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Invited Review

Putative functional pathogenic autoantibodies in systemic sclerosis

Devis Benfaremo, Silvia Svegliati Baroni, Lucia Manfredi, Gianluca Moroncini, Armando Gabrielli

Abstract

Systemic sclerosis (scleroderma, SSc) is a systemic disease characterized by vascular lesions, fibrosis, and circulating autoantibodies. A complex interplay between innate and adaptive immunity, and with regard to the latter, between humoral and cellular immunity, is believed to be involved in SSc pathogenesis. Lately, close attention has been paid to the role of B cells which, once activated, release profibrotic cytokines, promote profibrotic Th2 differentiation, and produce autoantibodies. Several novel interesting autoantibodies, targeting antigens within the extracellular matrix or on the cell surface, rather than the nuclear antigens of canonical SSc-autoantibodies, have been recently described in patients with SSc. As they show stimulatory or inhibitory activity or react with structures involved in the pathogenesis of SSc lesions, they can be considered as potentially pathogenic. In this paper, we will review those which have been better characterized.

Keywords: Systemic sclerosis, autoantibodies, scleroderma, platelet-derived growth factor, endothelial cells

Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by vascular alterations, progressive and extensive fibrosis, and the presence of circulating autoantibodies directed to several cellular and extracellular autoantigens (1). The pathogenic mechanisms involved in SSc are unclear, despite the recent progress in the treatment of its complications (2). Several genetic susceptibility loci, encoding cytokines, cytokine receptors, chemokines, and extracellular proteins have been reported to be associated with SSc (3-6), as well as environmental factors (7) and infectious agents (8). The presence of autoantibodies is one of the most common manifestations in SSc, being observed in more than 90% of patients (9). Several autoantibodies have been recognized for their value in the diagnosis of SSc, clinical subset classification, and for predicting organ involvement. Antibodies to DNA topoisomerase I (ATA), also known as anti-Scl-70, are associated with diffuse cutaneous SSc (dcSSc), severe fibrosis, interstitial lung disease, and digital ulcerations.

Anti-centromere antibodies (ACA) are most commonly associated with limited cutaneous SSc (lcSSc), and may be associated with pulmonary arterial hypertension (PAH). Anti-RNA polymerase III is also an important biomarker, associated with severe accelerated dcSSc, risk of scleroderma renal crisis, gastric antral vascular ectasias and malignancy (10, 11).

Other autoantibodies, including anti-polymyositis scleroderma (PM-Scl), anti-fibrillarin (U3 RNP), and anti-Th/To ribonucleoprotein (Th/To) are sometimes associated with overlap myositis and severe lung disease characterized by both fibrosis and vascular diseases.

Although the diagnostic and prognostic value of ATA, ACA, and other antibodies is clear (12), their role in the pathophysiology of vasculopathy, tissue fibrosis, and organ dysfunction has not been completely elucidated.

Autoantibodies can be considered pathogenic when they contribute to the development of an autoimmune disease and mediate the disease manifestations (13). Functional autoantibodies are pathogenic when they bind to their cognate autoantigen, stimulating (agonistic effect) or inhibiting (antagonistic effect) a specific molecular pathway.

ORCID iDs of the authors:
D.B. 0000-0002-9867-2360;
S.S.B. 0000-0001-6863-2600;
L.M. 0000-0002-9313-2197;
G.M. 0000-0002-0380-0105;
A.G. 0000-0002-4509-8772.

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Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy

Address for Correspondence: Armando Gabrielli; Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy

E-mail: a.gabrielli@staff.univpm.it

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Several criteria that should be fulfilled in order to demonstrate the putative pathogenicity of autoantibodies have been proposed (14). In general, the experimental evidence should derive from both *in vitro* and *in vivo* studies. The autoantibodies in question should be specific to the disease, precede its development, and cause disease manifestations when introduced into a healthy subject (or an experimental animal model).

In this review, we will discuss the existing evidence regarding the putative pathogenicity of autoantibodies in SSc.

Autoantibodies against endothelial cells

Anti-endothelial cell antibodies (AECA) have been identified in the sera of patients with autoimmune and connective tissue diseases as well as in patients with diabetes mellitus, multiple sclerosis, and pre-eclampsia (15). Although AECA are not specific to SSc, several studies have reported their association with lung and vascular involvement in patients with SSc (16-18), and the capacity of immunoglobulin G (IgG) from AECA-positive patients with SSc with PAH to induce activation *in vitro* of human umbilical vein endothelial cell (HUVEC) with higher expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin; and the production of interleukin (IL)-6, IL-8, and C-C motif chemokine ligand 2 was considered as evidence for the role of AECA to cause vascular damage and inflammation. *In vivo*, transfer of AECA-positive serum from University of California at Davis Line 200 chickens, an animal model of human SSc, but not AECA-negative serum, resulted in induction of endothelial cell apoptosis in healthy chicken embryos (19).

The interpretation of this study, as of others discussed below, is not immediate because of the experimental use of total serum IgG that includes a multitude of different antibodies targeting many different antigens.

