatase SHP-2.[64]

IL-6 activation of the JAK/STAT pathway leads to the expression of proliferative genes, anti-apoptotic genes, acute-phase protein genes, and regulators of inflammation.[25,63] The expression of the suppressor of cytokine signaling 3 (SOCS3) protein is upregulated upon activation of the JAK/STAT pathway and it subsequently acts as a negative regulator of signaling by inhibiting JAKs.[63] Activation of the JAK/STAT and MAPK pathways also induces the expression of genes such as matrix metalloproteinases (MMPs) and receptor activator of nuclear factor κB ligand (RANKL), which contribute to the cartilage degradation and bone resorption, respectively, that are characteristic of the structural damage associated with RA.[64]

TNF-α and IL-6 activate distinct but overlapping intracellular signaling pathways **(Figure 2)**.[3] When bound to its membrane-bound receptor (TNF-R1), TNF-α primarily activates the nuclear factor κB (NFκB) pathway, in contrast to IL-6, which primarily activates the JAK/STAT pathway through its receptor complex.[3] However, both TNF-α and IL-6 can activate the MAPK signaling pathways.[3] In addition, interactions between the NFκB and JAK/STAT pathways exist, as STAT3 can bind directly to NFκB and prevent it from activating its target genes.[68] These signaling features may help to explain both the overlapping and the distinct roles TNF-α and IL-6 play in RA pathogenesis.

## The soluble form of IL-6R allows for IL-6 *trans*-signaling in any cell that expresses gp130

IL-6R is also expressed as a soluble form (sIL-6R) in serum and synovial fluid, which serves to increase the variety of cells able to respond to IL-6 in a process referred to as *trans*-signaling **(Figure 1B)**.[62,69] sIL-6R lacks transmembrane and cytoplasmic components and can be generated by two different mechanisms: 1) limited proteolysis of the membrane-bound receptor by the metalloproteinase ADAM metallopeptidase domain 17 (ADAM17), and 2) translation of a differentially spliced mRNA.[64] sIL-6R binds

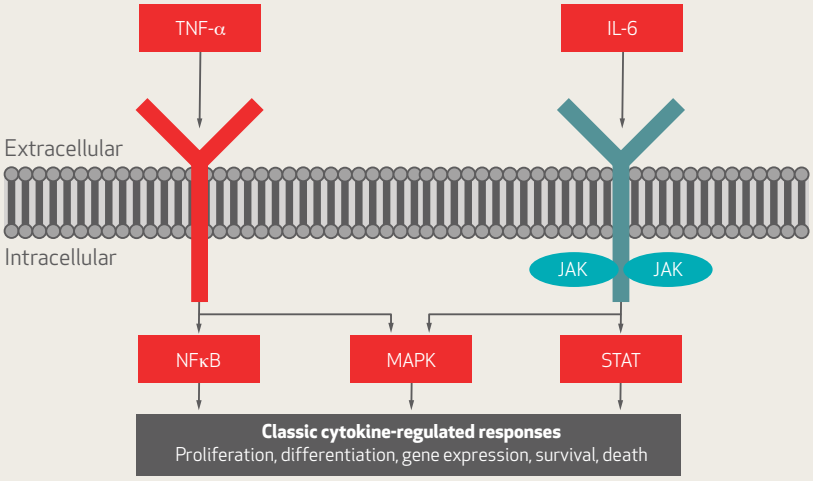


**Figure 2. IL-6 and TNF-α activate distinct but overlapping intracellular signaling pathways.** IL-6 and TNF-α bind to distinct cell surface receptors that activate distinct signaling pathways. TNF-α activates the NFκB pathway, whereas IL-6 activates the JAK/STAT pathway. Both TNF-α and IL-6 can also activate the MAPK pathway to a lesser extent. Each of these signaling pathways regulates the expression of genes involved in proliferation, differentiation, cell survival, and other functions. Figure adapted from Choy E. *Nat Rev Rheumatology*. 2013;9:154-163.

IL-6 with the same affinity as mIL-6R, and when bound to IL-6, it can interact with any cell that expresses gp130.[63] Once IL-6/sIL-6R associates with membrane-bound gp130, the complex initiates the same downstream pathways that occur in *cis*-signaling.[63]

