

The role of Interleukin 6 in the pathophysiology of rheumatoid arthritis

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Abstract: Interleukin 6 (IL-6) is a pleiotropic cytokine with a pivotal role in the pathophysiology of rheumatoid arthritis (RA). It is found in abundance in the synovial fluid and serum of patients with RA and the level correlates with the disease activity and joint destruction. IL-6 can promote synovitis and joint destruction by stimulating neutrophil migration, osteoclast maturation and vascular endothelial growth factor (VEGF)-stimulated pannus proliferation. IL-6 may also be mediating many of the systematic manifestations of RA including inducing the acute-phase reaction [including C-reactive protein (CRP)], anaemia through hepcidin production, fatigue via the hypothalamic–pituitary–adrenal (HPA) axis) and osteoporosis from its effect on osteoclasts. In addition, IL-6 may contribute to the induction and maintenance of the autoimmune process through B-cell maturation and TH-17 differentiation. All of the above makes IL-6 blockade a desirable therapeutic option in the treatment of RA. Following successful animal studies, a humanized anti-interleukin-6 receptor (anti-IL-6R) monoclonal antibody, tocilizumab (TCZ), entered into clinical trials and it has been shown to be an effective treatment in several large phase III clinical trials in RA with rapid and sustained improvement in disease activity, reducing radiographic joint damage and improving physical function.

Keywords: interleukin 6, pathophysiology, receptor blockade, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder affecting about 1% of the population worldwide, most commonly middle-aged women. It is characterized by chronic inflammation of the synovium, particularly of small joints, which often leads to destruction of articular cartilage and juxta-articular bone. It is often accompanied with systemic manifestations such as anaemia, fatigue and osteoporosis.

RA pathogenesis involves complex humoral and cellular reactions including immune-complex formation, vascular reactions and infiltration of lymphocytes and monocytes into the synovium. These infiltrating cells and synoviocytes release pro-inflammatory mediators, including interleukin (IL)-6, which perpetuate inflammation and destruction through effects on other cell types in the synovium and periarticular structures (Figure 1)

RA is thought to be initiated by immunity against an unknown antigen and later to a sustained inflammatory process [McInnes and

Schett, 2007]. The presence of auto-antibodies such as anticyclic citrullinated peptide [Avouac *et al.* 2006] and increased C-reactive protein (CRP) level [Nielen *et al.* 2006] many years before the appearance of clinical symptoms suggest a role for dysregulation of the immune response in the pathogenesis of this disease.

In RA the cytokine network is complex with numerous cytokines present both in blood and in synovial joints. One of these is IL-6, which is a pleiotropic cytokine important in B-cell maturation and therefore the production of auto-antibodies, as well as the direct stimulation of CRP from hepatocytes, so it may play a significant role in RA pathogenesis [Rose-John *et al.* 2006]. In animal models of autoimmune diseases, IL-6 also plays a critical role in the generation of Th17 pro-inflammatory lymphocytes [Chen and O'Shea, 2008]. In patients with established RA, many of the articular and systemic manifestations could be explained by the biologic effect of IL-6. In this review we aim to look into the role of IL-6 in the pathophysiology of RA.

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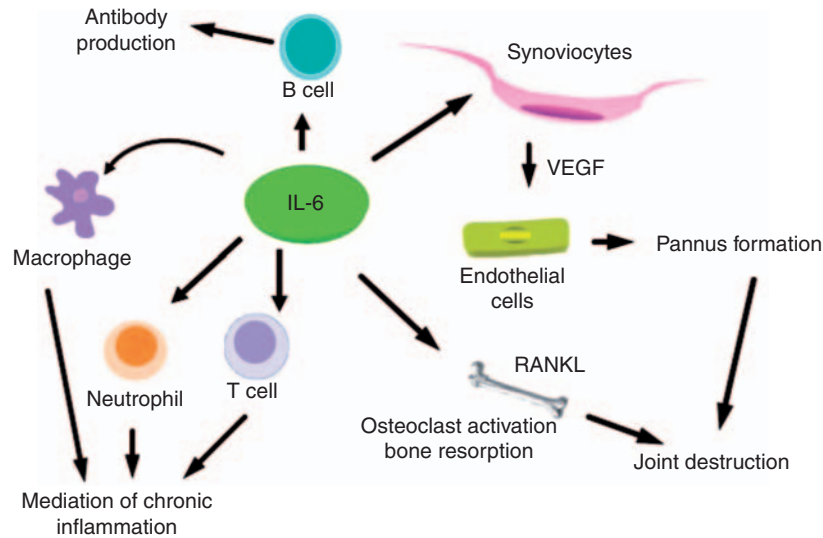


Figure 1. Inflammatory pathways activated by interleukin (IL)-6. In the joints IL-6 induces pannus formation, osteoclast activation and mediates chronic synovitis.

