REVIEW

Current perspectives on the immunopathogenesis of systemic sclerosis

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Abstract: Systemic sclerosis (SSc or scleroderma) is a progressive and highly debilitating autoimmune disorder characterized by inflammation, vasculopathy, and extensive fibrosis. SSc is highly heterogeneous in its clinical presentation, extent and severity of skin and internal organ involvement, and clinical course and has the highest fatality rate among connective tissue diseases. While clinical outcomes have improved in recent years, no current therapy is able to reverse or slow the natural progression of SSc, a reflection of its complex pathogenesis. Although activation of the immune system has long been recognized, the mechanisms responsible for the initiation of autoimmunity and the role of immune effector pathways in the pathogenesis of SSc remain incompletely understood. This review summarizes recent progress in disease pathogenesis with particular focus on the immunopathogenetic mechanisms of SSc.

Keywords: scleroderma, immune mediators, inflammation, autoimmunity

Introduction

Systemic sclerosis (SSc) is a rare disease with a prevalence ranging from 150 to 300 cases per million.[1,2] Although SSc has a worldwide distribution, prevalence varies substantially around the world, with lower estimates (<150 per million) in Northern Europe and Japan and higher estimates (276–443 per million) in Southern Europe, North America, and Australia.[2] As in many other autoimmune diseases, women are at higher risk than men (4:1 ratio over men),[3–5] and ethnicity plays a critical role in disease manifestations and mortality.[6] The etiology of SSc remains elusive, but it likely involves an interaction between environmental factors in a genetic predisposing background. Although SSc is not an inherited disease,[7] genetic factors contribute to its suscepti-bility,[8,9] as shown by a 60-fold higher occurrence of the disease in families (1.6%) than in the general population (0.026%).[8] Genetic linkage studies and genome-wide association studies have identified polymorphisms associated with the predisposition of patients to develop SSc.[10–15] These include genes of the major histocompatibility complex (MHC) class II,[9,14,16,17] as well as non-MHC genes,[13,18–24] such as genes associated with the metabolism of extracellular matrix (ECM) molecules[25–27] and genes coding for proteins involved in the control of innate immunity, macrophage activation, and T-cell functions.[10,14,28–32] Although progress has been made in the identification of genetic risk factors in SSc, the corresponding functional mechanisms remain elusive, except for the contribution of MHC class II to autoantibody specificity.[33–38] Functional studies of associated loci are thus an area of current focus. Environmental factors have been implicated as early triggers of disease processes. Viruses, including

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human cytomegalovirus,[39] parvovirus B19,[40] and Epstein–Barr virus,[41] are hypothesized to contribute to the development of SSc by inducing vascular damage and fibroblast proliferation.[42] Other environmental factors, such as drugs as well as environmental and occupational exposures to organic solvents including vinyl chloride, silica,[43] and nanoparticles from traffic-derived pollution,[44] have also been implicated as potential causative agents. SSc exhibits an extensive patient-to-patient variability. Heterogeneity has been observed in its clinical manifestations, clinical course, response to treatment, and survival.[3] Based on the extent of skin fibrosis and the pattern of internal organ involvement, patients with SSc are commonly classified into diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc) subsets.[45–47] Patients with dcSSc have rapidly progressive fibrosis of the skin, lungs, and other internal organs and present early development of visceral organ complications. In contrast, in lcSSc, the most prominent features are vascular manifestations, with generally mild skin and internal organ fibrosis. Classification criteria for SSc have been recently updated by a joint committee of the American College of Rheumatology and the European League Against Rheumatism.[48] The American College of Rheumatology/European League Against Rheumatism classification criteria are more sensitive and specific than the previous criteria and now include patients in the early stages of SSc and lcSSc. The expectation is that earlier and more specific diagnosis will enable timely treatment before irreversible organ damage occurs.

Mechanisms of pathogenesis

The pathogenesis of SSc is poorly understood, which has hampered the development of effective therapeutics for this complex connective tissue disease. Research effort in understanding the key pathogenetic pathways, cell types, and mediators underlying disease manifestations is crucial for the early diagnosis of SSc as well as for the development of targeted therapies. Pathogenesis of SSc is characterized by three hallmarks: small-vessel vasculopathy, dysregulation of innate and adaptive immunity, and extensive fibrosis of the skin and visceral organs. Fibrosis is a major contributor to the high level of morbidity and mortality in SSc and is believed to result from the interaction of immune mediators and other growth factors with responsive tissue fibroblasts, resulting in increased deposition of ECM in the skin and internal organs.[49,50] Although cutaneous fibrosis is the most characteristic feature of SSc, fibrosis of visceral organs results in organ damage and poor clinical outcome.

