The role of interleukin-6 signalling and its therapeutic blockage in skewing the T cell balance in rheumatoid arthritis

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Summary
Therapeutic blockage of cytokine signalling in autoimmune diseases has improved our understanding of the role of these cytokines in triggering, shaping and perpetuating autoimmune responses. In rheumatoid arthritis (RA), immunopathology is driven by a predominance of arthritogenic T helper cells secreting interferon-γ [T helper type 1 (Th1)] and interleukin (IL)-17 (Th17) over regulatory T cells (Treg). The pleiotropic cytokine IL-6 is crucial to the differentiation of Th17 cells and the balance between pathogenic Th17 and protective Treg. Targeting the IL-6 receptor (IL-6R) by humanized antibodies improves signs and symptoms of RA, and has provided new insights into the mechanisms of inflammation and immune regulation. Here we review current evidence on the role of IL-6 in the pathogenesis of RA and the molecular consequences of IL-6R blockage in disease, with special focus on the Th17/Treg balance and plasticity.

Keywords: interleukin 6, rheumatoid arthritis, T cell plasticity, Th17/Treg balance, tocilizumab

Introduction
Rheumatoid arthritis (RA) is a chronic autoimmune disorder which is characterized mainly by inflammation of the synovial tissues of the joints, but also potentially affects a variety of extra-articular organs. An important hallmark of RA is the progressive joint damage, evidenced by radiological findings such as joint space narrowing and bone erosions, which are related to functional disability [1]. Universally used disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX) and prednisone are able to reduce symptoms and control structural damage to some extent, especially if they are applied at early stages of the disease [2]. However, new therapeutic approaches, such as monoclonal antibodies and recombinant receptors that target the action of proinflammatory cytokines, are superior to conventional treatments. Treatment with biologics results in improved remission, as well as in the arrest of radiographic progression in RA patients, when used in combination with MTX [3]. In particular, blockage of the receptor for the pleiotropic cytokine interleukin (IL)-6 has been demonstrated to be more efficient than MTX in reducing RA activity [4]. Specific blockage of proinflammatory cytokines or its receptors in autoimmune diseases, particularly in RA, is improving our understanding of the role these cytokines play in shaping and perpetuating autoimmune responses. Within this review, we discuss the impact of IL-6 receptor (IL-6R) blockage on CD4⁺ T cell polarization and function in the context of RA immunopathogenesis.

Imbalance between CD4⁺ T cell subpopulations in RA pathogenesis

The pathogenesis of RA is driven by an inflammatory network, in which T and B cells, autoantibodies, cytokines and other inflammatory mediators are crucial components [5]. Nevertheless, the factors that lead ultimately to the breach of tolerance remain largely unknown. For years, there has been a general consensus that CD4⁺ T cells orchestrate the inflammatory cascade in RA, which is now supported by...
more recent evidence showing therapeutic efficacy of inhibitors of T cell co-stimulation in RA patients [6]. Activated CD4+ T cells exert their effect on a variety of cells that either reside in or infiltrate joints. Among others, CD4+ T cells help autoreactive B cells to differentiate into autoantibody-producing plasma cells, and stimulate macrophages and synovial fibroblasts to secrete cytokines that are crucial to the maintenance of a chronic inflammatory state in the joints, such as tumour necrosis factor (TNF), IL-1 and IL-6 [7]. These mediators promote the expression of chemokines and their receptors, leading to the recruitment of a new wave of cells to the joints, including T and B cells, plasma cells, neutrophils, mast cells, dendritic cells and natural killer cells [8].