IL-6 structure, family and receptors

IL-6 is a 26-kDa glycopeptide whose gene is found on chromosome 7. It is produced by various cell types, such as T cells, B cells, monocytes, fibroblasts, osteoblasts, keratinocytes, endothelial cells, mesangial cells and some tumour cells. IL-6 is one member of the IL-6 cytokine family which includes leukaemia inhibitory factor, ciliary neurotrophic factor, IL-11 and cardiotrophin-1. All of these cytokines require cell surface gp130 for cellular activation in addition to their respective cytokine receptors.

Previously, IL-6 has been known as hepatocyte stimulating factor, B-cell stimulatory factor 2, cytotoxic T-cell differentiation factor, B-cell differentiation factor, hybridoma/plasmacytoma growth factor, monocyte granulocyte inducer type 2 and thrombopoietin. The many names reflect the pleiotropism of IL-6 with important biologic effects on the liver, B cells, T cells, monocytes and platelets.

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 [Dayer and Choy, 2010]. The key role of trans-signalling in RA has been demonstrated 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 [Nowel *et al.* 2009]. These findings support earlier data showing restoration of experimental arthritis disease activity in an IL-6 knock-out mouse model when administered with a sIL-6R-IL-6 fusion protein [Nowell *et al.* 2003]. The increase in IL-6 and sIL-6R in synovial fluid increases the risk of joint destruction in RA [Kotake *et al.* 1996].

Role of IL-6 in the pathophysiology of RA

Adaptive immune response

IgM and IgG rheumatoid factors along with antibodies to citrullinated peptides are characteristically increased in RA. The therapeutic efficacy of B-cell depletion in RA demonstrates the impact of B-cell activity on synovial inflammation and joint damage. IL-6 stimulates B cells to differentiate into plasma cells to produce immunoglobulins [Muraguchi *et al.* 1988]. IL-6 induces B-cell differentiation [Jogo *et al.* 2001] and has been shown to induce B-cell antibody production [Dienz *et al.* 2009].

IL-6 influences T-cell development by stimulating the proliferation and differentiation of T lymphocytes into TH-17 cells which produce IL-17. In murine models of autoimmune diseases in the presence of IL-6 and transforming growth factor beta (TGF- β), naïve T cells develop into

Th17 cells. In humans this pathway is driven by IL-6 in combination with IL-1 β and IL-23 rather than TGF- β [Chizzolini *et al.* 2008; Acosta-Rodriguez *et al.* 2007; Rose-John *et al.* 2006]. All of this would suggest 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 role of IL-6 in the shift from acute to chronic inflammation

Neutrophil migration from blood to tissue is a characteristic feature of inflammation. Upon entry, activated neutrophils release proteolytic enzymes and reactive oxygen intermediates resulting in tissue destruction and joint damage in RA. Neutrophils express membrane-bound IL-6R and are activated by IL-6. When endothelial cells were cocultured with fibroblasts isolated from the synovium of RA patients, IL-6 levels increased and neutrophils adhered to the endothelium [Lally *et al.* 2005], a pivotal step prior to the migration of neutrophils into the joint. *In vivo*, IL-6-negative transgenic mice show impaired leukocyte accumulation in a subcutaneous air pouches model [Romano *et al.* 1997]. Conversely, an anti-IL-6 antibody in wild-type mice reduced leukocyte infiltration to levels observed in the IL-6 deficient mice.

