

Immunopathogenesis of Scleroderma – Evolving Concepts

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Abstract

Scleroderma, or systemic sclerosis, is a connective tissue disease which may affect various organ systems including skin, lungs, gastrointestinal tract, cardiovascular system and kidneys. While the etiology is not clear, it is currently believed that scleroderma may represent an autoimmune response to an unknown antigen. In this regard, there is evidence that both humoral and cellular immunity may play roles. The pathophysiology is complex and consists of three major features: (1) vascular damage; (2) mononuclear cellular infiltrates; and (3) massive deposition of newly synthesized connective tissue, mainly collagen. The major pathologic features of scleroderma and the roles of humoral and cellular immunity in its pathogenesis are reviewed and summarized.

Key Words: Scleroderma, systemic sclerosis, adhesion molecules, cytokines.

SCLERODERMA, OR SYSTEMIC SCLEROSIS, is a connective tissue disease which may affect various organ systems (1). While the etiology is not clear, it is currently believed that scleroderma may represent an autoimmune response to an unknown antigen. In this regard there is evidence that both humoral and cellular immunity may play a role. Autoantibodies have been demonstrated in the CREST (calcinosis, Raynaud's disease, esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome (antacentromere) and in the diffuse forms (scleroderma-70 and anti-nucleolar antibodies) (2, 3). There is also a possibility that scleroderma may represent an acquired disease secondary to a toxic chemical or an organism. Scleroderma-like syndromes have been reported following: Lyme disease; ingestion of adulterated oil (toxic oil syndrome) and tryptophan; inhalation of polyvinyl chloride; and the use of silicones for cosmetic procedures.

The pathophysiology is complex and consists of three major features: (a) vascular damage; (b) mononuclear cellular infiltrates; and (c) massive deposition of newly synthesized connective tissue, mainly collagen (4). It is very likely that these three events take place simultaneously, and that the ischemia and final fi-

brosis are responsible for the development of organ insufficiency. There is evidence that blood vessel damage and collagen deposition may be mediated by cytokines released by lymphocytes and monocytes (5). This paper reviews the main pathologic features of scleroderma, and summarizes the roles of humoral and cellular immunity in its pathogenesis.

Vascular Pathology

The vascular hypothesis suggests that the primary event in scleroderma occurs at the level of capillaries and small blood vessels. Blood vessel involvement in many organ systems is well documented (6, 7). The capillary network appears to be greatly diminished, as shown by capillaroscopy (8) and by immunofluorescence studies with laminin and type IV collagen antibodies (9). Electron microscopy showing damaged endothelial cells and reduplication of the basal lamina have verified capillary alterations (10, 11). Small arteries reveal thickening of the media, hyaline or fibrinoid degeneration, intimal thickening, and subintimal deposition of collagen, leading to narrowing of the lumen and ischemic necrosis (6, 7).

A striking feature in scleroderma is destruction of endothelial cells in capillaries (11). Recently, activation of endothelial cells in scleroderma skin has been demonstrated by increased expression of E-selectin (12). E-selectin, an adhesion molecule expressed in activated endothelial cells, is involved in the adhesion of these cells to lymphocytes, monocytes and neutrophils. More recently, it has been shown that

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E-selectin, tumor necrosis factor (TNF- α) and mast cell infiltrates could be noted in salivary glands in early stages of scleroderma where clinical features were restricted to Raynaud's phenomenon and abnormal capillaroscopic findings (13).

Mononuclear Cell Infiltrates

Mononuclear cell infiltration in various organ systems occurs early in the pathophysiology of scleroderma. These infiltrates have been noted in skin (14), smooth muscle (15), esophagus (16) ileum and jejunum (17), synovium (15) and liver (18). In the skin, cellular infiltrates consisting of lymphocytes, plasma cells and fibroblast-histiocytic types were found around small blood vessels, in eccrine sweat glands and in the subcutaneous tissue (14) (Fig. 1). In a study of 65 patients with systemic scleroderma,

