Scleroderma

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Abstract

The prototypic autoimmune diseases involving skin (lupus, dermatomyositis) typically result in epithelial injury and autoantibodies to characteristic cellular antigens. Disease-specific autoantibodies are also found in scleroderma, but scleroderma is different from other cutaneous autoimmune diseases because epithelial injury does not occur. Multiple factors and combinations of factors (immune system, vascular and extracellular matrix abnormalities) are the most likely triggers in an individual with a genetic predisposition to scleroderma. These lead to increased synthesis of normal collagen in skin, lungs and gut in the systemic form of scleroderma, systemic sclerosis. The hypotheses for the pathophysiology of scleroderma are diverse and include abnormal immunologic processes such as cytokine and chemokine dysregulation, abnormal T cell signaling, B cell dysfunction, injury due to autoantibodies to endothelial cells, persistent wound healing condition due to dysregulation of matrix homeostasis, abnormalities in the fibrinolytic system, polymorphisms in critical molecules of the immune system and matrix homeostasis, and microchimerism due to fetal/maternal placental exchange of HLA-compatible cells. Systemic sclerosis/scleroderma is chronic and progressive. To date, no definitive therapy is effective for any of the scleroderma variants, although agents for the vascular dysfunction have some utility. Hematopoietic bone marrow or stem cell transplantation before significant tissue fibrosis has occurred may be the most effective treatment.

Introduction to Scleroderma

Scleroderma as an Autoimmune Disease

Scleroderma (systemic sclerosis) is a debilitating chronic autoimmune disease of unknown etiology. It typically has an insidious onset and can lead to extensive progressive fibrosis of skin and viscera due to excessive production of normal collagen. The classic autoimmune features consist of autoantibodies to nuclear, nucleolar and cytoplasmic antigens, and to endothelial cells. The
autoantibodies are important for the classification of subsets of scleroderma and in some instances, they may be pathogenic.

Scleroderma is unlike other autoimmune diseases that involve skin because there appears to be no direct injury to epithelia as in lupus erythematosus, dermatomyositis or alopecia areata. Rather, abnormal upregulation of collagen synthesis and progressive vascular occlusion are the hallmarks of disease, accompanied by alterations in cytokines, chemokines and growth factors, and production of disease-specific autoantibodies. Except for the endothelial cell autoantibodies, the major scleroderma autoantibodies do not appear to cause direct injury to tissue, and no direct injury by T cells occurs. Rather, the tissue cytokine and chemokine environments, vascular injury and imbalance in the fibrinolytic system enhance collagen deposition and vascular occlusion. The result is skin, lung, gut fibrosis and renal vessel fibrosis in systemic forms of scleroderma. The clinical features can include sequelae of vascular compromise and ischemia such as Raynaud’s phenomenon and digital ulcers.

Severity and morbidity of disease depend on the degree of internal involvement and extent of cutaneous fibrosis. The typical clinical appearance of diffuse scleroderma is shown in figure 1a and the histology of skin fibrosis and prominent proliferative and obliteratorive vascular change in figures 1b and c. There are two other forms of scleroderma which cannot be distinguished from each other or from diffuse scleroderma histologically, but are quite distinct clinically. They are limited scleroderma (CREST) and linear scleroderma/morphea. For this review, we will concentrate on the immunopathophysiology and newest concepts of disease related to human systemic sclerosis/scleroderma, which has been best characterized and has the worst prognosis.

Fig. 1.  

**a** Typical clinical appearance of diffuse scleroderma (courtesy of Dr. Mamood Pazirandeh). **b, c** Histology of skin fibrosis and prominent proliferative and obliterative vascular change.
New data on the incidence in the US in black and Caucasian adults estimate scleroderma at approximately 276 cases per million adults (95% confidence interval 245–310) [1]. Women and blacks are more likely to have scleroderma, and blacks are twice as likely to have diffuse systemic disease as whites. Median survival in diffuse disease is 11 years. Male sex and older age at diagnosis are negative factors for survival [1].

What Causes Scleroderma?

