Therapeutic targeting of T cells in systemic sclerosis

Systemic sclerosis is an autoimmune disorder with an unknown cause. The cardinal features of the disease are autoimmunity, vasculopathy, inflammation and fibrosis. There appears to be a link between inflammation and inflammatory cells and the uncontrolled deposition of the extracellular matrix. In particular, T cells appear to play a prominent role in disease initiation and propagation through the secretion of a myriad of cytokines and growth factors. These T-cell-dependent products may drive the proliferation and activation of resident fibroblasts, which ultimately leads to fibrosis. This review summarizes the current literature of the role of T cells in systemic sclerosis and suggests that therapeutic targeting of T cells is a promising new avenue.

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Systemic sclerosis (SSc) is a rare, debilitating autoimmune disease of unknown origin characterized by inflammation and excessive deposition of extracellular matrix (ECM) components, particularly collagen [1–3]. SSc is prevalent worldwide, primarily affecting females, and there is currently no effective treatment [2,4,5]. Early activation of the immune system is thought to be involved in the pathogenesis, as reflected by the increased level of cytokines in the serum of patients and the infiltration of mononuclear cells in the dermis of patients, which is thought to stimulate the differentiation and proliferation of fibroblasts to myofibroblasts [6–8]. The increased expression of myofibroblasts is a marker of SSc; these cells express the protein αSMA, which increases the contractile force of the cells, thus increasing the deposition of ECM [9]. SSc patients can be classified into two subsets; limited and diffuse, each distinguishable by the level of fibrosis and the autoantibodies present in the serum of patients [10,11]. Diffuse SSc has a rapid onset with fibrosis occurring within the skin and in one or more internal organs, whereas limited SSc is milder, with fibrosis limited to the skin [11,12].

Despite the unknown etiology of SSc, several inflammatory mediators have been identified in SSc patients [13]. Analysis of the blood of patients has revealed the presence of autoantibodies, including anticentromere and anti-topoisomerase antibodies, an increase in the level of cytokines, including IL-1, IL-6 and IL-27, and the presence of activated immune cells, including macrophages, mast cells and T cells (TABLE 1) [14,15]. In addition, analysis of the inflammatory infiltrate in the affected tissue of SSc patients shows the presence of activated T cells and the increase in these cells correlates with the severity of disease and symptoms including skin thickening. Furthermore, examination of the peripheral blood of patients demonstrates a change in the composition of the T-cell population, with predominance for Th2 cells and a change in the phenotype of Tregs [16,17].

This review seeks to examine the role of T cells in the pathogenesis of SSc by looking into the origin of T cells and their role in the immune response; the authors will then review the evidence of an altered T-cell response in SSc patients and the possible role of the products of T cells in causing fibrosis. Finally, the authors will examine the therapeutic potential of targeting T cells in the treatment of this chronic disease, as there is currently no therapy to modify disease.

Origin of T cells

In order to generate an appropriate immune response against injury or infection an efficient immune system must be developed. There are two main branches of the immune system; the innate and the adaptive immune systems. T cells are an essential component of the adaptive immune system [18]. T lymphocytes are bone marrow-derived cells that mature in the thymus. The thymus is composed of two separate regions, the cortex and the medulla, which contain epithelial and mesenchymal tissue, providing the lymphocyte progenitor cells with a microenvironment to develop into a highly selective T-cell repertoire [18,19]. In order to ensure the development of specific

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Table 1. T-cell cytokines and chemokines that are elevated in systemic sclerosis patients.

Cytokine	Role	Ref.
IL-1	Increases fibrosis and fibroblast proliferation	[68]
IL-4	Increased in BALF of SSc patients Shown to be profibrotic and antifibrotic	[54–56]
IL-6	Increased in serum of SSc patients Presence correlates with skin thickness score and internal organ fibrosis	[60–62]
IL-13	Stimulate fibroblasts to produce ECMs	[38,39]
IL-17	IL-17 mRNA increases in SSc patients with lung fibrosis May induce proliferation of fibroblasts	[69,70]
TNF-α	Increased in SSc patients Presence correlates with skin thickening	[6]
MCP-1	Chemoattractant	[82]

BALF: Bronchoalveolar lavage fluid; ECM: Extracellular matrix component; SSc: Systemic sclerosis.

and nonself-reactive T cells, developing T cells undergo positive and negative selection in the thymus; this is termed 'thymic education' [18–20]. Upon infection or injury, T cells are recruited to the site of inflammation and, due to signals in the environment, it has been shown that CD4+ T cells further differentiate into subtypes. Figure 1 demonstrates the T-cell subsets. Studies on the differentiation of CD4+ cells in mice first identified the Th1 and Th2 subsets; these subsets can be identified according to the transcription factor that is activated and the cytokines that they produce [21,22]. Th1 cells develop in the presence of IL-12, which induces the activation of STAT4 and T-bet and the secretion of IFN-γ [21–23]. Whereas Th1 cells are important in the immune response against intracellular pathogens, Th2 cells develop owing to the presence of IL-4 and the activation of STAT6 and GATA-3, leading to the secretion of IL-4, -5, -6 and -13, and are important in helping B cells produce IgE and in clearing helminth infections [1,21,23,24]. The presence of TGF-β and IL-6 together in the inflammatory milieu induces the expression of RoRγt and the differentiation of naive CD4+ cells to Th17 cells [24–26]. Th17 cells secrete IL-17, -6, -23 and TNF-α, and express IL-23 receptor (R) on their surface [24,26]. IL-23 helps promotes the survival of Th17 cells [24]; however, it has also been shown that IL-23 can induce the expression of pathogenic Th17 cells. IL-23 has been shown to be important in the development of intestine inflammation and inflammatory bowel disease [27].

