Systemic sclerosis is a disease of unknown origin characterized by excessive deposition of collagen and other connective tissue macromolecules in skin and multiple internal organs, prominent and often severe alterations in the microvasculature, and humoral and cellular immunologic abnormalities (see Glossary). Systemic sclerosis is a complex and heterogeneous disease. Clinical forms range from limited skin involvement with minimal systemic alterations (limited cutaneous systemic sclerosis) to forms with diffuse skin sclerosis and severe internal organ disease (diffuse cutaneous systemic sclerosis) (1), and occasionally a fulminant course (fulminant systemic sclerosis) (2).

The most apparent and almost universal clinical features of systemic sclerosis are related to the progressive
Glossary

Allele: One of 2 or more different genes containing specific inheritable characteristics that occupy corresponding positions (loci) on paired chromosomes.

Allergenic: Having a different genetic constitution but belonging to the same species.

Apoptosis: Disintegration of cells into membrane-bound particles that are then phagocytosed by other cells.

Calcitonin gene-related peptide: A second product transcribed from the calcitonin gene. It is found in a number of tissues, including the nervous system. It can act as a vasodilator.

Cellular immunity: Involves the production of lymphocytes by the thymus (T cells) in response to exposure to an antigen.

Codon: A sequence of 3 nucleotides in a strand of DNA that provides the genetic code for a specific amino acid.

COL1A1: Gene encoding the α1 chain of type I collagen.

COL1A2: Gene encoding the α2 chain of type I collagen.

Connective tissue growth factor (CTGF): Growth factor that plays a critical role in tissue fibrosis as well as angiogenesis, axial development of the musculoskeletal system, structural organization of connective tissue, and embryo implantation.

Cytokines: Intercellular messenger proteins. Hormone-like products of many different cell types that are usually active within a small radius of the cells producing them.

DNAucleotide: The product of cleaving a polynucleotide (nucleic acid composed of ≥2 nucleotides).

Epitope: Any component of an antigen molecule that functions as an antigenic determinant by permitting the recognition and attachment of specific antibodies.

Extracellular matrix: The ground substance occupying the space outside cells in a specific tissue.

Exon: A portion of the DNA that is transcribed to RNA, which is then expressed.

Fibroblasts: Mesenchymal cells responsible for the production of fibrous tissue.

Fibronectin: A cell-binding glycoprotein.

Growth factors: Polypeptides produced by various cells of the body that promote growth and development by directing cell division, maturation, and differentiation and by mediating maintenance and repair of tissues.

Haplotypes: The genetic constitution of an individual with respect to 1 member of a pair of allele genes.

HLAs: Human leukocyte antigens. The cell surface molecules encoded by genes in the MHC on chromosome 6. These molecules are divided into 2 classes (class I and class II) on the basis of their structure. Class I and II molecules attach and present antigens to CD8+ (cytotoxic and suppressor cells) T cells and CD4+ (helper) T cells, respectively.

Humoral immunity: Immunologic system involved in the production of plasma lymphocytes (B cells) in response to antigen exposure with subsequent antibody production.

Intercellular adhesion molecule-1 (ICAM-1): A glycoprotein that is expressed on activated endothelial cells because of stimulation by local production of cytokines, which in turn causes adherence of leukocytes.

Interferons: A class of small glycoprotein cytokines produced by T cells, fibroblasts, and other cells in response to viral infection and other biological and synthetic stimuli. Their effects include inducing enzymes, suppressing cell proliferation, inhibiting viral proliferation, enhancing the phagocytic activity of macrophages, and augmenting the cytotoxic activity of T lymphocytes.

Interleukins: A large family of hormone-like messenger proteins produced by immune cells that act on leukocytes and other cells.

Intron: A portion of DNA that lies between two exons, is transcribed into RNA, but does not appear in mature messenger RNA because the intron is removed and the exons are spliced together, and so it is not expressed.

Lymphokine: A hormone-like intercellular messenger protein produced by lymphocytes.

Matrix metalloproteinases: A family of protein-hydrolyzing endopeptidases that hydrolyze extracellular proteins, especially collagens and elastin.

MHC: Major histocompatibility complex. The pair of genes on human chromosome 6 that encode the cell surface molecules known as HLAs.

Nucleotide: A combination of a nucleic acid (purine or pyrimidine), 1 sugar (ribose or deoxyribose), and a phosphoric group.

Phenotype: The observable characteristics at the physical, morphologic, or biochemical level of an individual, which are determined by the genetic sequence of the individual.

Platelet-derived growth factor (PDGF): A factor present in platelets that is mitogenic for cells at the site of a wound, causing proliferation of endothelial cells, fibroblasts, smooth-muscle cells, and glial cells.

Polymerase: Any enzyme catalyzing a polymerization, as of nucleotides to polynucleotides.

Polymorphism: The presence of alleles in a population at a frequency that is higher than expected and that cannot be explained by mutation, suggesting a positive selection mechanism.

Prostacyclin: A prostaglandin produced by endothelial cells that is a potent natural inhibitor of platelet aggregation and a powerful vasodilator.

