Interleukin-6: a new therapeutic target in systemic sclerosis?

Steven O'Reilly, Rachel Cant, Marzena Ciechomska and Jacob M van Laar

Interleukin-6 (IL-6) is a classic pro-inflammatory cytokine critical in mounting an effective immune response. It is secreted by a wide array of cell types; however, its effector cells are more restricted, owing to the fact that very few cells, except lymphocytes and hepatocytes, express the functional membrane IL-6 receptor thus reducing the number of IL-6-responsive cells. Trans-signalling, the shedding of the membrane-bound form of the IL-6 receptor into the local microenvironment, greatly increases the range of cells that can respond. IL-6 has been demonstrated to have a pivotal role in the pathogenesis of rheumatoid arthritis, Castleman's disease and Crohn's disease exemplified by the use of an anti-IL-6 biological therapy. However, IL-6 is also associated with the autoimmune disease systemic sclerosis (SSc) and has been shown to be directly fibrotic. Elevated levels of IL-6 are found in SSc patients and this correlates with skin thickness, suggesting a causal effect. This review focuses on the role of IL-6 in SSc, a chronic autoimmune disease with fibrosis. In particular, we will examine the evidence base of the role of IL-6 in fibrosis in this condition, especially the downstream effector pathways. We will then argue why molecular targeting of IL-6 is a promising therapeutic target in this fibrosing disease.

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Systemic sclerosis (SSc) is a heterogeneous chronic disease that is characterised by inflammation, vascular abnormalities and fibrosis of skin and inner organs of unknown aetiology. The disease is very heterogeneous and is broadly subdivided into limited and diffuse forms and is autoimmune in nature. The limited form of SSc is more mild and the diffuse form more aggressive, both subtypes have inflammation and fibrosis as clinical features. Fibrosis is the end point. Although great progress has been made in understanding the pathogenesis of the disease, there is currently no specific treatment. Raised acute phase proteins, such as C-reactive protein or interleukin-6, in peripheral blood are typically observed in early phases of the disease.1 These indicate that inflammation has a crucial role in the early stages of the disease that could lead to fibrosis.

Evidence for the involvement of the immune system in SSc is clear from studies demonstrating that in skin biopsies there are elevated numbers of monocytes and T cells, and that these are found before evidence of morphological fibrosis.2,3 Indeed, many of these infiltrating immune cells are found perivascularly suggesting that they have a role early in disease pathogenesis through the secretion of cytokines that amplify and perpetuate the fibrosis. IL-6 is a classic pro-inflammatory cytokine that is involved in the onset and maintenance of multiple diseases, including rheumatoid arthritis (RA),4 Castleman's disease5 and osteoporosis.6 Furthermore, IL-6 is central in various animal models of human autoimmune diseases, including type II collagen-induced arthritis, where IL-6-knockout (KO) mice are fully protected6 and also experimental autoimmune encephalomyelitis is abrogated in IL-6-KO mice underscoring its importance in autoimmune inflammatory conditions.7 In RA clinical trials of tocilizumab, an anti-IL-6 receptor antibody has shown therapeutic benefit. IL-6, however, has also been shown to have a pivotal role in the pathogenesis of SSc in both human and animal models, and high IL-6 levels have been reported in SSc.8 Increased expression of IL-6 has been reported in the serum and skin of SSc patients.8 The aim of this review is to discuss the role of IL-6 in SSc and argue that it is a valid novel therapeutic target owing to recent novel data in this area shedding light on its role.

IL-6

IL-6 is a pleiotropic cytokine that can be synthesised by a wide variety of cells, and aberrant IL-6 signalling has been implicated in many diseases.8 Many biological effects have been ascribed to IL-6, including immunoregulation, T-cell differentiation, angiogenesis and osteoclast formation. IL-6 has a molecular mass between 21–28 kDa depending on glycosylation of the protein. IL-6 exhibits functional pleiotropy and redundancy, and has a wide variety of effects depending on cell context. It was initially discovered and called B-cell differentiation factor because it was identified in tissue culture supernatants to induce B cells to produce immunoglobulins.9,10 It is composed of four main helices arranged in an up–up–down–down topology bundle. IL-6R (CD126) is an IL-6 receptor that contains the ligand-binding site.
Although IL-6R is sufficient for low-affinity binding, signal transduction requires the presence of the signalling molecule glycoprotein130 (gp130). This forms a functional signalling platform for which downstream signalling occurs.