IL-6 has also been implicated in the shift from acute to chronic inflammation [Kaplanski *et al.* 2003] by increase recruitment of monocytes. Neutrophils release sIL-6R as they reach the site of inflammation resulting in local recruitment of leukocytes through activation of adjacent endothelial cells and subsequent chemokine release [Lindemann *et al.* 2004; Hurst *et al.* 2001; Modur *et al.* 1997; Romano *et al.* 1997]. Trans-signalling via sIL-6R increases the amounts of monocyte-specific chemokines secreted by endothelial cells [Marin *et al.* 2001]. Thus, activation of endothelial cells through trans-signalling results in a shift from neutrophil to monocyte infiltration.

Role of IL-6 in the development of articular symptoms in RA

In the rheumatoid synovium, IL-6 is one of the most abundantly expressed cytokines [Madhok *et al.* 1993]. Levels of IL-6 and sIL-6R in synovial fluid correlate with histological characteristics of chronic synovitis in patients with RA [Sack *et al.* 1993] as does sIL-6R with leukocyte infiltration [Jones *et al.* 2005]. IL-6 can also promote joint

inflammation and damage through its effect on vascular endothelial growth factor (VEGF) levels in RA patients. VEGF is an important angiogenic mediator which promotes the migration and proliferation of endothelial cells, as well as inducing vascular permeability and mediating inflammation [Connolly *et al.* 1989; Keck *et al.* 1989]. Raised levels of VEGF correlate with disease activity in RA patients [Ballara *et al.* 2001]. IL-6 in the presence of sIL-6R increased VEGF levels in cultured synovial fibroblasts from RA patients and anti-IL-6R antibody significantly reduced VEGF concentration [Nakahara *et al.* 2003].

IL-6 and joint erosions

Erosions and joint space narrowing indicate destruction of bone and articular cartilage in RA. Osteoclasts are key cells involved in mediating erosions in inflammatory arthritis [Walsh *et al.* 2005]. IL-6 increases osteoclast recruitment by acting on hematopoietic stem cells from the granulocyte-macrophage lineage [Yoshitake *et al.* 2008; Liu *et al.* 2005; Otsuka *et al.* 1991]. The resorptive effects of IL-6 and their regulation of receptor activator of NF- κ B ligand (RANKL), receptor activator of NF- κ B (RANK), and osteoprotegerin (OPG) were studied in neonatal mouse calvaria where IL-6 in the presence of sIL-6R enhanced the expression of RANKL and OPG, but decreased RANK expression and induced bone resorption, which was decreased by osteoclast inhibitors, suggesting that sIL-6R trans-signalling influences osteoclastogenesis [Palmqvist *et al.* 2002]. In antigen-induced arthritis (AIA) IL-6 deficient mice had reduced severity of arthritis and fewer osteoclasts at sites of bone erosion along with lower IL-17 levels compared with wild-type mice [Wong *et al.* 2006]. In RA IL-6 and sIL-6R stimulated osteoclast-like cell formation, at the concentrations found within the synovial fluid of RA patients, which was inhibited by adding anti-IL-6R antibody [Kotake *et al.* 1996].

IL-6 is also implicated in damage to the articular cartilage in RA as sIL-6R and IL-6 markedly inhibit proteoglycan synthesis in cultures of human articular chondrocytes from patients with RA [Guerne *et al.* 1999].

The role of IL-6 in extracellular matrix turnover

Proteinases such as matrix metalloproteinases (MMPs) in RA are produced by synovial lining

cells, sublining fibroblasts and infiltrating leukocytes and macrophages [Murphy and Nagase, 2008; Yamanishi *et al.* 2002; Takizawa *et al.* 2000; Yamanaka *et al.* 2000; Ahrens *et al.* 1996; Hembry *et al.* 1995; Okada *et al.* 1990, 1989] and target the extracellular matrix. There is a correlation between articular cartilage destruction and the expression of MMPs [Ohta *et al.* 1998; Imai *et al.* 1997; Okada *et al.* 1992]. Cells lining the rheumatoid synovium have been shown to overproduce MMPs whose levels are higher in RA patients than controls [Chang *et al.* 2008; Yamanaka *et al.* 2000; Ahrens *et al.* 1996; Hauptmann *et al.* 1991; Seckinger *et al.* 1990; Okada *et al.* 1990, 1989]. In patients with early RA, IL-6 and CRP correlate with proMMP-3 [Roux-Lombard *et al.* 2001] suggesting a link between proteinase activity and IL-6. Tissue inhibitors of matrix metalloproteinases (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 [Silacci *et al.* 1998].

