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Review

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Anti-nuclear autoantibodies in systemic sclerosis : News and perspectives

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Abstract

Systemic sclerosis is a connective tissue disorder characterized by microvascular damage and excessive fibrosis of the skin and internal organs. One hallmark of the immunological abnormalities in systemic sclerosis is the presence of anti-nuclear antibodies, which are detected in more than 90% of patients with systemic sclerosis. Anti-centromere antibodies, anti-DNA topoisomerase I antibodies, and anti-RNA polymerase III antibodies are the predominant anti-nuclear antibodies found in systemic sclerosis patients. Other systemic sclerosis–related anti-nuclear antibodies include those targeted against U3 ribonucleoprotein, Th/To, U11/U12 ribonucleoprotein, and eukaryotic initiation factor 2B. Anti-U1 ribonucleoprotein, anti-Ku antibodies, anti-PM–Scl, and anti-RuvBL1/2 antibodies are associated with systemic sclerosis overlap syndrome. Anti-human upstream binding factor, anti-Ro52/TRIM21, anti-B23, and anti-centriole antibodies do not have specificity to systemic sclerosis, but are sometimes detected in sera from patients with systemic sclerosis. Identification of each systemic sclerosis–related antibody is useful to diagnose and predict organ involvement, since the particular type of systemic sclerosis–related antibodies is often predictive of clinical features, severity, and prognosis. The clinical phenotypes are largely influenced by ethnicity. Currently, an immunoprecipitation assay is necessary to detect most systemic sclerosis–related antibodies; therefore, the establishment of an easy, reliable, and simple screening system is warranted.

Keywords

Systemic sclerosis, anti-nuclear antibodies, autoantibodies, clinical features

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Introduction

Systemic sclerosis (SSc) is characterized by microvascular damage and excessive fibrosis of the skin and various internal organs.[1] The clinical phenotype of SSc varies among patients. For instance, there are two distinct subtypes of skin involvement, depending upon the extent of the area involved.[2] Limited cutaneous systemic sclerosis (lcSSc) includes those patients in whom skin thickening is restricted to the face and/or distal to the elbow and/or knee, and skin thickening of the upper extremity is limited to the fingers in many cases.[3] In contrast, patients with diffuse-type SSc (diffuse cutaneous systemic sclerosis (dcSSc)) have extensive skin lesions over the elbow and/or knee, and skin lesions on the trunk are often observed. With the exception of pulmonary arterial hypertension (PAH), lcSSc is associated with relatively mild internal organ involvement, whereas dcSSc often leads to more serious complications that include interstitial lung disease (ILD) and scleroderma renal crisis (SRC).

SSc is considered to have an autoimmune etiology due to the following reasons: anti-nuclear antibodies (ANAs) are detected in more than 90% of patients, several potentially pathogenic autoantibodies (autoAbs) that target autoantigens of various components are reported, and there are cases for which immunosuppressive therapies such as cyclophosphamide and autologous stem cell transplantation are effective. ANAs react against a variety of intracellular components.[4] SSc-related ANAs are classified into two subgroups: SSc-specific autoAbs and SSc-associated autoAbs. SSc-specific autoAbs are specifically detected in

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SSc patients and rarely found in other connective tissue diseases or healthy subjects. This subgroup includes anti-centromere antibodies (ACAs), anti-DNA topoisomerase I (anti-topo I) antibodies (Abs; formerly anti-Scl-70 Abs), anti-RNA polymerase (RNAP) III Abs, anti-U3 ribonucleoprotein (RNP) Abs, anti-Th/To Abs, anti-U11/U12 RNP Abs, anti-eukaryotic initiation factor 2B (eIF2B) Abs, anti-U1 RNP Abs, anti-PM–Scl Abs, anti-Ku Abs, and anti-RuvBL1/2 Abs. Among these autoAbs, anti-U1 RNP Abs, anti-PM–Scl Abs, anti-Ku Abs, and anti-RuvBL1/2 Abs are found in a clinically distinct group of patients with SSc–myositis overlap syndrome. SSc-associated autoAbs are not specific to SSc and occasionally coexist with other SSc-specific and/or other connective tissue disease–related autoAbs. This group includes Abs against human upstream binding factor (hUBF), B23, Ro52/tripartite motif (TRIM) 21, and the centriole proteins. ANAs in patients with SSc exhibit several interesting features. First, the production of ANAs is unique for each patient, and the coexistence of two or more ANAs rarely occurs.[5] Second, once a specific ANA develops, the type and titers of ANA do not change throughout the course of disease. In addition, other SSc-related ANAs do not arise.[6]

Both the mechanism by which ANAs are produced and the role of ANAs in the pathogenesis of SSc remain unknown. However, the identification of SSc-specific and SSc-associated autoAbs in each patient is clinically useful to diagnose and evaluate organ involvement, since the particular type of ANA is often indicative of clinical features, severity, and prognosis.

Ethnic differences need to be taken into account when considering the association of ANAs with clinical features, since mounting evidence has revealed that the patient's genetic background can affect the prevalence and clinical phenotype of SSc. For example, anti-PM–Scl Abs are extremely rare in Japanese SSc patients,[7,8] and the association of anti-PM–Scl Abs with SSc is weak in Japanese patients compared to other countries.[9]

In this review article, we will review the novel findings of ANAs in patients with SSc, as well as the association of SSc-related ANAs with clinical characteristics.

