Therapeutic uses of anti-interleukin-6 receptor antibody

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Abstract

Cytokine-targeted therapy has generated a paradigm shift in the treatment of several immune-mediated diseases. Interleukin-6 (IL-6), which was initially identified as B-cell stimulatory factor 2, is a prototypical cytokine with wide-ranging biological effects on immune cells such as B and T cells, on hepatocytes, hematopoietic cells, vascular endothelial cells and on many others. IL-6 is thus crucially involved in the regulation of immune responses, hematopoiesis and inflammation. When infections and tissue injuries occur, IL-6 is promptly synthesized and performs a protective role in host defense against such stresses and traumas. However, excessive production of IL-6 during this emergent process induces potentially fatal complications, including systemic inflammatory response syndrome (SIRS), and dysregulated, persistently high expression of IL-6 causes the onset or development of various chronic immune-mediated disorders. For these reasons, IL-6 blockade was expected to become a novel therapeutic strategy for various diseases characterized by IL-6 overproduction. Indeed, worldwide clinical trials of tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody, have successfully proved its outstanding efficacy against rheumatoid arthritis, juvenile idiopathic arthritis and Castleman disease, leading to the approval of tocilizumab for the treatment of these diseases. Moreover, various reports regarding off-label use of tocilizumab strongly suggest that it will be widely applicable for acute, severe complications such as SIRS and cytokine-release syndrome and other refractory chronic immune-mediated diseases.

Keywords: autoimmune diseases, chronic inflammatory diseases, IL-6, tocilizumab

Introduction

Cytokines are soluble regulators that facilitate intercellular communication in immune responses and hematopoiesis. Different from hormones, the characteristic features of cytokines are functional pleiotropy and redundancy (1). Although cytokines perform critical roles in host defense and maintenance of tissue homeostasis, abnormal production of cytokines causes the onset or development of acute and chronic diseases, so that novel therapeutic strategies using cytokines themselves or cytokine-targeted biologics have been developed and successfully used for the treatment of various diseases.

Interleukin-6 (IL-6), composed of 184 amino acids, was originally identified as B-cell stimulatory factor 2 (BSF-2) that promotes immunoglobulin synthesis by activated B cells (2); its complementary DNA was successfully cloned in 1986 (3). Later, IL-6 was found to be a prototypical cytokine with pleiotropic biological effects on immune responses, acute-phase responses and hematopoiesis, although some of these effects have been shown to be made redundant by those of other members of the IL-6 family of cytokines (4–6).

IL-6 is not expressed in healthy individuals, but when infections or tissue injuries occur, IL-6 is rapidly synthesized and contributes to host defense (7). However, excessive production of IL-6 during this process has been implicated in the development of acute, severe complications, including systemic inflammatory response syndrome (SIRS) and cytokine-release syndrome (CRS), and chronic dysregulated production of IL-6 plays a pathological role in the onset and development of chronic immune-mediated diseases (5, 8). For these reasons, it was anticipated that IL-6 blockade would constitute a novel therapeutic strategy for such diseases, resulting in the development of tocilizumab, a humanized anti-IL-6 receptor antibody (8–10). This review article focuses on recent findings of IL-6-related research and progress in IL-6-targeting therapy.
IL-6, a pleiotropic cytokine

IL-6 exerts various biological effects on different target cells and organs (Fig. 1). When acting on hepatocytes, IL-6 induces the synthesis of a wide range of acute-phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, hepcidin, and α1-antichymotrypsin, but inhibits expression of fibronectin, albumin, and transferrin (11, 12). At the site of infection or injury, IL-6 is rapidly generated from innate immune cells, including monocytes, macrophages, and dendritic cells, via molecules that recognize pathogen-associated molecular patterns or damage-associated molecular patterns, thus triggering innate immune responses.

