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Invited article

Autoantibodies in Scleroderma

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Abstract

Autoantibodies directed against nuclear, nucleolar, and a number of cytoplasmic components are described in the sera of scleroderma patients. Early studies of autoantibodies that relied on cryopreserved sections of rodent organ substrates showed that approximately 50% of scleroderma patients had anti-nuclear antibodies (ANA). More recent studies that have used tissue culture cell substrates have shown that up to 98% of scleroderma patients have a positive ANA. In all of these studies, the presence of different patterns of staining have suggested that scleroderma sera reacted with a variety of intracellular antigens. The use of molecular and immunochemical techniques has now shown that over 20 intracellular autoantigens are targets of autoantibodies in scleroderma sera. Clinical studies have shown that these autoantibodies are important diagnostic and prognostic markers in scleroderma. In the future, autoantibody testing may be used to monitor the patient's response to immunological therapies.

Key words: scleroderma; antibodies; autoantibodies; antinuclear antibodies; review

Introduction

For many years, autoantibodies were thought to occur in low frequency and low titer in the sera of patients with progressive systemic sclerosis (scleroderma). However, over the past decade with the aid of more sensitive diagnostic tests and molecular biological techniques, several unique autoantibody systems have been associated with this disease (Table 1) (reviewed in 1). These include antibodies to Scl-70, centromere and kinetochore components, RNA polymerase I, II and III, fibrillarin, and Th/To. Some autoantibody systems, such as PM-Scl and Ku, are characteristically seen in overlap syndromes (2). Other antibodies are directed to antigens in a variety of organelles such as mitochondria and centrioles.

It is important to interpret data on the frequency and association of autoantibodies with clinical features of scleroderma, because many of these autoantibodies appear to be variably expressed in different races. However, the identification of autoantibodies in patients suspected of having scleroderma has the potential of providing the physician with important clinical information. First, some antibodies are specific markers for scleroderma and for subsets of the disease. Second, some autoantibodies antedate full expression of the disease, permitting an early diagnosis and a prediction of the natural course of the disease. Third, as newer therapeutic strategies that target specific regulators of immune function are developed, autoantibodies may become valuable markers of disease activity.

Autoantibodies in Scleroderma and Scleroderma Overlap Syndromes

Scl-70/Topoisomerase I: Antibodies directed against Scl-70 were among the first autoantibodies identified that were specific for scleroderma. Indirect immunofluorescence studies of monospecific anti-Scl-70 sera revealed a minute and evenly distributed speckled staining pattern in tissue culture cell nuclei (3). The antigen, isolated from rat liver, was first identified as a 70 kD protein by SDS-polyacrylamide gel electrophoresis (4). Subsequent studies showed that the 70 kD protein was an

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Table 1. Autoantibodies and antigens in scleroderma

Specificity	Nature of antigen	Clinical association
Scl-70	DNA topoisomerase I 100 kD protein	acroosteolysis, pulmonary hypertension, diffuse scleroderma
Centromere	19, 50, 80 and 140 kD proteins	CREST, digital ulcers, telangiectasia
Fibrillarin	34 kD protein	diffuse skin involvement, multiple organ involvement, pulmonary hypertension
RNA polymerase	multiple subunits of three enzymes (Pol I, II and III)	high skin score, renal crisis
To/Th	40 kD protein	limited skin involvement
U1 RNP	70 kD protein A (33 kD) and C (22 kD)	overlap syndrome: MCTD
Ku	80 and 60 kD proteins	scleroderma/ polymyositis overlap
Ki	~30 kD protein	in PSS unknown
PM/Scl	75 kD acidic protein	scleroderma/ myositis overlap, sclerodactyly, telangiectasia, calcinosis, myositis
Wa	48 kD protein	Sjögren's syndrome
HMG 14/17	14 and 17 kD proteins	CREST: correlated with centromere antibodies
M-A, -B, -C	mitochondrial antigens	primary biliary cirrhosis

