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REVIEW

Spotlight on tocilizumab and its potential in the treatment of systemic sclerosis

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Lazaros I Sakkas

Department of Rheumatology and Clinical Immunology, Medical School, University of Thessaly, Larissa, Greece

Abstract: Systemic sclerosis (SSc) is a multisystem disease characterized by extensive collagen deposition in skin and internal organs, fibrointimal microvasculopathy, and activation of the immune system. T cells and B cells can promote fibrosis in SSc. Interleukin (IL)-6 is implicated in the pathogenesis of SSc. IL-6 is increased in the peripheral blood and lesional skin from patients with SSc, and induces fibroblast collagen production directly and indirectly by inducing profibrotic M2 macrophages. IL-6 also induces Th17 differentiation and promotes B cell differentiation toward Ig-producing plasma cells. IL-6 is also implicated in the pathogenesis of SSc in animal models as it is increased in mice with bleomycin-induced fibrosis, whereas neutralization of IL-6 in these mice prevents skin fibrosis. IL-6 acts on cells by binding to IL-6 receptor (IL-6R) which is transmembrane or soluble, and then recruits the signal-transducing glycoprotein 130 which is ubiquitously expressed. Tocilizumab is an anti-IL-6R humanized monoclonal antibody that blocks IL-6-mediated signaling. Tocilizumab has been approved for the treatment of moderate-to-severe rheumatoid arthritis, for polyarticular and systemic juvenile idiopathic arthritis, and for Castleman's disease, and is well tolerated. Case reports and a Phase II, randomized trial in SSc have shown some improvement of skin tightness and delayed deterioration of lung function. A Phase III randomized trial in SSc is anticipated.

Keywords: biologics, B cells, fibrosis, IL-6, IL-6 receptor

Systemic sclerosis

Systemic sclerosis (SSc) is a chronic disease characterized by extensive fibrosis in skin and internal organs, fibrointimal proliferation of small arteries, and activation of the immune system. The hallmark of the disease is increased extracellular matrix (ECM) deposition which is caused not only by increased ECM deposition by activated fibroblasts but also by decreased ECM degradation. Degradation of ECM components is caused by matrix metalloproteinases (MMPs), whereas tissue inhibitor of metalloproteinase 1 (TIMP-1) inhibits MMPs and thus regulates ECM turnover. In SSc, specific MMPs are inhibited by functional autoantibodies,[1] whereas serum TIMP-1 levels are elevated[2] and TIMP-1 expression is upregulated in SSc monocytes.[3] Thus, inhibition of ECM breakdown contributes to ECM accumulation.

Although the pathogenesis of SSc is complex, both adaptive and innate immunity appear to be implicated in the accumulation of ECM in this disease. There is convincing evidence for a pathogenic role of both T cells and B cells in SSc. T cells produce profibrotic cytokines interleukin (IL)-4, IL-13, and IL-17, whereas B cells are hyperactivated and promote fibrosis through autoantibodies, profibrotic cytokines IL-6 and TGFβ, and cell–cell contact with fibroblasts.[1,4–8]

Toll-like receptors (TLRs), receptors utilized by innate immune system, appear to be involved in SSc. TLRs recognize exogenous pathogen-associated molecular patterns

Correspondence: Lazaros I Sakkas
Department of Rheumatology and Clinical Immunology, Medical School, University of Thessaly, Biopolis, Larissa 41110, Greece
Tel +30 241 350 2813
Fax +30 241 350 1016
Email lsakkas@med.uth.gr

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and endogenous damage-associated molecular patterns. An endogenous ligand of TLR4, S100A8/A9 complex, is found to be increased in plasma from patients with SSc by proteomics analysis.[9] TLR agonists appear to contribute to fibrosis. Hyaluronan, a component of ECM and enhanced in mouse skin with bleomycin-induced fibrosis, stimulates B cell production of IL-6 and TGFβ primarily through TLR4.[10] TLR8 stimulation by ssRNA increases the production of TIMP-1 in monocytes from SSc patients or healthy controls, and thus inhibits the breakdown of ECM.[3] Nucleosome, which can stimulate TLR9, induces T cell expression of IL-4 and IL-17, and B cell Ig production.[11] Serum nucleosome levels are elevated in SSc, and TLR9 is upregulated in T cells and B cells from SSc patients.[11] TLR7 and TLR9 induce type I IFN-regulated genes, such as Siglec in SSc monocytes, and Siglec expression is increased in tissue macrophages in SSc.[12]