Main Points

- Systemic Sclerosis (SSc) is an autoimmune disorder characterized by complex pathogenetic mechanisms that have not been completely elucidated.
- Recent studies have demonstrated that several autoantibodies against different targets may play a pathogenic role in SSc.
- The identification of such functional pathogenic autoantibodies may shed light into the intricate pathogenetic landscape of SSc.

The exact nature of the antigen targeted by AECA has been elusive, preventing a precise identification of the mechanisms involved in the vascular damage occurring in SSc. Hill et al. (20) reported that the antibody reacting with the HUVEC membrane possesses anti-centromere activity, and Servettaz et al. (21), using a quantitative immunoblotting technique, showed that centromere protein B may be the main target of AECA in patients with lcSSc. Interestingly, Lunardi et al. (22) reported the presence of circulating antibodies recognizing the human cytomegalovirus (CMV) late protein UL94 in patients with SSc. These antibodies induced endothelial cell apoptosis *in vitro* by cross reacting with the cell surface tetraspanin transmembrane 4 superfamily member 7 (Nag-2) molecule, suggesting a link between CMV infection and anti-EC humoral immunity. The same group subsequently showed that Nag-2 is also expressed on dermal fibroblasts and that anti-Nag-2 antibodies, upon binding to fibroblasts, induced upregulation of 989 transcripts including genes involved in extracellular matrix (ECM) deposition and encoding growth factors, chemokines and cytokines (23). Vascular damage, fibrosis, and autoantibodies were, thus, connected. No evidence has been so far provided in experimental animals that these mechanisms are active *in vivo*.

Another potential pathogenic role of AECA is their ability to induce apoptosis in human dermal microvascular endothelial cells, but not in HUVEC, in the presence of activated NK cells via the Fas pathway (24).

It is also worth recollecting the report on anti-ICAM-1 antibodies in 32% of patients with dcSSc and 39% of patients with lcSSc by enzyme-linked immunosorbent assay. Interestingly, the exposure of HUVEC to anti-ICAM-1 antibodies induced increased generation of reactive oxygen species (ROS) and VCAM-1 expression (25). All these findings suggest that AECA also include antibodies targeting ICAM-1, which are responsible for pro-inflammatory activation of HUVEC, and thus may contribute to SSc vascular lesions.

A general mechanism by which AECA may cause SSc lesions linking endothelial cell apoptosis and fibrosis has been proposed (26). Apoptotic endothelial cells would release soluble mediators responsible for the induction of an anti-apoptotic phenotype in fibroblasts. Besides resistance to apoptosis, dermal fibroblasts would acquire a myofibroblast phenotype that constitutes the cellular basis of a persistent pro-fibrotic response. Interestingly, in the same study, human fibroblasts derived from SSc skin lesions were found to be more sensitive to the anti-apoptotic activities of mediators produced by apoptotic endothelial cells than normal fibroblasts. The molecular pattern of resistance to apoptosis in fibroblasts was reproduced by a synthetic peptide containing an endothelial growth factor (EGF) motif present on the C-terminal fragment of perlecan. Thus, persistent apoptosis of endothelial cells would induce and maintain fibrosis in SSc.

Autoantibodies against angiotensin II type 1 receptor and endothelin-1 type a receptor

Angiotensin II type 1 receptor (AT1R) and endothelin-1 type A receptor (ETAR) are widely expressed on cells of the vascular system and on immune cells. Functional anti-AT1R autoantibodies were first isolated from the sera of women with pre-eclampsia, and their agonistic activity was demonstrated by the chronotropic effect induced on cultured, spontaneously beating, neonatal rat cardiomyocytes, which was completely inhibited by the selective receptor antagonist losartan (27, 28). Subsequent studies showed that anti-AT1R autoantibodies stimulate AT1R on several cell types, inducing biological responses relevant to the pathophysiology of vascular diseases, such as malignant hypertension, renovascular diseases, and renal allograft rejection (29, 30). Anti-ETAR autoantibodies were first identified in sera from patients with idiopathic PAH (31).

Later on, anti-AT1R and anti-ETAR were also identified in patients with SSc. Using a solid phase assay, Riemekasten et al. (32) found anti-AT1R and anti-ETAR antibodies in about 85% of patients with SSc. The antibody levels strongly correlated with each other and showed cross-reactivity for both receptors. At the clinical level, higher levels of anti-AT1R and anti-ETAR antibodies were associated with severe SSc vascular manifestations such as digital ulcers and PAH (32). Becker et al. (33) subsequently reported that anti-AT1R and anti-ETAR antibodies were more frequent in PAH associated with SSc or other connective tissue diseases compared with other forms of pulmonary hypertension and might serve as prognostic and predictive biomarkers for cardiovascular complications and mortality.

Interestingly, these autoantibodies-induced extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) phosphorylation and increased the expression of transforming growth factor beta (TGFβ) in human dermal microvascular endothelial cells (32). Furthermore, when exposed to anti-AT1R and anti-ETAR antibodies, human microvascular endothelial cells showed evidence of increased expression of IL-18 and

VCAM-1 (34). In the subsequent studies, the presence of AT1R and ETAR was also described on human peripheral T cells, B cells, and monocytes, and their interaction with IgG from patients with SSc was responsible for increased production of IL-8 and the CC-chemokine ligand 18 (35).