There are many hypotheses for the triggering events leading to scleroderma, but none completely address the spectrum of clinical and molecular findings. Some of these hypotheses are listed in Table 1 and include abnormal immunologic processes such as cytokine and chemokine dysregulation, abnormal T cell signaling, B cell dysfunction, injury due to autoantibodies to endothelial cells, persistent wound healing condition due to dysregulation of matrix homeostasis, abnormalities in the fibrinolytic system, and microchimerism due to fetal/maternal placental exchange of HLA-compatible cells. Genetic studies on scleroderma in the Oklahoma Choctaw Indians and in well-defined ethnic populations of individuals with scleroderma (American blacks, Japanese), as well as linked HLA haplotypes and autoantibody phenotypes suggest a genetic predisposition to scleroderma. There are also gene polymorphisms involving molecules of the immune and cellular regulatory system that are associated with scleroderma in

\[\text{Table 1. Factors that contribute to pathogenesis in scleroderma}\]

| Immune dysregulation | – growth factor, cytokine and chemokine environments  
| – T cell signaling  
| – B cell abnormalities  
| – autoantibodies |
| Extracellular matrix dysregulation | – differentiation to myofibroblasts  
| – fibroblast signaling pathways  
| – altered fibroblast apoptosis  
| – altered metalloproteinases |
| Vascular injury | – anti-endothelial cell antibodies  
| – cytomegalovirus infection |
| Genetic factors | – HLA associations  
| – polymorphisms |
| Microchimerism of fetal/maternal cells | |
| Environmental triggers | – silica  
| – solvents |
subgroups of individuals. These all point to the critical and central role that genetics may play in scleroderma. Lastly, there are environmental triggers such as silica, toxic oils and solvents that have been implicated in scleroderma-like disease and are reviewed by Nietert and Silver [2].

Therefore, multiple factors and combinations of factors (immune system, vascular and extracellular matrix abnormalities) are the most likely triggers in an individual with a genetic predisposition to scleroderma. The variety of suspects suggests a complex multigenic disease, possibly with many overlapping pathways to fibrosis.

**In vitro Studies**

Information on molecular events in autoimmune fibrosing disease has come mainly from in vitro studies of fibroblasts isolated from skin of individuals with scleroderma. Mapping out the molecular pathways leading to the excessive collagen production by scleroderma fibroblasts (TGF-β, Smad signaling pathway) has increased our understanding of the fibroblast component of disease. A caveat for the fibroblast studies is that gene array profiles from cultured human scleroderma skin fibroblasts are incomplete compared with freshly isolated scleroderma skin cells [3].

**Animal Models**

Studies on several useful animal models reviewed separately have broadened our understanding of scleroderma [for review, see 4–6], including murine bleomycin-induced fibrosis [7, 8], murine sclerodermatous graft-versus-host disease [9–16], the UC Davis (UCD 200) chicken model [17, 18], the tight skin (TSK1) mouse [19] and several transgenic and knockout mouse models [6]. Backcrosses with mutant strains, conditional reporter transgenic lines for the TSK1 line and TGF-β receptor transgenic mice provide an exciting opportunity to dissect out the key events and pathways in scleroderma [20, 21]. Again, no single one of these demonstrates the entire spectrum of clinical findings in scleroderma patients. Rather, each one displays subsets of features, much like the clinical spectrum of scleroderma. The presence of multiple animal models, each representing a different process leading to the end point of tissue fibrosis, also suggests multiple possible etiologies of autoimmune fibrosing disease.

**New Developments in Scleroderma Research**

There are exciting new advances in other areas that relate to scleroderma. For instance, the concept of fibroblast patterning (different classes of cutaneous fibroblasts based on gene array analysis of skin fibroblasts from different regions of the body) [22] helps to explain the localization of early scleroderma to hands, feet and face, which all contain a distal-type fibroblast.
The new developments in scleroderma research occur in these diverse areas: immune dysregulation including chronic B cell activation, matrix dysregulation, molecular characterization of autoantibodies, microvascular dysfunction, genetic polymorphisms, and microchimerism (table 1). Linking the new concepts and findings in a coherent hypothesis for disease remains the challenge in our understanding of scleroderma [6, 23–31].