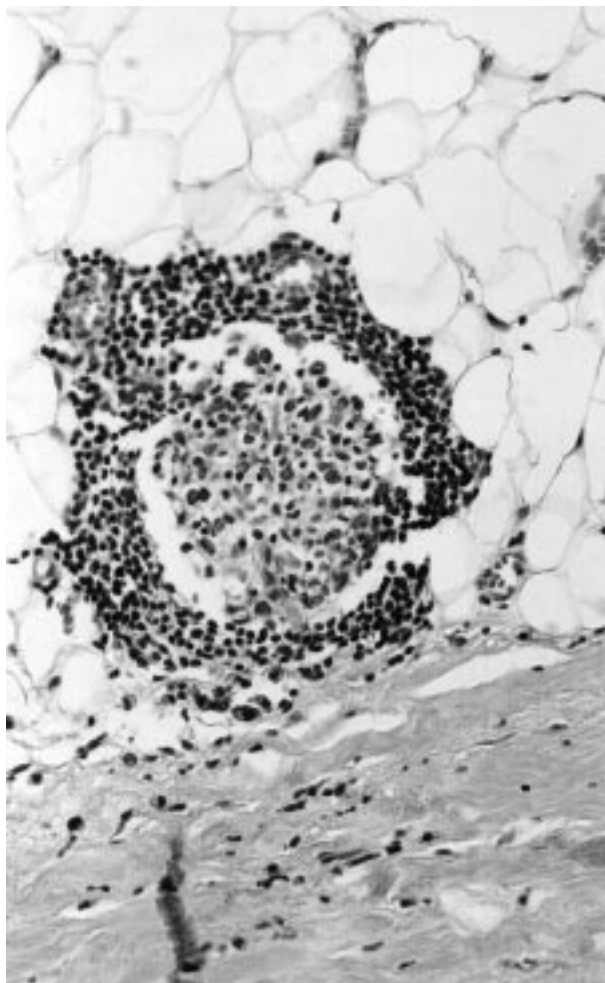


Fig. 1. Systemic scleroderma. Note mononuclear cell infiltrate surrounding a capillary and invading adipose tissue (hematoxylin and eosin; original magnification $\times 200$) (14).

49% of the biopsy specimens demonstrated a cellular infiltrate. The distribution of the infiltrate was perivascular in 26% and diffuse in 23% of the patients studied. By contrast, in 43 patients with localized scleroderma, infiltrates were seen in 84% of the patients studied (14). The cells found in the infiltrates fell into three groups: (a) mature lymphocytes, T-lymphocytes, immature plasma cells and plasma cells; (b) fibroblasts, fibrocytes, and fibroblast-like cells; and (c) macrophages, undifferentiated mesenchymal cells and monocytes (19). The high frequency and the severity of these infiltrates, particularly in localized scleroderma, suggest that these infiltrates represent an early stage preceding the development of fibrosis. Subsets of mononuclear cells have been identified in the skin and peripheral blood of patients with systemic sclerosis by means of monoclonal antibodies (20). Almost all the cells in scleroderma skin biopsies are activated T lymphocytes, as evidenced by the expression of HLA class II molecules. While the preponderance of cells in the skin are CD4⁺ (21), the majority of those in bronchiolar lavage specimens from patients with alveolitis are CD8⁺ (22). An elevated T-helper/T-suppressor lymphocyte ratio was found in the peripheral blood samples from scleroderma patients. It has been suggested that the antigenic stimulus for the dermal inflammation observed in scleroderma could be the exposure of basement membrane type IV collagen (23).

Fibrosis

The most striking feature of scleroderma pathology is the deposition of newly synthesized connective tissue, leading to fibrosis. In the skin, fibrosis involves the dermis and subcutaneous tissue. During the early stage, there is an increase in the fibroblast population. The collagen appears homogeneous and stains lightly with trichrome stains.

In addition, there is deposition of glycosaminoglycans and glycoproteins, as demonstrated by Alcian blue and PAS stains. Early deposition of type III collagen within cellular infiltrates in scleroderma skin has been observed by means of immunofluorescence microscopy (24, 25). This collagen can be stained with silver and probably represents newly synthesized collagen or reticulin fibrils. Types I and III collagen can be demonstrated throughout the dermis and subcutaneous tissue.

