Editorial

The Pathogenic Role of Autoantibodies to Nuclear Autoantigens in Systemic Sclerosis (Scleroderma)



Systemic sclerosis (scleroderma) is an incurable and potentially life-threatening systemic autoimmune disease of unknown cause characterized by cutaneous and visceral fibrosis, microvascular obliteration, and highly specific serum autoantibodies (aAb) to nuclear autoantigens[1,2]. As shown in Table 1, these aAb include anticentromere (ACA, anti-CENP-B), anti-topoisomerase I (anti-topo I), anti-RNA polymerase I/III, and anti-Th/To[2-36]. Together, these account for 80% to 85% of antinuclear aAb in pure SSc.

Pathogenic aAb are broadly defined as immunoglobulins contributing to the pathophysiologic mechanisms resulting in the development of a disease and its manifestations[37]. Systemic lupus erythematosus (SLE) is the prototypical systemic autoimmune disease associated with pathogenic aAb. For example, aAb to double-stranded DNA (dsDNA), nucleosomes, and Ro autoantigens contribute to disease manifestations in SLE by distinct immune mechanisms[38-40]. No such pathogenic mechanisms have been described thus far for SSc aAb to nuclear autoantigens. Indeed, reference texts state that "SSc aAb do not have a pathogenic role"[41]. A major reason for this perception is that SSc nuclear autoantigens are sequestered intracellularly and therefore are presumably unaccessible to circulating aAb. Further, even if SSc aAb gained access to the cytoplasm, it would be difficult to explain how they could then be transported across the nuclear envelope, bind to their cognate antigen, and disrupt cell function.

In this brief editorial, we hypothesize that some SSc aAb are pathogenic. We review indirect and direct scientific data that support this hypothesis. In particular, we present provocative data showing that anti-topo I can react with the cell surface, and, moreover, that this reactivity is highly targeted to a crucial cellular population in SSc pathogenesis, namely fibroblasts.

Our review is based on a number of *a priori* statements. (1) As stated by Jimenez and Derk, the pathogenesis of SSc is extremely complex[1,42], and we acknowledge that pathogenic aAb are only one facet of that complexity. (2) Defining a pathogenic role for SSc aAb and elucidating their origins are 2 different issues, and only the former will be addressed here (for recent reviews on how SSc aAb originate, see References[1,43-45]). (3) Although aAb to fibroblasts and endothelial cells have been described in SSc, unless specified otherwise, we restrict the term "aAb" here to designate the archetypal immunoglobulins associated with systemic autoimmune diseases, i.e., those directed against nuclear autoantigens. (4) We will focus primarily on anti-topo I and ACA, because these are the only SSc aAb for which extensive basic and clinical research data are available from international cohorts. (5) We will use as guidelines for defining pathogenic aAb the stringent criteria outlined by Naparstek and Plotz[46].

SSc IS ASSOCIATED WITH HIGHLY SPECIFIC AUTOANTIBODIES

A first clue to a potential pathogenic role for SSc aAb is their high disease specificity. Indeed, SSc aAb are rarely observed in healthy subjects or various disease controls[47]. In clinical practice, these aAb are routinely used as diagnostic markers for SSc. Although the respective frequencies of SSc aAb in defined populations are modulated by ethnic and geographic factors, their occurrence is universal (Table 1)[2,48-53]. Occasionally, these aAb are found in patients with features of other connective tissue diseases, either as a harbinger of SSc or as a marker of concurrent SSc[54-56]. Clinicians can usually recognize in such patients the presence of Raynaud's phenomenon (RP) and subtle manifestations of SSc (such as telangiectasias) and/or nailfold capillary abnormalities typical of SSc, thus allowing the recognition of early or discrete SSc[57,58]. Anti-topo I have been reported in SLE[59], but our experience is that significant titers of anti-topo I are not observed in SLE unless it overlaps with SSc[7,60]. Occasional instances of ACA immunofluorescent patterns in patients without SSc or RP are due to reactivity with minor centromere epitopes and not with the major CENP autoantigens characteristic of true positive ACA (Table 1)[61].

SSc aAb CLUSTER WITH DISTINCT CLINICAL PHENOTYPES

A second clue in strong support of a pathogenic role for SSc aAb is that they strongly cluster with clinically distinct phenotypes, in terms of both SSc subsets and selective visceral

Table 1. Major autoantibodies to nuclear autoantigens in systemic sclerosis.

