Antibodies in Scleroderma: Direct Pathogenicity and Phenotypic Associations

Lorinda Chung, MD and Paul J. Utz, MD*

Address
*Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, 1000 Welch Road, Suite 203, Stanford, CA 94305, USA.
E-mail: lorindachung@hotmail.com

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Scleroderma is an autoimmune disease involving endothelial cell damage and fibroblast overproduction of extracellular matrix. Several autoantibodies present in the sera of patients with scleroderma, including anti-endothelial cell, antifibroblast, anti–matrix metalloproteinase, and antifibrillin-1 antibodies, may directly contribute to disease pathogenesis. Scleroderma also is characterized by the presence of antinuclear and antinucleolar antibodies, which correlate with particular phenotypes. These include antitopoisomerase-I, anticentromere, antihistone, anti-polymyositis/scleroderma, anti-Th/To, anti-U3-small nucleolar ribonucleoprotein particle, anti-U1-small nuclear ribonucleoprotein particle, anti-RNA polymerase, and anti-B23 antibodies. Other antibodies classically associated with other autoimmune diseases, such as antiphospholipid, antineutrophil cytoplasmic, and antimitochondrial antibodies, also have been described in patients with scleroderma. This review will summarize the various autoantibodies associated with scleroderma, their putative pathogenic roles, and their phenotypic correlations.

Introduction

Scleroderma is a systemic connective tissue disease characterized by vascular damage and fibrosis involving the skin and other internal organs. Disease course, severity, and organ involvement are variable from patient to patient, and overlap syndromes with other autoimmune conditions can occur [1]. The pathogenesis of this disease is thought to involve three processes: 1) endothelial cell damage and intimal hyperplasia; 2) fibroblast activation and overproduction of extracellular matrix components; and 3) stimulation of the immune response, leading to production of autoantibodies [2]. The vascular pathology manifests as Raynaud's phenomenon and ischemic digital loss, while the excessive collagen deposition results in skin thickening and fibrosis in various organs, such as the lungs, heart, kidneys, or gastrointestinal tract [3•]. B cells from patients with scleroderma overexpress CD19, resulting in hyper-responsiveness and autoantibody formation [4]. Although the pathogenic role of certain autoantibodies associated with scleroderma can be explained on a molecular level, it is unclear whether other autoantibodies are primary drivers of pathology or represent a secondary response. A subset of autoantibodies correlates with clinical expression of disease and these may serve as prognostic markers.

Several clinical subtypes of scleroderma have been identified and are associated with distinct autoantibody patterns. Diffuse scleroderma is clinically characterized by skin thickening involving the face, neck, trunk, and proximal upper and lower extremities, as well as significant internal organ involvement, including the lungs, heart, gastrointestinal tract, and kidney. Diffuse scleroderma is classically associated with antitopoisomerase-I antibodies (anti-topo-I or anti-Scl-70) and the absence of anticentromere antibodies (ACA) [5]. Limited scleroderma involves skin thickening in the face, neck, and distal extremities, with frequent development of isolated pulmonary hypertension and ischemic digital loss. A form of limited scleroderma with calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias (CREST) is characterized by the presence of ACA [5]. Overlap syndromes, such as mixed connective tissue disease (MCTD), with features of systemic lupus erythematosus (SLE), polymyositis (PM), rheumatoid arthritis (RA), and scleroderma are associated with anti-U1-small nuclear ribonucleoprotein particle (U1-snRNP) antibodies [5]. This review summarizes the classical and novel autoantibodies associated with scleroderma, their putative pathogenic functions, and their correlation with disease expression.

Antibodies Involved in Disease Pathogenesis

Several autoantibodies have been discovered in the sera of patients with scleroderma that may be directly patho-

Table 1. Autoantibodies with direct pathogenicity in scleroderma

Autoantibody	Role in pathogenesis	Clinical associations	Study
Anti-endothelial cell	Incite vascular injury by inducing endothelial cell apoptosis	Ischemic digital infarcts; pulmonary arterial hypertension	Worda *et al.* [6] and Negi *et al.* [7]
Antifibroblast	Induce fibroblast production of ICAM-1 and IL-6, leading to vascular damage and ECM production	Limited scleroderma	Chizzolini *et al.* [8]
Anti–matrix metalloproteinase	Inhibit MMP-1 collagenase activity	Diffuse scleroderma	Sato *et al.* [9]
Antifibrillin-1	Induce instability of microfibrils resulting in ECM accumulation	Choctaw American Indian and Japanese ethnic backgrounds	Tan *et al.* [11] and Wallis *et al.* [12]