Table 2. Frequency of autoantibodies in scleroderma and polymyositis-scleroderma overlap

Autoantibody	Frequency (%)		Reference
	Sclero-derma	PM/Scl overlap*	
Scl-70-topoisomerase I	8–75	12	(2, 8, 119)
Fibrillarin (U3-RNP)	7–20	—	(68)
RNA Polymerases I, II, III	4–20	—	(40–42)
Centromere/ kinetochore	18	—	(119)
Th/To	4–8	0–3	(68, 73)
U1-RNP	2–12	13	(68, 119)
U2-RNP	0	4	(68)
U4/U6-RNP	rare	rare	(102)
PM-Scl	2–5	24	(37, 51, 53)
Ku	<5	26–55	(2, 68, 75)
Ki	7	7–19	(2, 87, 120)
Wa	3	—	(100)

*PM-Scl=polymyositis-scleroderma overlap syndrome

antigenically active proteolytic fragment of a native 100 kD molecule identified as topoisomerase I (5–7). In early studies, anti-Scl-70 was identified in 20% of scleroderma sera (3), but, with the use of a more stable antigen, other studies reported the frequency of this antibody to be 75% in this disease (8). Less than 1% of patients with other connective tissue diseases have anti-Scl-70 antibodies (9). In addition, studies of patients with Scl-70 antibodies have suggested that these patients are at higher risk of developing acroosteolysis and pulmonary involvement (8, 10, 11). Therefore, the presence of Scl-70 antibodies tends to convey a poor prognosis.

Recent studies have shown the association of specific amino acid sequences in the first domain of HLA-DQβ1 with Scl-70 antibodies (12). These observations are probably important in explaining racial differences in the frequency of autoantibodies. For example, precipitating Scl-70 antibodies were detected in 76% of Thai scleroderma patients but in only 26% of Australian patients (13).

Centromere-Kinetochore: In 1980, antibodies

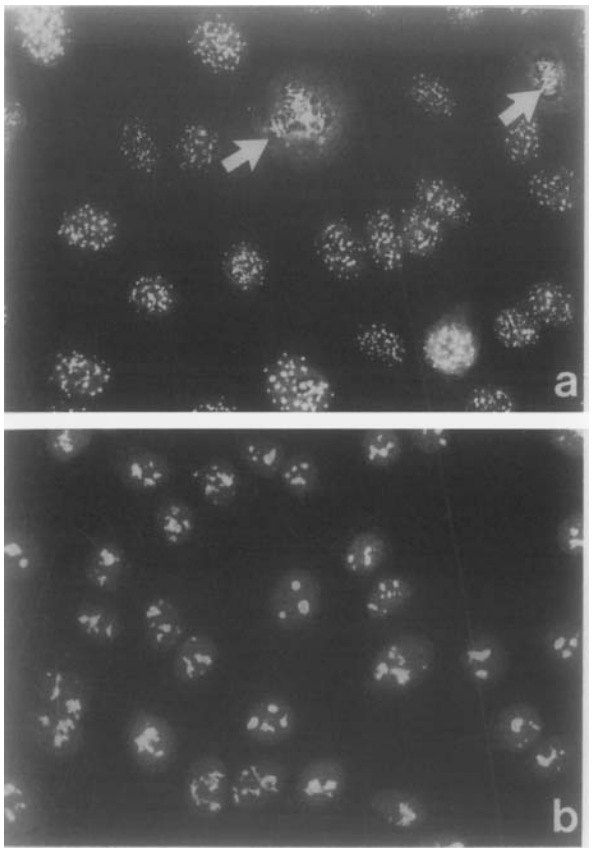

Fig. 1. Indirect immunofluorescence using HEp-2 cells as substrate. (a) Anti-centromere antibodies are characterized by a punctate speckled staining pattern that is dispersed in the interphase nucleus but localized to the metaphase plate (arrows) in dividing cells. (b) Antibodies directed against fibrillarin (U3-RNP) are represented by a clumpy pattern of nucleolar staining. In some nuclei, small speckles representing nucleolar bodies may be seen adjacent to the nucleolus.

directed to centromere antigens were identified in the sera of patients with the CREST variant of scleroderma (9, 14). The indirect immunofluorescence pattern of staining on tissue culture cells is typically a finite number of punctate spots that are dispersed in the interphase nucleus but localized to the primary constriction (centromere) on metaphase chromosomes (Fig. 1a) (9). Recent studies have confirmed that some centromeres are intimately associated with the nucleolus (15). This supports the concept that the autoimmune response in scleroderma is commonly directed to either structural or functional nucleolar components.