Autoantibody	Major Autoantigens	Autoantigen Cellular Localization	Sensitivity*, %	Current Methods of Identification**	References
		Pure Systemic Sclerosis			
Anticentromere	CENP-B (less commonly CENP A, C, D, E)	Centromeres (nucleolarly juxtaposed)	40–50	IFI, CENP-B ELISA	2–11
Anti-topoisomerase I (topo I)	DNA topoisomerase I	Nucleoli, nucleoplasm, fibroblast surface?	15–20	ELISA, IB	2, 7, 11, 12
Anti-RNA polymerase I/III	RNA polymerases I/III	Nucleoli, nucleoplasm	10–20	ELISA, IPP	13–16
Anti-Th/To	Proteins of the RNase MRP and RNase P ribonucleoprotein complexes	Nucleoli	5	IPP	17–21
		SSc in Association with Manifestations of Other CTD (e.g., myositis)			
Anti-PM-Scl	PM-Scl-100 and PM-Scl-75 proteins of the human exosome	Nucleoli	< 5	PM-Scl-100 and PM-Scl-75 ELISA, IPP, IFI	22–27
Anti-U3RNP	Fibrillarin and other U3RNP components	Nucleoli	< 5	ELISA, IPP, IFI	27–31
Anti-U1RNP	70 kDa, A and C polypeptides of U1 snRNP	Nuclear	5	ELISA, IB, IPP, IFI	32, 33
		Association with SSc, other CTD, and Cancer			
Anti-hUBF (NOR-90)	Human upstream binding factor	Nucleoli	< 5	IFI, IB	34
Anti-B23	Nucleophosmin	Nucleoli	< 5	B23 ELISA, IPP	35, 36

* Sensitivities vary according to ethnicity and geographic factors. IB: immunoblotting; IFI: indirect immunofluorescence on HEp-2 cells; IPP: immunoprecipitation.

involvement. For example, ACA are strongly associated with the slowly progressive, limited, and intermediate cutaneous SSc subsets[2,4,7,49,50]. In contrast, anti-topo I and anti-RNA polymerase I/III typically cluster with the more rapidly progressive and diffuse form of cutaneous SSc[2,7,47]. Thus, in clinical practice, these aAb are used as markers for SSc subsets. Because these subsets are associated with different survival times, as determined by standardized mortality ratios and survival curves, these aAb are also utilized as prognostic markers[2,41].

Moreover, a striking feature of visceral involvement in SSc is the association with specific aAb. For example, the presence of anti-RNA polymerase I/III is strongly associated with renal crisis, but not with pulmonary fibrosis[13-15]. In contrast, anti-topo I are not associated with renal crisis but are strongly associated with lung fibrosis and/or restrictive syndrome[2,13-15,62]. Also, patients with anti-topo I appear protected against isolated pulmonary arterial hypertension (PAH)[30], whereas PAH is a common complication observed in patients with ACA and anti-Th/To[20]. This selective visceral involvement is reminiscent of the association of glomerulonephritis with SLE and heart conduction defects with neonatal lupus as a result of pathogenic anti-dsDNA and anti-Ro aAb, respectively[38,39]. However, in contrast with SLE, SSc aAb are mutually exclusive, with rare exceptions[2,63]: only a single aAb is present in any given SSc patient during her lifetime.

Taken together, these data strongly suggest that if SSc aAb are indeed pathogenic, their respective pathogenicities are exerted preferentially in certain organs and tissues and via different mechanistic pathways.

SSc aAb ARE ALREADY PRESENT AT DISEASE ONSET

A third clue to pathogenicity is that SSc aAb are almost invariably present in high titers extremely early in the disease process, suggesting not only that they are a reflection of this process but that they actually contribute to it[54]. For example, limited cutaneous SSc is characteristically preceded by several years or even decades of isolated RP during which SSc aAb are already present[4,64]. We have shown that prospectively followed patients with isolated RP who express ACA or anti-topo I are 63 times more likely to develop SSc than patients without these aAb[65]. Further, the presence of high titers of ACA, anti-topo I, or anti-RNA polymerase I/III in isolated RP has been proposed as diagnostic of early SSc[58].

CORRELATION BETWEEN aAb TITERS AND SSc ACTIVITY AND SEVERITY

A fourth clue to the pathogenicity of SSc aAb is the correlation between their serum concentration and SSc activity and severity, a major criterion for pathogenicity[46]. Two recent reports have highlighted the close relationship between anti-

topo I levels and associated clinical phenotypes. In a study of 59 patients with diffuse cutaneous SSc from the United States, IgG anti-topo I titers determined by ELISA using recombinant topo I correlated strongly with disease severity, as assessed by total skin score (TSS) measurements ($r = 0.61$, $p < 0.001$)[66]. Moreover, mean anti-topo I titers were higher in patients with active versus inactive disease ($p < 0.001$) as determined from clinical examination and laboratory data[66]. Strikingly, in 8 of 11 patients analyzed longitudinally, anti-topo I titers fluctuated in parallel with the TSS; in some patients increasing titers actually preceded increases in TSS[66]. These important data expanded a previous study by Kuwana, *et al* where Japanese patients who had lost anti-topo I experienced significant improvement in pulmonary function and survival, in comparison with patients with persistent anti-topo I[67]. Together, these studies are consistent with another criterion for pathogenicity stating that "removal of a pathogenic aAb should ameliorate the disease process"[46].