Protease: Descriptive term for proteolytic enzymes, both endopeptidases and exopeptidases, which hydrolyze polypeptide chains.

Sequence homology: The degree of similarity between the nucleotide sequences of strands of DNA.

Smad: A family of messenger/transcription factor proteins that are involved in signal transduction by transforming growth factor-β.

Topoisomerases: A group of enzymes capable of converting 1 topologic version of DNA into another. They act by catalyzing the breakage and reformation of DNA phosphodiester linkages.

Transcript: Same as messenger RNA.

Transcription: Transfer of genetic code information from one kind of nucleic acid to another. Commonly used to refer to transfer of genetic information from DNA to RNA.

Transcription factors: Protein factors that interact with promoters and upstream regulatory elements on the DNA of the protein that will be expressed.

Transduction: Transfer of genetic material from one cell to another. Also, transmission of cell surface signals into the nucleus.

Transforming growth factor-β (TGF-β): A regulatory cytokine that has multifunctional properties and can enhance or inhibit many cellular functions, including interfering with the production of other cytokines and enhancing collagen deposition.

Tumor necrosis factor (TNF): A polypeptide cytokine produced by endotoxin-activated macrophages that has the ability to modulate adipocyte metabolism, lyse tumor cells in vitro, and induce hemorrhagic necrosis of certain transplantable tumors in vivo.
thickening and fibrosis of the skin (3). However, multiple internal organs are always involved in some degree even when it is not clinically apparent. The affected skin is tight, indurated, and firmly bound to the subcutaneous tissue. Hair follicles and sweat and sebaceous glands atrophy. The skin over the hands and face is most frequently involved; as the disease progresses, the sclerotic changes extend and may affect the entire body. In many cases, the cutaneous involvement is confined to the digits and the dorsum of the hands and feet (acro sclerosis), and progression of the sclerotic process is relatively slow. This form of disease was previously known as the CREST syndrome (Calcinosis, long-standing Raynaud phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectases) (4). Skin ulcerations, usually localized to fingertips or knuckles, and peculiar pigmentary changes are frequent. Calcinosis is most commonly found in fingertips and periarticular tissues.

The Raynaud phenomenon is the second most common manifestation of systemic sclerosis and is present in more than 85% of patients (5). It usually appears simultaneously with other manifestations but may antedate them by several years. Often, the initial vasoconstriction and blanching are followed by a dusky cyanosis and are accompanied by paresthesias and numbness. With return of blood flow, reactive erythema occurs. In some cases, larger blood vessels are affected and luminal narrowing and occlusion can result in ischemic necrosis.

Musculoskeletal symptoms are often the initial manifestations and may vary from mild polyarthralgias to frank arthritis (6). Synovitis with a moderate effusion occurs occasionally; however, the synovial fluid is only mildly inflammatory. In more advanced stages, thickening and fibrosis of periarticular tissues result in severe flexion contractures and distal phalangeal resorption. Muscle involvement can reveal either an inflammatory myopathy or a more indolent noninflammatory form due to muscle infiltration with fibrotic tissue.

The gastrointestinal tract is the most common internal organ system involved (7). Esophageal symptoms resulting from reduced lower esophageal sphincter pressure and dysmotility of the lower two thirds of the esophagus include reflux, heartburn, and dysphagia to solid foods. In severe cases, chronic esophagitis can lead to stricture and odynophagia. Poor gastric emptying and small intestine involvement may cause abdominal distention, bloating, nausea, and pain, and, in many instances, bacterial overgrowth with secondary malabsorption, diarrhea, and weight loss.

Pulmonary involvement has emerged as potentially the most serious visceral lesion in systemic sclerosis. Because no reliable ameliorating treatment is available for such involvement, it frequently leads to severe respiratory disability and often death (8). The most prominent symptoms are tachypnea and exertional dyspnea, secondary to either pulmonary fibrosis or pulmonary hypertension. Many patients remain asymptomatic despite evidence of fibrotic involve ment of the lung parenchyma. In some cases, fulminant pulmonary hypertension may lead to rapid death (9).

Cardiac involvement is not uncommon (9, 10). Chest pain, arrhythmias, various degrees of heart block, and myocardiopathy with left ventricular or biventricular failure may result from myocardial fibrosis. These alterations occur almost exclusively in patients with diffuse systemic sclerosis. Cor pulmonale can develop in patients with pulmonary hypertension. Pericardial involvement is usually asymptomatic; however, pericardial effusions are found by echocardiography or autopsy examination in approximately one half of patients.

Until recently, renal disease was the most rapidly fatal visceral involvement of systemic sclerosis. It is known as scleroderma renal crisis and is typically characterized by the abrupt onset of malignant hypertension and rapidly progressive renal insufficiency (11). It is often heralded by severe headache; visual symptoms from hypertensive retinopathy; seizures and other central nervous system symptoms; or myocardial ischemia, infarction, or left ventricular failure. Prompt aggressive treatment now can usually reverse this process, which otherwise is fatal.

