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IL-6 Revisited: From Rheumatoid Arthritis to CAR T Cell Therapy and COVID-19

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Abstract

The diverse biological activity of interleukin-6 (IL-6) contributes to the maintenance of homeostasis. Emergent infection or tissue injury induces rapid production of IL-6 and activates host defense through augmentation of acute-phase proteins and immune responses. However, excessive IL-6 production and uncontrolled IL-6 receptor signaling are critical to pathogenesis. Over the years, therapeutic agents targeting IL-6 signaling, such as tocilizumab, a humanized anti-IL-6 receptor antibody, have shown remarkable efficacy for rheumatoid arthritis, Castleman disease, and juvenile idiopathic arthritis, and their efficacy in other diseases is continually being reported. Emerging evidence has demonstrated the benefit of tocilizumab for several types of acute inflammatory diseases, including cytokine storms induced by chimeric antigen receptor T cell therapy and coronavirus disease 2019 (COVID-19). Here, we refocus attention on the biology of IL-6 and summarize the distinct pathological roles of IL-6 signaling in several acute and chronic inflammatory diseases.

INTRODUCTION

Cytokines are small proteins (15–20 kDa) that are key modulators in homeostasis and inflammation. Great progress has been made in identifying individual cytokines and their signaling cascades, which have subsequently been targeted by clinical therapies for immune disorders. A prominent example of a cytokine relevant to inflammation is interleukin-6 (IL-6).

In 1973, IL-6 was discovered as a factor that regulated antibody responses (1–3). Further extensive, fundamental research has revealed the biology of IL-6 and other IL-6 family members (4). Since its discovery almost 50 years ago, more than 90,000 reports relevant to IL-6 have been published in several biological fields, including immunology, cancer, metabolism, and neurology.

IL-6 has pleiotropic activities in diverse biological processes. IL-6 regulation and functions are a central theme of immune regulation but also have important implications in the pathogenesis of various diseases. Excessive production of IL-6 and overactivation of IL-6 receptor (IL-6R) signaling contribute to the development of several acute and chronic inflammatory disorders, including cytokine storms, rheumatoid arthritis (RA), Castleman diseases, giant cell arteritis, and several other autoimmune diseases.

Therapies that target IL-6 and its receptors have been developed. The first therapeutic antibody developed, tocilizumab, targets IL-6R. Others include sarilumab, satralizumab, and the IL-6-neutralizing antibody siltuximab (5). On the basis of its remarkable efficacy, tocilizumab has been approved for acute and chronic inflammatory diseases, and it might be applicable to other diseases. Over the last five decades, basic and translational research of IL-6 and its signaling in several cell types has driven the development of clinical reagents that are beneficial for various diseases. Moreover, its efficacy has been highlighted during the current coronavirus disease 2019 (COVID-19) pandemic. In this review, we revisit the discovery of IL-6 and its receptor signaling pathway and draw attention to the clinical implications of IL-6-targeted therapies of inflammatory diseases from RA to several types of cytokine storms.

DISCOVERY OF IL-6 AND ITS RECEPTORS

The history of IL-6 research originates with the study of antibody production by B lymphocytes. In the early 1970s, Kishimoto and Ishizaka were looking for soluble factors produced by T lymphocytes that would augment B lymphocyte production of IgG and IgE in antigen-specific responses. Although they could not provide a plausible explanation at that time, they suggested the presence of isotype-specific regulators acting on B lymphocytes (1–3). Then Kishimoto concentrated on identifying a factor that could stimulate human B lymphocytes and demonstrated that a soluble factor(s) from T cells after antigen-specific activation induced immunoglobulin production in activated B cells (6). From the 1970s to the early 1980s, many reports described a factor that was active in B lymphocyte responses. In 1983, the International Congress of Immunology named it B cell stimulatory factor (BSF). On the basis of the available data, the 20-kDa mouse B cell–specific growth factor (BCGF) was designated BSF-1 (7), and human B cell differentiation factor (BCDF) was designated BSF-2. A few years later, BSF-2 complementary DNA (cDNA) was cloned (8) and BSF-2 was produced by cardiac myxoma cells of a patient who exhibited several inflammatory symptoms, including fever, anemia, joint swelling, and autoantibodies. Consequently, BSF-2 was determined to be the same protein as plasmacytoma growth factor, IFN-β2, and hepatocyte-stimulating factor (HSF), suggesting diverse biological activities. BSF-2 was designated IL-6 at the 1988 conference Regulation of the Acute Phase and Immune Responses: a New Cytokine (9). These developments led to the expansion of IL-6 research, including identification of its receptors and intracellular signaling molecules as well as clarification of its role in the pathogenesis of various diseases.

STRUCTURAL FEATURES OF IL-6 AND ITS RECEPTOR MOLECULES

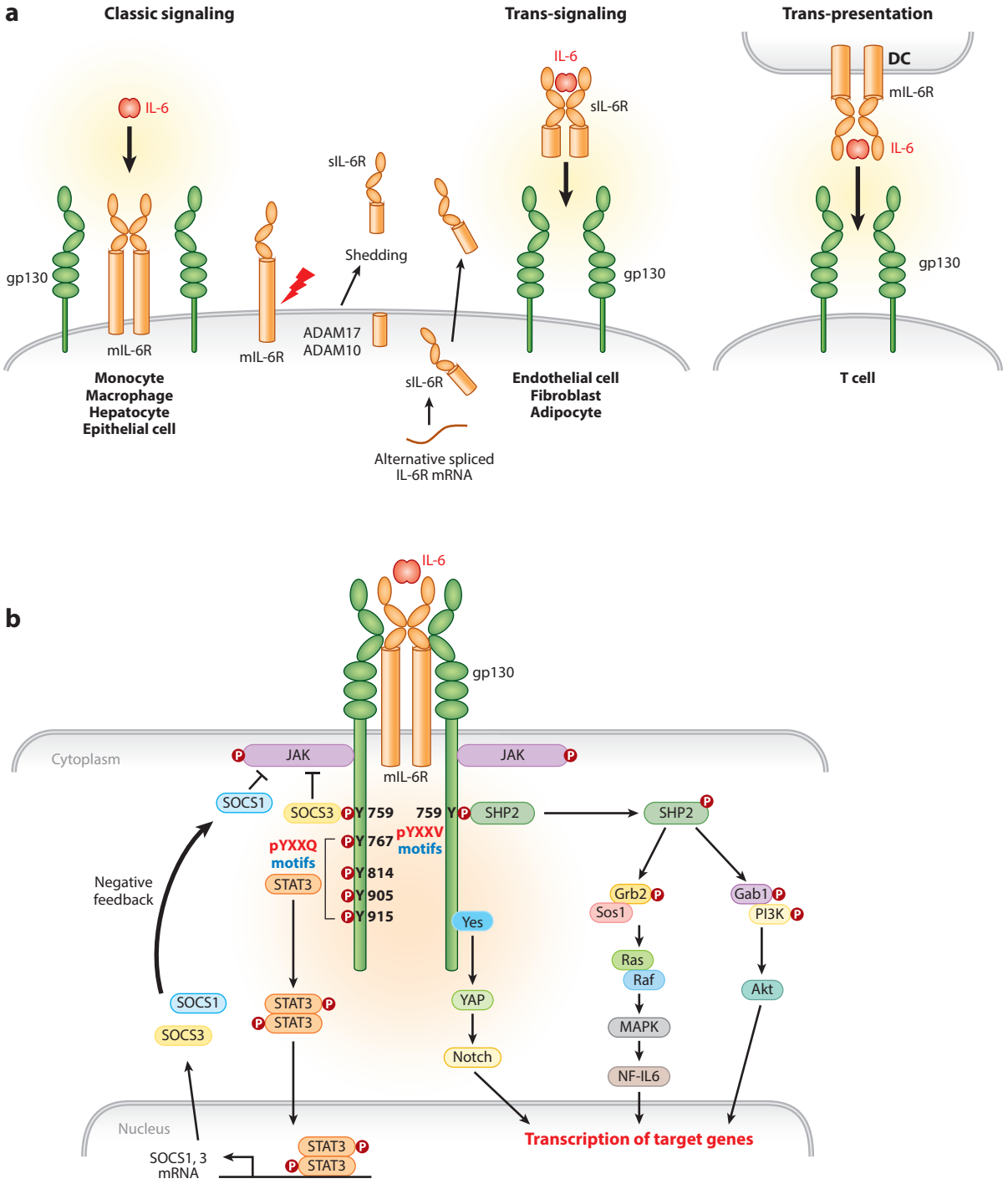
Mature human IL-6 is approximately 21 kDa and consists of 212 amino acids containing signal peptides with N- and O-glycosylation sites and four cysteine residues (8). Homology between human and murine IL-6 is 65% at the DNA sequence level and 42% at the amino acid sequence level, with a conserved cysteine-rich middle region (with four cysteine residues), suggesting a pivotal role in mediating the activity of IL-6 (10). IL-6 contains four α-helix bundles with ribbon forms that are similar in structure to granulocyte-macrophage colony-stimulating factor (GM-CSF) and growth hormone (11). IL-6 binds to IL-6R and gp130 and transmits the signals via hexamer formation. IL-6R is an 80-kDa glycoprotein that is also called CD126. It consists of 468 amino acids with a single transmembrane segment. IL-6R contains four cysteine residues at the N terminus and a Trp-Ser-Xaa-Trp-Ser (WSXWS) motif in the extracellular domain. This conserved region is required for mature IL-6 activity (12), whereas the intracytoplasmic region of IL-6R contains 82 amino acids but no tyrosine kinase domain. The binding of IL-6 to IL-6R initiates an association with gp130 to form a hexameric complex. gp130 is a 130-kDa transmembrane protein that is also called CD130. It forms immunoglobulin-like domains and contains four cysteine conserved residues or a WSXWS motif in the extracellular region, as well as several unique binding sites in the cytoplasmic region for other signaling pathways (**Figure 1**). gp130 cDNA was cloned in 1990 (13–15), and after two years it was identified as a common receptor of the IL-6 cytokine family, which includes leukemia inhibitory factor, cardiotrophin 1, ciliary neurotropic factor, IL-11, and oncostatin M (4).

