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IMMUNOLOGY OF SYSTEMIC SCLEROSIS

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1. ABSTRACT

Systemic sclerosis (scleroderma; SSc) is an autoimmune disorder of unknown etiology characterized by severe and progressive cutaneous and visceral fibrosis, pronounced alterations in the microvasculature, and numerous cellular and humoral immunological abnormalities. Clinically, systemic sclerosis is very heterogeneous, ranging from mild limited forms of skin sclerosis (LcSSc) with minimal internal organ involvement to severe skin to multiple internal organ fibrosis and extensive skin fibrosis (DcSSc). Mortality and morbidity in systemic sclerosis is very high and are directly related to the extent of the fibrotic and microvascular alterations. The interactions between blood vessels, fibroblast activity, and immunological processes play an important role in the pathogenesis of SSc.

2. ETIOLOGY AND PATHOGENESIS OF SSc

The name scleroderma, more recently termed systemic sclerosis, was derived from the Greek "skleros" which means hard and "derm" meaning skin. SSc manifests clinically by progressive cutaneous and visceral fibrosis. Age, race and genetic factors have all been found to influence the development and course of SSc. SSc has variable stages in disease progression and the pathological changes reflect these progressions. Firstly, there is a chronic inflammatory process in the early stages of disease, which is characterized by infiltration of mononuclear cells, mostly of T cell lineage. Then microvascular involvement develops, which results in intimal proliferation, narrowing and thrombosis of the vessel lumen. Progression of vascular and fibrotic changes leads to end-stage fibrosis, with a decrease in the inflammatory component and