With respect to ACA, however, a similar correlation between aAb titers and SSc activity and severity has yet to be demonstrated[5]. In contrast to the more dynamic anti-topo I-associated clinical phenotypes, ACA-associated phenotypes are slowly progressive, commonly evolving over several years or even decades, if the date of onset of RP is taken as the date of SSc onset. This slow clinical evolution is mirrored pathophysiologically by the slow development of nailfold capillary damage: we have shown that the slowest rates of moderate or severe capillary loss and of severe capillary dilatations are observed in ACA-associated SSc subsets, i.e., limited and intermediate cutaneous SSc[2]. Thus, we hypothesize that pathogenic ACA are characterized by low-grade but ongoing, slowly cumulative, pathogenicity.

SSc aAb SHARE THE FEATURES OF PATHOGENIC IMMUNOGLOBULINS

A fifth clue is that SSc aAb display the immunoglobulin features that are characteristic of pathogenic aAb. These features are those of antigen-driven and T cell-dependent immune responses, namely high titers of immunoglobulins that undergo IgM to IgG isotype switch and maturation, leading to high affinity IgG that undergo intramolecular and intermolecular epitope spreading and bind to highly specific and conserved autoepitopes[34,37,42,63,68-72]. Characteristically, pathogenic aAb target functional domains, resulting in inhibition of molecular function. Thus, purified human anti-topo I aAb inhibit relaxation of superhelical DNA[73], anti-RNA polymerase I/III aAb inhibit RNA transcription[74], and ACA disrupt mitosis[75,76].

TOWARD OVERCOMING MAJOR OBJECTIONS TO PATHOGENICITY

Although these data provide robust evidence in favor of a pathogenic role for SSc aAb (Table 2), this evidence is indirect and does not overcome the scientific objection that SSc nuclear autoantigens are inaccessible to circulating aAb, and hence such aAb are unlikely to be pathogenic. In addition, criteria for pathogenicity explicitly state that "the aAb should be capable of causing in experimental systems the lesions attributed to it"[46]. Also, "the aAb should be found along with a plausible target antigen at the site of tissue damage"[46]. As shown in Table 2, we present evidence suggesting that these obstacles are in the process of being overcome.

ANTI-TOPO I aAb BIND DIRECTLY TO THE FIBROBLAST CELL SURFACE

A key objection would be overruled if a major SSc aAb was shown to react specifically with extracellular epitopes. Indeed, we have shown for the first time that anti-topo I affinity-purified from the serum of SSc patients bind directly to the cell surface of fibroblasts, a crucial cellular population in SSc pathogenesis[60].

Our study was based on reports that aAb to fibroblasts (AFA) present in the sera of SSc patients induce a proadhesive and proinflammatory phenotype in fibroblasts, and are specifically internalized by these cells[77,78]. These findings suggest that the role of AFA in SSc may be of greater importance than previously thought. Hence, our aim was to further characterize the fibroblast binding activity of aAb from SSc sera and to explore the association of these AFA with major aAb to nuclear autoantigens.

Briefly, we found by flow cytometry that AFA of IgG isotype were significantly more common in SSc patients ($n = 26/99$, 26.3%) than in rheumatologic disease or healthy controls ($n = 5/123$, 4%; $p < 0.0001$, OR 8.4, 95% CI 3–22)[60]. AFA-positive sera from SSc patients bound to all fibroblast types tested, but not to human primary endothelial or smooth muscle cells. A striking and unexpected finding was the extensive correlation between the presence of AFA and anti-topo I aAb in SSc. Specifically, all SSc sera with AFA strongly reacted with topo I by ELISA and immunoblotting. Moreover, the mean anti-topo I reactivity was much higher in AFA-positive sera than in AFA-negative sera ($p < 0.0001$). A strong correlation was noted by flow cytometry between AFA binding intensity and anti-topo I reactivity by ELISA ($r = 0.65$, $p < 0.0001$). Also, the binding intensity of SSc AFA correlated strongly with reactivity against topo I on immunoblots of fibroblast extracts. Lastly but most significantly, total IgG and affinity-purified anti-topo I from AFA-positive SSc sera were found to react with the surface of unpermeabilized fibroblasts by flow cytometry as well as by immunofluorescence and confocal microscopy[60].