Clinical symptoms and histological data indicate that vascular injury and endothelial damage are the earliest pathogenic events in SSc,[51,52] possibly initiated by viruses, autoantibodies, chemicals, or oxidative products.[52,53] Activated endothelial cells upregulate the expression of adhesion molecules,[54] such as vascular cell adhesion protein 1, intercellular adhesion molecule, and E-selectin, as well as chemokines, such as MCP-1, MIP-1α, and MIP-1β, resulting in the recruitment of inflammatory cells. Endothelial cells also produce endothelin-1 and connective tissue growth factor, which stimulate vascular smooth-muscle cell proliferation and ECM production.[55,56] Progressive thickening of the vessel wall results in a narrowing of the lumen of the capillaries and in the loss of the microvasculature, which leads to tissue hypoxia and oxidative stress.[51] Moreover, vascular repair and angiogenesis are found defective in SSc, promoting the chronic disease state.[57] Infiltration of inflammatory cells is prominent in patients with early-stage disease[58,59] and is often seen in a perivascular distribution and preceding the development of vasculopathy and fibrosis.

A schematic representation of SSc pathogenesis is illustrated in Figure 1. This review focuses on the immune dysregulation processes associated with SSc pathogenesis and discusses the recent advances.

Immunopathogenesis of SSc

Immunological abnormalities of innate and adaptive immune system have long been recognized in SSc, including chronic mononuclear cell infiltration of affected tissues, dysregulation of cytokine and growth factor production, and production of autoantibodies.[60,61] In addition, numerous genetic association studies have identified several polymorphisms in genes relevant for innate and adaptive immune responses that confer susceptibility to SSc.[10,14] Polymorphisms in genes of the innate immune system include *PLD4*,[62] *toll-like receptor (TLR)2*,[31] *NLRP1*,[63] and *ATG5*.[13] Other polymorphisms associated with SSc are in genes that play important roles in T-cell differentiation, proliferation, and/or activation. Among those are *STAT4*,[64,65] *TBX21*,[65] *PTPN22*,[66] *tumor necrosis factor (TNF)SF4*,[67,68] *interleukin (IL)-21*,[69] *CD247*,[28,70] and *CD226*.[71] Polymorphisms in gene regulators of interferon (IFN) types I and II, such as IFN-regulatory factor (*IRF*) 5[28,72] and *IRF8*,[11] are also associated with SSc susceptibility. Other cytokines and chemokine genes associated with SSc include *TNFAIP3*,[73,74] *MIF*,[75] *IL-6*,[76] *CXCL8*,[77] and *CCR6*.[78] However, the mechanisms responsible for the initiation of autoimmunity leading to fibrosis and the role of immune effector
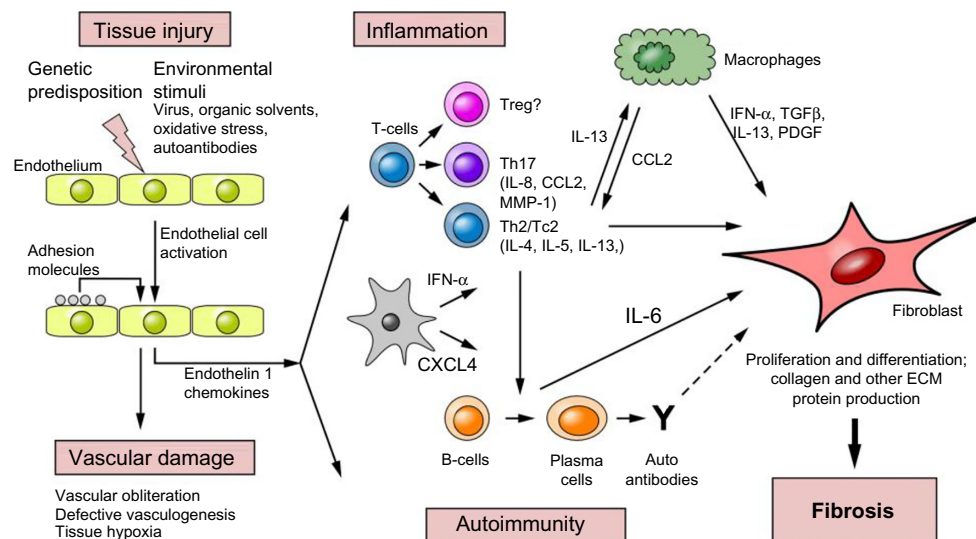
Figure 1 Etiopathogenesis of SSc.
Notes: Environmental and genetic factors contribute to the etiology of SSc. The pathogenesis of SSc involves an interplay between vascular, immunological, and fibrotic processes. Vascular injury and endothelial damage are the earliest events in the pathogenesis of SSc. Activated endothelial cells upregulate the expression of adhesion molecules and secrete chemokines, leading to inflammation and autoimmunity. Macrophages and T-cells are the predominant inflammatory cell types of the inflammatory infiltrates and produce cytokines and growth factors that drive the synthesis of extracellular matrix proteins by fibroblasts, resulting in progressive fibrosis. T-cells have also been implicated in autoantibodies production.
Abbreviations: ECM, extracellular matrix; IFN, interferon; IL, interleukin; MMP-1, matrix metalloproteinases-1; PDGF, platelet-derived growth factor; SSc, systemic sclerosis; TGFβ, transforming growth factor beta; Treg, T-regulatory cell; ?, role unknown.