Western immunoblotting of anti-centromere sera has identified at least four different centromere protein antigens: CENP-A, CENP-B, CENP-C, CENP-D (16–22). Antibodies to CENP-B, an 80 kD protein, are almost universally found in sera that demonstrate the centromere pattern of staining. CENP-A (19 kD), followed by CENP-C (140 kD) antibodies, are the next most common. Most scleroderma with anti-centromere antibodies demonstrate reactivity to at least two of these antigens (18). However, the clinical relevance of reactivity to different centromere antigens has not been thoroughly studied. Antibodies to centromere antigens are commonly seen in the CREST variant of the disease and are generally associated with a better prognosis (23). On the other hand, studies in our laboratory have shown that patients who have antibodies to centromere antigens and to nuclear histones have severe end organ involvement (24). In North American patients, the presence of centromere antibodies has been correlated with the presence of polar amino acids at position 26 of the HLA-DQβ1 first domain (25). Approximately 25% of unselected patients with idiopathic Raynaud's phenomenon have anti-centromere antibodies (26, 27). The value of testing patients with Raynaud's phenomenon for autoantibodies, including anti-centromere antibodies, was shown by a recent study which showed that the antinuclear antibody was the single most valuable test in predicting the clinical evolution of Raynaud's phenomenon into specific systemic rheumatic diseases (28).

Fibrillarin (U3-RNP): The naming of this antigen is based on immunoelectron microscopic studies which localized the antigen to the fibrillar region of the nucleolus, hence the name fibrillarin (29, 30). By indirect immunofluorescence, a clumpy pattern of nucleolar staining is commonly observed (Fig. 1b) (1, 31). Fibrillarin is a 34 kD basic protein associated with the U3 RNP particle of the nucleolus (32–35). The fibrillarin antigen is most reliably detected by immunoprecipitation of radiolabeled cell extracts. When the less sensitive

Western immunoblotting assay was used, fibrillarin antibodies were detected in 8% of scleroderma sera (33). Because of the relatively low frequency of anti-fibrillarin antibodies in scleroderma, clinical correlations with this autoantibody are not clearly defined in many racial cohorts. In a comprehensive study, 27/416 (~7%) scleroderma patients demonstrated precipitating U3-RNP antibodies (36). Of interest, the frequency of U3-RNP antibodies was approximately 10 fold higher in black patients than in whites and was associated with a higher frequency of pulmonary hypertension, radiographic small bowel involvement, and diffuse skin disease. A lower frequency of arthritis (37) and the widespread presence of telangiectasia and diffuse skin involvement (38) have also been reported in a few patients. Of interest, certain strains of mice treated with mercury chloride produce fibrillarin antibodies (39).

RNA Polymerases: The commonly identified RNA polymerases in mammalian cells include RNA polymerase I (Pol I), RNA polymerase II (Pol II), and RNA polymerase III (Pol III). Pol I is involved in the transcription of ribosomal RNA genes, Pol II, in the transcription of heterogeneous RNA and messenger RNA genes, and Pol III, in the transcription of transfer RNA and a number of other low molecular weight RNA species. Antibodies to Pol I were the first to be recognized in scleroderma sera (40, 41). Current evidence suggests that Pol I antibodies are commonly associated with antibodies to Pol II and Pol III (41, 42). Initial studies that analyzed sera with high titer anti-nucleolar antibodies suggested that the frequency of Pol I antibodies in scleroderma was 3% (40). More recent immunoprecipitation studies analyzing 252 unselected sera have suggested the frequency of Pol antibodies in scleroderma approximates 20% (42). Taken together, the frequency of antibodies to Pol I, II and/or III is reported to be approximately equal to the frequency of anti-Scl-70 or anti-centromere antibodies in systemic sclerosis (41, 42). Antibodies to the Pol system are important because they are associated with diffuse skin involvement (*e.g.* high skin scores), a high prevalence of internal organ involvement, and renal crisis (37).