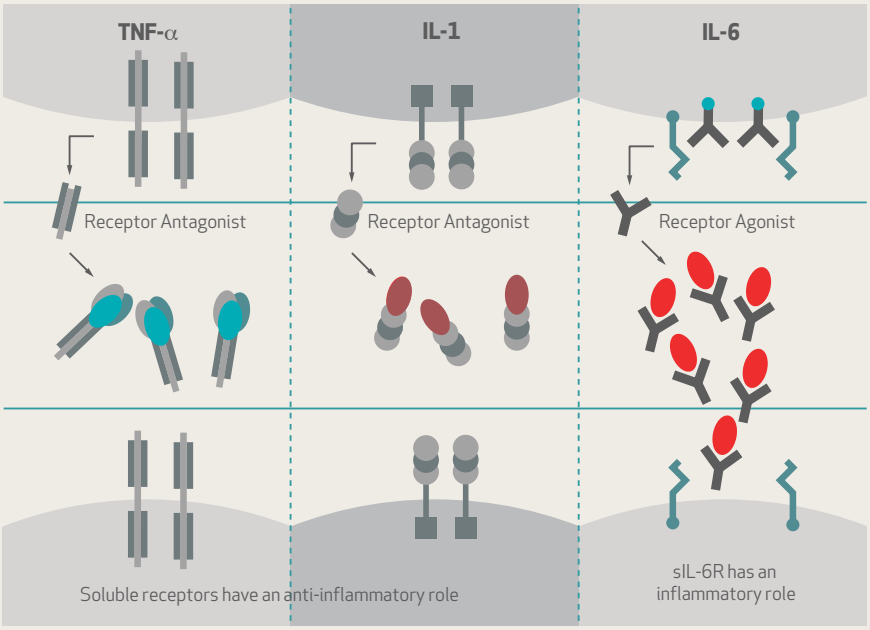


**Figure 3. Unique function of the IL-6 soluble receptor (sIL-6R).** When bound to IL-6, the sIL-6R/IL-6 complex can signal in virtually any cell type, through association with the universally expressed gp130 membrane protein. In contrast, soluble receptors for TNF and IL-1 sequester these cytokines and block signaling. Therefore, sIL-6R has an inflammatory role by expanding the range of IL-6 biologic activity, whereas soluble receptors for TNF-α and IL-1 have anti-inflammatory roles. Figure adapted from Colmegna I et al. *Clin Pharmacol*. 2012;91:607-620.

Receptors for TNF-α or IL-1 are also expressed as membrane-bound and soluble forms.[17,18] However, in contrast to IL-6, the soluble TNF-α and IL-1 receptors do not interact with gp130, or an analogous ubiquitously expressed membrane protein **(Figure 3)**.[17,18] As a result, these soluble receptors are nonfunctional and sequester TNF-α or IL-1, blocking their signaling pathways.[17,18]

IL-6 *trans*-signaling seems to play a key role in the transition from acute to chronic inflammation in RA, which is characterized by a shift from neutrophil to monocyte infiltration of the synovia **(Figure 4)**.[1,70] IL-6R is shed from neutrophils, which are the first cells to arrive at a site of infection. Shedding of sIL-6R allows for the stimulation of endothelial cells by IL-6 , which does not express mIL-6R, and is otherwise unresponsive to the cytokine.[1] Stimulation of endothelial cells by the IL-6/sIL-6R complex causes release of the mononuclear cell attracting cytokine monocyte chemoattractant protein-1 (MCP-1), leading to increased monocyte recruitment.[24,71,72]

## IL-6 *trans*-signaling contributes to RA joint damage through effects on fibroblast-like synoviocytes

FLSs—cells of the intimal, or inner, synovial lining—play a key role in chronic inflammation and joint destruction in RA.[14,73,74] These cells do not express mIL-6R but are highly responsive to IL-6 via *trans*-signaling.[1] IL-6 both activates and is produced by FLSs, establishing a positive feedback loop.[14,73,74] FLSs in the intimal lining are the primary source of IL-6 in joint synovia, as shown by in situ hybridization and immunohistochemistry studies.[75] The increase in IL-6 and sIL-6R in synovial fluid increases the risk of joint destruction in RA.[12]