FUTURE RESEARCH QUESTIONS

Several exciting questions stem from these data. What is the molecular identity of the fibroblast surface antigen recognized by anti-topo I aAb? Is it topo I itself, or are anti-topo

Table 2. Evidence supporting a pathogenic role for autoantibodies to nuclear autoantigens in systemic sclerosis.*

- The aAb are highly specific for systemic sclerosis
- The aAb cluster with distinct clinical phenotypes, in terms of both disease subsets and selective visceral involvements
- The aAb are almost invariably present in high titers extremely early in the disease process, suggesting that they contribute to it
- The serum concentration of anti-topo I aAb correlates with disease activity and severity
- The aAb share the features characteristic of pathogenic immunoglobulins
- AFA present in the sera of SSc patients induce a proadhesive and proinflammatory phenotype in fibroblasts and are specifically internalized by these cells
- AFA and anti-topo I aAb are strongly correlated:
  - all sera with AFA display strong topo I reactivity
  - AFA binding intensity by flow cytometry strongly correlates with anti-topo I reactivity by ELISA
  - Affinity-purified anti-topo I bind directly to the fibroblast cell surface

* Based on aAb to topoisomerase I, centromeres, RNA polymerases I/III, and Th/To autoantigens. aAb: autoantibodies; AFA: anti-fibroblast autoantibodies; topo I: DNA topoisomerase I.

I aAb cross-reactive with an integral or a peripheral fibroblast plasma membrane protein? To our knowledge, the presence of topo I on the plasma membrane of normal human fibroblasts has not been reported. Interestingly, a subset of anti-dsDNA aAb has been shown to cross-react with the N-methyl-D-aspartate glutamate receptor from neurons of the central nervous system in SLE[79]. Similarly, the fibroblast specificity of anti-topo I aAb may point to cross-reactivity with a fibroblast-specific protein on the cell surface. However, the molecular identity of the cell-surface antigen recognized by anti-topo I remains to be determined.

In keeping with a previously stated criterion for pathogenicity, does binding of anti-topo I to the fibroblast surface perturb fibroblast functions and contribute to SSc fibrosis? Results thus far point to their influence on the cellular activation state via direct interaction with an undefined fibroblast surface target[77,78]. Determining whether topo I itself is the fibroblast surface antigen will be of paramount importance to understand mechanistically how anti-topo I may perturb cell function. Certain autoantigens exert more than one biological function. For example, the myositis intracellular autoantigens histidyl-tRNA synthetase and asparaginyl-tRNA synthetase act extracellularly as chemoattractants by activating chemokine receptors on T lymphocytes and immature dendritic cells[80]. Whether topo I itself is such a bifunctional molecule, and whether its putative extracellular function is disturbed by anti-topo I aAb, are fascinating questions currently being examined by our group.

Could SSc aAb to other nuclear autoantigens bind to the surface of key cell lines involved in the pathogenesis of SSc, such as endothelial cells? Given on the one hand the relationship between certain SSc aAb with specific clinical phenotypes and visceral involvement, and on the other, the fibroblast selectivity of anti-topo I, it will be of great interest to examine the selective interaction of other aAb, such as ACA, with specific cell populations.

Finally, research teams will need to determine whether SSc aAb are present along with a plausible target antigen at the site of tissue damage. Interestingly, SSc-associated autoantigen genes are selectively overexpressed in SSc dermal fibroblasts[81]. Also, an *in vivo* antinuclear antibody phenomenon has been described in epidermal cells from skin biopsies of SSc patients[82]. Moreover, in patients with circulating antinucleolar aAb, *in vivo* nucleolar epidermal fluorescence was observed[83], an intriguing finding given the preferential nucleolar localization of many SSc autoantigens (Table 1). The relationship between this *in vivo* antinuclear antibody phenomenon and pathogenic SSc aAb remains to be examined.

CONCLUSION

SSc is an incurable and potentially life-threatening disease, often associated with a high degree of morbidity and suffering. Although therapeutic advances have been made, no therapy specifically targeting the self-perpetuating biologic cascades leading to characteristic fibrotic and vascular obliterative lesions is yet available. Thus in patients with active disease, ongoing end-organ damage is difficult to stop. Moreover, in patients with markers of poor survival[2,84], progression cannot be prevented. This reality is in striking contrast with rheumatoid arthritis, where highly targeted biologic treatments such as the anti-tumor necrosis factor-α agents have provided major therapeutic advances.

We have seen that a growing body of compelling evidence is now pointing to a pathogenic role for certain SSc aAb such as anti-topo I. Although their pathogenic role has not yet been proven beyond a doubt, exciting new research avenues are opening that will unravel the mystery. It is our hope that the molecular analysis of the pathogenic role of these aAb will identify new therapeutic targets that lead to the arrest of SSc disease processes and even prevent its morbid and life-threatening manifestations.