**Therapy for Scleroderma**

Evaluation of therapy is difficult because the clinical disease is heterogeneous and can wax and wane. Global immunosuppression with corticosteroids or other immunosuppressants is not effective and leads to unwanted side effects with long-term use. D-penicillamine, minocycline, recombinant relaxin, tamoxifen, extracorporeal photopheresis, interferon (IFN)-α and IFN-γ all failed as effective therapies in clinical trials [http://www.sclero.org/medical/research/clinical-trials/a-to-z.html]. A few agents are useful for the vascular dysfunction in selected patients. Current effective therapies for scleroderma are as follows [32]:

- Renal crisis: angiotensin-converting enzyme (ACE) inhibitors alter the rennin-angiotensin axis
- Pulmonary hypertension:
  - epoprostenol, treprostenil and ilopost supply prostaglandins not provided by the pulmonary arterial vasculature
  - sildanefil citrate (Viagra) elevates nitric oxide, a vasodilator
  - bosentan inhibits endothelin which is abnormally increased in pulmonary hypertension
- Pulmonary fibrosis: cyclophosphamide, an alkylating cytotoxic agent, affects cell replication but is also an immunosuppressant
- Raynaud’s disease: calcium channel blocking agents affect movement of calcium in endothelium and act as vasodilators
- Digital ulcers: bosentan prevents new ulcers
- Skin fibrosis: methotrexate inhibits immune cell activation and inflammation

Examples of clinical trials using therapeutic agents for vascular disease are:

- Bosentan in a variety of clinical trials for digital ulcers, pulmonary fibrosis, pulmonary hypertension, Raynaud’s disease and skin fibrosis (multiple centers)
- Quinipril (ACE inhibitor) for systemic sclerosis (Gwynedd Hospital, North Wales)

In addition, several different novel therapies (such as monoclonal antibodies to cytokines and cytokine receptors, immunosuppressive regimens and phototherapy) that alter cytokine and chemokine environments are reported in small patient cohorts. The benefits must always be weighed against the risks of
altering homeostasis in the complex networks of the immune system [33]. Examples of trials using these immunomodulatory agents are summarized below (http://www.sclero.org/medical/research/clinical-trials/a-to-z.html):

- Recombinant human anti-TGF-β antibody (CAT 192) is no better than placebo (Royal Free Hospital, England)
- Infliximab (TNF-α inhibitor) stabilizes systemic disease parameters but has serious side effects including pulmonary hypertension, digital ulcers and Raynaud’s disease (Royal Free Hospital, England)
- Intravenous immunoglobulin for immunosuppression (multiple centers)
- PVAC, a therapy derived from delipidated, deglycolipidated *Mycobacterium vaccae* for diffuse scleroderma (Stanford University, University of San Diego)
- Thalidomide for diffuse scleroderma (New York University)
- PUVA UVA-1 for diffuse and limited scleroderma and morphea (multiple centers)

The variety of agents in trials points to the refractory nature of scleroderma to treatment. At present, scleroderma is typically a chronic, debilitating, relentlessly progressive disease with few if any definitive therapies. Bone marrow or stem cell transplantation may be the only definite cure for scleroderma, and this option is available in experimental trials. It is effective in early but not late disease when significant tissue injury is already present [34]. Some examples of clinical trials using transplantation are summarized below (http://www.sclero.org/medical/research/clinical-trials/a-to-z.html):

- SCOT clinical trial comparing stem cell transplantation or high-dose cyclophosphamide (Johns Hopkins University)
- Allogeneic hematopoietic stem cell transplantation of sibling donor stem cells into scleroderma individuals previously treated with cyclophosphamide, fludarabine and Campath 1H for immunosuppression (Northwestern University)
- ASTIS study: high-dose immunoablation and hematopoietic stem cell transplantation versus monthly intravenous pulse therapy cyclophosphamide [European Group for Blood and Marrow Transplantation (EBMT)/European League Against Rheumatism (EULAR) Scleroderma Study Group]

How Do We Know When Therapy Works?
The groundwork for evaluation of potential therapies for scleroderma was laid by Metzger and Steen in the 1970s. They developed an extensive longitudinal database with demographics, clinical information and outcomes for scleroderma patients treated at the University of Pittsburgh. Multicenter trials developed in the 1980s and 1990s provided the power of additional patient
numbers and standardized therapy protocols. These important advances led to
the 6th and 7th Outcome Measures in Rheumatology Clinical Trials (OMER-
ACT), which provided guidelines for different outcome measurements for clini-
cal trials in scleroderma [35, 36]; however, at present there is no consensus on
how to evaluate disease activity or prognostic criteria in general clinical practice.