In vivo, repeated intravenous administrations of total IgG from patients with SSc (positive for anti-AT1R and anti-ETAR antibodies) induced thickening of airway vessels and elevated the cell density in interstitial tissue in healthy mice. Moreover, increased neutrophil count was found in the bronchoalveolar lavage of SSc IgG-treated mice as compared with HC IgG-treated mice, whereas no differences were observed for macrophages or lymphocytes (34).

Although interesting, these studies did not evaluate collagen expression and, more importantly, the total SSc IgG preparation that was administered might contain other agonistic antibody specificities in addition to the anti-AT1R and anti-ETAR.

Autoantibodies against muscarinic-3 receptor

Gastrointestinal (GI) involvement leading to dysmotility is frequent in SSc as a result of disturbance of cholinergic neurotransmission and smooth muscle atrophy. Acetylcholine secreted after stimulation of the muscarinic-3 receptor (M3R) is the principal excitatory mediator of GI tract motility acting on intrinsic neurons in the myenteric plexus. Antibodies blocking M3R would, therefore, inhibit excitatory enteric neurotransmission causing dysmotility.

Following this hypothesis, preliminary studies found a high prevalence of anti-myenteric neuronal antibodies in the sera of patients with SSc with GI symptoms (36) and demonstrated that passive transfer of these antibodies into a rat model significantly disrupted intestinal myoelectric activity (37). Finally, IgG from patients with SSc attenuated the M3R activation in smooth muscle cells of a rat internal anal sphincter (38).

Moreover, the administration of IgG fractions from patients with SSc (and also from patients with Sjögren's syndrome) into mice inhibited the contraction of the colonic smooth muscle caused by carbachol-induced activation of M3R in a concentration dependent fashion, further supporting earlier observations on the possible antagonistic function of anti-M3R autoantibodies (39). Similar results have been reported by Kawaguchi et al. (40) using an enzyme immunoassay. In this study, anti-M3R antibodies were found in 9/14 early-onset patients with SSc with severe GI tract involvement compared with only 3 positive out of 62 early-onset patients with SSc without severe GI tract involvement.

However, 43 patients had GI tract involvement but no circulating anti-M3R autoantibodies, implying that other mechanisms might be responsible for intestinal involvement in patients with SSc. Interestingly, Kumar et al. (41) demonstrated that in the early stage of the disease, patients with SSc had antibodies blocking the ganglionic cholinergic neurotransmission but as the disease progressed, inhibition of acetylcholine action occurred at smooth muscle cell membrane. How these events lead then to fibrosis remains to be elucidated.

Interestingly, using intact rat colon smooth muscle strips, SSc-associated GI dysfunction at both the neuropathic and myopathic stages may be potentially reversible with the administration of intravenous immunoglobulin (41, 42).

However, using a novel luminescence-based assay to detect functionally active antibodies to M3R failed to find antibodies inhibiting carbachol-induced activation of M3R in a cohort of 47 patients with SSc (43).

Autoantibodies against platelet-derived growth factor receptor

Platelet-derived growth factor receptor (PDGFR) is a cell surface tyrosine kinase receptor mediating the activation of different cell types that are involved in SSc pathogenesis, including fibroblasts and smooth muscle cells. In normal fibroblasts, the activation of PDGFR by PDGF triggers an increased production of ROS, which, in turn, activates ERK1/2 pathway and the downstream viral Harvey rat sarcoma (Ha-Ras) gene. Activation of ERK1/2 and high ROS levels stabilize the Ha-Ras protein by inhibiting proteasomal degradation. As compared with normal cells, fibroblasts in SSc are characterized by an amplified and persistent ROS-ERK1/2-Ha-Ras signaling loop, which stimulates excessive collagen synthesis (44, 45). Moreover, the accumulation of collagen I consequent to the activation of the Ha-Ras pathway in SSc fibroblasts is independent of TGFβ stimulation (46).

Thus, it was hypothesized that the presence of a factor unrelated to PDGF or TGFβ, such as an agonistic autoantibody against PDGFR, could sustain the profibrotic phenotype of SSc fibroblasts.

Anti-PDGFR antibodies were actually detected in total serum IgG purified from the sera of 46 patients with SSc (47). These autoantibodies were able to immunoprecipitate PDGFR from human fibroblasts, stimulate production of ROS, and activate the ROS-ERK1/2-Ha-Ras loop in mouse embryo fibroblasts expressing human PDGFRα (Fα) but not in PDGFRα negative cells (F-/-). Importantly, IgG purified from the serum of patients with other connective tissue diseases, used as negative controls, did not display these binding and stimulatory activities.

As other groups failed to replicate these data (48, 49), mostly because of the use of different read-out methods based on different cell lines (50), a more reliable characterization of these anti-PDGFR antibodies was necessary.

