Systemic Sclerosis - An Update

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Abstract
Systemic sclerosis (SSc) is a clinically heterogeneous, systemic disorder affecting the connective tissue of skin, internal organs, and walls of blood vessels. It is characterized by alterations of the microvasculature in the form of hypoxia, digital ulcers, and pulmonary arterial hypertension; disturbances of the immune system, including dysbalance of cytokine expression, autoantibodies (Auto-ab), and abnormalities of blood progenitor and/or effector cells; and by massive deposition of collagen in the connective tissue of the skin and various internal organs. This review discusses epidemiology and survival; clinical features including subsets and internal organ involvement; pathophysiology including genetics, microvasculature, immunobiology, fibroblasts (FBs), and connective tissue metabolism; and environmental factors. Early diagnosis and individually tailored therapy help to manage this disorder. Therapy involves immunomodulation and targeting of blood vessels and fibrosis.

Key concepts include: therapy involves immunomodulation and targeting of blood vessels and fibrosis.

Keywords: immunology, dermatopathology, systemic sclerosis, endothelial cells, T lymphocytes, autoantibodies, fibroblasts, collagen

Systemic sclerosis (SSc) is a clinically heterogeneous generalized disorder affecting the connective tissue of the skin, blood vessel wall, and internal organs such as the gastrointestinal tract, lungs, heart, and kidneys. It is characterized by alterations of the microvasculature, disturbances of the immune system, and by massive deposition of collagen in the connective tissue. The spectrum of sclerodermatous diseases comprises a wide variety of clinical entities such as morphea (patchy, linear, generalized), pseudo-scleroderma, and the overlap-syndromes with similar cutaneous and histopathologic manifestations as compared to SSc. Skin tightening, contractures, calcification, and ulcers frequently lead to functional impairment and changes in appearance.

Due to the complexity of the internal organ involvement, SSc has attracted attention from many disciplines, including rheumatology, pulmonology, cardiology, nephrology, and targeting of blood vessels and fibrosis.

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gastroenterology, and dermatology. Many aspects of diagnosis and therapy require close cooperation between several disciplines. In addition, the complex pathophysiology of SSc, involving genetic factors, environmental influences, vascular and immune system functions, as well as PBs and matrix substances, makes SSc attractive to study the pathophysiology of autoimmune connective tissue disorders. Basic functions of various cell types (endothelial cells [EC], T lymphocytes, monocytes, FB, mast cells) as well as the production and effects of cytokines, growth factors, and adhesion molecules have been studied. In addition, animal models have been developed to give closer insights into the pathophysiology of this disease.

Definition and Criteria

The American College of Rheumatology (formerly American Rheumatism Association [ARA]) has defined criteria that are 97% sensitive and 98% specific for SSc as follows:1

Major criterion:

- Proximal diffuse (truncal) sclerosis (skin tightness, thickening, non-fraying induration)

Minor criteria:

- Sclerodactyly (only fingers and/or toes)
- Digital pitting scars or loss of substance of the digital finger pads (pulp loss)
- Bibasilar pulmonary fibrosis.

The patient should fulfill the major criterion or 2 of the 3 minor criteria.

Raynaud’s phenomenon is observed in 90%-98% of SSc patients. Raynaud’s phenomenon is a vasospastic disorder causing discoloration of the fingers and toes. It includes cyclic color changes: 1) starting with pale or white color (pallor) of the skin, which becomes cold and numb due to vasoconstriction followed by 2) blue color (cyanosis) due to depleted oxygen supply and turning into 3) red (rubic) due to reactive hyperemia. In extreme cases (eg, in SSc), Raynaud’s phenomenon may progress to necrosis or gangrene of the fingertips (rat bite necrosis). Raynaud’s phenomenon may precede SSc for years, and its presence may have a predictive value for the subsequent development of SSc, in particular in association with abnormal nailfold capillaries and the occurrence of antinuclear antibodies (ANA).2,3

As a result, the American College of Rheumatology criteria from 1980 is in need of possible revision, particularly to more adequately incorporate patients with limited SSc who do not meet the criteria established in 1980. Therefore, simple clinical variables, such as nail capillary microscopy and anti-centromere antibody (ACA) positivity, should be added as novel minor criteria. With these 2 new criteria, the sensitivity of ARA preliminary criteria was improved from 33% to 97%.4,5

Classification

During the last 20 years, the majority of researchers have used the classification into limited vs diffuse cutaneous SSc (dSSc) according to LeRoy and colleagues.6 These categories are described as follows (Table 1):

1. More than 50% of SSc patients belong to the limited cutaneous SSc. They have a more insidious onset of illness, a long history of Raynaud’s phenomenon and swelling of digits, a more benign course, and a lower incidence of renal involvement and restrictive pulmonary disease with a much better prognosis.7 There are 45% to 60% of cases that are associated with ACAs. The CREST-syndrome (calcinosis, Raynaud’s phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) belongs to this subset.

