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Review

Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor

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Abstract

RA is a chronic, debilitating disease in which articular inflammation and joint destruction are accompanied by systemic manifestations including anaemia, fatigue and osteoporosis. IL-6 is expressed abundantly in the SF of RA patients and is thought to mediate many of the local and systemic effects of this disease. Unlike a number of other cytokines, IL-6 can activate cells through both membrane-bound (IL-6R) and soluble receptors (sIL-6R), thus widening the number of cell types responsive to this cytokine. Indeed, trans-signalling, where IL-6 binds to the sIL-6R, homodimerizes with glycoprotein 130 subunits and induces signal transduction, has been found to play a key role in acute and chronic inflammation. Elevated levels of IL-6 and sIL-6R in the SF of RA patients can increase the risk of joint destruction and, at the joint level, IL-6/sIL-6R can stimulate pannus development through increased VEGF expression and increase bone resorption as a result of osteoclastogenesis. Systemic effects of IL-6, albeit through conventional or trans-signalling, include regulation of acute-phase protein synthesis, as well as hepcidin production and stimulation of the hypothalamo-pituitary-adrenal axis, the latter two actions potentially leading to anaemia and fatigue, respectively. This review aims to provide an insight into the biological effects of IL-6 in RA, examining how IL-6 can induce the articular and systemic effects of this disease.

Key words: Interleukin-6, Rheumatoid arthritis, Trans-signalling, Joint destruction, Systemic effects, Immune response, Receptor inhibition.

REVIEW

Introduction

RA is a chronic inflammatory disease characterized by articular inflammation leading to joint destruction. RA pathogenesis involves complex humoral and cellular reactions including IC formation, vascular reactions and infiltration of lymphocytes and monocytes into the synovium. These infiltrating cells and synoviocytes release pro-inflammatory mediators, including IL-6, which perpetuate inflammation and destruction through effects on other cell types in the synovium and peri-articular structures (Fig. 1).

It is thought that RA is linked initially to immunity against an unknown antigen and later to self-maintained inflammatory processes [1]. The presence of autoantibodies such as anti-cyclic citrullinated peptide [2] and increased CRP levels [3] many years before the appearance of clinical symptoms suggests a role for dysregulation of the immune response in the pathogenesis of this disease. Since IL-6 is important in B-cell maturation and therefore production of autoantibodies, as well as the direct stimulation of CRP from hepatocytes, it may play a significant role in RA pathogenesis [4]. In animal models of autoimmune diseases, IL-6 plays a critical role in the generation of Th17 pro-inflammatory lymphocytes, thus increasing this possibility further [5]. In patients with established RA, many of the articular and systematic manifestations could be explained by the effect of IL-6.

The combination of articular and systemic effects of IL-6 makes inhibition of the IL-6R a logical target for treatment of patients with RA. This review will provide a perspective on how IL-6 induces the articular and systemic symptoms of RA.

IL-6 biology

Multi-target cytokine

IL-6 is a 26-kDa glycopeptide whose gene is found on chromosome 7. It has previously been known as

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FIG. 1 Inflammatory pathways activated by IL-6. At joint level, IL-6 induces pannus formation, osteoclast activation and mediates chronic synovitis.