Indeed, the generation of human monoclonal antibodies using memory B cells from a single patient with SSc allowed the identification of the major epitopes recognized by anti-PDGFRα antibodies and the development of a specific enzyme immunoassay (51). Surprisingly, four different monoclonal antibodies were identified, with similar heavy chains but different light chains. Each monoclonal antibody showed a peculiar binding and functional activity toward PDGFRα, ranging from low affinity binding without agonistic activity toward fibroblasts to high affinity binding with induction of the ROS-ERK1/2-Ha-Ras loop and increased collagen gene transcription in human fibroblasts *in vitro* (51, 52). Using a large PDGFRα peptide library, it was possible to identify the conformational PDGFRα epitope recognized by the antibody with agonistic activity, i.e., a discontinuous motif encompassing three distinct sequences across the second and third extracellular PDGFRα domains, largely overlapping the PDGF binding region. On the contrary, the non-agonistic antibodies targeted one linear aminoacidic sequence in the first extracellular PDGFRα domain (52).

In addition, anti-PDGFRα antibodies with receptor affinity as high as that of the collagen-inducing monoclonal autoantibody can be detected by ELISA in the sera of patients with SSc. Using this technique, anti-PDGFRα antibodies were detected in 66 of 70 patients with SSc (94.3%), 63 of 130 healthy controls (48.5%), 11 of 26 patients with primary Raynaud's phenomenon (42.3%), and 11 of 29 patients with SLE (37.9%). Importantly, IgG purified from ELISA-positive SSc serum samples turned out to be positive in the ROS bioassay also, whereas IgG purified from ELISA-positive healthy controls serum samples did not (51).

Overall, these findings indicate that both agonistic and non-agonistic anti-PDGFRα autoan-

tibodies may be produced, and even coexist, in the same patient with SSc. Moreover, the anti-PDGFRα autoantibodies that can also be detected in healthy subjects or patients affected by other connective tissue diseases are non-agonistic, and thus they should be considered as natural autoantibodies (53). Conversely, agonistic anti-PDGFRα autoantibodies recognize specific conformational epitopes, largely overlapping the PDGF binding region, suggesting their pathogenic role in the SSc-specific, unbalanced autoimmune response against cellular antigens.

A small clinical study provided the first indirect evidence of anti-PDGFRα antibody pathogenicity *in vivo*. Six patients with SSc with severe skin fibrosis, unresponsive to canonical immunosuppressive therapies, were treated with 375 mg/m^2 per week of rituximab for a total of four doses. A good clinical response, evaluated as decrease of the skin score and improvement of the disability indices observed in all patients, was observed, as well as a significant reduction of ROS stimulatory activity *in vitro* by IgG purified from the patients' sera. Furthermore, fibroblasts derived from skin biopsies performed at baseline and after 3 and 6 months showed downregulation of specific intracellular signaling pathways and type I collagen gene expression (54).

A subsequent study provided the first direct *in vivo* evidence of anti-PDGFR antibody pathogenicity. Three-dimensional bioengineered skin samples containing human keratinocytes and fibroblasts isolated from skin biopsies of healthy donors were generated and grafted onto the back of severe combined immunodeficiency mice. The dermis of the skin grafts was then injected with total IgG purified from the serum of either patients with SSc (SSc IgG) or healthy controls (HC IgG), i.e., either with the agonistic, collagen-inducing anti-PDGFRα monoclonal antibody or with the non-agonistic one. Strikingly, the injection of SSc IgG, but not of HC IgG, induced increased deposition of type I collagen and upregulation of fibroblast activation markers in healthy donor skin grafts. These findings demonstrated that the agonistic anti-PDGFRα antibodies, and not the non-agonistic ones, are profibrotic *in vivo* (55).

Agonistic anti-PDGFRα autoantibodies (both total IgG and the collagen-inducing monoclonal antibody mentioned earlier) have also been recently demonstrated to induce proliferation and migration of human pulmonary vascular smooth muscle cells (VSMCs) *in vitro* (56). The activation of PDGFR by SSc IgG was both selective and ROS dependent. Similar findings showing that serum IgG from patients with SSc induces contraction of VSMCs in a collagen matrix, in contrast with IgG from healthy controls, had been previously reported (57, 58), although in the latter study, SSc IgG, even if engaging PDGFR, leads to the activation of the EGFR through a PDGFR-independent pathway (58).

These findings are important as they further corroborate the hypothesis that anti-PDGFRa antibodies have agonistic activity and, therefore, they may contribute to the pathogenesis of SSc and, potentially, of SSc-associated vasculopathy.

Other autoantibodies

Several additional putative pathogenic autoantibodies have been described, but evidence is currently limited to clinical associations and *in vitro* experimental studies.

Anti-fibrillin antibodies have been investigated in several studies. In 1999, Tan et al. (59) reported a high prevalence of anti-fibrillin antibodies in Japanese patients with SSc; however, their presence was much lower in Caucasian and African-American subjects. Ethnic differences in epitope specificity of anti-fibrillin antibodies were later reported (60), but the presence of these autoantibodies was not associated with specific subsets of the disease or clinical manifestations. Experimental data *in vitro* showed that affinity-purified anti-fibrillin antibodies from patients with SSc induced increased expression of collagen and several other ECM components in normal human fibroblasts, and neutralization of TGF-β1 significantly diminished their activation (61). Other groups failed to replicate these findings in the Caucasian patients with SSc (62).

Weigold et al. (63) first reported the detection of anti-CXCR3 and anti-CXCR4 antibodies in patients with SSc. These autoantibodies were associated with dcSSC and ATA positivity and negatively correlated with lung function parameters, thus predicting a less severe lung disease. Experimental data are limited to the observation that autoantibodies from patients with SSc preferentially bind intracellular epitopes of CXCR3, whereas in HC, they target an extracellular epitope (64).