Traditionally, RA was considered an autoimmune process driven by CD4+ T helper (Th) cells that secrete interferon (IFN)-γ (Th1), which predominate over the Th2 subset that secretes IL-4, IL-5 and IL-13 and exerts preferably regulatory functions [9]. However, since 2003 this dogma has been questioned based on experiments performed in animal models of RA, such as collagen-induced arthritis (CIA). These studies demonstrated that a population of CD4+ T cells, characterized by the secretion of IL-17 and dependent upon IL-23 for its expansion, was responsible for the progressive damage observed in the joints of these animals [10]. This population, referred to as Th17 cells, was shown to be involved in other models of autoimmune diseases, and has been considered a distinct lineage, mutually exclusive with Th1 [11]. Human Th17 cells are characterized by the expression of the lineage-specific transcription factor retinoid acid-related orphan receptor C2 (RORC2) [12], as well as IL-23 receptor, chemokine receptor CCR6 [13] and lectin receptor CD161 [14]. Besides IL-17, Th17 cells produce IL-21, IL-22, IL-26, TNF and granulocyte–macrophage colony-stimulating factor (GM-CSF) [15]. Th17-derived cytokines attract different cell types through the induction of other cytokines and chemokines, such as IL-6, GM-CSF, CC chemokine ligand (CCL)-20 and IL-8 [16]. In particular, IL-17A and IL-17F are key cytokines for the recruitment and/or activation of numerous cell types involved in the pathogenesis of RA, including neutrophils, monocytes, macrophages, synovial fibroblasts, chondrocytes and osteoclasts [17]. Also, IL-21 produced by Th17 cells promotes B cell differentiation to autoantibody-producing plasma cells [18], and amplifies the Th17 response in an autocrine manner [19]. These findings led to clinical trials using neutralizing antibodies to IL-17 in patients with RA, which proved to be well tolerated and safe but failed, however, to improve clinical response compared to placebo [20,21].

The isolation of CD4+ T cells secreting both IFN-γ and IL-17 from synovial tissues of RA patients has challenged the Th1/Th17 dichotomy [22]. This fact has been corroborated in joints of patients with juvenile arthritis, where co-expression of Th17 and Th1 lineage-specific transcription factors, RORC2 and T-bet, has been demonstrated in Th17 cells which converted to ‘non-classical’ Th17/Th1 as a consequence of the proinflammatory environment [23,24].

Conversely, it has been postulated that autoimmune processes triggering RA are caused by an imbalance between CD4+ effector T cells and regulatory T cells (Treg), with a pre-eminence of the former [25,26]. Treg from peripheral blood of RA patients have been described to exhibit an impaired ability to suppress proinflammatory cytokine production by effector T cells and monocytes, a defect that has been attributed to epigenetic modifications and to the effect of TNF on Treg among other mechanisms [27,28]. This view has been challenged by other groups, which have shown that the impairment is due to the resistance of RA effector T cells to be suppressed by Treg [29,30]. Similarly, there is contradictory evidence regarding the functional proficiency of Treg from RA synovial fluid, where Treg are enriched compared to peripheral blood [30].

The pleiotropic cytokine IL-6 and its role in RA

IL-6 is secreted by a large number of cell types, including T and B cells, monocytes, fibroblasts and synoviocytes. IL-6 exerts its effects once it has bound to a receptor complex formed by the ligand-binding IL-6Rα chain (CD126) and the signal-transducing β-subunit gp130 (CD130) [31]. Binding of IL-6 to its receptor induces homodimerization of gp130 [32], leading to phosphorylation of tyrosine kinases of the Janus kinase (JAK) family, as well as the recruitment and activation of signal transducers and activators of transcription (STAT)-1 and STAT-3 [33]. Importantly, IL-6R as a membrane-bound receptor, is restricted to hepatocytes, leucocytes and megakaryocytes, and as a soluble form (sIL-6R) is present in circulation and at sites of inflammation [34]. Accordingly, there are two mechanisms through which IL-6 exerts its biological effects: classical IL-6R signal transduction via the membrane-bound IL-6R and IL-6 ‘trans-signalling’ [34], a process in which sIL-6R binds IL-6 and thereby prolongs its circulating half-life and bioavailability [35]. The gp130 subunit is expressed ubiquitously, so many cells that lack IL-6R can still respond to IL-6 through trans-signalling, accounting for the pleiotropic effects of IL-6 [36].