IL-6 is also important for adaptive immune responses by promoting B- and T-cell differentiation. As originally reported, IL-6 helps activated B cells to differentiate into antibody-producing cells, and it also promotes the growth of myelomas/plasmacytomas and enhances the survival of plasmablasts (13, 14). As one of its most important functions, IL-6 regulates the differentiation of naive CD4+ helper T cells into effector subsets. IL-6, together with TGF-β, preferentially induces differentiation of the naive CD4+ helper T cells into Treg cells, which produce the inflammatory cytokine IL-17, but IL-6 inhibits TGF-β-induced differentiation of those cells into Th17 cells. This results in an immunological imbalance between Treg/T17 subsets, which is believed to be an important pathological mechanism for the development of autoimmune and chronic inflammatory diseases (15).

Other actions of IL-6 are the promotion of T-foveal helper cell differentiation and IL-21 production (16), as well as induction of cytotoxic CD8+ T-cell differentiation by augmenting expression of IL-2 and its receptor. During inflammation, IL-6 increases the level of adhesion molecules and molecules that regulate migration, such as monocyte chemoattractant protein-1 in endothelial cells (17). Finally, during hematopoiesis, IL-6, together with IL-3, synergistically affects the formation of pleiotropic blast cell precursors, which support the formation of macrophage and megakaryocyte differentiation.

Besides its crucial involvement in the immune system, IL-6 is also involved in bone homeostasis. When released from bone marrow stromal cells, it is required for the expression of the receptor activator of the NF-κB ligand (RANKL), and subsequently promotes osteoclast differentiation, through which systemic as well as peri-articular osteoporosis and joint destruction is induced, as seen, for example, in patients with rheumatoid arthritis (RA) (18). In fact, it has been found that an increase in serum IL-6 levels strongly correlates with severity of radiographically detectable joint destruction (19). Robust angiogenesis and vascular permeability are characteristic features of RA synovial tissues and these characteristics are mediated by excessive production of vascular endothelial growth factor (VEGF), whose synthesis is also up-regulated by IL-6 (20). In dermal tissue, IL-6 appears to promote keratinocyte proliferation and collagen production in dermal fibroblasts. Finally, IL-6 has been demonstrated to

Fig. 1. Pleiotropic activity of IL-6 and the pathological implications in disease. IL-6 exerts a variety of biological effects by acting on various cells, and overexpression of IL-6 is pathologically involved in the status of various diseases, including immunological disorders, bone and cartilage destruction, persistent acute-phase responses and angiogenesis.
interact with many other cells and organ systems, including vascular endothelial cells, the neuropsychological system and the hypothalamic–pituitary–adrenal endocrinal system.

The IL-6 signaling system: IL-6 receptor and gp130

IL-6 is structurally classified as a member of the four-helix-bundle family of cytokines (21) and interacts with two different types of molecules, namely, IL-6 receptor (IL-6R, CD126) (22) and the signal-transducing receptor subunit gp130 (23). IL-6R exists in two forms, an 80-kDa transmembrane form and a 50–55-kDa soluble form. Transmembrane IL-6R, with a short cytoplasmic domain, interacts with gp130 and transduces the signal upon binding of IL-6, which is known as the classic IL-6 signaling pathway (Fig. 2). Due to the limited expression of transmembrane IL-6R on hepatocytes, monocytes, macrophages and lymphocytes, cell activation through the classic pathway is limited (24).

Soluble IL-6R, which lacks a cytoplasmic region, can also form a complex with IL-6, leading to homodimerization of gp130 and subsequent triggering of the downstream signaling cascade, which is known as the trans-signaling pathway. Soluble IL-6R is found in serum and tissue fluids, and gp130 is ubiquitously expressed in various cells, so that this trans-signaling pathway is widely used, and the pleiotropic activity of IL-6 can be explained by the broad range expression of gp130 (6, 25). gp130 is also used as a common signal-transducing molecule for the IL-6 family cytokines, including leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, IL-11, cardiotrophin 1, cardiotrophin-like cytokine, IL-27 and IL-35. These mechanisms account for the redundancy of characteristic features of cytokines at the molecular level (26, 27).