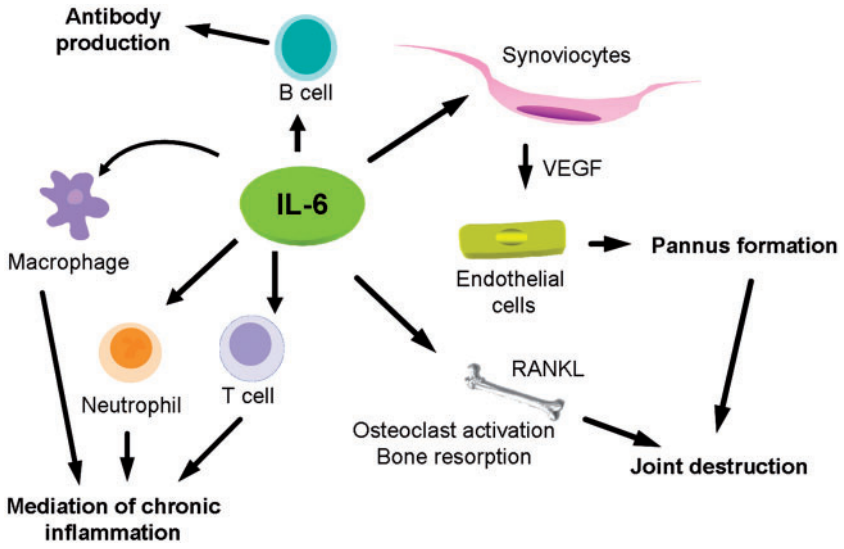
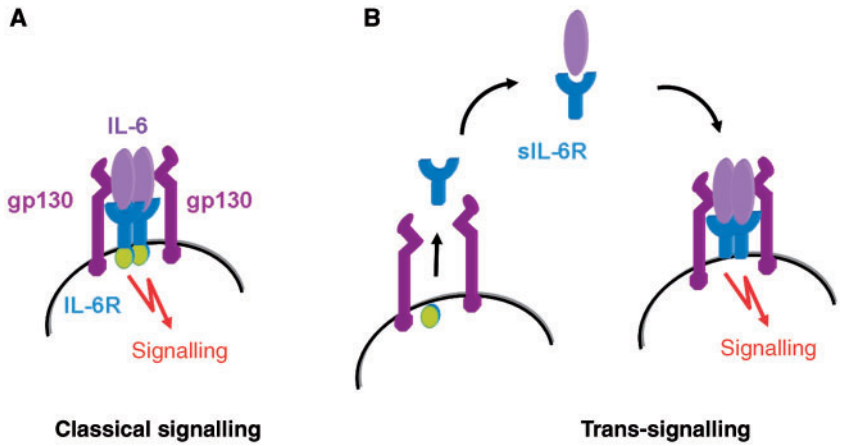


FIG. 2 IL-6 signalling mechanism. IL-6-mediated signal transduction through classical (**A**) and trans-signalling (**B**) pathways. In IL-6 trans-signalling, sIL-6R is generated either by limited proteolysis of the membrane-bound IL-6R or by alternative mRNA splicing. In both classical and trans-signalling, responses are elicited through engagement with membrane-bound gp130.



hepatocyte-stimulating factor, cytotoxic T-cell differentiation factor, B-cell differentiation factor, B-cell stimulatory factor 2, hybridoma/plasmacytoma growth factor, monocyte granulocyte inducer type 2 and thrombopoietin. The many names reflect the pleiotropism of IL-6. The IL-6-like family of cytokines has more than 10 members, including IL-11 and leukaemia inhibitory factor [4, 6].

Receptor binding

IL-6 signals primarily through a protein complex including the membrane-bound, non-signalling α-receptor subunit (IL-6R) and two signal-transducing gp130 subunits (Fig. 2A) [4]. While gp130 is expressed ubiquitously, IL-6R is predominantly expressed on hepatocytes, neutrophils, monocytes/macrophages and some lymphocytes [4]. However, IL-6 can also signal via a soluble receptor (sIL-6R) that lacks transmembrane and cytoplasmic components. Activated sIL-6R binds to membrane-bound gp130 subunits in a process known as trans-signalling (Fig. 2B) [4, 7]. sIL-6R is generated either by limited proteolysis of the membrane-bound IL-6R or by alternative mRNA splicing. sIL-6R is transported in bodily fluids and increases the variety of cells able to

respond to IL-6 [4]. For example, endothelial cells and synoviocytes express gp130 but not IL-6R, and can therefore only respond to IL-6 in the presence of sIL-6R. Indeed, IL-6 and sIL-6R induce tubule formation in fibroblast-like synovial cells from RA patients, an effect not observed with IL-6 alone or indeed TNF-α [8].

The key role of trans-signalling in RA has been demonstrated recently in a murine experimental arthritis model in which blocking IL-6 trans-signalling using a variant soluble gp130 molecule resulted in a marked clinical improvement in systemic arthritis [9]. These findings support earlier data showing restoration of experimental arthritis disease activity in an IL-6 knock-out mouse model when administered with an sIL-6R–IL-6 fusion protein [10].