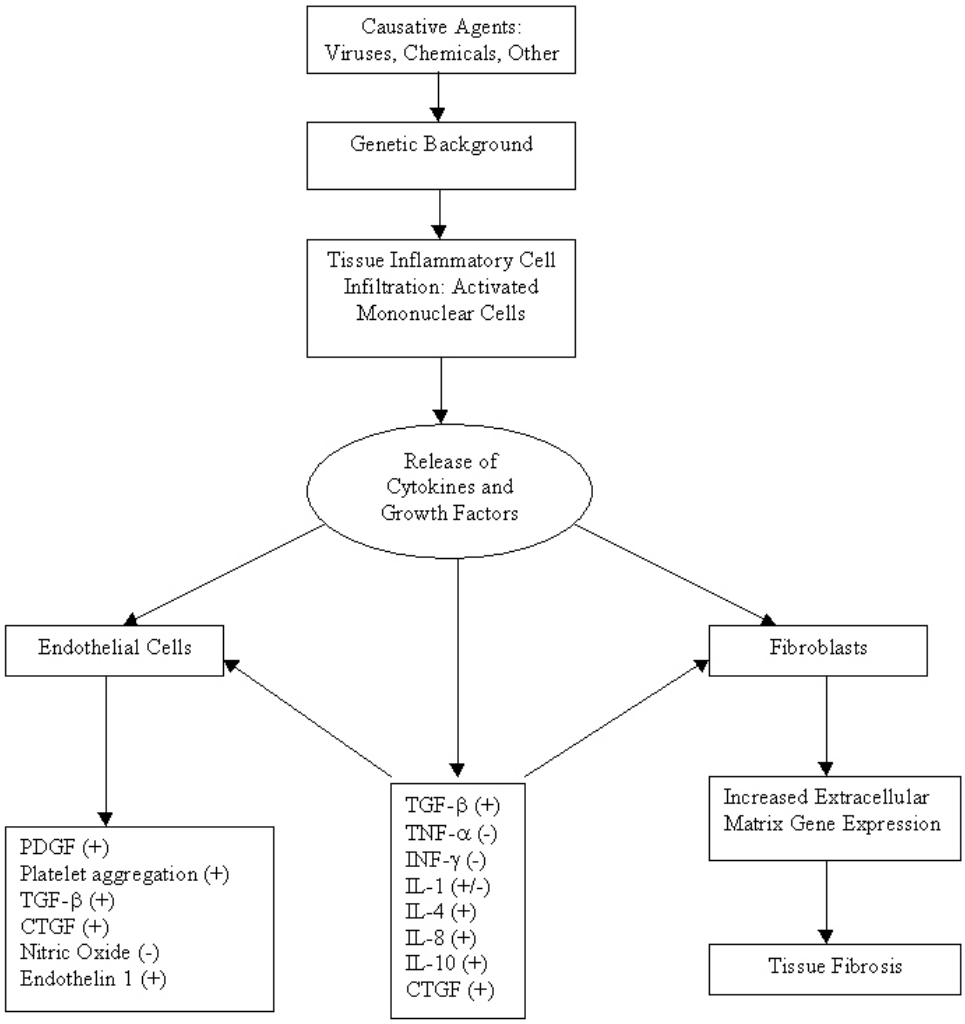

Figure 1. Diagram of the Pathogenesis of Systemic Sclerosis.

atrophy of the affected organs. The etiology of SSc remains unknown but its occurrence has been associated with numerous risk factors including various environmental agents, genetic influences, and viruses (Figure 1). At present, there is no single unifying hypothesis that explains all aspects of the pathogenesis of SSc.

2.1. Historical Account of SSc

An extensive historical account of SSc was published by Rodnan and Benedek in 1962 (1) and is summarized in the following. Descriptions of skin diseases similar to SSc were reported as early as Hippocrates (460 – 370 B.C.); however, the first credible description was by Carlo Curzio in 1755 (2). He convincingly reported a 17-year old woman who had excessive tension and hardness of skin that she could hardly move her limbs. On examination, he found her skin to be "hard like wood, or a dry hide" but the muscles under the skin were not affected. She was evidently treated with warm milk, vapor baths, bleeding and "small doses of quicksilver" and after 11 months, her skin was soft and flexible (2). Over 20 descriptive names have been proffered in the 19th century before the condition was designated as sclerodermie or scleroderma, where it gradually became the accepted term. In 1924, Matsui (3) described sclerosis in the lungs, gastrointestinal tract and kidneys and physicians realized that scleroderma is a disease that affects the entire connective tissue system including the tendons, tendon sheaths, muscles, joints and bones. Gradually increasing awareness by physicians altered the thinking that scleroderma was just a cutaneous disease and was a truly generalized manifestation. In 1945, Goetz proposed the name progressive systemic sclerosis or systemic sclerosis (4), which has now become the accepted term for this disease.

2.2. Epidemiology – Worldwide Statistics

The prevalence of disease varies by geographic region and by ethnic background. The prevalence in the United States is 242 to 286 cases per million persons (5), however in the Choctaw Indians in Oklahoma, the incidence is 469 cases per million (6). Recent population studies suggest that SSc occurs more frequently in the United States than in continental Europe where there is 158 cases per million in France (7), 11 cases per million in Italy (8), and 126 cases per million in Denmark (9). The United Kingdom has one of the lowest incidences of SSc with 82 –

88 cases per million in the United Kingdom (10). Japan has the lowest prevalence with approximately 10 cases per million (11). The incidence of SSc in Australia approximates that of the US with 230 cases per million (12). SSc has been found to cluster around industrial areas suggesting an environmental association. In southeast London, near Gatwick airport, there has been reported a significant increase in the prevalence of SSc to 146 cases per million people (13), and in southwestern Ontario it is 280 cases per million (14).

2.3. Environmental Factors

Of all the major autoimmune diseases, SSc is thought to have an environmental component in disease susceptibility and SSc or SSc-like illnesses have been associated with the exposure to a variety of chemicals. These chemicals include aromatic hydrocarbons such as toluene, xylene and benzene (15, 16), vinyl chloride, trichloroethylene, bleomycin, and silica (15, 17). Adulterated rapeseed oil and certain tryptophan products were associated with SSc-like illnesses such as toxic oil syndrome and eosinophilia-myalgia syndrome, respectively (16). Silica and silicone have been associated with the onset of SSc, however these are inert compounds. It is thought that they act as adjuvants facilitating the development of SSc (18). Vinyl chloride has been reported to induce many of the classic features of SSc including Raynaud's, sclerodactyly, pulmonary fibrosis, and capillary abnormalities in susceptible individuals (19). There have also been reports of circulating immune complexes in patients with vinyl chloride disease (20).