IL-6

IL-6, a 184-aminoacid glycoprotein, is a pleiotropic cytokine produced by various cell types, predominantly macrophages and fibroblasts. IL-6 binds to IL-6 receptor (IL-6R), which is transmembrane (tIL-6R) or soluble (sIL-6R), and constitutes the α subunit of the receptor. IL-6 bound to tIL-6R or sIL-6R recruits two molecules of glycoprotein 130 (gp130). gp130 constitutes the β subunit of the complex and is the signal-transducing subunit of the receptor. IL-6R is expressed on hepatocytes, on subpopulations of leukocytes, and on megakaryocytes, whereas gp130 is ubiquitously expressed. Other IL-6 family cytokine members, such as leukemia inhibitory factor, IL-11, oncostatin M, ciliary neurotrophic factor, cardiotrophin-1, IL-27, and IL-35 bind to their specific α subunit of receptors and mediate their signal transduction via the common gp130 β subunit.

IL-6 induces hepatocyte production of acute-phase proteins, such as CRP, serum amyloid A, haptoglobin, fibrinogen, and α1-antichymotrypsin, whereas it reduces the production of albumin and transferrin. IL-6 also induces the production of hepcidin, which blocks the iron transporter ferroportin-1 in gut and thus reduces serum iron levels. In bone marrow, IL-6 promotes maturation of megakaryocytes and release of platelets into the circulation.[13] IL-6 also plays an important role in adaptive immunity. IL-6 in combination with TGFβ induces Th17 differentiation from naïve CD4 T cells, and inhibits TGFβ-induced Tregs differentiation. IL-6 also promotes B cell differentiation toward IgG-producing plasma cells. IL-6 signaling via the sIL-6R is called trans-signaling and mediates predominantly pro-inflammatory signals, whereas IL-6 signaling via the tIL-6R is called classic signaling and mediates regulatory/anti-inflammatory signals.[13–15] For instance, transgenic mice overexpressing soluble gp130 which specifically blocks sIL-6R exhibit no inflammatory processes.[16]

IL-6 is a unique cytokine in two ways: first, it acts on cells that do not express IL-6R, and second, unlike other cytokines, its binding to sIL-6R exerts an agonist effect.

IL-6 in SSc

Serum levels of IL-6 are elevated in patients with early diffuse cutaneous SSc (dcSSc),[17–21] particularly in those with lung fibrosis.[17–19] Serum IL-6 levels are strongly correlated with the extent of skin fibrosis.[18] Serum IL-6 levels are predictive of early functional decline and mortality in SSc-associated interstitial lung disease[22] and reduced long-term survival.[20] IL-6 is also increased in phytohemagglutinin-stimulated peripheral blood mononuclear cells and in T cell lines from SSc patients.[19]

In SSc, skin IL-6 staining is detected in perivascular inflammatory infiltrates, endothelial cells, fibroblasts, and epidermis.[20,21] High skin IL-6 expression in skin of patients with early dcSSc is associated with more severe skin involvement at 3 years, and worse long-term survival.[20] Fibroblasts from affected SSc skin spontaneously produce 30-fold more IL-6 than normal fibroblasts.[23] B cells appear not only to be a source of IL-6 in SSc but also to induce IL-6 production by fibroblasts. Thus, SSc B cells stimulated with BAFF increase IL-6 production.[24] It is noted that serum BAFF levels are elevated in SSc patients and correlated with the extent of skin fibrosis.[24] Also, coculture of a B cell line from SSc lesional lung tissue with fibroblasts induces extraordinarily high IL-6 production by fibroblasts.[25]

IL-6 promotes fibrosis. In fibroblast cultures, IL-6 induces collagen production[20,26] through JAK2/STAT3- and ERK-dependent signaling.[20] This fibroblast collagen production is also found to be through IL-6R trans-signaling, STAT3 activation, and TGFβ induction via the Gremlin protein.[27] In addition, IL-6 induces the generation of profibrotic M2 macrophages.[28]

IL-6 in animal models of SSc

As mentioned, IL-6 in combination with TGFβ induces differentiation of Th17 cells. Th17 cells produce profibrotic IL-17A.[8] Serum levels of IL-6 are elevated in murine bleomycin-induced model of scleroderma, whereas neutralization of IL-6 improves dermal fibrosis. Thus, dermal fibrosis is reduced in IL-6 knockout mice.[29] Similarly, anti-IL-6R monoclonal antibody (MoAb)[21,29] or active immunization

with an IL-6 peptide[21] reduces dermal thickness in bleomycin-induced mouse model of SSc, and this improvement is associated with reduced T cell skin infiltrates.[21] However, anti-IL-6R MoAb has no effect on fibrosis in tight-skin mice, another model of SSc.[21] IL-6 induces α-SMA expression in IL-6 knockout mouse-derived fibroblasts in a dose-dependent manner, and this is inhibited by anti-IL-6R MoAb.[29]