**New Concepts in Scleroderma Pathophysiology**

*Immune Dysregulation in Scleroderma*

Historically, established scleroderma was thought to be predominantly a T
cell process with increased activated T cells in the blood and tissue. These bear
restricted T cell receptor repertoires and are associated with Th2-like cytokine
profiles based on RT-PCR of cells from blood and tissue and by ELISA
[37–39].

**Cytokines**

TGF-β, which is the prototypic profibrotic cytokine, increases collagen
synthesis by fibroblasts and downregulates extracellular matrix degradation.
TGF-β is upregulated in scleroderma, driving collagen type I synthesis via con-
nective tissue growth factor (CTGF), a downstream regulator of collagen syn-
thesis [40]. Other profibrotic cytokines that may play a role in scleroderma are
IL-4, IL-6 and IL-13 [for review, see 39, 41]. Cytokine-directed therapy is pro-
posed as a logical extension of the extensive knowledge base on cytokines and
scleroderma [42] but has not been effective, to date.

**Chemokines**

Individuals with scleroderma have increased serum monocyte chemoat-
tractant factor-1 (MCP-1) [43], cutaneous T cell-attracting chemokine (CTAC)
[44], regulated on activation normal T cell expressed and secreted (RANTES)
and IP-10 by a variety of methods [for review, see 39, 45]. Chemokine receptors
such as the MCP-1 receptor are also upregulated [46]. The combination of
chemokines for both T cells and macrophages is consistent with the presence of
both types of immune cells in scleroderma skin.

Transcriptional profiles for global gene expression of peripheral blood
cells have revealed some novel immune markers for scleroderma. Of note, a
large number of IFN-γ-inducible and vasculotrophic genes are upregulated
compared to normal controls. Several signaling pathways are also upregulated
compared to controls: insulin growth factor-1 and insulin, epidermal growth
factor, insulin, antiapoptosis factors, and platelet-derived growth factor, among
others [47]. Evaluation of these pathways in vivo and in vitro is ongoing.
B Cell Abnormalities and Scleroderma

The role of B cells in the normal immune response is to make immunoglobulin and participate in immune regulation. In the past, scleroderma was thought to be mainly a T cell disorder. However, it has been shown recently that individuals with systemic sclerosis have expanded naïve B cells and decreased but activated memory T cells compared to normal individuals. Several abnormalities in the B cell immune axis may explain this phenomenon.

- Individuals with diffuse scleroderma overexpress CD19, a marker for B cell regulation, by approximately 20%, which may produce chronic B cell activation and lead to autoantibody production [48].
- Polymorphisms of the promoter for CD19 may explain in part the chronic B cell activation and increased production of IL-6, a profibrotic cytokine elevated in scleroderma. Abnormal B cell function may also influence T cell activation and function, also leading to a tissue environment that enhances fibrosis [49, 50].
- Serum levels of B cell-activating factor (BAFF, a member of the TNF-α family of ligands and a survival factor for B cells) and BAFF receptor on B cells are elevated in diffuse scleroderma. Increased serum BAFF correlates with the extent and exacerbation of disease. Similarly, elevated mRNA for BAFF is seen in early diffuse scleroderma but not in normal skin. B cells isolated from individuals with diffuse scleroderma have an enhanced ability to produce IL-6 and immunoglobulin when exposed to BAFF [51].

The abnormalities in multiple cytokines, chemokines and growth factors in scleroderma suggest a complex regulatory immune network that involves multiple pathways and molecules. Interfering with one molecule may inadvertently change the balance in another. The challenge in scleroderma therapy is to target the critical pathways in fibrosing disease without disruption of the normal ones and subsequent adverse effects.

