IL-6/IL-6 receptor system and its role in physiological and pathological conditions

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ABSTRACT

IL (interleukin)-6, which was originally identified as a B-cell differentiation factor, is a multifunctional cytokine that regulates the immune response, haemopoiesis, the acute phase response and inflammation. IL-6 is produced by various types of cell and influences various cell types, and has multiple biological activities through its unique receptor system. IL-6 exerts its biological activities through two molecules: IL-6R (IL-6 receptor) and gp130. When IL-6 binds to mIL-6R (membrane-bound form of IL-6R), homodimerization of gp130 is induced and a high-affinity functional receptor complex of IL-6, IL-6R and gp130 is formed. Interestingly, sIL-6R (soluble form of IL-6R) also binds with IL-6, and the IL-6–sIL-6R complex can then form a complex with gp130. The homodimerization of receptor complex activates JAKs (Janus kinases) that then phosphorylate tyrosine residues in the cytoplasmic domain of gp130. The gp130-mediated JAK activation by IL-6 triggers two main signalling pathways: the gp130 Tyr759-derived SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase-2)/ERK (extracellular-signal-regulated kinase) MAPK (mitogen-activated protein kinase) pathway and the gp130 YXXQ-mediated JAK/STAT (signal transducer and activator of transcription) pathway. Increased IL-6 levels are observed in several human inflammatory diseases, such as rheumatoid arthritis, Castleman’s disease and systemic juvenile idiopathic arthritis. IL-6 is also critically involved in experimentally induced autoimmune diseases. All clinical findings and animal models suggest that IL-6 plays a number of critical roles in the pathogenesis of autoimmune diseases. In the present review, we first summarize the IL-6/IL-6R system and IL-6 signal transduction, and then go on to discuss the physiological and pathological roles of IL-6.

Key words: autoimmune disease, bone metabolism, immune response, inflammation, interleukin-6 (IL-6) signalling.
Abbreviations: AA, amyloid A; ACD, anaemia of chronic disease; ADAM, a disintegrin and metalloproteinase; ADAMTS, ADAM with thrombospondin motifs; α1-AGP, α1-acid glycoprotein; AMD, age-related macular degeneration; APP, acute-phase protein; APR, acute-phase response; AR, androgen receptor; BSF, B-cell stimulatory factor; CAC, colitis-associated cancer; CCL, CC chemokine ligand; CD62L, CD62 ligand; CNV, choroidal neovascularization; CRP, C-reactive protein; ERK, extracellular-signal-regulated kinase; FAS, fatty acid synthase; GM-CSF, granulocyte/macrophage colony-stimulating factor; HDL, high-density lipoprotein; ICAM, intercellular adhesion molecule; IFN, interferon; IGF-1, insulin-like growth factor-1; IL, interleukin; IL-6R, IL-6 receptor; JAK, Janus kinases; JIA, juvenile idiopathic arthritis; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; mgp130, membrane-bound gp130; mM, membrane-bound IL-6R; MM, multiple myeloma; MMP, matrix metalloproteinase; PB, pre-B-colony-enhancing factor; PPAR, peroxisome-proliferator-activated receptor; RA, rheumatoid arthritis; RA-FLS, fibroblast-like synoviocytes from RA patients; RANKL, receptor activator of nuclear factor κB ligand; ROR, retinoic acid-receptor-related orphan receptor; SAA, serum AA; sgp130, soluble gp130; SHP-2, Src homology domain-containing protein tyrosine phosphatase-2; sIL-6R, soluble IL-6R; sJIA, systemic JIA; SOCS, suppressor of cytokine signalling; SREBP, sterol-regulatory-element-binding protein; STAT, signal transducer and activator of transcription; TAG, triacylglycerol; TCZ, tocilizumab; TGF, transforming growth factor; TNF, tumour necrosis factor; Treg, regulatory T-cell; VEGF, vascular endothelial growth factor; VLDL, very-low-density lipoprotein.
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INTRODUCTION

IL (interleukin)-6 was discovered in 1986 as a B-cell differentiation factor that differentiates activated B-cells into immunoglobulin-producing cells [1]; it was therefore named BSF (B-cell stimulatory factor)-2. It was subsequently found to be identical with other factors described as IFN (interferon)-β2 [2], 26 kDa protein [3], hybridoma/plasmacytoma growth factor [4] and hepatocyte-stimulating factor [4].

IL-6 is produced by various types of cell, such as T-cells, B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, adipocytes and some tumour cells. IL-6R (IL-6 receptor) is mainly expressed in haemopoietic cells, such as T-cells, monocytes, activated B-cells and neutrophils. Interestingly, IL-6 influences various cell types and has multiple biological activities through its unique receptor systems. Elevated production of IL-6 contributes to the pathogenesis of various autoimmune and inflammatory diseases, and IL-6 blockade by TCZ [tocilizumab; a humanized anti-(human IL-6R) monoclonal antibody] has been proven to improve symptoms of RA (rheumatoid arthritis), Castleman’s disease and sJIA [systemic JIA (juvenile idiopathic arthritis)] [5]. IL-6 is a growth factor for certain tumours, such as MM (multiple myeloma) and renal carcinoma cells. Moreover, IL-6 is suggested to be involved in cancer cachexia. Cancer cachexia is a metabolic state that is seen in several malignant disorders and it is commonly recognized as progressive weight loss with depletion of host reserves of adipose tissue and skeletal muscle.

