Review Article

Interleukin-6; pathogenesis and treatment of autoimmune inflammatory diseases

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Interleukin-6 (IL-6), originally identified as a B cell stimulatory factor-2, is a typical cytokine featuring redundancy and pleiotropic activity. A transient expression of IL-6 participates in host defense against acute environmental stress such as infections and injuries by activating immune responses, hematopoiesis and acute phase reactions. However, its abnormal, persistent production plays an important pathological role in the development of various autoimmune inflammatory diseases, so that it was hypothesized that IL-6 blockade would constitute a novel strategy for the treatment of such diseases and to this purpose tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody, was developed. Clinical trials have indeed proved the efficacy and tolerable safety of tocilizumab for patients with moderate to severe rheumatoid arthritis, and it is now used as a "made-in-Japan" innovative biologic for rheumatoid arthritis in more than 90 countries worldwide, as well as for patients with Castleman's disease and systemic and polyarticular juvenile idiopathic arthritis. Moreover, favorable results of recent clinical trials or case reports of off-label use with tocilizumab indicate that it is likely to be broadly applicable for the treatment of various autoimmune inflammatory diseases. Its wider application for various diseases as well as clarification of the mechanism(s) through which IL-6 blockade becomes clinically efficacious and of the etiology of dysregulated persistent IL-6 production in such diseases are important issues for future studies.

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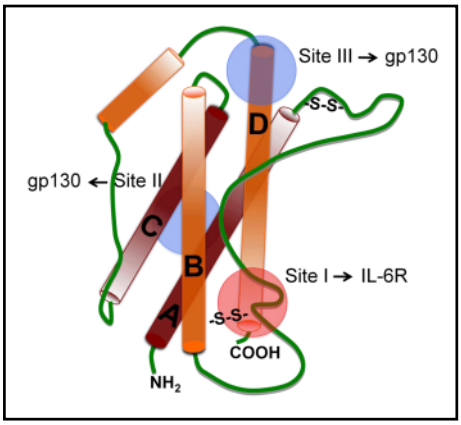

Fig.1 The human IL-6 structure

Schematic representation of the human IL-6 structure shows the four long α-helices, from A to D and three connecting loops. Two disulfide bonds are contained in loop A-B. Site I on the C-terminal end of helix D interacts with IL-6R, site II, located on helices A and C, interacts with one gp130, while site III on the N-terminal end of helix D interacts with another gp130.

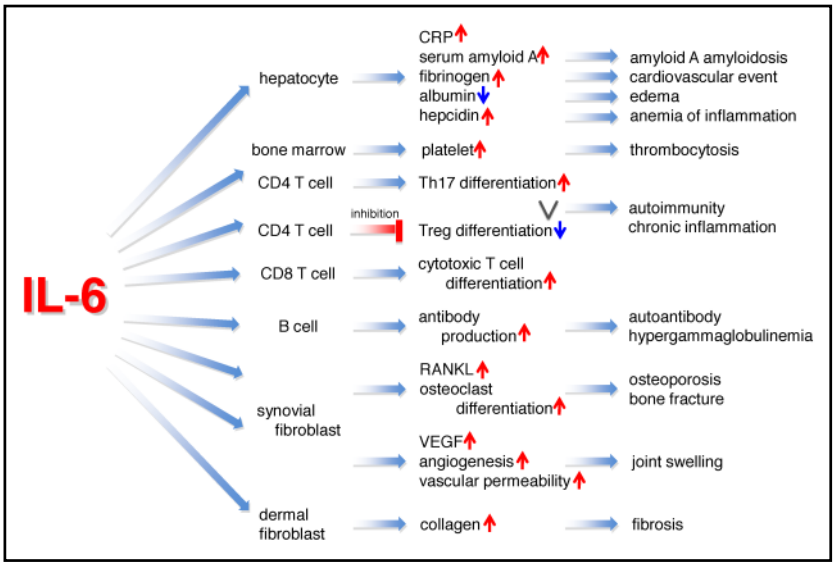

Fig.2 Pleiotropic activity of IL-6

IL-6 induces specific gene expression and cell differentiation. It induces production of acute phase proteins such as CRP, serum myloid A, fibrinogen, and hepcidin, whereas it reduces synthesis of albumin in hepatocytes. In bone marrow, IL-6 induces maturation of megakaryocytes into platelets. In addition, IL-6 induces Th17 differentiation from naïve CD4-positive T cells, whereas it inhibits Treg differentiation. It also induces cytotoxic T cell differentiation from CD8-positive T cells, and immunoglobulin synthesis in activated B cells. Moreover, IL-6 acts on synovial fibroblasts to produce RANKL and VEGF, which promote differentiation of osteoclasts and angiogenesis, respectively. Finally, IL-6 stimulates dermal fibroblasts to produce collagen. CRP: C-reactive protein, Treg: regulatory T cells, RANKL: receptor activator of NF-κB ligand, VEGF: vascular endothelial growth factor