JEAN-LUC SENÉCAL, MD, FRCPC, FACP,
Professor of Medicine;
JILL HÉNAULT, MSc,
PhD Candidate;
YVES RAYMOND, PhD,
Professor of Medicine,
Laboratory for Research in Autoimmunity,
Department of Medicine, Division of Rheumatology,
University of Montreal School of Medicine,
Montreal, Quebec, Canada.

Supported in part by grant MOP-68966 from the Canadian Institutes of Health Research and by a studentship from Fonds de la Recherche en Santé du Québec and Sclérodermie Québec.
Address reprint requests to Dr. J-L. Senécal, Laboratory for Research in Autoimmunity, Division of Rheumatic Diseases, Hôpital Notre-Dame, CHUM, 1560 East Sherbrooke Street, Montreal, Quebec H2L 4M1, Canada. E-mail: jeanluc.senecal@sympatico.ca

REFERENCES

1. Jimenez SA, Derk CT. Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. Ann Intern Med 2004;140:37-50.
2. Lonzetti L, Joyal F, Raynauld JP, et al. Predicting mortality in systemic sclerosis. Analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. Medicine 2002;81:154-67.
3. Moroi Y, Peebles C, Fritzler MJ, Steigerwald J, Tan EM. Autoantibody to centromere (kinetochore) in scleroderma sera. Proc Natl Acad Sci USA 1980;77:1627-31.
4. Fritzler MJ, Kinsella TD. The CREST syndrome: a distinct serologic entity with anticentromere antibodies. Am J Med 1980;69:520-6.
5. Earnshaw W, Bordwell B, Marino C, Rothfield N. Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. J Clin Invest 1986;77:426-30.
6. Rothfield N, Whitaker D, Bordwell B, Weiner E, Senécal JL, Earnshaw W. Detection of anticentromere antibodies using cloned autoantigen, CENP-B. Arthritis Rheum 1987;30:1416-9.
7. Weiner ES, Earnshaw WC, Senécal JL, Bordwell B, Johnson P, Rothfield NF. Clinical associations of anticentromere antibodies and antibodies to topoisomerase I: a study of 355 patients. Arthritis Rheum 1988;31:378-85.
8. Kingwell B, Rattner JB. Mammalian kinetochore/centromere composition: a 50 kDa antigen is present in the mammalian kinetochore/centromere. Chromosoma 1987;95:403-7.
9. Rattner JB, Rees J, Arnett FC, Reveille JD, Goldstein R, Fritzler MJ. The centromere kinesin-like protein, CENP-E. An autoantigen in systemic sclerosis. Arthritis Rheum 1996;39:1355-61.
10. Moroi Y, Hartman AL, Nakane PK, Tan EM. Distribution of kinetochore (centromere) antigen in mammalian cell nuclei. J Cell Biol 1981;90:254-9.
11. Pluta AF, Earnshaw WC. Specific interaction between human kinetochore protein CENP-C and a nucleolar transcriptional regulator. J Biol Chem 1996;271:18767-74.
12. Shero JH, Bordwell B, Rothfield NF, Earnshaw WC. High titers of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science 1986;231:737-40.
13. Okano Y, Steen VD, Medsger TA. Autoantibody reactive with RNA polymerase III in systemic sclerosis. Ann Intern Med 1993;119:1005-13.
14. Kuwana M, Kaburaki J, Mimori T, Homma M. Autoantibody reactive with three classes of RNA polymerases in sera from patients with systemic sclerosis. J Clin Invest 1993;91:1399-404.
15. Bunn CC, Denton CP, Shi-Wen X, Knight C, Black CM. Anti-RNA polymerases and other autoantibody specificities in systemic sclerosis. Br J Rheumatol 1998;37:15-20.
16. Kuwana M, Kimura K, Kawakami Y. Identification of an immunodominant epitope on RNA polymerase III recognized by systemic sclerosis sera: application to enzyme-linked immunosorbent assay. Arthritis Rheum 2002;46:2742-7.
17. Okano Y, Medsger TA Jr. Autoantibody to Th ribonucleoprotein (nucleolar 7-2 RNA protein particle) in patients with systemic sclerosis. Arthritis Rheum 1990;33:1822-8.
18. Falkner D, Wilson J, Medsger TA Jr, Morel PA. HLA and clinical associations in systemic sclerosis patients with anti-Th/To antibodies. Arthritis Rheum 1998;41:74-80.
19. Kuwana M, Kimura K, Hirakata M, Kawakami Y, Ikeda Y. Differences in autoantibody response to Th/To between systemic sclerosis and other autoimmune diseases. Ann Rheum Dis 2002;61:842-6.
20. Mitri GM, Lucas M, Fertig N, Steen VD, Medsger TA Jr. A comparison between anti-Th/To- and anticentromere antibody-positive systemic sclerosis patients with limited cutaneous involvement. Arthritis Rheum 2003;48:203-9.
21. Van Eenennaam H, Vogelzangs JH, Lugtenberg D, Van Den Hoogen FH, Van Venrooij WJ, Pruijn GJ. Identity of the RNase MRP- and RNase P-associated Th/To autoantigen. Arthritis Rheum 2002;46:3266-72.
22. Reimer G, Scheer U, Peters JM, Tan EM. Immunolocalization and partial characterization of a nucleolar autoantigen (PM-Scl) associated with polymyositis/scleroderma overlap syndromes. J Immunol 1986;137:3802-8.
23. Alderuccio F, Chan EK, Tan EM. Molecular characterization of an autoantigen of PM-Scl in the polymyositis/scleroderma overlap syndrome: a unique and complete human cDNA encoding an apparent 75-kD acidic protein of the nucleolar complex. J Exp Med 1991;173:941-52.
24. Oddis CV, Okano Y, Rudert WA, Trucco M, Duquesnoy RJ, Medsger TA Jr. Serum autoantibody to the nucleolar antigen PM-Scl. Clinical and immunogenetic associations. Arthritis Rheum 1992;35:1211-7.
25. Targoff IN. Idiopathic inflammatory myopathy: autoantibody update. Curr Rheumatol Rep 2002;4:434-41.
26. Raijmakers R, Renz M, Wiemann C, et al. PM-Scl-75 is the main autoantigen in patients with the polymyositis/scleroderma overlap syndrome. Arthritis Rheum 2004;50:565-9.
27. Mahler M, Bluthner M, Pollard KM. Advances in B-cell epitope analysis of autoantigens in connective tissue diseases. Clin Immunol 2003;107:65-79.
28. Okano Y, Steen VD, Medsger TA Jr. Autoantibody to U3 nucleolar ribonucleoprotein (fibrillarin) in patients with systemic sclerosis. Arthritis Rheum 1992;35:95-100.
29. Sacks DG, Okano Y, Steen VD, Curtiss E, Shapiro LS, Medsger TA Jr. Isolated pulmonary hypertension in systemic sclerosis with diffuse cutaneous involvement: association with serum anti-U3RNP antibody. J Rheumatol 1996;23:639-42.
30. Steen V, Medsger TA Jr. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. Arthritis Rheum 2003;48:516-22.
31. Yang JM, Hildebrandt B, Luderschmidt C, Pollard KM. Human scleroderma sera contain autoantibodies to protein components specific to the U3 small nucleolar RNP complex. Arthritis Rheum 2003;48:210-7.
32. von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 1995;24:323-58.
33. Burdt MA, Hoffman RW, Deutscher SL, Wang GS, Johnson JC, Sharp GC. Long-term outcome in mixed connective tissue disease:

longitudinal clinical and serologic findings. Arthritis Rheum 1999;42:899-909.
34. Dagher J, Voit R, Grummt I, Scheer U, Raymond Y, Senécal JL. Autoantibodies to NOR 90/hUBF: Long-term clinical and serological followup from childhood to adulthood in a patient with limited systemic sclerosis suggests an antigen-driven immune response. J Rheumatol 2002;29:1543-7.
35. Ulanet DB, Wigley FM, Gelber AC, Rosen A. Autoantibodies against B23, a nucleolar phosphoprotein, occur in scleroderma and are associated with pulmonary hypertension. Arthritis Rheum 2003;49:85-92.
36. Brankin B, Skaar TC, Brotzman M, Trock B, Clarke R. Autoantibodies to the nuclear phosphoprotein nucleophosmin in breast cancer patients. Cancer Epidemiol Biomarkers Prev 1998;7:1109-15.
37. Senecal JL, Ichiki S, Girard D, Raymond Y. Autoantibodies to nuclear lamins and to intermediate filament proteins: natural, pathologic or pathogenic? J Rheumatol 1993;20:211-9.
38. Hahn BH, Tsao BP. Antibodies to DNA. In: Wallace DJ, Hahn BH, editors. Dubois' lupus erythematosus. Philadelphia: Lippincott, Williams & Wilkins; 2002:423-45.
39. Clancy RM, Askanase AD, Kapur RP, et al. Transdifferentiation of cardiac fibroblasts, a fetal factor in anti-SSA/Ro-SSB/La antibody-mediated congenital heart block. J Immunol 2002;169:2156-63.
40. Senecal JL, Raymond Y. The pathogenesis of neuropsychiatric manifestations in systemic lupus erythematosus: a disease in search of autoantibodies, or autoantibodies in search of a disease? J Rheumatol 2004;31:2093-8.
41. Steen VD. Epidemiology of systemic sclerosis. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Reumatology. Toronto: Mosby; 2003:1455-79.
42. Harris ML, Rosen A. Autoimmunity in scleroderma: the origin, pathogenic role, and clinical significance of autoantibodies. Curr Opin Rheumatol 2003;15:778-84.
43. Casciola-Rosen L, Wigley F, Rosen A. Scleroderma autoantigens are uniquely fragmented by metal-catalyzed oxidation reactions: implications for pathogenesis. J Exp Med 1997;185:71-9.
44. Ulanet DB, Flavahan NA, Casciola-Rosen L, Rosen A. Selective cleavage of nucleolar autoantigen B23 by granzyme B in differentiated vascular smooth muscle cells: insights into the association of specific autoantibodies with distinct disease phenotypes. Arthritis Rheum 2004;50:233-41.
45. Sato S, Fujimoto M, Hasegawa M, Takehara K, Tedder TF. Altered B lymphocyte function induces systemic autoimmunity in systemic sclerosis. Mol Immunol 2004;41:1123-33.
46. Naparstek Y, Plotz PH. The role of autoantibodies in autoimmune diseases. Annu Rev Immunol 1993;11:79-104.
47. Reveille JD, Solomon DH, The American College of Rheumatology ad hoc Committee on Immunologic Testing Guidelines. Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl-70, and nucleolar antibodies. Arthritis Care Res 2003;49:399-412.
48. Arnett FC, Howard RF, Tan F, et al. Increased prevalence of systemic sclerosis in a Native American tribe in Oklahoma. Association with an Amerindian HLA haplotype. Arthritis Rheum 1996;39:1362-70.
49. Giordano M, Valentini G, Migliaresi S, Picillo U, Vatti M. Different antibody patterns and different prognoses in patients with scleroderma with various extent of skin sclerosis. J Rheumatol 1986;13:911–6.
50. Ferri C, Bernini L, Cecchetti R, et al. Cutaneous and serologic subsets of systemic sclerosis. J Rheumatol 1991;18:1826–32.
51. Barnett AJ, Miller MH, Littlejohn GO. A survival study of patients with scleroderma diagnosed over 30 years (1953–1983): The value of a simple cutaneous classification in the early stages of the disease. J Rheumatol 1988;15:276–83.