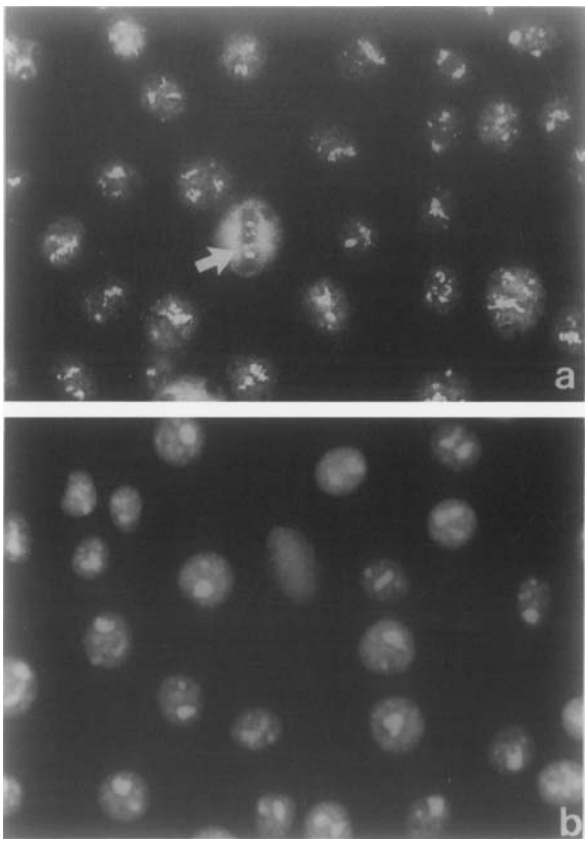

Fig. 2. (a) Antibodies to RNA polymerase I (Pol I) produce a punctate pattern of nucleolar staining in interphase cells and may also demonstrate punctate staining of metaphase chromatin (arrows). The staining of metaphase chromosomes is localized to the nucleolar organizer region. (b) A homogeneous nucleolar pattern of staining is characteristic of PM/Scl antibodies. Some sera also stain the nucleoplasm as represented by a finely speckled pattern.

Antibodies directed against Pol I give a punctate pattern of nucleolar staining in interphase cells, but many sera also stain the nucleolar organizer region of metaphase chromosomes (Fig. 2a) (40). Like fibrillarin, Pol I has been localized to the fibrillar centers of nucleoli (29). Sera from scleroderma patients were used to identify a 90 kD nucleolar organizer protein known as NOR-90 (43). This antigen has been identified as the human upstream binding factor (hUBF) (44). However,

two separate studies have suggested that NOR-90/hUBF antibodies are found in a variety of diseases and are probably rare in scleroderma (45, 46).

The polymerase antigens comprise a large number of peptides ranging from 240 to 12 kD. Pol I can be identified as a 210 protein and as 180, 80, 64, and 18 kD phosphoproteins (40). Pol II was identified as 240 and 220 kD phosphorylated and unphosphorylated proteins, respectively (41). A dominant antigenic motif in Pol II is a highly repetitive heptamer motif (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) in the carboxyl terminus (41).