THREE MODES OF IL-6R SIGNALING

Four decades after IL-6 was cloned in the 1980s, we now understand the principles of the three binding modes of IL-6 and its receptor molecules, which promote diverse biological activities. As described above, IL-6 binds to IL-6R, and then this complex binds to gp130. IL-6 binds to IL-6R but not gp130 (14) and therefore requires the formation of a complex with IL-6R. gp130 is expressed in most cell types, whereas the membrane form of IL-6R (mIL-6R) is expressed in few cell types, including macrophages, T cells, and hepatocytes (13), which indicates the diverse effects mediated by IL-6. The binding of IL-6 to the membrane form of IL-6R together with formation of a complex with gp130 is referred to as classic signaling (16) (**Figure 1*a***). In inflammatory states such as that caused by infection, IL-6R is cleaved from the cell surface by the metalloprotease ADAM10/ADAM17, and a soluble form of IL-6R (sIL-6R) can be generated. To a minor extent, an alternative spliced mRNA generates sIL-6R in humans only (17). sIL-6R can bind to IL-6, and the IL-6–sIL-6R complex binds to gp130, which is expressed ubiquitously in cells to induce signaling. This process is called trans-signaling and induces rapid responses in endothelial cells, epithelial cells, and smooth muscle cells during diseases. The third mode of signaling, termed trans-presentation, was recently reported in antigen-specific interactions between dendritic cells and T cells. The IL-6–mIL-6R complex on dendritic cells signals through gp130 on T cells to initiate pathogenic Th17 responses (18). Generally, classic signaling is involved in the antibacterial and resolution activities of IL-6, whereas trans-signaling and trans-presentation mediate proinflammatory responses (19, 20). These three signaling modes mediate the diverse biological activities of IL-6.

BUFFERING SYSTEM OF IL-6 ACTIVITY

A new concept of IL-6 activity is its buffering function in vivo. IL-6 binds to the membrane or soluble form of IL-6R at 1 nM, whereas the IL-6–sIL-6R complex has a higher affinity for gp130



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Three modes of IL-6 interaction with its receptor and the downstream intracellular signaling pathways. (*a*) Classic signaling is mediated by the binding of IL-6 to the mIL-6R, which is subsequently associated with gp130 on the surface of cells. This signaling is predominant in monocytes, macrophages, hepatocytes, and epithelial cells. The trans-signaling mode uses sIL-6R, which forms a complex with IL-6 before converging on gp130, and then this complex acts only on cells expressing gp130. Trans-signaling activates endothelial cells, fibroblasts, and adipocytes. In the third mode, trans-presentation, which is specialized for antigen presentation, IL-6–mIL-6R is presented on the surface of a cell and induces intracellular signaling via gp130 expressed on another cell. (*b*) Signal transduction pathway of IL-6R. Binding of IL-6 to its receptors induces the activation and phosphorylation of JAK family proteins. JAK, in turn, phosphorylates the cytoplasmic tail of gp130, which contains YXXV (Y759) and YXXQ (Y767, Y814, Y905, Y915) motifs and can therefore bind to STAT1/3 and SHP2, activating the MAPK pathway. Activation of the PI3K-Akt pathway triggers the binding of Gab to SHP2. YAP-Notch signaling is activated by Yes functions in the regeneration of intestinal epithelial cells by a STAT3-independent pathway. As negative feedback for gp130 signaling, SOCS1 and SOCS3 terminate this signaling pathway by inhibiting JAK activity and competing with SHP2 for binding to the phosphorylated site, respectively. Abbreviations: DC, dendritic cell; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; mIL-6R, membrane form of IL-6R; NF-IL6, nuclear factor IL-6; PI3K, phosphatidylinositol 3-kinase; sIL-6R, soluble form of IL-6R; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transduction.

at 10 pM. Therefore, IL-6 preferentially forms a complex with sIL-6R, which then binds to gp130. In some cases, soluble gp130 (sgp130) is generated by alternative splicing and forms the IL-6–sIL-6R–sgp130 complex (19). In the healthy state, serum concentrations of IL-6 are undetectable, whereas the concentration of sIL-6R or sgp130 is in molar excess. The affinity of IL-6 for sIL-6R is in the nanomolar range, and the affinity of the IL-6–sIL-6R complex for sgp130 is much higher, in the picomolar range. Interactions of sgp130 with the IL-6–sIL-6R complex have inhibitory effects on IL-6 trans-signaling but not classic signaling or trans-presentation, suggesting that the concentration of IL-6 is regulated (21). In the inflammatory state, the sIL-6R level is elevated, but it has been shown that the serum level of sgp130 is comparable, indicating the activation of IL-6 trans-signaling. Thus, buffering activity of IL-6 through sIL-6R and sgp130 may be more effective for IL-6R trans-signaling.

THREE MAIN INTRACELLULAR PATHWAYS OF gp130 SIGNALING AND THEIR REGULATION

The binding of IL-6–sIL-6R or mIL-6R initiates gp130 homodimerization, which transduces the intracellular signals of gp130 by the phosphorylation of the intracellular region and proteins. The downstream signaling of gp130 is mainly mediated by three well-known proteins, nuclear factor IL-6 (NF-IL6), signal transducer and activator of transduction 3 (STAT3), and suppressor of cytokine signaling (SOCS) (22–24) (**Figure 1*b***). The cytoplasmic region of gp130 is activated via five tyrosine phosphorylation sites. The binding of IL-6–IL-6R to gp130 activates the Janus kinase (JAK), which in turn, augments the three signaling pathways. The first signaling pathway involves the JAK and STAT3 cascades. Although gp130 constitutively binds to JAK1, JAK2, and Tyk2, the primary target of gp130 is JAK1. After gp130 binds to IL-6–IL-6R, the intercellular region of gp130 contains two consensus tyrosine motifs, YXXQ and YXXV, which function as docking sites for STAT1/3 and SHP2, respectively. Subsequently, phosphorylated STAT3 forms a homodimer and is translocated into the nucleus, where it functions as a transcriptional factor. Depending on the types of ligand, STAT1 is transiently phosphorylated, resulting in cytokine specificity (25). STAT1 and STAT3 form a heterodimer that binds to the cognate tyrosine residue and subsequently mediates the transcription of genes. The second signaling pathway involves the JAK and SHP-MAPK (mitogen-activated protein kinase) cascade. Activated JAK initiates the phosphorylation of tyrosine 759, which induces the phosphorylation of SHP2, which induces hyperphosphorylation of the MAPK pathway via the adaptor proteins Grb2 and Gab (26, 27). JAK-SHP2

augments the PI3K (phosphatidylinositol 3-kinase)-Akt cascade. Activated JAK-SHP2 signaling mediates PI3K phosphorylation, which activates Akt, which contributes to NF-κB activation (28). The third signaling pathway involves gp130, which augments YAP-NOTCH signaling by the Src kinase family member Yes, which directly binds to the gp130 cytoplasmic domain in carcinogenesis (29). These signaling cascades lead to varied pathological conditions in several diseases.

IL-6R signaling is regulated by negative-feedback signaling through the STAT3 and SOCS proteins. Augmented gp130 activates the transcriptional activities of STAT3, which targets the SOCS1 or SOCS3 gene. SOCS1 directly binds to JAK protein and inhibits its catalytic activity, whereas SOCS3 interacts with the phosphorylation site at tyrosine 759 of gp130, which is essential for termination of its activity. Collectively, the SOCS protein family attenuates IL-6R signaling cascades as a negative-feedback loop.

