Rheum

THE FINE SPECIFICITY OF RHEUMATIC DISEASE ASSOCIATED AUTOANTIBODIES: AN AGING CLINICAL IMMUNOLOGIST'S EDUCATION IN MODERN CELL BIOLOGY

UT Southwestern Internal Medicine Grand Rounds

January 28, 1988

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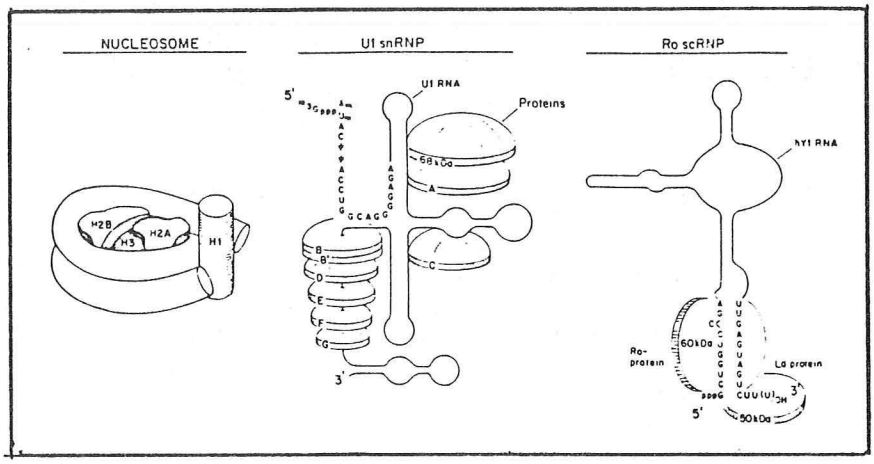
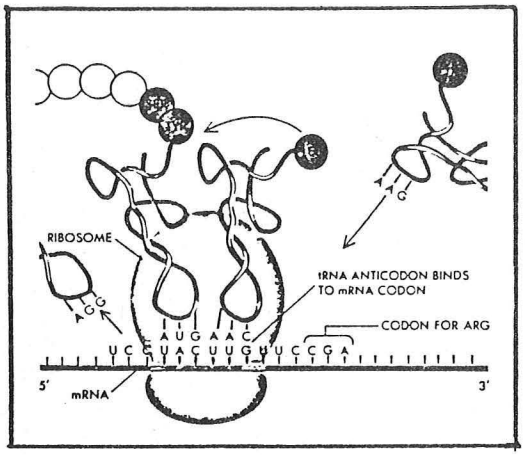
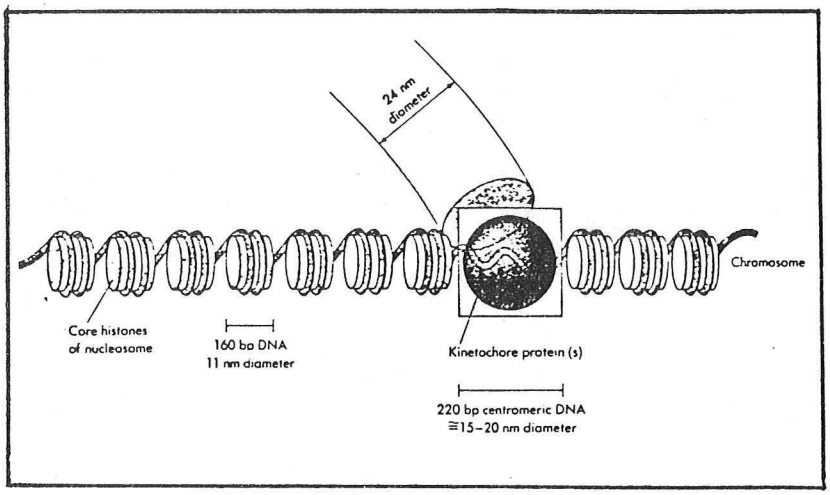


SLE



DM/PM



PSS

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1.0 INTRODUCTION

The birth of modern Rheumatology was closely linked with the ability to better diagnose and classify the various heterogeneous rheumatic syndromes that laboratory markers of humoral autoimmunity made possible. In addition, the maturation of this medical subspeciality has been facilitated by improvements in the assays used to identify and quantitate circulating autoantibodies. Thus, one might view the development of Rheumatology as a sequence of eras defined by increasing sophistication of autoantibody detection and autoantigen identification.