PM/Scl: An autoantibody that is typified by a homogeneous nucleolar pattern of staining is directed against the PM/Scl antigen (Fig. 2b). In 1977 Wolfe et al. first described this system as one of several PM-1 antibody systems and noted that 67% of patients with PM-1 antibodies had systemic sclerosis and myositis, 22% had myositis alone, and 11% had systemic sclerosis alone (47). One of these PM-1 systems was described in more detail by Reichlin and other investigators (48). In this study of 20 patients with a common precipitating antibody, 19 had polymyositis and 10 had scleroderma. Hence the autoantibody was designated PM/Scl. Other reports have confirmed that at least one-third of patients with PM/Scl antibodies are characterized by a scleroderma-polymyositis overlap syndrome (37).

Of interest, this antibody may show strong racial variation in frequency. For example, Nishikai et al. (49) could find no patients with PM/Scl antibodies in a study of 72 Japanese patients with inflammatory muscle disease. Several studies agree that there is a strong association of this autoantibody with HLA-DR3 (50–52).

In a recent study of 32 patients from three hospitals in the United Kingdom, it was noted that all patients had Raynaud's phenomenon, 31/32 had features of scleroderma, 28/32 had myositis, 15/32 had calcinosis, and 25/32 had lung restriction (52). Seventeen of the patients had limited skin involvement compared to 14 with a generalized cutaneous distribution. Other cutaneous features included telangiectasia in 20/32, hyperpigmentation in 4/32, patchy hypopigmentation in 5/32, and acro-osteolysis in 10/32. An interesting feature of these patients was the presence of arthritis in 31/32 patients, of which 9/32 had erosions. It has also been observed that these patients tend to have a favorable outcome with treatment (51).

Although anti-PM/Scl antibodies were initially described in the polymyositis/scleroderma overlap syndrome, they are also found in approximately 5% of unselected scleroderma or polymyositis sera (53). A nucleolar pattern of staining is most commonly seen in anti-PMScl sera when they are tested by IIF on HEp-2 cell substrates (52). The PM/Scl antigen system appears to be represented by 11–16 polypeptides with molecular weights ranging from 110–20 kD that are primarily located in the nucleolus and nucleoplasm (37, 51, 53, 54). When PM/Scl sera are tested by immunoblotting, they react with 100 or 80 kD saline-extractable proteins (37). Recently, 75 and 100 kD nucleolar proteins that represent the PM/Scl system have been cloned and sequenced (55–57).

tRNA Synthetases: Antibodies directed to several tRNA synthetases have been described (reviewed in 58). One of these systems was designated Jo-1 (59–61) and was subsequently identified as histidyl tRNA synthetase (reviewed in 58). Although this autoantibody system is most commonly associated with myositis, it is worthy of mention in the context of scleroderma because some of these patients have an overlap syndrome that includes features of scleroderma. In the patients with scleroderma, the skin involvement is typically limited to sclerodactyly of the hands and telangiectasia of the face (62). Patients with anti-tRNA-synthetase antibodies tend to have poor prognosis because of pulmonary involvement (62).

U1-RNP: Antibodies directed against U1-RNP identify patients with mixed connective tissue disease (63). Although the distinctiveness of this syndrome is controversial, criteria have been proposed that aid in the classification of a subset of patients that have anti-U1-RNP as a key serological feature (64). Reappraisal of the

patients reported in the original study has suggested that up to 50% may eventually meet criteria for the diagnosis of systemic lupus erythematosus (65, 66).

U2-RNP: Antibodies directed against U2-RNP appear to be rare, are found in association with U1-RNP antibodies, and are primarily found in patients with overlap syndromes (67). In one reported series, all patients with this autoantibody had myositis and sclerodactyly but no evidence of interstitial lung disease (68).

To/Th: Th antibodies were first described in 1982 (69), and later the anti-To system (34) in sera with nucleolar antibodies was shown to be identical (34, 70). The reactive determinants were found on 7-2 RNP and a cytoplasmic 8-2 RNP. A relationship between the 7-2 and 8-2 RNPs was suggested by observations that both of these molecules were bound by antibodies in the same sera (71). In 1988, the RNA component of the 8-2 RNA was identified as RNase P, the enzyme involved in processing transfer RNA (71). Later, it was shown that the 7-2 RNA was identical to a mitochondrial RNase MRP particle (72). Further evidence that the mitochondrial RNase MRP particle shared RNA and protein determinants with the Th nucleolar antigen was provided when Th antisera absorbed RNase MRP activity from cell extracts. Thus, an autoantigenic epitope on Th is distributed in different cellular compartments (nucleus, nucleolus, cytoplasm, mitochondria) and on different proteins.