52. Kuwana M, Okano Y, Kaburaki J, Tojo T, Medsger TA. Racial differences in the distribution of systemic sclerosis-related serum antinuclear autoantibodies. Arthritis Rheum 1994;37:902-6.
53. Kuwana M, Kaburaki J, Arnett FC, Howard RF, Medsger TA Jr, Wright TM. Influence of ethnic background on clinical and serologic features in patients with systemic sclerosis and anti-DNA topoisomerase I antibody. Arthritis Rheum 1999;42:465-74.
54. Tramposch HD, Smith CD, Senécal JL, Rothfield N. A long-term longitudinal study of anticentromere antibodies. Arthritis Rheum 1984;27:121-4.
55. Zimmerman C, Steiner G, Skriner K, Hassfeld W, Petera P, Smolen JS. The concurrence of rheumatoid arthritis and limited systemic sclerosis. Arthritis Rheum 1998;41:1938-45.
56. Ford AL, Kurien BT, Harley JB, Scofield RH. Anticentromere autoantibody in a patient evolving from a lupus/Sjögren's overlap to the CREST variant of scleroderma. J Rheumatol 1998;25:1419-24.
57. Lonzetti L, Joyal F, Raynauld JP, et al. Updating the American College of Rheumatology preliminary classification criteria for systemic sclerosis. Arthritis Rheum 2001;44:735-6.
58. LeRoy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol 2001;28:1573-6.
59. Gussin HA, Ignat GP, Varga J, Teodorescu M. Anti-topoisomerase I (anti-Scl-70) antibodies in patients with systemic lupus erythematosus. Arthritis Rheum 2001;44:376-83.
60. Henault J, Tremblay M, Clément I, Raymond Y, Senécal JL. Direct binding of anti-DNA topoisomerase I autoantibodies to the cell surface of fibroblasts in patients with systemic sclerosis. Arthritis Rheum 2004;50:3265-74.
61. Vazquez-Abad D, Grodzicky T, Senécal JL. Anticentromere antibodies in patients without Raynaud's disease or systemic sclerosis. Clin Immunol 1999;90:182-9.
62. Diot E, Giraudeau B, Diot P, et al. Is anti-topoisomerase I a serum marker of pulmonary involvement in systemic sclerosis? Chest 1999;116:715-20.
63. Mahler M, Mierau R, Genth E, Blüthner M. Development of a CENP-A/CENP-B–specific immune response in a patient with systemic sclerosis. Arthritis Rheum 2002;46:1866-72.
64. Gerbracht DD, Steen VD, Ziegler GL, Medsger TA Jr, Rodnan GP. Evolution of primary Raynaud's phenomenon (Raynaud's disease) to connective tissue disease. Arthritis Rheum 1985;28:87-92.
65. Weiner E, Hildebrandt S, Senécal JL, et al. Prognostic significance of anticentromere antibodies and anti-topoisomerase I antibodies in Raynaud's disease: a prospective study. Arthritis Rheum 1991;34:68-77.
66. Hu PQ, Fertig N, Medsger TA, Wright TM. Correlation of serum anti-DNA topoisomerase I antibody levels with disease severity and activity in systemic sclerosis. Arthritis Rheum 2003:48:1363-73.
67. Kuwana M, Kaburaki J, Mimori T, Kawakami Y, Tojo T. Longitudinal analysis of autoantibody response to topoisomerase I in systemic sclerosis. Arthritis Rheum 2000;43:1074-84.
68. Eisenberg RA, Earnshaw WC, Bordwell BJ, Craven SY, Cheek R, Rothfield NF. Isotype analysis of the anti-CENP-B anticentromere autoantibody: evidence for restricted clonality. Arthritis Rheum 1989;32:1315-8.
69. Earnshaw WC, Machlin PS, Bordwell BJ, Rothfield NF, Cleveland DW. Analysis of anticentromere autoantibodies using cloned autoantigen CENP-B. Proc Natl Acad Sci USA 1987;84:4979-83.
70. Vazquez-Abad D, Monteon V, Senécal JL, Walsh S, Rothfield N. Analysis of IgG subclasses of human antitopoisomerase I autoantibodies suggests chronic B cell stimulation. Clin Immunol Immunopathol 1997;84:65-72.
71. Henry PA, Atamas SP, Yurovsky VV, Luzina I, Wigley F, White B.