2. Patients with dSSc have a short history. These patients often have acral sclerosis, arthritis, Raynaud’s phenomenon, and rapid progression of skin involvement including arms and trunk. In addition, they have a higher incidence of renal, cardiac, pulmonary disease, and tendon friction rub.7 Anti-topoisomerase antibodies (ATA) or anti-fibrillarin antibodies (against U3 RNA-associated protein) may be present; ATA exist in about 55% of cases. When associated with anti-RNA polymerase (RNAP) antibodies, patients with diffuse SSc have the shortest survival time and worst prognosis.11

Table 1. Systemic Sclerosis Subsets According to LeRoy and Colleagues6

<table>
<thead>
<tr>
<th>Limited Cutaneous SSc</th>
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<tbody>
<tr>
<td>- Raynaud’s phenomenon for years at presentation</td>
</tr>
<tr>
<td>- Skin sclerosis limited to hands, feet, face, and forearms, or absent</td>
</tr>
<tr>
<td>- Significant late incidence of PHT, trigeminal neuralgia, calcinosis, and telangectasias</td>
</tr>
<tr>
<td>- Dilated nailfold capillary loops, usually without capillary dropouts detected by widefield nailfold capillaroscopy</td>
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<table>
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<tr>
<th>Diffuse SSc</th>
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<tbody>
<tr>
<td>- Onset of Raynaud’s phenomenon within 1 year of onset of skin changes</td>
</tr>
<tr>
<td>- Truncal and acral skin involvement</td>
</tr>
<tr>
<td>- Presence of tendon friction rubs</td>
</tr>
<tr>
<td>- Early and significant incidence of interstitial lung disease, oliguric renal failure, diffuse gastrointestinal disease, and myocardial involvement</td>
</tr>
<tr>
<td>- Presence of anti-DNA topoisomerase I (anti-Scl-70) antibodies</td>
</tr>
<tr>
<td>- Absence of anti-centromere antibodies</td>
</tr>
<tr>
<td>- Nailfold capillary dilatation and destruction detected by widefield nailfold capillaroscopy</td>
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</table>
Out of 646 patients studied before angiotensin-converting enzyme (ACE) inhibitor treatment, none of the 24 patients with kidney disease at onset survived for 6 years, and the 1-year survival was only 25%.12 Cancer mortality particularly of the lung is also increased.12

Genetics

Systemic sclerosis is a complex disorder with strong aspects of genetic predisposition. The most prominent genetic factor is gender (female: male = 3:1 to 6:1). Another is the human major histocompatibility complex (MHC). In SSc, the prevalence in first-degree relatives is 1.6% and in monozygotic twins is 4.7% as compared to 0.026% in the general population.19 The increased spontaneous and clastogen-induced chromosomal damage rates indicate that SSc lymphocytes may be susceptible to DNA damage caused by free radicals.20

In addition to associations between Auto-ab (ACA, ATA) and various HLA-alleles,22 the more consistent susceptibility genes reflect the associations of single nucleotide polymorphisms (SNP) in genes involved in autoimmune regulation (including innate immunity, T cell differentiation, and immune-cell signaling), vascular function, and extracellular matrix (ECM) with SSc. They are partly shared with other autoimmune diseases. Systemic sclerosis has been linked to interferon regulatory factor 5 (IRF5), signal transducer and activator of transcription 4 (Stat4), protein tyrosine phosphatase, non-receptor type 22 (PTPN22) and B cell scaffold protein with ankyrin repeats 1 (BANK1), connective tissue growth factor (CTGF), T-box transcription factor (TBX21), Corf13-BLK, interleukin 10 receptor (IL-10R), interleukin 23 receptor (IL-23R), and tumor necrosis factor superfamily (TNFSF4).19,21 Polymorphisms in the fibrillin gene have been described in the Choctaw Indians in association with the ATA-positive diffuse SSc.22 In the future, we need a better understanding of how these polymorphisms lead to the development of SSc and how the gene-gene as well as gene-environment interactions function.

Microchimerism

In female SSc patients, the presence of fetal CD3-positive T cells in the maternal circulation23 and of fetal cells in affected tissues24 has been acknowledged by identifying positive T cells in the maternal circulation and of fetal cells. Microchimerism is involved in the development of SSc and how the gene-gene as well as gene-environment interactions function.