2.4. Genetics of Men vs. Women

The incidence of SSc in females is in great excess compared to its incidence in males, ranging from 3:1 to 8:1 (21, 22). The onset of SSc most frequently occurs between 45-55 years, however, children and the elderly can be affected. Genetic factors have also been associated with the onset of SSc. Primary interest has centered on the HLA region on chromosome 6 and most associations have been found with class II genes (23), and more recently with allograft inflammatory factor-1 in class III (24). Vinyl chloride-induced disease was found to have a DRβ1*11 association in those individuals who progressed to SSc (25) and specific HLA alleles have been associated with the autoantibody expressed (26). More recently, autoantibodies were found to be associated with the class III TNF-α gene (27).

2.5. Viruses

Viruses have been implicated in the pathogenesis of SSc and recent interest has focused on cytomegalovirus (CMV) and parvovirus (28-30). Furthermore, sequence homologies have been identified between several retroviruses and topoisomerase I, the target antigen of the SSc-specific Scl-70 autoantibody (31) and it has been shown that sera from SSc patients who have the anti Scl-70 autoantibody were able to recognize the retrovirus responsible for equine infectious anemia (32). There is some tantalizing evidence for the involvement of a pathogen in SSc, however, CMV and parvovirus are frequently found in normal individuals without disease. Evidence shows that the onset of some autoimmune diseases, is strongly associated with viral or bacterial infections (33). Viral infections may be the initial trigger for an autoimmune response (34) and/or maintain the chronicity of the autoimmune process (35). The initiation of autoimmunity may result from the activation of B and T cells via antigenic determinants shared between the pathogen and the host (molecular mimicry) (36). Cross-reactions between pathogen-derived and self-antigens have been described for multiple sclerosis (37), type 1 diabetes (38), and Guillian-Barre syndrome (39). Furthermore, the autoantibody Scl-70 that corresponds to topoisomerase I is frequently found in patients with SSc (40, 41). Topoisomerase I can be found in Vaccinia virus (42, 43) and in the virus responsible for equine infectious anemia. It has properties of eukaryotic topoisomerase I enzyme (43). In the case of molecular mimicry, the trigger for the autoimmunity would be eliminated when the pathogen is cleared, however, recurrent infections could elicit a chronic relapsing autoimmune disease. Alternatively, immune responses against unknown, or yet, unidentified viruses may appear as an autoimmune reaction when the immune system targets mainly viral antigens. Therefore, it is important to distinguish between a genuine autoimmune response directed against self-antigens and immune responses directed against persisting viral antigens that often mimic autoimmune tissue destruction, but are in fact, virus-induced immunopathology.

2.5.1. Cytomegalovirus

The putative role of CMV as an etiologic agent for the vascular alterations in SSc has been recently reviewed (29). It was proposed in 1973 that microvascular injury was one of the earliest events in the pathogenesis of SSc (44). CMV preferentially infects endothelial cells causing cellular dysfunction, leading to intimal proliferation and occlusion. CMV infection is a leading complication in allograft rejection and restenosis. In SSc IgG autoantibodies were found to bind the CMV late protein UL94 and the antibodies induced apoptosis in endothelial cells (45). Moreover, CMV was recently associated with molecular mimicry in some patients with SSc 29,45 and antibodies to CMV were found in these patients (46). However, it is not known if CMV mediates SSc, or SSc patients are more susceptible to CMV infections (46).

2.5.2. Parvovirus

Recent studies have shown the association of parvovirus in some patients with SSc. Of interest, scleroderma is considered a relatively new disease with the first convincingly described case in 1753 and this coincides with the reintroduction of parvovirus B19 into Europe (47). Furthermore, for parvovirus, it was found that the chronicity of infection was associated with particular TGF-β1 polymorphisms (48) and SSc is associated with these TGF-β1 polymorphisms (49). In all SSc patients tested, parvovirus DNA was identified by in situ PCR in the endothelia, fibroblasts, mast cells and perivascular inflammatory cells (30). The finding of parvovirus in the tissues was confirmed in another study (50). This avenue of research is provocative, however, it does not explain the

individuals who are positive for parvovirus but do not have disease.