Discovery of interleukin-6

Interleukin-6 (IL-6) is a soluble mediator with a wide range of biological activities. In the early stages of research, various and distinct functions of IL-6 were being studied and each research group had its own name for this cytokine, based on the function their research focussed on. One name was B cell stimulatory factor 2 (BSF-2), since it induces B cell differentiation into antibody (Ab)-producing cells[1], another was hepatocyte-stimulating factor (HSF), derived from the activity of acute phase protein synthesis in hepatocytes, and yet another was hybridoma growth factor (HGF) reflecting its promotion of growth of fusion cells with myeloma, or interferon (IFN) β2 because of its IFN anti-viral activity. In 1986, the cDNA of BSF-2 was successfully cloned[2], and since the cytokines with their various names were found to be identical, it was then renamed IL-6[3]. Human IL-6 consists of 212 amino acids including a 28-amino-acid signal peptide and its gene has been mapped to chromosome 7p21. The structure of IL-6 includes four helix bundles in an up-up-down-down topology (labelled A to D in Fig.1) and three loops (two long ones, A-B and C-D, and a short one, B-C)[4].

Biological function of IL-6

In infected lesions, IL-6 is produced by monocytes and macrophages after stimulation by Toll-like receptors (TLRs) with their microbial motifs preserved. In noninfectious inflammations such as burn or trauma, damage-associated molecular patterns (DAMPs) from damaged or dying cells stimulate TLRs to produce IL-6. IL-6 then mediates inflammatory signals from localized lesions to other parts of the body to provide protection against emergent events. The IL-6 carried via the bloodstream to the liver rapidly induces a wide spectrum of acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, haptoglobin and α1-antichymotrypsin, but also reduces the production of fibronectin, albumin and transferrin (Fig.2)[5]. These biological actions on hepatocytes were initially studied when the focus was on HSF. Induction of hepcidin by IL-6 causes hypoferremia because it blocks iron transporter

ferroportin 1 on gut, leading to anemia of inflammation. When IL-6 reaches bone marrow, it promotes maturation of megakaryocytes, which instigates the release of platelets. These changes in acute phase protein levels and red blood cell and platelet counts are used in clinical laboratory tests for the diagnosis and evaluation of inflammatory severity. IL-6 also performs an important function in acquired immunity by promoting specific differentiation of T and B cells. As for T cells, IL-6 together with transforming growth factor (TGF)-β has been shown to be essential for Th17 differentiation from naïve CD4-positive T cells, whereas IL-6 inhibits TGF-β-induced regulatory T cell (Treg) differentiation. The resultant up-regulation of the Th17/Treg balance breaks immunological tolerance and thus leads to the development of autoimmune or chronic inflammatory diseases[6]. IL-6 also acts on CD8-positive T cells to induce cytotoxic T cells. As for B cells, IL-6 induces activated B cells to differentiate into Ab-producing plasma cells. This function was a property of IL-6 discovered when the focus was on BSF-2. Besides its effects on hepatocytes and lymphocytes, IL-6 has various other effects that are associated with inflammatory symptoms[3, 5]. For example, IL-6 production in bone marrow stromal cells induces receptor activator of the nuclear factor kappa B (NF-κB) ligand (RANKL), which is an essential molecule for the differentiation and activation of osteoclasts that induce bone resorption. Furthermore, enhanced angiogenesis and vascular permeability are pathological features of inflammatory sites such as synovial tissues of rheumatoid arthritis (RA), and these pathological changes are due to the excess production of vascular endothelial growth factor (VEGF), which is also induced by IL-6. It has also been demonstrated that IL-6 promotes the proliferation of keratinocytes or collagen production in dermal fibroblasts.

Signaling system of IL-6

The IL-6 receptor (IL-6R) system components are made up of two chains, the IL-6-binding chain IL-6R and the signal-transducing chain gp130, both of which have a Trp-Ser-X-Trp-Ser motif and belong to the cytokine receptor family[7-10]. The soluble form of IL-6R (sIL-6R) without the cytoplasmic region is present in human serum[11]. IL-6 also binds to sIL-6R and evokes the IL-6 signal on cells expressing gp130 but not transmembrane IL-6R, while the gp130 expression on the broad range of cells explains why IL-6 has pleiotropic effects on various organs. After binding of