CD4+ T cells can also differentiate into Tregs, which are essential in the prevention of autoimmune disease and the maintenance of self-tolerance [28,29]. It is now well established that there

are two distinct subsets of Tregs: natural regulatory cells (CD4+CD25+), which develop in the thymus, and inducible Treg cells, which develop in the periphery after exposure to cognate antigen [30]. Treg cells usually express the transcription factor FoxP3; however, this is not a reliable marker for Treg cells as some FoxP3 cells are not Tregs [31]. Interestingly, it has recently been shown that following exposure to IL-17, Tregs can differentiate into Th17 cells [30].

T cells in SSc

Evidence for the role of T cells in the pathogenesis of SSc initially comes from the presence of the inflammatory infiltrate in the dermis, gastric mucosa and other affected tissue of patients with SSc, which appears to drive fibrosis [1,32–34]. Analysis of skin infiltrates shows that there is an increase in CD3+ cells in SSc patients [1]. Another study has shown that the infiltrate contains a higher proportion of CD4+ T cells, implicating a role of these cells in causing fibrosis of the skin [33]. CD4+ T cells have been associated with the pathogenesis of other autoimmune diseases, including rheumatoid arthritis [35]. However, in these diseases there is a bias toward a Th1 response, whereas in SSc patients there appears to be a bias toward a Th2 response, as shown by the increase in Th2 cytokines, such as IL-4, -6 and -13 in the serum, skin and lung of bleomycin-treated mice (a mouse model of fibrosis) and in SSc patients [3,17,36–39]. Furthermore, in radiation-induced fibrosis in rats, there is a predominance of CD4+ T cells at sites of fibrosis, which are mainly comprised of Th2 subset [40]. The role of Th2 cells in inducing fibrosis is controversial since a recent study has shown that these cells block collagen production in healthy dermal fibroblasts; however, it was also shown that fibroblasts derived from SSc patients were less susceptible to Th2 inhibition of collagen production [41]. In addition, mice that genetically lack CD28 on T cells, the molecule required for full T-cell activation, have much reduced lung fibrosis compared with wild-type mice after bleomycin challenge. Importantly, adoptive transfer of CD28+ T cells from wild-type mice recovered the blocked fibrosis in the CD28-deficient mice [42].
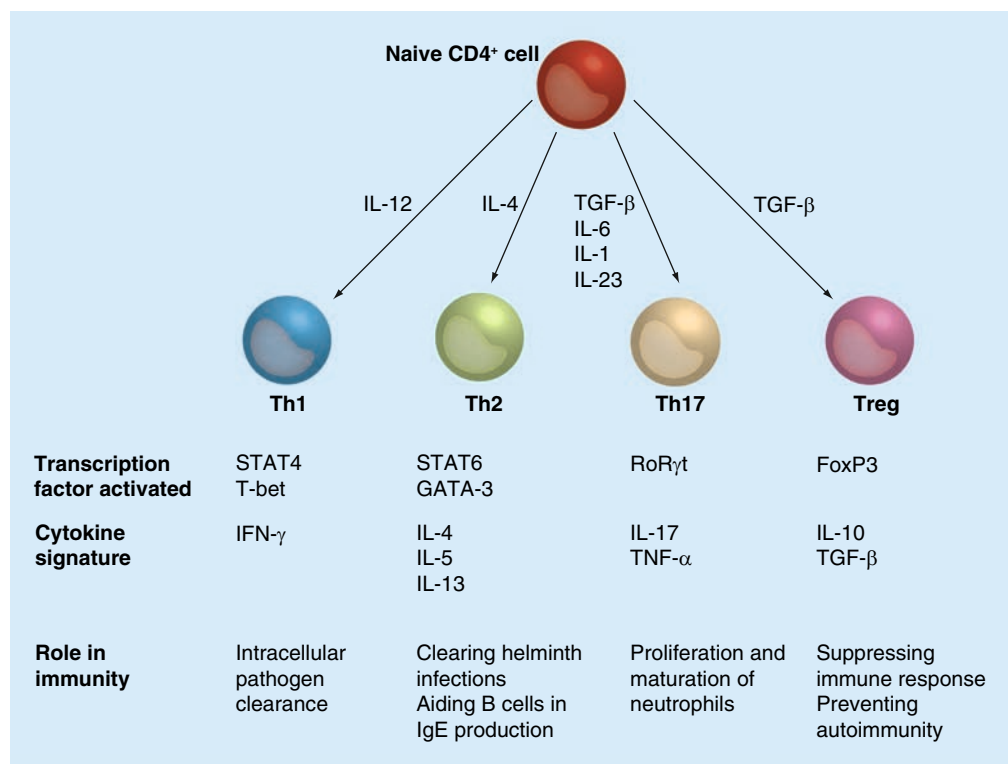
There is also evidence of a change in the proportion of Th17 and Treg cells in causing fibrosis in SSc patients, although this evidence is somewhat controversial. Examination of the skin and peripheral blood of SSc patients demonstrates an increase in Th17 cells and a decrease in Tregs [17,32,36,43–46]. Mathian *et al.* demonstrated that

Treg cells from SSc patients can inhibit anti-CD3-induced T-cell proliferation, thus suggesting that the immunosuppressive function of Treg cells is preserved [32]. However, when examining the total number of Treg cells in patients, it was shown that there is a decrease in these cells and FoxP3 expression compared with controls, and this correlates with diagnosis of disease [32]. This study also examines the levels of IL-17 and -22 in the serum of patients. Although there is no change in the concentration of IL-17 in SSc patients compared with controls, an increase in IL-22 is observed [32]. This suggests a differentiation of Treg cells to Th17 cells, resulting in fibroblast activation and, thus, fibrosis [32]. These observations have been confirmed [45,47], with an increase in CD4+ cells observed but a decrease in Treg cell numbers. However, several other studies have shown an increase or no change in Treg numbers in the peripheral blood or skin of SSc patients compared with healthy controls [48,49].