2.5.3. Other Viruses Associated with SSc

SSc has been found to be associated with other viruses, however these reports are isolated and may be a result of coincidence. For example, a patient with chronic infection of hepatitis C was found to developed SSc (51) and five children developed localized SSc after exposure Epstein-Barr virus resulting in infectious mononucleosis (52).

Amino acid sequence homologies have been identified between autoantibodies and immunosuppressive viruses including herpes simplex virus 1 (HVS-1) and human immunodeficiency virus 1 (HIV-1) (53). The CENP-B protein (one of the anticentromere antibodies) has homology at 10 sites with HVS-1 and HIV-1 and the homologous sites are clustered in the C terminal domains of the autoantibodies. This finding suggests that co-expression of immunosuppressive viruses may generate autoantibodies that cross-react to certain nuclear proteins (53). Interesting it was found that certain retroviral products from complex retroviruses (HIV-1, HTLV-1, HSRV) were found to upregulate the expression of extracellular matrix genes (28), also suggesting that retroviruses may be involved in the pathogenesis of SSc.

2.6. SSc and Microchimerism

SSc has many clinical features similar to those of chronic graft-versus-host disease (GVHD) (22, 54-64) and, therefore, it has been postulated that SSc may be a form of chronic GVHD (59, 63-65). Skin, lung, and esophageal involvement are prominent features of both chronic GVHD (54, 55, 66) and SSc. Furthermore, immune activation and lymphocytic infiltration in affected tissues (67-69), upregulation of inflammatory cytokines (70-72), and fibrosis in the dermis and visceral organs (73, 74) characterize both diseases. Early SSc lesions appear to be similar histologically to early chronic GVHD in rats and mice. Mononuclear cells localized deep within the dermis are associated topographically with fibroblasts, which have increased collagen production with loss of dermal appendages (75).

The activation of the immune system appears to be an early event in both SSc and chronic GVHD and T cells are central to the development of tissue damage, dominate the inflammatory infiltrates. Furthermore, a recent analysis of antinuclear antibodies in chronic GVHD identified the SSc-associated antibodies, Scl-70 and Pm-Scl, in 32% of individuals with chronic GVHD who presented with clinical symptoms similar to those of patients with DcSSc (76). Anti-mitochondrial antibodies are also found in patients with SSc and in 81% of patients with chronic GVHD (77).

The hypothesis involving microchimerism in SSc was first proposed by Scott Pereira in 1989 (59). In the original publication, the authors stated that "Scleroderma has been postulated as a type of chronic GVHD resulting from transplacental transfer of cells between mother and fetus (59)…" and in a companion paper they stated that "… this could lead to a state of microchimerism and activation of such [microchimeric] cells to cause a chronic GVHD-type of disease" (65). Subsequently, other investigators (78) proposed and presented some evidence suggesting the involvement of fetal cells in the pathogenesis of SSc. However, it was not until the discovery of the presence of male fetal cells in a normal woman 27 years after the birth of her infant (79) that the involvement of fetal cells in the pathogenesis of SSc was given credence (63, 64).

The microchimeric cells found within the lesions are more likely to be T cells. The microchimeric cells in SSc biopsies were found to express T cell phenotypes (CD3, CD4, CD8, CD45RO, and CD45RA) and the activated T cell markers, class II (HLA-DR/DQ/DP) and IL-2 receptor (CD25) (80). None of the microchimeric cells in the biopsies expressed CD56 or CD68. Autologous T cells were also found recruited to the lesion site and microchimeric cells comprised between 0.2 – 6.7% of the positively stained cells (80). These frequencies of the microchimeric cells in the lesions were comparable to the frequencies of microchimeric cells in transfusion-associated GVHD (81, 82). In an elegant study by Scaletti et al. (83), microchimeric cells were isolated from the biopsy from a patient with SSc who had had a male fetus. These microchimeric cells were found to be CD4+ and reactive against the HLA of the patient (83). Clones from male-offspring cells were found to have a higher IL-4 production in response to stimulation with maternal HLA (83).