Clinical Features

In general SSc affects the connective tissue, predominately of the skin and vessel wall and, to a lesser extent, the gastrointestinal tract, heart, lungs, and kidneys. Cutaneous symptoms, often associated or preceded by Raynaud’s phenomenon and finger arthralgias, are usually early signs in the course of SSc and therefore are helpful to establish diagnosis and initiate therapy. The following 3 phases of dermal involvement can be distinguished:25 1) edematous phase (stiff, puffy fingers), 2) indurative phase (hard, tight, hidebound), 3) atrophic phase (softened skin, burned out).

The usefulness of the skin thickness score has been confirmed as a predictor and correlate of the outcome in SSc.25 An abnormal nailfold capillary pattern on capillaroscopy with dilatation of all 3 parts of the capillary loop (arterial, apical, and venular) and loss of capillaries (drop out) either diffusely, or in localized areas, is an early and important diagnostic sign for SSc.

The prognosis of SSc largely depends on involvement of internal organs, particularly the lungs, heart, and kidneys. Involvement of the gastrointestinal tract, although most frequent, is less life-threatening. Severe organ involvement often occurs early in the course of diffuse SSc.27 As their survival is markedly reduced, these patients should be monitored very closely during the first 3 years and potential disease-modifying therapies must be initiated early.

Pathophysiology

The course and even the initial events in the pathogenesis of SSc are still poorly understood. The microvasculature (EC, platelets, capillaries) is 1 of the first affected systems, sometimes preceding the outbreak of the disease even by years (Raynaud’s phenomenon). There is evidence that the disease may be immunologically triggered, again as an early event.28 T lymphocytes in collaboration with monocytes, EC, platelets, and mast cells act as mediators and targets in the pathophysiological network. These cells express and release adhesion molecules, ILs, and growth factors that act on FBs. In addition, hypoxia causes oxidative stress in various organs. The excessive tissue fibrosis is due to an expansion of fibrogenic clones of tissue FBs and their transformation into myofibroblasts that behave relatively autonomously and over-express genes encoding ECM components.29 Tyrosine kinases have been shown to regulate the release and activity of various cytokines and growth factors such as transforming growth factor β (TGFβ) and platelet-derived growth factor (PDGF). This leads to excessive deposition of collagen and other connective tissue matrix proteins in the skin and internal organs as well as in the walls of blood vessels.

As known from many other autoimmune diseases, the pathogenesis of SSc is partly based on genetic background and modulated by environmental factors.30 The following sections will discuss the 3 main pathogenetic pathways. These include microvasculature abnormalities, abnormal immune response, and dysregulation of FB activity (Figure 1).

Microvasculature

Raynaud’s phenomenon, increased vascular wall thickness, vascular occlusion, devascularization, and thickening of the basement membrane are features that were described many years ago. Changes in the nailfold capillaries are 1 of the first signs in SSc.31,32 Furthermore, vascular injury is the basis for the major clinical manifestations of SSc, including pulmonary arterial hypertension, myocardial dysfunction, and renal involvement (scleroderma renal crisis). In internal organs, particularly the kidney, arterioles are characterized by intimal proliferation, thinning of the media, and fibrosis of
the adventitia, and exhibit accumulation of proteoglycans and collagens\textsuperscript{33} that are probably produced by myofibroblasts. In addition, the vascular pathology is associated with altered vascular function, with increased vasospasm, reduced vasodilatory capacity, hypoxia, oxidative stress, and increased adhesiveness of the blood vessels to platelets and lymphocytes.

**Endothelial Cells**

The role of EC is still poorly understood (Figure 2). On the 1 hand, EC are targets of immune activity. On the other hand, they may act as immune co-stimulators.\textsuperscript{34} One hypothesis suggests that SSc starts following repeated insults to the vascular endothelium, in particular after cold exposure. The vascular abnormality may be caused by repeated episodes of vasoconstriction leading to hypoxia, ischemia, and intravascular occlusion. Moreover, an imbalance in endothelial signals (increased vasoconstrictory endothelin release), impaired vasodilatory mechanisms (nitric oxide [NO] corresponding to endothelial dependent relaxation factor [EDRF]), enhanced platelet aggregation, and deficient neutrophile levels lead to the well recognized vasospastic propensity. The deficient endothelial dependent relaxation in SSc is suggested by impaired maximal responses to endothelial dependent vasodilators such as bradykinin and substance P in conjunction with defective endothelial production of the vasodilator NO.\textsuperscript{35} Presumably, the endothelial NO synthase gene expression is inhibited, in particular by TGFβ.\textsuperscript{36} Thus, the impaired NO production may contribute to platelet activation and to oxidative injury of EC, as well as promote inflammation and enhance the arteriolar internal proliferation in SSc.\textsuperscript{37}