Epigenetic modification (inheritable changes in gene expression that are not due to modification of DNA sequences) of inflammatory genes in T cells has been shown to have a role in the pathogenesis of SSc [4,5,50]. Jiang et al. have shown a role for the epigenetic modification of the CD70 promoter TNFSF7 [50]. CD70 is a costimulatory molecule expressed by activated immune cells; its expression is tightly regulated by epigenetic modifications of its promoter region. CD70 interacts with CD27 to induce plasma cell proliferation and antibody production on B cells, it enhances the survival of antigen-specific T cells, and hence the interaction has a key role in the inflammatory process [5,50]. It has been shown that CD70 expression is increased in SSc patients and bisulphite sequencing revealed that the overexpression of CD70 on SSc T cells is due to the hypomethylation of TNFSF7 promotor region [50]. Epigenetic modification of the CD40 ligand promoter region has also been observed in SSc patients and has been suggested as a reason for the predominance of SSc in females [4]. CD40 ligand is present on the X chromosome; in order to prevent overexpression of proteins encoded on the X chromosome in females, one chromosome is silenced by epigenetic modifications, such as DNA methylation [4,5]. It has also been shown that in female SSc patients, there is an increase in CD40 ligand compared with healthy female controls, owing to the demethylation of the promoter region on the X chromosome. Interestingly, there was no change observed in the levels of methylation between male SSc patients and healthy male controls [4].

During infection or injury, leukocytes infiltrate the affected tissue due to the presence of

	Th1	Th2	Th17	Treg
Transcription factor activated	STAT4 T-bet	STAT6 GATA-3	RoRγt	FoxP3
Cytokine signature	IFN-γ	IL-4 IL-5 IL-13	IL-17 TNF-α	IL-10 TGF-β
Role in immunity	Intracellular pathogen clearance	Clearing helminth infections Aiding B cells in IgE production	Proliferation and maturation of neutrophils	Suppressing immune response Preventing autoimmunity

Figure 1. Following an antigen encounter, CD4+ cells can differentiate into further subtypes according to the cytokines present.

adhesion molecules at the inflammatory site and on the surface of lymphocytes. The importance of the adhesion molecules L-selectin (expressed on leukocytes) and ICAM-1 (expressed by endothelial cells and fibroblasts) in causing fibrosis has been demonstrated in a bleomycin mouse model [36]. Yoshizaki et al. showed in ICAM-1 and L-selectin-knockout bleomycin mice that ICAM-1 and L-selectin are essential for: T-cell infiltration to skin and the lungs; differentiation of T-helper cells to their Th17 and Th2 subtypes; and the increase in proinflammatory cytokine expression, which is usually observed in the bleomycin mouse model [36]. The cell-adhesion molecule DNAX-1 (CD226) is increased in SSc patients' skin and in a majority of cells and, using bleomycin DNAX-/- mice, DNAX-1 has shown to be involved in the development of dermal thickness and T-cell infiltration into the skin [51].

T-cell products that drive fibrosis

Clearly, the presence of these T cells at the site of fibrosis in patients with SSc implicates a role for the T-cell repertoire in the pathogenesis of the disease. The distorted T-cell population is reflected in the polarized cytokine milieu present in SSc patients. The increase in Th2 and Th17 cells, plus the apparently nonresponsive Treg population, results in an increase in Th2 and Th17 cytokines including IL-4, -6, -13, -17 and TNF-α in the serum of SSc patients, which may induce fibrosis, directly or indirectly.

IL-4 is a pleiotropic cytokine involved in the differentiation of T cells that can induce the expression of MHC class II molecules and IgE secretion on B cells [52,53]. IL-4 signals via its receptor, which consists of the Il-4Rα chain, that binds IL-4 with high affinity, and the γ common chain, which contains the signaling domain. Binding of IL-4 to IL-4R can result in activation of the Janus kinase (JAK) and the STAT signaling cascades [52]. It has been shown that there is an increase in IL-4-producing T cells in the bronchoalveolar lavage fluid of SSc patients [54]. However, the role of IL-4 in causing fibrosis is controversial, since opposing studies suggest either an antifibrotic or a profibrotic role for IL-4. Izbicki et al. used an IL-4 knockout and IL-4 transgenic bleomycin mouse model to demonstrate the effect that the presence or absence of IL-4 has in causing fibrosis [55]. In bleomycin-treated IL-4-/- mice, an increase in lung fibrosis was observed, whereas in mice overexpressing IL-4 lung fibrosis was decreased, suggesting an antifibrotic function of IL-4 [55]. Huaux et al. demonstrated the pleiotropic characteristics of IL-4 using an IL-4-/- bleomycin-treated mouse model and examining the extent of pulmonary fibrosis [56]. In this model, an increase in lymphocytes was observed in the bronchoalveolar lavage fluid but pulmonary fibrosis was decreased [56]. However, Huaux et al. also showed that stimulation of pulmonary fibroblasts with IL-4 did not induce proliferation of these cells [56]. In contrast to these observations, it has been shown that stimulation of healthy dermal fibroblasts with IL-4 results in an increase in collagen production by these cells due to an activation of the ERK pathway [57]. In addition, Postlethwaite et al. demonstrated that stimulation of dermal fibroblasts with recombinant IL-4 induces collagen production, which is inhibited by the addition of IL-4 antibody [53]. Furthermore, use of the tight skin model of SSc implicates a role for IL-4 in causing dermal fibrosis [58,59]. In IL-4-/- tight skin models, a decrease in skin fibrosis is observed [59]. Further to this, it was found that the stimulation of tight skin dermal fibroblasts with IL-4 induced collagen production, which was attenuated with the addition of anti-IL-4 antibody [58].