ECM—extracellular matrix; ICAM-1—intercellular adhesion molecule-1; IL-6—interleukin-6; MMP-1—matrix metalloproteinase-1.

genic (Table 1). These include anti-endothelial cell, antifibroblast, anti–matrix metalloproteinase (MMP), and antifibrillin antibodies. Currently, these autoantibodies are not routinely analyzed in the clinical setting. Further studies on large cohorts of scleroderma patients need to be performed to define the sensitivity and specificity of these autoantibodies.

Anti-endothelial cell antibodies

Anti-endothelial cell antibodies have been found in 28% to 71% of sera from patients with scleroderma [6] and are clinically associated with ischemic digital infarcts and pulmonary arterial hypertension [7]. These antibodies may have a direct pathogenic role by inciting vascular injury. Worda *et al.* [6] recently demonstrated that transfer of anti-endothelial cell antibody–positive sera into normal chicken embryos induced endothelial cell apoptosis in vivo. However, a sclerodermatous phenotype did not develop in these animals, implying that factors other than anti-endothelial cell antibodies are required for disease expression [6].

Antifibroblast antibodies

Autoantibodies directed against fibroblasts have been recently observed in 58% of sera from 69 patients with diffuse and limited scleroderma. These antibodies were reported to induce fibroblast activation in vitro, resulting in increased expression of intercellular adhesion molecule-1 and production of the proinflammatory cytokine interleukin-6 [8]. Intercellular adhesion molecule-1 mediates adhesion and transendothelial migration of mononuclear cells, leading to vascular damage and inflammation, whereas interleukin-6 release is associated with increased extracellular matrix production [8].

Anti–matrix metalloproteinase antibodies

The degradation of extracellular matrix is dependent on a family of endopeptidases referred to as the MMP. MMP-1 is responsible for initiating the breakdown of collagen types I, II, and III, which are overexpressed in the skin of patients with scleroderma [9]. Sato *et al.* [9] demonstrated increased levels of anti-MMP-1 immunoglobulin (Ig) G in sera from patients with scleroderma compared with healthy control subjects and patients with other autoimmune diseases. The antibody titers correlated with the degree of fibrosis in the involved tissues, with higher titers in patients with diffuse compared with limited disease, presumptively by inhibiting MMP-1–mediated collagenase activity [9].

Antifibrillin-1 antibodies

Elastic microfibrils of the extracellular matrix are partially composed of a glycoprotein component called *fibrillin-1*. The tight skin mouse represents a genetic model for human scleroderma. These mice have a mutation within the fibrillin-1 gene that is thought to be responsible for increased collagen deposition and thickening of the skin. The tight skin mice also generate autoantibodies to fibrillin-1, implicating this protein as a possible target in disease pathogenesis [10]. Tan *et al.* [11] reported the presence of antifibrillin antibodies in approximately 80% of sera from scleroderma patients of Choctaw American Indian and Japanese ethnic backgrounds; however, less than 50% of Caucasian patients possessed antibodies to fibrillin-1 antigens. Wallis *et al.* [12] showed that microfibrils from fibroblasts of patients with scleroderma are unstable, possibly because of antibodies against fibrillin-1, with resultant dysregulation of extracellular matrix accumulation.

Antibodies Associated with Clinical Phenotypes

Antinuclear antibodies

Antinuclear antibodies (ANA) are present in the sera of more than 95% of scleroderma patients, with certain clinical phenotypes associated with specific ANAs [13•]. Anti-topo-I and ACA are classically associated with scleroderma, and respectively demonstrate nucleolar/homogeneous and centromeric staining patterns on ANA screening by immunofluorescence [14]. Antihistone antibodies (AHA), most commonly associated with drug-induced lupus, can occur in patients with scleroderma,

and demonstrate a homogenous staining pattern on immunofluorescence [14]. These three autoantibodies are widely available in the clinical setting, whereas the less-common antinucleolar antibodies have limited use in the routine clinical diagnosis of scleroderma [15•].