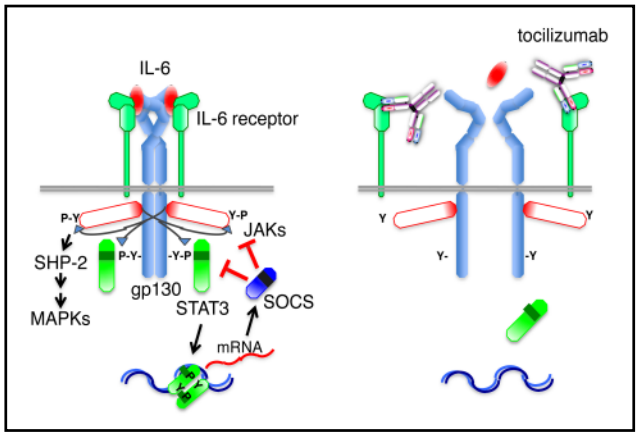

Fig.3 The IL-6 receptor system

The figure on the left shows the IL-6 receptor system. After binding of IL-6 to the IL-6 receptor (IL-6R), the IL-6/IL-6R complex associates with gp130 and induces homodimerization of gp130, which triggers activation of JAKs and tyrosine-phosphorylation of cytoplasmic part of gp130. The phosphorylated gp130 then recruits STAT3 via the SH2-domain. Tyrosine-phosphorylated gp130 also recruits SHP-2 and activates the MAPK pathway. Next, activated STAT3 translocates into the nucleus and regulates transcription for various sets of genes including SOCS, which binds to JAK or gp130 and turns off IL-6 signals. The figure on the right shows that tocilizumab binds to transmembrane and soluble IL-6R and inhibits IL-6 binding to both types of IL-6R. JAKs: Janus kinase family tyrosine kinases, STAT3: signal transducer and activator of transcription 3, SHP-2: SH2-domain containing protein tyrosine phosphatase-2, MAPKs: mitogen-activated protein kinase, SOCS: suppressor of cytokine signaling

IL-6 to IL-6R, the resultant IL-6/IL-6R complex in turn induces homodimerization of gp130 and triggers a downstream signalling cascade (Fig.3). The activated IL-6 receptor complex is formed as a hexameric structure comprising two molecules each of IL-6, IL-6R and gp130[12]. For formation of this complex, IL-6 provides one IL-6R binding site (site I) and two gp130 binding sites (sites II and III) (Fig.1) and these are assembled into a hexameric structure. IL-6R is an unique binding-receptor for IL-6, whereas signal-transducing chain gp130 is shared by members of the IL-6 family of cytokines[9, 10]. These members include leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), IL-11, cardiotrophin 1 (CTF1), cardiotrophin-like cytokine (CLC), IL-27 and IL-35. These cytokines bind to their specific binding receptors but use the gp130 they have in common to transduce their signal. The only exception is virus-encoded IL-6 (vIL-6), which is the product of Kaposi's sarcoma-associated herpes virus (also known as human herpes virus 8), and di-

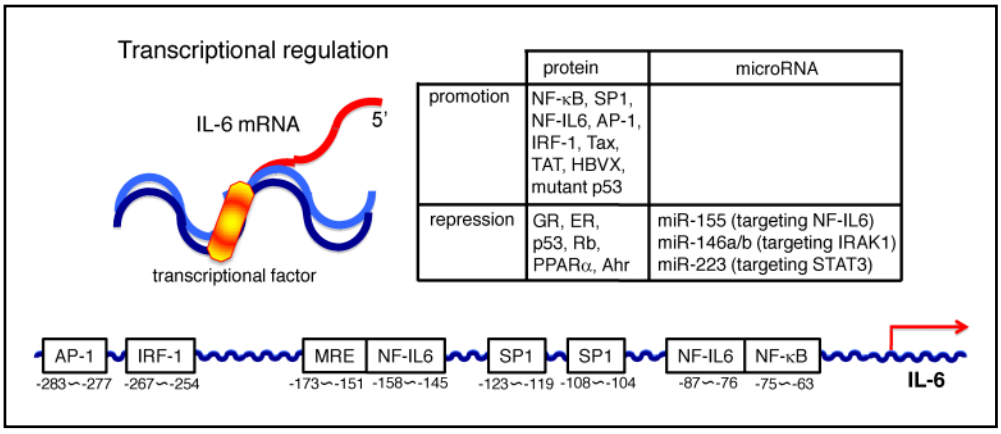

Fig.4 Transcriptional regulation of IL-6 gene

IL-6 gene activation is positively or negatively regulated by transcriptional factors or microRNAs. Several cis-acting elements are shown. NF-κB: nuclear factor kappa B, SP1: specificity protein 1, NF-IL6: nuclear factor IL-6, AP-1: activator protein 1, IRF-1: interferon regulatory factor 1, Tax: HTLV-1-derived transactivator protein, TAT: HIV-1 transactivator of the transcription, HBVX: hepatitis B virus X protein, GR: glucocorticoid receptor, ER: estrogen receptor, Rb: retinoblastoma, PPARα: peroxisome proliferator-activated receptor α, Ahr: aryl hydrocarbon receptor, miR: microRNA, IRAK1: interleukin-1 receptor-associated kinase 1, STAT3: signal transducer and activator of transcription 3, MRE: multiple response element

rectly binds to and activates gp130[13]. The model in which the IL-6 cytokine family members use the common signal-transducing chain makes it clear why these members show functional redundancy. Activated gp130 triggers activation of the Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3) pathway and the JAK-SH2-domain containing protein tyrosine phosphatase-2 (SHP-2)-mitogen-activated protein (MAP) kinase pathway. In this context, STAT3 is the transcriptional factor that regulates various sets of IL-6 responsive genes including acute phase proteins. STAT3 also induces a suppressor of cytokine signaling 1 (SOCS1) and SOCS3 which possess the SH2-domain. SOCS1 then binds to tyrosine-phosphorylated JAK, whereas SOCS3 binds to tyrosine-phosphorylated gp130, to attenuate the IL-6 signal in the negative feedback loop[14, 15].