Receptor activation

The increase in IL-6 and sIL-6R in SF increases the risk of joint destruction in RA [11]. Once IL-6 binds its receptor and gp130 homodimerization occurs, a signalling cascade is triggered. The Janus kinases are activated, followed by the recruitment of signal transducers and activators of transcriptions (STATs). Phosphorylated STATs translocate to the nucleus where they activate gene transcription [12, 13]. IL-6 also activates the mitogen-activated protein kinase cascade, which is upstream of various molecules involved in cell survival and stress responses [14]. IL-6R stimulation also recruits other signal transduction molecules, including SH2 domain-containing tyrosine phosphatase (SHP2) and suppressor of cytokine signalling (SOCS). Both SHP2 and SOCS may subsequently down-regulate IL-6 signalling [12].

The role of IL-6 in the adaptive immune response

RA is characterized by an increase in IgM and IgG RFs and antibodies to citrullinated peptides in both serum and joints. B-cell depletion is of therapeutic benefit in RA and demonstrates the impact of B-cell activity on synovial inflammation and joint damage in this disease. IL-6 was originally identified as a B-cell differentiation factor; it plays an important role in the development of antibody-producing plasma B cells [15]. IL-6 induces B-cell differentiation through its action on plasmablasts [16] and more recently has been shown to induce B-cell antibody production indirectly by promoting the B-cell helper properties of $CD4^+$ T cells via the production of IL-21 [17].

In addition to B-cell development, IL-6 influences T-cell development. When activated, naïve T cells develop into either effector or regulatory T cells [18]. Effector T cells are further subdivided into Th1, -2 and -17 cells, all of which have pro-inflammatory properties. Animal studies have shown that Th17 cells are important mediators in autoimmune diseases and the host defense against extracellular pathogens. Th17 cells produce IL-17, -12 and -22. In murine models of autoimmune diseases, differentiation of Th17 is regulated by the cytokine milieu. In the presence of TGF-β alone, naïve T cells differentiate into regulatory T cells and development of Th17 cells is suppressed. When IL-6 is present together with TGF-β, naïve T cells develop into Th17 cells through activation of STAT3 and induction of the transcription factor retinoic acid-related orphan receptor (RORgammat). IL-23, which also activates STAT3, is important in the maintenance of Th17 cells. In humans, however, it appears this pathway, and is driven by IL-6 in combination with IL-1β and -23 rather than TGF-β [5, 19, 20]. In addition, *in vitro* activated monocytes as well as *in vivo* activated monocytes from the rheumatoid joint drive Th17 induction from memory T cells via the production or expression of inflammatory mediators [21, 22].

Th17 cells are also involved in the host defense response against bacteria and fungi, suggesting that IL-6 may contribute indirectly to fighting infection through Th17 cell development [5, 23]. For example, IL-6-induced activation of STAT proteins is important in the recruitment of neutrophils during *Escherichia coli* pneumonia infection [24]. Differences between pro-inflammatory cytokines have been observed in a number of infections. For example, although TNF-α has been found to be involved in the formation and maintenance of granulomas during infection with *Mycobacterium tuberculosis*, there is little evidence to suggest involvement of IL-6 in granuloma preservation [25–28]. Overall, these findings demonstrate that IL-6 has an important role in the development of the adaptive immune response and may be involved in the pathogenesis of RA.

The effects of IL-6 at joint level

The role of IL-6 in the shift from acute to chronic inflammation

Neutrophils are important mediators of inflammation and joint destruction in RA due to their ability to secrete proteolytic enzymes and reactive oxygen intermediates. IL-6 acts directly on neutrophils through membrane-bound IL-6R. When endothelial cells were co-cultured with fibroblasts isolated from the synovium of RA patients, IL-6 levels increased and neutrophils adhered [29]; anti-IL-6 antibodies prevented this neutrophil adhesion. *In vivo*, IL-6-negative transgenic mice show defective leucocyte recruitment into the air pouch [30]. Similarly, in wild-type mice, introduction of an anti-IL-6 antibody reduced leucocyte infiltration to levels observed in the transgenic mice. Other reported effects of IL-6 on neutrophils include survival, activation of proliferation through inflammatory cytokines, mobilization of marginated neutrophils into the circulation and transit of neutrophils from the bone marrow [31–35].

During acute inflammation in RA, monocytes, macrophages and endothelial cells release IL-6, accompanied by an increase in neutrophils in SFs. As disease progresses, IL-6 is thought to influence the shift from acute to chronic inflammation [36], marked by an increase in the recruitment of monocytes.