Functional thyroid abnormalities, including elevated levels of antithyroid autoantibodies and clinical and subclinical hypothyroidism, are common (12). Impotence caused by erectile failure may be an early feature of systemic sclerosis (13), and some degree of erectile dysfunction ultimately develops in many affected patients. Patients may develop the sicca syndrome (keratoconjunctivitis sicca and xerostomia) caused by fibrosis and lymphocytic infiltration of the salivary and lacrimal glands (14). Unusual clinical manifestations include urinary bladder involvement, pneumatosis cystoides intestinialis with pneumoperitoneum, trigeminal neuralgia, and hoarseness from vocal chord involvement.

The pathologic changes in systemic sclerosis encompass a spectrum reflecting variable stages of development and progression of 3 major processes in the affected tissues: 1) severe tissue fibrosis with exaggerated deposition of collagen and other connective tissue components in the extracellular matrix (see Glossary); 2) chronic inflammation, occurring predominantly in the early stages of disease and characterized by infiltration with mononuclear cells, mostly of the macrophage and T-cell lineages; and 3) microvascular disease, characterized by intimal proliferation, concentric subendothelial deposition of collagen and mucinous material, and narrowing and thrombosis of the vessel lumen. Progression of the vascular and fibrotic changes and a decrease in the inflammatory component lead to end-stage fibrosis and atrophy of the affected organs.

The histopathologic findings in the skin include marked thickening of the dermis with massive accumulation of dense collagen, causing epidermal atrophy, flattening of the rete pegs, and replacement of sebaceous and sweat glands as well as hair follicles. A prominent inflammatory infiltrate is often present at the dermal–adipose
tissue interphase, especially in early lesions (15, 16). The small vessels of the lower dermis show fibrous thickening but no evidence of vasculitis. In the lungs, the architecture of the alveolocapillary membrane and the parenchymal interstitium are markedly disrupted by fibrosis and severe mononuclear-cell infiltration. Prominent vascular abnormalities with intimal proliferation causing narrowing or complete obliteration of small vessels are frequent. The renal lesions display severe narrowing and obliteration of the medium-size arterioles because of subintimal accumulation of loose connective tissue and intimal and perivascular fibrosis. The glomeruli frequently appear ischemic, and there is no evidence of glomerulitis. Severe interstitial, perivascular, and periglomerular fibrosis may be present in cases of long duration. Other affected organs display variable degrees of fibrosis, mononuclear-cell infiltration, and microvascular obliteration and fibrosis.

**PATHOGENESIS**

The pathogenesis of systemic sclerosis is extremely complex. At present, no single unifying hypothesis explains all aspects of its pathogenesis. However, fundamental abnormalities in at least 3 types of cells are intimately involved in the development of the clinical and pathologic manifestations of the disease: 1) fibroblasts (see Glossary); 2) endothelial cells; and 3) cells of the immune system, particularly T and B lymphocytes. The profound functional alterations in these cells result in the characteristic triad of pathologic changes in systemic sclerosis: severe and often progressive cutaneous and visceral fibrosis; obliteration of the lumen of small arteries and arterioles; and humoral and cellular immunologic abnormalities, which include the production of numerous autoantibodies (some very highly specific for the disease), the chronic mononuclear-cell infiltration of affected tissues, and the dysregulation of lymphokine and growth factor production (see Glossary). These dysfunctional cellular processes and their resulting effects on the affected tissues are illustrated in Figure 1. At present, it is not clear which of these alterations is of primary importance or how they interrelate to cause the progressive fibrotic process in systemic sclerosis. However, a crucial component in the pathogenesis of the disorder is the persistent and unregulated activation of genes encoding various collagens and other extracellular matrix proteins in systemic sclerosis fibroblasts. This is the most important difference between normal fibroblasts, which promote normal wound healing, and systemic sclerosis fibroblasts, which demonstrate an uncontrolled production and tissue deposition of collagen resulting in pathologic organ fibrosis. This paper reviews some of the numerous components of the complex puzzle of systemic sclerosis pathogenesis, as illustrated in Figure 1, and at-
tempts to form hypotheses that may provide frameworks for the development of novel and effective therapies. Because we were not able to provide a comprehensive discussion of the abundant literature published, we refer readers to recent reviews for a more complete and detailed description.