Antitopoisomerase-I antibodies

Antitopoisomerase-I antibodies are 100% specific for scleroderma compared with normal control subjects, and 99.5% specific compared with patients with other connective tissue diseases [15•]. The sensitivity of various assays for anti-topo-I antibodies, including immunodiffusion, immunoblotting, and enzyme-linked immunosorbent assay, varies from 20% to 43% [15•]. Patients with anti-topo-I antibodies usually have diffuse cutaneous involvement, and frequently develop peripheral vascular disease, pulmonary fibrosis, cardiac involvement, and coexisting malignancies [13•]. Although no definitive role in pathogenesis has been determined for anti-topo-I antibodies, Hu *et al.* [13•] recently demonstrated that anti-topo I IgG titers fluctuate with disease activity and total skin score. These antibodies may provide prognostic information and serve as markers of disease activity.

Anticentromere antibodies

Anticentromere antibodies also are highly specific for scleroderma, though patients with primary Raynaud's phenomenon alone or with other connective tissue diseases can develop ACA [15•]. These antibodies are particularly common in patients with the subset of limited scleroderma referred to as CREST syndrome, requiring only two of the five criteria for the diagnosis [15•]. ACAs also have been associated with an increased prevalence of ischemic digital loss in patients with limited cutaneous involvement [16•]. Schachna *et al.* [16•] recently demonstrated that fragments of centromeric proteins (specifically CENP-C) produced by granzyme B–mediated cleavage in cytotoxic T lymphocytes serve as the antigenic targets of ACA. The sera used in this study were from patients with limited cutaneous scleroderma and ischemic digital loss, implicating anti–CENP-C antibodies in the pathogenesis of ischemic digital loss. Compared with anti-topo I antibodies, ACAs are associated with isolated pulmonary hypertension without pulmonary fibrosis, as well as renal involvement [14].

Antihistone antibodies

Antihistone antibodies are frequently seen in patients with drug-induced lupus and SLE. AHAs have been reported in up to 29% of patients with scleroderma and are associated with severe pulmonary fibrosis [14]. Hesselstrand *et al.* [14] observed an association with pulmonary, cardiac, and renal disease in a subset of AHA-positive patients. The presence of AHAs in patients with scleroderma may serve as a poor prognostic indicator associated with internal organ involvement and reduced survival [14].

Antinucleolar antibodies

Antinucleolar antibodies have been reported in 15% to 40% of patients with scleroderma [15•]. Although these antibodies are considered specific for scleroderma, antinucleolar antibodies can occur in other autoimmune diseases, such as SLE, PM or dermatomyositis, and RA [17]. The first of the antinucleolar antibodies to be characterized in 1977 were the anti-polymyositis/scleroderma (anti-PM/Scl) antibodies. Over the next 16 years, anti-Th/To, anti-U3-small nucleolar ribonucleoprotein particle (U3-snoRNP), anti-U1-snRNP, and anti–RNA polymerase antibodies were discovered in sera from scleroderma patients [15•]. The nucleolar protein B23 recently has been described as a novel autoantigen in scleroderma [18]. Other than anti-U1-snRNP antibodies, individual antinucleolar antibodies currently are not routinely tested for in clinical practice.

Anti-polymyositis/scleroderma antibodies

Anti-PM/Scl antibodies may be detected in sera of patients with myositis (5% to 8%), scleroderma (3%), or a PM/scleroderma overlap syndrome (24%), as well as other autoimmune diseases [19]. These patients have a high incidence of Raynaud's phenomenon, arthritis, pulmonary disease, and calcinosis. They tend to have a good response to immunosuppressive therapy and follow a benign course [19]. The antigenic complex recognized by anti-PM/Scl antibodies consists of 11 to 16 proteins, referred to as the human exosome, that are thought to play a role in ribosome synthesis in the nucleolus [20]. Two subunits have been identified as the primary targets, PM/Scl-75 and PM/Scl-100, which are homologous to exoribonucleases in yeast and *Escherichia coli* [20]. Novel components of the human exosome have been identified as targets of anti-PM/Scl antibodies, including hRrp4p and hRrp42p, possibly through epitope spreading after an initial immune response to PM/Scl-100 and PM/Scl-75 [20]. The development of autoantibodies to other antigens of the human exosome may occur as the disease progresses. Further studies to characterize the clinical associations with antibodies to the novel antigens of the PM/Scl complex should be performed.