IL-6/IL-6R SYSTEM

IL-6 exerts its biological activities through two molecules: IL-6R (also known as IL-6Ra, gp80 or CD126) and gp130 (also referred to as IL-6Rβ or CD130) [6]. IL-6R is important for ligand binding, but it only has 82 amino acids in its cytoplasmic domain, indicating that it can play only a minor role in signal transduction. However, the cytoplasmic tail of the IL-6R may play a decisive role in basolateral sorting, which is an important function in polarized epithelial cells [7]. In contrast, the cytoplasmic domain of gp130 contains several potential motifs for intracellular signalling, such as the YSTV sequence for SHP-2 (Src homology domain-containing protein tyrosine phosphatase-2) recruitment and YXXQ motifs (where X is any amino acid) for STAT (signal transducer and activator of transcription) activation. gp130 does not have an intrinsic kinase domain; instead, like other cytokine receptors, the cytoplasmic domain of gp130 contains regions required for its association with a non-receptor tyrosine kinase called JAK (Janus kinase), through which downstream signalling cascades are initiated.

When IL-6 binds to mIL-6R (membrane-bound IL-6R), homodimerization of gp130 is induced, and a high-affinity functional receptor complex of IL-6, IL-6R and gp130 is formed. sIL-6R (soluble IL-6R), which lacks the intracytoplasmic portion of mIL-6R and is produced either by the enzymatic cleavage of mIL-6R by ADAM (a disintegrin and metalloproteinase)-17 or by alternative splicing, can also bind with IL-6, and then the complex of IL-6 and sIL-6R can form a complex with gp130 (Figure 1). This unique receptor signalling system is termed IL-6 trans-signalling [8].

mgp130 (membrane-bound gp130) is expressed ubiquitously in the body. Therefore the IL-6–sIL-6R complex could, theoretically, stimulate most cells in the body. However, this trans-signalling is thought to be highly regulated by sgp130 (soluble gp130), which exists in higher concentrations in circulating blood. sgp130 binds to the IL-6–sIL-6R complex and thereby inhibits the binding of the IL-6–sIL-6R complex to mgp130 [9,10]. Thus sgp130 is a natural inhibitor of IL-6 signalling. IL-6 binds to IL-6R with an affinity of $10^{-9}$ to $10^{-10}$ mol/l and gp130 binds to IL-6–sIL-6R complex with an affinity of $10^{-11}$ mol/l [6,11–13]. In fact, TCZ can dissociate the IL-6–sIL-6R complex, but not the IL-6–sIL-6R–sgp130 complex, suggesting that the IL-6–sIL-6R complex is more rigid than the IL-6–sIL-6R–sgp130 complex [14].

gp130 is used in the signalling of many other members of the IL-6 family of cytokines, including LIF (leukaemia inhibitory factor), CNTF (ciliary neurotrophic factor), OSM (oncostatin M), CT-1 (cardiotrophin-1), CLC (cardiotrophin-like related cytokine), also known as NNT-1 (novel neurotrophin-1) or BSF-3, NPN (neuropoietin), IL-11 and IL-27 [15–17]. The sharing of the gp130 molecule by other IL-6 superfamily cytokines may explain their functional redundancy. A similar system can also be seen within the other cytokine...
families; for example, the group of cytokines comprising IL-3, IL-5 and GM-CSF (granulocyte/macrophage colony-stimulating factor) uses a common β receptor, and the group of ILs including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 uses a common γ receptor component [18].

The structure of the gp130–IL-6R–IL-6 complex has been solved by X-ray crystallography: it is a hexamer comprising two IL-6, two IL-6R and two gp130 proteins [19]. It has been argued, however, that the signalling complex is built of one IL-6–IL-6R complex bound to two gp130 proteins [20].

**IL-6 SIGNAL TRANSDUCTION**

It is thought that receptor homodimerization brings the JAKs into close proximity, resulting in their mutual transactivation. The activated JAKs phosphorylate tyrosine residues in the cytoplasmic domain of gp130. There are six tyrosine residues in the human gp130 cytoplasmic domain. The gp130-mediated JAK activation by IL-6 triggers two main signalling pathways: the gp130 Tyr759-derived SHP-2/ERK (extracellular-signal-regulated kinase) MAPK (mitogen-activated protein kinase) pathway and the gp130 YXXQ-mediated JAK/STAT pathway (Figure 2) [21].

In the gp130 Tyr759-derived SHP-2/ERK MAPK pathway, upon IL-6 stimulation, SHP-2 is recruited to the phosphorylated Tyr759 residue of gp130. After being recruited, SHP-2 is phosphorylated by JAKs and then interacts with Grb2 (growth-factor-receptor-bound protein 2), which is constitutively associated with Sos (son-of-sevenless), a GDT/GTP exchanger for Ras. The GTP form of Ras transmits signals that lead to activation of the ERK MAPK cascade, which activates transcription factors such as NF-IL-6 [C/EBPβ (CCAAT/enhancer-binding protein β)] that can act through their own cognate response elements in the genome.

In the gp130 YXXQ-mediated JAK/STAT pathway, upon IL-6 stimulation, STAT proteins are recruited to the phosphorylated YXXQ/YXPQ motifs and are then phosphorylated by JAKs. The activated STAT proteins form a heterodimer (STAT1–STAT3) or homodimers (STAT1–STAT1 and/or STAT3–STAT3), subsequently translocate to the nucleus and activate the transcription of target genes. Interestingly, SOCS (suppressor of cytokine signalling) is one of the target genes of the JAK/STAT pathway. SOCS inhibits JAK activity and thus negatively regulates the signals [22], suggesting the existence of an autoregulatory mechanism for this signalling pathway.

These two signals independently induce a variety of biological activities. For example, we have reported that the SHP-2/ERK MAPK pathway induces MMP (matrix metalloproteinase) production and the JAK/STAT pathway induces RANKL (receptor activator of nuclear factor κB ligand) expression in synovial cells [23,24].

**PHYSIOLOGICAL AND PATHOLOGICAL ROLES OF IL-6**

**Acute-phase response**

The APR (acute-phase response) is rapidly induced by inflammation associated with infection, injury and other factors. This reaction neutralizes pathogens and prevents their further invasion, and also minimizes tissue damage. Briefly, the APR is involved in the body’s recovery from an unwanted inflammatory state. The APR consists of fever, an increase in vascular permeability and the production of APPs (acute-phase proteins) by hepatocytes.