Other damaging influences, such as toxic factors, proteases (granzyme 1), lipoxygenases, and IgG antienothelial Auto-ab may contribute to this process.\textsuperscript{38} In addition, SNPs of hypoxia-induced factor (HIF)1A gene are associated with SSc. Although persisting hypoxia is a major stimulus for angiogenesis, it does not occur in SSc, probably due to an insufficient response to the elevated levels of vascular endothelial growth factor (VEGF).\textsuperscript{39} The increased release of endotheš, thromboxane, factor VIII antigen, and thrombomodulin are signs of an injury to EC, partly also mediated by antiendotheš cell antibodies (AECA).\textsuperscript{40,41}

**Endothelial Cell Apoptosis**

Endothelial cell apoptosis may also be a primary event in SSc.\textsuperscript{42} It may be related to viral infection, including cytomegalovirus (CMV), in view of the increased levels of anti-CMV antibodies measured in SSc and the remarkable similarities between CMV vasculopathies and SSc vascular disease.\textsuperscript{33} On the other hand, endothelial apoptosis may be related to immune reactions, to antiendothešal antibodies, to environmental factors, and/or reperfusion injury.\textsuperscript{44}

**Endothelial Progenitor Cells**

In contrast to angiogenesis, vasculogenesis is defined by formation of new vessels. However, functionally impaired circulating endothelial progenitor cells, party apoptotic, occur in increased numbers in SSc.\textsuperscript{45} Autologous stem cells and progenitor cells are theoretically an ideal tool to counteract and repair dysfunctional cells and tissue, given the fact that these cells home to the affected sites of SSc.

**Endothelial-Derived Proteins With Pathophysiological Relevance**

**Endothelin**

Increased plasma endothelin levels have been associated with Raynaud’s phenomenon and SSc, particularly diffuse SSc.\textsuperscript{46} Endothelin exerts a prolonged vasoconstriction and is fibrogenic as well, enhancing FB proliferation and collagen synthesis.\textsuperscript{66} Thus, it may be a major link between vascular pathology and the abundant deposition of connective tissue matrix materials in SSc. Increased endothelin expression in microvascular EC of the upper dermis in association with an increased number of endothelin-binding sites is also reported in SSc.\textsuperscript{44}

**Angiotensin-Converting Enzyme**

Angiotensin-converting enzyme is located on the luminal surface of the endothelium. Decreased plasma ACE activity has been reported in SSc patients.\textsuperscript{47} Angiotensin-converting
enzyme levels are inversely related to levels of von Willebrand factor (vWF) and have been proposed as markers of EC injury. Further indicators of vascular injury include increased serotonin-induced platelet aggregation. In addition, platelets release thromboglobulin, platelet factor 4, cytokines, and growth factors (PDGF and TGF) that can themselves activate EC. This indicates an imbalance between endothelial and platelet function.

Integrins

In SSc, EC expressing increased numbers of ligands of 1-integrins as well as various cell adhesion molecules such as MadCAM1, CD34, ELAM-1, and intercellular adhesion molecule (ICAM)-1 facilitate the interaction with lymphocytes expressing 1- and 2-integrins. In this way the trans-capillary migration of inflammatory cells is enhanced, leading to prominent T cell infiltrates around blood vessels in early skin lesions. The ACEA also induce leukocyte adhesion to EC. In regulating levels of endothelin-1, P-selectin, E-selectin (E-sel), vascular cell adhesion molecule (VCAM)-1, and ICAM-1 are useful markers of vascular and fibrotic change in SSc. They correlate well with their in situ activity.

Immune System

T lymphocytes

The location of inflammatory infiltrates, mainly CD4-T cells, around blood vessels and at sites of active connective tissue formation suggests a pathogenetic role for them. Obviously, regulatory (suppressor) T cells are functionally impaired. Levels of lymphocyte- and monocyte-derived cytokines, such as IL-1, IL-2, IL-4, IL-6, and receptors (rec), such as soluble CD4 and IL-2R, are elevated in the circulation (Table 2). Interleukin-2 can induce up to 40-fold elevations in the secretion of active TGFβ by monocytes. Activated T cells may produce IL-2, which upregulates TGFβ in monocytes that in turn activates FBs to secrete and organize the elements of the ECM. Lymphocyte and monocyte ligands, L-selectin, sialated glycoproteins, lymphocyte function-associated antigen 1 (LFA-1) (CD11a, CD18), and Mac1 (CD11b, CD18) bind to the EC rec and modulate the migration of these cells. In addition, lymphocytes respond to chemotactic stimuli, activate FBs via ICAM-1, and bind to non-cellular integrins expressed on collagen and fibronectin via surface VLA-1 (CD49a, CD29) and VLA-4 (CD49d, CD29). These processes explain the reciprocal activation of immune cells and FBs by direct cell contact as well as by the indirect effects of soluble cytokines.