As schematically shown in Fig. 2, IL-6 stimulation causes tyrosine phosphorylation of a cytoplasmic protein, the kinase JAK that is constitutively bound with gp130, and then activates two main signal transduction pathways, the STAT3 pathway and the MAPK pathway, through phosphorylation of the SH2-domain containing protein tyrosine phosphatase-2 (SHP-2). STAT3 activated by JAK forms a dimer, translocates into the nucleus, and then acts as a transcription factor to induce expression of IL-6 responsive genes. Furthermore, STAT3 activated by IL-6–IL-6R interaction also induces SOCS1 and SOCS3 expression (28, 29). SOCS1 binds directly to JAK, which attenuates its catalytic activity. SOCS3, on the other hand, inhibits the signaling through directly binding to gp130. Putting these findings together, IL-6 signaling is controlled by SOCSs as negative feedback regulators.

![Diagram of IL-6 signaling system](https://example.com/diagram)

**Fig. 2.** The classic IL-6 signaling system. IL-6 is capable of activating two major pathways: the STAT3 and MAPK pathways. These pathways activate downstream signaling of IL-6R, leading to induction of a variety of gene expression. This signaling also induces SOCS1 and SOCS3 expression, which in turn suppress the IL-6 signaling pathway.
Regulation of IL-6 production

When infections or tissue damage occur, IL-6 synthesis is promptly induced to contribute to host defense. After removal of such stresses from the host, the IL-6 production ends as the result of strict control of IL-6 synthesis at the transcriptional and post-transcriptional stages. A number of cis-transcription factors have been identified to be involved in IL-6 gene activation, including NF-κB, specificity protein 1 (SP1), nuclear factor IL-6 (NF-IL6), activator protein 1 (AP-1) and interferon regulatory factor 1 (IRF-1) (Table 1). Stimulation with TNF-α, IL-1, and even IL-6 activates the functional cis-regulatory factors to bind to their specific elements at the 5′-flanking region in the IL-6 gene. IL-6 in liver in particular can strongly induce NF-IL6 activation, suggesting that NF-IL6 might be a main transcriptional factor for acute-phase protein expression. In addition to inflammatory cytokines, some virus species such as the human T-lymphotropic virus 1-derived transactivator of the transcription protein (Tax) have been shown to be able to modulate the DNA-binding activity of NF-κB and NF-IL6 to the IL-6 promoter region.

On the other hand, inflammatory stimuli also modulate the expression of factors involved in IL-6 transcription suppression, including the aryl hydrocarbon receptor (Ahr) (Table 1). Ahr is a ligand-activated transcription factor, which acts as a receptor for several exogenous toxins. It forms a complex with NF-κB and STAT1, leading to inhibition of the promoter activity of IL-6 in macrophages, so that Ahr-deficient macrophages feature enhanced production of IL-6 (30). In addition, a number of studies have recently demonstrated that Ahr activation may also act on various immune cells such as T cells, B cells and dendritic cells, and thus affect their function in immune responses (31-34). For example, the specific depletion of Ahr in T cells inhibits T,17 cell differentiation and the development of collagen-induced arthritis (33), and Ahr-deficient dendritic cells produce less of the anti-inflammatory cytokine, IL-10 (34). In addition, some micro-RNAs (miRs) reportedly modulate, either directly or indirectly, the binding activities of several transcription factors to the IL-6 gene. Most post-transcriptional control mechanisms of cytokines target the 5′-untranslated region (UTR) or 3′-UTR of mRNAs to modulate initiation of translation or stability, respectively (35). Modification of post-transcription of IL-6 mRNA primarily occurs at AU-rich elements located in the 3′-UTR region, and a number of RNA-binding proteins or miRs are involved in the regulation of IL-6 mRNA stabilization/degradation (Table 1). Interestingly, some recent studies have identified two counteractive molecules that regulate IL-6 mRNA stability (Fig. 3); regulatory RNase-1 (Regnase-1, also known as Zc3h12a) (36) and AT-rich interactive domain-containing protein 5a (Arid5a) (37).