Anti-estrogen receptor (ER) antibodies have been detected in a variety of autoimmune diseases, including SSc, where they have been associated with dcSSC, positive ATA, and disease activity (65). *In vitro*, the percentage of activated T regulatory cells was significantly higher in anti-ER positive than in anti-ER negative patients, suggesting that these autoantibodies may act as functional modulators of the immune system.

Functional anti-CD22 antibodies have been detected by the ELISA assay in 22% of patients with SSc, although they are not specific to SSc (66). *In vitro* assays suggested that anti-CD22 antibodies were able to stimulate B cell response in both in patients with SSc and SLE.

Finally, Sato et al. (67) first reported the presence of anti-matrix metalloproteinase-1 (MMP-1) and anti-MMP-3 antibodies in patients with SSc (68). *In vitro* studies showed that total serum IgG from anti-MMP-1 or anti-MMP-3 positive patients with SSc inhibited the activity of MMP-1 and MMP-3, respectively, suggesting their potential role in the impaired ECM turnover in the pathogenesis of SSc.

Conclusion

The detection of several putative pathogenic serum autoantibodies in patients with SSc supports the hypothesis that targeting the production of autoantibodies or preventing their functional activity may provide advantages in the management of this heterogeneous condition lacking disease-modifying therapies (69-71).

The identification of autoantibodies with specific functional properties may help identify different clinical variants of the disease as well as shed light on SSc pathogenesis. Unraveling the pathways triggered or inhibited by functional autoantibodies may, in turn, pave the way for novel therapeutic approaches. These therapeutic strategies should preferably rely on selective targeting of the receptors rather than on an unselective block of total receptor activity, possibly burdened with significant adverse effects.

Further studies with robust animal models are needed before definitive conclusions on the putative pathogenicity of these autoantibodies can be drawn.