The release of sIL-6R is thought to play a key role in the regulation of acute and chronic inflammation. Indeed, sIL-6R release from neutrophils as they reach the site of

inflammation results in local recruitment of leucocytes through activation of adjacent endothelial cells and subsequent chemokine release [30, 37–39]. This may be an important rate-limiting step for inflammation. In particular, sIL-6R signalling increases the amounts of monocyte-specific, but not neutrophil-specific chemo-attractants secreted by endothelial cells [40]. Thus, activation of endothelial cells through trans-signalling results in a shift from neutrophil to monocyte infiltration.

The role of IL-6 in extracellular matrix turnover

Extracellular matrix is the target of proteinases such as MMPs and disintegrin-metalloproteinases with thrombospondin motifs. Proteinases in RA are produced by synovial lining cells, sublining fibroblasts and infiltrating leucocytes and macrophages [41–48]. Several studies have shown a correlation between articular cartilage destruction and the expression of MMPs [49–51]. Cells lining the synovium in RA have been shown to overproduce MMPs, with plasma levels of MMP-2 and -9 higher in RA patients than controls [41, 44, 45, 47, 52]. Both IL-6 and sIL-6R increased collagenase-3 mRNA and protein levels in rat osteoblast cultures [53, 54]. However, this situation has not been demonstrated in humans. Although some studies suggest that IL-6 does not stimulate proteinase production or activity [55, 56], correlations between IL-6 and CRP, as well as CRP and proMMP-3, have been identified in patients with early RA [57]. These findings suggest a link between proteinase activity and IL-6 levels.

Tissue inhibitors of MMPs (TIMPs) are endogenous inhibitors of MMPs. IL-6, in the presence of sIL-6R, induced TIMP-1 mRNA and protein expression in cultured human chondrocytes and synovial fibroblasts [58]. The ability of culture supernatants from IL-6/sIL-6R-stimulated cells to inhibit collagen digestion in IL-1-stimulated synovial cells further supports IL-6-induced TIMP production and the role of IL-6 in extracellular matrix turnover [58].

The role of IL-6 in the development of articular symptoms of RA

IL-6 is abundantly expressed in the synovium in RA [59]. Levels of IL-6 and sIL-6R in SF correlate significantly with local joint measures of chronic synovitis and the severity of joint destruction in patients with RA [60], as does sIL-6R with leucocyte infiltration [61]. Moreover, the sIL-6R:IL-6 ratio is significantly higher in patients with Stage 1 and 2 disease, according to Mallya and Mace [62, 63] assessment of disease activity, compared with patients with Stage 4 disease.

IL-6 can also promote joint inflammation and damage through its effect on VEGF levels in RA patients. VEGF is a potent angiogenic factor that promotes the migration and proliferation of endothelial cells, as well as inducing vascular permeability and mediating inflammation [64, 65]. Significant increases in VEGF levels in RA patients correlate with disease activity, suggesting that VEGF is implicated in RA pathogenesis, particularly in pannus formation [66]. IL-6, in the presence of sIL-6R, increased VEGF levels in cultured synovial fibroblasts from RA patients [67]. In these cell cultures, anti-IL-6R antibody significantly reduced VEGF concentration.

Effects of IL-6 on erosion

Joint damage in RA is characterized by erosions and joint space narrowing indicating destruction of bone and articular cartilage. In human and animal studies, osteoclasts have been identified as the key cell type-mediating erosions in inflammatory arthritis [68]. IL-6 increases osteoclast recruitment by acting on haematopoietic stem cells from the granulocyte–macrophage lineage [69–71].