Detection of antibody

When SSc-related ANAs are suspected, the first step is to confirm the existence of ANA. Indirect immunofluorescence (IIF) staining using HEp-2 cells is recommended for screening for ANA,[10] since the staining pattern and titer help to estimate SSc-related ANA specificities (Figure 1). ACAs and anti-centriole Abs can be identified by IIF, because both Abs exhibit a characteristic staining pattern (Table 1). Anti-U3 RNP Abs, anti-Th/To Abs, anti-PM–Scl Abs, and anti-hUBF Abs produce a nucleolar pattern that is commonly associated with SSc (Table 1). A cytoplasmic pattern is often indicative of myositis-specific autoAbs. While this pattern has been ignored in SSc, it is now necessary to pay attention to this pattern, since anti-eIF2B Abs produce a cytoplasmic staining pattern.[11] Except for ACA and anti-centriole Abs, additional techniques are required to confirm ANA specificities in patients' sera. Enzyme-linked immunosorbent assay (ELISA) is widely used in routine clinical practice. Although ELISA is excellent in convenience and cost, it is a disadvantage that only limited SSc-related ANAs can be measured and false positives occur frequently. Currently, ELISA systems for ACAs, anti-topo I Abs, anti-RNAP III Abs, and anti-U1 RNP Abs are commercially available. Immunodiffusion assay is another reliable test. Immunoprecipitation (IP) assay is a gold standard for identifying SSc-related ANAs, but only limited facilities adopt this assay due to the complicated procedure. Recently, a line blot assay was used to identify SSc-related ANAs in several studies. Although a line blot assay is easy to apply, physicians should be cautious about interpreting the results, since clinical usefulness of a line blot assay has not been fully established. It is necessary to use validated kits if the results obtained from ELISA and/or a line blot assay are inconsistent with clinical presentation, and re-evaluation using additional detection methods, such as IP assay, is highly recommended.[12]

Clinical usefulness of autoAbs in diagnosing early SSc

SSc remains an intractable disease for which no curative therapy has been developed.[13] Once the condition is fully established, SSc is often difficult to treat. Therefore, it is desirable to diagnose and intervene in the SSc patients at an early stage. The Joint Committee of the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) developed classification criteria for SSc to overcome the disadvantages of the 1980 ACR preliminary classification criteria.[14,15] The ACR/EULAR classification criteria include the presence of ACA, anti-topo I Abs, and anti-RNAP III Abs as one of the items. The presence of SSc-specific autoAbs is a predictive marker in identifying patients in a pre-SSc state who do not yet meet classification criteria for SSc, since SSc-specific autoAbs and nailfold capillary abnormalities are recognized as independent predictors for the future development of SSc in patients with Raynaud's phenomenon, but without any features of connective tissue diseases.[16] Valentini et al.[17] demonstrated faster progression of SSc in SSc-specific autoAb-positive patients, particularly in those with preclinical internal organ involvement at baseline, compared to SSc-specific autoAb-negative patients.

SSc-specific autoAbs

ACAs

ACAs were first reported by Moroi et al.[18] in 1980. ACAs can be identified by IIF, as they produce a characteristic