B Lymphocytes

B cells are the sources of the Auto-ab representing hallmarks of SSc. Beside this, B cells secrete B cell activating factor (BAFF). Increased serum levels of BAFF positively correlate with the severity of skin fibrosis. CD19, a critical cell-surface signal transduction molecule, is relatively specifically...
over-expressed in naïve and memory B cells in SSc, indicating an abnormal regulation of the response regulator function and expression that may lead to loss of tolerance and to autoimmunity. In addition, this may be interpreted as linkage between autoimmunity and fibrosis.

**Autoantibodies**

In the majority of SSc sera, Auto-ab to intracellular antigens are detectable (Table 3). However, an individual patient’s serum contains only a limited number of Auto-ab, often in a disease-specific manner. The particular autoantibody present is often indicative of clinical expression, disease course, and overall severity. In SSc with highly variable clinical features, such information is a valuable aid to the diagnosis and prognosis of an individual patient.

Antinuclear antibodies are detected in approximately 85% of SSc patients. With repeated investigations during the course of the disease, they have been found in approximately 98%. The following 3 Auto-ab are of diagnostic importance:

1. Anti-topoisomerase antibodies correlate with dSSc. The association of ATA with DR3 and DRW52a means a significantly higher risk of pulmonary interstitial fibrosis. While ATA I positive idiopathic SSc cases were associated with DR2 and DR5, an association between ATA I positive silica-associated SSc (SI-SSc) and the HLA-A-DR3 alleles has been shown.

2. The ACA group has the best prognosis of the 3 with the longest cumulative survival times and the lowest frequency of dSSc, pulmonary involvement, and renal disease.

3. Patients with anti-RNA polymerase III antibodies exhibit the greatest risk of dSSc, the highest mean maximum skin thickness score, the shortest cumulative survival times, and the greatest likelihood of renal involvement compared with patients in either of the other 2 groups.

Autoantibodies are important for the early diagnosis of the specific type of SSc and then the initiation of the appropriate therapy. Consequently, it is now possible to classify more than 85% of SSc patients by these main 3 Auto-ab. Antigen-driven and molecular mimicry hypotheses have been proposed for ANA induction in SSc. Some Auto-ab are homologous to certain mammalian p30gag retroviral proteins. 

The pathogenetic role of these Auto-ab is mostly unknown. Although some of these SSc-specific Auto-ab are capable of inhibiting the cellular function of the autoantigens they recognize in vitro, they are unlikely to have access to the intracellular locations of these antigens in vivo. On the other hand, some of the Auto-ab may recognize extracellular antigens or those exposed on the cell surface. These Auto-ab could be involved in the disease pathogenesis.

Thus, the EC-antibodies (AECA) are able to activate EC; to increase the synthesis and release of coagulation factors such as factor VIII, vascular adhesion molecules, endothelin-1, and thrombomodulin; as well as induce EC apoptosis; the last of these possibly triggering the fibrosing process in SSc. Altogether AECA may affect microvessels more distinctly than macrovessels. Their prevalence in SSc is between 28% and 86%. For example, AECA have been associated with vascular involvement, digital ischemic ulcers, gangrene, alveolocapillary impairment, and pulmonary arterial hypertension, and they correlate with apoptotic progenitors. In addition, they can mediate EC cytotoxicity by direct complement activation (antibody dependent cell-mediated cytotoxicity).

### Table 2. Cytokines, Chemokines, Growth Factors Involved in Systemic Sclerosis

<table>
<thead>
<tr>
<th>Mediators</th>
<th>Findings in SSc</th>
</tr>
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<tbody>
<tr>
<td>Proinflammatory</td>
<td></td>
</tr>
<tr>
<td>IL-2, IL-2R</td>
<td>Elevated in serum</td>
</tr>
<tr>
<td>IL-6</td>
<td>Elevated in serum</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Elevated in lesions, correlation with disease activity</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Increased in serum and tissue, up-regulation of TNF-α converting enzyme (TACE) in monocytes, inhibition of collagen synthesis in FB</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Increased in tissue, monocyte activation</td>
</tr>
<tr>
<td>Pro-fibrotic</td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>Increased in tissue, reduced in serum, increased TRα1 receptor expression, increased activation of latent TGF-β by integrins</td>
</tr>
<tr>
<td>IL-4, IL-13</td>
<td>Increased collagen synthesis, myofibroblast differentiation</td>
</tr>
<tr>
<td>PDGF</td>
<td>Receptor stimulation by Auto-ab via tyrosine phosphorylation and stimulation of the ERK pathway</td>
</tr>
<tr>
<td>BAFF</td>
<td>Increased in serum</td>
</tr>
</tbody>
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### Table 3. Autoantibodies in Systemic Sclerosis