The interpretation of the above data may be subject to revision in view of findings suggest-

ing that type I and type III collagen molecules may be hybridized within single collagen fibrils (26, 27). In one study, staining for the aminopropeptide of type I procollagen was noted at the epidermodermal junction (which is normal), around capillaries, and in the lower dermis at the junction of the reticular dermis and subcutaneous tissue (9). Staining for the aminopropeptide of type III procollagen was intense throughout the dermis.

In addition, deposition of fibronectin has been shown in the lower dermis and subcutaneous tissue. It is likely that the focal increase in fibronectin will correspond to areas of active collagen synthesis, since fibronectin is a chemotactic factor for fibroblasts. An increase in fibronectin has also been demonstrated in scleroderma skin and in cultured scleroderma fibroblasts when performing chemical analysis.

Electron microscopy of dermal fibrosis reveals areas consisting of fine collagen fibrils, about 10–30 nm in diameter, embedded in abundant ground substance; areas showing a bimodal distribution of 40 nm and 80 nm collagen fibrils; and areas of tightly packed, thick collagen fibrils about 80–100 nm in diameter (25, 28). Considerable disorganization of collagen bundles is seen throughout the areas of fibrosis. The nature of these fibrils is still not well understood, although it has been suggested that they represent type III collagen or early immature type I collagen. Immunoelectron microscopy has been performed with antibodies directed against the aminopropeptides of type I and type III procollagens. These studies showed that although most thin fibrils retain the aminopropeptide of type III procollagen (pN-collagen), some also label for the aminopropeptide of type I procollagen (29).

Chemical analysis of scleroderma skin by ELISA also revealed a 2.5-fold increase in pN-III collagen and a 3-fold increase in fibronectin, thus corroborating previous immunofluorescence studies. The above data suggest that most thin collagen fibrils in scleroderma skin represent type III collagen, which retains its aminopropeptide. However, this interpretation may be subject to revision in light of recent data showing that collagen fibrils may be hybrids of type I and type III collagens (26, 27). One may speculate that in scleroderma, retention of the aminopropeptide of type III collagen may, by steric hindrance, stop the growth of the fibril. It is noteworthy that pN-type III propeptides have been found to be increased in scleroderma serum and that the increase correlated with fibrotic activity in those patients (30).

Fibroblast Heterogeneity

Scleroderma fibroblasts show an increase in collagen synthesis as compared to normal controls (31); this metabolic change is most striking in those fibroblasts derived from the lower dermis (32, 33). When fibroblasts were cultured from various levels of involved scleroderma skin, considerable metabolic heterogeneity (collagen and fibronectin synthesis) was observed, suggesting the presence of different clones. The highest collagen-producing populations were found at the lower levels of the dermis. Furthermore, several cultures revealed a synchronized increase in type I and type III collagens, and fibronectin (33). Activated fibroblasts in scleroderma skin have been demonstrated by *in-situ* hybridization with $\alpha_1(I)$ collagen cDNA clones. These studies showed that fibroblasts at the lower levels of the dermis and around blood vessels were the most active, again suggesting possible clonal selection (34).

There is considerable doubt that fibroblasts grown in a monolayer (culture) accurately reflect the *in-vivo* situation. To address this issue, *in-situ* hybridization was performed with specific antisense RNAs on frozen sections of skin from patients with localized and systemic scleroderma (34). Enhanced messenger RNA levels for type I and type III collagen were demonstrated, indicating increased transcriptional activity for these collagens in scleroderma fibroblasts (35).

Increased collagen can be due to overproduction or decreased breakdown. Collagenase activity was found to be decreased in scleroderma skin, suggesting a possible reduction in collagen breakdown (36). However, this finding may be secondary to a reduction in the dermal cell population during the fibrotic stage. Furthermore, collagenase activity and collagen degradation were normal in cultures of scleroderma fibroblasts.