IL-6 ACTIVITIES

Acute-Phase Response and Regeneration

IL-6 was originally found as a hepatocyte-stimulating factor (30). It was demonstrated to be a prototypical cytokine with pleiotropic activity related to inflammation, immune homeostasis, and hematopoiesis (**Figure 2**). In the liver, hepatocytes express mIL-6R and gp130, and the binding of IL-6 to these receptors activates the classic signaling pathway in hepatocytes to mediate the production of a broad range of acute-phase proteins including C-reactive protein (CRP), serum amyloid protein A (SAA), complement C3, fibrinogen, hepcidin, haptoglobin, thrombopoietin, and α_1-antichymotrypsin, as well as to reduce the levels of albumin and cytochrome P450 (30, 31). These proteins mediate the early phase of acute-phase responses as a host defense mechanism against infection or tissue injury. Elevated levels of these proteins are often measured in the clinic because they reflect the inflammatory states. Indeed, increased levels of hepcidin were related to the pathogenesis of hypoferremia and anemia by inhibiting the iron transporter ferroportin 1 in hepatocytes, macrophages, and intestinal epithelial cells (32). Sustained production of SAA causes amyloid A amyloidosis, which is a frequent complication of RA or bronchiectasis (33).

Additionally, IL-6 has effects on regeneration after liver injury. The IL-6 classic and trans-signaling pathways in hepatocytes promote liver regeneration via STAT3 proteins (34). IL-6-deficient mice exhibited necroptosis in the liver resulting in impaired tissue regeneration. However, administration of IL-6 promoted hepatocyte proliferation by inducing Mcl-1, an antiapoptotic member of the Bcl-2 family, which prevented liver damage (35). The alternative gp130 signaling pathway, which involves the YAP-NOTCH axis, contributed to tumor growth in human hepatocellular carcinoma (HCC) after liver cirrhosis in murine hepatocytes (36), although the precise mechanisms underlying this signaling in liver regeneration are not clear. In the clinic, elevation of serum IL-6 levels correlate with the progression of HCC in patients with chronic hepatitis B infection (37).

Immune Responses

Originally, IL-6 was shown to have B cell stimulatory activity related to the differentiation of B cells into antibody-producing plasma cells (38). Regarding the role of IL-6 in pathogenesis, the overexpression of IL-6 induces autoantibodies such as anti–aquaporin 4 (AQP4) and hypergammaglobulinemia (39). High levels of gamma globulin are decreased by the blockade of IL-6R signaling. In T cells, IL-6 is involved in the differentiation of several Th cell subsets from naive $CD4^+$ T cells. The combination of TGF-β and IL-6 promotes IL-17-producing Th17 cells, which contributes to the pathogenesis of several types of autoimmune diseases (40). By contrast,

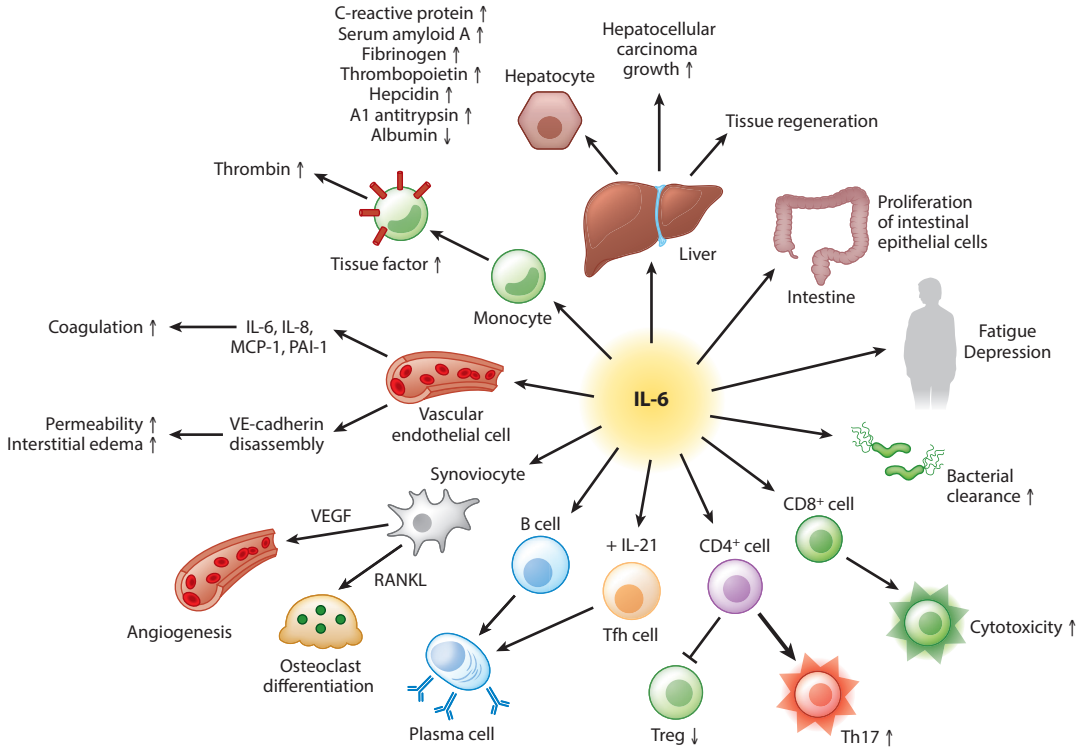


Figure 2

Pleiotropic activities of IL-6. IL-6 acts on various cells and exerts diverse biological activities. Appropriate expression of IL-6 contributes to host defense against infections or tissue injury; however, dysregulated (excessive or persistent) IL-6 production causes various diseases, including acute and chronic immune disorders. IL-6 activates macrophages to promote bactericidal activity. In addition to regulating the immune system, IL-6 regulates mood, bone turnover, and tissue regeneration. In the liver, IL-6 induces production of several acute-phase response proteins. IL-6 also activates vascular endothelial cells to produce IL-6, IL-8, MCP-1, PAI-1, and VE-cadherin disassembly. IL-6 also induces tissue factor expression in monocytes, which contributes to thrombin formation. In the intestine, IL-6 is required for proliferation of intestinal epithelial cells. Additionally, IL-6 affects mental disorders such as depression; the serum level of IL-6 is higher in depressed patients. Abbreviations: PAI-1, plasminogen activator inhibitor 1; RANKL, receptor activator of NF-κB ligand; Tfh, follicular helper T; Th17, helper T 17; Treg, regulatory T cell; VE-cadherin, vascular endothelial cadherin; VEGF; vascular endothelial growth factor.

IL-6 inhibited the differentiation of regulatory T cells (Tregs) by suppressing the inhibitory activity of Foxp3 (40), suggesting that abundant IL-6 production leads to an aberrant ratio of Th17 to Tregs and the development of autoimmune diseases (41). In addition, IL-2 treatment partially abrogated IL-6R and gp130 expression under the condition of Th17 differentiation (42). IL-6 and IL-21 are coordinately required for the differentiation of follicular helper T (Tfh) cells, which contribute to immunoglobulin production (43). Following infection by lymphocytic choriomeningitis virus (LCMV), IL-6 produced by follicular helper dendritic cells induced the development of Tfh cells to generate anti-LCMV antibodies that eliminated the pathogenic virus (44). In addition, IL-6 promotes the cytotoxic effects of $CD8^+$ T cells (45). Furthermore, IL-6 has critical roles in innate immune responses, although excessive IL-6 production causes severe acute inflammatory diseases. Regarding this point, we focus on the pathogenic activities of IL-6 in relation to cytokine storm, vascular injury, and coagulation disorders in the section titled Inhibition of IL-6 Signaling in Cytokine Storm.

Bone Homeostasis

IL-6 has an important role in bone homeostasis. It is produced by bone marrow stromal cells, where it stimulates fibroblast-like synoviocytes to increase expression of receptor activator of the NF-κB ligand (RANKL), an inducer of osteoclastogenesis that contributes to bone homeostasis (46). Of note, IL-6-overproducing mice exhibited osteoporosis related to an increase in osteoclasts and a decrease in osteoblasts (47). Moreover, IL-6 produced by synovial fibroblasts promoted abundant production of vascular endothelial growth factor (VEGF). This axis of enhanced angiogenesis and increased vascular permeability in synovial tissues is frequently observed in RA patients (48).

