Interleukin-6: From arthritis to CAR-T cell therapy and COVID-19

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Abstract

Blockade of interleukin (IL)-6 function by an anti-IL-6 receptor (IL-6R) antibody (tocilizumab, trade name Actemra) has been shown to be effective for the treatment of chronic autoimmune inflammatory diseases including rheumatoid arthritis. Interestingly, treatment with tocilizumab has also been found to alleviate the cytokine storm induced by chimeric antigen receptor (CAR)-T cell therapy. Patients with serious cases of coronavirus disease 2019 (COVID-19) exhibit cytokine release syndrome (CRS), which suggested that tocilizumab might be an effective therapeutic for serious cases of COVID-19. In the first part of this short review, the therapeutic effect of tocilizumab for the disease induced by IL-6 overproduction is described. CRS induced by CAR-T cell therapy and COVID-19 is then discussed.

Keywords: cytokine storm, tocilizumab, cytokine release syndrome

Introduction: pleiotropic functions and the unique receptor system of interleukin 6

Interleukin (IL)-6 was originally discovered as a B-cell differentiation factor that induces B cells to become antibody-producing cells (1). After the cDNA encoding this molecule was first isolated in 1986 (2), subsequent studies with recombinant IL-6 and anti-IL-6 antibodies revealed that this molecule has a wide range of biological functions (3). As shown in Figure 1, IL-6 acts not only on the immune system but also in nearly all tissues and cells, including liver, bone, muscle, and hematopoietic cells and neuronal tissue. In liver cells, IL-6 acts as a hepatocyte-stimulating factor (HSF), induces various acute phase proteins—such as C-reactive protein (CRP), fibrinogen and hepcidin—and inhibits albumin production.

Although the originally identified 80-kDa IL-6 receptor (IL-6R) (4) is expressed on only immune-related tissues and cells, IL-6 is able to have a wide variety of biological functions throughout the body because of the unique IL-6 receptor system (Fig. 2). The IL-6 receptor system is composed of two polypeptide chains. The 80-kDa IL-6R has a short intracytoplasmic portion that is unable to transduce signals into cells. A separate 130-kDa receptor component called gp130 (5) is expressed by all tissues and cells. When IL-6 binds to the 80-kDa IL-6R, the resulting IL-6–receptor complex dimerizes with gp130, after which signals can be transduced through gp130. Additionally, the proteinase ADAM metalloprotease 17 (ADAM17) cleaves membrane-bound IL-6R into soluble IL-6R, which can form a soluble complex of IL-6 and IL-6R. This soluble complex can also bind with gp130 to form a hexamer complex (composed of dimers of IL-6, IL-6R and gp130) that can transduce IL-6 signals.

Because of its unique receptor system, IL-6 is a pleiotropic cytokine. As described below, elevated levels of both IL-6 and soluble IL-6R are found in the serum of patients with various

autoimmune inflammatory diseases or CRSs. Furthermore, as shown in Figure 2, gp130 functions as a signal transducer for not only IL-6 but also several other cytokines, such as leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neutrophic factor (CNTF), IL-11, IL-27, IL-31 and cardiotrophin (CTF). Consequently, all these cytokines show similar redundant and pleiotropic functions (6).

Antibody-based therapy of autoimmune inflammatory diseases

Several clinical observations have suggested that IL-6 overproduction induces various inflammatory phenomena. For example, in a patient with cardiac myxoma (7) who showed a high fever and elevated CRP levels, all their inflammatory symptoms disappeared after the myxoma was surgically removed, and the myxoma tissue showed a high IL-6 titer. Additionally, in a case of Castleman's disease (8) where the patient exhibited high fever and anemia with an elevated CRP level and swelling of multiple lymph nodes, a biopsy of the affected lymph nodes revealed a high IL-6 titer in B cells within the germinal centers. Since these initial observations, IL-6 overproduction has been described in various autoimmune inflammatory diseases (Table 1).

An antibody directed against the 80-kDa IL-6R was prepared to treat these chronic inflammatory diseases. The humanized anti-IL-6R antibody is called tocilizumab. As shown in Figure 2, tocilizumab can block the binding of IL-6 with IL-6R as well as interfere with the formation of IL-6–soluble IL-6R complexes and inhibit gp130 dimerization. This signaling system is called IL-6 trans-signaling (3), and tocilizumab almost completely blocks IL-6 signals by these two pathways (Fig. 2). The clinical effect of tocilizumab is dramatic. It has been successfully used to treat patients with Castleman's disease, juvenile idiopathic arthritis

(9), rheumatoid arthritis (10) and giant cell arteritis (11). Currently, over one million patients in more than 100 countries are treated with this antibody.