**GP130 SIGNALLING**

The gp130 system comprises a wide variety of ligands and receptors that elicit a response by binding of ligands that all contain at least one copy of the transmembrane protein gp130 by forming a hexameric complex. The complex is comprised of a gp130 homodimer, together with two ligands and two respective ligand-binding receptor α-subunits. The expression of gp130 is ubiquitous. Mice that have been genetically altered to overexpress gp130 develop multiple haematopoietic abnormalities, including an expansion of immature and committed progenitor cells in the bone marrow and spleen resulting in lymphadenopathy. The gp130 signalling subunit is a common shared subunit utilised by multiple cytokines, including IL-6, and use of this common subunit induces redundancy of cytokines. Although these cytokines share the common gp130 subunit to signal and while many of the cytokines have redundant functions they exert non-redundant functions as well. There are a family of gp130 cytokines that include IL-6, IL-11, leukaemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotrophin-1, cardiotrophin-like cytokine, IL-27 and IL-31.

**IL-6 SIGNALLLING**

IL-6 signalling commences when the soluble IL-6 binds to IL-6R (CD126), which dimerises with the common subunit gp130. It is important to note that while gp130 might be expressed widely in isolation it cannot transduce a signal without a receptor, and this signalling can be determined by the relative abundance of the receptor. Downstream signalling occurs through Janus kinases (JAK1 and JAK2), subsequent phosphorylation of the receptor subunit and activation of signal transducers and activators of transcription 1 and 3 (STATs1/3), and also the RAS/MAPK and also the PI-3 kinase pathway. Once STATs are activated it induces their dimerisation, nuclear accumulation and binding to DNA. STATs regulate a variety of cellular processes, including development and proliferation. Thus, STAT3 target genes are a reflection of the process needed and are cell type specific. Figure 1 illustrates the signalling pathways of IL-6.

Nearly every cell that expresses the signalling subunit gp130 can respond to IL-6 via soluble IL-6 receptor (sIL-6R) released by proteolytic cleavage of membrane-bound IL-6R or by translation of an alternatively spliced messenger RNA. This is so called ‘trans-signalling’. Trans-signalling provides a platform for multiple cells to respond to IL-6 that would not otherwise be responsive to IL-6 itself. That sIL-6R works as an agonist is therefore unusual. An exception to the rule is that human osteoblasts have a high expression of membrane-bound IL-6R, but cannot respond to IL-6 and downstream signalling unless it is auto cleaved. The reason that osteoblasts have such high levels of receptor expression, yet cannot respond without cleavage of the IL-6R is unclear.

**SIL-6R SHEDDING AND REGULATION**

Regulating the immune response is critical in halting aberrant inflammation leading to unwanted tissue damage. One process of negative feedback of the immune response in vivo is the liberation of soluble cytokine receptors that lead to negation of soluble cytokine signals. This provides a mechanism to prevent excessive immune responses. However, the sIL-6R when bound to IL-6 is agonistic, not antagonistic. The regulation of sIL-6R shedding from cells is through two independent processes. The first mechanism of production of sIL-6R is through shedding’ via proteolytic cleavage of the membrane-bound form of IL-6R mediated by a disintegrin and metalloprotease 17 (ADAM17) and to a lesser degree ADAM10. ADAM17 was initially identified as the enzyme responsible for the liberation of tumour necrosis factor-α. Purification of ADAM17 was based on hydrolysis of tumour necrosis factor-α substrates. Another mechanism of sIL-6R being released is through a splice variant. This alternative splice variant lacks the transmembrane domain. It is noteworthy that multiple diverse stimuli lead to cleavage and release of sIL-6R from different cells including the phorbol ester phorbol-12-myristate-12-acetate, a potent T-cell activator and mitogen.