Regulatory mechanism of IL-6 production

IL-6 is produced by not only immune-mediated cells but also mesenchymal cells, endothelial cells, fibroblasts and many other cells in response to various stimuli including TLR ligands, IL-1 and tumor necrosis factor (TNF)[16]. Since IL-6 provides an SOS signal to indicate occurrence of an emergency, the synthesis of IL-6 is strictly regulated through both gene transcription and posttranscription levels[16-18]. A number of transcription factors have been shown to regulate the IL-6 gene activation (Fig.4). Functional cis-regulatory elements in the human IL-6 gene 5' flanking region include NF-κB, specificity protein 1 (SP1), nuclear factor IL6 (NF-IL6) (also know as CAAT/enhancer-binding protein beta (C/EBPβ)), activator protein 1 (AP-1), and interferon regulatory factor 1 (IRF-1) binding sites. Stimulation with IL-1, TNF, TLR-mediated signal and forskolin activate cis-regulatory elements to activate IL-6 promoter. Some virus products also modulate the DNA binding activity of NF-κB and NF-IL6 to enhance transcription of IL-6 mRNA. For example, human T lymphotropic virus 1 (HTLV-1) activates IL-6 production via the interaction of the virus-derived transactivator protein (Tax) with NF-κB. Human immunodeficiency virus 1 (HIV-1) transactivator of the transcription (TAT) protein enhances NF-κB and NF-IL6 DNA binding activity. Moreover, the human hepatitis B virus X protein enhances the DNA binding of NF-IL6. On the other hand, some transcription factors suppress IL-6 expression. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors consisting of three subtypes, α, β and γ. Fibrate-activated PPARα interacts with c-Jun and the p65 NF-κB subunit, which negatively regulates IL-6 transcription. Some hormone receptors reportedly suppress IL-6 expression. For instance, the activation of glucocorticoid receptor can suppress IL-6 expression, which is one of the mechanisms responsible for

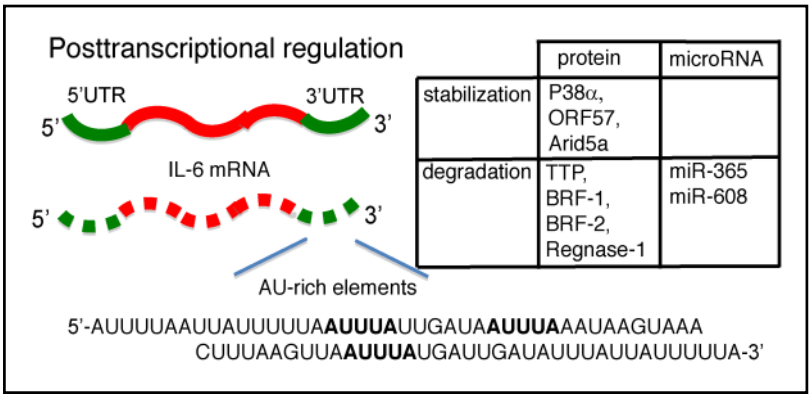

Fig.5 Posttranscriptional regulation of IL-6 mRNA

IL-6 expression is posttransciptionally regulated by several RNA binding proteins or microRNAs. AU-rich elements (AREs) located in 3'untranslated region (UTR) of IL-6 mRNA are shown. ORF: open reading frame, TTP: tristetraprolin, BRF-1: butyrate response factor-1, miR: microRNA

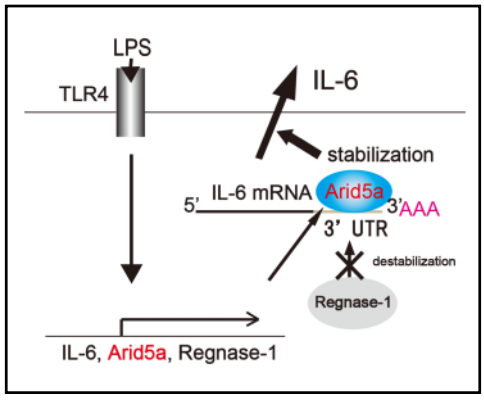

Fig.6 Arid5a counteracts the destabilizing effect of regnase-1 on IL-6 mRNA

Regnase-1 accerates IL-6 mRNA degradation, whereas arid5a conteracts the destabilizing effect of regnase-1. The balance between arid5a and regnase-1 plays an important role in IL-6 mRNA stability. LPS: lipopolysaccharide, TLR4: Toll-like receptor 4, UTR: untranslated region

the anti-inflammatory effects of corticosteroids, while suppression of IL-6 expression by estrogen receptors is associated with an increase in serum IL-6 after menopause or ovarectomy. Retinoblastoma protein and p53 have been demonstrated to repress the IL-6 gene promoter, whereas mutant p53 upregulates the IL-6 promoter. It was also found that the aryl hydrocarbon receptor (Ahr), a ligand-activated transcriptional regulator that binds to dioxin and other exogenous contaminants, forms a complex with NF-κB and inhibits the promoter activity of IL-6. In addition, some microRNAs regulate the activity of transcription factors directly or indirectly. MicroRNA-155 interacts with the 3'-untranslated region (UTR) of NF-IL6 and suppresses transcription of NF-IL6, while microRNA-146a/b and -223 suppress transcription of IL-6 indirectly by targeting interleukin-1 receptor-associated kinase 1 (IRAK1) and STAT3.