One regulatory mechanism of IL-6 production occurs at the mRNA level. Two molecules, endonucleases Regnase-1 (12) and Arid5A (13), act at the stem–loop of the 3′-untranslated region of IL-6 mRNA. Regnase-1 is involved in the degradation of mRNA, and it downregulates IL-6 production. Arid5A binds to the same portion of the IL-6 mRNA, where it competes with Regnase-1 to protect the mRNA from degradation. Under normal conditions, Arid5A is present mainly in nuclei, but when inflammation occurs, it moves to the cytoplasm and stabilizes IL-6 mRNA, resulting in the overproduction of IL-6.

Cytokine release syndrome by chimeric antigen receptor T cell therapy

B precursor-acute lymphoblastic leukemia (B-ALL) is one of the most common childhood malignancies. The recently reported strategy of using chimeric antigen receptor (CAR)-modified T cells directed against the B-cell antigen cluster differentiation 19 (CD19) can effectively treat refractory B-ALL in children (14). CAR binding with CD19 B-ALL results in T-cell activation, antigen-mediated cell killing, and T-cell proliferation.

The robust expansion and sustained proliferation of the modified CAR-T cell population can eradicate leukemic cells and appears to sustain remission in many patients. However, this robust T-cell proliferation also activates macrophages to produce inflammatory cytokines, particularly IL-6, which causes a unique, significant side effect of CAR-T cell therapy, cytokine release syndrome (CRS). However, tocilizumab administration was found to effectively treat the CAR-T cell-induced CRS, which likely saved CAR-T therapy. In the initial case for which the use of tocilizumab was tested, the patient's high fever went down,

low blood pressure went up, and serum IL-6 and CRP levels returned to normal only a couple of hours after tocilizumab administration (15).

As shown in Figure 3, tocilizumab blocks the function of large amounts of IL-6 secreted from T cell-stimulated macrophages and prevents CRS. Importantly, although steroid hormones such as dexamethasone can also prevent CRS, they block the antitumor effect of CAR-T cells. CAR-T cell therapy has now been approved for clinical use in combination with tocilizumab for preventing CRS (15).

CRS induced by systemic inflammatory responses to infection

Excessive immune responses to bacterial or viral infections or tissue injury induce the overproduction of various cytokines, including IL-6, IL-10, interferon (IFN)-γ, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor (TNF)-α, IL-2 and IL-8. Elevated levels of these cytokines are associated with the clinical manifestation of CRS. As shown in Figure 4, patients with sepsis, acute respiratory distress syndrome (ARDS) or burns showed strikingly high serum levels of the cytokines IL-6, IL-8, MCP-1, and IL-10, while the levels of TNF-α, IL-1β, IL-12p40, IFN-α, IFN-γ, IL-17, and IL-4 were not significantly different from those in the healthy controls. The clinical severity of systemic inflammation is associated with vascular endothelial injuries and coagulopathy owing to the induction of vascular leakage and tissue hypoxia, which results in hypotension and multiple organ failure.

Plasminogen activator inhibitor-1 (PAI-1) levels are elevated in cases of systemic inflammation, especially sepsis-induced intravascular coagulation (16), suggesting that the PAI-1 concentration is a predictor of CRS disease progression (Fig. 4). Importantly, serum IL-6 levels were positively correlated with elevated IL-8, IL-10, MCP-1 and PAI-1 levels in

cases of sepsis, ARDS and burns. The elevated PAI-1 levels in CRS from various causes, such as sepsis, ARDS and burns, indicate that vascular endothelial cells play a major role in CRS. Indeed, *in vitro* stimulation of vascular endothelial cells with IL-6 and soluble IL-6R was found to induce increases in the levels of PAI-1, IL-6 and several other cytokines.

Because vascular endothelial cells do not express IL-6R, a complex of IL-6 and soluble IL-6R stimulates the gp130 expressed by endothelial cells via IL-6 trans-signaling. This mechanism can explain the inhibitory effect of tocilizumab on vascular endothelial cells. Thus, tocilizumab blocks the production of IL-6 and several other cytokines, as well as that of PAI-1, by the mechanism of IL-6 trans-signaling. Our *in vivo* and *in vitro* studies showed that over-reactive endothelial cells are a critical factor in acute and systemic inflammation and multiple organ disorders.