Possible Causative Agents

The cause of systemic sclerosis has remained elusive despite intense investigations. Although the disease is not inherited in a classical Mendelian pattern, there is strong evidence that genetic factors contribute to its development and clinical manifestations, as discussed in more detail shortly. However, it has become apparent that environmental agents play a crucial and more important role than genetic influences. One study reported a remarkably low concordance in the development of systemic sclerosis among homozygous twins, indicating that the heritability component of the disease was very low and that the most important factor was of an environmental or acquired origin (17). Many infectious, chemical, and physical agents have been postulated as being involved in the cause of the disease. The hypothesis that infectious agents may cause systemic sclerosis has been studied extensively. Some researchers have suggested that the production of specific autoantibodies in systemic sclerosis is the result of an antigen-driven response caused by “molecular mimicry.” The concept of “molecular mimicry” proposes that antibodies against self-antigens are produced because these antigens contain epitopes (see Glossary) that share structural similarities with viral or bacterial proteins. In the immunopathogenesis of systemic sclerosis, herpesviruses, retroviruses, and human cytomegalovirus infections, among others, have been suggested as possible causative agents. Evidence supporting the role of retroviruses includes the demonstration of sequence homologies (see Glossary) between certain retroviral proteins and the topoisomerase I antigen (see Glossary), which is the target of anti–Scl-70 antibodies in patients with systemic sclerosis (18). In addition, it has been shown that the induced expression of retroviral proteins in normal human dermal fibroblasts results in the acquisition of a systemic sclerosis–like phenotype (see Glossary) in the production of extracellular matrix proteins (18). Furthermore, antibodies to retroviral proteins have been detected in serum specimens from patients with systemic sclerosis (19). Another hypothesis has suggested that human cytomegalovirus may be involved in the initial events of systemic sclerosis. This hypothesis is supported by the observations of a higher prevalence of IgA antihuman cytomegalovirus antibodies in patients with systemic sclerosis, which are capable of inducing apoptosis (see Glossary) in human endothelial cells; the increased prevalence of anticytomegalovirus IgA antibodies in patients positive for Scl-70 autoantibodies; and the severe fibroproliferative vascular changes and the increased occurrence of antinuclear antibodies with an immunofluorescence pattern similar to that present in serum specimens from patients with systemic sclerosis in human cytomegalovirus infections (20, 21). Despite intensive study, however, there is no definitive evidence to conclude that systemic sclerosis has a viral origin.

Environmental agents have also been implicated in the development of systemic sclerosis (22, 23). Silica and metal dust exposure had been shown in case studies to be related to systemic sclerosis (22–24), although some studies have failed to confirm an association. On the other hand, organic solvent exposure may eventually be proven to be an important environmental factor in triggering this disease. Indeed, persons exposed to vinyl chloride have an increased risk for skin thickening, the Raynaud phenomenon, and digital ulcers (23), and recent epidemiologic studies have found a higher frequency of organic solvent exposure in patients with systemic sclerosis than in normal controls (24). Several other environmental exposures have been associated with the development of systemic sclerosis, including certain pesticides, hair dyes, and fuel-derived or industrial fumes (22, 23).

Role of Genetic Factors

The contribution of genetic factors in the development and expression of systemic sclerosis is strongly supported by the observation of familial clustering of the disease, the high frequency of autoimmune disorders and autoantibodies in family members of patients with systemic sclerosis, differences in prevalence and clinical manifestations among different ethnic groups, and the increased prevalence of certain HLA and MHC alleles (see Glossary) among different ethnic groups and among patients with different clinical subsets of the disease or with different patterns of autoantibodies (25). Strong evidence indicates that genetic factors largely determine the production of specific autoantibodies in systemic sclerosis. Although the concordance of systemic sclerosis among identical twins is only 4.2% and is not significantly different from the concordance of disease in dizygotic twins (5.9%), the concordance for the presence of specific autoantibodies is substantially higher (17). These observations indicate that inherited genetic factors are important for the production of autoantibodies but are not sufficient for development of disease.

Prevalence of the disease varies by geographic region and by ethnic background; the prevalence in the United States is 242 to 286 cases per million persons in the population. However, in Native American persons of the Choctaw tribe in Oklahoma, one of the best-studied groups for the role of genetic factors in systemic sclerosis, the prevalence is 469 cases per million persons in the population (26). The differences of MHC and HLA allele expression are evident when comparing the different haplotypes (see Glossary) identified as being linked to disease expression, in particular to the pattern of autoantibody response, among ethnic groups (27). Disease expression
also appears to differ among ethnic groups. African-American persons are more likely to have anti-topoisomerase I antibodies and more severe visceral manifestations, including a higher frequency of pulmonary fibrosis. In contrast, anticentromere antibodies are more common in white persons, who are also more likely to have limited disease with less severe systemic manifestations (27).

In addition to these genetic characteristics, which may predispose persons to systemic sclerosis, other genetic factors may influence the expression of the disease. Recent studies have examined mutations or polymorphisms (see Glossary) in relevant genes that may result in their increased expression. For example, in patients with systemic sclerosis, the upstream region of COL1A2 (see Glossary), the region of the gene preceding the initiation of gene transcription, harbors numerous dinucleotide repeats that may increase gene activity (see Glossary) (28). Similarly, 2 single nucleotide polymorphisms at codon 10 of the transforming growth factor (TGF)-β1 gene were found in statistically significant higher frequency in patients with systemic sclerosis (see Glossary) (29). This suggests a genetic predisposition to higher production of TGF-β1 (29). Furthermore, patients who had the TGF-β1 polymorphism had a higher frequency of pulmonary fibrosis and graft fibrosis following lung transplantation (30). As discussed later in more detail, TGF-β1 is a potent profibrotic cytokine (see Glossary) secreted from macrophages and T lymphocytes that may play a central role in the pathogenesis of tissue fibrosis in systemic sclerosis.