Animal experiments have shown that central IL-6 is necessary for the fever response: body temperature rose significantly after intracerebroventricular injection of IL-6 in rats; however, when administered intravenously or intraperitoneally, IL-6 had no effect on body temperature [25]. However, injection of recombinant human IL-6 caused influenza-like symptoms, including fever in human breast cancer and lung cancer patients [26]. Thermal stress promotes lymphocyte trafficking via the enhanced expression of ICAM (intercellular adhesion molecule)-1 and CCL (CC chemokine ligand) 21 in endothelial cells. Interestingly, IL-6 trans-signalling involves the thermal induction of ICAM-1 [27,28]. This system is important during inflammation by augmenting lymphocyte trafficking to inflamed tissues.

When human IL-6 (1–10 μg/kg of body weight per day) was administered into humans by daily subcutaneous injection for 7 days, increases were observed in the positive APPs, such as SAA [serum
AA (amyloid A)], CRP (C-reactive protein), α1-AGP (α1-acid glycoprotein), α1-antitrypsin, haptoglobin, α1-antitrypsin, fibrinogen, complement component C3 and caeruloplasmin, with the greatest incremental changes and fastest responses being observed for CRP and SAA. Negative APPs, such as transferrin, transthyretin and retinol-binding protein, fell to a nadir within 48–96 h after the first IL-6 injection [29]. With TCZ treatment of patients with RA or other inflammatory diseases, and in TCZ treatment of monkey arthritis, CRP levels rapidly decreased and became negative, indicating that IL-6 is the main inducer of CRP in primates [30–32]. In a mouse study, the production of APPs (haptoglobin, α1-AGP and SAA) was remarkably increased by the injection of turpentine, Listeria monocytogenes or LPS (lipopolysaccharide). In contrast, APP production by turpentine and L. monocytogenes was not observed in IL-6-deficient mice. In the case of LPS, APP production was only slightly reduced compared with wild-type mice. This result suggests that LPS systemically induces inflammatory mediators other than IL-6 [33] (Figure 3).

AA amyloidosis is a severe complication of chronic inflammatory diseases and chronic infections [34,35]. AA amyloidosis is caused by the deposition of insoluble fibrils containing AA protein, which is derived from its precursor, SAA [36]. SAA is an APP that is synthesized predominantly in the liver after stimulation by IL-6. SAA circulates as an apolipoprotein to HDL (high-density lipoprotein). When SAA is dissociated from HDL and degraded, it is deposited as AA fibrils in the extracellular space of vital tissues, leading to organ dysfunction such as renal failure and gastrointestinal tract dysfunction. Therefore it is thought that IL-6 blockade will be a useful therapy for AA amyloidosis. In fact, IL-6 blockade has been shown to dramatically ameliorate AA amyloidosis in animal models and in patients with sJIA and RA [37–39].

The liver has the ability to regenerate, allowing for rapid recovery after partial hepatectomy, liver transplantation or toxic injury. Liver regeneration leads to hepatocyte and non-parenchymal cell proliferation. IL-6-deficient mice had impaired liver regeneration characterized by liver necrosis and failure. There was a blunted DNA synthetic response in hepatocytes of these mice, but not in non-parenchymal liver cells [40]. This fact suggests that IL-6 is an important factor for homoeostasis of the liver.

**Angiogenesis**

Angiogenesis is an essential component of inflammation and its resolution. Inflammatory cells, such as monocytes/macrophages, T-cells and monocytes, fully participate in the angiogenic process by secreting pro- and anti-inflammatory cytokines that can control endothelial cell proliferation, survival and apoptosis, as well as their migration and activation. It is reported that a number of growth factors and cytokines have angiogenic activity; these include VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor), EGF (epidermal growth factor), TGF (transforming growth factor)-β1, IL-6, IL-8, IL-1 and TNF (tumour necrosis factor)-α.

In RA patients, changes in the synovium are marked by neovascularization, infiltration of inflammatory cells and synoviocyte hyperplasia, which together produce a pannus (inflamed vascular tissue). Newly formed blood vessels are thought to be involved in the development and maintenance of synovitis because they support the infiltration of inflammatory cells and the growth and survival of synovial cells. Significant increases in VEGF levels in RA patients correlate with disease activity, suggesting that VEGF is implicated in RA pathogenesis [41]. Treatment with TCZ significantly lowers VEGF levels [42]. IL-6 induced tubule formation in a co-culture system of HUVECs (human umbilical venous endothelial cells) and RA-FLS (fibroblast-like synoviocytes from RA patients), and this angiogenesis was completely inhibited by an anti-VEGF antibody, indicating that VEGF plays a crucial role in IL-6-induced angiogenesis [43]. IL-6 induces VEGF production from synovial cells [42,43], and semi-quantitative assessment by ultrasonography indicated that TCZ significantly decreases neovascularization in patients with RA [44].

AMD (age-related macular degeneration) is complicated by CNV (choroidal neovascularization), leading to severe vision loss and blindness. CNV observed in AMD develops with chronic inflammation adjacent to the retinal pigment epithelium, Bruch’s membrane and choriocapillaris. Studies have indicated that VEGF is a critical factor for promoting CNV [45,46]. Interestingly, increased serum levels of IL-6 and CRP have proven to be correlated with the progression of AMD [47]. CNV induction by laser treatment stimulated IL-6 expression in the retinal pigment epithelium–choroid complex, and blockade of IL-6 signalling led to a significant suppression.
of CNV [48]. These lines of evidence suggest that VEGF induced by IL-6 plays a role in CNV.

**Neutrophil trafficking**

Under conditions of inflammation, neutrophils migrate into the inflamed sites and the number of neutrophils in the circulating blood is substantially increased.