A number of *in vitro* and *in vivo* studies have looked at the effects of IL-6 and sIL-6R on osteoclastogenesis and bone resorption. In an *in vitro* study, IL-6-induced osteoclast differentiation is indirect and appears to be mediated via interaction with osteoblasts through the sIL-6R, resulting in PGE2 synthesis. PGE2 acts in an autocrine manner to induce the RANK-ligand expression and down-regulate osteoprogerin expression leading to enhanced osteoclastogenesis [11, 72, 73]. In mouse calvarial bone cultures, IL-6, in the presence of sIL-6R, induced bone resorption, which was decreased by osteoclast inhibitors, suggesting that sIL-6R trans-signalling influences osteoclastogenesis [72]. *In vivo*, a reduction in the severity of antigen-induced arthritis was observed in IL-6-deficient mice compared with wild-type mice [73]. In addition, the IL-6-deficient mice had markedly reduced osteoclast recruitment to the sites of joint disease, as well as lower IL-17 levels. In humans, however, the effect of IL-6 on PGE2 production is not established. Moreover, it has recently been suggested that, under normal conditions, IL-6 suppresses bone resorption by specifically inhibiting the RANK signalling pathway. In RA, IL-6 and sIL-6R induced osteoclastogenesis in osteoclast-like multinucleated cells obtained from RA patients, at the concentrations found within the SF of RA patients. This process was inhibited by anti-IL-6 [11].

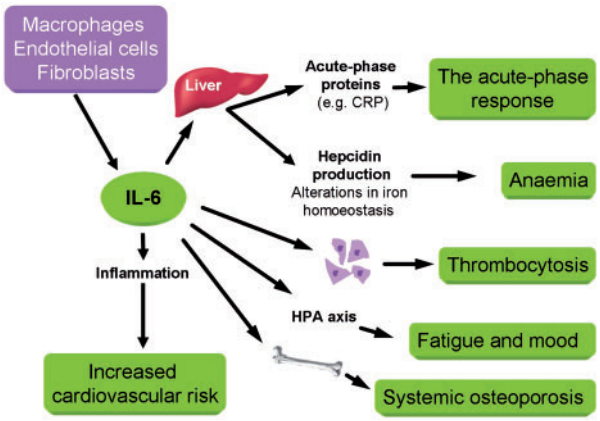
IL-6 also exerts effects on the components of the articular cartilage. Proteoglycans are the principal component of articular cartilage and the depletion of these in RA contributes to cartilage degradation. In cultures of human articular chondrocytes from patients with RA [74], either IL-6 or sIL-6R alone induced a small inhibitory effect on proteoglycan synthesis. However, the combination of sIL-6R and IL-6 markedly increased the inhibition, again suggesting a key role for IL-6 in causing joint damage in RA.

The role of IL-6 in the development of systemic symptoms of RA

Acute-phase response

The acute-phase response, one of the body's first reactions to injury, is characterized by a number of systemic changes. These include the release of pro-inflammatory cytokines and alterations in the level of acute-phase proteins in the plasma [75, 76]. Acute-phase proteins are

Fig. 3 The systemic effects of IL-6. Systemically, IL-6 actions include stimulation of acute-phase proteins and hepatocyte proliferation in the liver, induction of anaemia and effects on lipids and lipid metabolism, impairment of HPA axis and osteoporosis.



produced in the liver and are classed as either positive or negative, depending on whether their concentrations increase during inflammation (e.g. CRP and TIMPs) or decrease (e.g. apolipoprotein A1 and transferrin). IL-6 is a principal stimulator of acute-phase protein synthesis through hepatocyte stimulation. In patients with RA, serum IL-6 levels correlate with CRP levels [59]. As IL-6 is easily measured in biological fluids, it can be used as a biomarker of inflammation and disease activity [59, 77] (Fig. 3).

Anaemia of chronic inflammation

Anaemia is the most common systemic manifestation of RA. Defined as haemoglobin (Hb) levels <13 g/dl in men and <12 g/dl in women, anaemia is present in more than a third of RA patients and in a quarter of patients within the first year of disease [78, 79]. Patients with RA and anaemia have more severe physical disability compared with non-anaemic patients (Hb >14 g/dl) [78].

The peptide hepcidin is produced by hepatocytes and regulates iron metabolism by preventing iron transport and the release of iron from macrophages [80]. *In vitro*, IL-6 stimulation of human hepatoma cells induced hepcidin expression [81]. In wild-type mice, turpentine stimulation of the inflammatory response resulted in marked increase in liver hepcidin expression accompanied by a decrease in serum iron, whereas in IL-6 knock-out mice, hepcidin levels were below baseline levels and iron levels increased slightly [81]. Moreover, IL-6 infusion resulted in a rapid 7.5-fold increase in hepcidin secretion in healthy volunteers [81]. Thus, the IL-6–hepcidin axis has a vital role in the anaemia of inflammation observed in many RA patients.