Regnase-1 is a nuclease and binds to the 3′-UTR of IL-6 mRNA, resulting in the destabilization of this mRNA. This resulted in the development of spontaneous autoimmune diseases, accompanied by splenomegaly and lymphadenopathy, in Regnase-1-deficient mice (36). Stimulation with an IL-1R/TLR agonist induces phosphorylation of Regnase-1, while the inhibitor of the NF-κB (IkB) kinase (IKK) complex destabilizes IL-6 mRNA. Phosphorylated Regnase-1 is subject to both ubiquitination and degradation. Regnase-1 also binds to the stem-loop site of the 3′-UTR of Regnase-1 mRNA and functions itself as a negative regulator. These findings demonstrate that IKK induces not only phosphorylation of IkB but also that of Regnase-1 to prevent IL-6 mRNA expression (38).

We recently identified a novel RNA-binding protein, Arid5a (37) and found that its expression is rapidly induced and degraded within 6 h in macrophages upon LPS, IL-1β or IL-6 stimulation. Arid5a binds to the 3′-UTR of IL-6 mRNA and selectively stabilizes IL-6 but not TNF-α or IL-12 mRNA. Arid5a-deficient mice show a striking reduction in serum IL-6 levels, induced by LPS injection, and in T,17 cell development in a mouse model—experimental autoimmune encephalomyelitis (EAE). Interestingly, Arid5a can interfere with the destabilizing effect of Regnase-1 on IL-6 mRNA, thus demonstrating that the balance between Arid5a and Regnase-1 is canonical for the stability of IL-6 mRNA, and also suggesting that predominance of Arid5a over Regnase-1 may give rise to the development of autoimmune inflammatory diseases (Fig. 3) (39). A deeper understanding of the role of these RNA-binding proteins may thus result in novel therapeutic approaches to target IL-6 mRNA stability in various diseases.

Pathological involvement of IL-6 in diseases

The immediate and transient expression of IL-6 contributes to host defense against environmental stress factors. When the source of stress is removed from the host, the production of IL-6 is terminated and results in normalization of serum levels of acute-phase proteins such as CRP and SAA. However, IL-6 is also involved in disease development (4, 5). First, during immune response against infectious agents, excessive production of IL-6 induces potentially fatal complications, such as SIRS and CRS. Second, chronic, dysregulated

Table 1. Transcriptional and post-transcriptional regulation of IL-6 gene expression

<table>
<thead>
<tr>
<th>Function</th>
<th>Protein</th>
<th>microRNA</th>
</tr>
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<tbody>
<tr>
<td>Transcription</td>
<td>Promotion</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>NF-κB, SP1, NF-IL6, AP-1, IRF-1, Tax, TAT, HBVX, mutant p53</td>
<td>—</td>
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<tr>
<td></td>
<td>Repression</td>
<td>miR-155 (targeting NF-IL6), miR-146a/b (targeting IRAK1), miR-223 (targeting STAT3)</td>
</tr>
<tr>
<td></td>
<td>P38α, GRF, ORF57, Arid5a</td>
<td>miR-365, miR-608</td>
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</tbody>
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Transcription of IL-6 is regulated by transcription factors or by miRs, and several RNA-binding proteins or miRs are post-transcriptional regulators for IL-6 mRNA. BRF, butyrate response factor; ER, estrogen receptor; GR, glucocorticoid receptor; HBVX, hepatitis B virus X protein; IRAK1, IL-1 receptor-associated kinase 1; ORF, open reading frame; PPARα, peroxisome proliferator-activated receptor α; Rb, retinoblastoma; TAT, human immunodeficiency virus 1-derived transactivator of the transcription protein; TTP, tristetraprolin.
IL-6 production by particular cell populations leads to the development of various chronic immune-mediated diseases. However, at present the reason(s) for excessive or chronic IL-6 production remains unknown; it may be partly due to an imbalance between Arid5a and Regnase-1.

The first evidence that IL-6 was pathologically involved in disease development was observed in a case of cardiac myxoma. The culture fluid obtained from the myxoma tissue of a patient with unclassified connective tissue disease, who had presented with fever, polyarthritis, increased serum CRP level, anemia and hypergammaglobulinemia with positivity for anti-nuclear factor, contained a large quantity of IL-6 and the myxoma tissues could be positively stained with anti-IL-6 antibody. Subsequent studies have demonstrated that excessive expression of IL-6 is found in synovial cells of RA, germinal center B cells in swollen lymph nodes of Castleman disease, myeloma cells and peripheral blood cells or infiltrating cells in tissues involved in various other diseases, as well as in many tumor cells.