Major Autoantibodies in Scleroderma

Individuals with scleroderma can be grouped into several nonoverlapping categories based on the presence of characteristic disease-specific autoantibodies in their sera: anti-centromere (limited cutaneous disease and pulmonary vascular disease), anti-Scl-70/topoisomerase I (diffuse systemic disease and diffuse pulmonary fibrosis), anti-fibrillarin/anti-U3 nucleolar antigens (diffuse systemic disease) and anti-RNA polymerase I–III (diffuse systemic disease) [for review, see 52–56]. The anti-fibrillarin/U3 and anti-RNA polymerase antibodies are less commonly found in scleroderma sera. Other autoantibodies historically described in small groups of scleroderma patients are Pm/Scl (limited cutaneous disease) and anti-Th/To (limited cutaneous disease but likely a marker for pulmonary hypertension).
Less Common Autoantibodies in Scleroderma

There are rare autoantibodies in scleroderma sera that are also found in other autoimmune diseases and are not useful for clinical diagnosis or correlation with disease activity: anti-Ku which is also found in lupus erythematosus [52] and anti-U1 which is found in mixed connective tissue disease [57]. Anti-Ro autoantibodies are occasionally found in scleroderma patients and are a marker for sicca syndrome. A list of other rare autoantibodies reported in the last 5 years in scleroderma is given in table 2. Some are associated with subgroups of individuals with scleroderma, but none have yet been shown to be directly pathogenic. They may simply reflect immune system dysfunction in autoimmunity. They are worth noting because the autoantibodies in scleroderma and other autoimmune diseases have been used as tools to identify, characterize and in some cases, purify important molecules of the cell. The autoantibodies of scleroderma have been the basis for important advances in molecular and cell biology because they target well-conserved critical molecules essential to the cell.

A few autoantibodies in scleroderma may be pathogenic [30]. They are directed to endothelial cells, fibroblasts, fibrillin, matrix metalloproteinases and platelet-derived growth factor (PDGF).

Anti-Endothelial Cell Antibodies

Anti-endothelial cell antibodies (AECA) are a newly described group of antibodies that may play a major role in pathogenesis of scleroderma. They are found mainly in limited scleroderma and bind to the ubiquitous centromeric nuclear protein CENP-B [58]. AECA have been identified in vivo in serum and directly in fibrotic lung tissue in scleroderma [59].

Anti-Fibroblast Antibodies

Anti-fibroblast antibodies have also been described in scleroderma sera and may be pathogenic. They bind to the surface of human and rodent fibroblasts. Anti-topoisomerase antibodies also have some anti-fibroblast binding properties. The binding of purified topoisomerase antigen to fibroblasts in culture and subsequent binding of anti-topoisomerase antibodies causes activation and adhesion of cocultured macrophages. This suggests a pathogenic role for the anti-topoisomerase antibodies in amplification of the fibrogenic cascade [60].

Anti-PDGF and PDGF Receptor Antibodies

PDGF stimulates production of reactive oxygen species, which can damage vascular endothelium [61, 62].
Table 2. Rare new autoantibodies in scleroderma

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Function</th>
<th>Association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECM molecules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrillin-1</td>
<td>Component of ECM microfibrils and regulator of TGF-β by sequestration in ECM; duplicated in TSK1 mouse</td>
<td>Choctaw Indians with scleroderma</td>
<td>[100, 101]</td>
</tr>
<tr>
<td>MMP-1 and MMP-3</td>
<td>Enzymes that degrade ECM</td>
<td>Rheumatoid arthritis</td>
<td>[102–104]</td>
</tr>
<tr>
<td>PHET</td>
<td>Ectopic expression in scleroderma fibroblasts</td>
<td>Lung disease</td>
<td>[105]</td>
</tr>
<tr>
<td><strong>Nuclear and nucleolar regulatory molecules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ReqQ3 RNA/DNA helicase (WRN)</td>
<td>Nuclear enzyme mutated in Werner syndrome</td>
<td></td>
<td>[106]</td>
</tr>
<tr>
<td>U1 RNP</td>
<td>Component of the small nuclear RNP involved in mRNA splicing</td>
<td>Esophageal and lung disease</td>
<td>[57, 107, 108]</td>
</tr>
<tr>
<td>U3 RNP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B23</td>
<td>Nucleolar phosphoprotein</td>
<td>Pulmonary hypertension</td>
<td>[109]</td>
</tr>
<tr>
<td>HSP47</td>
<td>Collagen-specific molecular chaperone with role in collagen homeostasis in fibroblasts</td>
<td></td>
<td>[110]</td>
</tr>
<tr>
<td>Ufd2 complex</td>
<td>E4 polyubiquitylating enzyme, regulates chromosome and separation in mitosis condensation</td>
<td></td>
<td>[111]</td>
</tr>
<tr>
<td><strong>Vascular antigens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphotidyl serine prothrombin complex Cardiolipin</td>
<td>β2 glycoprotein in resting endothelial cells</td>
<td>Thrombo-embolism, peripheral ischemia and pulmonary hypertension</td>
<td>[54, 112]</td>
</tr>
</tbody>
</table>