In the murine sclerodermatous chronic graft-versus-host disease model of SSc, serum IL-6 levels are elevated, whereas administration of anti-IL-6R MoAb prevents fibrosis and this is mediated through increase in CD4+CD25+FoxP3+ Tregs.[30] However, anti-IL-6R MoAb has no effect on fibrosis in established chronic graft-versus-host disease model of SSc.[30]

IL-6 also appears to be involved in pulmonary arterial hypertension (PAH), a serious manifestation of SSc. Transgenic mice with lung-specific overexpression of IL-6 spontaneously develop PAH with distal arterial neointimal proliferative changes composed of endothelial cells and T cells.[31] IL-6 also appears to be involved in chronic hypoxia-induced PAH. Mice lacking IL-6 develop less severe PAH compared to wild-type mice after 2 weeks of hypoxia.[32]

Tocilizumab

Tocilizumab (TCZ) is an IgG1 humanized anti-IL-6R MoAb that binds to both sIL-6R and tIL-6R, and blocks IL-6-mediated signaling, both classic signaling and trans-signaling (Figure 1). TCZ is generated by inserting the complementarity-determining regions of a mouse anti-human IL-6R antibody into IgG1κ at the cDNA level.[33]

TCZ has been proved as an effective therapy for various autoimmune inflammatory diseases. It is approved for the treatment of moderate-to-severe rheumatoid arthritis (RA), polyarticular and systemic juvenile idiopathic arthritis, and multicentric Castleman's disease, a multicentric disease of non-clonal lymphadenopathy. TCZ has been off-label used for the treatment of refractory cases of polymyalgia rheumatica/giant cell arteritis, Takayasu arteritis, and adult-onset Still's disease. In RA, TCZ is used at a dose of 8 mg/kg intravenously every 4 weeks or 162 mg subcutaneously every 2 weeks.

In patients with RA, TCZ decreases peripheral blood activated B cells and T cells[34] and the frequency of memory B cells.[35] Also, in patients with RA, clinical improvement after TCZ treatment is associated with expansion of TGFβ+ CD25 high B cells considered to be regulatory B cells.[36]

TCZ is well tolerated. Adverse effects reported include risk of infections (mostly pneumonia and cellulitis), neutropenia, thrombocytopenia, elevated liver enzymes, elevated total cholesterol, low-density lipoprotein, and triglycerides.[37] TCZ restores the depression by IL-6 activity of CYP450 enzymes in RA and decreases bioavailability of drugs that are substrates of CYP450 enzymes. Thus, simvastatin levels decrease by 57% after TCZ infusion. Therefore, close monitoring is required for drugs that are metabolized by CYP450 enzymes and have a narrow therapeutic window, such as warfarin.

Serum IL-6 levels are elevated in patients with RA or Castleman's disease treated with TCZ because sIL-6R bound to TCZ hinders IL-6 clearance from the circulation.[38]

TCZ in SSc

SSc is a complex disease with frequent serious manifestations that reduce life expectancy. Once fibrosis causes symptoms, there is no effective therapy. Thus far, there are

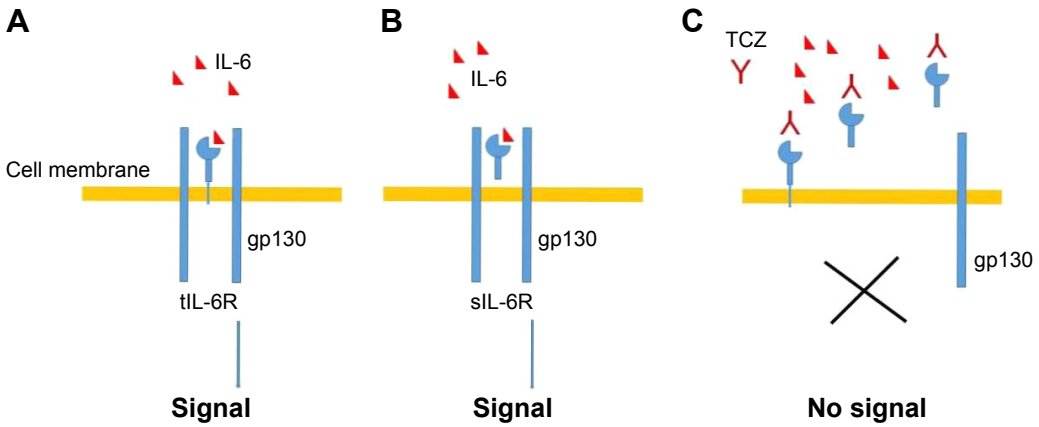

Figure 1 TCZ: mechanism of action.
Notes: IL-6 binds to tIL-6R (**A**) and sIL-6R (**B**), and this recruits two molecules of signal-transducing gp130 to activate cells. (**C**) TCZ binds to both tIL-6R and sIL-6R and blocks the action of IL-6.
Abbreviations: TCZ, tocilizumab; IL-6, interleukin-6; tIL-6R, transmembrane IL-6 receptor; sIL-6R, soluble IL-6 receptor; gp130, glycoprotein 130.