While classical IL-6R signalling controls central homeostatic processes and immunological outcomes, including acute-phase response, glucose metabolism, haematopoiesis and regulation of the neuroendocrine system, IL-6 trans-signalling plays a role in the recruitment and apoptosis of leucocytes, the maintenance of T cell effector functions and activation of stromal tissues [37]. sIL-6R is released upon activation from T cells, monocytes and neutrophils via IL-6R ectodomain shedding through adamalysin proteases ADAM metallopeptidase domain 17 (ADAM17) and ADAM10 [38–40]. Therefore, it is not surprising that serum concentrations of sIL-6R are elevated during
Additionally, there are soluble forms of gp130 (sgp130) that are released by differential splicing [41] and bind to the IL-6-sIL-6R complex to block trans-signalling [42]. However, high serum concentrations of sgp130 remain largely unaltered during inflammation, suggesting a buffering effect on IL-6 trans-signalling [37,42].

Serum concentrations of IL-6 are elevated rapidly in the context of inflammation in response to IL-1β and TNF (major activators of IL-6 expression), Toll-like receptors, prostaglandins and mediators of stress response [37]. IL-6 expression is also induced by IL-32, a cytokine that is over-expressed in RA patients and associated highly with synovial inflammation [43,44].

In RA, IL-6 signalling triggers a variety of degenerative and inflammatory processes involving multiple mechanisms (Fig. 1). A relevant effect of IL-6 in the pathogenesis of RA is its capacity to trigger systemic inflammatory events, such as increase in thermogenesis and synthesis of acute-phase proteins by hepatocytes, resulting in manifestations such as fever, fatigue and anaemia. In the synovial tissue of the joints, IL-6 also induces vascularization, infiltration of inflammatory cells, such as neutrophils and monocytes/macrophages, and expansion of synovial fibroblasts leading to RA tissue destruction and pannus formation, respectively. IL-6 also promotes cartilage damage, the differentiation of osteoclasts and bone resorption by stimulating the expression of matrix metalloproteinases (MMPs) and receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL). Furthermore, IL-6 promotes the expansion of CD4+ T cells and, together with cytokines secreted by inflammatory dendritic cells, induces the differentiation into interferon (IFN)-γ and/or IL-17-producing T effector cells which, in turn, activate macrophages and attract neutrophils. IL-6 drives also the differentiation of B cells to autoantibody-producing plasma cells. Binding of autoantibodies to Fc receptors further triggers activation and release of inflammatory mediators by macrophages. TGF = transforming growth factor; TNF = tumour necrosis factor.
CD4+ T cells to follicular Th cells that promote immunoglobulin class-switching in B cell follicles [49]. Direct evidence of a relationship between IL-6 and chronic joint inflammation in RA arises from the findings that IL-6 levels are elevated in serum and synovial fluid of RA patients, and that IL-6 serum concentration correlates positively with disease activity [50,51]. In accordance, it has been reported that IL-6-deficient mice are resistant to the induction of arthritis [52], and that blocking the IL-6R reduces the incidence of arthritis in mice [53].

**IL-6 as critical factor in the Th17/Treg balance**

Experiments performed with murine naive CD4+ T cells have revealed that Treg and Th17 cells share a common pathway for their generation: both lineages require TGF-β to differentiate (Fig. 2) [54]. However, addition of IL-6 represses Treg differentiation [55] while favouring the development of Th17 cells, an effect that is potentiated by other proinflammatory cytokines, including IL-1β, IL-21, IL-23 and TNF [12,54,56]. The full acquisition of pathogenic functions by effector Th17 cells is mediated by IL-23 rather than IL-6 and TGF-β, because apart from their role in driving the initial lineage commitment, IL-6 and TGF-β are capable of restraining the inflammatory response of activated Th17 cells by inducing IL-10 production [57]. However, a shift in the balance between proinflammatory and regulatory signals, for example through decreased numbers of Treg, might lead to a reduced IL-10 production, concomitant with an up-regulation of proinflammatory mediators produced by unrestrained Th17 cells. It has been shown that IL-6-deficient mice fail to develop a Th17 response, while their peripheral repertoire is dominated by forkhead box protein 3 (FoxP3) Treg [58]. Interestingly, deletion of Treg in this model leads to the reappearance of Th17 cells, suggesting an additional pathway of Th17 generation in vivo, which involves IL-21 and TGF-β [58]. In addition, IL-6 over-expression in vivo was shown to inhibit the generation of induced Treg (iTreg), but had no impact on thymus-derived natural Treg (nTreg) [59].