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Target, Function</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATA</td>
<td>Topoisomerase, FB surface 100kD molecule, mediates relaxation of supercoiled DNA</td>
<td>Unknown</td>
</tr>
<tr>
<td>ACA</td>
<td>Centromeres A,B,C,D kinetochore, interacts with cell division</td>
<td>Unknown</td>
</tr>
<tr>
<td>A-RNA-P-A</td>
<td>RNA-polymerase I, II, III protein biosynthesis by ribosomes</td>
<td>Unknown</td>
</tr>
<tr>
<td>A-U3RNAP(fibrillarin)-A</td>
<td>Fibrillarin, 34kD basic protein, processing of peri-ribosomal RNA</td>
<td>Unknown</td>
</tr>
<tr>
<td>AECA</td>
<td>NAG-2, topoisomerase I centromeric protein B</td>
<td>Apoptosis, ECM expression, EC cytotoxicity by direct complement activation</td>
</tr>
<tr>
<td>A-FIB-A</td>
<td>Fibrillarin-1, NAG-2</td>
<td>ECM expression via TGF-β pathway, FB activation/ECM expression</td>
</tr>
<tr>
<td>A-MMP-A</td>
<td>MMP-1 and -3</td>
<td>Inhibition of ECM degradation</td>
</tr>
<tr>
<td>A-PDGF-A</td>
<td>PDGF-R</td>
<td>Collagen expression, production of ROS, tyrosine phosphorylation, and stimulation of the ERK pathway</td>
</tr>
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</table>
2) Anti-matrix metalloproteinase (MMP) antibodies are directed against MMP1 and 3 that block ECM degradation and may promote fibrosis due to imbalance between synthesis and degradation of collagen.64

3) Anti-FB antibodies are directed against fibrillin-1 and activate FBs via the TGFβ pathway, resulting in increased collagen synthesis.

4) Autoantibodies against the PDGF rec activate the platelet derived growth factor rec (PDGFR) and stimulate collagen synthesis as well as reactive oxygen species (ROS).65 The PDGFR-Auto-ab are profibrotic, appearing to be relevant not only for matrix formation but also for vascular pathology. They stimulate tyrosine phosphorylation and up-regulate the intracellular Ha-Ras-extracellular-signal-regulated kinases 1 and 2 (ERK1/2) pathway.66

**Fibroblasts**

**Cytokines and Growth Factors**

Activated FBs release cytokines and growth factors, such as IL-1, IL-6, prostaglandin E, TGFβ, CTGF, and PDGF. Both TGFβ and CTGF may exercise self-activation via an autocrine loop.67,68 In this way ICAM-1 is expressed on FBs, which augments adhesion and retention of immune cells within the tissue.69,70

Several cytokines and growth factors, such as IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17, TGFβ, PDGF, tumor necrosis factor-alpha (TNF-α), interferon (IFN)-γ, and particularly the high-affinity IL-2 rec, can also be found elevated in the serum of SSc patients.71,72 To some extent they are correlated with the degree of organ involvement and disease activity. Transforming growth factor beta clearly activates FBs to produce increased amounts of ECM components such as collagen I, III, V, and VII, and fibronectin.67 The presence of TGFβ1 prior to the onset of fibrosis indicates an early involvement of this factor in the pathogenesis of SSc.73 The TGFβ1 increases the promoter activity and type I collagen mRNA and protein synthesis in FBs via SMAD transactivation factor pathway. Connective tissue growth factor exhibits a permanent over-expression of mRNA in fibrotic lesions of SSc skin and in isolated FBs.75

**Matrix Protein Metabolism**

In SSc, abundant deposits of collagen type I are found in the perivascular region of the dermis and at the border between deep dermis and subcutis, as demonstrated by immunohistochemistry and in situ hybridization. In addition, collagen types III, V, VI, VII, as well as fibronectin and tenascin are also overexpressed in the skin.76 Recently, an increased formation of bone-type cross-links due to activation of lysyl hydroxylase 2 has been reported in SSc skin as well as bone-type degradation products in the serum (collagen telopeptides may be used as a surrogate marker of disease activity). Hypoxia may contribute in the process of transdifferentiation of FBs to an altered bone-like phenotype.77,78

Increased collagen synthesis and decreased collagenase expression may result in excessive accumulation of collagen, indicating that the balance between these synthesizing and degrading processes (MMPs) is crucial and may be modulated by TIMP-1 expression.79 On the other hand, increase in the proteolytic activity (cathepsin K) could indicate major remodeling as a counter-regulatory mechanism.80

**Fibrogenic Phenotype**

One of the most interesting phenomena in the pathophysiology of SSc is the persistence of the fibrogenic phenotype. The protease nexin-1 (PN-1) is over-expressed in SSc. This may play a role in increasing collagen gene transcription.81 This altered gene expression is due to turning on autocrine signals in the FBs, that, once activated, stimulate a continuous feedback loop.