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References

1. Gabrielli A, Avvedimento E V, Krieg T. Scleroderma. N Engl J Med 2009; 360: 1989-2003. [Crossref]
2. Varga J, Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: Recent insights of molecular and cellular mechanisms and therapeutic opportunities. J Scleroderma Relat Disord 2017; 2: 137-52. [Crossref]
3. Márquez A, Kerick M, Zhernakova A, Gutierrez-Achury J, Chen WM, Onengut-Gumuscu S, et al. Meta-analysis of Immunochip data of four autoimmune diseases reveals novel single-disease and cross-phenotype associations. Genome Med 2018; 100: 97. [Crossref]
4. López-Isac E, Martín JE, Assassi S, Simeón CP, Carreira P, Ortego-Centeno N, et al. Brief report: Irf4 newly identified as a common susceptibility locus for systemic sclerosis and rheumatoid arthritis in a cross-disease meta-analysis of genome-wide association studies. Arthritis Rheumatol 2016; 68: 2338-44. [Crossref]
5. López-Isac E, Campillo-Davo D, Bossini-Castillo L, Guerra SG, Assassi S, Simeón CP, et al. Influence of TYK2 in systemic sclerosis susceptibility: A new locus in the IL-12 pathway. Ann Rheum Dis 2016; 75: 1521-6. [Crossref]
6. López-Isac E, Bossini-Castillo L, Palma AB, Assassi S, Mayes MD, Simeón CP, et al. Analysis of ATP8B4 F436L missense variant in a large systemic sclerosis cohort. Arthritis Rheumatol 2017; 691: 337-8. [Crossref]
7. Marie I, Gehanno J-F. Environmental risk factors of systemic sclerosis. Semin Immunopathol 2015; 37: 463-73. [Crossref]
8. Moroncini G, Mori S, Tonnini C, Gabrielli A. Role of viral infections in the etiopathogenesis of systemic sclerosis. Clin Exp Rheumatol 2013; 31: S3-7.
9. Domsic RT. Scleroderma: The role of serum autoantibodies in defining specific clinical phenotypes and organ system involvement. Curr Opin Rheumatol 2014; 26: 646-52. [Crossref]
10. Moinzadeh P, Fonseca C, Hellmich M, Shah AA, Chighizola C, Denton CP, et al. Association of anti-RNA polymerase III autoantibodies and cancer in scleroderma. Arthritis Res Ther 2014; 16: R53. [Crossref]
11. Ceribelli A, Cavazzana I, Airò P, Franceschini F. Anti-RNA polymerase III antibodies as a risk marker for early Gastric Antral Vascular Ectasia (GAVE) in systemic sclerosis. J Rheumatol 2010; 37: 1544. [Crossref]
12. Koenig M, Dieudé M, Senécal J-L. Predictive value of antinuclear autoantibodies: The lessons of the systemic sclerosis autoantibodies. Autoimmun Rev 2008; 7: 588-93. [Crossref]
13. Moroncini G, Svegliati Baroni S, Gabrielli A. Agonistic antibodies in systemic sclerosis. Immunol Lett 2018; 195: 83-7. [Crossref]
14. Naparstek Y, Plotz PH. The Role of autoantibodies in autoimmune disease. Annu Rev Immunol 2003; 11: 79-104. [Crossref]
15. Belizna C, Cohen Tervaert JW. Specificity, pathogenecity, and clinical value of antiendothelial cell antibodies. Semin Arthritis Rheum 1997; 27: 98-109. [Crossref]
16. Carvalho D, Savage COS, Black CM, Pearson JD. IgG antiendothelial cell autoantibodies from scleroderma patients induce leukocyte adhesion to human vascular endothelial cells in vitro: Induction of adhesion molecule expression and involvement of endothelium-derived cytokines. J Clin Invest 1996; 97: 111-9. [Crossref]
17. Salojin KV, Le Tonquèze M, Saraux A, Nassonov EL, Dueymes M, Piette JC, et al. Antiendothelial cell antibodies: Useful markers of systemic sclerosis. Am J Med 1997; 102: 178-85. [Crossref]
18. Pignone A, Scaletti C, Matucci-Cerinic M, Vázquez-Abad D, Meroni PL, Del Papa N, et al. Anti-endothelial cell antibodies in systemic sclerosis: Significant association with vascular involvement and alveolo-capillary impairment. Clin Exp Rheumatol 1998; 16: 527-32.
19. Worda M, Sgonc R, Dietrich H, Niederegger H, Sundick RS, Gershwin ME, et al. In vivo analysis of the apoptosis-inducing effect of anti-endothelial cell antibodies in systemic sclerosis by the chorionallantoic membrane assay. Arthritis Rheum 2003; 48: 2605-14. [Crossref]
20. Hill MB, Phipps JL, Cartwright RJ, Milford Ward A, Greaves M, Hughes P. Antibodies to membranes of endothelial cells and fibroblasts in scleroderma. Clin Exp Immunol 1996; 106: 491-7. [Crossref]