pathways in the pathogenesis of SSc remain incompletely understood.

Mononuclear cell infiltrates

Histological studies indicate that a perivascular inflammatory infiltrate accompanies endothelial cell damage in very early stages of SSc.[58,59] Macrophages and T-lymphocytes are the predominant inflammatory cell types and are believed to produce cytokines and other immune mediators with proinflammatory and profibrotic function. Interestingly, in situ hybridization studies have demonstrated that collagen-synthesizing fibroblasts are located in close proximity to small blood vessels and to the perivascular inflammatory infiltrate,[60,79] consistent with the hypothesis that inflammatory cells provide important stimuli that drive collagen synthesis in fibroblasts. Indeed, multiple studies in patients with early disease have demonstrated an association between macrophages, inflammation, and skin[80] and lung fibrosis.[81] Tissue-resident macrophages become profibrotic through "alternative activation" by type 2 cytokines, such as IL-13 (M2 macrophages), and produce transforming growth factor beta (TGFβ) with profibrotic function.[82] Indeed, increased levels of soluble CD163, a marker for M2 macrophages, were found in the blood and in the affected tissues of patients with early SSc.[83] Infiltrating T-cells in SSc-affected tissues

exhibit increased expression of activation markers and express an oligoclonal T-cell receptor repertoire suggestive of an antigen-driven expansion.[84,85] While their antigen specificity is not known, T-cell-derived cytokines have been implicated in the induction of fibrosis.[86] T-cells have also been found necessary for the production of autoantibodies[87] and in driving inflammatory responses, which can involve concurrently fibroblasts as well as endothelial and epithelial cells. CD4+ and CD8+ T-cell subsets were both found in the skin[88] and lungs[89] of patients with SSc. However, we observed that CD8+ lymphocytes are more abundant than CD4+ T-cells in the skin of patients with early SSc, while in late-stage disease more CD4+ lymphocytes are found,[88] suggesting that CD8+ T-cells are involved in early disease processes. We and others established that SSc CD4+ and CD8+ are characterized by a predominant type 2 phenotype[86,88] and produce type 2 cytokines, such as IL-13. Moreover, we demonstrated that IL-13-producing CD8+ lymphocytes are abundant in the skin lesions of patients with early-stage disease and induce a profibrotic phenotype in fibroblasts.[88] Although Th17 cells have been found in the skin of patients,[90] several studies indicate that they do not play a direct role in skin fibrosis but contribute in boosting the inflammatory response in SSc.[91]

Dysfunction of T-regulatory cells (Tregs) also seems to contribute to altered immune homeostasis in SSc, with some

studies implicating a Treg-deficient suppressive function[92–94] and/or reduced number[95–97] and other studies indicating a redirected function of Tregs favoring fibrosis.[98]

An activated B-cell signature has been found in lesional skin and affected the lung tissues of patients with SSc,[99,100] with upregulation of cell-surface expression of CD19 and CD21,[101] costimulatory molecules, such as CD80 and CD86,[101] and B-cell activating factor,[102] a B-cell stimulatory molecule that induces B-cell proliferation, and immuno-globulin secretion.[103] CD19 associates with CD21 and positively regulates B-cell function.[104] CD19 overexpression induces the production of autoantibodies[105] and skin fibrosis[106] in transgenic mouse models. Significantly, a single nucleotide polymorphism in the CD19 gene promoter (−499G>T) has been associated with higher CD19 expression in B-cells and with susceptibility to SSc,[107] consistent with a role in B-cell activation in SSc. Furthermore, recent studies have shown that SSc B-cells can induce contact-dependent human dermal fibroblasts activation and upregulation of type I collagen[108] and that depletion of B-cells in a mouse model of scleroderma led to reduced fibrosis.[109] Therefore, B-cell activation and overactivity is not only involved in autoantibodies production in SSc but might also contribute to the fibrotic process.