In addition, altered FBs’ susceptibility to apoptosis may play a role in the pathogenesis of FB abnormalities. As a result, SSc FBs are more resistant to Fas-mediated apoptosis than normal FBs.82 Consistently, there are no data showing apoptosis of FBs in SSc lesions or in culture. This is in agreement with results reporting unchanged numbers of FBs in SSc lesions compared to healthy skin.83 Therefore, the fibrotic events in SSc seem due to increased synthesis of matrix proteins rather than to increased cell numbers. In addition, endothelin-1, which is over-expressed in SSc, protects FBs from c-myc-dependent apoptosis.84,85 Finally, hypoxia can also select for apoptosis-resistant cells by inducing apoptosis in c-myc-overexpressing cells.85 Trans-acting nuclear factors binding to cis-acting elements in enhancer (intronic) and promoter regions of the genes modulate the basal and inducible transcriptional activity of collagen genes.86

Fibroblasts subjected to mechanical strain proliferate, elongate, and become bipolar and oriented along the plane of the force.87 Prominent action stress fibers develop within the cells as myofibroblasts.

There are several candidates inhibiting the fibrotic process, such as antibodies to TGFβ88 or lysyl hydroxylase inhibitors that interact with cross-link formation between the collagen chains. An exciting approach might be the direct inhibition of transcription factors by antisense oligonucleotides that interact with specific DNA elements controlling the activity of these genes in FBs. The complexity of FBs in the pathophysiology of SSc is shown in Figure 3.

**Environmental Factors**

Environmental factors, including chemical compounds such as solvents or drugs (bleomycin is best known), can induce SSc-like diseases upon exposure.89,57 that can be distinguished from SSc by the following features:89

- Cessation of toxic damage
- Absence of Auto-ab.

Environmental factors, including chemical compounds such as solvents or drugs (bleomycin is best known), can induce SSc-like diseases upon exposure.89,57 that can be distinguished from SSc by the following features:89

- Type of skin manifestation, in particular acrosclerosis, circumscribed and generalized morphea, fibrotic nodules, joint contractures.
- Visceral involvement due to toxic damage of the liver, kidney, nervous system, and muscles, as well as angiosarcoma of the liver.
- Laboratory findings of partial thrombocytopenia, and absence of Auto-ab.
- Cessation or reversibility of the disease process after early discontinuation of the exposure.

In contrast to these chemical substances, silica is able to induce a form of SSc-like disease indistinguishable from idiopathic SSc90,91 as shown by clinical and laboratory data and

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similar pathophysiology. Therefore, silica should be accepted as an inducer of SSc. This is in agreement with Rodnan and colleagues and Rustin and colleagues. Our experimental data concerning the exposure of various cell-(co)cultures such as macrophages/monocytes, EC, and FBs to silica support the hypothesis that silica-mediated cell activation can play a role in the pathogenesis of SSc. However, the long exposure times needed for the onset of silica-induced SSc and the fact that not all exposed persons develop silicosis and SSc suggest silica alone does not cause SSc. The development of SSc in a silica-exposed individual depends not only on the length and concentration of exposure but also on the individual genetic background of the host. Several cases have been published in which SSc, after long-term exposure with silica, was acknowledged as an occupational disease, mostly by an individual decision. In addition, exacerbation of SSc, or even the new onset of diffuse SSc cases, has been observed after X-ray treatment.

**Treatment**

Therapy must be planned individually due to the inadequate knowledge of the point at which therapeutic action is appropriate and the difficulty of obtaining objective measurements of the treatment results. Beside basic recommendations, physiotherapeutic activities and competent psychological guidance are important. The therapy is directed at 3 pathogenic compartments:

- Vascular system
- Immune system (inflammation, immunomodulation, autoimmunity)
- Fibrosis

Clinical trials in SSc must (semi)quantitatively define disease activity, specific stage of disease, joint motility, and level of internal organ involvement as published by Medsger and colleagues. They developed a severity grading scale from 0 (no documented involvement) to 4 (endstage disease) for 9 potentially affected organ systems and tested it in 579 SSc patients. In 1995, the European Scleroderma Study Group initiated a multicenter prospective 1-year study for this reason. Systemic sclerosis is non-curable but treatable, although the response to therapy is generally slow.