21. Servettaz A, Tamby MC, Guilpain P, Reinbolt J, Garcia de la Penã-Lefebvre P, Allanore Y, et al. Anti-endothelial cell antibodies from patients with limited cutaneous systemic sclerosis bind to centromeric protein B (CENP-B). Clin Immunol 2006; 120: 212-9. [Crossref]
22. Lunardi C, Bason C, Navone R, Millo E, Damonte G, Corrocher R, et al. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. Nat Med 2000; 6: 1183-6. [Crossref]
23. Puccetti A, Dolci M, Peterlana D, Navone R, Bason C, Lunardi C. Antibodies against human cytomegalovirus in the pathogenesis of atherosclerosis: A gene array approach. Clin Immunol 2007; 123: S12. [Crossref]
24. Sgonc R, Gruschwitz MS, Boeck G, Sepp N, Gruber J, Wick G. Endothelial cell apoptosis in systemic sclerosis is induced by antibody-dependent cell-mediated cytotoxicity via CD95. Arthritis Rheum 2000; 43: 2550-62.
25. Wolf SI, Howat S, Abraham DJ, Pearson JD, Lawson C. Agonistic anti-ICAM-1 antibodies in scleroderma: Activation of endothelial pro-inflammatory cascades. Vascul Pharmacol 2013; 59: 19-26. [Crossref]
26. Laplante P, Raymond M-A, Gagnon G, Vigneault N, Sasseville AM-J, Langelier Y, et al. Novel fibrogenic pathways are activated in response to endothelial apoptosis: Implications in the pathophysiology of systemic sclerosis. J Immunol 2014; 174: 5740-9. [Crossref]
27. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jüpner A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest 1999; 103: 945-52. [Crossref]
28. Xia Y, Kellems RE. Is preeclampsia an autoimmune disease? Clin Immunol 2009; 133: 1-12. [Crossref]
29. Fu MLX, Herlitz H, Schulze W, Wallukat G, Micke P, Eftekhari P, et al. Autoantibodies against the angiotensin receptor (AT 1) in patients with hypertension. J Hypertens 2000; 18: 945-53. [Crossref]
30. Ansari MJ, Tinckam K, Chandraker A. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. N Engl J Med 2005; 352: 2027-8. [Crossref]
31. Wallukat G, Schimke I. Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases. Semin Immunopathol 2014; 36: 351-63. [Crossref]
32. Riemekasten G, Philippe A, Näther M, Slowinski T, Müller DN, Heidecke H, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis 2011; 70: 530-6. [Crossref]
33. Becker MO, Kill A, Kutsche M, Guenther J, Rose A, Tabeling C, et al. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. Am J Respir Crit Care Med 2014; 190: 808-17. [Crossref]
34. Kill A, Tabeling C, Undeutsch R, Kühl AA, Günther J, Radic M, et al. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. Arthritis Res Ther 2014; 16: R29. [Crossref]
35. Günther J, Kill A, Becker MO, Heidecke H, Rademacher J, Siegert E, et al. Angiotensin receptor type 1 and endothelin receptor type A on immune cells mediate migration and the expression of IL-8 and CCL18 when stimulated by autoantibodies from systemic sclerosis patients. Arthritis Res Ther 2014; 16: 1-14. [Crossref]
36. Howe S, Eaker EY, Sallustio JE, Peebles C, Tan EM, Williams RC. Antimyenteric neuronal antibodies in scleroderma. J Clin Invest 1994; 94: 761-70. [Crossref]
37. Eaker EY, Kuldau JG, Verne GN, Ross SO, Sallustio JE. Myenteric neuronal antibodies in scleroderma: Passive transfer evokes alterations in intestinal myoelectric activity in a rat model. J Lab Clin Med 1999; 133: 551-6. [Crossref]
38. Singh J, Mehendiratta V, Del Galdo F, Jimenez SA, Cohen S, DiMarino AJ, et al. Immunoglobulins from scleroderma patients inhibit the muscarinic receptor activation in internal anal sphincter smooth muscle cells. Am J Physiol Liver Physiol 2009; 297: G1206-13. [Crossref]
39. Goldblatt F, Gordon TP, Waterman SA. Antibody-mediated gastrointestinal dysmotility in scleroderma. Gastroenterology 2002; 123: 1144-50. [Crossref]
40. Kawaguchi Y, Nakamura Y, Matsumoto I, Nishimagi E, Satoh T, Kuwana M, et al. Muscarinic-3 acetylcholine receptor autoantibody in patients with systemic sclerosis: Contribution to severe gastrointestinal tract dysmotility. Ann Rheum Dis 2009; 68: 710-4. [Crossref]
41. Kumar S, Singh J, Kedika R, Mendoza F, Jimenez SA, Blomain ES, et al. Role of muscarinic-3 re-