Antibodies to small nucleolar ribonucleoprotein particles

Small nucleolar ribonucleoprotein particles are important factors in ribosomal RNA processing and modification. The particles can be divided into three groups according to conserved RNA sequences—box ribonuclease multidrug resistance protein/ribonuclease P, box C/D, and box H/ACA snoRNPs [17].

Anti-Th/To antibodies

The Th/To antigen is comprised of the two RNA processing enzymes, ribonuclease multidrug resistance protein/ribonuclease P, and 10 associated proteins [21]. Anti-Th/To antibodies occur in 4% to 13% of patients with sclero-

derma [21], and produce a bright nucleolar or nucleolar and speckled pattern on ANA testing [22•]. Previous studies had shown an association of anti-Th/To antibodies with puffy fingers, small bowel involvement, hypothyroidism, and poor survival [23]. Like ACAs, anti-Th/To antibodies are associated with limited cutaneous disease; however, these antibodies very rarely occur together [24]. Mitri *et al.* [22•] compared patients with limited scleroderma who were anti-Th/To–positive to those who were ACA-positive. In this study, anti-Th/To–positive patients had lower maximum total skin scores and less digital ischemic disease than ACA-positive patients. However, anti-Th/To–positive patients had an increased risk of pulmonary fibrosis and renal crisis [22•]. Anti-Th/To antibodies can be seen in other autoimmune diseases, such as SLE or PM [17], and may indicate the future development of an overlap syndrome with bowel, pulmonary, or renal involvement.

Anti-U3-snoRNP antibodies

The most abundant nucleolar snoRNP in mammalian cells is U3 snoRNP, which is part of the box C/D family of snoRNPs [25]. The primary target of anti-U3-snoRNP antibodies is the fibrillarin protein complex. Antifibrillarin antibodies have been considered synonymous with anti-U3-snoRNP antibodies. Antifibrillarin antibodies are up to 96% specific for diffuse cutaneous involvement [15•], although the low sensitivity associated with these antibodies, occurring in 12% to 48% of antinucleolar antibody–positive patients, limits their usefulness in diagnosis [25]. Antifibrillarin antibodies have been associated with severe disease, especially in African-Americans and men, with more frequent cardiac, renal, or gastrointestinal involvement [26]. In a study of limited scleroderma patients, those with isolated pulmonary hypertension were significantly more likely to have anti-U3-snoRNP antibodies than patients without isolated pulmonary hypertension [27]. Because antifibrillarin antibodies correlate specifically with diffuse scleroderma, this subset of anti-U3-snoRNP–positive patients with limited disease implies that fibrillarin is not the only target for these antibodies. van Eenennaam *et al.* [17] demonstrated that anti-U3-snoRNP–positive sera without reactivity against fibrillarin exists. Yang *et al.* [25] found that autoantibodies to other U3-snoRNP proteins, Mpp10 and hU3-55K, exist in sera from patients with scleroderma. Anti-hU3-55K antibodies always occurred in association with antifibrillarin antibodies, whereas a small group of patients were anti-Mpp10–positive alone. Two-thirds of these anti-Mpp10–positive patients had significant esophageal and lung involvement [25]. Other U3-snoRNP–specific proteins, such as Imp3 and Imp4, also may serve as antigenic targets of anti-U3-snoRNP antibodies [25].

Anti-U1-snRNP antibodies

The U1-snRNP complex serves as a common antigenic target in patients with scleroderma. Patients with anti-U1-snRNP antibodies usually manifest clinical features of lupus in an overlap syndrome—MCTD. Loss of these antibodies occurs during prolonged disease remission [28]. Patients who are anti-U1-snRNP–positive frequently suffer from Raynaud's phenomenon. Greidinger *et al.* [29] hypothesized that Raynaud's phenomenon leads to ischemia reperfusion, with the production of reactive oxidant species that modify the U1-70 kd antigen. They showed that sera from patients with anti-U1-snRNP antibodies and Raynaud's phenomenon had increased immune reactivity to oxidative fragments of the antigen compared with those patients without Raynaud's phenomenon [29]. This study implies that certain autoantibodies develop as a result of the disease process, leading to modification of target antigens.