Several recent studies implicate TLR signaling as one of the early steps during inflammatory and fibrotic processes of SSc.[110,111] TLR activation in SSc innate immune cells is believed to be triggered by microbial and endogenous ligands, such as products released from cells upon damage, necrosis, or stress.[110] A recent study showed that TLR2 is upregulated in SSc fibroblasts and responds to the acute-phase reactant serum amyloid A, resulting in increased IL-6 secretion by fibroblasts.[112,113] Interestingly, a rare polymorphism in the gene for TLR2 is associated with the SSc phenotype and induces the production of inflammatory mediators.[31] TLR4 is overexpressed in SSc skin and lung biopsies,[114–116] and its levels correlate with progressive skin disease.[114] Although the canonical ligand of TLR4 is LPS, numerous endogenous ligands have been shown to activate TLR4.[117] TLR4 can also respond to the alternative spliced fibronectin domain A (Fn-EDA), which is markedly upregulated in response to tissue damage and wound healing.[118] Interestingly, Fn-EDA was shown to be upregulated in the serum and skin biopsies of patients with SSc.[116] High levels of Fn-EDA were also found in idiopathic pulmonary fibrosis[119] and cardiac allograft fibrosis.[120] Significantly, in vitro and in vivo studies demonstrated that Fn-EDA promoted cutaneous fibrosis through TLR4 signaling, whereas its blockade led to reduced experimental fibrosis,[115,116] supporting a model

of endogenous Fn-EDA–TLR4 signaling axis in cutaneous fibrosis. Intracellular TLRs, such as TLR3, TLR7, TLR8, and TLR9, are sensors for nucleic acids, often of viral origin,[121] and have been implicated in driving inflammation and fibrosis in SSc.[110,111] Of interest, Farina et al[41] reported an association between Epstein–Barr virus infection of SSc dermal fibroblasts and endothelial cells, activation of TLR, and upregulation of selected IRFs, IFN-stimulated genes, TGFβ, and several markers of fibroblast activation, such as smooth-muscle actin and endothelin-1. These results suggest that persistent injury following a viral infection of nonimmune cells might cause chronic inflammation and fibrosis. TLR activation in SSc immune cells triggers the production of several inflammatory cytokines, particularly type I IFNs. Indeed, an increased gene expression IFN "signature" has been found in peripheral blood mononuclear cells and in the skin of patients with SSc[31,122,123] along with evidence suggesting TLR activity in SSc sera.[124] Moreover, TLR activation of dendritic cells and macrophages also stimulates IL-1, TNFα, and IL-6 production, and these or other undefined mediators might drive inflammation and fibrosis in SSc.

Immune mediators

Several cytokines and growth factors are released by immune cells and are believed to play a critical role in the inflammatory and fibrotic processes of SSc.[125,126] Abnormal levels of cytokines, such as TGFβ,[127] TNFα, and IL-6, IL-10,[128] IL-17,[129] IL-4, and IL-13,[130] have been found in the serum and affected tissues of patients with SSc. Among other functions, these cytokines are thought to promote overproduction of collagen by fibroblasts, resulting in excessive fibrosis.[131] Monocytes and macrophages mainly produce TGFβ, IFN-α, IL-13, TNFα, and IL-1. Lesional macrophages are also a main source of platelet-derived growth factor (PDGF). Numerous studies have implicated PDGF working in concert with TGFβ in the development of organ fibrosis in SSc. These findings demonstrated the existence of a TGFβ and IL-1α-dependent autocrine PDGF-A/PDGF receptor α signaling loop in scleroderma skin and lung fibroblasts, which promotes fibrogenesis.[132,133]

IL-6 is also involved in the pathogenesis of SSc. A recent study reported that serum and skin levels of IL-6 are significantly increased in patients with early dcSSc and that a monoclonal anti-IL-6-receptor antibody prevents the development of bleomycin-induced dermal fibrosis in mice.[134] A clinical trial with an anti-IL-6-receptor antibody (tocilizumab) in SSc is completed, but the results have not

been released (NCT01532869). B-cells are a main source of IL-6 in SSc,[135] but endothelial cells and fibroblasts also produce high levels of IL-6.[136]

Recent studies have focused on TGFβ and to a lesser extent on IL-13 as major profibrotic factors in the pathogenesis of SSc. Recent advances in these studies are outlined below.