IL-6 is another pleiotropic cytokine that is increased in the serum of SSc patients [60–62]. IL-6 signaling requires the expression of two receptors: IL-6R and gp130 [16]. IL-6R is only expressed on hepatocytes, neutrophils, monocytes and leukocytes, whereas gp130 is ubiquitously expressed [63]. gp130 is a critical shared component of IL-6-related cytokine ligands. A soluble form of the receptor (sIL-6R) also exists, owing to the cleaving of IL-6R from activated lymphocytes or hepatocytes by the enzyme ADAM17 [16]. IL-6 signaling can occur via classical signaling, in which two IL-6 molecules bind two IL-6R chains, this in turn recruits two gp130 molecules; or via trans-signaling, in which two IL-6 molecules bind to two sIL-6R molecules. This IL-6–sIL-6R complex then binds to gp130, which contains the phosphorylation domains, resulting in signaling via the JAK–STAT and RAS–MAPK pathways [16,61,63]. Thus, trans-signaling enables IL-6 to signal to cells that do not usually express IL-6R. As previously stated, IL-6 is increased in the serum of SSc patients and correlates with modified skin thickness score and internal organ fibrosis in diffuse SSc [60,62]. The same study demonstrated that IL-6 induction of collagen production was dependent on the JAK2–STAT3 signaling cascades and that inhibiting these pathways attenuates collagen production [60]. It has also been shown that stimulated T cells from SSc patients, incubated with TNF-α, produced higher levels

of IL-6 and sIL-6R. These T cells also induced an increase in collagen production by healthy dermal fibroblasts, which can be inhibited with the addition of anti-IL-6 antibody, thus implicating trans-signaling in causing fibrosis [6]. Duncan *et al.* demonstrated that addition of recombinant IL-6 to healthy dermal fibroblasts results in an increase in collagen production, which can be inhibited by the addition of an anti-IL-6 antibody [64]. A bleomycin mouse model of fibrosis shows that there is an increase in IL-6 in the serum and IL-6 mRNA in these mice [37]. Treatment of bleomycin mice with MR16-1 (an anti-IL-6R antibody) results in a decrease in dermal thickening, myofibroblast proliferation and αSMA production [37]. In addition, in an IL-6$^{-/-}$ bleomycin mouse model, a decrease in dermal sclerosis and αSMA was observed (**FIGURE 2**) [37].

IL-13 is a 33 amino acid-long cytokine that has similar properties to IL-4 and shares a common receptor subunit [65]. IL-13 binds the IL-13R and signals via JAK kinases to activate STAT6 signaling [38,65,66]. IL-13 can stimulate fibroblasts to increase ECM production; however, conflicting studies have suggested that IL-13 stimulated fibrosis may be TGF-β dependent or independent. Fichtner-Feigl *et al.* demonstrated that IL-13 and TNF-α induced TGF-β production via IL-13Rα, which resulted in fibrosis [38]. However, Kaviratne *et al.* suggested that liver fibrosis caused by IL-13 was independent of TGF-β [39]. This study used IL-13$^{-/-}$ mice and showed a decrease in fibrosis of the liver after infection with *Schistosoma mansoni* [39]. Fuschiotti *et al.* implicated a role of CD8$^+$ T cells in the production of IL-13, as CD8$^+$ IL-13-secreting cells were found elevated in the skin of patients with SSc [67]. Culturing these CD8$^+$ T cells with fibroblasts resulted in an increase in ECM production, which correlated with IL-13 levels. In addition, inhibition of IL-13 with an IL-13 antibody decreased the collagen mRNA production by these cells [67]. Although CD8$^+$ T cells are best known for their ability to lyse virally infected cells, it is now known that they have a much broader role in the immune system.

TNF-α is another pleiotropic cytokine involved in the pathogenesis of SSc [6,68]. TNF-α has two forms; a membrane-bound (mTNF-α) form and a soluble (sTNF-α) form. TNF-α can signal via two receptors. TNFR1 is a ubiquitously expressed receptor, which both sTNF-α and mTNF-α can bind to, whereas TNFR2 only binds mTNF-α and is only expressed by immune cells. TNF-α is increased in patients
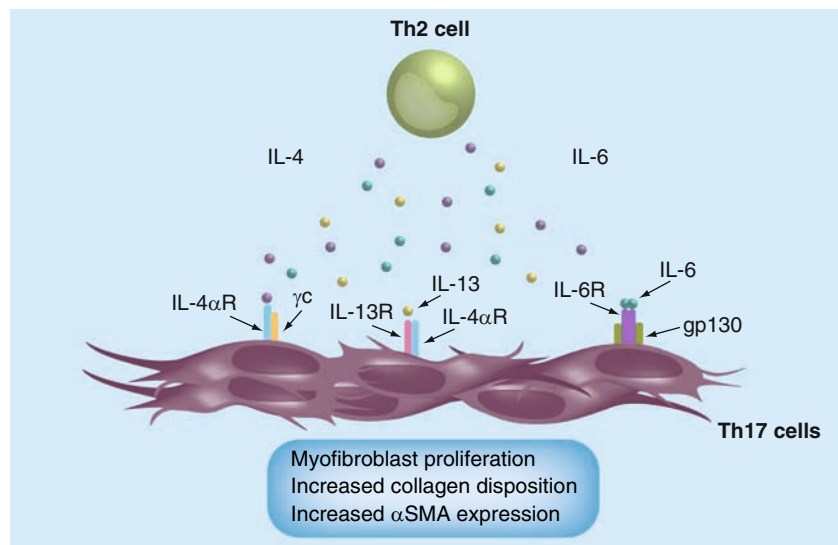
Figure 2. In systemic sclerosis patients, there is a skewed T-cell repertoire, with an increase in Th2 and Th17 cells observed. Cytokines, including IL-4, -6, -13 and -17, are secreted by T cells and can interact with fibroblasts, resulting in activation of the cell and its differentiation into a myofibroblast. This results in an increase in extracellular matrix components disposition, resulting in the characteristic fibrosis that is observed in systemic sclerosis patients. gp130 is the common cytokine shared subunit for IL-6 and associates with IL-6R to form a functional receptor complex. The box indicates downstream effects of T-cell products in dermal fibroblasts.
R: Receptor.

with SSc and T cells from SSc patients have increased expression of TNFR1 and 2 on their surface and this correlates with skin thickening. In addition, activation of these T cells prior to stimulation of TNF-α increases IL-6 and IL-13 secretion, resulting in an increase in collagen production by dermal fibroblasts [6]. In addition, as previously stated, DNAX-1 has been shown to be involved in the development of dermal thickness and T-cell infiltration into the skin. DNAX-1 knockout mice have decreased TNF-α expression, which is thought to decrease T-cell infiltration and dermal thickening [51].

