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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/To²⁻³⁶. 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 manifestations³⁷. 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 mechanisms³⁸⁻⁴⁰. 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 inaccessible 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 Plotz⁴⁶.

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 controls⁴⁷. 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 SSc⁵⁴⁻⁵⁶. 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 SLE⁵⁹, 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/To²⁰. 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 it⁵⁴. 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 aAb⁶⁵. 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 SSc⁵⁸.

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 pathogenicity⁴⁶. 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 data⁶⁶. 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 TSS⁶⁶. 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 I⁶⁷. 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 demonstrated⁵. 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 nail-fold 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 SSc². 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 DNA⁷³, anti-RNA polymerase I/III aAb inhibit RNA transcription⁷⁴, 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 indi-

rect 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 pathogenesis⁶⁰.

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 microscopy⁶⁰.

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 SLE⁷⁹. 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 cells⁸⁰. 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 fibroblasts⁸¹. Also, an *in vivo* antinuclear antibody phenomenon has been described in epidermal cells from skin biopsies of SSc patients⁸². Moreover, in patients with circulating antinucleolar aAb, *in vivo* nucleolar epidermal fluorescence was observed⁸³, 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.

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