The elevated metabolic activity of scleroderma fibroblasts could be attributed to several factors: (1) a defect in the regulation of collagen synthesis; (2) selection of fibroblast clones that express a phenotype for high matrix protein synthesis, and (3) release of cytokines that may activate monocytes-macrophages, T-lymphocytes and platelets.

Immunopathology

The etiology of scleroderma remains unknown, although it has been suggested that it

may represent an autoimmune process which involves both humoral and cellular immunomechanisms. Circulating autoantibodies are common in scleroderma, including those against centromere, topoisomerase, nucleolus, and smooth muscle cells. It is uncertain what the role of these antibodies is, since in some patients typical scleroderma without circulating autoantibodies can be seen. Furthermore, there is no strong correlation between autoantibody titers and severity of the disease. Autoantibodies against type I collagen, type IV collagen and laminin have been reported, although these autoantibodies may represent a secondary phenomenon. Moreover, there are no consistent HLA-phenotypes to support an autoimmune process. Perhaps the most convincing evidence for an autoimmune process in scleroderma is its clinical similarity with chronic graft-versus-host disease.

The mononuclear cell infiltrates, perhaps triggered by vascular damage or some unknown antigen, result in the recruitment of lymphocytes (T and B cells), macrophages and fibroblasts. An increase in the T-helper population may explain: (a) the increase in B-cell activity with formation of autoantibodies and (b) an expansion of reactive T-cell clones. These activated T-cell clones may release lymphokines that are chemotactic for fibroblasts. Moreover, they may stimulate fibroblast proliferation as well as collagen and glycosaminoglycan synthesis. Monocytes could also be stimulated to release monokines such as fibronectin and interleukin-1. Fibronectin is chemotactic for fibroblasts, and interleukin-1 may stimulate cell proliferation and increase collagen, collagenase and hyaluronate production by fibroblasts. In addition, specific lymphokines released by T-cells may be responsible for endothelial cell damage.

Humoral Immunity

Autoantibodies can be demonstrated in about 70-95% of patients with systemic sclerosis, provided the proper substrate (Hep-2 cells) is used. These circulating antibodies give different patterns by indirect immunofluorescence microscopy: homogeneous, speckled, centromere distribution and nucleolar. Five types of autoantibodies have been described in systemic sclerosis: anticentromere, scleroderma-70 (Scl-70), nucleolar, anti-Fc γ receptors and antimitochondrial (Table 1).

TABLE 1
Autoantibodies in Scleroderma

Antibody	Clinical Significance
1. Scleroderma-70	diffuse form
2. Anticentromere	CREST
3. Nucleolar	
(a) Fibrillar	diffuse form
(b) RNA Polymerase	diffuse form
(c) PM-Scl	moderate severity
(d) Th/To	mild form
4. Fc γ -receptor	may be indicator of disease activity
5. Anti-mitochondrial	primary biliary cirrhosis

Anticentromere Autoantibody

This autoantibody is present in 99% of patients with the CREST syndrome. By immunofluorescence microscopy, it gives a discrete speckled pattern (37, 38). The anticentromere autoantibody (ACA) is usually seen in patients with sclerodactyly, without facial involvement or acrosclerosis. However, the ACA has been reported in patients with Sjögren's syndrome and diffuse scleroderma. The ACA has also been reported in 20% of patients with Raynaud's disease; they may eventually develop systemic sclerosis. Interestingly enough, the ACA may also be present in Raynaud's phenomenon associated with rheumatoid arthritis and Hashimoto thyroiditis. Thus, the ACA, while not present in all cases, could also be regarded as a marker for Raynaud's phenomenon. Since the ACA is so frequently associated with CREST, it may predict a good prognosis as it relates to progression of the disease. However, it should be stressed that the CREST syndrome can be associated with two serious complications, namely biliary cirrhosis and pulmonary hypertension.