IL-17 is a proinflammatory cytokine produced by Th17 cells that has been found to be increased in the serum, bronchoalveolar lavage fluid, skin lesions and lungs of patients with SSc [17,44,69–71]. IL-17 expression correlates with interstitial lung disease in SSc patients [17]. Kurasawa *et al.* showed that IL-17 mRNA is increased in SSc patients with lung fibrosis and when IL-17 is incubated with dermal fibroblasts from both healthy controls and SSc patients, proliferation of these cells is observed [69]. However, it was not shown that IL-17 increases collagen production by these cells. More recently it has been shown that the presence of IL-17-expressing T cells and mast cells, in SSc patients, colocalizes with areas of myofibroblast proliferation [70]. However,

Truchetet *et al.* also confirmed that IL-17 does not induce collagen production, rather IL-17 seems to inhibit myofibroblast generation [70].

Therapeutic targeting of T cells

The increased presence of T cells in the affected tissue of SSc patients and the increased levels of Th2 cytokines in the serum of patients with SSc suggests that that the targeting of T cells in the treatment of SSc is a promising avenue in this chronic disease. Targeting of T cells has been used in the treatment of other inflammatory diseases. First, alemtuzumab (Campath-1H; Genzyme, MA, USA), a humanized antibody that targets CD52 resulting in depletion of B and T cells, has previously been used in the treatment of rheumatoid arthritis (RA) patients refractory to standard treatment. Administration of alemtuzumab to RA patients resulted in improvements; however, trials were cut short owing to therapy-induced lymphopenia [72,73]. Importantly, a 12-year follow-up of patients revealed no excess mortality after treatment [73]. Indeed, a case report showed that a patient with diffuse cutaneous SSc treated with alemtuzumab had a rapid and sustained improvement in skin fibrosis [74].

The administration of abatacept could also be used in the treatment of SSc. Abatacept is a soluble, fully humanized fusion protein comprised of the extracellular domain of human CTLA4 and the Fc domain of IgG1. Its mechanism of action involves competing with CD28 to bind to CD80/CD86. CD28 is required for the full activation of T cells through binding of CD80/86 on the antigen-presenting cell; this signal is called 'costimulation'. Abatacept is licensed for RA and may actually be most useful in early undifferentiated RA, where the T cell is binding to its putative autoantigen presented by an antigen-presenting cell in the joint.

An alternative approach may be to target T-cell-derived cytokines, such as IL-13 and -6, using targeted antibodies. Tocilizumab is an anti-IL-6R monoclonal antibody that inhibits IL-6 signaling. This antibody has been used to treat patients with juvenile idiopathic arthritis and RA to good effect [75,76]. A case report of two patients with diffuse SSc who were administered tocilizumab at 8 mg/kg for 6 months demonstrated clinical improvement and a reduction in skin thickness [76]. Thus, the blockade of IL-6 signaling is a possible therapeutic option, although a randomized clinical trial is necessary.

Autologous hematopoietic stem cell transplantation is a promising new treatment in SSc. During autologous hematopoietic stem cell transplantation, the patient's CD34+ cells are isolated before their lymphocytes are depleted using cyclophosphamide and antithymocyte globulin. After depletion of lymphocytes, the patient's stem cells are transplanted, resulting in the repopulation of the lymphocytes [77]. The mechanism may be a restoration of the patients' immune system that is now 'toleregenic'. Indeed, clinical data suggest that autologous hematopoietic stem cell transplantation is effective in improving patient mortality, decreasing the skin thickness score and improving pulmonary function [77–79]. Extracorporeal photochemotherapy (ECP) is another possible therapeutic option in the treatment of SSc. ECP uses apheresis to separate leukocytes from the blood before treatment of leukocytes with psorlen 8-methoxypsoralan and ultraviolet light A. In the presence of ultraviolet light A, 8-methoxypsoralan crosslinks to DNA preventing the proliferation of cells. The treated cells are then reinfused into the patient. In patients with SSc, ECP treatment has been shown to improve dermal thickness, modified Rodman skin thickness score and joint involvement. In addition, ECP treatment of SSc resulted in a decrease in the number of Th17 cells and an increase in Treg cell numbers [10,80,81].

Conclusion

SSc is an autoimmune disease in which activated T cells from the adaptive immune system play a prominent role. Accumulating evidence points toward T cells playing a role in the initiation and progression of the disease. T cells, once activated, produce a myriad of cytokines that either directly or indirectly influences the deposition of ECM; a hallmark of SSc. The therapeutic targeting of T cells may hold promise as a future therapy of SSc, as there are currently no treatments that modify the disease outcome. However, it must be borne in mind that any therapeutic depletion of T cells, while beneficial to reduce the role of these cells in driving the pathogenesis in SSc, may result in greater risk of infection. The relative risk of infection versus progression of disease must be carefully considered.

Future perspective

Over the next 5–10 years, research will further investigate the precise contribution of T cells to SSc pathogenesis. The use of novel animal models that faithfully recapitulate the disease will aid in investigations of the role of T cells and their products and will help evaluate the use of agents that specifically target T cells and/or their products. T-cell-targeted therapies may be useful in other autoimmune diseases, not only SSc.

Executive summary

Background
- Systemic sclerosis (SSc) is an autoimmune disease characterized by inflammation and fibrosis.
- T cells are thought to contribute to disease initiation and progression.