In a study investigating prior pregnancies and the onset of disease (84), it was found that patients who had never been pregnant were more likely to have disease onset at a younger age, with more severe lung involvement and a higher rate of demise (84). Further, the number of gestations prior to disease onset appeared to delay the age at which the disease manifested, but did not affect the disease progression as a whole. Many authors believe that microchimeric cells from fetuses play a part in the pathogenesis of SSc. Within this hypothesis, the cause of SSc in women who have never been pregnant is unclear, however, we speculate that there are maternal microchimeric cells from their mother mediating the disease.

More recently, another mouse model for SSc was described employing the immunodeficient RAG-2KO mouse strain transplanted with B10.D2 spleen cells (85). This model closely mimicked SSc in all clinical, histopathological, and immunological aspects. The animals showed fibrosis of the skin, gastrointestinal tract, heart, lung, and kidney, immune activation, and typical SSc-like vascular changes. Furthermore, the expression of TGF-β, a growth factor indicated in the pathogenesis of fibrosis in SSc was found to be elevated in affected tissues (85). Antinuclear antibodies were present in the majority of mice and displayed the Scl-70 specific profile. This mouse model in which there is replacement of T and B cells with chimeric cells, suggests that microchimeric cells may indeed contribute to the pathogenesis of SSc by

Table 1. Autoantibodies in Scleroderma

Antigen	Immunofluorescence Pattern	Identity	Disease and Frequency
Scl-70 (topoisomerase I)	Nuclear (diffuse fine speckles)	100 kD protein that degrades to 70 kD	30% of DcSSc 21% chronic GVHD
Kinetochore (centromere)	Centromere	18 - 400 kD proteins at the inner and out kinetochore plates	70 – 80% of CREST
RNA Polymerase I & III	Nucleolar (speckled)	13 proteins 12 – 210 kD	4% of dcSSc with internal organ involvement 22% of female Caucasian SSc
RNA Polymerase II	Nucleolar	220 kD CTD heptapeptide	SSc/SLE overlap
Fibrillarin	Nucleolar (clumpy)	34 kD protein component of U3 RNP	8% of men with SSc 48% SSc
Fibrillin 1	Nucleolar	30 kD protein	High prevalence in American Indians, Japanese and Blacks with SSc
Mitochondrial M2	Cytoplasmic (rod like)	70, 50 and 45 kD proteins	25% CREST 95% primary biliary cirrhosis High prevalence in SLE 81% chronic GVHD
PM-Scl	Nucleolar (homogeneous)	11 proteins 20 – 110 kD	17% SSc 3% SSc/myositis overlap 11% chronic GVHD

Adapted from (93).

establishing an immune activation against the host. However, in contrast with the RAG-2KO mice, which are deficient in T and B cells, patients with SSc have both T and B cells and the proportion of microchimeric cells is extremely low in number. Whether such a small population of foreign cells can mediate a systemic disease in an immunocompetent host is yet to be determined.

3. HUMORAL IMMUNOLOGY in SSc

Serologic alterations were among the first immune abnormalities noted in patients with SSc. They are one of the most common manifestations of the disease, where almost 90% of patients have anti-nuclear antibodies. Recent evidence has confirmed that sera from the majority of SSc patients contain one of three mutually exclusive, SSc-associated autoantibodies: topoisomerase I antibody (Scl-70), anti-centromere antibodies (ACA) or anti-RNA polymerase III antibody (RNAP) (26, 86). Each autoantibody is associated with the presence of distinct clinical features (40, 87-89) and certain HLA alleles (90-92). African Americans are more likely to have the DcSSc subset with Scl-70 antibody and more severe visceral complications including pulmonary fibrosis, whereas Caucasian patients are more likely to have the LcSSc subset with ACA antibody and less severe complications. Thus, while these SSc-associated autoantibodies do not appear to be directly involved in disease pathogenesis, they are associated with disease-specific pathologic phenomena. ANAs have also been detected in the relatives and spouses of patients with SSc, and some have antigens specific for scleroderma (92). More recently, the autoantibodies have been associated with TNF-α alleles (27), however, it is not clear how the TNF-α polymorphisms restrict antibody production. Antibodies most commonly identified in SSc have been outlined in Table 1 (adapted from 93).

Scl-70 has been associated with DcSSc with pulmonary interstitial fibrosis and peripheral vascular disease (40, 87), and is significantly increased in Japanese (94, 95). The differences observed in the frequency of Scl-70 in SSc are most likely due to HLA risk factors and ethnic variability.