Table 1

AUTOANTIBODY "ERAS" OF MODERN RHEUMATOLOGY

1948 - Pathologically-Defined Autoantibody Era	(eg, LE Prep)
1957 - Immunofluorescence-Defined Autoantibody Era	(eg, ANA Patterns)
1969 - Immunodiffusion-Defined Autoantibody Era	(eg, ENA antibodies)
1979 - Molecularly-Defined Autoantibody Era	(eg, U1RNP)

Three and one half years ago at these rounds, I reviewed certain concepts and misconceptions concerning the technical and clinical aspects of the Immunofluorescence-Defined and Immunodiffusion-Defined Autoantibody Eras. Since then, the current era of Molecularly-Defined Autoantibodies has really begun to blossom. With the tools of modern molecular biology and genetics, the fine specificities of a number of rheumatic disease-associated antinuclear and anticytoplasmic autoantibodies have been identified and the genes for several of these autoantigens have been cloned, allowing more sensitive and specific autoantibody assays to be developed. In addition, the use of autoantibodies as molecular probes has allowed the discovery of heretofore unknown cellular structures. Part of the rationale of such studies has been the hope that a better understanding of the antigens to which autoantibodies develop could yield insight into the etiology and pathogenesis of the autoimmune states which underly these disorders. In fact, some of the traditional views of human autoimmunity are currently being challenged based upon insight which has come from the molecular characterization of these intracellular autoantigens.

Today, I would like to review for you the recent progress which has been made in this area, focusing particularly on the three major, dermatologically-relevant rheumatic disease categories: systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS) and dermatomyositis-polymyositis (DM-PM).

1.1 Scope of current presentation

Patients with rheumatic diseases can make autoantibodies to products secreted by cells (eg, rheumatoid factor → immunoglobulins), constituents of the plasma membrane (eg, phospholipids, insulin receptors) as well as intracellular components. Because of the constraints of time I will be able to discuss only the cytoplasmic and nuclear autoantigens today. Of the 10^4 macromolecules which can be found inside a cell, only about 30 are the targets of autoantibody production (1). Table 2 lists the major intracellular autoantigens and the frequencies with which autoantibodies to them can be found in various clinical autoimmune states.

Table 2

DISTRIBUTION OF AUTOANTIBODIES TO INTRACELLULAR ANTIGENS IN AUTOIMMUNE DISEASE*

Disease (No. of patients) Antibody system	SLE (270) %	MCTD (30) %	SS (55) %	Myositis (36) %	PSS (161) %	RA (70) %	ITP (110) %	PBC (135) %	CAH (50) %	Other (194) %	Controls (70) %
Sm (20)	7.4	6.7	—	—	—	—	—	—	—	—	—
RNP (103)	23	100	3.6	14	2.5	—	—	—	—	—	—
Ro (134)	24	17	75	8.3	4.3	2.9	7.3	5.9	4.0	—	—
La (50)	8.5	3.3	42	—	1.2	—	0.9	0.7	—	—	—
Jo-1 (10)	0.4	3.3	—	25	0.6	—	—	—	—	—	—
SL (22)	6.3	3.3	—	—	—	2.9	0.9	0.7	—	—	—
rRNP (11)	3.3	—	1.8	—	0.6	—	—	—	—	—	—
PL-4 (6)	2.2	—	—	—	—	—	—	—	—	—	—
PCNA (7)	2.6	—	—	—	—	—	—	—	—	—	—
Pm-Scl (9)	—	—	—	11	3.0	—	—	—	—	—	—
PL-7 (1)	—	—	—	2.7	—	—	—	—	—	—	—
PL-8 (2)	0.7	—	—	—	—	—	—	—	—	—	—
Ku	~5	—	—	~1	—	—	—	—	—	—	—
XR (25)	—	—	—	—	—	—	—	9.6	24	—	—
XH (11)	—	—	—	—	—	—	—	7.4	2.0	—	—
Scl-70 (27)	—	—	—	—	16	—	—	—	—	—	—
Centromere (65)	1.8	—	—	—	29	—	nd**	8.0	—	—	1.4
Nuclear dots (27)	5.0	8.0	—	8.0	6.2	nd**	nd**	1.5	8.0	0.8	—
Multiple nuclear dots (22)	1.7	—	1.8	—	—	nd**	nd*	13	—	—	—
Nucleoli (99)	6.7	—	18	8.0	37	nd**	nd**	5.9	6.0	4.8	3.0
Golgi apparatus (1)	—	—	—	—	—	nd**	nd**	—	—	—	1.4
Mitochondria (122)	—	—	3.6	—	—	nd**	nd**	88	2.0	—	nd**

Disease abbreviations: systemic lupus erythematosus, SLE: mixed connective tissue disease, MCTD; primary sicca syndrome, SS; polymyositis and dermatomyositis, myositis; progressive systemic sclerosis, PSS: rheumatoid arthritis, PA; idiopathic thrombocytopenic purpura, ITP; primary biliary cirrhosis, PBC; chronic active hepatitis CAH

** not determined

*- From reference #1.