Cytokine mRNA is regulated posttranscriptionally through both the 5'- and 3'- UTRs[17, 18]. The 5'UTR dictates initiation of mRNA translation, while the 3'UTR determines the stability of mRNA. IL-6 mRNA is regulated by modulation of AU-rich elements (AREs) located in the 3'UTR region. A number of RNA-binding proteins and microRNAs bind to the 3'UTRs and regulate the stability of IL-6 mRNA (Fig.5). For example, MAP kinase (MAPK) p38α promotes IL-6 mRNA stabilization via 3'UTR of the IL-6, and KSHV open reading frame (ORF) 57 promotes the stabilization of both viral and human IL-6 mRNA by competing with the binding of microRNA-1293 to the viral or of microRNA-608 to the human IL-6 mRNA. Conversely, RNA-binding proteins such as tristetraprolin (TTP), butyrate response factor-1 (BRF-1) and BRF-2 promote IL-6 mRNA degradation, while microRNAs such as microRNA-365 and -608 reduce IL-6 mRNA levels through direct interaction with IL-6 3'UTR.

Recently it was found that a regulatory RNase-1 (regnase-1) (also known as Zc3h12a) is a nuclease involved in destabilization of IL-6 mRNA and the knockout mice developed spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy[19]. The inhibitor of NF-κB (IκB) kinase (IKK) complex controls IL-6 mRNA stability by phosphorylating regnase-1 in response to IL-1R/TLR stimulation. Phosphorylated regnase-1 underwent ubiquitination and degradation. Regnase-1 re-expressed in IL-1R/TLR-activated cells exhibited delayed kinetics, and regnase-1 mRNA was found to be negatively regulated by regnase-1 itself via a stem-loop region present in the regnase-1 3'UTR. These findings demonstrate that IKK complex phosphorylates not only IκBα, activating transcription, but also regnase-1, releasing the brake on IL-6 mRNA expression[20]. Moreover, we have recently identified a novel RNA-binding protein, AT-rich interactive domain-containing protein 5a (arid5a), which selectively stabilizes IL-6 but not TNF-α or IL-12 mRNA through binding on the 3'UTR of IL-6 mRNA (Masuda T, Kishimoto T, et al. Manuscript in submission). Arid5a was enhanced in mac-

rophages in response to LPS, IL-1β and IL-6, and also induced under Th17 polarizing conditions. Arid5a gene deficiency developed elevation of IL-6 level in lipopolysaccharide (LPS)-injected mice, and preferential Th17 cell development in naïve T cells. Finally, arid5a counteracted the destabilizing function of regnase-1, indicating that the balance between arid5a and regnase-1 plays an important role in IL-6 mRNA stability and predominance of arid5a over regnase-1 promotes inflammatory processes and possibly induces the development of autoimmune inflammatory diseases (Fig.6).

Pathogenesis of IL-6 in the development of autoimmune inflammatory diseases

When IL-6 is synthesized transiently, it promptly participates in the host defense against environmental stress such as infections and injuries by triggering a broad spectrum of biological events. Once the source of stress is removed from the host, IL-6-mediated activation of the signal transduction cascade is terminated by negatively regulatory mechanisms in conjunction with the normalization of serum IL-6 and CRP levels. However, dysregulated, persistent IL-6 production has been implicated in the development of various autoimmune and chronic inflammatory diseases and even cancers[3, 5]. The pathological significance of IL-6 for diseases was first demonstrated in a case of cardiac myxoma. The patient presented with fever, arthritis with positivity for anti-nuclear factor, an elevated CRP level and hypergammaglobulinemia and was diagnosed with undifferentiated connective tissue disease. The culture fluid obtained from the myxoma tissues of this patient contained a large quantity of IL-6[21], which suggested that IL-6 might pathologically contribute to chronic inflammation and autoimmunity. Subsequent studies have shown that dysregulation of IL-6 production is implicated in the pathogenesis of Castleman's disease, RA, multiple myelomas and various other autoimmune, inflammatory and malignant diseases[3, 5, 22]. The fact that all clinical symptoms and laboratory findings become normalized after surgical removal of myxoma tissue in cardiac myxoma[23] or involved lymph nodes in Castleman's disease cases[24] indicates that IL-6 production by these tissues plays a central role in the development of systemic inflammation and autoimmunity. However, the reason(s) why such dysregulated continuous IL-6 production is induced in various diseases remains to be clarified and elucidation of the mechanism(s) underlying persistent IL-6 synthesis is of particular importance to gain a better understanding of their pathogenesis.

Furthermore, numerous animal models of diseases have also disclosed the pathological role of IL-6 in disease development and that IL-6 blockade by means of gene-knockout or administration of anti-IL-6 or anti-IL-6R Ab can suppress such disease development either preventively or therapeutically[5, 18, 25]. For example, IL-6 blockade demonstrably limited susceptibility to Castleman's disease-like symptoms in IL-6 transgenic mice, as well as in various mouse models of RA, systemic lupus erythematosus, systemic sclerosis, polymyositis, uveitis, multiple sclerosis, asthma, and many other diseases.