Anti-Th antibodies have been found in 4% of scleroderma sera (73) and in a patient with Raynaud's phenomenon and undifferentiated connective tissue disease (74). Analysis of subsets of the disease showed that anti-Th antibodies are almost exclusively found in patients with limited cutaneous involvement and in certain patients with Raynaud's phenomenon whose disease evolved to limited cutaneous involvement (73). However, despite limited skin involvement, the cumulative survival of patients with this antibody was only 78% at 10 years, compared to 91% for patients with other autoantibodies.

Ku: The Ku antigen was described by Mimori and his colleagues (75) in the serum of patients with a polymyositis-scleroderma overlap syndrome. The Ku system is comprised of two nonhistone proteins of 80 and 70 kD. The 80 and 70 kD proteins exist as non-covalently linked heterodimers that are distinct in their structures and functions. The 70 kD protein that binds to the blunt end of dsDNA (76) and the 80 protein have been cloned (77–79). Clinical studies have shown that anti-Ku antibodies are found in 26% of patients with polymyositis-scleroderma overlap but only in 1% of systemic sclerosis and SLE sera (68). Therefore, this autoantibody is a relatively specific marker for the polymyositis-scleroderma overlap syndrome. In a recent study, anti-Ku antibodies were found in 23% of patients with primary pulmonary hypertension as compared to only 4% of patients with secondary pulmonary hypertension (80).

A report by Birdi et al. (81) identified Ku antibodies in a patient with morphea who progressed to develop a polymyositis-scleroderma overlap syndrome approximately 2 years later. Of interest, the myositis in this patient was identified by elevated muscle enzymes (*e.g.* CPK), but the patient did not have remarkable weakness and the muscle biopsy did not demonstrate a cellular infiltrate in the muscle. These observations are in keeping with early reports which suggested similar features of myositis in some patients and a good prognosis (75).

Similar to observations with the PM/Scl, Scl-70, and fibrillarin systems, there appear to be racial differences in the frequency and disease association of Ku antibodies. For example, overlap syndromes were not noted in North American patients with anti-Ku antibodies (82, 83).

Ki: In 1981, Tojo and his colleagues identified Ki as a precipitating antigen-antibody system directed to a soluble nuclear antigen (84). Ki is believed to be identical to the sicca lupus (SL) antigen described by Harmon and her colleagues (85) and later by Bernstein et al. (86). The Ki antigen was initially identified as a 32 kD protein in rabbit thymus extracts (87). Antibodies to the Ki antigen were identified in approximately 7% of systemic sclerosis sera and

in up to 22% of SLE sera. In SLE, anti-Ki antibodies are associated with a higher frequency of central nervous system disease (87). No clinical characteristics of anti-Ki positive systemic sclerosis patients have been reported. Eventually, the Ki antigen was cloned from bovine and human cDNA libraries (88) and was shown to be a novel protein with a molecular weight of 29.5 kD.

Mitochondrial antigens: In 1988, Hirakata et al. described precipitating antibodies to mitochondrial antigens in patients with a limited form of scleroderma (CREST) and primary biliary cirrhosis (89). In these patients, three precipitating systems previously termed M-A, M-B, and M-C (90) were identified. Antibodies to mitochondrial antigens in systemic sclerosis patients with primary biliary cirrhosis had been described by indirect immunofluorescence in a number of studies (91–94). In a study of 341 Japanese sera with anti-mitochondrial antibodies, it was suggested that centromere antibodies tend to be more frequently associated with anti-mitochondrial antibodies than other nuclear autoantibodies (95).