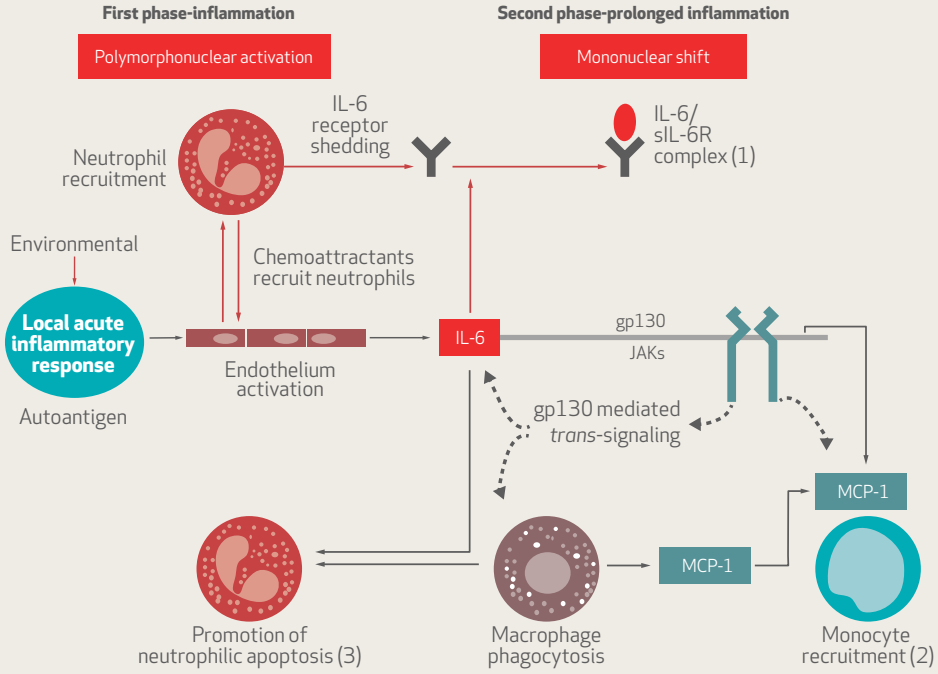


**Figure 4. IL-6 and the transition from acute to chronic inflammation.** IL-6 is thought to contribute to the transition from acute to chronic inflammation. In response to environmental stimuli or autoantigens, neutrophils are recruited to local sites of inflammation. Neutrophil shedding of mIL-6R results in increased IL-6 *trans*-signaling and production of the chemoattractant MCP-1. These local signaling changes are thought to lead to a shift from neutrophil recruitment to monocyte recruitment, which reflects a transition to chronic inflammation. Figure adapted from Gabay C. *Arthritis Res Ther*. 2006;8(suppl 2):S3.

## Relative concentrations of IL-6 signaling components regulate IL-6 signaling

Because all cells in the body express gp130, any cell can, theoretically, be activated by the IL-6/sIL-6R complex, and there needs to be a control mechanism to prevent IL-6 *trans*-signaling under steady-state conditions. This regulation is achieved in part by the relative concentrations of IL-6, sIL-6R, and a naturally occurring, nonfunctional, soluble form of gp130 (sgp130).[76] Under steady-state conditions, levels of sIL-6R and sgp130 are roughly 1000 times higher than IL-6 levels.[25] At these concentrations, IL-6, once secreted, will bind to sIL-6R in the plasma, and this complex will be neutralized by associating with sgp130, which acts as a buffer.[25] When IL-6 levels are elevated, the buffering capacity of the system can be overcome, allowing for IL-6 signaling.[25]

In humans, genetic association studies have identified an interesting link between levels of IL-6R/sIL-6R and disease.[77,78] Researchers have identified a single nucleotide polymorphism (SNP) in the human IL6R gene that causes a substitution of asparagine to alanine at amino acid 358 (Asp358Ala) in the IL 6R protein.[79] Interestingly, this amino acid is located at the ADAM17 cleavage site of mIL-6R, and carriers with the 358 sequence have markedly elevated levels of sIL-6R in the blood, suggesting increased shedding of mIL-6R.[77,79] These carriers have reduced levels of the acute-phase reactants C-reactive protein (CRP) and fibrinogen.[77,78] It has been proposed that this IL-6R SNP causes a loss of mIL-6R and therefore decreases *cis*-signaling.[80] Another hypothesis, which is not mutually exclusive with the prior hypothesis, is that the increase in sIL-6R levels raises the buffering capacity of sIL-6R/sgp130 in the blood, leading to an overall reduction of IL-6 activity.[25]

# How IL-6 Signaling May Impact Disease Manifestations in RA Patients

Through its dual signaling mechanism, IL-6 impacts many of the manifestations displayed by RA patients in clinical practice. For example, structural damage contributes to pain and impaired function in patients with RA. Cartilage degradation is a key component of structural damage and is mediated largely by FLSs, which, as mentioned earlier, respond to and produce IL-6.[75,81] Bone erosion and systemic decreases in bone density are also impacted by IL-6 signaling.[1] IL-6 can increase expression of RANKL on both osteoblasts and FLSs, leading to osteoclast activation and bone resorption by these cells.[82-84] IL-6 is also the major inducer of CRP—an acute phase reactant whose measurement is used in part to assess RA disease activity.[8,85] CRP is produced in the liver via hepatocytes, which express mIL-6R and thus may respond to IL-6 via either *cis*- or *trans*-signaling.[1] IL-6, originally discovered as a B-cell differentiation factor, also activates B cells, which are responsible for producing rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA)—autoantibodies found in the circulation of most RA patients.[30,86] In fact, many patients are ACPA-positive before the appearance of RA signs and symptoms.[87]This evidence suggests that cytokine-driven activation of the innate and adaptive immune systems establishes chronic inflammation early in the course of the disease.

These select examples represent some, but not all, of the ways in which IL-6 signaling, either by *cis*- or *trans*-signaling mechanisms, contributes to manifestations of RA relevant to clinical practice.

## Conclusions

Persistently elevated IL-6 levels can contribute to the disruption of homeostasis in many cell types and physiologic processes throughout the body. The widespread effects of IL-6 stem from its versatile signaling, which allows it to interact with a broad range of cells and tissues.[1] IL-6 can signal through both membrane and soluble forms of its receptors and when its levels are persistently elevated, the dual signaling mechanism allows IL-6 to make important contributions to both articular and systemic manifestations of RA.[1,7-10,88-90] Continued research on the many functions of IL-6 may further delineate the pathological origins and underpinnings of RA.

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