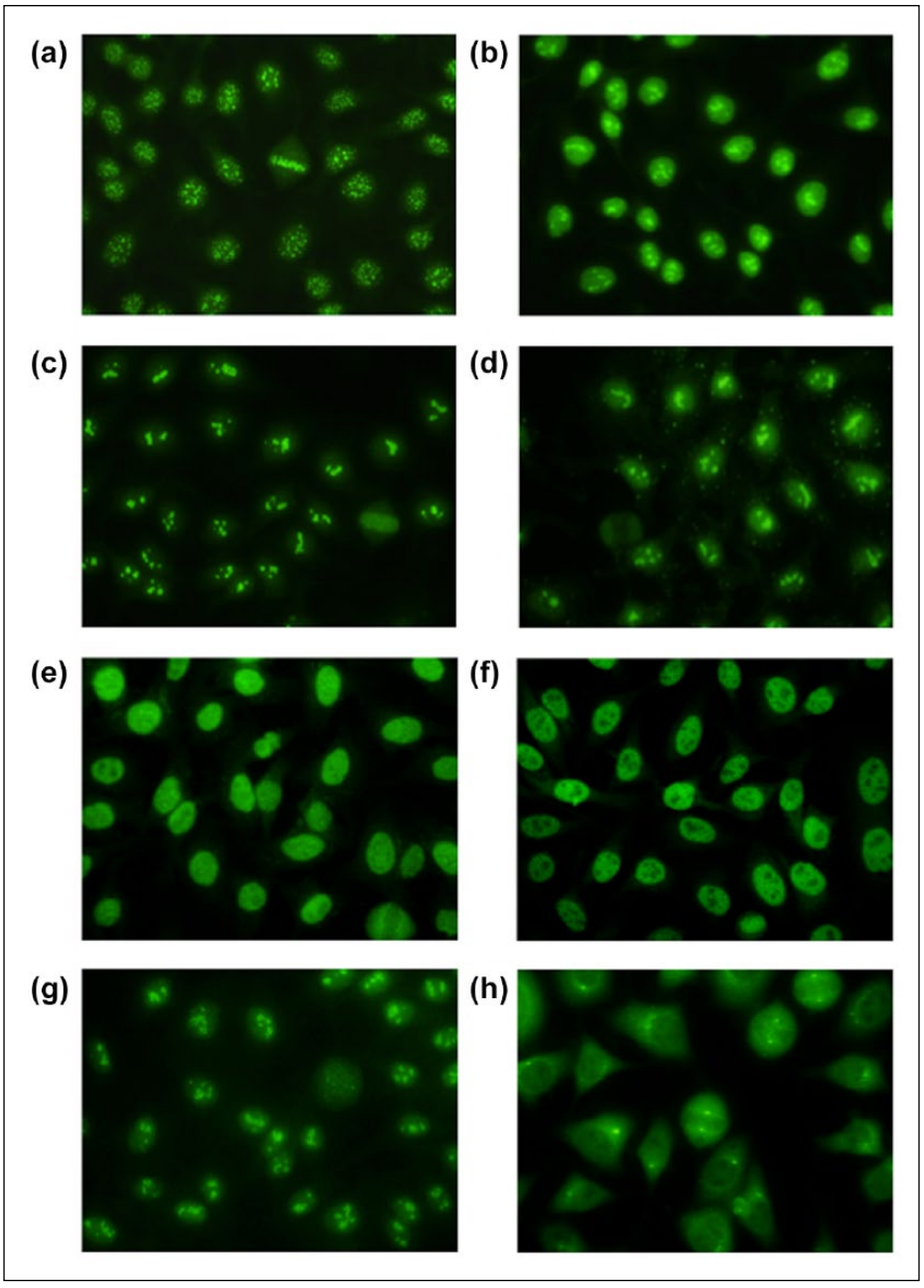

Figure 1. Indirect immunofluorescence patterns observed on HEp-2 cells stained with (a) anti-centromere antibody (Ab), (b) anti-topoisomerase I Ab, (c) anti-U3 RNP Ab, (d) anti-Th/To Ab, (e) anti-Ku Ab, (f) anti-RuvBL1/2 Ab, (g) anti-hUBF Ab, and (h) anti-centriole Ab sera (original magnification 400×).

staining pattern of punctate spots dispersed in the interphase nucleus, localized to the constriction on metaphase chromosomes (Figure 1(a)). This staining pattern is also termed discrete-speckled (DC; Table 1). There are at least six centromeric polypeptides, CENP-A–F, of which CENP-B is the major autoantigen that reacts with virtually all ACA-positive sera[19,20] (Table 1). The CENP-B antigen is used in commercially available ELISA systems with adequate sensitivity and specificity.[21,22] ACAs are occasionally detected in patients with primary biliary cirrhosis[23] or systemic lupus erythematosus (SLE).[24] ACAs are sometimes found even in healthy individuals without any connective tissue disease–related symptoms.[25] When ACAs are found in patients with Raynaud's phenomenon without skin thickening, it is predictive of the future development of lcSSc.[26,27]

The overall frequency of ACAs in SSc patients is 20%–30%. Approximately 30% of Caucasian SSc patients are positive for ACAs, whereas the frequency is lower in African American and Thai patients.[28,29]

Table 1. Systemic sclerosis–specific autoantibodies.

Autoantibody	Major autoantigen	IIF
Anti-centromere	CENP-A, -B, and -C	DC
Anti-topoisomerase I	DNA topoisomerase I	Ho + N or H + Sp
Anti-RNA polymerase	RNA polymerase I, II, and III	Sp and/or N
Anti-U3 RNP	Fibrillarin and other U3 RNP components	N
Anti-Th/To	H1/8-2 and Th/7-2 RNA	N
Anti-U11/U12 RNP	U11/U12 RNP complex	Sp
Anti-eIF2B	Eukaryotic initiation factor 2B	Cyto
Anti-U1 RNP	70 kDa, A and C polypeptides of U1 snRNP	Sp
Anti-PM–Scl	PM–Scl-75 and 100 proteins of the human exosome	N
Anti-Ku	80- and 70-kDa DNA binding dimeric protein	Sp
Anti-RuvBL1/2	RuvBL1 and RuvBL2 complex	Sp

IIF: indirect immunofluorescence staining pattern on HEp-2 cells; snRNP: small nuclear ribonucleoprotein; DC: discrete-speckled; Ho: Homogeneous; N: Nucleolar; Sp: speckled; Cyto: cytoplasmic.

ACAs are associated with limited skin involvement, peripheral vasculopathy, calcinosis, and PAH[30] (Table 2). The frequency and degree of peripheral ischemia vary among different ethnicities. Pitting scars or ulcers occurred in 42%–61% of Caucasian and/or African American patients[31–33] and, in contrast, only 11%–17% in Japanese patients.[7,8] Digital gangrene is also observed more frequently in Caucasian and/or African American patients. Severe internal organ involvement, such as ILD or SRC, seldom occurs. However, the presence of ACAs can be predictive of the development of PAH at a late stage[10,34] and the DETECT algorithm to identify SSc patients at high risk of developing PAH includes ACAs as one of the indices.[35] Generally, ACA-positive SSc patients have a more favorable prognosis than patients with other SSc-related autoAbs,[34] unless ACA-positive patients are suffering from PAH.