It is interesting that C-reactive protein itself can induce proteolytic shedding of membrane IL-6R into a soluble receptor. It is known that IL-6 stimulates the acute phase amount of C-reactive protein and now this could work by then shedding the receptor to alter responsive cells to facilitate wound healing. Therefore, IL-6 signalling may serve to help the wound healing response, whatever the stimuli, but a failure of resolution of IL-6 may yield pro-fibrotic pathways. C-reactive protein is elevated in inflammatory fibrosing conditions, including SSC, and correlates with many disease indices. Matthews et al. showed that cholesterol depletion triggers membrane-bound IL-6R shedding, and this is mediated via the ‘sheddase’ ADAM17 also called TACE and to lesser degree ADAM10 and bacterial metalloproteases induces IL-6R shedding. Interestingly, a recent study using the bleomycin model of fibrosis demonstrated a reduction in skin fibrosis when pretreated with TAPI, a chemical inhibitor of ADAM17. This raises the possibility that inhibition of ADAM17 leads to reduced cleavage of sIL-6R and hence release of sIL-R, and therefore less trans-signalling mediating fibrosis. A recent paper using primary neutrophils demonstrated that apoptosis (induced by a wide variety of chemical agents) induced IL-6R
shading mediated by apoptosis and was ADAM17-dependent, and sIL-6R helped facilitate signalling in neighbouring non-apoptotic cells. This suggests that apoptotic cells can facilitate a wound healing phenotype by releasing sIL-6R thereby rendering local cells responsive (that would otherwise not be) to IL-6 and the fibrotic response. In other words, local cell dying switches the local microenvironment from pro-inflammatory to a more wound healing environment necessary to promote repair. It has been speculated in SSc apoptosis of endothelial cells could act as a ‘triggering’ event in the early stages of the disease. It is tempting to speculate that this endothelial apoptosis leads to inflammation and the recruitment and retention of mononuclear cells to the site of apoptosis, and that after the initial inflammation the ‘spent’ leukocytes apoptose and release sIL-6R into the local environment.

**EVIDENCE FOR A ROLE OF IL-6 IN SSc**

Multiple lines of evidence indicate that IL-6 is critical in SSc pathogenesis. A recent study demonstrated that polymorphisms in the IL-6 gene are important in susceptibility to SSc development. IL-6 is normally present in the blood at levels of 1–5 ng·l−1. IL-6 levels are elevated in SSc, and also spontaneous production of IL-6 and sIL-6R by peripheral blood leukocytes from SSc patients is elevated compared with healthy controls. Furthermore, IL-6 levels predicted worse outcome for SSc patients. Indeed as well as PBMCs that secrete IL-6 it has been shown that epidermis stains very strongly for IL-6 and also fibroblasts in the dermis from SSc patients.

Interestingly, we also found that unmanipulated CD3+ T cells secreted higher levels of sIL-6R in basal conditions from SSc donors than T cells from controls, indicating both higher secretion basally and activation of CD3+ T cells. Thus, trans-signalling appears to be the dominant pathway utilised for collagen synthesis in dermal fibroblasts. IL-6 levels have also been found to be elevated in bronchoalveolar lavage fluid from patients with SSc. IL-6 levels also correlate with the modified Rodnan skin score, hinting at a possible causal relationship between the two variables.

In animal models of SSc, such as the bleomycin mouse model, IL-6 has been demonstrated to have a critical role in disease pathogenesis. The bleomycin animal model is inflammation driven and requires leukocytes. Interestingly, mice treated with topoisomerase I and Freund’s complete adjuvant developed autoimmunity, and dermal fibrosis strikingly resembling SSc and genetic deletion of IL-6 in this murine model of SSc reduced autoimmunity and subsequent fibrosis. Interestingly, IL-6 deletion also altered the number and frequency of Th17 regulatory cells (Tregs). Saito et al demonstrated a critical role of IL-6 mediating the phenotype in the bleomycin model of SSc. They used IL-6-KO mice and demonstrated reduced fibrotic changes induced by bleomycin associated with reduced CCL3 levels. Indeed fibroblasts isolated from idiopathic pulmonary fibrosis, a fibrotic disease, were found to proliferate in response to IL-6; however, fibroblasts isolated from normal lungs and exposed to IL-6 showed the opposite effect. This indicates that fibroblasts from the fibrotic site have a fundamental aberration.