The prevalence of ACA in SSc varies but has been found to be strongly associated with the LcSSc (87). However, ACA has also been identified in normal individuals (96) and patients with Raynaud's (40, 96), SLE (96), primary biliary cirrhosis (97), and morphea (97).

Anti-RNAP I and III antibodies are associated with female Caucasian patients with DcSSc, particularly in those patient who have internal organ involvement resulting in a poor prognosis (98-101). However, anti-RNAP I antibodies have also been identified in patients with rheumatoid arthritis (102). Antibodies to RNAP III have been detected in 45% of sera from SSc patients with DcSSc and only 6% of patients with LcSSc, and in some instances occur more frequently than Scl-70 (101).

Antibodies directed against other proteins have been reported in SSc. Pm-Scl is found in approximately 17% of patients with SSc and identifies a group of SSc patients with a high prevalence of myositis and renal involvement (98). Fibrillin 1 antibodies were detected in the majority of Japanese, African American and Choctaw Indian patients, however there are ethnic differences in antigenic epitope specificity. Fibrillarin antibodies occur in patients who develop severe fatal primary arterial hypertension (103, 104) and have been identified in a large proportion of patients with other autoimmune diseases including rheumatoid arthritis, SLE and mixed connective tissue diseases (105). Anti-mitochondrial antibodies are found in a high proportion of SSc patients with other connective tissue diseases, however, reactivity to the mitochondrial peptides appears to be disease specific (106). Antibodies to non-histone nuclear proteins or nucleolar antigens have been described and have been termed Ku, Ro (SS-A), La (SS-B) Sm, nRNP, and Jo-1 have been found in subgroups of patients with SSc (107).

4. HLA AND IMMUNOGENETICS

4.1. HLA Class II Associations

Immunogenetic studies support a more specific defect in SSc and many patients have been found to produce antibodies with well-defined target epitopes. HLA class II genes (DRB1*01, DRB1*17, DRB1*11) have been implicated as strong risk factors in the pathogenesis of SSc (reviewed in 108) and that these alleles are associated with

particular subsets and autoantibody profiles. Although DRB1*01, DRB1*17 and DRB1*11 are risk factors with SSc across several continents, it is the subsets of the disease that particularly show variation in the level of risk with HLA alleles.

Frequently, the autoantibody pattern has been found to be associated with particular HLA risk factors. Studies suggest that there is a primary association between Scl-70 (topoisomerase I antibody) and HLA class II (26, 41, 90-92, 109). Risk factors have been identified with DQB1* alleles (91, 92, 110) and DRB1*11 (26, 111) in Caucasians, DRB1*11 in Blacks (109), DRB1*15, DQB1*0601 and DQB1*0301 in Japanese (41, 109) and DRB1*16 in American Indians (109). Furthermore, a recent study has shown DPB1*1301 to be a very strong risk factor for Scl-70 (112). Anti-centromere antibody (ACA) is associated with the HLA class II DRB1*01 and DRB1*11 (87, 90, 113) or DQB1* alleles (110, 114). DRB1*01 has been found to be strong risk factor for ACA in Japanese (109).

4.2. HLA Class III Associations

Class III risk factors have centered on complement 4 null alleles, however, these alleles are in tight linkage disequilibrium with class II alleles, particularly DRB1*17, which is also a significant risk factor for SSc (115-117).

Allograft inflammatory factor-1 (AIF1) is a newly identified protein that was mapped to the short arm of chromosome 6 and clusters with other genes involved in inflammation in the class III region, within 50 Kb of the TNF-α gene. Because of the association of the AIF1 gene with the HLA region and the previously identified HLA class II associations with SSc, AIF1 was investigated in SSc. AIF1 expression was found in the active lesions in patients with SSc. Inflammatory foci within the lesions were found to strongly express AIF1. There was also weaker staining of the endothelium of small vessels. Inflammatory foci expressing AIF1 were usually found in close proximity to vessels. The normal skin did not express AIF1. AIF1 protein was found to co-localize with CD3+ cells in the lesions from patients with SSc with about 80% of CD3+ cells expressing AIF1. AIF1+/CD3+ cells were found to localize around vessels in the upper dermis and sub dermis. In contrast, normal skin contained a small number of CD3+ lymphocytes (0 – 37) in the sections analyzed, however, these cells did not express AIF1 (24).