Systemic osteoporosis

Osteoporosis, a common systemic manifestation of RA, is also linked with IL-6. Bone remodelling requires a careful balance between the actions of bone-resorbing osteoclasts and bone-forming osteoblasts. Dysregulation of this process can lead to overall bone loss. Transgenic mice over-expressing IL-6 have been shown to have decreased osteoblast and increased osteoclast numbers [82]. Accelerated bone resorption, reduced bone formation and defective ossification were also reported, suggesting that IL-6 over-expression results in osteopaenia due to osteoclast and osteoblast dysregulation.

Fatigue and hypothalamo-pituitary-adrenal (HPA) axis

Fatigue is a commonly reported problem in patients with RA, with 41% experiencing clinically important levels of fatigue [83–85]. For a number of years, IL-6 has been known to influence fatigue and sleep, with healthy volunteers recording increased fatigue, inactivity and lack of concentration following IL-6 administration *vs* placebo [86]. These IL-6-induced effects were found to correspond with HPA axis function. More recently, IL-6 production has been correlated with reports of fatigue in patients with RA, providing further evidence of the link between IL-6 and fatigue [87].

IL-6, lipids and inflammation

Patients with RA are at increased risk of cardiovascular disease. The atherogenic effects of systemic inflammation manifest themselves at different levels, including endothelial dysfunction and dyslipidaemia [88–90]. Elevated CRP levels are associated with increased risk of cardiovascular disease [91], hospitalization and hospital mortality, although more research is required to determine the direct role of CRP [92, 93].

Inflammation through the effects of IL-6 reduces circulating lipid levels. When IL-6 was administrated to normal healthy volunteers [94], within 24 h of IL-6 administration, total cholesterol, apolipoprotein B and triglyceride were reduced. The exact mechanism by which IL-6 induces these changes remains unknown. However, IL-6 has been shown to affect lipid metabolism by stimulating hepatic fatty acid synthesis and adipose tissue lipolysis. In addition, IL-6 increases cholesterol synthesis while decreasing cholesterol secretion [95, 96].

Independent of the effect on lipids, IL-6 and CRP have been associated with increased cardiovascular risk in apparently normal healthy males [97] and females [98]. Furthermore, IL-6 is associated with increased mortality in patients with acute coronary syndromes [99]. Serum IL-6 levels were significantly higher in patients with a complicated in-hospital course, compared with those demonstrating an uncomplicated course. In addition, decreases in IL-6 within 48 h were associated with uncomplicated outcomes, whereas increases in IL-6 were associated with complications. These data implicate IL-6 in the development of coronary artery disease.

IL-6R inhibition in the treatment of RA

Recent advances in understanding of RA pathogenesis have identified a number of potential targets for

intervention. Indeed, three TNF inhibitors are now available in clinical practice. Other therapeutic targets include the B cell, which is deleted by the anti-CD20 monoclonal antibody rituximab and the CD80/CD86–CD28 co-stimulatory signal required for T-cell activation, which is blocked by the recombinant fusion protein abatacept. However, RA treatment is still far from satisfactory as ~20–40% of the patients do not respond to TNF inhibitors administered either alone or in combination with DMARDs [100].

Although there are few head-to-head comparisons for TNF, IL-1 and -6 activity in the same biological system, there is evidence of complex interactions between these cytokines. Table 1 summarizes, in the authors' opinion, the characteristics that distinguish IL-6 activity from other pro-inflammatory cytokines. Given these differences and the multiple roles of IL-6 in the immune response and inflammation, therapies that disrupt IL-6 signalling offer an important treatment option for RA (Fig. 4). Indeed, results from Phase III clinical trials of IL-6 inhibition with the monoclonal antibody tocilizumab in patients with RA are confirming this promise [101–103].