Another genetic polymorphism that may influence expression of systemic sclerosis has been described in the gene encoding fibrillin-1, a large extracellular matrix protein that is a structural component of connective tissue microfibrils. The polymorphism is located in the upstream region (vide supra) of the gene, and its presence is strongly associated with the occurrence of systemic sclerosis in Native American persons from the Choctaw tribe and in Japanese persons (31). These collective studies on the influence of genetic polymorphisms on the development or expression of systemic sclerosis, however, must be performed in larger cohorts to corroborate their relevance.

**Microchimerism**

Microchimerism is a novel and provocative hypothesis of the cause of systemic sclerosis (32–34). The hypothesis suggests that, during pregnancy, allogenic (see Glossary) fetal or maternal cells cross the placenta in bidirectional traffic and persist in the circulation and tissues of the mother or child, respectively, as a result of HLA II (DRB1) compatibility between the mother and the fetus. These engrafted foreign cells may become activated by a second event and may mount a graft-versus-host reaction, which manifests as systemic sclerosis. The remarkable similarities in clinical, histopathologic, and serologic features between graft-versus-host disease and systemic sclerosis, including esophageal, lung, and skin involvement; lymphocytic infiltration and fibrosis of affected organs; and production of autoantibodies, strongly support this hypothesis. Researchers suggested this theory when fetal cells were found in the circulation of normal women several decades after the birth of their children and fetal DNA and fetal cells were subsequently identified in affected skin samples from women with systemic sclerosis who had previously been pregnant with a male fetus (32). Microchimeric cells of maternal origin have also been identified in the circulation of offspring (35). These findings might explain the occurrence of systemic sclerosis in nulliparous women and men.

Although some studies found that the frequency of male chromosome sequences in the skin of women with systemic sclerosis who had male offspring was similar to that of normal controls, quantification of microchimeric fetal cells showed a significant difference in the amount of male DNA in affected skin between the 2 groups (36). This indicates that it is the quantity and not only the mere presence of fetal cells that might contribute to the pathogenesis of systemic sclerosis. A more recent study confirmed the quantitative difference in the number of microchimeric cells between patients with systemic sclerosis and healthy individuals and also showed that immunologic stimulation expected to trigger antigen-specific T cells caused a potent amplification of the microchimeric cells in patients with systemic sclerosis but not in controls (37). Despite these observations, at present, the role of microchimerism in the pathogenesis of systemic sclerosis remains undetermined.

**Humoral Immune System Alterations**

The presence of specific autoantibodies is one of the most common manifestations of systemic sclerosis. More than 90% of patients with systemic sclerosis harbor antinuclear antibodies in their serum. Numerous autoantibodies, some of which are extremely specific for systemic sclerosis, have been described in patients with the disease; other autoantibodies are associated with different clinical manifestations (38).

Anti–Scl-70 antibodies have been shown to react with DNA topoisoisomerase I and are almost exclusively present in serum specimens from patients with the diffuse form of systemic sclerosis, although only about 30% to 40% of these patients harbor these autoantibodies. Anticientromere antibodies are present in 80% to 90% of patients with the limited form of systemic sclerosis but are found in fewer than 10% of patients with diffuse systemic sclerosis. These 2 autoantibodies rarely coexist in the same patient.

Other autoantibodies are less common in patients with systemic sclerosis. They include anti-RNA polymerases I and III antibodies in patients with rapidly progressive disease and severe internal organ involvement; antifibrillarin antibodies, which are commonly found in diffuse systemic sclerosis; and anti–PM-Scl antibodies, which are usually found in patients with systemic sclerosis who develop an inflammatory myopathy. Although autoantibodies are
common in systemic sclerosis, they are not directly involved in the clinical manifestations of the disease. However, owing to their high frequency and their specificity for certain clinical disease subsets, their presence is very helpful in establishing the diagnosis and predicting a probable pattern of organ involvement, severity, and disease progression (38).

**Tissue Inflammation: An Early Event in the Cascade?**

Some of the earliest evidence suggesting that chronic and persistent inflammation may play a role in the pathogenesis of systemic sclerosis was provided by the demonstration of lymphocytic infiltrates in affected skin from patients with disease of recent onset (15, 16). Subsequently, researchers found that the extent of lymphocytic infiltration correlated with the severity and the progression of skin sclerosis (39). The mononuclear cells within the skin infiltrates are predominantly CD4+ T cells and express the activation marker class II MHC antigen DR (40, 41). Subsequent expansion of these cells within the tissue appears to be oligoclonal, as shown in studies of T-cell receptor transcripts (see Glossary) in the skin of patients with systemic sclerosis (42). These results suggest an antigen-driven response, although at present there is no information on the putative antigen or antigens that may be involved. The expanded populations of inflammatory cells in affected tissues release cytokines and growth factors that initiate or perpetuate the fibrotic process as well as the endothelial and vascular alterations. Indeed, clones of T cells established from infiltrating lymphocytes isolated from affected skin of patients with systemic sclerosis produce cytokines that can stimulate fibroblast collagen production (43).

The mechanisms responsible for the migration of distinct populations of T cells from peripheral blood to skin and other affected tissues in patients with systemic sclerosis are not completely understood. However, it is likely that their retention and accumulation in such tissues result from specific interactions between certain T-cell subsets and fibroblast membrane proteins or extracellular matrix, which are mediated by integrins and cell adhesion molecules such as the adhesive protein intercellular adhesion molecule-1 (see Glossary) (44).