Anti-topo I Abs

The autoAbs against a 70- to 100-kDa chromatin-associated protein in patients with SSc were first identified as anti-Scl-70 Abs.[46] This protein was later identified as DNA topoisomerase I[47] (Table 1). The IIF staining pattern of anti-topo I Abs is a combination of a homogeneous with a nucleolar or a homogeneous with a speckled pattern (Figure 1(b)). Anti-topo I Abs were found in about 40% of patients with SSc, although this varies widely among different ethnicities, ranging from 28% to 70%.[7,48] Anti-topo I Abs are highly specific to SSc and are rarely found in healthy individuals or in patients with other connective tissue diseases.[48] Coexistence with ACAs occurs in about 0.5% of SSc patients only.[49] Anti-topo I Abs are detected in about 40% of patients with dcSSc, and another study demonstrated that one third of anti-topo I Ab-positive patients had lcSSc.[50]

It is well recognized that anti-topo I Abs are strongly associated with a higher risk for severe ILD, resulting in increased mortality (Table 2).[51] ILD occurs in >70% of patients with anti-topo I Abs during the disease course, and about 25% develop severe disease that requires oxygen supplementation. Wells et al.[52] have reported that the percent predicted diffusing capacity of the lungs for carbon monoxide reflected the extent of ILD and, therefore, routine measurement was recommended. SRC, cardiomyopathy, and severe peripheral vasculopathy including digital ulcers/gangrene are also important organ involvement outcomes in patients with anti-topo I Abs.[7,31] Anti-topo I Abs are considered to be a marker for poor prognosis, since a uniformly effective therapy for the treatment of ILD has not yet been established.

Serial measurements of antibody titers are not generally considered to be useful for monitoring disease severity in SSc patients. However, several studies have found that anti-topo I Ab levels, as determined by ELISA, were related to disease severity and that conversion to seronegativity resulted in disease remission.[53–55]

Anti-RNAP III Abs

Major targets of anti-RNAP Abs are RNAP I, II, and III[6,56,57] (Table 1). There are four patterns of combination observed for reactivity to anti-RNAP Abs: RNAP I/III, RNAP I/II/III, RNAP III, and RNAP II.[58] Abs to RNAP I and III routinely coexist and this pattern is highly specific for SSc.[58] Anti-RNAP I/III Ab-positive patients sometimes have anti-RNAP II Abs (anti-RNAP I/II/III Abs). Anti-RNAP III Abs alone may be present in only a few cases. Anti-RNAP II Abs alone are detected at a low frequency in SSc patients in combination with anti-topo I Abs, as well as in sera from patients with other connective tissue diseases, including SLE or an overlap syndrome.[59,60]

The prevalence of anti-RNAP III Abs is influenced by patient ethnicity. In Caucasians in North America and the United Kingdom, 20%–25% of SSc patients are positive for anti-RNAP III Abs, whereas 15.3% (69 out of 451) of

Table 2. Clinical characteristics associated with systemic sclerosis–specific autoantibodies.

Autoantibody	Subtype	Clinical manifestations							Reference
		Digital ulcer/ gangrene	ILD	PAH	Heart	Renal	Muscle	Joint	
Anti-centromere	lcSSc	11–61	6–20	0–19	2–16	0–1	0–3	20–60	Kuwana et al.,[7] Steen,[31] and Mitri et al.[32]
Anti-topoisomerase I	dcSSc	41–63	67–89	0–14	9–20	4–10	6–13	36–86	Kuwana et al.,[7] Steen,[31] Satoh et al.,[36] and Cavazzana et al.[37]
Anti-RNA polymerase	dcSSc	7–51	7–66	4–10	7–50	14–43	0–4	14–88	Kuwana et al.,[7] Steen,[31] Nikpour et al.,[38] and Meyer et al.[39]
Anti-U3 RNP	dc=lc	40–58	12–36	0–31	10–23	6–12	10–33	10–89	Kuwana et al.,[7] Steen,[31] Tormey et al.,[40] and Aggarwal et al.[41]
Anti-Th/To	lcSSc	24–29	16–48	28–32	7–21	4–5	6	60	Kuwana et al.,[7] Steen,[31] and Mitri et al.[32]
Anti-U11/U12 RNP	dc=lc	NR	79	0	24	6	6	79	Fertig et al.[42]
Anti-eIF2B	dcSSc	50	86	0	67	NR	29	NR	Betteridge et al.[11]
Anti-U1 RNP	lcSSc	30-49	22–49	14–18	3–11	0–7	20–27	52–94	Kuwana et al.[7] and Steen[31]
Anti-PM–Scl	lcSSc	47	17–50	3–8	6–11	4–8	35–58	58–75	Steen,[31] D'Aoust et al.,[43] and Kaji et al.[44]
Anti-Ku	lcSSc	0–29	43–57	14–46	0–21	0–2	43–100	43	Kuwana et al.,[7] Kaji et al.,[44] and Rozman et al.[45]
Anti-RuvBL1/2	dcSSc	86	56	12	30	3	57	NR	Kaji et al.[44]

ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; RNP: ribonucleoprotein; lcSSc: limited cutaneous systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; NR: not reported.
Values are given as percentages.