IL-6 targeting as strategy for autoimmune inflammatory diseases

Because of the pathological role of IL-6 in various autoimmune inflammatory diseases, it was hypothesized that IL-6 blockade would constitute a novel treatment strategy for such diseases. For this reason, tocilizumab, a humanized anti-IL-6R monoclonal Ab of the IgG1 class was generated by grafting the complementarity determining regions of a mouse anti-human IL-6R Ab onto human IgG[26]. Tocilizumab blocks IL-6-mediated signal transduction by inhibiting IL-6 binding to transmembrane and soluble IL-6R (Fig.3). If free serum tocilizumab concentration is maintained at more than 1 μg/ml, CRP remains negative, so that the serum CRP level is a hallmark for *in vivo* confirmation of whether IL-6 activity is completely blocked[27]. The first clinical study of tocilizumab was performed with seven patients with multicentric plasma cell or mixed type Castleman's disease[28]. Immediately after administration of tocilizumab, fever and fatigue disappeared, while anemia as well as serum levels of CRP, fibrinogen, and albumin started to improve. After three months of treatment, hypergammaglobulinemia and lymphadenopathy were markedly alleviated. These findings indicate that IL-6 contributes significantly to the development of Castleman's disease and that IL-6 blockade by anti-IL-6R Ab is a promising therapeutic strategy for IL-6-related diseases. Various subsequent clinical trials of tocilizumab have indeed verified its outstanding efficacy and tolerable safety profile, which have resulted in its approval for the treatment of several diseases. Tocilizumab is currently approved for the treatment of RA in more than 90 countries worldwide, of

systemic juvenile idiopathic arthritis (JIA) in Japan, India, the EU and the USA, and of polyarticular JIA and Castleman's disease in Japan and India[5, 18, 25].

Efficacy of tocilizumab for rheumatoid arthritis

RA is a chronic, progressive inflammatory diseases of the joints and surrounding tissues accompanied by intense pain, irreversible joint destruction and systemic complications such as fatigue, anemia and fever. IL-6 plays a major role in RA development since it has been shown that IL-6 causes immunological abnormalities such as an imbalance between Th17 and Treg as well as autoantibody production including rheumatoid factor and anti-citrullinated peptide Ab, systemic inflammation and local inflammation causing joint destruction[29]. IL-6 can activate endothelial cells to produce IL-8, monocyte chemoattractant protein, expression of adhesion molecules, and recruitment of leukocytes to involved joints. As described elsewhere, IL-6 can induce synoviocyte proliferation, osteoclast differentiation and VEGF production, while in combination with IL-1 it enhances the production of matrix metalloproteinases from synovial cells, which may lead to cartilage and joint destruction. We also found that the synovial fluids from patients with RA contained more IL-6 than those from patients with osteoarthritis[30]. Moreover, in antigen-induced arthritis, an experimental animal model of RA, IL-6 gene-knock-out mice did not show any significant inflammation in the joints[31]. These findings, indicating that IL-6 is an essential molecule for the development of RA, have led to clinical trials of tocilizumab for treatment of RA.

In a multicenter, double-blind, placebo-controlled phase II trial in Japan, 164 patients with refractory RA were randomized to receive either tocilizumab (4 or 8 mg/kg) or placebo once every 4 weeks[32]. After 12 weeks, American College of Rheumatology (ACR) 20% improvement response was observed in 78%, 57% and 11% of RA patients treated with 8, 4 mg/kg of tocilizumab and the placebo, respectively. Seven phase III trials of tocilizumab that were subsequently performed globally as well as in Japan demonstrated its outstanding efficacy and tolerable safety profile either as monotherapy or in combination with disease-modifying antirheumatic drugs (DMARDs) for adult patients with moderate to severe RA. The SAMURAI and the LITHE trials demonstrated that progress of joint destruction was significantly suppressed by tocilizumab treatment[33, 34]. Moreover, the RADIATE study showed that at 24 weeks ACR20 response was attained in response to 8 mg/kg, 4 mg/kg of tocilizumab and placebo by 50.0%, 30.4% and 10.1%, respectively, of patients with RA refractory to TNF inhibitor therapy[35]. Currently tocilizumab is approved in more than 90 countries for the treatment of RA, and actual medical practice has confirmed its efficacy[29, 36].

Efficacy of tocilizumab for systemic juvenile idiopathic arthritis

JIA is not a single disease, but a term that encompasses all forms of arthritis that begin before a patient is 16 years old, persist for more than 6 weeks and are of unknown origin. Systemic JIA (SJIA) is characterized by systemic features such as daily spiking fever, salmon-colored macular rash, serositis, lymphadenopathy and hepatosplenomegaly as well as arthritis. Although its precise pathogenesis remains to be determined, IL-6 was found to play a major role in its development[37]. Serum IL-6 levels are elevated in patients with SJIA and correlate with systemic disease activity, and peripheral blood mononuclear cells from patients with SJIA were observed to produce an increase in IL-6. Following the outstanding efficacy and tolerable safety profiles of tocilizumab for SJIA demonstrated in phase II clinical trials in Japan, the UK and France, a phase III trial was conducted in Japan to determine the efficacy and safety of tocilizumab for 56 patients with SJIA who had been refractory to conventional treatment[38]. The study consisted of three phases: an open-label lead-in phase of 6 weeks, a double-blind, randomized, placebo-controlled phase of 12 weeks, and an open-label extension phase of at least 48 weeks. Tocilizumab was administered intravenously at 8 mg/kg every 2 weeks. At the end of the first open-label phase, the American College of Rheumatology pediatric criteria (ACR Pedi) 30, 50 and 70% responses were achieved for 91, 86 and 68% of the enrolled patients, respectively. In the double-blind, placebo-controlled phase, 17% of children in the placebo group maintained an ACR Pedi 30% response, compared with 80% of children in the tocilizumab group. By week 48 of the open-label extension phase, ACR Pedi 30, 50 and 70% responses were achieved for 98, 94 and 90% of the 48 patients, respectively. Improvements in osteoporosis and catch-up growth by children with retarded growth were also observed. In addition, pre-treatment radiographic findings for joint destruction, such as joint space narrowing, sub-