REGULATION OF IL-6 SYNTHESIS

Supplemental Material >

Overproduction of IL-6 causes various inflammatory diseases. Thus, IL-6 expression should be tightly regulated by transcriptional or posttranscriptional mechanisms (**Supplemental Figure 1**). In addition, changes in the chromatin structure of the IL-6 promoter initiated the transcription of factors related to monocyte differentiation (49). Polymorphisms in the human IL-6-flanking region also affected IL-6 transcriptional activity, which was associated with the pathogenesis of several inflammatory diseases (50, 51).

Transcription Factors Related to IL-6 Expression

The human IL-6 gene contains functional *cis*-regulatory elements in its promoter and enhancer regions. Several transcription factors, including the NF-κB binding region (–74/–63 from the transcription start site), NF-IL6 or CCAAT/enhancer-binding protein β (C/EBPβ) (–87/–76 or –159/–145), specificity protein 1 (SP-1) (–109/–104 and –123/–119), cyclic AMP response element–binding protein (CREB) (–165/–158), interferon regulatory factor 1 (IRF-1) (–267/–254), and activator protein 1 (AP-1) (–284/–277), regulate transcription of the IL-6 gene (52). Stimulation by cytokines such as IL-1 and IL-6 primarily induces transcription of IL-6 through NF-IL6 (53). During viral infection, human T-lymphotropic virus 1–derived transactivator protein (TAX) interacts with NF-κB to activate IL-6 transcription (54). In addition, HIV-1-derived transactivator of the transcription protein (TAT) enhanced DNA-binding activity of both NF-κB and NF-IL6 (55). However, suppressive factors such as peroxisome proliferator–activated receptor α, aryl hydrocarbon receptor (Ahr), estrogen receptor, and glucocorticoid receptor are also involved in regulation of IL-6 transcription (56). In lipopolysaccharide (LPS)-stimulated macrophages, the Ahr/STAT1 heterodimer suppresses the transcriptional activity of NF-κB to regulate IL-6 expression. In line with this, Ahr-deficient mice showed increased IL-6 expression and decreased Th17 cell differentiation (57, 58).

Posttranscriptional Regulation of IL-6 Expression

After transcription, the initiation of *Il6* mRNA translation is regulated by the 5′ untranslated region (UTR), and its stability is controlled by the 3′ UTR by the modulation of AU-rich elements. This regulation is mediated by microRNA (miRNA) and RNA-binding protein (RBP), which target the 3′ UTR of the mRNA. Several miRNAs target upstream activators of IL-6, and these are indirectly involved in the suppression of IL-6 transcription: miRNA-26 for IL-6 and NF-κB 3′ UTRs, miRNA-155 for the NF-IL6 3′ UTR, and miRNA-365 for the IL-6 3′ UTR (59–61).

RBPs bind to *cis*-elements of the 3′ UTR region of *Il6* mRNA, including AU-rich elements and stem-loop structures, which regulate the stability of *Il6* mRNA. A nuclease known as regulatory RNase 1 (Regnase-1) (also known as ZC3H12A or MCPIP-1) degrades *Il6* mRNA by binding to

the IL-6 3′ UTR region via a stem-loop structure in the cytoplasm, endoplasmic reticulum, and ribosomes (62). Regnase-1-deficient mice exhibited excessive IL-6 production in an endotoxin shock model and spontaneously developed autoimmune-like diseases including splenomegaly and lymphadenopathy (63). Moreover, Regnase-1 also promotes the decay of *Ox40* and *Icos* mRNAs, which contributes to differentiation of Th1, Th2, and Th17 subsets (64). Roquin, another RBP, recognizes the stem-loop structure of target mRNAs and has similar activity to Regnase-1 (65). Roquin regulates the decay of *Il6* mRNA with a distinct spatiotemporal state via its location in stress granules and processing bodies. By contrast, our group identified AT-rich interactive domain–containing protein 5a (Arid5a), which is an RBP that contributes to *Il6* mRNA stability. Arid5a recognizes the stem-loop structure of the IL-6 mRNA 3′ UTR and contributes to its stabilization (66). The engagement of IL-1R, IL-6R, and Toll-like receptor 4 (TLR4) induces Arid5a expression in macrophages. Arid5a then induces robust IL-6 production, but not transcription of *Tnf* or *Il12* mRNA, indicating its role in the positive-feedback loop of IL-6 production. *Arid5a*-deficient mice were resistant to endotoxin shock because of inhibition of IL-6 production. Of note, Arid5a counteracts the endonuclease activity of Reganse-1 in the stem-loop structure of *Il6* mRNA. Moreover, LPS stimulation induces translocation of Arid5a from the nucleus to the cytoplasm via chromosomal region maintenance 1 (CRM1), and it is also imported into the nucleus via an importin-α/β-dependent mechanism to terminate IL-6 production. Thus, these data indicate that the dynamic translocation of Arid5a is important for controlling the quantity of *Il6* mRNA. In addition, Arid5a stabilizes *Tbet* and *Stat3* mRNA under conditions of Th1 and Th17 differentiation (67). Furthermore, *Arid5a*-deficient mice exhibited impaired IL-6 production and reduced Th17 populations, which promoted their resistance to development of experimental autoimmune encephalomyelitis, a model of multiple sclerosis. Regarding pathogenesis, the inhibition of IL-6R signaling by tocilizumab treatment decreased Arid5a expression in peripheral $CD4^+$ cells derived from RA patients (68). Collectively, these findings indicate that a balance between Arid5a and Regnase-1 is required for immune homeostasis, and dysregulation of the balance of these proteins contributes to the pathogenesis of several acute and chronic inflammatory diseases.

CONGENITAL DEFICIENCY OF TWO IL-6 RECEPTORS AND THEIR SIGNALING GENES IN HUMANS

Congenital deficiency of IL-6R and its related signal protein genes in humans indicates that they function in diverse biological processes. A genome-wide association study reported that variants rs412967 and rs2228145 of the human IL-6R gene correlated with a high risk of asthma (69, 70). Of note, variant rs2228145 of IL-6R increased the activity of ADAM protease, leading to the increased shedding rate of mIL-6R and elevated serum sIL-6R levels (71), which decreased the mIL-6R levels and reduced classic signaling causing the asthma. In addition, increased serum sIL-6R concentrations promoted formation of the IL-6–sIL-6R–sgp130 complex, which inhibited the function of IL-6 as a buffering system (72, 73). Mutations of the gp130 and STAT3 genes caused symptoms that were similar to those related to a deficiency in IL-6R. A patient with a causative homozygous mutation in the gp130 gene (*IL6ST*) presented with immunodeficiency and skeletal abnormalities including craniosynostosis that were related to deficiencies in the IL-6, IL-11, IL-27, and oncostatin M signaling pathways, although leukemia inhibitory factor signaling was intact (74). A dominant negative mutation on STAT3 in humans was associated with hyper-IgE syndrome, which is characterized by marked elevation of IgE, staphylococcal abscesses, and pneumonia (75, 76). Variations in the range of hyper-IgE syndrome (HIES) symptoms are a common feature related to the phenotypes of the mutated IL-6R, gp130, and STAT3 genes, suggesting critical roles of congenital IL-6 signaling.

Recently, two cases of homozygous mutations of the IL-6R gene were reported (77). These patients exhibited recurrent infections, but not significant viral infection, and presented with severe asthma but moderate atopic dermatitis. These mutations cause severe clinical symptoms including elevated serum IgE levels, marked increase in IL-6 but not CRP levels, marked eosinophilia but not neutrophilia during infection, and elevated levels of Th2 subsets and Tregs in peripheral blood. This report suggests toxicity related to inhibition of IL-6R signaling.

In addition, a homozygous mutation in the human STAT1 gene enhances susceptibility to herpes simplex virus 1 (HSV-1) infection that is related to defective IFN-α/β production (78). Deletion of the ADAM17 gene impaired TNF-α production and caused severe skin and bowel inflammation, as well as myocarditis (79). Moreover, a congenital deficiency in TYK2 impaired IL-12 and IFN-α/β release and enhanced susceptibility to mycobacterial and viral infections without hyper-IgE syndrome (80). Unlike congenital IL-6R deficiency, congenital loss of function of STAT1, ADAM17, or TYK2 is primarily linked to IFN-α/β, IL-12, or TNF-α production.

THERAPEUTICS TARGETING THE IL-6 RECEPTOR—FROM RHEUMATOID ARTHRITIS TO CYTOKINE STORM

Over the last two decades, it has been established that IL-6 has functions in diverse biological responses and is a major cytokine in health and diseases (81). IL-6 has homeostatic functions, including proliferation and differentiation of immune cells, regulation of metabolic activity, and proinflammatory effects related to dysregulation of the homeostatic state (5). In contrast, excessive and/or sustained production of IL-6 is proinflammatory and is often correlated with severity or progression of disease. Thus, inhibition of IL-6 activity is associated with control of diseases.