The relative contribution between IL-6 signalling and IL-6 ‘trans-signalling’ may be important in disease pathogenesis. A recent paper has used a monoclonal rat anti-mouse IL-6 receptor antibody to specifically inhibit trans-signalling in vivo after bleomycin treatment to mimic SSc, and the authors found that there was an amelioration of dermal fibrosis. The authors also found that in the anti–IL-6R-treated bleomycin group along with reduced skin thickening also decreased numbers of myofibroblasts expressing α-sm, suggesting that blockade of sIL-R was the predominant mechanism mediating reductions in myofibroblasts.

IL-6 can also rescue T cells from apoptosis, which would serve to propagate the inflammatory insult in the tissue by increasing T-cell numbers. Soluble gp130 is the natural negative regulator of IL-6 trans-signalling. It has no affinity for IL-6 or sIL-6R alone but binds at high affinity for the IL-6/sIL-6R complex, thus is a negative regulator. Elevated levels of sgp130 have been described in localised SSc patient’s serum; this may reflect a negative feedback loop to dampen IL-6 trans-signalling in this disease.

STAT-3 is the central downstream transcription factor activated by IL-6 and this has been found to be highly activated in many autoimmune diseases, including RA. Indeed, STAT3 is considered a viable drug target in RA. We have recently demonstrated elevated levels of STAT-3 in SSc-derived fibroblasts and preferential usage in IL-6-dependent collagen production. Elevated phosphoSTAT-3 has been demonstrated in skin biopsies from SSc patients. Furthermore, blockade of JAK2, which lies upstream of STAT-3 in the bleomycin model of SSc, reduced fibrosis in this model significantly, therefore indicating the pivotal role of the transcription factor STAT-3. We have demonstrated using a small molecular inhibitor of STAT3 in vitro that IL-6 trans-signalling leading to excessive collagen I messenger RNA expression is STAT3 mediated; however, IL-13-mediated collagen I gene expression is STAT3-independent. Indeed, genetic ablation of STAT3 in mice protects mice from bleomycin-induced fibrosis.

**DIRECT FIBROTIC ACTIONS OF IL-6**

Fibrosis is a pathological situation when the normal wound healing response has become aberrant. IL-6 and fibrotic events may be mediated directly via direct transcriptional activation of collagen or through the upregulation of other cytokines that act in an autocrine manner. In SSc, the primary issue is increased collagen deposition and it has been shown that the addition of IL-6 to dermal fibroblasts leads to upregulation of collagen. Indeed, IL-6 has been shown to induce synthesis of collagen in human tendon. However, IL-6-KO mice have a relatively mild phenotype likely indicating a level of redundancy. In contrast, gp130-deleted mice die before birth, thus underlining the importance of gp130 signalling. Gp130 has numerous cytokines as its ligand. However, IL-6-KO mice do show a delayed wound healing phenotype. In IL-6-KO mice, the delay in wound healing after wounding is also accompanied by a reduction in transforming growth factor-β1 (TGF-β1) expression. TGF-β1 is one of the most potent fibrotic cytokines known and is associated with multiple fibrotic diseases. Indeed, TGF-β1 is a key drug target in fibrosis in general and mediates its effects primarily via the transcription factor Smad3. Interestingly, differences in wound healing between IL-6 total and IL-6R-KO mice were noted with IL-6R-KO mice having less severe reduction in wound healing compared with IL-6 KO as compared with control. It was also noted that in IL-6R-deleted mice, phosphorylated extracellular signal-related kinase was elevated, and this may be a compensatory mechanism as chemical inhibition of MEK in the IL-6R-KO mice reduced wound closure time. Extracellular signal-related kinase can be activated in response to IL-6 in fibroblasts. In vitro cultures of dermal fibroblast cultured with anti–IL-6 antibodies attenuated collagen I levels in dermal fibroblasts derived from SSc donors compared with nonspecific isotype-matched antibody. However, despite a large amount of
research investigating this area, it is rather surprising that it is still unknown at the molecular level how IL-6 leads to fibrosis and which downstream signalling pathway contributes to this extracellular matrix (ECM) deposition. Further examination of the signalling nodes downstream of IL-6 receptor engagement may delineate this pathway further and lead to novel anti-fibrotic therapeutics.