TGFβ has long been implicated in the pathogenesis of SSc.[137] Based on extensive in vitro and animal data and the correlation observed between disease activity and increased expression of TGFβ-regulated genes in fibrotic skin and lungs of patients with SSc,[137] TGFβ is considered as a key mediator of fibrosis in SSc. TGFβ promotes collagen synthesis, secretion, processing, and cross-linking,[137] as well as secretion of other matrix molecules, such as fibronectin and thrombospondin.[137] Although inhibition of TGFβ represents an ideal therapeutic approach in SSc, a recent clinical trial using the anti-TGFβ mAb CAT-192 failed to show any change in skin thickening, measured by the Modified Rodnan Skin Score, between treatment groups.[138] However, striking results were recently obtained in an open-label trial that used fresolimumab, a high-affinity neutralizing antibody, that targets all three TGFβ isoforms.[139] Patients with early-stage dcSSc treated with fresolimumab showed a rapid and significant decrease in Modified Rodnan Skin Score, which correlated closely with the inhibition of TGFβ-regulated gene expression. Thus, this study shows that fresolimumab reverses markers of skin fibrosis and holds promise as a potent antifibrotic agent.

Multiple studies indicate that the immunopathological response in SSc is dominated by type 2 cytokines, such as IL-4 and IL-13.[86,126] Type 2 cytokines are important regulators of ECM remodeling, leading to enhanced collagen deposition and tissue fibrosis. Animal studies provide support for the role of a polarized immune response in the pathogenesis of fibrosis.[140,141] Transcriptome analysis in animal models of inflammation has shown that genes involved in wound healing and fibrosis are associated with Th2-polarized responses,[142,143] and IL-13 was shown to have an important role in the mouse model of bleomycin-induced fibrosis.[141] Increased levels of type 2 cytokines have been found in the serum and affected tissues of patients,[88,125,130] and we and others established that T-lymphocytes[88] and macrophages[144] are the major cellular source in SSc. We demonstrated that dysregulated production of profibrotic IL-13 by peripheral blood effector CD8+ T-cells correlates with more severe skin thickening in SSc[145] and is associated with defects in the molecular control of IL-13 production, such as the aberrant expression of the transcription

factor GATA-3.[146] Circulating CD8+IL-13+ T-cells express skin-homing receptors and induce a profibrotic phenotype in normal dermal fibroblasts, which is inhibited by an anti-IL-13 antibody.[88] High number of CD8+IL-13+ T-cells were also found in the skin lesions of patients, particularly in the early inflammatory phase of the disease[88] and potentially contributing to the development of sustained profibrotic and inflammatory autoimmune responses. Two Phase II double-blind, randomized, placebo-controlled trials were started in SSc-related interstitial lung disease (ILD) and IPF with a fully human monoclonal antibody against human IL-13 (Clinical Trial Registration Number: NCT00581997). However, the study was terminated early due to concerns with risks associated with the bronchoscopy procedure involved, and no results ensued.

Chemokines play a crucial role in the inflammatory, vascular, and fibrotic processes of SSc. In all cases, chemokines provide a chemotactic signal to cells by binding to their specific cell-surface receptors. Chemokines, such as CCL18, CCL19, and CXCL13, were found upregulated in the skin of patients with dcSSc. Expression of CCL19 correlated with markers of vascular inflammation and macrophage recruitment and may represent a marker for the perivascular inflammation and immune cell recruitment in dcSSc skin disease.[147] Serum and tissue levels of CCL2, CCL3, and IL-8 are also increased in patients with SSc and correlate with disease severity and can predict progression.[148–150] Plasmacytoid dendritic cells from patients were found to secrete high levels of CXCL4 (or platelet factor 4),[151] a chemokine with antiangiogenic function. Plasma levels of CXCL4 are increased in SSc and correlate with disease severity,[151] including lung fibrosis and pulmonary arterial hypertension.[151] A recent study shows that the expression and function of CCR1, CCR2, and CCR3 are upregulated in monocytes from patients with SSc via molecular mechanisms involving caveolin-1, Src/Lyn, and MEK/ERK signaling and represent promising targets for novel treatments for fibrotic diseases, such as SSc.[152]

Autoantibodies

Serum autoantibodies directed against a variety of intracellular antigens are present in nearly all patients and are considered a hallmark of SSc.[153] Many of these autoantibodies are specific to nuclear antigens and play no role in the pathogenesis of the disease. However, they represent important diagnostic and prognostic agents and exhibit a strong association with distinct clinical subsets, which has been confirmed in many independent patient cohorts.[153] More recently, autoantibodies

targeting cell-surface antigens and/or extracellular proteins have been detected in the serum of patients with SSc. These autoantibodies have been shown in some studies to be functional, as they were capable of triggering receptor activation and eliciting profibrotic responses. Several patients have been reported to have circulating autoantibodies against the PDGF receptor.[154] These antibodies were shown to generate reactive oxygen species and stimulate myofibroblast differentiation and type 1 collagen production. Autoantibodies against the angiotensin II receptor type 1 and endothelin receptor type A have been recently identified in patients with SSc and are believed to stimulate production of IL-18 and CCL18 by mononuclear blood cells.[155] Antiendothelial cell antibodies have been detected in the sera of some patients with SSc and have been shown to induce endothelial cell apoptosis in vitro.[156–158] Finally, antibodies against fibroblasts,[159,160] fibrillin,[161] and matrix metalloproteinases-1[162] and -3[163] were found, which are also believed to carry biological activities.