IL-6 acts as a stimulus for myelopoiesis and causes accompanying changes in the numbers of peripheral neutrophils [49]. Briefly, an injection of IL-6 caused biphasic neutrophilia with an initial peak at 1.5 h and a second sustained wave of neutrophilia between 4 and 12 h. The bone marrow showed no significant changes at 1.5 h, suggesting that the peripheral neutrophilia is caused by demargination of intravascular neutrophils. On the other hand, the bone marrow at 12 h showed a mild myeloid hyperplasia of myeloblasts and promyelocytes.

Migrated neutrophils in inflamed sites are deeply involved in the onset and maintenance of inflammation through their robust production of inflammatory mediators, such as prostaglandins, ROS (reactive oxygen species), complements, proteases and cytokines. Many investigations have shown that adhesion molecules and chemokines are essential for neutrophil transmigration [50]. IL-6 augments the expression of adhesion molecules such as VCAM (vascular cell adhesion molecule)-1 and ICAM-1 in inflamed sites and endothelial cells, and induces the production of chemokines such as CXCL (CXC chemokine ligand) 8/IL-8, CCL2/MCP (monocyte chemoattractant protein)-1, CCL8/MCP-3 and CCL20 from many kinds of cells [51–54]. In fact, IL-6 blockade reduced transmigration of neutrophils into inflamed site in arthritis and in the air pouch model [32,55].

On the other hand, circulating neutrophils also play an essential role in the course of inflammation, because removal of neutrophils from circulating blood ameliorates inflammation in RA and inflammatory bowel disease patients [56–58]. One mechanism of neutrophilia is thought to be the mobilization of neutrophils from the marginal pool into the circulation. In the marginal pool, leucocyte rolling and adhesion is mediated by the sequential interaction of adhesion molecules on the leucocyte with counter-ligands on the vascular endothelium and extra-vascular structures [59]. Once neutrophils are activated, the expression levels of CD162/PSGL (P-selectin glycoprotein ligand)-1, CD62L (CD62 ligand)/L-selectin and CD11b are changed, and neutrophils then translocate from the marginal pool into the circulating blood. Suwa et al. [60] reported that IL-6 injection decreased CD62L expression on circulating neutrophils in rabits. We also found that IL-6 reduces CD162 expression on circulating neutrophils in monkeys, but CD162 expression was induced by IL-8 and GM-CSF was induced by IL-6 [61].

IL-6 blockade rapidly decreased the number of circulating neutrophils in arthritic animals and RA patients [62,63], suggesting that IL-6 plays a dominant role in neutrophilia in arthritis and that IL-6 blockade inhibits demargination of intravascular neutrophils into circulating blood.

**Immune responses**

IL-6 was originally identified as a B-cell differentiation factor. IL-6 enhances the production of IgM, IgG and IgA in B-cells activated with *Staphylococcus aureus* Cowan I or pokeweed mitogen. Conversely, anti-IL-6 antibodies inhibit pokeweed mitogen-induced Ig production from peripheral blood mononuclear cells without affecting cell proliferation, indicating that IL-6 is required for antibody production in B-cells [64]. IL-6 promotes antibody production by promoting the B-cell helper capabilities of CD4+ T-cells through increased IL-21 production, suggesting that IL-6 could be a potential co-adjuvant to enhance humoral immunity [65].

Autoantibody production is induced by the overproduction of IL-6. In patients with cardiac myxoma, a benign intra-atrial heart tumour that produces a large amount of IL-6, autoimmune symptoms are ameliorated when the tumour cells are resected [66]. In transgenic mice that overproduce IL-6, hypergammaglobulinemia and autoantibody production are observed [67]. These facts suggest that IL-6 causes B-cell abnormalities associated with the inflammatory process. Moreover, IL-6 blockade suppresses autoantibody production in lupus-prone NZB/NZW F1 mice, despite the fact that they do not have elevated IL-6 levels [68]. This phenomenon can be explained by the fact that B-cells in this strain of mice show hypersensitivity to IL-6 [69]. A similar phenomenon is reported in patients with systemic lupus erythematosus [70].

B-cells play an essential role in the pathogenesis of RA, because B-cell depletion therapy by an anti-CD20 antibody is effective in RA therapy [71]. IL-6 trans-signalling has been shown to induce PBEF (pre-B-cell enhancing factor) in synovial fibroblast cells [72]. PBEF was discovered as a cytokine for the differentiation of B-cells [73]. Therefore B-cell maturation induced by IL-6 itself and IL-6-induced PBEF may play important roles in the pathogenesis of RA.

IL-6 is involved in the proliferation and differentiation of helper T-cells. IL-6 significantly enhances the proliferation of T-cells when T-cells are stimulated with a mitogen [74,75]. In addition, IL-6 blockade suppresses anti-CD3 antibody-induced and anti-CD28 antibody-induced CD4+ T-cell proliferation, and this suppression is achieved by the inhibition of IL-2 production and the induction of Tregs (regulatory T-cells) [76].

CD4+ helper T-cells have been classified as Th1 and Th2 cells on the basis of their cytokine production profiles, and IL-6 promotes IL-4-induced Th2 differentiation and inhibits IL-12-induced Th1 differentiation [77]. Th17 cells, which produce IL-17 in autoimmune pathology,
have become recognized as a separate subset. In vitro studies in mice have shown that the co-stimulation of IL-6 and TGF-β is essential for the differentiation of T\textsubscript{h}17 cells from naïve CD4\textsuperscript{+} T-cells [78]. Furthermore, the involvement of IL-6 in induction of T\textsubscript{h}17 cells has been reported in several autoimmune disease models: an anti-IL-6R antibody suppressed the onset of disease and been reported in several autoimmune disease models: an anti-IL-6R antibody suppressed the onset of disease and concomitantly inhibited the appearance of Th17 cells [79–83] (Figure 4).