Australian Caucasian SSc patients have anti-RNAP III Abs.[38,61] On the other hand, only 6%–9% were positive in a French cohort.[61,62] An Italian cohort study also reported a low frequency of anti-RNAP III Abs: only 16 of 466 (3%) have anti-RNAP III Abs.[63] In a Japanese population, anti-RNAP III Abs are found in 6%–10%[7,8,36] of SSc patients. An ELISA system using recombinant RNAP III as the antigen is currently available and widely used in clinical settings.[36,64]

Most SSc patients with anti-RNAP III Abs present a diffuse cutaneous form with rapidly progressive skin thickening (Table 2). Nevertheless, many patients experience rapid regression of skin thickening over time, even without treatment.[12] Anti-RNAP III Abs are also strongly associated with SRC independent of ethnicity.[31,48,56,65] SRC occurred in 25% of patients with anti-RNAP III Abs in contrast to 12% in other patients.[31] A recent study clarified clinical and immunological predictors of SRC and found that anti-RNAP I/II/III Ab positivity and a higher anti-RNAP III Ab titer, as measured by ELISA, were the independent factors associated with the development of SRC.[65] It has been reported that two anti-RNAP III Ab-positive patients without skin thickness developed SRC. Skin sclerosis was absent at SRC onset, but the two patients eventually developed diffuse and rapidly progressive skin thickening.[66] Conversely, ILD that requires aggressive therapy and severe peripheral ulcers/gangrene rarely occurred in this subgroup. The prognosis of patients with anti-RNAP III Abs was the worst in all SSc patients until angiotensin-converting enzyme (ACE) inhibitors were developed. However, mortality rates in patients with anti-RNAP III Abs have dramatically improved; currently, prognoses for patients with anti-RNAP III Abs are better than those with anti-topo I or anti-U3 RNP Abs.[31] This is due to the fact that patients with anti-RNAP III Abs have a low risk of suffering ILD, and SRC is now more readily treated with ACE inhibitors.[67] Regular, daily monitoring of blood pressure can help with the early diagnosis of SRC, and the prompt introduction of an ACE inhibitor can result in reduced mortality. Another clinical characteristic of patients with anti-RNAP III Abs is gastric antral vascular ectasia (GAVE), also known as watermelon stomach. A case–control study identified anti-RNAP III Abs as a risk factor for GAVE.[68]

A recent topic of interest is the association of anti-RNAP III Abs with malignancy.[69] Shah et al.[70] reported a

close temporal relationship between the onset of cancer and anti-RNAP I/III positivity in SSc patients. The median duration of SSc at the time of cancer diagnosis is significantly different among SSc-related Ab-based subgroups: –1.2 years for anti-RNAP I/III Abs, +13.4 years for anti-topo I Abs, +11.1 years for ACAs, and +2.3 years for the SSc-specific Ab-negative group. This group also reported that anti-RNAP III positivity and older age at scleroderma onset were significantly associated with a short cancer–scleroderma interval.[71] Joseph et al.[72] reported that novel antigens were encoded by somatically mutated genes in SSc patients with cancer. Genetic alterations of the *POLR3A* locus that encodes RPC1 were found in anti-RNAP III Ab-positive SSc patients with Abs to RPC1 but not in those without Abs to RPC1, and *POLR3A* mutations triggered cellular immunity and cross-reactive humoral immune responses in anti-RNAP III Ab-positive patients with cancers.

Anti-U3 RNP Abs

Anti-U3 RNP Abs were first found in sera from SSc patients in 1985.[73] The major autoantigen of anti-U3 RNP Abs is identified as fibrillarin, which is a 34-kDa protein and a component of the nucleolar U3 RNP complex (Table 1). Anti-U3 RNP Abs produce a nucleolar, clumpy IIF staining pattern (Figure 1(c)).[40]

The frequency of anti-U3 RNP Abs is around 4%–10% of SSc patients.[7,8,31,40,41] Anti-U3 RNP Abs are generally specific to SSc, but have also been described in patients with SLE.[10,74] Two thirds of patients with anti-U3 RNP Abs have dcSSc, but one third have the limited cutaneous form. In African American SSc patients, approximately 30% are positive for anti-U3 RNP Abs.[31] Severe internal organ involvement, such as ILD, PAH, cardiomyopathy, and SRC are common in anti-U3 RNP Ab-positive patients, irrespective of dcSSc or lcSSc (Table 2). Anti-U3 RNP Abs were reported to be an independent risk factor for the development of PAH,[75] and PAH is the most common cause of death, leading to an increased mortality in this subgroup.[41] Nishimagi et al.[76] reported that 5 out of 14 patients who experienced severe gastrointestinal tract involvement, including malabsorption syndrome and/or pseudo-obstruction within 2 years of onset of SSc, had anti-U3 RNP Abs. Prognosis in patients with anti-U3 RNP Abs is poor and comparable to that in SSc patients with anti-topo I Abs.