Moreover, the concept of the pathological role of IL-6 in disease development has been supported by numerous experimental findings that IL-6 blockade by means of gene knockout or injection of neutralizing anti-IL-6 or anti-IL-6R antibody could prevent the onset or ameliorate the severity of diseases in animal models. For instance, IL-6 blockade resulted in a striking reduction in susceptibility to Castleman disease-like symptoms in IL-6 transgenic mice, and also suppressed disease development in models of RA, systemic lupus erythematosus, systemic sclerosis, polymyositis, EAE, experimental autoimmune uveoretinitis and other autoimmune inflammatory diseases.

On the basis of these findings, IL-6 targeting was expected to constitute a novel therapeutic strategy against immune-mediated diseases. This led to the development of tocilizumab, a humanized monoclonal antibody, with the CDR of a mouse anti-IL-6R grafted on to human IgG1 molecule. Tocilizumab can block both classic and trans-signaling pathways by inhibiting IL-6 binding to transmembrane IL-6R and soluble IL-6R. The production of CRP in hepatocytes is mediated by the classic signaling pathway, and if the free concentration of tocilizumab is maintained in serum at more than 1 μg ml⁻¹, CRP remains negative.

**Fig. 3.** Regulatory mechanisms of IL-6 production. Regnase-1 promotes degradation of IL-6 mRNA, whereas Arid5a counteracts this by destabilizing the effect of Regnase-1. The functional balance between Arid5a and Regnase-1 determines IL-6 mRNA stability. IRAK1, IL-1 receptor-associated kinase 1; NEMO, NF-κB essential modulator; TRAF6, TNF receptor-associated factor 6; Ub, ubiquitination.

**Therapeutic uses of the anti-IL-6R antibody, tocilizumab**

The first clinical trial of tocilizumab was performed with seven patients with Castleman disease, a chronic lymphoproliferative
disorder characterized by multiple lymph node swellings with massive infiltration of mature plasma cells and persistent production of IL-6 in germinal center B cells. These patients had presented with severe inflammatory symptoms and laboratory findings such as high fever, anemia, increased levels of acute-phase proteins, hypoalbuminemia and hypergammaglobulinemia, but the administration of tocilizumab promptly ameliorated clinical symptoms together with normalization of serum CRP levels and improvement of anemia, serum albumin concentration and hypergammaglobulinemia (43). The outstanding efficacy of tocilizumab was subsequently confirmed in another clinical trial with an enrollment of 28 patients with Castleman disease (44), and this resulted in the approval of tocilizumab as an orphan drug in 2005 in Japan.

The first randomized controlled trial of tocilizumab for RA was performed in 45 patients, who were sequentially allocated to receive a single intravenous dose of either 0.1, 1, 5 or 10 mg kg\(^{-1}\) of tocilizumab or placebo (45). At week 2, a significant difference was observed between the group treated with 5 mg kg\(^{-1}\) of tocilizumab and the placebo group, with five patients (56%) in the tocilizumab cohort and none in the placebo cohort achieving a 20% improvement in the defined range of symptoms formulated by the American College of Rheumatology (ACR20%).

A 12-week, multicenter, double-blind, placebo-controlled late phase II trial was performed in Japan (46). In this trial, 164 patients with refractory RA were randomized to receive either 8 or 4 mg kg\(^{-1}\) of tocilizumab every 4 weeks or placebo. At week 12, an ACR20% response was observed in 78, 57 and 11% of RA patients treated with 8 mg kg\(^{-1}\) of tocilizumab, 4 mg kg\(^{-1}\) of tocilizumab and placebo, respectively, while 40% of patients in the 8 mg kg\(^{-1}\) group and 1.9% in the placebo group achieved an ACR50% response. Subsequently, seven phase III clinical trials verified the outstanding efficacy of tocilizumab in the suppression of disease activity and progression of joint destruction associated with RA, and this drug is currently approved for the treatment of RA in more than 130 countries (47).