T\textsubscript{h}17 cells have also been identified in human blood and inflamed tissues, but they seem to exhibit different features from murine T\textsubscript{h}17 cells [84]. First, human T\textsubscript{h}17 cells express CCR6 and IL-23R, but also IL-12Rβ2 and CD161. Secondly, human T\textsubscript{h}17 cells express T-bet in addition to ROR (retinoic acid-receptor-related orphan receptor) γt and can be induced to produce IFN-γ in addition to IL-17A in the presence of IL-12. Finally, although murine T\textsubscript{h}17 cells originate from a precursor common to Fox (forkhead box) p3 + Tregs by IL-6 and TGF-β stimulation, human T\textsubscript{h}17 cells originate from CD161\textsuperscript{+}CD4\textsuperscript{+} precursors, which constitutively express RORγt and IL-23R, in response to the combined activity of IL-1β and IL-23. TGF-β does not play a direct role in human T\textsubscript{h}17 differentiation. It is reported that IL-6 enhances IL-1β-induced T\textsubscript{h}17 polarization of human naïve CD4\textsuperscript{+} T-cells [85,86]. Further studies are necessary to clarify the involvement of IL-6 in the development of human T\textsubscript{h}17 cells, especially in vivo.

**Bone metabolism**
The skeleton is a dynamic organ in which mineralized bone is continuously resorbed by osteoclasts and new bone is formed by osteoblasts. This process, known as bone remodelling, is normally highly regulated to maintain a normal amount of bone.

A study using IL-6-deficient mice showed that neither gross structural abnormalities nor significant differences in trabecular bone volume were observed in the bones of the mutant mice [87]. Moreover, the IL-6-deficient mice had a normal number of osteoclasts and normally active bone resorption, as measured by the extent of eroded bone surface containing osteoclasts and the number of osteoclast precursors in the bone marrow [CFU-GM (colony-forming unit-granulocyte, monocyte)] was normal [87]. These lines of evidence suggest that IL-6 is not required for normal bone development and is not essential for promoting osteoclastogenesis and osteoclastic bone-resorbing activity.

Oestrogen deficiency is a cause of bone loss in patients with postmenopausal osteoporosis. In ovariectomized mice, an animal model of postmenopausal osteoporosis, treatment with an anti-IL-6 antibody failed to prevent bone loss or the increase in bone resorption and osteoclastogenesis [88]. Although oestrogen is thought to regulate the secretion of cytokines that are produced in the bone microenvironment and influence bone remodelling, there is a study showing that IL-6 expression was not induced in bone-related tissues after ovariectomy [89]. In contrast, there is a report showing that ovariectomy did not induce bone loss in IL-6-deficient mice [87]. Therefore the involvement of IL-6 in postmenopausal osteoporosis is still controversial.

However, IL-6 is suggested to have a pathogenetic role in the abnormal bone resorption in RA, MM and other bone diseases characterized by excessive osteoclastogenesis and focal osteolytic lesions. Synovial fluids from RA patients contain high levels of IL-6 and sIL-6R, and the IL-6–sIL-6R complex stimulates osteoclast formation in a co-culture system of mouse bone marrow cells and osteoblastic cells [90]. This may be due to the induction of RANKL on the surface of stromal/osteoblastic cells by IL-6 + sIL-6R stimulation [91].

We have also reported that IL-6 + sIL-6R stimulation induces RANKL expression in RA-FLS. In a co-culture of RA-FLS and osteoclast precursor cells (RAW 264.7 cells) in the presence of IL-6 + sIL-6R, the RAW 264.7 cells differentiated into mature osteoclasts, and this phenomenon was blocked by an anti-RANKL antibody [24] (Figure 5).

On the other hand, IL-6 acts directly on osteoclast precursor cells and suppresses their differentiation by regulating the transcription of specific genes related to MAPK phosphatases and the ubiquitin pathway [92]. However, we found that ICAM-1/LAF (leucocyte function-associated antigen)-1 signalling may be involved in the discrepancy between the effects of IL-6 on osteoclast differentiation in a mono-culture system and in a co-culture system. Briefly, addition of sICAM-1 (soluble ICAM-1) reverses the IL-6-induced suppression of osteoclast formation in a mono-culture system [93]. Collectively, IL-6 induces abnormal osteoclastogenesis in the inflamed joints of RA patients via the induction of RANKL expression in osteoblastic cells and synovial cells in the presence of ICAM-1. TCZ therapy in RA...
Figure 5  Effect of IL-6 on bone metabolism in RA synovium

patients significantly prevents the progression of joint destruction, as assessed by radiography, and improves levels of the bone resorption marker CTX-1 (C-terminal cross-linking telopeptide of type I collagen) [94].

Cartilage metabolism

Growth impairment is a common manifestation of several childhood diseases with chronic inflammation and/or severe recurrent infections, such as juvenile rheumatoid arthritis, Crohn’s disease, cystic fibrosis and immunodeficiencies, all of which are characterized by elevated levels of IL-6 and decreased levels of IGF-1 (insulin-like growth factor-1). IL-6 transgenic mice (NSE/hIL-6 strain) represent a model of the growth impairment associated with childhood chronic inflammatory diseases. Complete correction of the growth defects, as well as normalization of the IGF-1 levels, is obtained by the neutralization of IL-6 [95]. Longitudinal bone growth occurs at the growth plates by endochondral ossification, which involves the two steps of chondrogenesis and ossification. These are regulated by a multitude of genetic and hormonal factors, growth factors, environment and nutrition. The GH (growth hormone)/IGF-1 axis is considered to have a particularly important regulatory effect on growth plate chondrogenesis. Nakajima et al. [96] reported that IL-6 directly inhibits insulin-induced differentiation of chondrogenic progenitor cells. These results strongly suggest that IL-6 inhibits chondrogenesis via reduction in IGF-1 levels and via inhibiting differentiation of chondrogenic progenitor cells.