ECM = Extracellular matrix; MMP = matrix metalloproteinase; PHET = protein highly expressed in testis; RNP = ribonucleoprotein; HSP = heat shock protein.
Autoantigens unique to diffuse scleroderma (that is, topoisomerase I) have metal binding sites, and are susceptible to metal ion-associated oxidation reactions and cleavage. These data support the vascular injury/reperfusion hypothesis for scleroderma [63, 64]. The search for an abnormality in metal ion status in scleroderma has not yielded any candidate metal ions, however.

Innate Immunity
Very little is known about the role of innate immunity in scleroderma.

Extracellular Matrix Dysregulation
There is an extensive literature on fibroblast biology in scleroderma [for review, see 41, 65–67]. Fibroblasts are collected from scleroderma skin or lung in individuals with established disease and cultured for 6–8 passages for in vitro studies. The concepts distilled from these experiments have been important in understanding molecular pathways that could lead to the abnormal collagen synthesis of scleroderma. However, recent data from gene array studies comparing cultured scleroderma fibroblasts and freshly isolated scleroderma skin cells have shown that the cultured fibroblasts provide an incomplete picture of in vivo cell transcription profiles [3]. With that caveat in mind, several critical pathways in fibroblast biology and collagen regulation have been identified.

There are several interesting populations of fibroblast-related cells that may be involved in abnormal skin fibrosis in scleroderma.
• Fibrocytes: the peripheral blood contains a population of CD34+ collagen-positive cells that may function in wound healing and may have a role in fibrosing disorders such as scleroderma [68]. Fibrocytes may be a source of myofibroblasts.
• The myofibroblast, proposed to originate from fibroblasts, pericytes and possibly monocytes, expresses α smooth muscle actin, Thy-1 and EDA-fibronectin [69]. TGF-β induces the differentiation of myofibroblasts; increased numbers of myofibroblasts are seen in scleroderma but not in control skin [66, 68]. TGF-β also inhibits the apoptosis of fibroblasts and myofibroblast lineage cells, suggested as a mechanism for the abnormal scleroderma phenotype [27, 70, 71].
Smads are intracellular signal-transducing molecules and activators of the fibroblast collagen transcription regulating pathway [72]. TGF-β interacts with the transmembrane type I activin-like receptor kinase (ALK5) TGF-β receptor. The receptor is linked to Smad 2/3 proteins which activate the pathway. Smad 7 inhibits the pathway.
• Protein and mRNA levels of Smad 3 but not Smad 4 or Smad 7 are increased in scleroderma fibroblasts compared to matched normal controls, suggesting
that the Smads play a critical role in maintenance of the scleroderma fibroblast phenotype [73].

- Others have shown deficient Smad 7 in scleroderma fibroblasts [74].

Endothelin-1, a vasoconstrictor peptide derived from endothelium and also synthesized by fibroblasts, is also important for extracellular matrix homeostasis via endothelin receptors. Endothelin-1 in vitro promotes fibroblast synthesis of collagen, inhibits metalloproteinase synthesis, induces myofibroblast differentiation and upregulates ICAM-1 which could affect immune cell-fibroblast adhesion [75–77]. TGF-β can induce endothelin synthesis by fibroblasts via the JNK/AP-1 signaling pathway, providing an autocrine endothelin loop that may promote fibrosis in scleroderma [78]. The combination of increased endothelin in plasma in scleroderma [79], linked TGF-β-endothelin activation of fibroblast collagen synthesis, elevated endothelin-1 in scleroderma fibroblasts compared with normal fibroblasts, and endothelin receptor polymorphisms (see below) are proposed as evidence for the contribution of endothelin to fibrosis in scleroderma.