Immunosuppressive and immunomodulatory therapies in SSc

Therapeutic options in SSc are limited due to the multisystem involvement of this disease and the wide spectrum of clinical features. Current therapeutic strategies include general immunosuppression and organ-based therapies for the improvement of symptoms. More specific therapies for SSc are currently unavailable. Current or completed clinical studies of immunotherapeutic candidates are reported in Table 1.

Immunosuppressive therapy has been commonly used to control the inflammatory phase of patients with progressive or early-stage disease. However, multiple studies have demonstrated the inefficacy of this therapy in affecting the fibrotic manifestations and the potential for severe secondary effects. Immunosuppressive agents have been used on more aggressive forms of SSc skin disease, such as early dcSSc.[164] However, no conclusive trials are currently available to guide management of SSc skin involvement. This is due to the lack of sensitive and specific outcome measures and to the normally variable history of SSc.[165] An improvement in skin score was observed in two multicenter, randomized, controlled trials in which methotrexate[166] and cyclophosphamide[167] were used. In addition, two case series at scleroderma centers indicated the efficacy of mycophenolate mofetil in the treatment of skin disease.[168,169]

Immunosuppressive therapy has shown benefits in the treatment of SSc-ILD in those patients with severe lung involvement. Several clinical trials have shown the efficacy of cyclophosphamide in improving[167,170,171] and/or stabilizing[172–176] lung function parameters. Other frequently used immunosuppressants in ILD include mycophenolate mofetil and azathioprine.[177] While immunosuppressive therapy is advised for patients with early stage, progressive SSc, lung transplantation can be considered for end-stage disease.[178]

Imatinib is a powerful inhibitor of PDGF and TGFβ signaling pathways and has been evaluated in multiple clinical studies to establish effectiveness on skin[179,180] and lung[181,182] fibrosis. Outcomes from these studies provided controversial results on efficacy and demonstrated the poor tolerability of this drug. In a recent study, low-dose imatinib was used in a cohort of patients with SSc-ILD with active pulmonary disease and unresponsive to cyclophosphamide.[182] Of note, 73% of the 30 patients treated had improved or stabilized pulmonary disease after 6 months' treatment. Despite these encouraging results, the risk/benefit ratio for the use of imatinib needs to be determined in larger controlled trials.

Rituximab, an inhibitor of B-cell function, has also shown promise as a new therapeutic option in various manifestations of SSc, particularly ILD. In an open-label clinical trial, rituximab treatment improved skin scores and preserved the pulmonary function of patients with early progressive dcSSc.[183] Moreover, rituximab was well tolerated by patients even after repeated courses of treatment.[183] As no control group was used in this study, a double-blind, randomized control trial is going on to confirm these results. Efficacy on skin thickness and lung function was also observed after rituximab treatment in a case–control study using the European Scleroderma Trial and Research cohort.[184]

Intravenous immunoglobulin (IVIG) is another potential agent for skin involvement. The role of IVIG in SSc is currently unknown. However, IVIG has demonstrated to have immunomodulatory and anti-inflammatory effects in other autoimmune disorders as well as an antifibrotic effect in several animal models.[185] Multiple courses of IVIG treatment were employed in a multicenter, randomized, controlled clinical trial.[186] The outcome of this study demonstrated a beneficial effect on skin score. Similarly, improvement in skin involvement was also observed in a single-center retrospective study, in which patients with active refractory dcSSc received monthly courses of IVIG (with or without immunosuppressive therapies).[187]

High-dose immunosuppressive therapy followed by autologous hematopoietic stem-cell transplantation (HSCT) is an emerging treatment option for patients with early progressive SSc who are refractory to conventional