The inflammatory changes in affected tissues are also reflected in quantitative and functional alterations in peripheral blood cells (45). For example, the proportions and absolute numbers of total CD4+ CD45RA+ (suppressor-inducer) T cells and CD8+/CD11b+ suppressor T cells are decreased. Thus, the balance between immunoregulatory T-cell populations appears to be substantially impaired. Peripheral blood T cells in systemic sclerosis manifest evidence of previous activation, indicated by the spontaneous expression of high affinity interleukin-2 receptor (IL-2R) on their membranes (see Glossary). Several observations further support the evidence for in vivo T-cell activation in systemic sclerosis. First, serum specimens from patients with systemic sclerosis contain approximately 3-fold higher levels of soluble IL-2R than specimens from normal controls. In addition, mononuclear cells obtained from bronchoalveolar lavage fluid in patients with systemic sclerosis display ongoing activation. Finally, elevated levels of serum interleukin-2 and increased production of this cytokine by T cells in vitro have also been demonstrated in patients with systemic sclerosis. Abnormalities in numerous other cytokines have also been described (45). However, whether the alterations in the proportions of regulatory T-cell subsets and the various cytokines are secondary to disease activity or reflect a more fundamental pathogenic event is unknown.

**Role of Transforming Growth Factor-β, Connective Tissue Growth Factor, and Smad Proteins**

At present, it is unknown whether the exaggerated connective tissue production by systemic sclerosis fibroblasts is a response to an unknown injury, thus representing an abnormal regulation of a physiologic process, or whether the primary event is an alteration in the regulation of expression of the relevant matrix protein genes. It has been postulated that alterations in the regulation of extracellular matrix gene expression may be induced by cytokines and growth factors released from the tissue-infiltrating inflammatory cells. Numerous alterations in the expression of cytokines and growth factors with potent effects on fibroblast collagen synthesis, endothelial cell functions, and T-cell responses have been demonstrated in patients with systemic sclerosis (45). Many studies have examined the effects of various products of inflammatory cells, including TGF-β, connective tissue growth factor, platelet-derived growth factor, interleukin-1, interleukin-2, interleukin-4, interferons, and tumor necrosis factor, on many different aspects of fibroblast biology (see Glossary) (46). These proteins exert stimulatory or inhibitory effects on fibroblast proliferation and collagen production and sometimes exert bifunctional effects, depending on their concentrations and on the context of the other cytokines and growth factors present simultaneously in the pericellular environment.

One of the growth factors that appears to play a crucial role in the fibrosis that accompanies systemic sclerosis is TGF-β. The 3 functionally and structurally similar human isoforms of TGF-β play important roles in embryonic development, in immune responses, and in the regulation of tissue repair following injury (47). One of the most important effects of TGF-β is the stimulation of extracellular matrix synthesis by stimulating the production of various collagens and other matrix proteins, including fibronectin (see Glossary) (48). Small amounts of TGF-β appear to sensitize fibroblasts and maintain them in a persistently activated state involving an autocrine signaling mechanism that causes further production of TGF-β. Systemic sclerosis fibroblasts also seem to express increased levels of TGF-β receptors on their surface. This might account for the increased TGF-β–induced signaling and the resulting...
increased collagen production by these fibroblasts (49). In addition to its stimulatory effects on extracellular matrix synthesis, TGF-β also decreases the production of collagen-degrading metalloproteinases and stimulates the production of protease (see Glossary) inhibitors, such as tissue inhibitor of metalloproteinases-1, which prevent breakdown of the extracellular matrix (46).

Recent studies have provided a clearer picture of the intricate pathways that mediate the stimulation of collagen gene expression by TGF-β (50, 51). These include the binding of TGF-β to specific cell surface receptors and the transduction (see Glossary) of the binding signal into the nucleus to influence the activity and expression of TGF-β-responsive genes, such as those encoding extracellular matrix proteins. Briefly, TGF-β is secreted from activated lymphocytes or monocytes in an inactive form and requires complex events to become activated. Once active TGF-β comes into contact with a given target cell, it binds to a specific TGF-βII receptor on the target cell surface, which in turn recruits and phosphorylates a TGF-βI receptor to form an active receptor complex. Subsequent signaling to the nucleus then occurs through the Smad family of proteins (see Glossary) (50, 51). Smad 2 or Smad 3 binds to the TGF-β receptor complex and then becomes phosphorylated and able to form a complex with Smad 4, a cytoplasmic protein involved in the translocation of the Smad complex into the nucleus. Smad 7 is an inhibitory Smad that can bind to the TGF-β receptor complex and prevent Smad 2 or Smad 3 phosphorylation. It appears that both interferon-γ and tumor necrosis factor-α, 2 potent collagen synthesis inhibitory cytokines, stimulate increased expression of Smad 7. This results in decreased signaling of TGF-β–induced collagen gene expression. Following nuclear translocation, the stimulatory Smad complexes bind to specific promoter sites in target genes and activate their expression. The pathways involved in the activation of collagen genes following TGF-β binding to target cell surface receptors are shown in Figure 2. The potential involvement of Smad proteins in the pathogenesis of systemic sclerosis has been reviewed elsewhere (52). Furthermore, a recent study described substantially reduced levels of Smad 7 in systemic sclerosis (53). The reduction of Smad 7 inhibitory effects may be responsible for an exaggerated and unregulated TGF-β signaling cascade resulting in excessive extracellular matrix tissue accumulation.