Because IL-6 has pleiotropic functions, it is difficult to develop a specific therapeutic reagent to inhibit its disease-related effects while preserving its beneficial effects. Several drugs that target IL-6, its receptors, or related signaling molecules have been developed (**Figure 3**). Siltuximab, an IL-6-neutralizing antibody, inhibits classic signaling and trans-signaling. Tocilizumab, a humanized monoclonal anti-IL-6R antibody, blocks all IL-6R signaling (5) and is used to treat several chronic inflammatory diseases and distinct types of cytokine storm. Soluble gp130-Fc (olamkicept) blocks the trans-signaling and trans-presentation pathways by binding to the IL-6–sIL-6R or IL-6–mIL-6R complex (82, 83). In addition, baricitinib, tofacitinib, upadacitinib, filgotinib, and peficitinib target the IL-6R intracellular signaling molecules, JAK proteins. Small molecules with inhibitory activity against STAT3 have also been developed. Several therapies targeting IL-6 signaling have been developed to treat chronic inflammatory diseases such as RA; however, numerous clinical trials have also demonstrated the efficacy of these therapies for cytokine storm. Thus, we highlight the pathological roles of IL-6 and its signaling pathways as well as their beneficial effects in acute and chronic diseases.

Inhibition of IL-6 Signaling in Chronic Inflammatory Diseases

Blockade of IL-6 signaling has been successful in treating chronic inflammatory diseases (**Tables 1**, **2**). This therapy has been approved in several countries.

IL-6 inhibition in rheumatoid arthritis. RA is a chronic autoimmune disorder characterized by joint manifestations, including osteoporosis, swelling, and joint destruction, and increased risk of cardiovascular diseases. IL-6 and TNF-α are critical in the pathogenesis of RA. Several biological therapies that target these cytokines' signaling pathways have been developed to treat RA. As described above, IL-6 is a primary cytokine RA pathogenesis, and increased levels of IL-6 in the

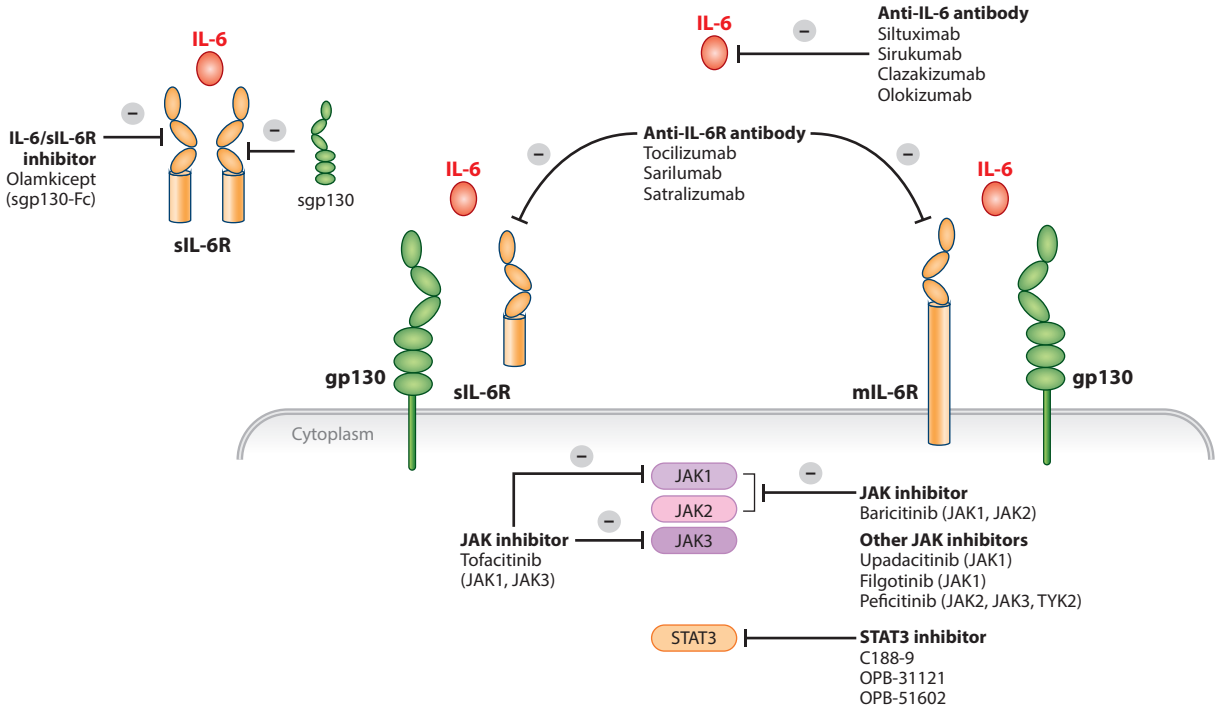


Figure 3

Pharmacological approaches for targeting IL-6 signaling. The neutralizing antibodies siltuximab, sirukumab, clazakizumab, and olokizumab directly bind to IL-6. IL-6R can be inhibited at the IL-6–IL-6R binding site by the monoclonal antibodies tocilizumab, sarilumab, and satralizumab. The soluble form of gp130 associates with the IL-6–sIL-6R complex to block trans-signaling, and sgp130-Fc, also known as olamkicept, has an inhibitory function specific for IL-6 trans-signaling. As inhibitors of intracellular signaling molecules, JAKs can be blocked by tofacitinib, baricitinib, upadacitinib, filgotinib and peficitinib. STAT3 activities can be blocked by small-molecule inhibitors. Abbreviations: JAK, Janus kinase; mIL-6R, membrane form of IL-6R; sgp130, soluble form of gp130; sIL-6R, soluble form of IL-6R; STAT, signal transducer and activator of transcription.

Table 1 Approved IL-6-inhibition biologics

Disease	Target	Name	Country (Year)
Castleman diseases	IL-6R	Tocilizumab	Japan (2005)
	IL-6	Siltuximab	EU, US (2014)
Rheumatoid arthritis	IL-6R	Tocilizumab	Japan (2008), EU (2009), US (2010)
	IL-6R	Sarilumab	Japan, EU, US (2017)
Systemic juvenile idiopathic arthritis	IL-6R	Tocilizumab	Japan (2008), EU, US (2011)
Giant cell arteritis	IL-6R	Tocilizumab	Japan, EU, US (2017)
Takayasu arteritis	IL-6R	Tocilizumab	Japan (2017)
Adult-onset Still diseases	IL-6R	Tocilizumab	Japan (2019)
Neuromyelitis optica spectrum disorder	IL-6R	Satralizumab	Japan, US (2020), EU (2021)
Cytokine storm in CAR T cell therapy	IL-6R	Tocilizumab	US (2017), Japan (2019)
Severe COVID-19 pneumonia	IL-6R	Tocilizumab	US, EU (2021), Japan (2022)

Abbreviations: CAR, chimeric antigen receptor; COVID-19, coronavirus disease 2019; EU, European Union; sIL-6R, soluble form of IL-6R.

Table 2 Clinical trials of IL-6-inhibition biologics

Disease	Target	Name	Trial phase
Lupus nephritis	IL-6	Sirukumab	II
Rheumatoid arthritis	IL-6	Olokizumab	III
Organ transplant rejection	IL-6	Clazakizumab	II
Systemic lupus erythematosus	IL-6R	Tocilizumab	I
Ulcerative colitis and IBD	IL-6/sIL-6R	Olamkicept	II
Crohn disease	IL-6	Olokizumab	II

Abbreviation: IBD, inflammatory bowel disease.

serum and synovial fluid of RA patients are correlated with disease activity (84–87). In the early phase of RA, IL-6 induces neutrophil recruitment to the joints (88), which mediates the subsequent infiltration of monocytes into the synovial fluid (89). Excessive and sustained joint inflammation causes tissue destruction via activation of osteoclasts, bone erosion, and cartilage damage. Moreover, IL-6 also directly stimulates osteoclast activity by inducing RANKL production in synovial cells (46).