Scleroderma-70 (Scl-70)

The Scl-70 autoantibody is usually seen in association with diffuse scleroderma. The Scl-70 and ACA autoantibodies are exclusive of each other. The antigen for Scl-70 antibody has been identified as the nuclear enzyme DNA topoisomerase, which regulates chromosomal super-coiling during the cell cycle. The Scl-70 autoantibody demonstrates, by immunofluorescence, a fine speckled pattern with or without nucleolar staining, and is present in 20% of pa-

tients with systemic sclerosis. This autoantibody is a marker for systemic sclerosis and is usually seen with diffuse skin involvement, sometimes associated with acroosteolysis and pulmonary hypertension. It has also been observed with idiopathic Raynaud's phenomenon and the CREST syndrome.

Nucleolar Antibodies

Antibodies directed against nucleolar components are present in about 50% of patients with systemic sclerosis. The three major antibodies in this class are U3-RNP, Th/To and PM-Scl. Several antigens have been isolated, including 4-6 S nucleolar RNA, anti-RNA polymerase I, U3-RNP and nucleolar organizer. The antifibrillar or U3-RNP antibody is usually associated with severe scleroderma, including diffuse skin involvement, digital infarcts and ulcers, extensive telangiectasias, calcinosis, muscle and small bowel involvement, and pulmonary hypertension. Those patients with anti-RNA polymerase I antibodies also have severe disease, including: diffuse skin involvement; frequent tendon and joint changes; and frequent internal organ involvement, including renal crises. The PM-Scl antigen is a complex of 11 to 16 proteins that range from 20 to 110 kilodalton (kd) in molecular weight and whose function is not yet known (39). PM-1 antibodies, first identified in polymyositis patients in 1977 (40), were later demonstrated to contain two or more antibodies. One of these was named PM-Scl because it was frequently found in sera of patients with polymyositis-scleroderma overlap syndrome (41). The antibody was later demonstrated in patients with pure polymyositis / dermatomyositis and in patients with scleroderma without myositis (41, 42).

In a study involving 617 patients, PM-Scl antibodies were found in 24% of myositis-scleroderma overlap patients, 5% of patients with polymyositis/dermatomyositis, and 2% of scleroderma patients (without myositis) (42). The antibody identifies a subset of scleroderma patients with limited cutaneous involvement, who are more likely to have myositis and/or arthritis (39). These patients generally have no serious visceral involvement and show a favorable response to therapy (43, 44).

The Th/To antigen is a complex of 6 proteins associated with 2 RNAs referred to as 7-2 and 8-2 (39). The 7-2 RNA is identical to the component of the mitochondrial RNA-process-

ing enzyme known as Rnase MRP (45). The 8-2 RNA is identical to the RNA component of Rnase P, a processing enzyme for all precursor transfer RNA transcripts (46). The antibody is specific for scleroderma or for scleroderma-related disorders such as Raynaud's phenomenon (47). Approximately 2–4% of scleroderma patients are positive for this antibody, and these patients have limited skin involvement (47, 48).

Fc γ Receptor Autoantibodies

It has recently been shown that autoantibodies against the Fc γ receptor are present in the serum of patients with systemic lupus erythematosus, Sjögren's syndrome and scleroderma (49). Fc γ receptors are members of the immunoglobulin supergene family and are present in a variety of cells, including macrophages, natural killer cells, monocytes, neutrophils, platelets and B-lymphocytes. The Fc γ receptor is specific for immunoglobulin G (IgG) and plays an important role in removing immune complexes from the circulation. Furthermore, when Fc γ receptors bind to the ligands, they stimulate cells to release various cytokines (interleukin [IL]-2, IgG, TNF, etc.) and a variety of prostaglandins. In a recent study, it was shown that 66% of patients with systemic scleroderma and 32% with localized scleroderma have circulating antibodies against the Fc γ receptor (50). Autoantibodies against the Fc γ receptor were found in association with Scl-70 or anticentromere autoantibodies. However, the presence of Fc γ receptor autoantibodies did not correlate with duration of disease, clinical picture or other laboratory data. Furthermore, these autoantibodies are not markers for scleroderma. Yet, they may play a role in its pathogenesis by causing the release of cytokines that may contribute to vascular changes and fibrosis.