chondral bone cysts and erosion, all improved after tocilizumab treatment. Based on its outstanding efficacy and tolerable safety for SJIA, tocilizumab was approved in 2008 for the treatment of SJIA in Japan. Moreover, a global double-blind, placebo controlled, randomized phase III TENDER trial demonstrated that at 12 weeks, 85% of the participants receiving tocilizumab achieved ACR Pedi 30% response, compared with 24% of the children receiving placebo, and that 1-year results of the TENDER study showed that 88% of tocilizumab-treated patients attained ACR Pedi 30% + absence of fever, 89% an ACR Pedi 70% response and 65% an ACR Pedi 90% response[39]. In view of its outstanding efficacy and tolerable safety profile, the US Food and Drug Administration (FDA) approved tocilizumab in April 2011 as the first biological drug for the treatment of patients two years of age and older with active SJIA.

Efficacy of tocilizumab for Castleman's disease

Castleman's disease is a lymphoproliferative disease with benign hyperplastic lymph nodes characterized by follicular hyperplasia and capillary proliferation accompanied by endothelial hyperplasia. Dysregulated IL-6 expression generated by transgenic mice produced a syndrome resembling Castleman's disease, while anti-IL-6R Ab suppressed Castleman's disease-like symptoms which had developed in IL-6 transgenic mice. Further, IL-6 was highly expressed in hyperplastic lymph nodes of patients with Castleman's disease and surgical removal of a solitary involved lymph node led to clinical improvement and a reduction in serum IL-6 concentration[24].

The first evidence of the beneficial effect of IL-6 blockade was observed in a patient with Castleman's disease treated with a mouse anti-IL-6 Ab[40]. As described before, the initial clinical study of tocilizumab in Castleman's disease showed promising efficacy[28] and subsequently, an open-label multicenter clinical trial of tocilizumab was performed for 28 patients with multicentric Castleman's disease[41]. The initial dosing period consisted of eight infusions of 8 mg/kg tocilizumab administered biweekly. After 16 weeks, adjustments in dose and treatment interval were allowed during an extension phase. Within 16 weeks, tocilizumab treatment alleviated lymphadenopathy and inflammatory parameters, while chronic inflammatory symptoms were successfully managed over 60 weeks. For eight patients (28.6%), the tocilizumab dose was reduced or the treatment interval extended without exacerbation. This outstanding efficacy led to approval in 2005 of tocilizumab as an orphan drug for Castleman's disease in Japan. At present a clinical trial of tocilizumab for KSHV-associated Castleman's disease is ongoing in the USA.

IL-6 targeting as strategy for various other autoimmune inflammatory diseases

Tocilizumab is now used as an innovative drug for RA, JIA and Castleman's disease. Because of the pathological role of IL-6 in various other autoimmune inflammatory diseases, tocilizumab is expected to find wide application for their treatment, and favorable results of recent clinical trials and case reports have suggested that this is indeed a realistic expectation[5, 18, 25]. These diseases include systemic autoimmune diseases such as systemic lupus erythematosus, systemic sclerosis, polymyositis, vasculitis syndrome and relapsing polychondritis, in addtion to organ specific autoimmune diseases including autoimmune hemolytic anemia, acquired hemophilia A and neuromyelitis optica, as well as chronic inflammatory diseases such as adult-onset Still's disease, Crohn's disease, polymyalgia rheumatica, amyloid A amyloidosis, remitting seronegative, symmetrical synovitis with pitting edema, graft-versus-host disease, Behcet's disease, uveitis, TNF-associated periodic syndrome, pulmonary arterial hypertension, and sciatica, and atopic dermatitis. Some case studies have reported that tocilizumab is efficacious for spondyloarthritides such as ankylosing spondylitis and reactive arthritis, although recent clinical trials of tocilizumab as well as of sarilumab, a fully human anti-IL-6R Ab, could not detect any beneficial effect for ankylosing spondylitis. An observational study of RA patients complicated with type 2 diabetes mellitus treated with tocilizumab led to an average reduction in HbA1c levels, and a study of 11 non-diabetic RA patients detected a significant decrease in insulin resistance indices such as the homeostasis model assessment of insulin resistance (HOMA-IR) and the leptin-to-adiponectin ratio after 3 months of tocilizumab treatment, while serum levels of reactive oxygen metabolites diminished in RA patients treated with tocilizumab. It can thus be expected that long-term tocilizumab treatment may offer protection against the progression of atherosclerosis leading to cardiovascular events[42]. A randomized, open-label, parallel-group, multicenter study is in progress to de-