There is also a certain plasticity between Th17 and Treg lineages. It has been demonstrated that stimulating Treg with IL-6 destabilizes the transcription factor FoxP3, while increasing IL-17 and RORγt expression [60,61]. Furthermore, CD4+ T cells that co-express IL-17 and FoxP3 have been found in the synovium of RA patients, suggesting that Treg instability contributes to the pathogenesis of RA [62]. There is evidence that IL-6-driven CD4+ T cell activation
via STAT-3 occurs as an early event in the pathogenesis of RA, especially in seronegative patients [63]. Thus, a proinflammatory environment enriched with IL-6 could favour the de-novo generation of highly pathogenic Th17 lymphocytes, and also suppress iTreg generation and function, amplifying the inflammatory process further.

Blockage of IL-6R for the treatment of rheumatoid arthritis

Based on the important role that IL-6 plays in triggering systemic inflammation, it is not surprising that IL-6 antagonists have been tested for the treatment of RA and other inflammatory diseases. Tocilizumab (TCZ) is a humanized monoclonal antibody that blocks the IL-6 binding site of the IL-6R [37]. TCZ, used as monotherapy as well as in combination with MTX, showed an acceptable safety profile, and was proved effective in reducing disease activity, delaying joint destruction and improving physical function, especially in RA patients who were refractory to other anti-rheumatic therapies, including TNF inhibitors [4,64–68]. Moreover, clinical studies have demonstrated a similar efficacy of TCZ compared to other biological therapies [69,70]. Furthermore, it has been demonstrated recently that TCZ is effective in treating early-diagnosed RA patients who had not been treated previously with DMARDs [71]. These results led to the approval of TCZ for RA treatment and the successful use of TCZ on other rheumatic diseases such as giant cell arteritis [72], and also encouraged the development of more biological drugs targeting IL-6 or its receptor.

Sarilumab, a monoclonal human antibody directed against anti-IL-6Rα, has shown efficacy and safety profiles similar to TCZ as monotherapy as well as in combination with MTX [73,74]. Like TCZ, sarilumab has been demonstrated to be superior to adalimumab when used as monotherapy [75]. Monoclonal antibodies directed against IL-6, such as clazakizumab, olokizumab, and sirukumab, have been shown in Phase II clinical trials to be well tolerated and to reduce disease activity in RA patients with inadequate response to MTX or TNF inhibitors [76–78].

JAK inhibitors, such as tofacitinib (JAK1/3 inhibitor) and baricitinib (JAK1/2 inhibitor) which, as a consequence of their mechanism of action, interfere with IL-6 secretion and downstream pathway activation, have also demonstrated efficacy in reducing signs and symptoms of RA in combination with MTX as well as monotherapy in Phase III clinical studies with patients who did not respond to MTX or TNF inhibitors [79–81].

The mechanisms by which IL-6 inhibitors achieve this symptomatic improvement in RA patients have not yet been clarified entirely. However, some effects which are related directly to the known functions of IL-6 have been described, such as normalization of bone formation/resorption markers and cartilage replacement, reduction of chemokine concentrations in serum and decrease of plasma cells and circulating autoantibodies [82–84]. It has also been suggested that CD56+ natural killer (NK) cells play a protective role in RA and that high levels of NK cells are associated with RA remission upon treatment with TCZ [85]. It is likely that new data on immunological parameters able to predict an adequate response to IL-6 inhibitors begin to appear [86,87]. Evidence regarding the immunological impact of IL-6R blockage has been obtained mainly through application of TCZ in clinical practice. Because sarilumab and anti-IL-6 monoclonal antibodies show a clinical efficacy similar to TCZ, it is conceivable that they may trigger similar immunological effects; however, this assert must be tested.