Cellular Immunity

The role of cellular immunity in the pathophysiology of scleroderma has received considerable attention over the past decade. Several factors suggest that cellular immunity is operative; these include the presence of mononuclear cell infiltrates, altered function of T-helper cells and monocytes, reduced natural killer (NK) cell activity, release of various cytokines, and the presence of autoantigens against various components of the connective tissue (51).

Adhesion molecules play a crucial role in the regulation of the inflammatory reaction.

These molecules, located on endothelial cells, lymphocytes and fibroblasts, mediate cell-cell and cell-matrix interactions (Fig. 2). Recruitment of activated lymphocytes into the tissues affected by scleroderma may be explained, in part, by aberrant expression of these molecules.

Expression of Adhesion Molecules

Three major families of adhesion molecules have been defined: the selectins, integrins and members of the immunoglobulin (Ig) superfamily. Each family demonstrates increased expression in scleroderma, indicating an increase in the activity of the cells bearing those molecules.

Selectins

The initial contact of leukocytes with endothelial cells is mediated primarily by the selectin superfamily of adhesion molecules. Rapid expression of E-selectin and P-selectin occurs after initial stimulation of endothelial cells by a leukocyte (Fig. 2). Endothelial cells in the skin and minor salivary glands of patients with scleroderma show an increased expression of E-selectin. The intensity of the mononuclear cell infiltrate in the skin of patients with scleroderma correlates with endothelial cell expression of this adhesion molecule (52). Sera from

patients with scleroderma show an increase in soluble E-selectin and P-selectin (53).

Integrins

Integrins are a family of heterodimeric transmembrane glycoprotein molecules that are assembled from one α and one β subunit. The ligand that binds to a specific integrin receptor is determined by the precise combination of α and β subunits. Integrins serve as a means of communication between extracellular matrix molecules such as collagen, laminin, fibronectin, thrombospondin and vitronectin, through the cell membrane to the intracellular compartment. They also bind receptors of the Ig gene superfamily.

Expression of integrins is increased on endothelial cells, mononuclear infiltrating cells, fibroblasts and dendritic cells in scleroderma skin (52). Endothelial cells have increased expression of $\beta 1$ integrins, very-late antigen (VLA)-2 and VLA-4 in acute and chronic scleroderma. A moderate number of mononuclear cells in chronic scleroderma and nearly all inflammatory cells in the acute phase of scleroderma express leukocyte function associated antigen (LFA)-1 α and/or LFA-1 β , members of the $\beta 2$ integrin family. In contrast, these same integrins are expressed on scattered mononuclear cells in healthy skin. $\beta 1$ integrin expression, particularly those with the $\alpha 3$ epitope, is increased in dermal fibroblasts and dendritic cells of macrophage origin.

Immunoglobulin Gene Superfamily

Increased expression of integrin receptors on endothelial cells and immunoglobulin (Ig) adhesion molecules on T cells plays a substantial role in the recruitment of T cells to peripheral tissues. Intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are members of the Ig gene superfamily. These molecules play a significant role in the interactions between lymphocytes and endothelial cells or fibroblasts (Fig. 2). While ICAM-1 is located on the cell surface of both endothelial cells and fibroblasts, VCAM-1 is located only on endothelial cells.

Firm adhesion of activated lymphocytes to endothelial cells occurs by interactions between integrin and Ig adhesion molecules. LFA-1 and VLA-4, integrin receptors on lymphocytes, bind to ICAM-1 and VCAM-1, respectively, on endothelial cells. The infiltrating T cells contact and activate fibroblasts by interactions be-