It will be impossible to fully discuss all of the current data pertaining to even this limited set of autoantigens. I will therefore

limit this discussion to those intracellular antigens to which SLE, PSS or DM-PM patients make autoantibodies, focusing particularly on those specificities which are most commonly involved in these three disorders. The discussion will be concluded by an attempt to integrate this new knowledge of the molecular nature of autoantigens into a paradigm for considering the pathomechanisms of the autoimmune state associated with rheumatic disorders such as these.

2.0 SYSTEMIC LUPUS ERYTHEMATOSUS AUTOANTIGENS

An overview of the intracellular autoantigens of SLE is shown in Table 3. These antigens are ranked in descending order according to the frequency with which autoantibodies to them are produced by SLE patients.

Table 3

SYSTEMIC LUPUS ERYTHEMATOSUS INTRACELLULAR AUTOANTIGENS[1]

	Autoantibody				
	Frequency				
Antigen Name	ID[2]	SPA/RIA[3]	Disease Specificity	Binding Specificity	Antigen's Function
ssDNA		60%	Low	denatured DNA	?
dsDNA		60%	High	native DNA	genetic code
Histones		50%	Low	histones	DNA binding
U1RNP	25%		Low	ribonucleoprotein	RNA processing
Ro/SS-A	25%		Low	ribonucleoprotein	?
Ki	10%		High	protein	DNA binding
La/SS-B	8%		Low	ribonucleoprotein	RNA transport
Sm	7%		High	ribonucleoprotein	RNA processing
SL	6%		Low	protein	?
rRNP	3%		High	ribosomal P protein	protein synthesis
PCNA	3%		High	cyclin	cell proliferation

1 - adapted from references (#1, 124).
2 - immunodiffusion
3 - solid phase assay (ELISA) or radioimmunoassay

2.1 Double-stranded DNA and Histones: The Nucleosome Antigens

2.1.1 Historical perspective and clinical correlations

Autoantibodies to double-stranded (ds) DNA occur in 50-70% of SLE patients and are highly specific for this disease (2). This autoantibody is also occassionally seen in clinical settings where SLE overlaps with other rheumatic diseases (eg, mixed connective tissue disease) (2). Some workers have occassionally reported the occurrence of this autoantibody specificity in non-rheumatic clinical disorders. The basis of such reports has often proven to be contamination of the ds-DNA substrate used in the antibody assay with single-stranded DNA regions. The kinetoplast of the hemoflagellate, Crithidia luciliae, offers a very pure source of ds-DNA that can be employed in a convenient indirect immunofluorescence assay to detect ds-DNA autoantibodies (2). Circulating ds-DNA antibody levels fluctuate with systemic disease activity, particularly renal involvement (3-4), and this autoantibody specificity has been implicated in the pathogenesis of the more aggressive forms of lupus nephritis (5-6).

Rubin and Waga (7) have pointed out that anti-histone antibodies probably represent the oldest class of SLE related autoantibodies, since the opsonin of nuclear debris responsible for the LE cell phenomenon was shown by absorption studies not to be anti-DNA, but rather antibodies to histone containing targets (8-10). Holman et al (11) and Kunkel et al (12) first directly demonstrated that isolated histones are antigenic. It was initially felt, based upon microcomplement fixation assays (13), that anti-histone antibodies were relatively infrequent in SLE patients. Recent studies using more sensitive solid phase immunoassays have documented that anti-histone antibodies can be found in about 50% of the sera of unselected SLE patients (14-16) and in approximately 80% of patients with active disease (16). Autoantibodies reactive to histones are also found in other conditions such as rheumatoid arthritis, mixed connective tissue disease and progressive systemic sclerosis. Virtually all patients with procainamide or hydralazine induced lupus produce antihistone antibodies (17-18). It is now clear that separate autoantibodies occur to the five different histone molecules and that different profiles of these autoantibodies are produced in different clinical settings (Figure 1).