Recently, coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was found to be associated with a broad spectrum of clinical features, such as interstitial pneumonia. Patients with COVID-19 develop ARDS and thrombosis, leading to severe vasculopathy. Notably, a critical feature of COVID-19 is the vascular changes associated with disease severity. For cases reported in Wuhan, IL-6 was one of the pivotal cytokines associated with lung damage progression during the COVID-19-induced cytokine storm (17). This observation suggested that an inhibition of IL-6 signaling could be effective for treating COVID-19-related pneumonia as well as CRS. The first trial of tocilizumab in severe COVID-19 patients, which occurred in Wuhan, indicated that tocilizumab has potential as an effective treatment; it improved the clinical symptoms, computed tomography (CT) opacity changes, and CRP concentrations of the treated patients (17).

Our retrospective data also indicate that patients with severe COVID-19 display a mild elevation in their levels of IL-6 and several other cytokines, along with a striking elevation of PAI-1 levels similar to those seen in patients with bacterial sepsis or ARDS (Fig. 4). Notably, tocilizumab treatment decreased the levels of both IL-6 and PAI-1 and also improved some clinical pneumonia symptoms in patients with severe COVID-19 (16). These data indicate that IL-6 signaling in the vascular endothelium plays pivotal roles in coagulation activation and thrombosis during the cytokine storm observed in patients with COVID-19.

Numerous clinical trials of tocilizumab as a COVID-19 treatment have now been conducted, but their results are inconsistent. In patients with moderate cases of COVID-19, no support was found for beneficial effects of tocilizumab treatment (18). For patients who were critically ill with COVID-19, the EMPACTA trial reported that tocilizumab treatment was associated with a reduction in the requirement for mechanical ventilation but did not improve the overall mortality (19). However, the REMAP-CAP trials in the United Kingdom, which assessed the effectiveness of a blockade of IL-6R signaling via tocilizumab or another anti-IL-6R antibody, sarilumab, found that both antibodies improved survival in patients with severe COVID-19 (21). These results suggest the beneficial efficacy of IL-6R antagonists in patients with COVID-19.

As mentioned, a complex of IL-6 and IL-6R transduces signals into the inside of cells through gp130. Dimerization of gp130 activates JAK–STAT3 signaling pathways. As expected, blockade of JAK–STAT3 signaling pathway by the anti-JAK kinase, baricitinib shows beneficial effects together with remdesivir in hospitalized patients (20).

Compared with the CRS associated with CAR-T cell therapy and other causes, the elevated serum IL-6 levels in patients with COVID-19 are 100-fold lower, but the PAI-1

levels are comparable. Thus, although the beneficial effect of tocilizumab for treating COVID-19 remains controversial, the treatment appears to be effective for vascular damage. The network of signal transduction between IL-6 and PAI-1 remains an interesting question to be addressed in future work.

Conclusions

As described here, IL-6 not only exhibits a wide variety of biological activities but also is involved in chronic autoimmune inflammation and can induce the acute shock condition known as a cytokine storm. A blockade of IL-6 signals produces significant clinical benefits for both autoimmune diseases and CRSs. In this respect, the blockade of IL-6 signals with a steroid hormone has similar effects, as demonstrated by the clinical benefit of dexamethasone in the treatment of severe COVID-19. However, unlike treatment with steroid hormones, IL-6 signal inhibition does not globally suppress immune function. At present, how IL-6 can induce both acute and chronic inflammation remains incompletely understood. It is also unknown how IL-6 production is regulated, which is important because constitutive IL-6 production occurs in various diseases. This review conveys the contents of my lecture given for the Japanese Society of Immunology in 2020.

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Conflict of interest statement

The author declares that T.K. holds the patent for Actemra.

Abbreviations

CRS, cytokine release syndrome

IL, interleukin

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Figure legends

Figure 1. Pleiotropic functions of IL-6. IL-6 has multiple biological activities in immune cells and in various organs.

Figure 2. IL-6 receptor signaling pathways, and the action of anti-IL-6R antibody, which blocks both direct IL-6 binding and trans-signaling. The humanized anti-IL-6R antibody, tocilizumab inhibits both classic and trans-signaling of IL-6 receptor pathways.

Figure 3. Scheme of anti-IL-6R antibody treatment in the CAR-T cell induced cytokine storm. The anti-IL-6R antibody tocilizumab ameliorates CRS without impairing the cytotoxic effect of CAR-T cells.

Figure 4. Scheme of anti-IL-6R antibody treatment in the COVID-19 induced cytokine storm. SARS-CoV-2 directly infects vascular endothelial cells, leading to excessive pro-inflammatory cytokines and PAI-1 production. Tocilizumab treatment can inhibit these inflammatory circuits as an effective therapy for the cytokine storm.