AIF1 was originally thought to be an early inflammatory protein expressed only in monocytes and macrophages, however, it is also expressed in CD3+ lymphocytes in the skin lesions of patients with SSc. This is the first indication that this protein is involved in later inflammatory signaling. Thus, although the precise role of AIF1 in the inflammatory cascade is yet to be fully determined, it appears that AIF1 will be shown to be an important inflammatory protein in SSc. Microvascular alterations are one of the crucial components of the tissue damage in SSc and are responsible for some of the most severe clinical features. AIF1 expression in inflammatory cells surrounding the vessels may contribute to the severe microvascular alterations in SSc. Expression of AIF1 was observed in the endothelium of small vessels but not observed in vascular smooth muscle cells in SSc affected skin. Inflammatory cells surrounding the vessels had strong staining for the AIF1 protein and it is likely that this expression of AIF1 in close proximity to these vessels may induce the activation and proliferation of the vascular smooth muscle cells, thereby contributing to the vessel narrowing. Although the role of AIF1 in SSc pathogenesis is unknown, AIF1 may be an important inflammatory protein for vascular injury, which is the hallmark of SSc.

5. CELLULAR IMMUNOLOGY IN SSc

Immune activation is an early event in SSc however, it is not known whether it is the initiating event in SSc, or is secondary to other disease processes. T cells are important in the development of tissue damage and dominate the inflammatory infiltrates in the lesions early in disease. T cells regulate many of the functions of fibroblasts and endothelial cells by the production of soluble mediators or by cytotoxic effects. They also show evidence of selection and provide specificity to the immune response. There is accumulated evidence to suggest that SSc is T_H2 mediated. Although many studies have investigated T cells, T cell responses, cytokines, and the like, until recently there has not been a standardized criteria for the severity of SSc. Therefore many of the studies have been contradictory. This is because earlier research combined LcSSc and DcSSc subsets together, and early and late disease was combined. More recently, it has been recognized that early SSc is a predominantly inflammatory process, whereas in later disease course, it is more fibrotic. Therefore, a disease severity score for SSc has been established (118) and once investigators classify their patients according to the disease severity score, then consistencies in the data will likely occur.

5.1. T cells in peripheral blood

The T cell subsets in the blood from patients with SSc have been investigated extensively and the results are frequently contradictory. These contradictions may result from differences in T cell patterns in early and late stages of disease and/or with the clinical subsets. The clinical expression of SSc is heterogeneous and has different stages of expression and activation during the disease course.

In general, a larger CD4:CD8 ratio was found to be associated with a shorter disease duration and more extensive skin involvement (119). *In vivo* T cell responses to exogenous antigens appear to be normal in SSc and patients have a normal responses to immunization, cutaneous delayed-type hypersensitivity, and antibody production (120).

5.2. T cells in lesions

In early SSc lesions, lymphocytes infiltrate the skin and are scattered throughout the subcutaneous tissue and dermis or are localized around small vessels, nerves and skin appendages, whereas later in the course of disease, fibroblasts and collagen are more prominent (121-123). Immunohistochemical study of skin biopsies from patients

with SSc of recent onset have identified increased numbers of perivascular CD3+ T lymphocytes, which are mostly CD4+ helper cells expressing CD45RO+ memory phenotype and HLA class II. The T cell infiltrate precedes the findings of small vessel vasculopathy and alterations of interstitial tissues including fibroblast activation and proliferation (70). The degree of cellular infiltration correlates with the extent and progression of skin thickening (123). Fibroblasts displaying increased production of various collagens are located in close proximity to lymphocytes (124). *In vitro* studies have shown that extracellular matrix components including collagens type I, III, and VI, fibronectin, decorin and glycosaminoglycans are increased in SSc lesions (125-129).

Patients with active lung disease have increased numbers and percentages of T cells and macrophages in the interstitium (130-132) and in bronchoalveolar lavage (BAL) fluids (133-135). In contrast to the T cell repertoire in the skin, patients with alveolitis have more CD8+ T cells than CD4+ T cells in the BAL fluids.