In addition to antibodies directed against the protein constituents of the U1-snRNP (U1-70 kd, U1A, U1C), anti-U1-snRNA antibodies have been reported in approximately 60% of patients with scleroderma or MCTD who are anti-U1-snRNP–positive [30]. Anti-U1-snRNA antibodies are always associated with anti-U1-snRNP antibodies, supporting the concept of epitope spreading in the immunopathogenesis of scleroderma [30]. Asano *et al.* [30] showed that anti-U1-snRNA antibodies correlated with pulmonary fibrosis in patients with scleroderma or MCTD, suggesting that these antibodies may serve as an indicator for development of pulmonary fibrosis.

Anti-RNA polymerase antibodies

Antibodies to RNA polymerases (RNAP) I, II, and III have been found in sera from scleroderma patients with three typical patterns—anti-RNAP I+/III+ sera, anti-RNAP I/II/III sera, and anti-RNAP II+/topo-I+ sera [31]. Patients with RNAP antibodies frequently have diffuse cutaneous involvement and an increased incidence of renal crisis, with the highest correlation occurring in patients with anti-RNAP I/II/III specificity [31]. Although anti-RNAP+ sera can produce a nucleolar staining pattern on indirect immunofluorescence, other ANA patterns, such as speckled, can occur, making these antibodies difficult to detect [32]. Because of the correlation with high mortality related to renal disease, more sensitive assays to detect these antibodies are being developed, including an enzyme-linked immunosorbent assay using an epitope from an RNAP III subunit [32].

Anti-B23 antibodies

B23 is a nucleolar protein that functions in the regulation of cell proliferation. Anti-B23 antibodies previously had been described in association with various autoimmune diseases, including RA and SLE, as well as certain cancers [18]. Ulanet *et al.* [18] identified B23 as a novel nucleolar autoantigen in scleroderma, with antifibrillarin antibodies occurring in conjunction with anti-B23 antibodies 80% of the time. Phenotypically, B23-positive patients had an increased prevalence of pulmonary arterial hypertension

and occurred more commonly in patients with limited cutaneous disease, though they all were ACA negative. Given the association of anti-Scl-70 antibodies and anti-B23 antibodies with malignancies, one would have expected a high degree of correlation between these antibodies; however, only 20% of the B23-positive patients were anti-Scl-70–positive [18]. Studies to investigate the pathogenic role of B23 autoantibodies in scleroderma and associations with malignancy would be of great interest.

Other Autoantibodies Associated with Scleroderma

Antiphospholipid antibodies

The antiphospholipid syndrome is characterized by an increased risk of arterial and venous thromboses and recurrent fetal loss. Patients develop anticardiolipin antibodies, the most common being anti-beta2 glycoprotein I (GPI) antibodies, or lupus anticoagulants. This syndrome can be idiopathic or occur in association with a connective tissue disease, most commonly SLE [1]. Anticardiolipin antibodies have been reported in 0% to 25% of patients with scleroderma [33]. Anti-beta2-GPI antibodies occur in 10% to 23% of scleroderma patients and are significantly associated with isolated pulmonary hypertension [34]. Pope and Thompson [35] had shown previously that anticardiolipin antibodies in patients with scleroderma rarely manifest clinically with thrombotic events [35]. Antonioli *et al.* [36] performed a retrospective study on 60 patients with scleroderma and reported eight thrombotic events, five of which were associated with anticardiolipin or anti-beta2-GPI antibodies [36]. The authors did not comment on the lupus anticoagulant status of the other three patients with a history of thrombosis. Further studies should be done to investigate why patients with scleroderma have fewer thrombotic events associated with antiphospholipid antibodies than patients with SLE. One may hypothesize that the endothelial cell damage associated with scleroderma results in the release of antithrombotic factors.