Table 1 Clinical studies of immunotherapeutic candidates in systemic sclerosis

Candidate therapy	Mechanism of action	Main indication	Completed clinical studies	Ongoing clinical studies	Main finding
Cyclophosphamide	Immunosuppression	Early dcSSc		Observational/prospective (NCT02339441)	Improvement of lung function and skin score
		SSc-ILD and skin involvement	Multicenter/randomized[167,170] (NCT00004563)		Results not yet reported
		Skin involvement	Open label/Phase III (NCT00501995)		Benefit on skin score and lung stabilization
		Skin involvement/ILD	Single-center/nonplacebo controlled[172]		
		SSc-ILD	Observational (NCT01762449)		No results released
		SSc-ILD	Randomized/multicenter[171]		Stabilization lung function. No side effects
		SSc-ILD		Randomized/double-blind controlled (NCT01862926)	
		SSc-ILD		Randomized/Phase II (NCT00883129)	
		SSc-ILD		Randomized/Phase III (NCT01570764)	
		SSc-ILD		Observational/prospective (NCT01858259)	
Methotrexate	Immunosuppression	Early dcSSc		Observational/prospective NCT02339441	
		SSc-ILD		Randomized trial (NCT01858259)	Trend showing the modest improvement of skin score
Mycophenolate mofenil	Immunosuppression	Early dcSSc	Multicenter/randomized[166]	Observational/prospective NCT02339441	
		SSc-ILD	Open label/safety/efficacy (NCT00333437)		Small number of participants, unreliable or uninterpretable results
		SSc-ILD		Randomized/Phase II(NCT00883129)	
		Skin involvement/ILD	Observational/prospective[168]		Marked improvement in skin involvement and stabilization of pulmonary function
		SSc-ILD		Observational/prospective (NCT01858259)	
		SSc-ILD		Observational/prospective (NCT01858259)	
Azathioprine	Immunosuppression				
Imatinib	Tyrosine kinase inhibition	Skin involvement	Randomized/Phase II[180] (NCT01545427)		Adverse events, poor tolerability. Study terminated
		Skin	Randomized/Phase II[179] (NCT00479934)		No efficacy
		SSc-ILD	Open label/Phase I/II[181] (NCT00512902)		Improvement of lung function. Adverse events
		SSc-ILD	Nonrandomized/Phase II[182]		Stabilization lung function
		SSc-associated polyarthritis		Randomized/double blind (NCT01748084)	
Rituximab	B-cell CD20+ blockade	SSc-ILD	Open label/Phase II (NCT00936546)		Improvement of lung function
		SSc skin and lung fibrosis	Multicenter/nested case–control–EUSTAR cohort[184]		Improvement of skin and lung fibrosis
		SSc-ILD		Randomized/double-blind controlled (NCT01862926)	

(Continued)

Table I (*Continued*)

Candidate therapy	Mechanism of action	Main indication	Completed clinical studies	Ongoing clinical studies	Main finding
		Skin involvement	Open label/Phase I[135,183] (ISRCTN77554566)		Improvement skin score
		SSc-PAH		Randomized/Phase II/multicenter (NCT01086540)	
Abatacept	T-cell activation blockade	Joint, skin, and lung involvement		Randomized/Phase II/III (NCT01748084)	
High-dose IVIG	Immunomodulation	Skin involvement	Randomized/placebo controlled[186] (NCT00348296)	Randomized/Phase II (NCT02161406)	Effect on skin sclerosis
CAT-192	Anti-TGFβ	Skin involvement	Multicenter/randomized/Phase I/II trial[138] (NCT00043706)	Randomized/placebo controlled (NCT01785056)	No efficacy, excess adverse events
Fresolimumab	Anti-TGFβ	Skin involvement	Open label/Phase I[139] (NCT01284322)		
Tocilizumab	Anti-IL-6R mAb	Skin involvement	Multicenter/randomized/Phase III (NCT01532869)		Small number of participants lead to unreliable or uninterpretable data
Rilonacept	IL-1 inhibitor				
QAX576	Anti-IL-13 mAb	SSc-pulmonary fibrosis	Multicenter/randomized/placebo controlled (NCT00581997)	Randomized trial (NCT01538719)	Concerns about risk of bronchoscopy procedure in the selected patient population and frequency of severe adverse events
HSCT	Immunosuppression/ immunomodulation		Open label, randomized[188] (NCT00278525)	Multicenter randomized, open label, Phase II (NCT00114530)	Benefits on skin score and lung function
			Open label, randomized/Phase III[189] (ISRCTN54371254)	Multicenter/Phase II (NCT01413100)	Increased treatment-related mortality. Long-term event-free survival benefit
			Open label/Phase I (NCT00282425)		Study terminated. No study results posted
			Open label/nonrandomized/Phase I (NCT00058578)		No results reported
			Open label/nonrandomized/Phase I (NCT00849745)		Study terminated. No results posted
Mesenchymal stem-cell transplantation	Immunosuppression/ immunomodulation		Clinical report cases[203,204]	Open label/nonrandomized (NCT02213705)	Improvement on skin score. No major adverse events were detected