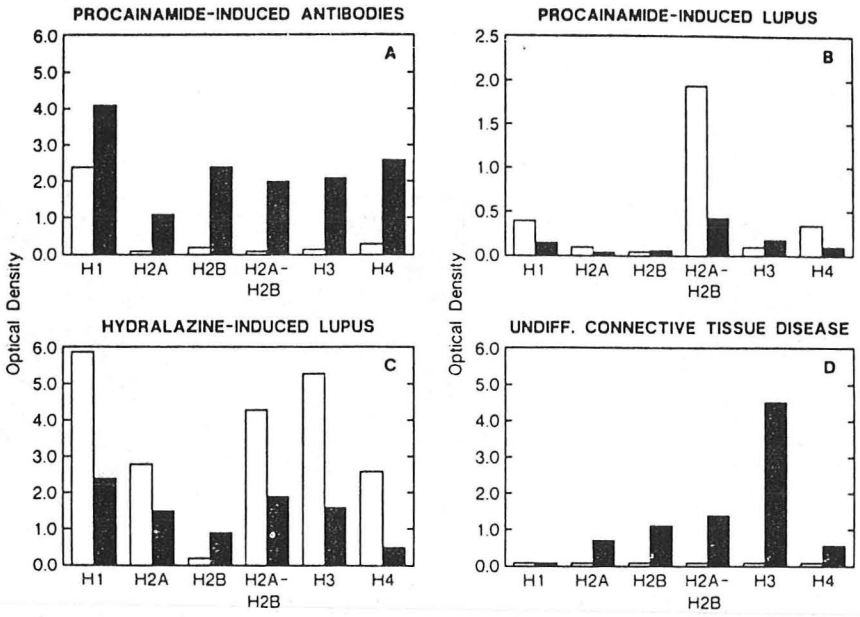

Figure 1 - Histone autoantibody profiles seen in different clinical settings (from reference #7).

2.1.2 Autoantibody binding specificity

Histones are found in all eukaryotic cells associated with genomic DNA. The subunit of this histone-DNA complex, termed the nucleosome, consists of two molecules of each of the "core" histones (H2A, H2B, H3 and H4), and one H1 molecules along with two turns of DNA of about 200 base pairs in length. The DNA is wrapped around an octamer of histones H2A, H2B, H3 and H4, and this "core" particle is connected to the adjacent core particle by a linker segment of DNA along with associated proteins including H1. The linear array of nucleosomes forms the primary chromatin fiber of about 100 angstroms in diameter. This fine filament has a tendency to supercoil into higher ordered solenoid-like structures of increasing diameter (7). The arrangement of histones within the core particle has recently been determined by x-ray crystallography and is illustrated schematically in Figure 2.

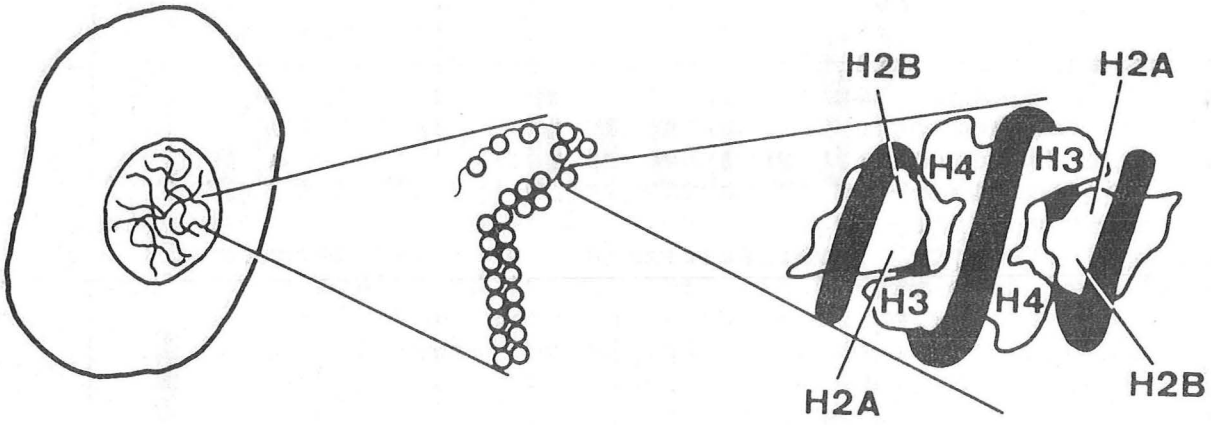

Figure 2 - Nucleosome structure (from reference #7).