Table 1. Diseases associated with IL-6 overproduction

Chronic immune diseases
Cardiac myxoma
Castleman's diseases
Rheumatoid arthritis
Systemic onset of JIA
Adult onset Still's diseases
Progressive sclerosis
Reactive arthritis
Polymyalgia rheumatica
Aortitis (Takayasu diseases)
Giant cell arteritis
Neuromyelitis optica

Figure 1

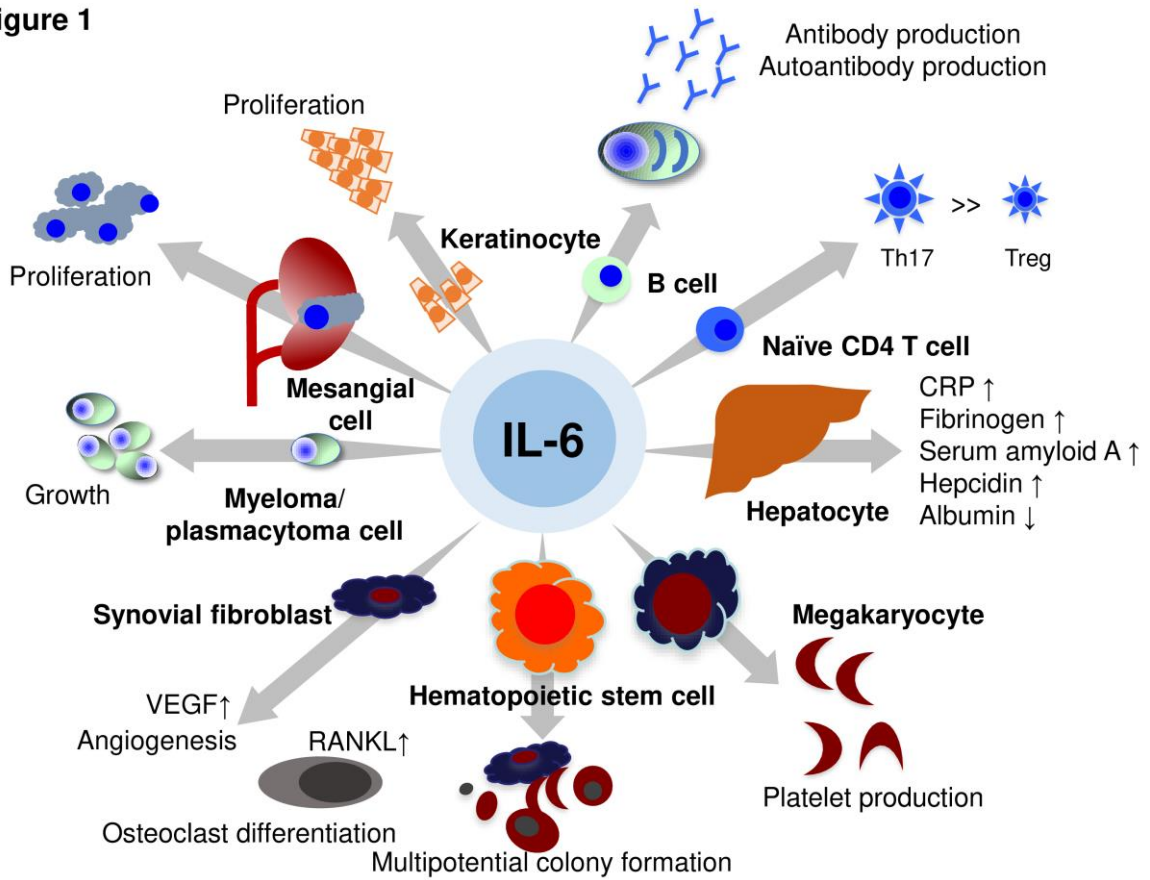
Antibody production
Autoantibody production
Proliferation
Proliferation
Keratinocyte
B cell
Th17
>>
Treg
Naïve CD4 T cell
Mesangial cell
IL-6
CRP ↑
Fibrinogen ↑
Serum amyloid A ↑
Hepcidin ↑
Albumin ↓
Hepatocyte
Growth
Myeloma/ plasmacytoma cell
Synovial fibroblast
Megakaryocyte
Hematopoietic stem cell
VEGF↑
Angiogenesis
RANKL↑
Osteoclast differentiation
Platelet production
Multipotential colony formation