Vascular Injury

Vascular injury is a prominent feature of scleroderma. There is proliferation of vascular intima, and differentiation of smooth muscle cells and possibly monocytes to myofibroblasts causing narrowing of the vascular lumen. Several possible triggers of injury have been proposed, including environmental triggers such as cytomegalovirus or parvovirus that cause vessel damage [80, 81]. Possible immunologic triggers include cytokines (TGF-β, CTGF) or growth factors (PDGF, VEGF) that produce impaired vasodilation, leading to ischemia-reperfusion vascular injury. AECA (see above) may be directly pathogenic.

Genetic Factors

HLA Associations

Early studies in cohorts of individuals with scleroderma revealed no HLA associations. Later studies in which subgroups of individuals with certain autoantibody profiles were analyzed were more revealing [82, 83]. Examples of some of the associations are given below:

- Anti-topoisomerase antibody is associated with HLA DRB 1*1101/1104 and DPB 1*1301 alleles [54, 84].
- RNAP 1/III (RNA polymerase) autoantibodies are associated with DRB1*405, DRB4*01 and DQB1*0401 (Japanese), and DRB3*02 (Caucasians) [85].
- Anticentromere antibodies are associated with DRB1*01, DRB1*04 and DQB1*05 [54].
- There are also disproportionate increases in certain HLA alleles in diffuse scleroderma (DRB1*1104) and limited scleroderma (DRB1*1101). Both
diffuse and limited scleroderma have increased DRB1*11 compared to controls [86].

Of interest have been the twin studies which show low concordance of monozygotic and dizygotic twins for scleroderma (5%), but a high concordance for HLA haplotypes, suggesting that genetic factors alone are not enough for the development of scleroderma [87, 88]. There is infrequent familial aggregation of scleroderma (1–1.5%), again supporting predisposition but not causality of genetic factors [89].

Polymorphisms

The wide variety of genetic polymorphisms (table 3) suggests that there may be multiple pathways to autoimmune fibrosing disease. A certain immunologic or matrix phenotype may affect disease expression in an individual with a predisposition to scleroderma. The genetic polymorphisms occur not only in the HLA molecules, but also in cytokines and chemokines, and in vascular and extracellular matrix molecules.

Microchimerism of Fetal/Maternal Cells

In pregnancy, the placenta allows two-way exchange of fetal and maternal cells. The result is microchimerism, which can persist for HLA-compatible stem cells and precursor immune cells for many years. For instance, CD34+ and CD34+CD68+ fetal cells are present in maternal circulation up to 27 years after a pregnancy [90]. Microchimerism has been proposed as a hypothesis for scleroderma [91–93] because (1) women with scleroderma are more likely to have had an HLA-compatible fetus than matched controls and (2) Y-chromosome-positive cells are found in skin of women with scleroderma many years after childbirth [94]. This hypothesis is still in debate because microchimerism is a common event in normal individuals as well as in those with autoimmune disease. Persistent fetal stem cells may in fact be beneficial rather than pathogenic, providing renewal stem cells and circulating to sites of inflammation to help with repair [95, 96].

Environmental Triggers

Multiple environmental agents, among them bleomycin, silica, vinyl chloride, epoxy resins, adulterated cooking oils, L-tryptophan contaminants and solvents, can produce autoimmune fibrosing disease [for summary, see 2, 97]. There are several intriguing reports of scleroderma associated with systemic therapy for other disorders, suggesting a predisposition to scleroderma that can be uncovered or exacerbated by manipulation of the cytokine environment. In one report, two individuals receiving IFN-α, one for chronic active hepatitis, developed diffuse scleroderma and lung fibrosis 6 months after initiation of the agent [98].
Table 3. Genetic polymorphisms in scleroderma