Table 1 Ongoing clinical trials of tocilizumab for treatment of diseases other than rheumatoid arthritis and juvenile idiopathic arthritis, registered with ClinicalTrials.gov

Targeted diseases	Status	Identifier
Adult-onset Still's disease	Unknown	NCT01002781
Type II diabetes mellitus, obesity	Recruiting	NCT01073826
Graves' ophthalmopathy	Not yet recruiting	NCT01297699
Cardiovascular disease in RA	Recruiting	NCT01331837
Polymyalgia rheumatic	Recruiting	NCT01396317
KSHV-associated Castleman's disease	Recruiting	NCT01441063
Giant cell arteritis	Recruiting	NCT01450137
Acute GVHD	Recruiting	NCT01475162
Non-ST elevation myocardial infarction	Recruiting	NCT01491074
Systemic sclerosis	Recruiting	NCT01532869
Transplant rates awaiting kidney transplantation	Recruiting	NCT01594424
SJIA-associated uveitis	Not yet recruiting	NCT01603355
Recurrent ovarian cancer	Recruiting	NCT01637532
Behcet's syndrome	Recruiting	NCT01693653
Schizophrenia	Recruiting	NCT01696929

Current clinical trials of tocilizumab are listed. RA: rheumatoid arthritis, KSHV: Kaposi's sarcoma herpes virus, GVHD: graft-versus-host disease, SJIA: systemic juvenile idiopathic arthritis

termine and assess the rate of cardiovascular events for patients with RA following treatment with tocilizumab in comparison to that with etanercept, a TNF inhibitor.

Conclusion and future prospects

Tocilizumab is currently approved worldwide for the treatment of RA, and in several countries for that of Castleman's disease, and systemic and polyarticular JIA. The success of the clinical application of tocilizumab for the treatment of these intractable diseases opened the door to the possibility that IL-6 blockade could be a new treatment strategy for various diseases, so that other IL-6 inhibitors are now being developed[43]. These include fully human anti-IL-6R Ab, anti-IL-6R nanobody, anti-IL-6 Ab, anti-IL-6/IgG-binding domain avimer protein, which consists of the IgG-binding domain fused to the N-terminus of a 3-domain IL-6-binding region, resulting in a 19-kDa heterotetrameric avimer, and soluble gp130-Fc fusion protein. Moreover, a new fully human Ab against IL-6R (SA237), generated from tocilizumab by Ab structural optimization technology was developed and this IgG2 class Ab significantly improved the pharmacokinetics and duration of CRP inhibition in cynomolgus monkeys[44]. These novel biologics are now being evaluated in clinical trials. In view of the favorable results of off-label use of tocilizumab and the pathological role of IL-6 in various autoimmune inflammatory diseases, tocilizumab as well as other IL-6 inhibitors are expected to find wide application for the treatment of such diseases. However, further clinical investigations are required to achieve this goal and ongoing clinical trials of tocilizumab are listed in Table 1.

In addition to the evaluation of the efficacy and safety of

tocilizumab for various diseases, the clarifications of the mechanism(s) through which tocilizumab exerts its beneficial clinical effects and of the etiology of dysregulated persistent IL-6 synthesis in various autoimmune inflammatory diseases are important issues for future studies. As mentioned above, the preventative effect of toclizumab on joint destruction in RA is mediated by the inhibition of IL-6-induced RANKL induction followed by osteoclastogenesis and suppression of IL-6-induced production of matrix metalloproteinases. Amyloid A amyloidosis and anemia of inflammation are complications of RA, and the dramatic improvement engendered by tocilizumab treatment is mediated through the inhibition of their respective resposible proteins, SAA and hepcidin[45, 46]. Moreover, it was recently shown that tocilizumab corrects Th17 ($CD4^+IL\text{-}17^+$)/Treg ($CD4^+CD25^{high}Foxp3^+$) imbalance in RA patients[47], while other studies found that tocilizumab could exert its clinical effect by inhibiting pathological autoantibody production, since tocilizumab treatment was found to lead to a reduction in the pathologic $CD38^{high}CD19^{low}IgD^{negative}$ plasma cells of systemic lupus erythematosus patients[48] and to diminish the survival of plasmablasts, in this case, $CD19^{int}$ $CD180^{negative}CD27^+$ $CD38^+$ cells, which produce the anti-aquaporin 4 Ab seen in neuromyelitis optica[49]. IL-6 synthesis is regulated by transcriptional levels and posttranscriptional mechanisms and it was demonstrated that some viral products could constitutively activate transcriptional activation of the IL-6 gene and/or inhibit its mRNA degradation. For these reasons, clarifications of the cell source and the mechanism of dysregulated persistent IL-6 production are expected to aid and facilitate investigations of the pathogenesis of a wide range of diseases.

Conflict of interest

Tadamitsu Kishimoto holds a patent of tocilizumab and receives royalities for Actemra. The other authors declare no conflict of interest.

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