Regarding joint cartilage, MMPs and ADAMTS (ADAM with thrombospondin motifs) are thought to play crucial roles in cartilage matrix degeneration. IL-6 induces the production of MMPs (MMP-1, MMP-3 and MMP-13) and ADAMTS-4 from chondrocytes and synovial cells [97,98]. Interestingly, IL-6 synergistically increases MMP production with IL-1 via the induction of IL-1R (IL-1 receptor) [98]. On the other hand, it is reported that IL-6 + sIL-6R induces the production of TIMPs (tissue inhibitors of MMPs), which are endogenous inhibitors of MMPs, in human chondrocytes and synovial fibroblasts, suggesting that IL-6 plays a role in extracellular matrix turnover [99]. Improvement in cartilage turnover markers [PIANP (N-terminal propeptide of type IIA collagen) and HELIX-II (type II collagen helical peptide)] has been reported in TCZ-treated RA patients [94]. Moreover, TCZ decreases serum MMP-3 levels [100]. These findings suggest that the preventive effect of TCZ on cartilage degeneration is mediated by suppression of the IL-6-induced production of MMPs and ADAMTS.

Anaemia of chronic diseases

Anaemia is often observed in patients with chronic inflammatory diseases, such as RA, inflammatory bowel disease and cancer, and is called ACD (anaemia of chronic disease). ACD is characterized by hypoferraemia in the presence of adequate iron stores. Inflammatory cytokines are thought to play important roles in ACD.

Anaemia induced in monkey collagen-induced arthritis is characterized by decreased serum iron and transferrin saturation and by elevated serum ferritin, and the severity of anaemia is correlated with serum IL-6 levels [101]. Hepcidin is a master regulator of iron homoeostasis in humans and other mammals [102]. It inhibits the absorption of iron in the small intestine and the release of recycled iron from macrophages, effectively decreasing the delivery of iron to maturing erythrocytes in the bone marrow [103]. In fact, mice genetically engineered to overproduce hepcidin die of severe iron deficiency shortly after birth [104].

IL-6 induces hepcidin production in liver cells [105]. Administration of TCZ to monkeys with collagen-induced arthritis rapidly improved anaemia and induced a rapid, but transient, reduction in serum hepcidin. Hepcidin mRNA expression was more potently induced by serum from arthritic monkeys than that from healthy animals, and this was inhibited by the addition of TCZ. These lines of evidence indicate that TCZ improves anaemia in monkey arthritis via the inhibition of IL-6-induced hepcidin production [106] (Figure 6).

Moreover, TCZ treatment resulted in a rapid reduction in serum hepcidin-25 in patients with Castleman’s disease. Long-term reductions, accompanied by progressive normalization of iron-related parameters and improvement in symptoms, were observed after the start of TCZ treatment, indicating that IL-6 plays an essential role in the induction of hepcidin in Castleman’s disease [107].

Lipid metabolism

IL-6 transgenic mice had low total cholesterol and TAG [triacylglycerol (triglyceride)] levels compared with littermates, and treatment with an anti-IL-6R antibody increased their lipid profiles to normal levels [108]. It has
been shown that high levels of TAG and VLDL (very-low-density lipoprotein) are present in IL-6-deficient mice [109]. B-cells in the germinal centres of hyperplastic lymph nodes in patients with Castleman’s disease have abnormal production of IL-6 [110], and also have hypolipidaemia [111]. Moreover, in patients with early active RA and high-grade inflammation, cholesterol and TAG levels appear normal or even low [112]. These lines of evidence clearly suggest that IL-6 plays an important role in lipid metabolism.

We have reported that IL-6 injection into mice decreased total cholesterol and TAG levels [113]. In this model, expression of the VLDL receptor, which plays a role in the delivery of fatty acids derived from VLDL-TAGs from the blood to peripheral tissues, was up-regulated in IL-6-treated mice. These results suggested that the induction of VLDL receptor by IL-6 may be related to the hypolipidaemia. Several reports have described the function of IL-6 in lipid metabolism in adipose tissue. Interstitial IL-6 concentrations in adipose tissue are approximately 100 times as high as in plasma, implying an important autocrine and paracrine regulatory function in this tissue [114]. IL-6 has lipolytic properties and increases lipolysis of adipose tissue and adipocytes in vitro [115,116]. Consistent with these in vitro studies, IL-6 infusion in humans increased non-esterified (‘free’) fatty acid and whole-body fat oxidation [117].

IL-6 treatment produced a significant induction of PPAR (peroxisome-proliferator-activated receptor) α and reduction of SREBP (sterol-regulatory-element-binding protein)-1c mRNA in the hepatoma cell line Hep3B-cells. PPARα regulates decomposition of fatty acids through mitochondrial and peroxisomal oxidation [118]. It has been shown that PPARα deficiency abolishes the normal diurnal variations in the expression of lipogenic genes, such as those encoding FAS (fatty acid synthase), ACC (acetyl-CoA carboxylase) and HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, in the liver [119]. On the other hand, the SREBP family is a group of transcription factors that activate genes encoding enzymes regulating cholesterol and fatty acid biosynthesis. In particular, SREBP-1c preferentially enhances transcription of genes associated with fatty acid synthesis, including FAS [120,121]. Furthermore, we have observed a similar phenomenon in mice treated with IL-6; i.e. in the liver from such mice, PPARα expression was indeed increased and SREBP-1c was decreased [122]. From these results, it can be seen that IL-6 accelerates decomposition of lipids and inhibits the synthesis of lipids, leading to a reduction in serum lipid levels (Figure 7).