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Allele</th>
<th>Role</th>
<th>Association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokines and chemokines</strong></td>
<td></td>
<td></td>
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<tr>
<td>IL-1α promoter</td>
<td>−889T</td>
<td>Proinflammatory</td>
<td>Lung disease and response to cyclophosphamide</td>
<td>[113, 114]</td>
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<tr>
<td>IL-6 promoter</td>
<td>−597</td>
<td>Profibrotic</td>
<td>Disease activity</td>
<td>[115]</td>
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<tr>
<td>IL-10</td>
<td>−3575</td>
<td>Anti-inflammatory</td>
<td></td>
<td>[116]</td>
</tr>
<tr>
<td>IL-13</td>
<td>−1055</td>
<td>Profibrotic</td>
<td></td>
<td>[117]</td>
</tr>
<tr>
<td>TNF-α promoter</td>
<td>−863</td>
<td>Proinflammatory</td>
<td></td>
<td>[88, 118]</td>
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<td>TNF-α receptor</td>
<td>−238</td>
<td>Regulatory</td>
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<td>MCP-1 promoter</td>
<td>−2518G</td>
<td>Proinflammatory</td>
<td></td>
<td>[119]</td>
</tr>
<tr>
<td>MIF-1</td>
<td>−173C</td>
<td>Anti-inflammatory</td>
<td>Lower in limited scleroderma</td>
<td>[120]</td>
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<td><strong>Immune cell markers</strong></td>
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<tr>
<td>CD86 (B7.2) promoter</td>
<td>−3479T</td>
<td>Antigen-presenting</td>
<td></td>
<td>[121]</td>
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<tr>
<td>CTLA-4 promoter</td>
<td>−1722C</td>
<td>T cell signaling</td>
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<td>[122]</td>
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<td>CD19 promoter</td>
<td>−499G/-318T</td>
<td>Regulation of B cell</td>
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<td>[123]</td>
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<td>3’ UT region (GT)(14)</td>
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<td><strong>Vascular and matrix molecules</strong></td>
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<td>Endothelin receptor A, B</td>
<td>Increased B-1a B-2a alleles; +69 and +105 of exon 6 of receptor A</td>
<td>Vasoconstriction, modulation and ECM turnover; promotes myofibroblast differentiation</td>
<td>Diffuse scleroderma RNA polymerase antibodies</td>
<td>[124]</td>
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<tr>
<td>NOS</td>
<td>−186C/894T</td>
<td>Regulation of the</td>
<td></td>
<td>[125, 126]</td>
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<tr>
<td>ACE</td>
<td>Insertion/deletion</td>
<td>Regulation of the microcirculation</td>
<td>Diffuse scleroderma microcirculation</td>
<td>[126]</td>
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<tr>
<td>Fibrillin</td>
<td>5’ UT region of exon1</td>
<td>Component of microfibrils in ECM</td>
<td>Choctaw Indians, Japanese</td>
<td>[127, 128]</td>
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In summary, scleroderma is a complex, probably multigenic, chronic and progressive autoimmune disease. It may be the end point of many different pathological processes involving the immune system, vessels and extracellular matrix in individuals with a genetic predisposition. The course of disease is dependent on the scleroderma variant, which can be identified with autoantibody profiles as well as clinical parameters. Systemic sclerosis/scleroderma has the highest morbidity and mortality. Molecular advances such as gene arrays, and more sensitive methods and reagents for detection of molecules and cells of the immune system, vessels and extracellular matrix have increased the body of knowledge about scleroderma. However, except for a few agents for the vascular abnormalities in scleroderma, no definitive therapy is available to date.

In one way, scleroderma is like the elephant in the ancient story about the 6 blind men and the elephant. Each blind man thought that the elephant was like the body part that he touched (such as the trunk, tail and foot). The elephant driver said to the blind men: ‘Even if you put all the parts you can feel together, still it will not be a complete elephant. Many parts are still missing. As you have seen, you cannot see the complete elephant by putting together parts. You must see the whole elephant to have a complete idea of the elephant’ [99]. For scleroderma, the whole elephant has not yet been seen and the diverse hypotheses about pathophysiology are summed up in this poem:

O how they cling and wrangle, some who claim  
For preacher and monk the honored name!  
For, quarreling, each to his view they cling.  
Such folk see only one side of a thing [99].

Fortunately the scleroderma community of investigators has a very productive forum for exchange of ideas and data every two years at the International Scleroderma Workshop, organized by Drs. Carol Black (Royal Free Hospital) and the late Joseph Korn (Boston University), and now by Robert Lafayatis (Boston University). Together, this group of researchers, clinicians and industry

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<tr>
<td>Molecule</td>
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<td>metalloproteinases</td>
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MIF-1 = Macrophage inhibitory factor; ECM = extracellular matrix; NOS = nitric oxide synthase; ACE = angiotensin-converting enzyme; UT = untranslated; AIF-1 = allograft inflammatory factor 1.
sponsors has moved the field forward toward a better understanding of the pathophysiology of scleroderma and toward better diagnosis and treatment of individuals with scleroderma.

References


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