In the early 1990s, cell-based studies revealed that increased levels of IL-6 might contribute to osteoporosis, cartilage destruction, and synovial inflammation, which are characteristic symptoms of RA (90–92). In a murine collagen-induced arthritis model, inhibition of IL-6 and IL-6R ameliorated inflammation in joints (93, 94). Furthermore, RA patients treated with an anti-IL-6 monoclonal antibody showed a transient improvement in inflammatory symptoms and CRP levels (95). Tocilizumab has demonstrated beneficial efficacy in refractory RA patients (96). After initial promising results, the first large-scale, double-blind, randomized, controlled trial of tocilizumab for RA demonstrated that tocilizumab treatment ameliorated disease activity by improving the clinical parameters and radiological manifestations (97–99). In the AMBITION study of the efficacy of tocilizumab compared with methotrexate (MTX), it was found that tocilizumab treatment was better than MTX, with increased response rate and remission rate and improved quality of life compared with MTX (100). Moreover, the ADACTA study revealed that tocilizumab monotherapy had a greater effect in decreasing RA activity compared with monotherapy with adalimumab, a TNF-α antagonist (101). Thus, tocilizumab was approved for RA treatment in Japan, the European Union (EU), and the United States in 2008, 2009, and 2010, respectively. An alternative anti-IL-6R antibody, sarilumab, which is a human monoclonal antibody, has higher affinity for human IL-6R than tocilizumab does. Combination treatment with sarilumab and MTX demonstrated marked beneficial efficacy for clinical symptoms and radiographic manifestations compared with placebo and MTX group (102). Therefore, sarilumab was approved for the treatment of RA in 2017 in Japan, the EU, and the United States. Clinical trials of other monoclonal antibodies against IL-6, including sirukumab, clazakizumab, and olokizumab, for RA are ongoing.

IL-6 inhibition in systemic juvenile idiopathic arthritis and adult-onset Still disease. Systemic juvenile idiopathic arthritis (sJIA) is a chronic form of arthritis in children younger than 16 years. Systemic symptoms include high fever, rash, and serositis, and there are several inflammatory signs. In severe cases, this disease is accompanied by joint destruction, functional disability, and growth retardation, leading to high morbidity and mortality (103). High levels of IL-6 were reported in the serum and synovial fluid of sJIA patients, and these correlated with the severity of disease, implicating IL-6 in the pathogenesis of sJIA (104). Several clinical trials of tocilizumab for sJIA provided evidence for its beneficial efficacy in reducing severe pediatric conditions, compared with conventional therapy (105). Moreover, long-term tocilizumab treatment

improved bone homeostasis and sJIA-associated growth retardation (106, 107). Tocilizumab was approved for sJIA treatment in 2008 in Japan and in 2011 in the EU and the United States.

Adult-onset Still disease (AOSD) and sJIA have similarities in systemic symptoms, although the age of onset is different (108). A double-blind, randomized, controlled trial with patients with AOSD refractory to glucocorticoids revealed that tocilizumab treatment improved clinical manifestations and reduced the glucocorticoid dose required (109). Thus, tocilizumab was approved for AOSD treatment in 2019 in Japan.

IL-6 inhibition in Castleman diseases and idiopathic multicentric Castleman diseases. Castleman diseases are heterogeneous chronic inflammatory diseases characterized by benign hyperplastic lymph nodes, infiltration of plasma cells, and capillary proliferation by vascular hyperplasia (110). Although the etiology of Castleman diseases is not well understood, we do know that sustained IL-6 production in the germinal centers of lymph nodes is linked to disease activity. Lymph node resection for unicentric Castleman disease patients reduced clinical symptoms, as well as serum IL-6 and CRP levels, indicating a critical role of IL-6 in the induction of hyperplastic lymph nodes (110). Human herpes virus 8 (HHV-8), or Kaposi sarcoma herpes virus, infection is implicated in the pathogenesis of multicentric Castleman diseases (MCD). HHV-8 encodes viral IL-6 (vIL-6), which is produced by HHV-8-infected plasmablasts. vIL-6 directly binds to gp130 in the absence of IL-6R and is involved in the pathogenesis of HHV-8 Castleman diseases. Several murine studies support this association. Transgenic mice expressing murine IL-6 or vIL-6 developed MCD-like symptoms including splenomegaly, hypergammaglobulinemia, multiple lymphadenopathy, and plasmacytosis (111, 112). However, vIL-6 gene transfer into IL-6-deficient mice did not induce any features of MCD, indicating that endogenous IL-6 expression is critical for the pathogenesis of HHV-8-associated MCD. In addition, HHV-8-encoded vIL-6 induced human IL-6 production in several cell lines and cells isolated from MCD patients and promoted angiogenesis due to release of VEGF, resulting in prominent capillary proliferation (113). Based on these findings, two open-label clinical trials of tocilizumab for Castleman disease were conducted. These studies found that tocilizumab treatment markedly reduced clinical symptoms and disease activity (114, 115). On the basis of these clinical trials and basic research, tocilizumab was first approved for Castleman diseases in 2005 in Japan.

IL-6 inhibition in giant cell arteritis and Takayasu arteritis. Giant cell arteritis (GCA) and Takayasu arteritis (TA) are chronic forms of systemic large-vessel vasculitis. GCA is a typical large-vessel vasculitis and cranial arteritis and usually affects individuals over the age of 50 years, whereas TA affects the arteries and their major branches and often begins in adolescents and in women under the age of 40 years. IL-6 has a critical role in the pathogenesis of GCA and TA. Although the pathogenesis of GCA and TA is unknown, serum IL-6 levels are correlated with disease activity (116, 117). In addition, some case reports indicate that tocilizumab is effective for refractory GCA and refractory TA. Glucocorticoids are used to treat GCA, although some patients exhibit disease flares upon dosage reduction. The phase 3 GiACTA study of GCA patients compared the combination treatment of tocilizumab and prednisone taper for 6 months to prednisone taper plus placebo. Fifty-six percent of patients receiving subcutaneous injection of tocilizumab weekly and 53% of patients receiving tocilizumab every two weeks achieved sustained glucocorticoid-free remission at 13 months compared to over 6 months for 14% of those who received prednisone taper alone (118). Consequently, Japan, the United States, and the EU approved tocilizumab for GCA treatment in 2017. A phase 3 trial of another anti-IL-6R antagonist, sarilumab, in GCA is ongoing.

A phase 3 TAKT trial investigated the efficacy of tocilizumab in relapsed TA patients receiving subcutaneous injection of tocilizumab weekly or placebo. Although the intended end point of

the study was not met, tocilizumab treatment delayed the time to relapse during glucocorticoid tapering (119). Based on these clinical trials, tocilizumab was approved for TA treatment in 2017 in Japan.

IL-6 inhibition in neuromyelitis optica. Neuromyelitis optica (NMO) is a chronic disease of the central nervous system (CNS) caused predominantly by inflammatory responses in the spinal cord and optic nerves (120). Typical NMO symptoms include marked elevation of autoantibodies against AQP4, an astrocyte water channel protein. Autoantibodies attack the optic nerve and spinal cord via activation of complement, causing demyelination and necroptosis (121). In addition, high concentrations of IL-6 in the cerebrospinal fluid are often found in patients with NMO. Moreover, increased numbers of plasmablasts ($CD19^{int}CD27^{hi}CD38^{hi}CD180^{neg}$) were found in the periphery in NMO patients, suggesting IL-6 affects plasmablast proliferation and the secretion of autoantibodies. Thus, the disease activity of NMO might be inhibited by targeting IL-6 signaling. A pilot study reported that tocilizumab treatment reduced anti-AQP4 antibody production in patients with NMO and prolonged the relapse rate and alleviated neuropathic pain in patients with refractory NMO (122). Moreover, a novel anti-IL-6R antibody, satralizumab, which is a humanized IgG2 recycling monoclonal antibody, showed beneficial efficacy in NMO patients. Satralizumab monotherapy reduced serum anti-AQP4 levels in patients with relapsed NMO. Consequently, satralizumab was approved as an orphan drug for NMO treatment in 2020 by US Food and Drug Administration (FDA) (123, 124).

Inhibition of IL-6 Signaling in Cytokine Storm

Recently, anti-IL-6R antibody has been found to be a valuable therapy for treating cytokine storm, a major complication of antitumor therapy and infectious diseases (**Tables 1**, **2**).

IL-6 as a biomarker of cytokine storm. Cytokine storm, or cytokine release syndrome, includes uncontrolled systemic inflammatory diseases such as graft-versus-host disease, severe infectious diseases including sepsis, and noninfectious diseases such as macrophage activation syndrome and hemophagocytic lymphohistiocytosis. These excessive immune responses can lead to vascular injury, coagulopathy, disseminated intravascular coagulation, multiple organ failure, and death.

Cytokine storm involves the rapid release of diverse cytokines and chemokines that have pathological roles in the progression of acute inflammatory responses. Although diverse cytokines are elevated in cytokine storm, IL-6 and its signaling pathway are emerging as key factors in the pathogenesis of this disease (**Figure 4**). Upon bacterial or viral infection, or tissue stress, IL-6 contributes to host defense by activating acute reactive responses, the immune system, and hematopoiesis (125). Innate immune cells such as monocytes and macrophages as well as vascular endothelial cells, stromal cells, fibroblasts, and many other types of cells can produce IL-6 when stimulated by TLR ligands or cytokines such as IL-1, TNF-α, or IL-6 itself (126). Monocytes and macrophages are the main producers of IL-6 after the recognition of pathogen-associated molecular patterns (PAMPs) at the site of infection or damage-associated molecular patterns (DAMPs) at the site of tissue injury (127, 128), and they mediate or initiate inflammatory responses. IL-6 rapidly acts on hepatocytes and induces acute-phase responses to production of CRP, hepcidin, fibrinogen, SAA, and antitrypsin, as well as reducing albumin levels (30, 129).