Origin of T cells
- T cells develop in the thymus, where they undergo positive and negative selection to produce a nonself-binding T-cell antigen receptor, and emerge as naive, single, positive T cells.
- After encountering antigen in the periphery and under the influence of the cytokine milieu present, CD4+ T cells can further differentiate into subtypes, including Th1, Th2 and Th17.

T cells in SSc
- T cells seem to be involved in the pathogenesis of SSc due to their infiltration to the affected skin in patients and the increased concentration of T-cell cytokines in SSc patients.
- There is an altered T-cell population in SSc patients, with an increased expression of Th2 and Th17 T cells and an altered phenotype in Treg cells.
- Epigenetic modification of the CD70 and CD40 ligand promoters is observed in SSc patients.
- Adhesion molecules, including L-selectin, ICAM-1 and DNAX-1, are thought to be important in T-cell infiltration and differentiation into the different T-cell subtypes.

T-cell products that drive fibrosis
- Th2 and Th17 cytokines, including IL-4, -6, -13 and -17, appear to be important in driving fibrosis.

Therapeutic targeting of T cells
- Therapeutic targeting of T cells and their products in the treatment of SSc is a promising avenue.
- Administration of alemtuzumab, a humanized antibody to target CD52, to a patient with diffuse cutaneous SSc resulted in sustained improvement in the skin.
- Tocilizumab, an anti-IL-6 receptor monoclonal antibody, administered to two SSc patients, resulted in a reduction in skin thickness.

References

1 O'Reilly S, Hügle T, van Laar JM. T cells in systemic sclerosis: a reappraisal. *Rheumatology* 51(9), 1540–1549 (2012).

2 Fuschiotti P. CD8+ T cells in systemic sclerosis. *Immunol. Res.* 50(2–3), 188–194 (2011).

3 Chizzolini C, Brembilla NC, Montanari E, Truchetet ME. Fibrosis and immune dysregulation in systemic sclerosis. *Autoimmun. Rev.* 10(5), 276–281 (2011).

4 Lian X, Xiao R, Hu X *et al*. DNA demethylation of CD40L in CD4+ T cells from women with systemic sclerosis: a possible explanation for female susceptibility. *Arthritis Rheum.* 64(7), 2338–2345 (2012).

5 Hedrich CM, Rauen T. Epigenetic patterns in systemic sclerosis and their contribution to attenuated CD70 signaling cascades. *Clin. Immunol.* 143(1), 1–3 (2012).

6 Hügle T, O'Reilly S, Simpson R *et al*. Tumor necrosis factor-costimulated T lymphocytes from patients with systemic sclerosis trigger collagen production in fibroblasts. *Arthritis Rheum.* 65(2), 481–491 (2013).

7 Dar SA, Bhattacharya SN, Das S, Ramachandran VG, Banerjee BD, Arora P. Response of T-cell subpopulations to superantigen and recall antigen stimulation in systemic sclerosis. *Indian J. Dermatol.* 57(3), 175–180 (2012).

8 Hussein MR, Hassan HI, Hofny ER *et al*. Alterations of mononuclear inflammatory cells, CD4/CD8+ T cells, interleukin 1β, and tumour necrosis factor α in the bronchoalveolar lavage fluid, peripheral blood, and skin of patients with systemic sclerosis. *J. Clin. Pathol.* 58(2), 178–184 (2005).

9 Beon M, Harley RA, Wessels A, Silver RM, Ludwicka-Bradley A. Myofibroblast induction and microvascular alteration in scleroderma lung fibrosis. *Clin. Exp. Rheumatol.* 22(6), 733–742 (2004).

10 Papp G, Barath S, Szegedi A, Szodoray P, Zeher M. The effects of extracorporeal photochemotherapy on T cell activation and regulatory mechanisms in patients with systemic sclerosis. *Clin. Rheumatol.* 31(9), 1293–1299 (2012).

11 Wei J, Bhattacharyya S, Tourtellotte WG, Varga J. Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. *Autoimmun. Rev.* 10(5), 267–275 (2011).

12 Gorlova O, Martin JE, Rueda B *et al*. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. *PLoS Genet.* 7(7), e1002178 (2011).

13 Moinzadeh P, Denton CP, Abraham D *et al*. Biomarkers for skin involvement and fibrotic activity in scleroderma. *J. Eur. Acad. Dermatol. Venereol.* 26(3), 267–276 (2012).

14 Yoshizaki A, Yanaba K, Iwata Y *et al*. Elevated serum interleukin-27 levels in patients with systemic sclerosis: association with T cell, B cell and fibroblast activation. *Ann. Rheum. Dis.* 70(1), 194–200 (2011).

15 Kuwana M, Medsger TA, Wright TM. T and B cell collaboration is essential for the autoantibody response to DNA topoisomerase I in systemic sclerosis. *J. Immunol.* 155(5), 2703–2714 (1995).

16   O'Reilly S, Ciechomska M, Cant R, Hugle T, van Laar JM. Interleukin-6, its role in fibrosing conditions. *Cytokine Growth Factor Rev.* 23(3), 99–107 (2012).

17   Truchetet ME, Brembilla N, Montanari E, Allanore Y, Chizzolini C. Increased frequency of circulating Th22 in addition to Th17 and Th2 lymphocytes in systemic sclerosis: association with interstitial lung disease. *Arthritis Res. Ther.* 13(5), R166 (2011).

18   Anderson G, Takahama Y. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends Immunol.* 33(6), 256–263 (2012).

19   Goldrath AW, Bevan MJ. Selecting and maintaining a diverse T-cell repertoire. *Nature* 402(6759), 255–262 (1999).

20   Gascoigne NR, Palmer E. Signaling in thymic selection. *Curr. Opin. Immunol.* 23(2), 207–212 (2011).

21   Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* 17(3), 138–146 (1996).

22   Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136(7), 2348–2357 (1986).

23   Liew FY. TH1 and TH2 cells: a historical perspective. *Nat. Rev. Immunol.* 2(1), 55–60 (2002).

24   Bettelli E, Oukka M, Kuchroo VK. TH-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 8(4), 345–350 (2007).

25   Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell-mediated tissue damage. *Nat. Med.* 13(2), 139–145 (2007).