Effects of IL-6R blockage on T cell subpopulations

Recent discoveries regarding the role that IL-6 plays in the generation and expansion of Th17 responses, while affecting Treg adversely, suggest another angle addressed by TCZ in rheumatological diseases which, unlike the aforementioned described effects, involve initial events of the inflammatory cascade. Years ago, precursor experiments in mice showed that IL-6R blockage affects the capacity of T cells to respond to antigenic stimuli [88]. This hypothesis has been proved in the murine model of CIA by inoculating an anti-IL-6R antibody at the time of disease induction [89]. The authors of this study observed that treated mice did not develop pathogenic Th17 activity without affecting the percentage of Treg, thus shifting the balance between these populations towards a protective response and, consequently, reducing the severity of arthritis [89]. However, this effect could not be reproduced in mice with already established disease [89]. Similar results have been obtained in other animal models of arthritis, both in spontaneous and in induced models [90,91].

Regarding clinical settings, data describing the behaviour of Th17 and Treg subpopulations during TCZ treatment are somewhat conflicting (Table 1). A study on RA patients described a decrease in Th17 frequency and an increase of Treg after 3 months of TCZ treatment [25]. As the authors did not observe changes in IL-6 serum levels upon TCZ treatment, they hypothesized that TCZ rather acts on restoration of the Th17/Treg balance than on the reduction of IL-6-induced inflammation [25]. In contrast, our group has shown that the percentages of peripheral Treg and Th17/Th1 cells increased, while the frequency of Th1 or Th17 cells remained unaltered in RA patients who received TCZ for 6 months [26]. This is in accordance with another study by Thioliat and co-workers reporting an expansion of Treg expressing the adenosine triphosphate (ATP)-hydrolysing ectonucleotidase CD39, but stable levels of Th17 cells after 3 months of TCZ treatment [93]. The stability of the Th17 subpopulation upon TCZ treatment, together with data from IL-6R blockage in murine arthritis, suggests that
IL-6 is necessary for Th17 induction, but might be dispensable for the maintenance of Th17 responses [94]. It is likely that recovering T_{reg} frequency and function is key in achieving a positive clinical outcome, as an increase in the T_{reg} population has also been described after therapeutic TNF blockage [95]. Anti-TNF-induced T_{reg} can mediate suppression via IL-10 and TGF-β and compensate for the defective natural T_{reg} in RA patients [96]. Interestingly, differentiation of T cells into T_{reg} instead of Th17 cells might be due partially to decreased IL-6 levels in response to anti-TNF treatment [97].

Unlike the studies described above, Guggino and colleagues reported that both 3-month treatment of early-diagnosed RA patients with TCZ and in vitro exposure of patient-derived peripheral blood mononuclear cells to TCZ induced a depletion of Th1, Th17 and T_{reg} and their related cytokines IL-12, IL-17 and IL-10, while increasing the Th2 subset and IL-4 secretion [98]. The authors suggest that blockage of IL-6 signalling reverts T cell resistance to Fas-mediated apoptosis [99], and that distinct sensitivities of activated Th17, Th1 and Th2 cells to Fas-mediated apoptosis might result in the depletion of Th1 and Th17, but not Th2, cells [100]. Nevertheless, this discrepancy might be due to the differences in duration of T cell stimulation between the studies.

Conclusions

Despite some differences, the studies presented herein support the hypothesis that anti-IL-6R treatment restores the physiological T_{reg}/Th17 balance and promotes the expansion of protective T_{reg} which may be important for the clinical response to anti-IL-6R observed in most RA patients. Although further studies are required to unravel the complete spectrum of effects that IL-6R blockage exerts in the human organism, we postulate that its activity upstream of the inflammatory cascade, re-establishing the T_{reg}/Th17 equilibrium, is crucial to prevent downstream inflammatory effects of IL-6, induced by a storm of pro-inflammatory cytokines and chemokines attracting and activating multiple cell populations, and therefore there lies a substantial proportion of its clinical efficacy.

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