5.3. T cell receptor (TCR) gene expression

Evidence of antigen driven TCR cell expansion in patients with SSc was determined by the analysis of the TCR gene families. White et al. showed an increase in the expression of Vδ1 gene on CD3+ T cells and γ/δ T cells in lungs (136). A restricted diversity of the TCR junctional regions were identified in Vδ1+ γ/δ T cells isolated from BAL and peripheral blood from patients with SSc compared to controls (137). Absolute numbers and percentages of Vδ1+ T cells were found to be increased in peripheral blood and in skin biopsies (138). Furthermore, these cells were activated and expressed HLA-DR and CD49d (138). In studies from the active lesions from patients with SSc with less than 18 months disease duration, Sakkas et al., found that the Vβ13, Vβ14 and Vβ21 TCR gene segments were more frequent (139). These results suggest that there is antigen-driven selection of the T cells in SSc patients.

5.4. Soluble mediators and adhesion molecules

The functions of fibroblasts and vascular cells are affected by the soluble mediators, which are secreted by cells of the immune system. Patients with SSc have increased levels of IL-2, IL-4, IL-6, IL-8, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α and platelet derived growth factor in the sera and tissues. These mediators have been implicated in the pathogenesis of SSc and not only affect fibroblasts, endothelial cells and vascular smooth muscle cells, but may also in turn be produced by some of these cells.

Along with the low numbers of CD8+ T cells patients with SSc also have low numbers of natural killer (NK) cells and natural killer cell activity is reduced (140, 141). SSc patients have diminished lymphokine-activated killer cell function in the presence of exogenous IL-2 (141, 142), and the increased serum levels of soluble IL-2 receptor may be contributory. The high levels of soluble IL-2 receptor in the serum of SSc patients could result in the chronic activation of immune cells leading to a rapid turnover of IL-2 resulting in the depletion of precursor cells responsive to IL-2 (141). Furthermore, patients with SSc have increased levels of soluble CD30 (143, 144). These levels correlate with skin score and erythrocyte sedimentation rate. Immunohistochemical analysis of the active lesions, has shown that CD4+ T cells found in the perivascular regions (138) express CD30 and IL-4 (144). CD30 is a member of the TNF receptor superfamily and the interaction of CD30 with its ligand induces proliferation of cells and activation of NF-kappa B, or cell death depending on the cell type. In SSc, increased expression of NF-kappa B in turn causes the increased expression of vascular endothelial growth factor (VEGF), thereby contributing to the vascular pathology of SSc (145). Increased VEGF is correlated with lung fibrosis (146).

The skin lesions show evidence of an active immune response associated with collagen overproduction. There is marked fibrosis with tightly packed collagen fibers and scattered infiltrates of mononuclear cells clustered around vessels, sweat glands and nerves (121, 147) with a co-ordinate increase in expression of collagens type I and type III genes in SSc fibroblasts (127). Indeed, not all fibroblasts over produce excessive collagen though, as there is a distinct fibroblast subset, which produces the high levels (148).

Fibroblasts express intercellular adhesion molecule (ICAM)-1 in response to TNF-α. Serum levels of ICAM-1 are higher in patients with DcSSc than in patients with LcSSc (149). Furthermore, *in vitro* studies of SSc fibroblasts demonstrated that ICAM-1 expression in response to cytokine stimulation (IL-1β, TNF-α, IFN-γ) was increased when compared to normal fibroblast cultures (150). ICAM-1 and HLA class II antigen can be detected by in situ hybridization on most endothelial cells and fibroblasts especially those located in areas of infiltrating lymphocytes (151).

6. PERSPECTIVE

This review gives an overview of the immunological advances in understanding the pathogenesis of SSc. In the past 30 years, SSc has evolved from the concept that it is a fibrotic disorder, to a disease that with a distinct and complex onset. Recent progress and technologies has allowed for some pieces of the puzzle to be placed, but to date, the etiology remains unknown. Regardless as to the onset of SSc, further research will uncover novel genes and processes and that these processes may lead to the development of effective therapeutic options for this incurable disease.

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