26   Stockinger B. Th17 cells: an orphan with influence. *Immunol. Cell Biol.* 85(2), 83–84 (2007).

27   Farge D, Labopin M, Tyndall A *et al.* Autologous hematopoietic stem cell transplantation for autoimmune diseases: an observational study on 12 years' experience from the European Group for Blood and Marrow Transplantation Working Party on Autoimmune Diseases. *Haematologica* 95(2), 284–292 (2010).

28   Belkaid Y. Regulatory T cells and infection: a dangerous necessity. *Nat. Rev. Immunol.* 7(11), 875–888 (2007).

29   Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. *Nat. Rev. Immunol.* 8(7), 523–532 (2008).

30   Kitani A, Xu L. Regulatory T cells and the induction of IL-17. *Mucosal Immunol.* 1(Suppl. 1), S43–S46 (2008).

31   Fazekas De St Groth B. Regulatory T-cell function: when suppressor cells can't suppress. *Immunol. Cell Biol.* 85(3), 179–181 (2007).

32   Mathian A, Parizot C, Dorgham K *et al.* Activated and resting regulatory T cell exhaustion concurs with high levels of interleukin-22 expression in systemic sclerosis lesions. *Ann. Rheum. Dis.* 71(7), 1227–1234 (2012).

33   Xie Y, Zhang X, Wakasugi S, Makino T, Inoue Y, Ihn H. Immunohistochemical characterization of the cellular infiltrate in localized scleroderma. *Int. J. Dermatol.* 47(5), 438–442 (2008).

34   Manetti M, Neumann E, Muller A *et al.* Endothelial/lymphocyte activation leads to prominent CD4⁺ T cell infiltration in the gastric mucosa of patients with systemic sclerosis. *Arthritis Rheum.* 58(9), 2866–2873 (2008).

35   Boissier MC, Semerano L, Challal S, Saidenberg-Kermanac'h N, Falgarone G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. *J. Autoimmun.* 39(3), 222–228 (2012).

36   Yoshizaki A, Yanaba K, Iwata Y *et al.* Cell adhesion molecules regulate fibrotic process via Th1/Th2/Th17 cell balance in a bleomycin-induced scleroderma model. *J. Immunol.* 185(4), 2502–2515 (2010).

37   Kitaba S, Murota H, Terao M *et al.* Blockade of interleukin-6 receptor alleviates disease in mouse model of scleroderma. *Am. J. Pathol.* 180(1), 165–176 (2012).

38   Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nat. Med.* 12(1), 99–106 (2006).

39   Kaviratne M, Hesse M, Leusink M *et al.* IL-13 activates a mechanism of tissue fibrosis that is completely TGF-β independent. *J. Immunol.* 173(6), 4020–4029 (2004).

40   Westermann W, Schöbl R, Rieber EP, Frank KH. Th2 cells as effectors in postirradiation pulmonary damage preceding fibrosis in the rat. *Int. J. Radiat. Biol.* 75(5), 629–638 (1999).

41   Chizzolini C, Parel Y, De Luca C *et al.* Systemic sclerosis Th2 cells inhibit collagen production by dermal fibroblasts via membrane-associated tumor necrosis factor α. *Arthritis Rheum.* 48(9), 2593–2604 (2003).

42   Okazaki T, Nakao A, Nakano H *et al.* Impairment of bleomycin-induced lung fibrosis in CD28-deficient mice. *J. Immunol.* 167(4), 1977–1981 (2001).

43   Fenoglio D, Bernuzzi F, Battaglia F *et al.* Th17 and regulatory T lymphocytes in primary biliary cirrhosis and systemic sclerosis as models of autoimmune fibrotic diseases. *Autoimmun. Rev.* 12(2), 300–304 (2012).

44   Truchetet ME, Allanore Y, Montanari E, Chizzolini C, Brembilla NC. Prostaglandin I2 analogues enhance already exuberant Th17 cell responses in systemic sclerosis. *Ann. Rheum. Dis.* 71(12), 2044–2050 (2012).

45   Fenoglio D, Battaglia F, Parodi A *et al.* Alteration of Th17 and Treg cell subpopulations co-exist in patients affected with systemic sclerosis. *Clin. Immunol.* 139(3), 249–257 (2011).

46   Papp G, Horvath IF, Barath S *et al.* Altered T-cell and regulatory cell repertoire in patients with diffuse cutaneous systemic sclerosis. *Scand. J. Rheumatol.* 40(3), 205–210 (2011).

47   Klein S, Kretz CC, Ruland V *et al.* Reduction of regulatory T cells in skin lesions but not in peripheral blood of patients with systemic scleroderma. *Ann. Rheum. Dis.* 70(8), 1475–1481 (2011).

48   Kalogerou A, Gelou E, Mountantonakis S, Settas L, Zafiriou E, Sakkas L. Early T cell activation in the skin from patients with systemic sclerosis. *Ann. Rheum. Dis.* 64(8), 1233–1235 (2005).

49   Slobodin G, Ahmad MS, Rosner I *et al.* Regulatory T cells (CD4⁺CD25(bright) FoxP3⁺) expansion in systemic sclerosis correlates with disease activity and severity. *Cell. Immunol.* 261(2), 77–80 (2010).

50   Jiang H, Xiao R, Lian X *et al.* Demethylation of TNFSF7 contributes to CD70 overexpression in CD4⁺ T cells from patients with systemic sclerosis. *Clin. Immunol.* 143(1), 39–44 (2012).

51   Avouac J, Elhai M, Tomcik M *et al.* Critical role of the adhesion receptor DNAX accessory molecule-1 (DNAM-1) in the development of inflammation-driven dermal fibrosis in a mouse model of systemic sclerosis. *Ann. Rheum. Dis.* 72(6), 1089–1098 (2013).

52   Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu. Rev. Immunol.* 17, 701–738 (1999).