Abbreviations: dcSSc, diffuse cutaneous systemic sclerosis; EUSTAR, European Scleroderma Trial and Research; HSCT, hemopoietic stem-cell transplantation; IL, interleukin; IVIG, intravenous immunoglobulin; PAH, pulmonary arterial hypertension; SSc-ILD, systemic sclerosis-related interstitial lung disease; TGFβ, transforming growth factor beta.

treatments.[177–181] Clinical trials, such as the American Scleroderma Stem Cell versus Immune Suppression Trial[188] and the Autologous Stem-Cell Transplantation International Scleroderma trial,[189] have shown efficacy in preventing disease progression. In both trials, HSCT was shown to cause an improvement in skin and lung involvement as well as vasculopathy and was able to correct immune abnormalities. Despite its potential benefits, HSCT is a dangerous therapeutic option, which is associated with a high risk of treatment-related mortality and an increase in serious adverse events. Its use, therefore, is limited to severe cases of SSc and administered only as a part of a research protocol. Two large multicenter trials are going on. One trial compares monthly intravenous cyclophosphamide to myeloablation with cyclophosphamide and total body irradiation (Scleroderma: Cyclophosphamide or Transplantation) (ClinicalTrials.gov identifier NCT00114530). The second trial (Scleroderma Treatment with Autologous Transplant) includes myeloablation followed by HSC transplantation and long-term immunosuppression (mycophenylate) for dcSSc (ClinicalTrials.gov identifier NCT01413100).

Mesenchymal stem cell (MSC)-based therapy represents an alternative potential therapeutic approach for SSc, with fewer long-term side effects.[190–192] Several in vitro studies have demonstrated that MSCs display specific immunomodulatory and immunosuppressive properties as well as regenerative potential.[193–195] Their most important immunosuppressive effects are on T-cell proliferation and dendritic cell differentiation[191,192,196–199] as well as the production of immunosuppressive mediators, such as TGFβ,[200] prostaglandin E2, and indoleamine 2,3-deoxygenase.[201] A recent report demonstrated that MSCs from patients with SSc while supporting normal hemopoiesis and retaining their immunosuppressive properties on T-cells also exhibit an increased expression of TGFβ receptor type II compared to MSCs from healthy donors, which leads to increased activation of TGFβ signaling and synthesis of COL1A1,[200,202] thereby contributing to SSc pathogenesis. While this defect limits the clinical use of autologous MSCs in SSc, it supports the use of allogeneic MSCs instead. Two recent clinical case studies[203,204] describe the use of allogeneic MSCs in patients with severe refractory SSc. In one study, a significant decrease in the number of digital ulcers and skin thickness was observed after 3 months and 6 months, respectively, from intravenous injection of a patient with MSCs.[203] The second study reported skin improvement in two out of the four cases analyzed and observed no major side effects for several months from MSCs' injections.[204] Although these results are encouraging, no conclusions about the efficacy of allogeneic MSCs in SSc can be yet drawn because of the limited number of patients tested. Moreover, additional studies are necessary to better understand the underlying MSC-immunomodulatory mechanisms as well as the role of MSCs in the pathogenesis of SSc. Furthermore, preclinical and clinical data that underlie the therapeutic potential of MSCs in patients with SSc are also necessary.

Conclusion

SSc is a complex multisystem disorder with heterogeneous clinical features that results from individual genetic background and exposure to environmental triggers. Pathogenesis of SSc is dominated by a complex interrelation between vascular, immunologic, and fibrotic processes, and it is poorly understood. Clinical outcomes in SSc have improved considerably in recent years, which may reflect improvements in the early detection and better management of significant complications, such as renal crisis or pulmonary arterial hypertension. However, SSc continues to exhibit high mortality, and it is still considered an incurable disease. Research efforts toward understanding the cellular and molecular basis of scleroderma aim to reveal novel molecular targets and diagnostic agents, leading to early and accurate diagnosis and innovative therapies against this disease. Next-generation sequencing and other cutting-edge technologies applied to affected tissues or cells will be crucial for the identification of biomarkers and pathways that are uniquely expressed in patients and are associated with disease form and/or stage. The development of preclinical models, including animal models that accurately recapitulate human disease, will be essential tools for the ultimate goal of finding a cure for this disease.

Personalized medicine offers interesting opportunities in SSc. Genomic and proteomic studies coupled with novel computational approaches have led to the identification of several biomarker signatures in patients with SSc,[15,205–208] which allowed the classification of related patients for more specific treatment. Advances in personalized medicine could be used for objective assessment of responses to clinical trials as well as for developing more effective therapies tailored to a patient's genome or to the molecular and cellular contents.

Disclosure

The author reports no conflicts of interest in this work.

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