Anti-Th/To Abs (known as anti-7-2RNA Abs)

Okano and Medsger[77] first reported Abs to Th/To (anti-Th/To Abs) in 1990. Anti-Th/To Abs produce a nucleolar, dotty IIF staining pattern (Figure 1(d)). Anti-Th/To autoantigens are RNPs associated with H1/8-2 and Th/7-2 RNAs (Table 1). H1/8-2 is a component of RNase P and TH/7-2 is a component of RNase mitochondrial RNA processing (MRP), and both are RNA processing enzymes[78–80] (Table 1). There are at least six subunits consisting of these complexes and a 120-kDa protein contains the major epitope.[81] Anti-Th/To Abs were originally specific for SSc or Raynaud's disease, but were subsequently detected in patients with localized scleroderma.[82]

Anti-Th/To Abs are found in 2%–5% of SSc patients. Anti-Th/To Abs are associated with lcSSc, but their overall prognosis is worse, since anti-Th/To Ab-positive patients have a higher risk for ILD and PAH[83,84] (Table 2). Mitri et al.[32] compared the clinical features between ACA and anti-Th/To Abs and found that patients with anti-Th/To Abs were younger and had a shorter disease duration at their first evaluation than those with ACAs. Both subgroups had a higher frequency of PAH (28% of anti-Th/To Abs and 19% of ACAs), but anti-Th/To Ab-positive patients had worse prognoses because anti-Th/To Ab-positive patients were more often suffering from ILD. However, in Japanese SSc patients with anti-Th/To Abs, internal organ involvement is not as severe as in Caucasian patients.[7,8]

Anti-U11/U12 RNP Abs

Fertig et al.[42] reported 33 patients with anti-U11/U12 RNP Abs who were identified by RNA-IP assay. Anti-U11/U12 RNP Abs produce a speckled nuclear IIF staining (Table 1). Anti-U11/U12 RNP Abs were found in 1%–3% of patients with SSc. The ratio of dcSSc and lcSSc in this cohort was almost 1:1. All 33 patients with anti-U11/U12 RNP Abs had Raynaud's phenomenon and 82% had gastrointestinal tract involvement. Although none of the 33 patients with this antibody had PAH, nearly 80% of patients with anti-U11/U12 RNP Abs had ILD, which is often severe and rapidly progressive, resulting in an increased mortality (Table 2).

Anti-eIF2B Abs

Anti-eIF2B Abs were recently identified by protein-IP assay.[11] The autoantigen targeted by anti-eIF2B Abs has a molecular weight of 30 kDa. Sera from patients with anti-eIF2B Abs produce a cytoplasmic IIF staining pattern (Table 1). Out of 548 SSc patients, 7 (1.3%) were positive for anti-eIF2B Abs, and six out of seven had dcSSc. ILD was confirmed in six out of seven patients with anti-eIF2B Abs (Table 2). Four out of seven anti-eIF2B Ab-positive patients had overlap features of either myositis or rheumatoid arthritis (RA) (two with myositis and two with RA). SSc specificity and clinical characteristics of anti-eIF2B Abs need to be confirmed in a larger cohort study among different ethnicities.

Anti-U1 RNP Abs

Anti-U1 RNP Abs are directed against the 70-kDa A and C proteins associated with U1 RNA (Table 1). Anti-U1 RNP

Abs yield a pure speckled pattern with a high antibody titer. Anti-U1 RNP Abs are the hallmark of mixed connective tissue disease (MCTD), and can occur in SLE in combination with anti-dsDNA Abs or anti-Sm Abs,[85,86] but are also detected in patients with SSc comorbidity (range 2%–14%).[7,31,48,87] Clinical features of anti-U1 RNP Abs include lcSSc, puffy fingers, Raynaud's phenomenon, arthritis, and esophageal dysfunction. PAH can occur and cause increased mortality (Table 2).[31] Anti-U1 RNP Abs are generally predictive of a better prognosis, but PAH is the most common cause of death.[31,88]

Anti-PM–Scl Abs

Anti-PM–Scl Abs were first identified in 1977 in patients with overlap syndrome of polymyositis (PM) and SSc.[89] The PM–Scl antigen consists of 11–16 polypeptides forming antigens 75–100 kDa in size[90] (Table 1). Anti-PM–Scl Abs have a homogeneous nucleolar staining pattern. Anti-PM–Scl Abs are found in 4%–11% of SSc patients overall, but the prevalence of anti-PM–Scl Abs is significantly associated with certain ethnicities, since anti-PM–Scl Abs are strongly associated with HLA DQA1*0501 and HLA DRB1*0301.[91] In fact, anti-PM–Scl Abs are rarely found in non-Caucasian patients.[92] In two Japanese cohort studies, none of the SSc patients were positive for anti-PM–Scl Abs.[7,8] Anti-PM–Scl Abs are detected in approximately 25% of SSc–myositis overlap patients, but in only 2% of SSc patients alone.[48,83,93] In a Japanese study, nine anti-PM–Scl Ab-positive patients were identified by IP assay, including 4 out of 16 (25%) with undifferentiated connective tissue disease, 3 out of 126 (2.4%) with dermatomyositis, 1 out of 223 (0.4%) with SSc, and 1 out of 88 (1.1%) with Sjögren's syndrome.[9] Anti-PM–Scl Ab-positive patients often present with subacute myositis, limited cutaneous form SSc, and less serious internal organ involvement[43] (Table 2). Previous reports revealed a good response to low or moderate dosage of corticosteroids, and there is a favorable prognosis in patients with anti-PM–Scl Abs.[94] The prevalence and SSc specificity may be different between Japanese and Caucasian patients. Alternatively, the clinical phenotype of anti-PM–Scl is probably different from "classical" SSc and has the descriptor of sclerodermatomyositis.