Other intracellular antigens: Antibodies to centrioles and the centrosome have been reported in scleroderma (96–98). One of the centriolar autoantigens has been identified as the glycolytic enzyme enolase (99).

A precipitating autoantibody system, referred to as Wa, was found in approximately 3% of scleroderma sera (100). Immunoblots and immunoprecipitation studies identified a 48 kD protein that is likely associated with tRNA. Two of the 4 patients in this report had Sjögren's syndrome.

Recent studies showed that antibodies to a group of chromatin-associated proteins known as high mobility group (HMG) proteins were seen in 77/196 (39%) of scleroderma sera (101). Further analysis showed that antibodies to HMG14/17 were correlated with the presence of centromere antibodies.

Last, a scleroderma patient with a history of exposure to polyvinylchloride had antibodies to proteins on U4/U6 RNP particles (102). Since this was the only patient identified in a screen of over 700 autoimmune sera, this autoantibody is likely rare.

Autoantibodies as Markers of Subsets of Scleroderma

Although anecdotal references to autoantibodies in other subsets of scleroderma are published, there are no definite associations of specific autoantibodies with linear scleroderma or morphea (103–108).

There has been considerable interest in the possible association of silicone breast implants (SBI) with scleroderma (109–112). Although a number of autoantibodies have been described in symptomatic SBI patients (113), there does not appear to be an autoantibody that distinguishes scleroderma patients with silicone breast implants from patients without implants. More detailed studies of autoantibodies in these clinical subsets may provide additional information.

Autoantibodies Antedate Disease

One of the most exciting aspects of autoantibody analysis is the prospect of identifying disease earlier and thereby promoting a better outcome (114, 115). Although careful and extensive studies are still required in this area, some autoantibodies have been identified in the pre-clinical phase of the disease (23, 116, 117). An example of a situation in which the use of autoantibody testing might be useful is in the patient who presents only with Raynaud's phenomenon (115). The majority of patients with centromere antibodies and Raynaud's phenomenon followed in our clinic went on to develop at least three features of the CREST syndrome over a ten year period. Prospective studies of clinical outcome in relatively asymptomatic patients with Scl-70 or fibrillarin antibodies are still required.

Autoantibodies in Scleroderma: Clues to Etiology?

For many years, research has attempted to determine if autoantibodies hold clues to the etiology of the disease. Clearly, the appearance of autoantibodies in the serum is not the result of a random production of autoantibodies within a single disease. It is becoming increas-

ingly clear that autoantibodies in systemic rheumatic diseases are associated with distinct themes related to cell physiology. For example, the autoantibodies in patients with SLE target antigens involved in gene regulation and processing messenger RNA. The autoantibody response in polymyositis patients is aimed at macromoleculaes involved in translation of messenger RNA (*e.g.* tRNA synthetases). Autoantibodies in scleroderma primarily target proteins and nucleic acids involved in ribosomal RNA processing. These observations should be considered when theories regarding the stimulus for autoantibody production in scleroderma are proposed. For example, it would be difficult to adhere to the concept of molecular mimicry as the basis for the origin of autoantibodies in scleroderma. It is difficult to imagine how molecular mimicry is repeated over and over again in one disease and results in autoantibodies to macromolecules involved in the processing of ribosomal RNA. Emerging evidence clearly shows that the antigens involved in inducing the autoimmune response in SLE and scleroderma are truly endogenous macromolecules (autoantigens) (for a review see 118). Studies of the role of the T cell receptor and the major histocompatibility complex hold promise for clarifying the alterations that occur in the humoral immune response that lead to the production of autoantibodies and the expression of disease.

The Future: Autoantibodies as Indicators of Effective Intervention?

Some market specialists predict that, in the coming years, the demand for autoantibody testing will increase. The reason for this increasing demand is the tremendous progress being made in therapeutics for autoimmune diseases such as scleroderma. As progress in the development of therapy for specific diseases increases, there will be a strong incentive to develop diagnostic tests that correlate with flares and remission of disease.

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