few randomized trials in SSc, and today, medications used in SSc include methotrexate for skin thickness, and cyclophosphamide or mycophenolate mofetil for interstitial lung disease with limited efficacy.[39–41] B cell-depleting therapy with anti-CD20 MoAb (rituximab) has also been shown to reduce skin thickness and stabilize lung function in a small series of patients.[42]

Blocking IL-6 signaling with TCZ offers an advantage over previous targeted treatments because IL-6 is involved in both innate and adaptive immunity. Even B cell depletion therapy in SSc may work through IL-6, by eliminating IL-6-producing B cells. For instance, mice with B cell-specific IL-6 deficiency were reported to exhibit less severe disease than mice with wild-type B cells, and B cell depletion treatment ameliorated experimental autoimmune encephalomyelitis only in mice with IL-6-producing B cells.[43] Additionally, rituximab nonresponders among RA patients were shown to exhibit persistent IL-6 elevated blood levels despite synovial B cell depletion,[44] and TCZ provided better efficacy than TNF inhibitors or abatacept after rituximab failure in RA patients.[45]

TCZ has exhibited encouraging results in case reports on SSc overlap syndromes[46,47] and case reports or small observational studies on SSc.[48–52] In a study, two patients with SSc, one with lung fibrosis and the other with chronic renal failure after scleroderma renal crisis, received TCZ for 6 months. There was significant reduction of skin tightness and histological thinning of skin collagen bundles in both patients, and also improvement of renal function. There was no change in lung function.[48] In a small observational 5-month study in patients with SSc and refractory polyarthritis, TCZ achieved a good European League Against Rheumatism response in ten out of 15 patients with arthritis.[49] In a patient with dcSSc, TCZ reduced Rodnan skin score from 35 to 7 and expanded range of motion of joints within 16 months.[51] In two out of three SSc patients with refractory disease, TCZ improved skin score and completely healed digital ulcers.[50] Recently, a Phase II, double-blind, placebo-controlled randomized trial of TCZ (162 mg administered subcutaneously per week) in 87 patients with early SSc (<5 years from first non-Raynaud's symptom) has been published.[53] Overall, TCZ decreased the modified Rodnan skin score but not statistically significantly so. However, more patients in the TCZ group (37%) achieved clinically important decrease in skin score (>4.7 units) compared to patients in the placebo group (25%). Fewer patients in the TCZ group than in the placebo group had worsening in the percent of forced vital capacity. At 48 weeks, 10% in the TCZ group and 23% in the placebo group had >10% (absolute) decrease in the percent predicted forced vital capacity values. Interestingly, TCZ treatment resulted in downregulation of genes associated with M2 macrophages in skin biopsies. Serious infections were more common in the TCZ group (16%) than in the placebo group (5%), and one patient in the TCZ group died. A 2-year Phase III randomized controlled trial of TCZ (162 mg administered subcutaneously per week) in SSc is underway (NCT02453256).

Although TCZ has a good record in terms of adverse effects, physicians should be alert for any new adverse effects when TCZ is used therapeutically for new diseases. For instance, patients with SSc often have digital ulcers that are frequently infected. TCZ may blunt CRP increase in these patients and delay diagnosis of infection. Another watchful area may be the gut. TCZ improved skin score in two patients with SSc, but both patients experienced bowel pseudo-obstruction.[52] This latter effect might be explained by the beneficial effect IL-6 may exert on gut epithelia after injury. For instance, in two murine models of gut injury, one with biopsy wound and the other with bacteria-induced colitis, inhibition of IL-6 resulted in decreased epithelial proliferation and impaired wound healing.[54]

Future perspectives

SSc is a frequently serious disease with devastating manifestations, and treatment of this disease thus far is suboptimal, to say the least. TCZ blocking the effects of IL-6, which is a profibrotic cytokine involved in both adaptive and innate immunity, raises expectations for efficacy in SSc. Early results from case series and case reports, and a Phase II study are encouraging. The publication of new classification criteria for SSc is expected to help in early diagnosis of SSc and implementing early treatment. Blocking IL-6 signaling early in the course of the disease, when inflammatory infiltrates are prominent, is expected to be more efficacious and is hoped to brighten the prognosis of this frequently devastating disease.

Disclosure

The author reports no conflicts of interest in this work.

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