ceptor antibody in systemic sclerosis: Correlation with disease duration and effects of IVIG. Am J Physiol Liver Physiol 2016; 310: G1052-60. [Crossref]
42. Singh J, Cohen S, Mehendiratta V, Mendoza F, Jimenez SA, Dimarino AJ, et al. Effects of scleroderma antibodies and pooled human immunoglobulin on anal sphincter and colonic smooth muscle function. Gastroenterology 2012; 143: 1308-18. [Crossref]
43. Preuss B, Tunaru S, Henes J, Offermanns S, Klein R. A novel luminescence-based method for the detection of functionally active antibodies to muscarinic acetylcholine receptors of the M3 type (mAchR3) in patients' sera. Clin Exp Immunol 2014; 177: 179-89. [Crossref]
44. Svegliati S, Spadoni T, Moroncini G, Gabrielli A. NADPH oxidase, oxidative stress and fibrosis in systemic sclerosis. Free Radic Biol Med 2018; 125: 90-7. [Crossref]
45. Svegliati S, Cancello R, Sambo P, Luchetti M, Paroncini P, Orlandini G, et al. Platelet-derived growth factor and reactive oxygen species (ROS) regulate Ras protein levels in primary human fibroblasts via ERK1/2. Amplification of ROS and Ras in systemic sclerosis fibroblasts. J Biol Chem 2005; 280: 36474-82. [Crossref]
46. Smaldone S, Olivieri J, Gusella GL, Moroncini G, Gabrielli A, Ramirez F. Ha-Ras stabilization mediates pro-fibrotic signals in dermal fibroblasts. Fibrogenesis Tissue Repair 2011; 4: 8. [Crossref]
47. Svegliati Baroni S, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006; 354: 2667-76. [Crossref]
48. Classen JF, Henrohn D, Rorsman F, Lennartsson J, Lauwerys BR, Wikström G, et al. Lack of evidence of stimulatory autoantibodies to platelet-derived growth factor receptor in patients with systemic sclerosis. Arthritis Rheum 2009; 60: 1137-44. [Crossref]
49. Loizos N, LaRiccia L, Weiner J, Griffith H, Boin F, Hummers L, et al. Lack of detection of agonist activity by antibodies to platelet-derived growth factor receptor α in a subset of normal and systemic sclerosis patient Sera. Arthritis Rheum 2009; 60: 1145-51. [Crossref]
50. Gabrielli A, Moroncini G, Svegliati S, Avvedimento EV. Autoantibodies against the platelet-derived growth factor receptor in scleroderma: Comment on the articles by Classen et al and Loizos et al. Arthritis Rheum 2009; 60: 3521-2. [Crossref]
51. Moroncini G, Grieco A, Nacci G, Paolini C, Tonnini C, Pozniak KN, et al. Epitope specificity determines pathogenicity and detectability of anti-platelet-derived growth factor receptor α autoantibodies in systemic sclerosis. Arthritis Rheumatol 2015; 67: 1891-903. [Crossref]
52. Moroncini G, Cuccioloni M, Mozzicafreddo M, Pozniak KN, Grieco A, Paolini C, et al. Characterization of binding and quantification of human autoantibodies to PDGFRα using a biosensor-based approach. Anal Biochem 2017; 528: 26-33. [Crossref]
53. Balada E, Simeón-Aznar CP, Ordi-Ros J, Rosa-Leyva M, Selva-O'Callaghan A, Pardos-Gea J, et al. Anti-PDGFR-α antibodies measured by non-bioactivity assays are not specific for -systemic sclerosis. Ann Rheum Dis 2008; 67: 1027-9. [Crossref]
54. Fraticelli P, De Vita S, Franzolini N, Svegliati S, Scott CA, Tonnini C, et al. Reduced type I collagen gene expression by skin fibroblasts of patients with systemic sclerosis after one treatment course with rituximab. Clin Exp Rheumatol 2015; 33: 160-7.
55. Luchetti MM, Moroncini G, Jose Escamez M, Svegliati Baroni S, Spadoni T, Grieco A, et al. Induction of scleroderma fibrosis in skin-humanized mice by administration of anti–platelet-derived growth factor receptor agonistic autoantibodies. Arthritis Rheumatol 2016; 68: 2263-73. [Crossref]
56. Svegliati S, Amico D, Spadoni T, Fischetti C, Finke D, Moroncini G, et al. Agonistic anti-PDGF receptor autoantibodies from patients with systemic sclerosis impact human pulmonary artery smooth muscle cells function in vitro Front Immunol 2017; 8: 75. [Crossref]
57. Bussone G, Tamby MC, Calzas C, Kherbeck N, Sahbatou Y, Sanson C, et al. IgG from patients with pulmonary arterial hypertension and/or systemic sclerosis binds to vascular smooth muscle cells and induces cell contraction. Ann Rheum Dis 2012; 71: 596-605. [Crossref]
58. Arts MR, Baron M, Chokr N, Fritzler MJ, (CSRG) the CSRG, Servant MJ. Systemic sclerosis immunoglobulin induces growth and a pro-fibrotic state in vascular smooth muscle cells through the epidermal growth factor receptor. PLoS One 2014; 9: e100035. [Crossref]
59. Tan FK, Arnett FC, Antohi S, Saito S, Mirarchi A, Spiera H, et al. Antiantibodies to the extracellular matrix microfibrillar protein, fibrillin-1, in patients with scleroderma and other connective tissue diseases. J Immunol 1999; 163: 1066-72.
60. Tan FK, Arnett FC, Reveille JD, Ahn C, Antohi S, Sasaki T, et al. Autoantibodies to fibrillin 1 in systemic sclerosis: Ethnic differences in antigen recognition and lack of correlation with specific clinical features or HLA alleles. Arthritis Rheum 2000; 43: 2464-71.
61. Zhou X, Tan FK, Milewicz DM, Guo X, Bona CA, Arnett FC. Autoantibodies to Fibrillin-1 activate normal human fibroblasts in culture through the tgf-β pathway to recapitulate the "scleroderma phenotype." J Immunol 2005; 175: 4555-60. [Crossref]
62. Brinckmann J, Hunzelmann N, El-Hallous E, Krieg T, Sakai LY, Krengel S, et al. Absence of autoantibodies against correctly folded recombinant fibrillin-1 protein in systemic sclerosis patients. Arthritis Res Ther 2005; 7: R1221-6. [Crossref]
63. Weigold F, Günther J, Pfeiffenberger M, Cabral-Marques O, Siegert E, Dragun D, et al. Antibodies against chemokine receptors CXCR3 and CXCR4 predict progressive deterioration of lung function in patients with systemic sclerosis. Arthritis Res Ther 2018; 20: 52. [Crossref]
64. Recke A, Regensburger AK, Weigold F, Müller A, Heidecke H, Marschner G, et al. Autoantibodies in serum of systemic scleroderma patients: Peptide-based epitope mapping indicates increased binding to cytoplasmic domains of CXCR3. Front Immunol 2018; 9: 428.[Crossref]
65. Giovannetti A, Maselli A, Colasanti T, Rosato E, Salsano F, Pisarri S, et al. Autoantibodies to estrogen receptor α in systemic sclerosis (SSc) as pathogenetic determinants and markers of progression. PLoS One 2013; 8: 4-9. [Crossref]
66. Odaka M, Hasegawa M, Hamaguchi Y, Ishiura N, Kumada S, Matsushita T, et al. Autoantibody-mediated regulation of B cell responses by functional anti-CD22 autoantibodies in patients with systemic sclerosis. Clin Exp Immunol 2010; 159: 176-84. [Crossref]
67. Sato S, Hayakawa I, Hasegawa M, Fujimoto M, Takehara K. Function blocking autoantibodies against matrix metalloproteinase-1 in patients with systemic sclerosis. J Invest Dermatol 2003; 120: 542-7. [Crossref]
68. Nishijima C, Hayakawa I, Matsushita T, Komura K, Hasegawa M, Takehara K, et al. Autoantibody against matrix metalloproteinase-3 in patients with systemic sclerosis. Clin Exp Immunol 2004; 138: 357-63.[Crossref]
69. Kowal-Bielecka O, Fransen J, Avouac J, Becker M, Kulak A, Allanore Y, et al. Update of EULAR recommendations for the treatment of systemic sclerosis. Ann Rheum Dis 2017; 76: 1327-39. [Crossref]
70. Iudici M, Moroncini G, Cipriani P, Giacomelli R, Gabrielli A, Valentini G. Where are we going in the management of interstitial lung disease in patients with systemic sclerosis? Autoimmun Rev 2015; 14: 575-8. [Crossref]
71. Moroncini G, Paolini C, Orlando F, Capelli C, Grieco A, Tonnini C, et al. Mesenchymal stromal cells from human umbilical cord prevent the development of lung fibrosis in immunocompetent mice. PLoS One 2018; 13: e0196048. [Crossref]