Diversity and plasticity of the anti-DNA topoisomerase I autoantibody response in scleroderma. Arthritis Rheum 2000;43:2733-42.
72. Plotz PH. The autoantibody repertoire: searching for order. Nature Rev Immunol 2003;3:73-8.
73. D'Arpa P, Machlin PS, Ratrie H 3rd, Rothfield NF, Cleveland DW, Earnshaw WC. cDNA cloning of human DNA topoisomerase I: catalytic activity of a 67.7-kDa carboxyl-terminal fragment. Proc Natl Acad Sci USA 1988;85:2543-7.
74. Kuwana M, Kaburaki J, Mimori T, Homma M. Autoantibody reactive with three classes of RNA polymerases in sera from patients with systemic sclerosis. J Clin Invest 1993;91:1399-404.
75. Cox JV, Schenk EA, Olmsted JB. Human anticentromere antibodies: distribution, characterization of antigens, and effect on microtubule organization. Cell 1983;35:331-9.
76. Bernat RL, Borisy GG, Rothfield NF, Earnshaw WC. Injection of anticentromere antibodies in interphase disrupts events required for chromosome movement at mitosis. J Cell Biol 1990;111:1519-33.
77. Chizzolini C, Rashi E, Rizzonico R, et al. Autoantibodies to fibroblasts induce a proadhesive and proinflammatory phenotype in patients with systemic sclerosis. Arthritis Rheum 2002;46:1602-13.
78. Ronda N, Gatti R, Giacosa R, Raschi E, Testoni C, Meroni PL. Antifibroblast antibodies from systemic sclerosis patients are internalized by fibroblasts via a caveolin-linked pathway. Arthritis Rheum 2002;46:1595-601.
79. DeGiorgio LA, Konstantinov K, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. Nature 2001;7:1189-93.
80. Howard OM, Dong HF, Yang D, et al. Histidyl–tRNA synthetase and asparaginyl–tRNA synthetase, autoantigens in myositis, activate chemokine receptors on T lymphocytes and immature dendritic cells. J Exp Med 2002;196:781-91.
81. Zhou X, Tan FK, Xiong M, et al. Systemic sclerosis (scleroderma): specific autoantigen genes are selectively overexpressed in scleroderma fibroblasts. J Immunol 2001;167:7126-33.
82. Velthuis PJ, Kater L, van der Tweel I, et al. In vivo antinuclear antibody of the skin: diagnostic significance and association with selective antinuclear antibodies. Ann Rheum Dis 1990;49:163-7.
83. Chen ZY, Dobson RL, Ainsworth SK, Silver RM, Rust PF, Maricq HR. Immunofluorescence in skin specimens from three different biopsy sites in patients with scleroderma. Clin Exp Rheumatol 1985;3:11-6.
84. Bryan C, Knight C, Black CM, Silman AJ. Prediction of five-year survival following presentation with scleroderma: development of a simple model using three disease factors at first visit. Arthritis Rheum 1999;42:2660-5.