IL-6 in the development of systemic symptoms of RA

Acute-phase response

During an acute-phase response there is release of pro-inflammatory cytokines and alterations in the level of acute-phase proteins in the plasma [Castell *et al.* 1989; Gaudie *et al.* 1987]. 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 [Madhok *et al.* 1993].

Anaemia of chronic inflammation

Anaemia of chronic inflammation is present in more than a third of RA patients and in a quarter of patients within the first year of disease [Nikolaisen *et al.* 2008; Han *et al.* 2007]. Anaemia is an independent factor contributing to physical disability in patients with RA [Han *et al.* 2007].

The peptide hepcidin produced by hepatocytes regulates iron metabolism by preventing iron transport and the release of iron from macrophages [Ganz, 2003]. IL-6 stimulation induced hepcidin expression has been noted in *in vitro* studies of human hepatoma cells [Nemeth *et al.* 2004]. Turpentine stimulation of the inflammatory response in wild-type mice resulted in marked increases in liver hepcidin expression

accompanied by a decrease in serum iron; however, in IL-6 knock-out mice, hepcidin levels were low and iron levels increased slightly [Nemeth *et al.* 2004]. This along with the observation that a rapid increase in hepcidin secretion occurs following IL-6 infusion in healthy volunteers suggests an important role of IL-6 in the anaemia of inflammation observed in many patients with RA.

Systemic osteoporosis

An imbalance between osteoblasts and osteoclasts results in dysregulation of bone remodelling resulting in osteoporosis. Osteoporosis is a common systemic manifestation of RA. *In vivo* studies with IL-6 transgenic mice showed increased osteoclastogenesis with reduced osteoblast activity resulting in accelerated bone resorption, reduced bone formation and defective ossification [De *et al.* 2006] suggesting that IL-6 overexpression results in osteopenia due to osteoclast and osteoblast dysregulation.

Fatigue and the hypothalamic–pituitary–adrenal axis

Fatigue is a commonly reported problem in RA, with 41% of patients experiencing clinically important levels of fatigue [Kirwan and Hewlett, 2007; Tubach *et al.* 2007; Wolfe *et al.* 1996]. The cause of fatigue in RA is unknown. Pain, cytokines, hormonal, psychological, metabolic factors, and medication may all contribute. Hypothalamic–pituitary–adrenal (HPA) axis abnormality has been linked to the development of fatigue in many diseases [Tsigos and Chrousos, 2002]. Cytokines such as IL-6 can modulate the HPA axis [Chrousos, 1995]. Indeed, following IL-6 administration, healthy volunteers recorded increased fatigue, inactivity, lack of concentration and altered sleep architecture. These effects were found to correspond with the HPA axis function [Spath-Schwalbe *et al.* 1998].

IL-6, lipids and inflammation

Cardiovascular mortality is increased in patients with RA. Systemic inflammation causes atherogenesis via endothelial dysfunction and dyslipidemia [van Leuven *et al.* 2008; Dessein *et al.* 2007; Niessner *et al.* 2007]. Elevated CRP levels are associated with an increased risk of cardiovascular disease [Yeh, 2004]. Inflammation through the effects of IL-6 reduces circulating lipid levels although mechanisms by which IL-6 induces these changes is not known [Papanicolaou

et al. 1998]. IL-6 has been shown to affect lipid metabolism by stimulating hepatic fatty-acid synthesis and adipose-tissue lipolysis. IL-6 also increases cholesterol synthesis while decreasing cholesterol secretion [Khovidhunkit *et al.* 2004; Nonogaki *et al.* 1995]. IL-6 and CRP have been associated with increased cardiovascular risk in apparently normal healthy males [Ridker *et al.* 2000] and females [Ridker and Cook, 2004] independent of their effects on lipids. IL-6 is associated with increased mortality in patients with acute coronary syndromes [Biasucci *et al.* 1999]. The above would implicate IL-6 in the development of coronary artery disease. A summary of the systemic effects of IL-6 is shown in Figure 2.