The European League Against Rheumatism now recommends tocilizumab as one of eight first-line biologics to be used for RA patients with an inadequate response to the standard disease-modifying antirheumatic drug (DMARD), methotrexate (MTX). First-line biologics include five TNF inhibitors (infliximab, adalimumab, golimumab, certolizumab and etanercept), a T-cell activation blocker (abatacept) and a B-cell depleter (rituximab) as well as tocilizumab. However, tocilizumab is the only biologic that has proved to be more efficacious as monotherapy than MTX or other DMARDs. TNF inhibitors require the concomitant use of MTX to achieve their maximal effects, but tocilizumab monotherapy is not inferior to the combination therapy of tocilizumab plus MTX in the suppression of disease activity (48).

Since it is observed that MTX treatment reduces the plasma level of IL-6 but not that of TNF-\(\alpha\) in patients with early RA (49), it is likely that the effect of MTX in RA is partly mediated via inhibition of IL-6 production and this may be one reason why tocilizumab does not require concomitant use of MTX for the maximal effect. Moreover, a direct comparison of tocilizumab and adalimumab, a fully human anti-TNF-\(\alpha\) antibody, demonstrated that monotherapy tocilizumab was superior to adalimumab, as evaluated by several indices of disease activity in RA patients (50). For instance, at week 24, the proportion of patients attaining remission assessed by DAS28 (disease activity score in 28 joints) was 39.9% with tocilizumab and 10.5% with adalimumab. ACR20%, ACR50% and ACR70% response rates were achieved in 65% and 49.4%, 47.2% and 27.8% and 32.5% and 17.9% of patients treated with tocilizumab and adalimumab, respectively. Thus, tocilizumab appears to be the most powerful antirheumatic biologic.

In addition to the outstanding clinical efficacy, tocilizumab has unique properties in comparison with other biologics. First, as described elsewhere, IL-6 promotes the synthesis of acute-phase proteins such as SAA and hepcidin, which are proteins responsible for the development of amyloid A amyloidosis and anemia of chronic disorder, respectively. Indeed, tocilizumab appears to be the most powerful suppressant to reduce the expression of these acute-phase proteins and the treatment reportedly produces prominent ameliorative effects on these complications (51, 52). Second, the predominance of T\(_{17}\) cells over T\(_{reg}\) cells in the effector CD4\(^+\) T-cell subsets, which is thought to be a fundamental immunological abnormality in RA, is possibly induced by continual expression of IL-6 (15); tocilizumab may correct this T\(_{17}\)/T\(_{reg}\) imbalance. The results of recent studies have demonstrated that inhibition of IL-6 function by tocilizumab could rectify the imbalance between T\(_{17}\) and T\(_{reg}\) cells in the peripheral CD4\(^+\) T-cell population (53-55).

The third disease for which tocilizumab is currently on label is systemic juvenile idiopathic arthritis (sJIA), which is a subtype of chronic childhood arthritis that leads to joint destruction, functional disability and growth impairment, and is accompanied by systemic inflammation. A clinical trial, composed of a 6-week open-label lead-in phase and a 12-week double-blind phase, was performed for 56 children with sJIA in Japan (56). At the end of the open-label phase, tocilizumab treatment (8 mg kg\(^{-1}\) every 2 weeks) resulted in ACR Pediatric 30, 50 and 70% responses for 91, 86 and 68% of the patients, respectively. Forty-three patients continued to the double-blind phase and 16 (80%) of 20 patients in the tocilizumab group could maintain an ACR Pediatric 30% response, compared with only 4 (17%) of 23 patients in the placebo group.

A global phase III trial, in which 112 children with active sJIA were enrolled, has also proved that tocilizumab is highly efficacious for the suppression of disease activity of sJIA (57), leading to the acknowledgement that a new era has started in the treatment of this disease, which had long been considered to be one of the most intractable juvenile diseases.