**IL-6 AND TIMPS**

In order to produce and maintain an appropriate ECM, there are precise and specific tissue remodelling mechanisms present in the body. These tissue remodelling mechanisms are co-ordinated by specific sets of enzymes, such as matrix metalloproteinases (MMPs). There are 24 MMPs present in mammals and they have a wide range of functions, including the catabolism of the ECM. MMP function is regulated by tissue inhibitors of metalloproteinases (TIMPs), which are 25–31 kDa proteins and bind to the active site of MMP, at a 1:1 ratio, inhibiting their function. There are four TIMP proteins in humans, and the ratio of TIMP and MMP is crucial in regulating the turnover of ECM. Altering the TIMP–MMP ratio can lead to pathological consequences as seen in diseases such as RA, SSc and idiopathic pulmonary fibrosis. Animal models have highlighted the importance in the maintenance of the TIMP–MMP ratio. Over-expression of TIMP resulted in increased ECM deposition, particularly an increase in collagen levels. Also, in a bleomycin lung fibrosis mouse model, an elevated level of TIMPs is observed at the site of lung fibrosis.45

Upon tissue damage, mononuclear cells infiltrate the site of injury secreting a milieu of cytokines and chemokines polarising the healing response. The composition of the cytokine milieu can change the composition of MMP and TIMP in the tissue, thus affecting the ECM composition. In SSc patient serum, elevated levels of TIMP-1 is observed. Stimulation of fibroblasts with cytokines including IL-1β, TGF-β and oncostatin M induces TIMP-1 expression. Of particular interest oncostatin M, like IL-6, signals via the gp130 subunit implicating a role of this signalling pathway in the induction of TIMP-1 expression. Indeed, IL-6 trans-signalling was shown to induce TIMP-1 production in synovial fibroblasts.46 IL-6 has also been shown to induce TIMP-1 production in synovial cells, chondrocytes and endothelial cells. This IL-6 induction of TIMP-1 has shown to be STAT3 dependent as transfection of a dominant-negative mutant of STAT3 abolished IL-6-induced TIMP-1 expression in hepatocellular cells.

The AP-1 transcription factor, JunD, binds to the AP-1-binding site and induces TIMP-1 and IL-6 expression in rat hepatic stellate cells.47 Overexpression of JunD has been shown to induce TIMP-1 and IL-6 production.47 Thus, it is possible that in SSc ligands that induce JunD expression may be elevated in SSc patients leading to the induction of IL-6 and TIMP-1 expression.

**IL-6 AND TH17 CELLS IN SSc**

The differentiation of naïve CD4+ T cells into effector cells begins with activation of the T-cell receptor, and specific cytokines polarise the cell to a particular T-cell lineage. The exact T-effector cell is determined by a number of factors but the local cytokine environment appears particularly important. However, the identification of IL-17A/F and that IL-17A/F producing T-helper cells were a unique cell subset forced us to look outside the Th1/Th2 paradigm. Th1 cells produce IFN-γ and Th2 cells that produce IL-4, IL-5, TGF-β and IL-13 and help clear extracellular pathogens.48 Th17 cells are a distinct class of effector T cells, characterised by high IL-17A expression. These Th17 cells are a class of effector T cells that can be polarised by a distinct set of cytokines and have ascribed to them specific transcription factors like retinoid-related orphan receptor gamma t. The transcription factor STAT3 (itself a target of IL-6) is also necessary for the generation of Th17 cells. IL-17 has intense pro-inflammatory effects and has been associated with a variety of autoimmune diseases, including RA. High frequencies of IL-17 T cells have been reported in RA and multiple sclerosis. Indeed, IL-17 gene-deleted mice are collagen-induced arthritis suppressed, suggesting a critical role of IL-17 in RA. The differentiation of Th17 cells from naïve T cells requires both IL-6 and TGF-β, both cytokines are elevated in SSc.49 Indeed in the absence of IL-6 and presence of TGF-β, FOXP3+ Treg cells are polarised and help maintain self-tolerance.50 However, in the presence of both TGF-β and IL-6, pathogenic pro-inflammatory Th17 cells predominate, indeed IL-6-KO mice have a deficit in production of Th17 cells.51 These Th17-deficient mice also are resistant to the development of experimental autoimmune encephalitis, a model of MS, thus demonstrating the critical role of IL-6 in Th17 induction and autoimmunity.51