Systemic inflammatory responses are characteristic of vasculitis and coagulopathy (130). Proinflammatory cytokines such as IL-6, IL-8, TNF-α, IFN-γ, and IL-1β activate monocytes to produce tissue factor (TF), also known as factor III, which is critical for the activation of the coagulation cascade. TF triggers the activation of factor VIIa to Xa and the subsequent formation of

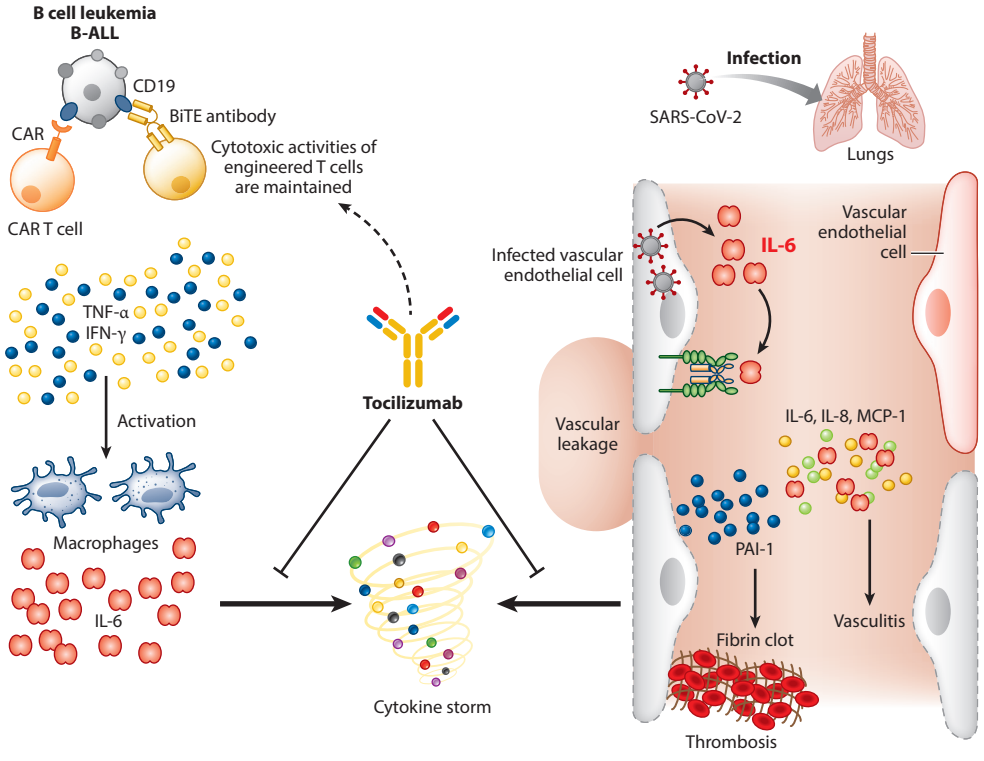


Figure 4

Pathological mechanisms of cytokine storm and treatment by IL-6 blockade using tocilizumab. Activation of T cells or CAR T cells induces high levels of IFN-γ and TNF-α. These cytokines activate macrophages, leading to increased levels of IL-6, which cause a cytokine storm. Tocilizumab treatment can block the development of a cytokine storm without blocking the cytotoxic activity of engineered T cells directed against B-ALL. In the setting of COVID-19, SARS-CoV-2 infection induces loss of vascular integrity, activation of coagulation, and amplification of inflammation through IL-6 trans-signaling. Tocilizumab acts on endothelial IL-6 trans-signaling and reduces COVID-19-induced cytokine storm. Abbreviations: B-ALL, B cell acute lymphoblastic leukemia; BiTE, bispecific, T cell–engaging; CAR, chimeric antigen receptor; COVID-19, coronavirus disease 2019; PAI-1, plasminogen activator inhibitor 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

thrombin, which generates fibrin clot formation. In addition, accumulated thrombin promotes IL-6 release from vascular endothelial cells (**Figure 2**). In turn, IL-6 signals trigger IL-8 and MCP-1 production as well as adhesion molecule expression, which mediates the recruitment of monocytes and neutrophils to vascular endothelial cells (131–133). In addition, under hypoxic conditions, vascular endothelial cells release IL-6, which promotes VEGF production from adipocytes or other cells (134, 135). IL-6 itself or IL-6-induced VEGF production mediates the internalization of vascular endothelial (VE)-cadherin molecules into endothelial cells and increases vascular permeability (136–138). The increase in vascular permeability causes interstitial edema and increases tissue pressure, leading to tissue damage. Moreover, IL-6 also activates the complement system by increasing expression of C5a receptor (C5aR) on endothelial cells, which enhances C5a-induced vascular permeability (139, 140). In addition, the IL-6–sIL-6R complex directly activates endothelial cells by secretion of IL-6, IL-8, MCP-1, and plasminogen activator inhibitor 1 (PAI-1), which mediates the infiltration of proinflammatory immune cells and the activation of coagulation

cascades (132). IL-6 also acts on papillary muscle cells to reduce their contraction, causing myocardial dysfunction in sepsis (141, 142). Collectively, pathological IL-6 activity contributes to tissue hypoxia, disseminated intravascular coagulation, myocardial dysfunction, and multiple organ failure, which are features of cytokine storm. Therefore, anti-IL-6 therapeutics are being investigated in several ongoing clinical trials for the treatment of severe cytokine storm, including COVID-19 cytokine storm. Measuring circulating IL-6 levels is difficult because peak IL-6 expression differs between different types of cytokine storm and depending on the timing of blood collection. Ultimately, serum IL-6 is the primary inducer of CRP production, and potentially the primary cause of deterioration of patients with cytokine storm, such as in sepsis and acute respiratory distress syndrome (ARDS). Of note, blockade of IL-6 signaling is beneficial for various types of cytokine storm, especially blockade by tocilizumab, which has been approved for CAR (chimeric antigen receptor) T cell–induced cytokine storm and COVID-19-induced cytokine storm.

Role of IL-6 signaling in CAR T cell–induced cytokine storm. During the last decade, the field of cancer immunotherapy has advanced. The success of new T cell–engaging immunotherapies has highlighted the field of cytokine storm, which is the most frequent serious adverse effect of these therapies. T cells engineered to recognize the CD19 antigen that is fused to the signaling region of the T cell receptor are referred to as CAR T cells (143). CAR T cells can recognize and eliminate $CD19^+$ B cell lineage cells and have high efficacy for malignant lymphoma cells. Blinatumomab is a bispecific, T cell–engaging (BiTE), single-chain antibody construct that interacts with $CD3^+$ T cells and $CD19^+$ cells and has impressive therapeutic activity against refractory B cell precursor acute lymphoblastic leukemia. Such T cell–engaging immunotherapies have direct cytotoxic, antitumor effects and marked efficacy in clinical trials. Indeed, some of these therapies have already been approved (**Figure 4**). However, engaged T cells release massive amounts of proinflammatory cytokines, such as IL-6, IL-8, MCP-1, IFN-γ, and IL-10, which can initiate a cytokine storm (143). After CAR T cell administration, a large amount of IFN-γ is produced by activated T cells or tumor cells and stimulates macrophages in the tumor environment via the IL-6 classic signaling pathway (144). These activated macrophages are primary cytokine producers and release IL-6, TNF-α, and IL-10, which causes a cytokine storm that can be detrimental. Recent studies reported that monocytes also produce high levels of cytokines and factors that promote the severity of cytokine storm in mice and that blockade of IL-6 or IL-1 signaling had different effects (145, 146).