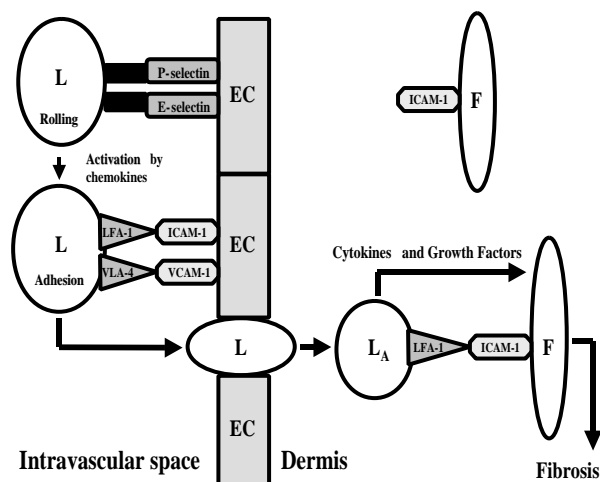


Fig. 2. Role of adhesion molecules in the regulation of the inflammatory reaction in scleroderma. These molecules, located on endothelial cells, leukocytes and fibroblasts, mediate cell-cell and cell-matrix interactions. Abbreviations: L = leukocyte, EC = endothelial cell, L_A = activated leukocyte, F = fibroblast, LFA-1 = leukocyte function associated antigen-1, VLA-4 = very late antigen-4, ICAM-1 = intracellular adhesion molecule-1, and VCAM-1 = vascular cell adhesion molecule-1.

tween LFA-1 (located on the activated T-cell) and ICAM-1 (located on the fibroblast).

In acute and chronic scleroderma, the expression of ICAM-1 is increased on both endothelial cells and fibroblasts (52, 54, 55). The increased expression of LFA-1 and ICAM-1 offers one explanation for the increased infiltration of mononuclear cells in biopsies of scleroderma skin (52) and for the increased binding of T-cells to scleroderma fibroblasts relative to control fibroblasts (56). The other major molecule in this family of adhesion molecules, VCAM-1, also shows increased expression on endothelial cells in the skin of patients with scleroderma (57).

Cytokines

It has been hypothesized that, following the activation of lymphocytes and monocytes by an unknown antigen, multiple cytokines are released. These lymphokines and monokines, released into tissues and in the circulation, may damage endothelial cells and modulate behavior of fibroblasts as it relates to proliferation, chemotaxis, migration and synthesis of extracellular matrix elements (Table 2). This leads to massive deposition of newly synthesized connective tissue, fibrosis and concomitant organ insufficiency.

Several cytokines are increased in scleroderma serums, including IL-1, IL-2, IL-4, IL-6, and IL-8. In addition, IL-4, IL-6 and IL-8 have been demonstrated in scleroderma skin. Also, a variety of growth factors were noted in scleroderma skin, including transforming growth factor- β (TGF- β), platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF) and endothelin-1 (ET-1). It should be noted

that although many of the cytokines and growth factors act to promote fibrosis, the effect of others is to hinder it. For example, interferon (IFN)- γ is a potent suppressor of collagen synthesis (Table 2). Finally, the effects of some cytokines are mixed. TNF stimulates the proliferation of fibroblasts. Moreover, it increases endothelial cell expression of adhesion molecules (E-selectin, ICAM-1, VCAM-1) and release of endothelin-1. On the other hand, it decreases fibroblast production of types I and III collagen while promoting collagenase gene induction.

TGF- β

Many studies have focused on the role of TGF- β in the pathogenesis of scleroderma. TGF- β is a growth factor that has pleiomorphic cellular actions, typified by its actions on fibroblasts and endothelial cells. *In vitro*, TGF- β stimulates the synthesis of extracellular matrix (ECM) components, including collagen types I and III, glycosaminoglycans and fibronectin (58–60). It further promotes fibrosis by augmenting the synthesis of inhibitors of matrix metalloproteinases (TIMPs), which inhibit collagenase activity (61). These inhibitors, particularly TIMP-1, are also elevated in the sera of patients with scleroderma (62).

TGF- β may also regulate collagen production indirectly, by its effect on other cytokines and growth factors. For example, PDGF is another growth factor that stimulates fibroblast proliferation and increases collagen synthesis. TGF- β produces upregulation of PDGF α receptors and mRNA upon continual stimulation of scleroderma fibroblasts (63). TGF- β also induces endothelial cell production of endothelin-1, a potent vasoconstrictor that also stimulates production of collagen by fibroblasts.