Connective tissue growth factor also appears to play a crucial role in fibrosis (54). It participates in angiogenesis, axial development of the musculoskeletal system, structural organization of connective tissues, and embryo implantation. Transforming growth factor-β causes potent stimulation of connective tissue growth factor synthesis in fibroblasts, vascular smooth-muscle cells, and endothelial cells. Connective tissue growth factor also seems to be involved in an autocrine loop stimulating its own production and, thus, maintaining a continuous or prolonged cycle of excessive fibrosis. Although studies on the role of connective
tissue growth factor in the pathogenesis of fibrotic diseases are just emerging, it is likely that this growth factor may become recognized as an important mediator in the tissue accumulation of extracellular matrix in systemic sclerosis.

Tissue and Vascular Fibrosis: The Final Step in the Pathogenic Pathway

The most prominent clinical manifestations of systemic sclerosis are caused by the exaggerated accumulation of collagen and other connective tissue components in the affected organs. The excessive collagen deposition in systemic sclerosis is due to overproduction of this protein by fibroblasts. Regardless of the etiologic event, the resulting alterations in the biosynthetic phenotype of collagen-producing cells are crucial in the pathogenesis of systemic sclerosis. Indeed, it is the persistent activation of the genes encoding various collagens in systemic sclerosis that is the hallmark of systemic sclerosis.

Despite recent advances in the understanding of the regulation of collagen gene expression under normal conditions or under the effects of various cytokines and growth factors, the intimate mechanisms responsible for the pathologic increase in the expression of collagen genes in systemic sclerosis remain obscure. The exaggerated production of extracellular matrix macromolecules by systemic sclerosis fibroblasts largely results from increased transcription rates of their corresponding genes (55, 56). The most important molecule in the fibrotic processes of systemic sclerosis is type I collagen, the prototype of the interstitial collagens, which is largely responsible for the functional failure of the affected organs. Normal fibroblasts are capable of regulating collagen production according to the requirements of the situation during development, differentiation, and repair. As for most eukaryotic genes, the regulation of transcription rates of collagen genes involves precise interactions between specific nucleotide sequences present in the promoters and, often, in their first introns (see Glossary) and various transcription factors (see Glossary) capable of recognizing and binding specifically to these sequences. The entire complement of regulatory elements in collagen genes is not yet known, and the number of identified transcription factors that interact with these elements continues to increase. Numerous transcription factors capable of regulating the expression of collagen genes have been identified (57).

One of the most extensively studied transcription factors is Sp1. Sp1 recognizes a specific sequence in the promoters of various Sp1-responsive genes. Sp1 appears to play a pivotal function in the increased expression of COL1A1 (see Glossary) in systemic sclerosis. Increased Sp1 binding activity has been demonstrated in systemic sclerosis fibroblast nuclear extracts in comparison with nuclear extracts from normal cells (58), and increased Sp1 phosphorylation associated with increased expression of type I collagen genes has also been found in these cells (59). Another study showed that activated hepatic stellate cells, which are the primary cells responsible for increased type I collagen production during liver fibrosis, also display increased Sp1 binding activity (60). Binding of Sp1 to its recognition sites has also been implicated in the regulation of expression of type I collagen genes under the influence of TGF-β. Furthermore, numerous studies have shown that specific inhibition of Sp1 binding to its cognate elements within collagen genes caused potent and selective inhibition of collagen production in normal fibroblasts and fibroblasts cultured from patients with systemic sclerosis (61, 62).

Another transcription factor that appears to play an important role in collagen gene regulation is the CCAAT-binding factor (CBF). Both CBF and Sp1 transcription factors are involved in a protein–protein interaction in the collagen gene promoter, and both appear to be crucial regulatory factors in the expression of collagen genes. The role of CBF in the regulation of collagen gene expression in systemic sclerosis has also been examined, and it has been shown that CBF binding activity is increased in systemic sclerosis fibroblasts (63). In contrast with the stimulatory effects of Sp1 and CBF, the transcription factor c-Krox mediates tissue-specific inhibition of collagen gene expression (64), although its role in pathologic fibrosis has not been investigated.