It has been reported that treatment of RA patients with TCZ increased blood levels of total cholesterol, TAG and HDL-cholesterol in a manner inversely related to the disease activity of RA [123]. It is also reported that blockade of TNF-α increased blood levels of total cholesterol, TAG and HDL-cholesterol, and that the persistent inflammatory condition reflected by elevated serum TNF-α levels results in low levels of total cholesterol and TAG in RA [124–126]. This strongly suggests that the inhibition of IL-6 production induced by TNF-α blockade results in an increase in lipids. However, although these drugs increase total cholesterol and TAG, neither TNF-α blockade nor TCZ changes the atherogenic index (total cholesterol/HDL) [127,128].

Cancer
Chronic inflammation plays an important role in human carcinogenesis. There are many reports describing elevated serum levels of IL-6 in cancer patients, which are related to disease severity and outcome [129–132]. IL-6 has been implicated in the modulation of growth and differentiation in many cancers, and is associated with poor prognosis in renal cell carcinoma, ovarian cancer, lymphoma, melanoma and prostate cancer [133]. By activating ERK1/2, IL-6 stimulates tumour cell proliferation [134–136]. IL-6 is an important regulator of cell survival, providing tumour cells with a mechanism to escape cell death induced by stress and cytotoxic drugs. Additionally, the physiological role of IL-6 has been shown to promote not only tumour proliferation, but also metastasis and symptoms of cachexia [137–140].

MM is a malignancy of plasma cells and is the most common malignant lymphoma in adults. It is characterized by localization of tumour cells to the bone marrow, where these cells disseminate and induce bone diseases. The interaction between MM cells and stromal cells in the bone marrow microenvironment stimulates the production of cytokines, growth factors and adhesion molecules, which together play an important role in the proliferation and localization of
MM cells in the bone marrow. MM cells cause osteolysis, leading to bone pain and hypercalcaemia. Kawano et al. [141] reported that IL-6 is a major growth factor for MM cells. In approximately half of all MM patients, proliferation of cultured MM cells is observed to be mediated by an autocrine loop, and it is now well known that IL-6 produced by the bone marrow environment is the major cytokine involved in the growth and survival of MM cells [142]. Moreover, IL-6 is well known to be an essential factor in the survival of MM cells, since it prevents apoptosis of MM cells induced by different stimuli such as dexamethasone, Fas and serum deprivation. The IL-6-sIL-6R complex is more potent than IL-6 alone in up-regulating both Bcl-xL and Mcl-1 in native MM cells, which do not express IL-6R on the cell surface [143].

The expression of IL-6 and IL-6R and the role of IL-6 as a growth factor in prostate cancer are well documented [144]. IL-6 is responsible for resistance to apoptosis and increased levels of an anti-apoptotic member of the Bcl-2 family in the advanced prostate cancer cell line LNCaP [141]. Moreover, since the growth of prostate cancer cells depends on the presence of androgens, almost all patients with advanced prostate cancer respond initially to androgen deprivation and anti-androgen therapy. Because IL-6 stimulates androgen synthesis and ARs (androgen receptors) in prostate cancer cells [145], it is possible that IL-6 diminishes the therapeutic effect of anti-androgen treatment in prostate cancer [145]. On the other hand, in AR-negative prostate cancer cells, IL-6 is known as an inhibitor of apoptosis [146].

Cancer-related anorexia and cachexia are serious complications associated with malignant disease [147]. The features of cachexia are anaemia, abnormalities of liver function, fatigue and vomiting. Elevated serum IL-6 in patients with pancreatic cancer and correlation with cachexia has been observed [148]. As described above, IL-6 is related to iron metabolism. Moreover, IL-6 also has a regulatory role related to excess glucose metabolism and muscle loss [149]. Previously, we have reported that IL-6 is essential for cancer cachexia in a syngeneic mouse model, in which treatment with an anti-IL-6 antibody prevented the induction of cancer cachexia [150]. In addition, in syngeneic mice, injection of IL-6 cDNA-transfected Lewis lung carcinoma cells resulted in unaltered net tumour growth rate, but caused weight loss and shortened survival [151]. It is reported that an anti-human IL-6 antibody (ALD518) reversed fatigue and reduced loss of lean body mass (−0.19 kg in patients taking ALD518 compared with −1.50 kg in those taking placebo) in patients with advanced non-small cell lung cancer [152], and that in these patients ALD518 increased haemoglobin, haematocrit, mean corpuscular haemoglobin and albumin, and raised haemoglobin levels to ≥12 g/dl in 58% of patients with haemoglobin levels of ≤11 g/dl at baseline. ALD518 may be a novel non-erythropoietic-stimulating agent for cancer-related anaemia [153].

Patients with long-standing ulcerative colitis carry a much higher risk of developing colon cancer, suggesting a role of the immune system as a tumour promoter in the colon. However, the reasons for this increased cancer occurrence are not fully understood. A study has shown that IL-6, which is produced in innate immune cells within the lamina propria in response to intestinal injury, enhances proliferation of tumour-initiating cells and protects normal and pre-malignant intestinal epithelial cells from apoptosis during acute colonic inflammation.
and CAC (colitis-associated cancer) induction [154]. A more recent study has also shown that, in azoxymethane-induced colonic tumours in ulcerative colitis models, the appearance of tumours was accompanied by the co-appearance of an F4/80$^+$CD11b$^{high}$Gr$^1_{low}$ (M2) macrophage subset, which is a source of tumour-promoting factors, including IL-6 [155]. These results suggest that IL-6 blockade could be an approach for the therapy of CAC.

**CLINICAL APPLICATION OF IL-6 BLOCKERS**

As described above, IL-6 has vast variety of biological activities and plays important roles in inflammation, the immune response, haemopoiesis etc. Therefore IL-6 blockade is anticipated to constitute a novel treatment strategy for inflammatory and autoimmune diseases, as well as for cancers. TCZ, a humanized anti-(human IL-6R) monoclonal antibody, was the first IL-6 blocker to be developed. TCZ blocks IL-6-mediated signal transduction through the inhibition of IL-6 binding to mIL-6R and sIL-6R [156]. TCZ is approved for the therapy of Castleman’s disease, RA, and JIA (polyarticular JIA and sJIA).