53   Postlethwaite AE, Holness MA, Katai H, Raghow R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. *J. Clin. Invest.* 90(4), 1479–1485 (1992).

54   Divekar AA, Khanna D, Abtin F *et al.* Treatment with imatinib results in reduced IL-4-producing T cells, but increased CD4⁺ T cells in the broncho–alveolar lavage of patients with systemic sclerosis. *Clin. Immunol.* 141(3), 293–303 (2011).

55   Izbicki G, Or R, Christensen TG *et al.* Bleomycin-induced lung fibrosis in IL-4-overexpressing and knockout mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 283(5), L1110–L1116 (2002).

56 Huaux F, Liu T, Mcgarry B, Ullenbruch M, Phan SH. Dual roles of IL-4 in lung injury and fibrosis. *J. Immunol.* 170(4), 2083–2092 (2003).

57 Bhogal RK, Bona CA. Regulatory effect of extracellular signal-regulated kinases (ERK) on type I collagen synthesis in human dermal fibroblasts stimulated by IL-4 and IL-13. *Int. Rev. Immunol.* 27(6), 472–496 (2008).

58 Ong C, Wong C, Roberts CR, Teh HS, Jirik FR. Anti-IL-4 treatment prevents dermal collagen deposition in the tight-skin mouse model of scleroderma. *Eur. J. Immunol.* 28(9), 2619–2629 (1998).

59 Ong CJ, Ip S, Teh SJ *et al.* A role for T helper 2 cells in mediating skin fibrosis in tight-skin mice. *Cell. Immunol.* 196(1), 60–68 (1999).

60 Khan K, Xu S, Nihtyanova S *et al.* Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. *Ann. Rheum. Dis.* 71(7), 1235–1242 (2012).

61 Kishimoto T. IL-6: from its discovery to clinical applications. *Int. Immunol.* 22(5), 347–352 (2010).

62 Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. *J. Dermatol. Sci.* 27(2), 140–146 (2001).

63 Rose-John S, Waetzig GH, Scheller J, Grotzinger J, Seegert D. The IL-6/sIL-6R complex as a novel target for therapeutic approaches. *Expert Opin. Ther. Targets* 11(5), 613–624 (2007).

64 Duncan MR, Berman B. Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6. *J. Invest. Dermatol.* 97(4), 686–692 (1991).

65 Mannon P, Reinisch W. Interleukin 13 and its role in gut defence and inflammation. *Gut* 61(12), 1765–1773 (2012).

66 Fuschiotti P. Role of IL-13 in systemic sclerosis. *Cytokine* 56(3), 544–549 (2011).

67 Fuschiotti P, Larregina AT, Ho J, Feghali-Bostwick C, Medsger TA Jr. Interleukin-13-producing CD8+ T cells mediate dermal fibrosis in patients with systemic sclerosis. *Arthritis Rheum.* 65(1), 236–246 (2013).

68 Jinnin M. Mechanisms of skin fibrosis in systemic sclerosis. *J. Dermatol.* 37(1), 11–25 (2010).

69 Kurasawa K, Hirose K, Sano H *et al.* Increased interleukin-17 production in patients with systemic sclerosis. *Arthritis Rheum.* 43(11), 2455–2463 (2000).

70 Truchetet ME, Brembilla NC, Montanari E *et al.* Interleukin-17A+ cells are increased in systemic sclerosis skin and their number is inversely correlated to the extent of skin involvement. *Arthritis Rheum.* 65(5), 1347–1356 (2013).

71 Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. *J. Leukoc. Biol.* 71(1), 1–8 (2002).

72 Isaacs JD, Greer S, Sharma S *et al.* Morbidity and mortality in rheumatoid arthritis patients with prolonged and profound therapy-induced lymphopenia. *Arthritis Rheum.* 44(9), 1998–2008 (2001).

73 Lorenzi AR, Clarke AM, Wooldridge T *et al.* Morbidity and mortality in rheumatoid arthritis patients with prolonged therapy-induced lymphopenia: twelve-year outcomes. *Arthritis Rheum.* 58(2), 370–375 (2008).

74 Isaacs JD, Hazleman BL, Chakravarty K, Grant JW, Hale G, Waldmann H. Monoclonal antibody therapy of diffuse

cutaneous scleroderma with CAMPATH-1H. *J. Rheumatol.* 23(6), 1103–1106 (1996).

75 Decelle K, Horton ER. Tocilizumab for the treatment of juvenile idiopathic arthritis. *Ann. Pharmacother.* 46(6), 822–829 (2012).

76 Shima Y, Kuwahara Y, Murota H *et al.* The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. *Rheumatology* 49(12), 2408–2412 (2010).

77 Zeher M, Papp G, Szodoray P. Autologous haemopoietic stem cell transplantation for autoimmune diseases. *Expert Opin. Biol. Ther.* 11(9), 1193–1201 (2011).

78 Burt RK, Shah SJ, Dill K *et al.* Autologous non-myeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised Phase 2 trial. *Lancet* 378(9790), 498–506 (2011).

79 Farge D, Passweg J, van Laar JM *et al.* Autologous stem cell transplantation in the treatment of systemic sclerosis: report from the EBMT/EULAR Registry. *Ann. Rheum. Dis.* 63(8), 974–981 (2004).

80 Oliven A, Shechter Y. Extracorporeal photopheresis: a review. *Blood Rev.* 15(2), 103–108 (2001).

81 Papp G, Horvath IF, Barath S *et al.* Immunomodulatory effects of extracorporeal photochemotherapy in systemic sclerosis. *Clin. Immunol.* 142(2), 150–159 (2012).

82 Distler O, Pap T, Kowal-Bielecka O *et al.* Overexpression of monocyte chemoattractant protein 1 in systemic sclerosis: role of platelet-derived growth factor and effects on monocyte chemotaxis and collagen synthesis. *Arthritis Rheum.* 44(11), 2665–2678 (2001).