In SSc, pathogenic Th17 cells have been found to be altered in the peripheral blood of patients compared with healthy controls.52 Increased serum IL-17 levels have also been observed in SSc compared with both healthy controls and SLE patients. And although there is an increased frequency of Treg cells in SSc patients, they are functionally compromised as demonstrated with functional Treg assays. Tregs are natural (or inducible) populations of T cells that influence the immune response and control the development of autoimmunity. We have also found that Tregs produce much lower levels of IL-10 in SSc patients compared with controls at the same time as elevated levels of T cell-derived IL-6.31 Th17 subset also produce the cytokine IL-22 and this has been found to be elevated in the lesional skin of SSc patients.53 As the cytokine milieu at the local site of induction of T-cell polarisation determines the distinct subset of T-effector cells, the overall balance between pro-inflammatory IL-6 and immune-modulating TGF-β determine the polarisation of naïve T cells toward a Th17 or T effector subset, thus targeting IL-6 would lead to suppression of deleterious IL-17-producing T cells while at the same time increasing the number of ‘good’ Treg cells.

**IL-6 AND FIBROBLASTS**

Trans differentiation of cardiac fibroblasts to the pathogenic myofibroblasts mediated by IL-6 has been reported.55 In a rat model of cardiac hypertrophy by artery ligation, collagen synthesis and hypertrophy occurs but inhibition of IL-6 or specific inhibition of STAT3 phosphorylation resulted in blockade of fibrosis and hypertrophy.54 This was also shown in in vitro cultures,56 suggesting that IL-6-mediated collagen synthesis is driving the fibrosis and hypertrophy seen in this model. Indeed SSc fibroblasts have elevated STAT3 phosphorylation constitutively compared with controls. It has recently been demonstrated that cardiac fibrosis induced by angiotensin II infusion in mice leads to IL-6-dependent fibrosis through activation of the pro-fibrogenic molecule TGF-β leading to collagen deposition mediated through Smad3 phosphorylation and activation.57

**CONCLUSION**

IL-6 is a pleiotropic cytokine whose aberrant expression is associated with a variety of diseases, including RA, Castleman’s disease and osteoporosis. Its role in SSc pathogenesis is now only beginning to be elucidated. It is well documented that IL-6 levels are elevated in SSc
serum and also localised in skin biopsies, it is especially prominent in early stages of disease. Moreover, IL-6 levels correlate tightly with skin thickness scores indicating a casual relationship. This gives a rationale for the targeting of IL-6 in this disease setting. To date, there is no study looking at serum levels of IL-6R in relation to duration of disease or subtype. Tocilizumab is a monoclonal antibody against the IL-6 receptor, which is very effective in RA, Castleman’s disease and juvenile idiopathic arthritis. It has been well tolerated and the safety profile is excellent. In a case report from Japan, two patients treated with tocilizumab showed a small improvement in skin score thickening. They also showed a reduction in tissue collagen levels. This was only a small study; however, and a placebo-controlled clinical trial is needed to corroborate these findings. It is worth bearing in mind that SSc has no effective treatment and is an unmet clinical need.

Sirukumab is another monoclonal IL-6 antibody that targets only soluble IL-6 and not the receptor, and should only target the ‘classical’ pathway of IL-6 and not so called ‘transsignalling’. This has been shown to be safe and well tolerated; however, no published data are available on the efficacy. Clinical trials are ongoing investigating this in RA. Thus, we can compare the efficacy between the canonical and non-canonical pathway in IL-6 signalling. If trans-signalling is the primary pathological hallmark in the disease, one would expect tocilizumab to have a greater clinical effect. The clinical benefits of anti-IL-6 treatment may be twofold: directly blocking the clear pro-fibrotic nature of IL-6 in fibroblasts and also blocking the inflammation part of SSc through the reduction of the polarisation of pathogenic Th17 cells and the upregulation of the tolerance Treg cells.

The reduction of sIL-6R through the neutralisation with tocilizumab has been a clinical breakthrough in the treatment of RA. We postulate that marked improvements in clinical status should be seen with tocilizumab in SSc. However, blockade together with other pro-fibrotic cytokines, such as IL-13 (lebrikizumab) or IL-4, may yield greater clinical responses. A current clinical trial assessing tocilizumab in SSc is underway.

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