Anti-Ku Abs

Mimori et al.[95] initially reported a case of PM/SSc overlap syndrome with anti-Ku Abs in 1994. The Ku autoantigen is now recognized as a heterodimer of 70-kD and 80-kD subunits (Table 1). Anti-Ku Abs present a speckled nuclear staining pattern, but can be distinguished from that produced by anti-U1 RNP Abs, since the nuclei are stained in a reticular pattern that spares the nucleoli (Figure 1(e)).[95] Anti-Ku Abs were originally considered to be specific to SSc, but subsequent studies reported that anti-Ku Abs are also detected in patients with other autoimmune connective tissue diseases, including SLE and overlap syndrome.[96,97] Franceschini et al.[97] reported 14 anti-Ku Ab-positive patients; one-half had an overlap syndrome (five with PM/SSc, one with PM/SLE/SSc, and one with PM/SLE). Skin sclerosis is mild in this subgroup, and internal organ involvement is less frequent and mild if present (Table 2). In addition, vascular complications, including digital ulcers or telangiectasia, are not common.[45] Myositis is usually mild with a good response to corticosteroids, leading to a favorable prognosis.

Anti-RuvBL1/2 Abs

Our group reported the clinical characteristics of 37 patients with newly identified anti-RuvBL1/2 Abs in 2014.[44] RuvBL1 and RuvBL2 are highly conserved eukaryotic proteins that form a double hexamer in the nucleoplasm (Table 1). Anti-RuvBL1/2 Abs have a speckled nuclear IIF staining pattern with a high antibody titer (Figure 1(f)). Anti-RuvBL1/2 Abs were detected in 10 out of 588 (1.7%) SSc patients in a Japanese cohort and 27 out of 585 (4.6%) SSc patients in a Pittsburg, PA cohort. Anti-RuvBL1/2 Abs were highly specific to SSc and strongly associated with SSc in overlap with myositis in both the Japanese and Pittsburgh cohorts. The diffuse type is dominant in patients with anti-RuvB1/2 Abs. Compared with other SSc–myositis overlap–related autoAbs (anti-PM–Scl Abs and anti-Ku Abs), anti-RuvBL1/2 Abs were distinctive in terms of its associations with older age at SSc onset, male gender, and a high frequency of the diffuse cutaneous form (Table 2).

SSc-associated autoAbs

Anti-hUBF Abs (formerly anti-NOR90 Abs)

AutoAbs reactive with nucleolus-organizing region (NOR) 90 were first reported in 1987.[98] Later, the autoantigen specificity of this autoAb was identified as hUBF.[99] IIF staining shows that staining is limited to nucleoli and has a coarse speckled pattern (Figure 1(g)).[98] Anti-hUBF Abs are detected not only in SSc, but also in other autoimmune connective tissue diseases such as Raynaud's disease, Sjögren's syndrome, RA, and SLE. Anti-hUBF Abs are also found in some malignancies.[100–102] Although reported cases with anti-hUBF Abs are limited, previous studies demonstrated that anti-hUBF Abs are probably related to lcSSc, mild organ involvement, and a favorable prognosis.[101] More cases are needed to clarify the clinical characteristics of anti-hUBF Abs.

Anti-Ro52/TRIM21 Abs

Anti-SSA/Ro Abs can occur in SSc patients with concomitant Sjögren's syndrome. Ro antigens consist of the proteins

Ro52 and Ro60.[103,104] Ro52 is also termed tripartite motif family of protein 21 (TRIM21). AutoAbs against Ro52/TRIM21 (anti-Ro/TRIM21 Abs) are detected in patients with various connective tissue diseases, especially in SLE and Sjögren's syndrome.[105] A multicenter cohort study revealed that anti-Ro52/TRIM21 Abs were detected in 20% of the patients and associated with ILD and overlap syndrome.[106] A larger cohort study involving 1574 SSc patients confirmed that 324 (20.6%) patients had anti-Ro52/TRIM21 Abs, an association with ILD and poor survival.[107]

Anti-B23 Abs

B23 is one of the most abundant proteins in the nucleolus. It is involved in pre-ribosomal RNA processing and ribosome assembly.[108,109] Anti-B23 Abs have a nucleolar IIF pattern. Anti-B23 Abs are detected in <11% of SSc patients and are associated with moderate to severe PAH and anti-U3 RNP Abs.[110]

Anti-centriole Abs

Moroi et al.[111] reported two cases of anti-centriole Abs, one with SSc and the other suffering from Raynaud's phenomenon. Anti-centriole Abs can also be detected by IIF due to a characteristic staining pattern in which each of two dots per cell is located at each side of the visibly grouped chromosomes in mitotic HEp-2 cells (Figure 1(h)). A recent study reported five patients with anti-centriole Abs,[112] all of which were female and had digital ulcers/gangrene. Four of the five (80%) patients had PAH and none of them had active ILD or developed SRC. Anti-centriole Abs may be a marker for a subgroup of severe vasculopathy, such as digital ulcers/gangrene and PAH. More studies are needed to confirm whether anti-centriole Abs are specific to SSc and to report on the clinical characteristics in patients with anti-centriole Abs.