Moreover, various case studies, series and pilot studies of off-label use with tocilizumab have produced favorable results, indicating that tocilizumab may be used for the treatment of various chronic, intractable immune-mediated diseases (9, 10, 39). These include systemic and organ-specific autoimmune diseases, chronic inflammatory diseases, including autoinflammatory syndromes, and other diseases such as atherosclerosis, type 2 diabetes mellitus, atopic dermatitis, sciatrica and amyotrophic lateral sclerosis (Fig. 4). In particular, accumulated evidence provides strong indications that tocilizumab looks highly promising for the treatment of
systemic sclerosis, large-vessel vasculitis, adult-onset Still's disease, amyloid A amyloidosis and polymyalgia rheumatica, for all of which clinical trials are in progress.

Neuromyelitis optica (NMO) is a chronic inflammatory demyelinating disease of the central nervous system that primarily affects the spinal cord and optic nerves. Autoantibodies against the astrocyte water channel protein, aquaporin-4 (AQP-4), play a pathological role in the disease development. Anti-AQP-4 antibodies are produced by the plasmablast population showing a CD19<sup>intermediate</sup>CD29<sup>high</sup>CD38<sup>high</sup>CD180<sup>−</sup> phenotype that is increased in the peripheral blood of NMO patients (58). IL-6 enhances the survival of the plasmablast population, whereas the addition of tocilizumab into the culture diminishes the survival (58). These findings suggest that an IL-6-blockage strategy is promising for the treatment of NMO by inhibiting anti-AQP-4 antibody production. Indeed, the prominent beneficial effects of tocilizumab have been recently reported in patients with NMO that is refractory to conventional treatment regimens (59–61), and a clinical trial of a new fully human antibody against IL-6R (SA237) (62)—generated from tocilizumab by techniques for structural antibody optimization—for NMO is in progress.

In addition to these indications of the potential of tocilizumab for the successful treatment of a variety of chronic diseases, an IL-6-blockade strategy could serve as rescue therapy for acute life-threatening conditions. CRS entails potentially fatal immediate complications and is sometimes induced by non-physiologic T-cell activation after therapies that engage T cells by using a chimeric, modified antigen-receptor or by using a CD19–CD3-bi-specific antibody (blinatumomab) (63). IL-6 and IL-10 as well as the effector cytokine, IFN-γ, have been shown to be markedly elevated in patients with CRS. Surprisingly, one administration of tocilizumab for three patients with CRS receiving T-cell-engaging therapies dramatically resolved their serious conditions (64, 65). These findings suggest that this IL-6R antibody may constitute a novel therapeutic drug for emergent fatal complications mediated by a cytokine storm, such as CRS, SIRS, macrophage activation syndrome, hemophagocytic syndrome or septic shock.

**Conclusions**

Following the successful cloning of the IL-6 gene, IL-6-related research has progressed rapidly (8). Clarification of the whole picture of the IL-6-mediated signaling system solved the long-standing mystery of the functional pleiotropy and redundancy of cytokines. In line with this progress, the pathological role
of IL-6 in various diseases has been thoroughly documented, and this resulted in the development of the humanized anti-IL-6R monoclonal antibody, tocilizumab.

Clinical trials of tocilizumab started in the late 1990s, and this biologic was first approved for the treatment of Castleman disease in Japan in 2005. During the following years, tocilizumab has been adopted as a first-line biologic for the treatment of RA and is currently being used in more than 130 countries. The outstanding efficacy of tocilizumab for sJIA heralded a new era for the treatment of this disease. Moreover, the success of tocilizumab has accelerated the development of other IL-6 inhibitors (24). It is expected that during the next decade IL-6 inhibitors will be widely used for the treatment of various as-yet-intractable diseases, and that their application is certain to overcome the refractory nature of such diseases.

However, a mystery remains as to why excessive or chronic production of IL-6 is associated with various diseases. The findings of further analyses of RNA-binding proteins such as Arid5a and Regnase-1, transcription factors and miRs that regulate IL-6 synthesis may solve this mystery, while clarification of the mechanism(s) involved is likely to result in the identification of more-specific target molecules and lead to further investigations into the pathogenesis of specific diseases.

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Therapeutic uses of anti-IL-6R, tocilizumab

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