IL-6 blockade is an effective treatment in animal models

Studies in IL-6 deficient mice have shown evidence that IL-6 is essential in the development of RA [Boe *et al.* 1999]. Characteristically, wild-type animals developed joint inflammation and swelling about 9 days after intra-articular injection of antigen. However, IL-6 knockout mice were resistant to AIA in that no inflammatory response or synovial inflammation was induced. Subcutaneous injections of IL-6 could induce arthritis in knockout animals. In DBA/1J mice,

immunization with bovine type II collagen leads to an inflammatory arthritis resembling RA clinically and histologically. Monoclonal antibody to IL-6R reduced the disease severity if given early in the disease process [Takagi *et al.* 1998]. In a primate model of RA, immunization with bovine type II collagen induces inflammatory arthritis in cynomolgus monkeys. Blocking IL-6 with anti-IL-6R monoclonal antibody led to disease improvement [Mihara *et al.* 2001].

IL-6 blockade is effective in RA

A humanized anti-IL-6R monoclonal antibody, tocilizumab (TCZ), entered into clinical trials following the successful use of anti-IL-6R antibodies in animal models of arthritis. The first clinical trial of TCZ was conducted in patients with established RA [Choy *et al.* 2002]. A total of 45 patients who had failed at least one disease-modifying antirheumatic drug (DMARD) were randomized to receive a single intravenous infusion of TCZ of 0.1, 1, 5, 10 mg/kg or placebo. Patients in the 5 and 10 mg/kg arms showed rapidly improvement in disease activity by week 1 that was statistically significant when compared with placebo-treated patients. Improvement was maintained until week 8. CRP normalized after treatment in the 5 and 10 mg/kg treated patients confirming IL-6 as the dominant cytokine in

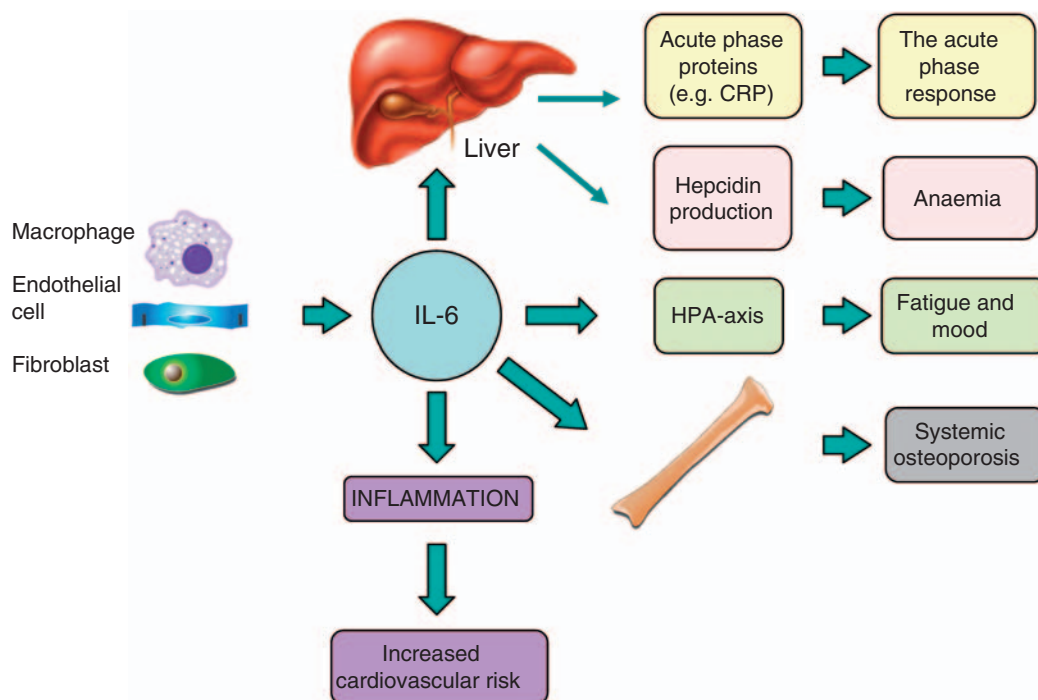


Figure 2. Systemic effects of IL-6.