**Basic Treatment**

Mostly symptomatic treatment consists of proton-pump inhibitors for reflux, prokinetic drugs, calcium channel blockers (nifedipine) for vasodilatation, and an ACE inhibitor (captopril, enalapril) or angiotensin (AT)-II rec antagonist (losartan) for the prevention of renal crisis. Aspirin and statins may reduce cardiovascular risk factors. When malabsorption is caused by bacterial overgrowth, rotating antibiotics are useful.

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**Figure 3.** Activation of Fibroblasts in SSc. Various ILs, growth factors, chemokines, thrombin, endothelin-1, ROS, activating antibodies (eg, PDGFR-ab) as well as tension trigger signal cascades in FBs: either SMAD signaling pathway or phosphokinase/ERK 1/2 pathway which can modulate transcription factors. As a result, synthesis of ECM protein, cytoskeleton, cytokines and cytokine receptors, as well as growth factors, and the growth factors in an autokrine loop (TGF, CTGF) are stimulated, thus maintaining the pathological FB activation. UV activates collagenases (matrix metalloproteinases). The increased lysyl hydroxylase 2 mediates the formation of bone-like collagen.
**Vasoactive Medication**

Continuous intravenous infusion of prostacyclin (PGI2)-like substances (iloprost, epoprostenol) decreases the frequency and severity of Raynaud’s attacks and induces healing of digital ulcers. Endothelin-1 antagonists such as bosentan or sitaxsentan are helpful in prevention as well as healing of digital ulcers and in controlling PHT (grade 2 to 4). They improve exercise capacity, functional class and some hemodynamic measures in PHT. In addition, sildenafil, a phosphodiesterase inhibitor, is also effective in the treatment of PHT and digital ulcers.

**Immunosuppressive Agents**

Cyclophosphamide and methotrexate exert significant effects on skin thickness and lung function. Cyclophosphamide is particularly recommended in the treatment of interstitial lung fibrosis; methotrexate is the treatment of choice in patients with SSC/myositis or SSC/arthritis. Recently mycophenolate mofetil has shown positive effects. There is no clear evidence for the efficacy of glucocorticoids. There is no clear evidence for the efficacy of glucocorticoids. Recently mycophenolate mofetil has shown promising results. Rituximab, a monoclonal antibody against the CD20 transmembrane protein on B cells, could not improve skin thickness or reduce autoantibody titres despite B cell depletion.

**Antifibrotic Agents**

Imatinib mesylate, an inhibitor of the tyrosine kinase, also targets the PDGFR kinase, thus inhibiting the ERK1/2 pathway of FB activation. Various clinical studies have shown it reduces fibrosis of different organs. Oral administration of 500 mg bovine collagen type I reduced skin thickness. The rationale is the induction of oral tolerance against the suspected autoantigen collagen as measured by T cell reactivity. However, the target of this therapy may only be a secondary mechanism. Attempts to treat with neutralizing anti-TGFβ antibodies (CAT-192) and with peptide inhibitors of TGFβ therapy may only be a secondary mechanism. Antifibrotic agents could not improve skin thickness or reduce autoantibody titres despite B cell depletion.

**Ultraviolet A**

Ultraviolet A (UVA) irradiation (320-400 nm) penetrates into deeper layers of the skin. Repetitive UVA irradiation alone or in conjunction with photosensitizing agents (PUVA) increases the expression, synthesis, and activation of matrix metalloproteinases and additionally of cathepsins; it decreases the synthesis of collagen in dermal FBs; and destroys collagen cross links. On the other hand, UVA exerts cutaneous and systemic immunosuppressive effects, such as apoptosis of T cells and induction of IL-10. These effects may contribute to the improvement of skin score in SSC.

**Cellular Therapy**

**Cellular Therapy Targets Immune System and Fibrosis**

The rationale of hematopoietic stem cell transplantation (HSCT) is to reset the dysregulated immune system with immunoaablative therapy (cytostatic agents, anti-lymphocyte globulin, total body irradiation) followed by reinfection of previously isolated hematopoietic stem cells. The main mechanism is achieved by eradication of autoaggressive “effector T and B cells” and the induction of regulatory T cells. The idea is to restore tolerance despite the use of autologous cells. With allogenic HSCT, the postulated graft vs autoimmunity effect may contribute to better results but also to more significant side effects. The patients treated with HSCT represent a negative selection of severe cases of short history and involvement of internal organs. Skin thickening and performance status were improved markedly and organ dysfunction stabilized.

The early evidence that fibrosis can, in principle, be reversed provides a hopeful outlook for future therapeutic advances.

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