Figure 2

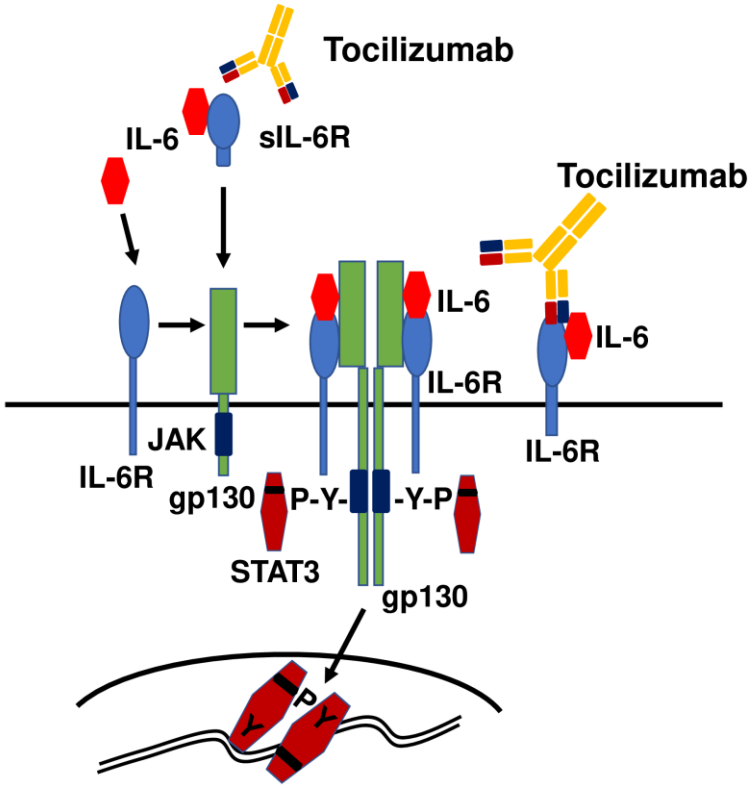

Figure 3

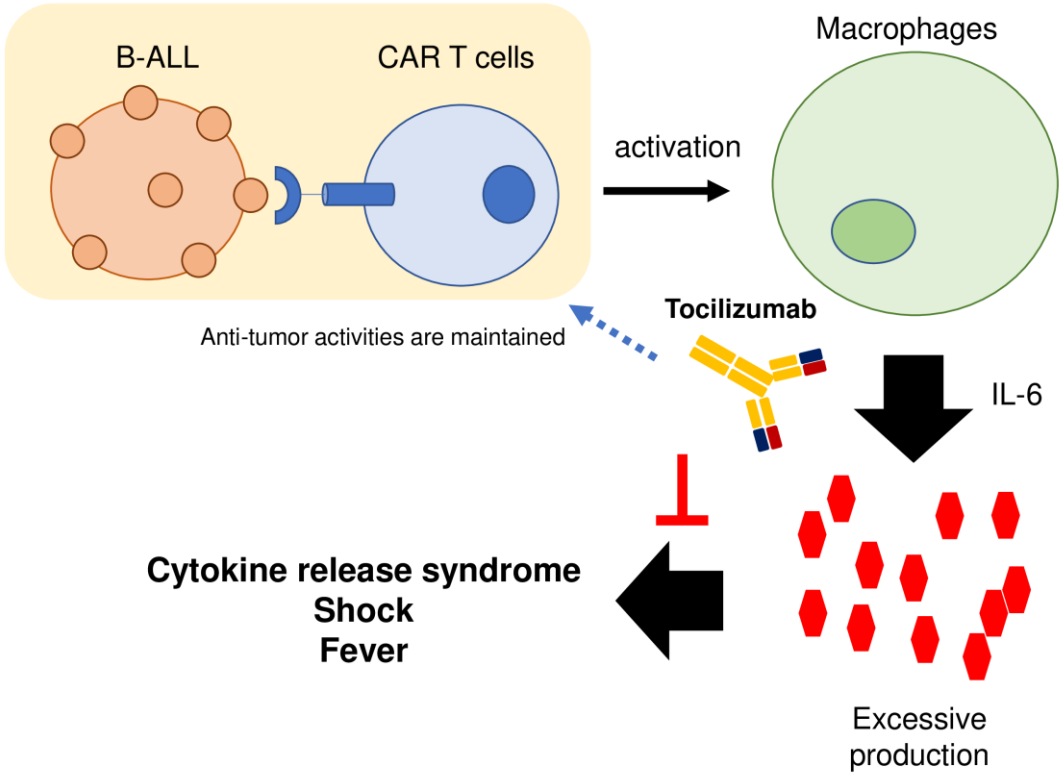

Figure 4