generating the acute-phase response in patients with RA. A double-blind, placebo-controlled trial in 164 RA patients demonstrated that the clinical response was maintained with repeated dosing of TCZ monotherapy [Nishimoto *et al.* 2004]. CHARISMA, a European study, examined not only the therapeutic effect of TCZ monotherapy in RA but also its efficacy when combined with methotrexate (MTX). The study recruited 359 RA patients with partial response to MTX. It was found that TCZ was efficacious as monotherapy or in combination with MTX although the latter appeared to enhance the benefit of TCZ [Maini *et al.* 2006]. Two phase III randomized placebo control trials confirmed the therapeutic benefit of TCZ in combination with MTX [Smolen *et al.* 2008] or DMARDs [Genovese *et al.* 2008] in RA, as compared, respectively, with placebo plus MTX and placebo plus DMARDs. In combination with MTX, TCZ has also been shown to be effective in patients with RA who were refractory to tumour necrosis factor (TNF) antagonists [Emery *et al.* 2008]. TCZ monotherapy [Nishimoto *et al.* 2007] or in combination with MTX [Fleischmann *et al.* 2009] has been shown to reduce radiographic joint damage in RA patients. Two-year data from the LITHE study show that giving the TCZ to patients who do not adequately respond to MTX results in sustained improvement in physical function and reduced radiographic joint damage [Fleischmann *et al.* 2009].

Risk of IL-6 blockade

Infection

In the controlled studies the rate of all infections reported with TCZ 8 mg/kg plus DMARD treatment was 127 events per 100 patient years compared with 112 events per 100 patient years in the placebo plus DMARD group. In controlled clinical studies, the rate of serious infections with TCZ 8 mg/kg plus DMARDs was 5.3 events per 100 patient years exposure compared with 3.9 events per 100 patient years exposure in the placebo plus DMARD group. In the monotherapy study the rate of serious infections was 3.6 events per 100 patient years of exposure in the TCZ group and 1.5 events per 100 patient years of exposure in the MTX group. In the long-term safety population (core and extension studies), the rate of serious infections observed with TCZ plus DMARD treatment was 3.9 events per 100 patient years exposure. Reported serious

infections included pneumonia, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis. Serious infections were rarely fatal. Screening for latent tuberculosis (TB) is recommended prior to starting therapy with initiation of standard antimycobacterial therapy for those patients with latent TB.

Increase liver transaminases

Transient or intermittent mild and moderate elevations of hepatic transaminases have been reported particularly when TCZ is used along with MTX. Caution should be exercised when considering initiation of TCZ treatment in patients with elevated alanine transaminase (ALT) or aspartate transaminase (AST) >1.5 x ULN (upper limit of normal). In patients with baseline ALT or AST >5 x ULN, treatment is not recommended.

Decrease in neutrophils

Decreases in neutrophil count have occurred following treatment with TCZ 8 mg/kg in combination with MTX. There may be an increased risk of neutropenia in patients who have previously been treated with a TNF antagonist.

Increase in lipids

In clinical trials increases in lipid parameters have been reported with 24% experiencing sustained elevation in total cholesterol >6.2 mmol/l and 15% experiencing a sustained increase in LDL to >4.1 mmol/l which responded to treatment with lipid lowering agents.

Summary

IL-6 is a pleiotropic cytokine with a pivotal role in the pathophysiology of RA. It is found in abundance in the synovial fluid and serum of patients with RA and the levels correlate with the disease activity and joint destruction. 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 through hepcidin production (anaemia), its 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 TH-17 differentiation. All of the above makes IL-6 blockade a desirable option in the treatment of RA. Following

successful animal studies a humanized anti-IL-6R monoclonal antibody, TCZ, entered into clinical trials and it has shown to be an effective choice in the long-term management of RA with rapid and sustained improvement in disease activity, reducing radiographic joint damage and improving physical function in patients with RA.

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Conflict of interest statement

E.C. has received research grants and honoraria from Roche and from Allergan, Boehringer Ingelheim, Chelsea Therapeutics, Eli Lilly, GSK, Jazz Pharmaceuticals, Merrimack Pharmaceutical, MSD, Pfizer, Pierre Fabre Medicament, Schering-Plough, UCB Celltech and Wyeth for advisory boards, consultancy and speaker bureaus. The other author has declared no conflicts of interest.

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