ANA-negative SSc

As described above, ANAs are positive in more than 90% of SSc patients. However, there is a small proportion of SSc patients who are negative for ANAs, and these patients appear to form one unique subgroup. Salazar et al.[113] reported that 208 of 3249 (6.4%) SSc patients were ANA negative in the Scleroderma Family Registry and DNA repository. ANA-negative SSc patients are more likely to be male, less frequently have vasculopathy, such as digital ulcers, telangiectasia, and PAH, and more frequently present with lower gastrointestinal involvement.[113] Meanwhile, the possibility that the ANA-negative patients have other autoAbs that have not been currently identified cannot be ruled out.

Potentially pathogenic autoAbs

Apart from autoAbs that have corresponding antigens in the nucleus or cytoplasm, it has been reported that several autoAbs may have a potentially pathogenic role in tissue fibrosis and vascular damage in SSc.[114–116] These autoAbs include autoAbs against the endothelial cell antigens, matrix metalloproteinases (MMPs), and the platelet-derived growth factor receptor (PDGFR).

Anti-endothelial cell Abs

AutoAbs directed to the endothelial cell antigens were initially reported in sera from patients with primary Raynaud's phenomenon and SSc.[117] Anti-endothelial cell antibodies (AECAs) were detected in 25%–85% of patients with SSc, but are also seen in other connective tissue diseases.[117–119] The presence of anti-endothelial cell Abs was associated with severe vascular complications including severe Raynaud's phenomenon, digital ulcers, and PAH. ILD also frequently occurs in patients with SSc with AECAs. AECA-induced apoptosis of endothelial cells, via activation of the caspase 3 pathway and expression of fibrillin-1, was linked to subsequent autoAb production to fibrillin-1.[120] In another study, purified IgG from AECA-positive SSc and SLE patients with PAH induced significantly higher expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin and production of interleukin (IL)-6, IL-8, and chemokine (C–C motif) ligand 2 (CCL2) on human umbilical vein endothelial cells compared to IgG from AECA-negative patients.[121] Therefore, AECAs may alter the endothelial cell function in SSc to increase adhesion molecules for leukocytes and pro-inflammatory cytokines and chemokines, leading to vascular damage.

Anti-MMP Abs

AutoAbs to MMP-1[122] and MMP-3[123] were reported in the sera of patients with SSc. Anti-MMP-1 and anti-MMP-3 Ab levels were significantly higher in dcSSc than those in lcSSc, and also correlated with fibrosis of the skin, lung, and renal blood vessels. Moreover, IgG anti-MMP-1 and anti-MMP-3 Abs in sera from patients with SSc inhibited MMP collagenase activity. Therefore, anti-MMP-1 and anti-MMP-3 Abs are serological markers that reflect the severity of SSc and contribute to the development of fibrosis by inhibiting collagenase activity and reducing extracellular matrix turnover.

Anti-PDGFR Abs

Baroni et al.[124] reported anti-PDGFR Abs in patients with SSc, but not in healthy controls or patients with SLE, RA, idiopathic pulmonary fibrosis, or primary Raynaud's

phenomenon. These autoAbs may have a pathogenic role, since PDGFR expression is increased by pathologic transforming growth factor (TGF)-β signaling, and binding of PDGFR to anti-PDGFR Abs results in the amplification of the Ras–ERK1/2–ROS cascade, leading to enhanced collagen production.[124–126] Further analysis revealed that anti-PDGFRα Abs recognize specific conformational epitopes, leading to a blockade of PDGFRα signaling in patients with SSc,[127] and anti-PDGFR Abs from patients with SSc induced fibrosis in skin-humanized mice.[128] These data provide important information that is critical to elucidating the pathophysiology of SSc. However, neither an agonistic role for anti-PDGFR Abs nor specificity of these autoAbs for SSc was found in other reports.[125,129] Therefore, the role of anti-PDGFR Abs in SSc remains to be confirmed.

Conclusion

Since clinical features and prognoses in SSc patients largely vary, it is clinically significant for classifying SSc patients into subgroups based on their autoAb status. Identification of autoAbs in SSc patients is also useful for early diagnosis. Novel findings for each SSc-specific autoAbs are being reported; however, these findings are virtually limited to the SSc-specific autoAbs for which an ELISA procedure has been developed. Most SSc-specific and SSc-associated autoAbs still require IP assays for identification. An easy, reliable, and simple screening system for ANA specificities is needed.

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