Antineutrophil cytoplasmic antibodies

Scleroderma is rarely associated with vasculitis, but several cases of antineutrophil cytoplasmic antibody (ANCA)-associated disease have been reported. Endo *et al.* [37] described six patients with normotensive renal failure who were perinuclear ANCA– (pANCA) and anti-myeloperoxidase (MPO)–positive, two of whom also had pulmonary hemorrhage [37]. Wutzl *et al.* [38] reported a case of pANCA/anti-MPO–associated pulmonary/renal syndrome in a patient with an 18-year history of diffuse scleroderma. Miyata *et al.* [39] described two cases of MPO-ANCA–associated rapidly progressive glomerulonephritis, one patient with CREST syndrome, and the other with diffuse systemic sclerosis. These reports suggest that ANCAs in scleroderma target MPO alone and correlate with renal disease. Casari *et al.* [40] performed a retrospective study on 168 patients with scleroderma or an overlap syndrome and found five patients who were positive for pANCA. One patient was initially cytoplasmic ANCA–positive, but later converted to pANCA positivity, and only three patients had anti-MPO antibodies, two of whom had renal disease. This study implies that antigenic targets other than MPO exist in ANCA-associated diseases in scleroderma, and that ANCA positivity does not necessarily correlate with glomerulonephritis. Khanna *et al.* [41] recently showed that ANCA-positive sera from scleroderma patients frequently demonstrate an atypical pattern of staining. The antigenic targets were against bactericidal/permeability-increasing protein, a protein that plays a role in bactericidal effects against gram-negative organisms, and cathepsin G, a neutrophil protease that functions in platelet activation and degranulation. In addition, none of the patients with anti-MPO antibodies in this study had renal disease [41]. Further studies on larger patient populations will help clarify the clinical phenotypes associated with ANCAs directed against the various antigens.

Antimitochondrial antibodies

Antimitochondrial antibodies (AMA) are present in the sera of 95% of patients with primary biliary cirrhosis (PBC), a progressive liver disease characterized by destruction of intrahepatic bile ducts resulting in liver failure [42]. Studies have shown that 3% to 17% of patients with PBC also have features of scleroderma, most commonly of the CREST variant [42]. Tojo *et al.* [43] described a subgroup of PBC patients who had associated CREST symptoms and a better prognosis than patients with PBC alone [43]. These patients develop AMA and ACA, with no crossreactivity between the two autoantibodies [44]. Conversely, 7.6% to 25% of patients with scleroderma have AMA, which target the 70-kd autoantigen of PBC [42]. Patients with scleroderma who have a positive AMA may be at risk for developing PBC in the future.

Novel autoantibodies

New autoantibodies associated with scleroderma have been recently described. Katsumata *et al.* [45] recently reported a case of severe thrombocytopenia with megakaryocytic hypoplasia in a patient with a 23-year history of limited scleroderma associated with pulmonary fibrosis and Raynaud's phenomenon. Amegakaryocytic thrombocytopenia is an extremely rare complication of scleroderma, with only five cases, including this one, reported over 25 years. The authors demonstrated high titers of anti-c-Mpl antibodies in the patient's serum, with substantial reduction in antibody titer after improvement in the platelet count with steroids and intravenous immunoglobulin therapy. Anti-c-Mpl antibodies block the binding of thrombopoietin to its receptor, c-Mpl, and thereby inhibit thrombopoiesis. Although amegakaryocytic thrombocytopenia rarely occurs in patients with scleroderma, anti-c-

Table 2. Autoantibodies and their clinical associations in patients with scleroderma

Autoantibody	Cutaneous involvement	Clinical associations	Study
Antitopoisomerase-I	Diffuse	Pulmonary fibrosis, peripheral vascular disease, cardiac involvement, and malignancies	Hu *et al.* [13•] and Reveille and Solomon [15•]
Anticentromere	Limited	CREST syndrome, ischemic digital loss, isolated pulmonary hypertension, and renal disease	Hesselstrand *et al.* [14], Reveille and Solomon [15•], and Schachna *et al.* [16•]
Antihistone	Limited>diffuse	Severe pulmonary fibrosis, cardiac disease, renal disease, and poor prognosis	Hesselstrand *et al.* [14]
Anti-polymyositis/ scleroderma	Diffuse or limited	Severe Raynaud's phenomenon, arthritis, pulmonary disease, calcinosis, myositis, and benign course	Brouwer *et al.* [19,20]
Anti-Th/To	Limited	Puffy fingers, small bowel involvement, hypothyroidism, pulmonary fibrosis, and renal crisis	van Eenennaam *et al.* [21], Mitri *et al.* [22•], Okano and Medsger [23], and Kuwana *et al.* [24]
Anti-U3-snoRNP (fibrillarin, Mpp10, hU3-55K)	Diffuse (fibrillarin) or limited	African-American ethnicity, cardiac disease, renal disease, and gastrointestinal involvement	Reveille and Solomon [15•], Yang *et al.* [25], Arnett *et al.* [26], and Steen and Medsger [27]
Anti-U1-snRNP proteins; anti-U1-snRNA	Diffuse or limited	Severe Raynaud's phenomenon, MCTD, and pulmonary fibrosis (U1-snRNA)	Burdt *et al.* [28], Greidinger *et al.* [29], and Asano *et al.* [30]
Anti-RNA polymerase	Diffuse	Renal crisis	Harvey *et al.* [31] and Kuwana *et al.* [32]
Anti-B23	Limited>diffuse	Pulmonary arterial hypertension and malignancies	Ulanet *et al.* [18]