Endothelial Cells

Vascular dysfunction is one of the earliest alterations of systemic sclerosis, and it has been suggested that it may represent the initiating event in its pathogenesis (65). Severe alterations in small blood vessels of skin and internal organs, including fibrosis and perivascular cellular infiltration with activated T cells, are almost always present in systemic sclerosis. Cytokines such as TGF-β are secreted by these activated lymphocytes and in turn injure the endothelial cells, inducing their expression of MHC class I and II antigens and adhesion ligand intercellular adhesion molecule-1. Transforming growth factor-β causes an upregulation of connective tissue growth factor, which also results in increased production of extracellular matrix components as well as an upregulation of platelet-derived growth factor. Elevated expression of platelet-derived growth factor causes increased endothelial cell proliferation and a downregulation of vascular endothelial growth factor, the endogenous growth factor that promotes neovascularization. Chemoattraction of fibroblasts into the vessel wall also occurs as a result of the effects of the cytokines released locally, leading to increased collagen synthesis and deposition in the vessel wall. Transforming growth factor-β may also induce transdifferentiation of vascular wall fibroblasts into myofibroblasts, which are phenotypically distinct cells with contractile properties and increased extracellular matrix biosynthetic ability.

The endothelial injury initiated by the release of cyto-
kines causes the subendothelium to become exposed to circulating platelets, which eventually adhere to it and initiate fibrin deposition and intravascular thrombus formation. It has also been suggested that endothelial cell damage in systemic sclerosis may be due to a direct effect of cytotoxic factors for endothelium, since serum specimens from patients with systemic sclerosis contain cytotoxic activity directed against endothelial cells (66). Other proposed mechanisms of endothelial cell injury in systemic sclerosis are those mediated by proteolytic activities present in serum (67) or by serum IgG antibodies that result in antibody-dependent cell-mediated cytotoxicity (68). These antibodies occasionally coexist with Clq-binding immune complexes and anticardiolipin antibodies, both of which are associated with thrombosis.

Vasodilation, which is controlled by both endothelial and nonendothelial (neural) mechanisms, also appears to be impaired in patients with systemic sclerosis. Endothelial cells normally secrete vasoactive substances that control vascular tone, such as the potent vasodilators prostacyclin, nitric oxide, and calcitonin gene–related peptide (see Glossary) (69). On the other hand, they also produce endothelin-1, a 21-amino acid polypeptide that has potent vasoconstrictor activities and stimulates extracellular matrix production and deposition in the vessel wall (70). Patients with systemic sclerosis appear to have a relative deficiency of vasodilators and a remarkable increase in levels of endothelin-1. This imbalance in turn causes further vascular hypoxia, which leads to increased collagen gene expression as well as endothelial injury, thus maintaining the vicious cycle of endothelial injury and fibrosis.

**DISCUSSION**

Although the exact mechanisms involved in the pathogenesis of systemic sclerosis are not known, there is sufficient evidence to allow cogent hypotheses about the possible pathway the disease takes from its causation to organ
fibrosis and resulting clinical manifestations. At present, there is no single unifying concept but rather several possible pathways, as shown in Figures 3, 4, and 5. The novel hypothesis of a graft-versus-host reaction secondary to engrafted microchimeric cells is illustrated in Figure 3. In this pathway, it is necessary to postulate an environmental agent (for example, physical, chemical, or infectious) acting as a required second event that will trigger this reaction. In contrast, in Figure 4, it is hypothesized that the environmental agent or agents is the inciting factor that acts on a genetically predisposed host and results in the subsequent recruitment and homing of macrophages and T cells to the affected tissues. The inflammatory cells would undergo selective proliferation and expansion, perhaps because of an antigen-driven response, and then release cytokines and growth factors that initiate the process of tissue and vascular fibrosis. Finally, in Figure 5, it is postulated that the environmental factor or factors, most likely of an infectious origin acting in a genetically susceptible host, cause a profound phenotypic change in various target cells of different lineages (immune cells, fibroblasts, and endothelial and vascular smooth-muscle cells). This phenotypic change could be caused by integration of genetic material (for example, of retroviral origin) within the genetic sequence of the target cells that through unknown mechanisms would induce the expression of specific regulatory genes, altering...
the function and behavior of the target cells. These alterations are manifested by increased collagen and extracellular matrix production in fibroblasts, generation of autoantibodies and cellular immune abnormalities in lymphocytes, and severe fibroproliferative and prothrombotic alterations in endothelial cells. The target cell effects of cytokines and growth factors, particularly TGF-β and connective tissue growth factor, and of the various components of their regulatory cascades are downstream components common to all 3 pathways.

All 3 hypotheses, and other plausible ones that can be construed on the basis of currently known molecular abnormalities in systemic sclerosis, may prove to be correct, since the disease exhibits a remarkably heterogeneous spectrum of clinical manifestations and its pathogenesis may be different in different individuals. Although the ultimate goal of research studies about the pathogenesis of a disease is to find a unifying mechanism, in the case of systemic sclerosis, a single common pathogenetic mechanism may not be the correct answer.

In the past 30 years, the study of systemic sclerosis has evolved from the concept that the disease was purely due to collagen overproduction by affected fibroblasts to the current stage, in which it is possible to postulate various distinct and extremely complex processes during the initial phases. Regardless of whether the answer to the puzzle of systemic sclerosis pathogenesis is a single, unified mechanism or a constellation of different ones, the knowledge
acquired in this search will undoubtedly uncover novel targets, including those involving regulatory transcription factors for collagen gene expression, such as Sp1; intracellular mediators, such as Smad proteins; and TGF-β-signaling cascades. These targets may lead to the development of long-awaited and potentially effective therapeutic options for this incurable disease.

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