**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. IL-6 is produced in high levels in the hyperplastic lymph nodes, and IL-6 is the key element responsible for the various clinical symptoms [107]. Two open-label clinical trials have shown that TCZ administered at 8 mg/kg of body weight every 2 weeks had a marked effect on clinical symptoms and on laboratory findings, as well as on histologically determined amelioration [108,157]. TCZ was approved as an orphan drug for Castleman’s disease in 2005 in Japan.

**Rheumatoid arthritis**

RA is a chronic progressive autoimmune inflammatory disease with unknown aetiology that particularly affects the joints of the hands and feet. The synovial tissue of affected joints is infiltrated by inflammatory cells, such as macrophages and lymphocytes, leading to hyperplasia with neovascularization, which causes joint swelling, stiffness and pain. This ultimately leads to cartilage destruction and bone resorption in the joints, with some patients suffering permanent disability. The biological activities of IL-6 and the elevation of IL-6 in the serum and the synovial fluids of RA patients indicate that IL-6 is one of the key cytokines involved in the development of RA [158,159]. Seven Phase III clinical trials of TCZ carried out in Japan and worldwide have revealed its efficacy, both as a monotherapy or combined with DMARDs (disease-modifying anti-rheumatic drugs), for adult patients with moderate-to-severe RA [94,160–165]. Moreover, SAMURAI (Study of Active Controlled Monotherapy Used for Rheumatoid Arthritis, an IL-6 Inhibitor) [94] and LITHE (Tocilizumab safety and THE prevention of structural joint damage trial) [164] proved that radiological damage of joints was significantly inhibited by TCZ treatment. As a result, TCZ has now been approved for the treatment of RA in many countries.

**Systemic juvenile idiopathic arthritis**

sJIA is a subtype of chronic childhood arthritis that leads to joint destruction and functional disability, accompanied by systemic inflammation. This long-lasting inflammation also causes spike fever, anaemia and impairment of growth. Moreover, the acute complication known as macrophage activation syndrome is associated with serious morbidity. IL-6 has been reported to be markedly elevated in blood and synovial fluid, and the IL-6 level has been shown to correlate with disease activity [166,167]. TCZ showed outstanding efficacy in a randomized double-blind placebo-controlled withdrawal Phase III trial for 56 patients with sJIA, who had been refractory to conventional treatment regimens [168]. It was approved in 2008 in Japan as the first biological drug for sJIA. This efficacy was maintained in a recent global Phase III trial (TENDER); 112 patients with severe sJIA refractory to conventional treatments, including TNF and IL-1 inhibitors, were enrolled [169].

As shown in Table 1, the treatment of many inflammatory diseases by TCZ shows beneficial effects

<table>
<thead>
<tr>
<th>Disease</th>
<th>Candidate disease target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic autoimmune disease</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>Organ-specific autoimmune disease</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>Chronic inflammatory disease</td>
<td>Reactive AA amyloidosis</td>
</tr>
</tbody>
</table>

**Table 1 Possible disease targets for IL-6 blockade by TCZ**

TCZ is an anti-IL-6 antibody produced by Chugai/Roche.
IL-6 signalling and its biological activities

Table 2  Novel IL-6 inhibitors in clinical trials

<table>
<thead>
<tr>
<th>Name of inhibitor (type of inhibitor; company)</th>
<th>Disease</th>
<th>Candidate disease target</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT0-328 (chimaeric anti-IL-6 antibody; Centocor)</td>
<td>Cancer</td>
<td>MM</td>
</tr>
<tr>
<td>CNT0-136 (humanized anti-IL-6 antibody/Center)</td>
<td>Systemic autoimmune disease</td>
<td>RA, Spondyloarthritis, Cancer</td>
</tr>
<tr>
<td>REGN-88 (anti-IL-6R antibody; Regeneron/Sanofi-Aventis)</td>
<td>Systemic autoimmune disease</td>
<td>RA, Spondyloarthritis</td>
</tr>
<tr>
<td>ALD518/BMS-945429 (anti-IL-6 antibody; Alder/BMS)</td>
<td>Systemic autoimmune disease</td>
<td>RA, Spondyloarthritis</td>
</tr>
<tr>
<td>CPD6038 (anti-IL-6 antibody; UCB)</td>
<td>Systemic autoimmune disease</td>
<td>RA</td>
</tr>
<tr>
<td>C326 (avimer protein; Avidia)</td>
<td>Systemic autoimmune disease</td>
<td>RA</td>
</tr>
<tr>
<td>FE301 (sgp130-Fc fusion protein; Ferring/Conaris Research Institute)</td>
<td>Systemic autoimmune disease</td>
<td>RA</td>
</tr>
</tbody>
</table>

in pilot studies and clinical practices [170]. Future clinical trials to evaluate the efficacy and safety of TCZ for these diseases are essential for the identification of new indications for TCZ. Basic research to clarify the mechanism(s) of how IL-6 blockade exerts its efficacy is also important.

In response to clinical success of TCZ, many other IL-6 blockers are being developed for these diseases (Table 2) [171,172]. However, to date, there is less information about the differences between TCZ and other IL-6 blockers. Further studies to address this issue are necessary.

CONCLUSIONS

IL-6 participates in a broad spectrum of biological events, such as immune responses, haemopoiesis and acute-phase reactions. In contrast, overproduction of IL-6 production has been implicated in the pathogenesis of a variety of diseases, including several chronic inflammatory diseases and cancer. Future studies to clarify the molecular mechanisms of IL-6 functions and the use of inhibitors of IL-6 signalling should provide information critical to a better understanding of the molecular mechanisms of diseases and the development of new therapeutic methods.

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