TABLE 2

Cytokines and Growth Factors Involved in the Regulation of the Biological Behavior of Fibroblasts (39)

Biological Effect	Cytokine / Growth Factor
1. Increased collagen synthesis	TGF- β ; PDGF; IL-1; IL-4; IL-6;
2. Decreased collagen synthesis	IFN- β ; IFN- γ , TNF- α and β
3. Fibroblast proliferation	IFN- γ and β ; TGF- β ; PDGF; TNF; IL-1; IL-4;
4. Chemoattraction	TGF- β ; IL-4; TNF; PDGF; IFN- γ
5. Glycosaminoglycan synthesis	IL-1; TGF- β ; TNF
6. Fibronectin synthesis	TGF- β ; IL-4
7. Endothelial cell injury	TNF (α , β); IFN- γ ; NK cell; IL-2; granzyme A
8. Reduction of collagenase synthesis	TGF- β
9. Collagenase gene induction	TNF- α

IL = interleukin

TGF = transforming growth factor

PDGF = platelet derived growth factor

TNF = tumor necrosis factor

IFN = interferon

NK = natural killer

However, other effects are less clear in the literature. Some *in vivo* studies have shown fibrotic skin to be lacking TGF- β expression (64), while others have reported an increase (65). There is conflicting data as to whether skin fibroblasts and platelets from patients with scleroderma produce increased amounts of TGF- β (66, 67). Other studies implicate endothelial cells as the source of TGF- β synthesis (68). Despite these inconsistencies in the literature, TGF- β remains a model candidate as a mediator of fibrosis in scleroderma.

Chemokines

Chemotactic cytokines, secreted primarily by leukocytes and related by a conserved four-cysteine motif, regulate the recruitment of specific leukocytes to inflammatory regions. Recent studies have elaborated abnormalities of several chemokines in patients with scleroderma. Serum levels and spontaneous production levels of monocyte chemoattractant protein-1 (MCP-1, an attractant for monocytes) and macrophage inflammatory protein-1 α and β (MIP-1 α and β , attractants for monocytes and type-1 T helper cells) are elevated in scleroderma patients (69). Elevated serum levels of MCP-1 and MIP-1 α correlate with the presence of pulmonary fibrosis (69).

MCP-1 shows greater expression in the epidermis, inflammatory mononuclear cells and endothelial cells of the sclerotic skin from patients with scleroderma compared to those with normal skin (69). Abnormal recruitment of macrophages and the consequent release of cytokines/growth factors by these cells is one possible mechanism by which MCP-1 may contribute to fibrosis of the skin. It is noteworthy that TGF- β is also secreted from macrophages (70). Upregulation of this growth factor has been proposed as the indirect method by which MCP-1 stimulates collagen synthesis by fibroblasts (71).

Cellular Cytotoxicity

Studies investigating cellular cytotoxicity in scleroderma are relatively few in number. Cytolytic CD8⁺ T cells may contribute to endothelial cell injury in scleroderma by releasing granzyme A. Levels of this serine protease have been found to be elevated in the sera of patients with scleroderma (72).

Pathogenesis of Scleroderma — Current Concepts

A recent study investigated the theory that fetal antimaternal graft versus host reactions may be involved in the pathogenesis of scleroderma in some women (73). The study demonstrated Y-chromosome sequences in DNA from peripheral-blood cells in 32 of 69 women with systemic sclerosis (46%), as compared with 1 of 25 normal women (4%). Moreover, skin biopsy specimens from 19 women with scleroderma demonstrated Y-chromosome sequences in 11 specimens. The authors postulated that scleroderma is initiated in some women by fetal cells that cross the placenta during pregnancy. A subsequent environmental exposure (viral or chemical) then activates these cells, which launch the series of events that results in scleroderma. Although the theory implicating cellular microchimerism is attractive, the study offered no data to explain the occurrence of scleroderma in men, or in women who have never had children.

Conclusions

Research continues to contribute to our understanding of the immunopathogenesis of scleroderma. Inevitably, additional adhesion molecules, cytokines, growth factors, and chemokines will be identified. Some of these may play more substantial roles than those presently being investigated. Further elucidation of the events that precipitate the initial activation of the immune system in this disease will be crucial for both basic research and clinical practice.

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