CREST—calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly, and telangiectasias; MCTD—mixed connective tissue disease; snRNA—small nuclear RNA: snRNP—small nuclear ribonucleoprotein particle; snoRNP—small nucleolar ribonucleoprotein particle.

Mpl antibodies could be useful in identifying patients with borderline thrombocytopenia who may be at risk for developing this complication.

Cytoplasmic linker protein (CLIP)-170 antibodies were described in one patient with CREST syndrome who demonstrated a speckled cytoplasmic staining pattern on indirect immunofluorescence. CLIP-170 enables the interaction between endosomal vesicles and cytoplasmic microtubules [46]. Although further studies are being conducted in large cohorts of patients with autoimmune diseases, anti–CLIP-170 antibodies may identify a subset of patients with CREST syndrome who are ANA- and ACA-negative.

Conclusions

Scleroderma is a disease characterized by the production of autoantibodies. The clinical relevance of autoantibodies in the diagnosis and prognosis of autoimmune diseases has been a topic of investigation for many years, highlighted by recent discoveries in three other autoimmune diseases. First, Berger *et al.* [47] showed that the presence of antimyelin oligodendrocyte glycoprotein and antimyelin basic protein antibodies in patients with clinically isolated syndrome (a precursor to multiple sclerosis) have predictive value in determining the risk to develop multiple sclerosis. Arbuckle *et al.* [48•] demonstrated that autoantibodies are present up to 9.4 years before the diagnosis of SLE. They demonstrated that the accumulation of specific autoantibodies occurs in a predictable manner, with antinuclear, anti-Ro, anti-La, and antiphospholipid antibodies preceding the development of anti–double-strand DNA, anti-Sm, and antinuclear ribonucleoprotein antibodies. Finally, Rantapaa-Dahlqvist *et al.* [49] demonstrated that anticyclic citrullinated peptide and IgA rheumatoid factor predict the development of RA, with increasing titers of the autoantibodies correlating with the onset of symptoms.

This review of autoantibodies in scleroderma highlights the possible use of these antibodies in clinical practice. Several autoantibodies, including anti-Scl-70, anticentromere, anti-RNAP I/III, antifibrillarin, and anti-Th/To antibodies, have been found to be specific for scleroderma, and therefore helpful in differentiating this disease from other connective tissue diseases [15•]. However, the low sensitivity of anti-Scl-70 and ACA assays, and the low frequency of the antinucleolar antibodies, limits their usefulness in diagnosis before clinical signs develop. Because Raynaud's phenomenon can be idiopathic, it would be

useful to determine the autoantibodies that occur early in the disease process and predict the risk to develop scleroderma. Table 2 shows the phenotypic associations with various autoantibodies in scleroderma. These clinical associations may be useful in determining the patients that will be more likely to develop severe disease. Titers of anti-MMP-1 and anti-topo-I antibodies have been shown to correlate with the degree of fibrosis and skin thickness [9,13•], implying that these antibodies may be useful as markers of disease activity.

Certain autoantibodies discussed here, including anti-endothelial cell, antifibroblast, anti-MMP, and antifibrillin antibodies, have been hypothesized to be directly pathogenic in scleroderma. Other antibodies, such as ACA and anti-U1-snRNP antibodies, may develop against autoantigens that have undergone modification as a result of the disease process. Autoantibodies also develop against new antigens through epitope spreading. Alternatively, several autoantibodies in scleroderma may exist as epiphenomena without any relationship to disease pathogenesis. Studies of serially collected sera from scleroderma patients over a span of years, before development of disease and after the clinical diagnosis is made, would clarify the autoantibodies that develop as a cause or result of the disease process. One would expect a predictable accumulation of autoantibodies over time, as has been shown to occur in SLE.

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