Increased serum IL-6 levels were observed in patients and mice with cytokine storm, suggesting IL-6 has a key role in the progression and severity of cytokine storm (129, 147–149). Indeed, the peak serum levels of IL-6, sIL-6R, sgp130, and IFN-γ had a significant correlation with the severity of cytokine storm in pediatric and adult B cell acute lymphoblastic leukemia patients who received CAR T cell therapy, suggesting a critical role of IL-6 trans-signaling (150). The first case of tocilizumab treatment to mitigate the symptoms of cytokine storm complicated by CAR T cell therapy in a pediatric patient with refractory acute lymphoblastic leukemia was reported (151). In this case, treatment with both tocilizumab and an anti-TNF inhibitor, etanercept, rapidly decreased serum proinflammatory cytokine levels but maintained the cytotoxic activity of the transferred CAR T cells. The second successful case of tocilizumab treatment was reported in a pediatric patient who received blinatumomab (148). This patient had severe hemophagocytic lymphohistiocytosis with multiple organ failure and high serum levels of IL-6, IL-10, IL-8, MCP-1, IL-2R, and IFN-γ. Cytokine levels were rapidly reduced within 3.5 days after tocilizumab treatment. A retrospective study included 30 patients with a wide range of cytokine symptoms, including respiratory failure, coagulopathy, and hypotension, after CAR T cell therapy (152). The serum levels of IL-6 in these patients were higher than 1,000 pg/mL, and the symptoms of

cytokine storm were profoundly improved by tocilizumab treatment. In addition, a recent postmortem study identified endothelial cells as the major IL-6-producing cells that were responsible for the development of cytokine storm in a patient who was administered CD19-targeted CAR T cell therapy (153). Another complication of CAR T cell therapy is neurotoxicity. High levels of IL-6 and MCP-1 correlated with severe neurotoxicity in patients who underwent CAR T cell infusion and were associated with vascular dysfunction due to endothelial activation (154). Furthermore, cytokine storm was observed after the administration of rituximab, gene therapy, or immune checkpoint inhibitors (155). Thus, treatment with tocilizumab, which inhibits the classic and trans-signaling pathways, has a beneficial effect on CAR T cell therapy–induced cytokine storm characterized by multiple organ dysfunction and respiratory failure. Finally, tocilizumab was approved for CAR T cell therapy–induced cytokine storm by the FDA in 2017.

Efficacy of IL-6 signaling in COVID-19-induced cytokine storm (viral sepsis). In December 2019, SARS-CoV-2 infectious disease appeared, causing severe respiratory dysfunction with high mortality. In March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. The clinical symptoms of COVID-19 range from mild to moderate in healthy individuals, but in severe cases, hyperreactive immune responses can lead to respiratory dysfunction including pneumonia, ARDS, and multiple organ failure (156, 157). Of note, serum levels of cytokines including IL-6, GM-CSF, IL-18, TNF, and interferons were commonly elevated in critically ill COVID-19 patients, as is common in bacterial sepsis. The poor outcomes of severe COVID-19 are closely associated with uncontrolled viral expansion and vascular health condition. Initially, corticosteroids were used to manage severe COVID-19. However, several studies indicated increased IL-6 levels in COVID-19 patients may be associated with the severity of disease (158). Of note, increased levels of IL-6 were correlated with a wide variety of inflammatory states, including sepsis (132). As described above, inhibition of IL-6 signaling is approved for the management of cytokine storm following CAR T cell therapy. Because there are several similar clinical parameters in patients with CAR T cell cytokine storm and those critically ill with COVID-19, there was a great interest in tocilizumab for managing severe COVID-19 in the early phase of this pandemic (158). Other anti–IL-6 signaling antibodies, including sarilumab and siltuximab, have also been studied for the treatment of COVID-19, but they have had, so far, lower efficacy compared with tocilizumab, suggesting a pathological role of IL-6R signaling in COVID-19-induced cytokine storm.

In the setting of SARS-CoV-2 infection, the main contributors to the interplay between inflammation and thrombosis are vascular endothelial cells. Of note, endothelial cells contributed to the development and propagation of ARDS by promoting vascular injury, endotheliitis, and a procoagulative state; thus, endothelial cells are emerging as a therapeutic target for COVID-19 (159). At the onset of SARS-CoV-2 infection, the virus directly infects endothelial cells or pericytes via ACE2 (angiotensin-converting enzyme 2) expressed in multiple organs (159). Subsequently, the infection causes the dysregulation or activation of endothelial cells, leading to coagulopathy. Indeed, thrombocytopenia and serum levels of D-dimer were elevated in hospitalized COVID-19 patients (160–162). In addition, patients with severe COVID-19 who required ICU care had high levels of von Willebrand Factor and PAI-1, indicators of coagulopathy (163, 164). Of note, serum concentrations of IL-6 and IL-1β in severe COVID-19 correlated with aberrant coagulation parameters and serum fibrinogen levels (165), indicating a close correlation between IL-6 and coagulation cascades. Consistent with this, IL-6 trans-signaling in endothelial cells forms an inflammatory circuit by releasing IL-6, MCP-1, and IL-8 and induces PAI-1 production. In addition, serum IL-6 levels showed a significant correlation with PAI-1 levels in patients with severe

COVID-19 and patients with other types of cytokine storm (132). Therefore, IL-6 signaling might be critical for vascular injury and coagulopathy in COVID-19 (**Figure 4**).

A preliminary study reported that dexamethasone treatment improved mortality in severe COVID-19 patients (166). Because IL-6 level is elevated in COVID-19 patients and is correlated with mortality and severity, tocilizumab might be used to manage COVID-19-induced cytokine storm. The first trial of tocilizumab treatment for patients with severe COVID-19 in Wuhan, China, was conducted as an open-label study. The outcome of this trial indicated that it improved several clinical parameters of COVID-19 pneumonia, including CRP levels and opacities on CT (computed tomography) scans (167). This suggests that inhibition of IL-6 signaling might be an effective therapeutic strategy for COVID-19-induced cytokine storm. Despite several observational studies suggesting the beneficial effects of tocilizumab on mortality of severe COVID-19 (168–170), the outcomes of randomized clinical trials of tocilizumab for COVID-19 have failed to prove its benefit. The first randomized trial of tocilizumab, COVACTA, failed to meet the primary end point of improved clinical status but met the secondary end point of improved mortality and shortened ICU stay for patients with severe COVID-19-associated pneumonia. Another randomized trial, EMPACTA, met the primary end point of reduced progress to mechanical ventilation or death of critically ill COVID-19 patients treated with tocilizumab compared with patients treated with placebo plus standard of care. However, tocilizumab treatment did not improve overall mortality or duration of hospitalization at day 28 (171). In early 2021, the United Kingdom–based trial REMAP-CAP, investigating the IL-6R antagonists tocilizumab and sarilumab, was conducted with a broad range of hospitalized COVID-19 patients (172). In the REMAP-CAP trial, treatment with IL-6R antagonists in addition to high-dose dexamethasone reduced the mortality of COVID-19 patients who required high-flow oxygen or mechanical ventilation. The RECOVERY trial reported that combination treatment with tocilizumab and steroids reached the study end point, which was modest reduction of mortality compared with treatment with steroids alone (173). Taken together, the findings indicate that the efficacy of tocilizumab with steroids is likely to be additive but not synergic. In this context, combination treatment of IL-6R antagonists and corticosteroids has a more positive impact than antiviral therapy. Thus, tocilizumab was approved by the United States and the EU in 2021 and very recently by Japan in 2022 for the treatment of severe cases of COVID-19 pneumonia. More recently, a prospective meta-analysis by the REACT group from the WHO demonstrated that tocilizumab with corticosteroids improved the outcomes of critically ill COVID-19 patients (174). Thus, tocilizumab is the second drug recommended by the WHO as a therapeutic reagent for severe COVID-19.

CONCLUDING REMARKS

This is my (T.K.) third time contributing a review article on IL-6 to the *Annual Review of Immunology* (87, 175). In 1985, I wrote a review on B cell stimulatory factors, one of which was later identified as IL-6. In the early 1970s, we identified a T cell–derived soluble factor(s) that activated B cells to produce antibodies. This result revealed one of the mechanisms of T and B cell interactions in antibody production. Several molecules were reported for B cell growth and differentiation. One of these molecules, BSF-2, was isolated in 1986. By using cDNA and an antibody, very interesting biological and medical fields were opened. This molecule has a wide variety of biological functions and is involved in various acute and chronic inflammatory diseases. In 2005, after completing studies on IL-6, IL-6R, and IL-6 signal transduction, I contributed a review article to the *Annual Review of Immunology* (87). However, our IL-6 study was not over. In the 1990s, we generated a humanized monoclonal anti-IL-6R antibody that blocked IL-6 signaling and prevented various diseases induced by overproduction of IL-6. This antibody,

tocilizumab, is widely used for diseases from RA to cytokine storm induced by CAR T cell therapy and COVID-19. More than a million RA patients have been treated with this antibody, and it is now being used for COVID-19 patients. Tocilizumab can treat patients with severe cytokine storm. The focus of studies on IL-6, while excluding the extended IL-6 family members, remains true to the importance of its pathogenic roles defined by my research of almost 50 years. I hope that this review of the basic features of IL-6 biology and the clinical implications of targeting its signaling pathway will aid our understanding of several inflammatory diseases.

DISCLOSURE STATEMENT

T.K. holds a patent for tocilizumab and has received royalties for Actemra. S.K. is not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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