Table 1 Summary of the characteristics that suggest a distinctive role of IL-6 in RA (based on the authors' extensive knowledge of the available literature)

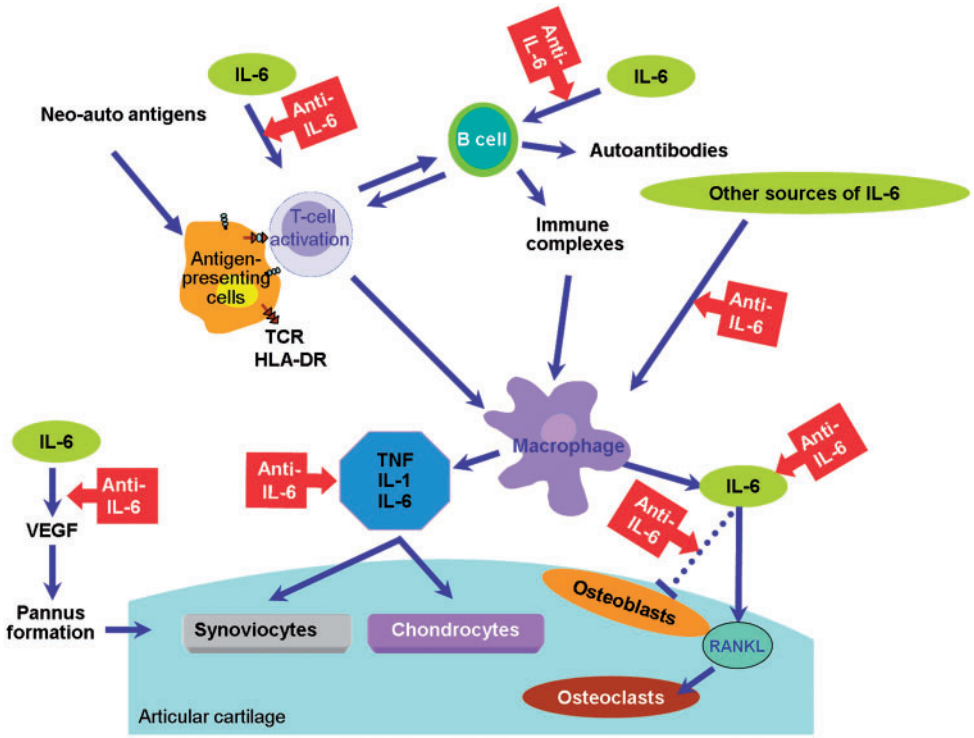
Features	IL-6	TNF	IL-1
Levels in blood and SF	++++	+	+
Local effects			
Endothelial activation	++	++	+
Polymorphonuclear cells (neutrophil migration)	++	+	+
Proteases, MMP secretion	+	++	+++
Bone-derived cell activity (osteoclast and osteoblast)	++	+	+
B-cell function and survival	+++	–	+
Th17 differentiation	++	–	++
Systemic effects			
Acute-phase protein production	+++	+	+
Bone marrow (anaemia)	++	+	+
CNS (fatigue)	+++	++	++

The level of activity is represented by crosses, with an increased number of crosses representing increased activity.

Summary

IL-6 is the most abundant cytokine in the serum and SF of patients with RA and levels correlate with both disease activity and joint destruction. IL-6 signalling occurs through both membrane-bound and soluble receptors. IL-6 is a multitarget cytokine with activity relevant to RA

Fig. 4 Potential sites for intervention in RA. Based on the present knowledge of RA pathogenesis, therapeutic strategies can influence the outcome of initial or late-phase processes. IL-6 inhibition can influence the initial autoimmune reaction between antigen-presenting cells and T and B cells or the later stage in tissue destruction when synovial cells, chondrocytes and bone-derived cells are involved.



at joint and systemic levels. At the joint, IL-6 has a pivotal role in the inflammatory process, in osteoclast-mediated bone resorption and in pannus development through increased VEGF expression. IL-6 is pro-inflammatory, induces acute-phase proteins (including CRP) and contributes to the systemic manifestations of RA though hepcidin production (anaemia), its potent action on the HPA axis (fatigue) and its impact on bone metabolism (osteoporosis). In addition, IL-6 may contribute to the induction and maintenance of the autoimmune process through B-cell modulation and Th17 cell differentiation. In combination, these findings make IL-6 activity a logical target for inhibition in patients with RA. This observation is supported by Phase III studies using an anti-IL-6R antibody, which have demonstrated benefits of IL-6R inhibition across a number of patient populations with RA.

Rheumatology key messages

- IL-6 is a pleiotropic cytokine.
- IL-6 plays a role in the local and systemic manifestations of RA.
- The IL-6 receptor offers an important treatment option for RA.

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