Craft and Hardin (19) have pointed out that lupus antibodies recognize histone H1 (external) and the amino terminal (external) but not the carboxyl (internal) segments of H2B and to a lesser extent the exposed segments of the other core proteins (20-21). The internal histone regions are rarely recognized by patient sera, although animal antisera to purified histones readily bind such sites. They also point out that since patients with SLE produced antibodies to DNA and the external histone determinants, they are responding primarily to the external features of relatively intact nucleosomal segments of chromatin. These observations might have relevance to the mechanism by which these autoantibodies are formed.

Antibodies to double stranded DNA are directed to some aspect of the double-helical conformation of double stranded DNA, the exact structure as yet being undetermined. Such antibodies do not bind the free purine and pyrimidine bases to which anti-single stranded DNA antibodies react.

2.2 U1RNP and Sm

2.2.1 Historical perspective and clinical correlations

Autoantibodies to Sm and nRNP antigens were originally identified in the sera of SLE patients (22-23). Subsequent investigations showed that anti-Sm antibodies were seen exclusively in SLE (24). However, anti-nRNP autoantibodies were particularly common in overlapping connective tissue disease syndromes such as mixed connective tissue disease. In addition, they were occasionally present in a number of other rheumatic conditions (SLE, PM/DM, PSS, Sjogren's syndrome, and rheumatoid arthritis). The highest levels of anti-nRNP antibodies have been seen in mixed connective tissue disease patients. It was initially felt that this autoantibody specificity was a serologic marker for a new, distinctive rheumatic condition (mixed connective

tissue disease) (25), however, subsequent studies suggest that many of these patients go on to evolve more typical features of progressive systemic sclerosis (26). It was initially felt that anti-Sm antibodies correlated closely with certain systemic manifestations of SLE such as cutaneous vasculitis, pleuropulmonary disease or cardiac manifestations (27); however, at this time the presence of antibodies to Sm do not clearly correlate with any particular subset of lupus manifestations. Some patients with overlapping features of SLE, PSS and rheumatoid arthritis make predominately anti-nRNP antibodies in high titer (mixed connective tissue disease), however, may patients with SLE could be shown to produce both anti-nRNP and anti-Sm antibodies simultaneously.

2.2.2 Autoantibody binding specificity

The earlier immunochemical characterizations of these two saline-soluble, nuclear antigens resulted in considerable controversy regarding their molecular structure (data reviewed in reference #28). Studies employing immunoaffinity purification of these antigens strongly suggested that nRNP and Sm antigens were bound together in some way in crude tissue extracts. The molecular nature of the nRNP and Sm ribonucleoproteins were greatly clarified by the studies of Lerner and Steitz (29-30). These workers used monospecific anti-nRNP and Sm antibody containing sera to immunoprecipitate intrinsically labeled RNA protein molecules from tumor cells and then characterized the particular RNA protein species bound by its molecular weight in urea gels as well as finger printing.

These and more recent similar studies (reviewed in references #19,31) have yeilded a molecular model of nRNP and Sm antigens which is illustrated in Figure 3.

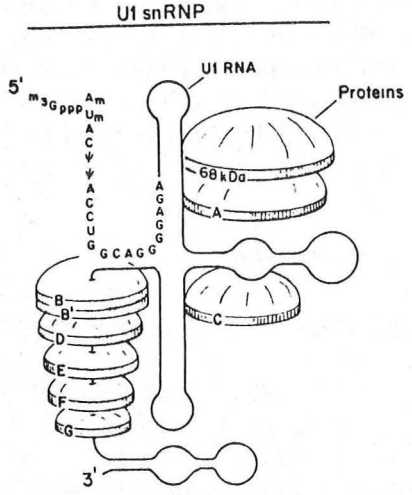

Figure 3 - Currently accepted model for U1snRNP structure (from reference #19).

Immunopercipitation studies demonstrated that nRNP and Sm antigens were present on a unique, new class of small nuclear ribonucleoproteins (snRNP's). These ribonucleoprotein particles were shown to consist of a unique, uridylic acid-rich (thus "U") class of RNA that was coupled to nRNP and Sm antigen-bearing polypeptides (32). This class of RNA is found predominately in the nucleoplasm and has been shown to be highly conserved during evolution. Five species of UsnRNA (URNA) have been identified: U1, U2, U4, U5, U6.

Precipatating nRNP autoantibodies have been shown to bind to a subset of ribonucleoprotein particles that contain only U1RNA. These particles consist of a complex of nine proteins in addition to the uridine-rich U1RNA (see figure 3) (32). These observations have resulted in a change in the name of anti-nRNP autoantibodies to the currently accepted designation, anti-U1RNP (or anti-U1nRNP).

Anti-U1RNP antibodies consists of a family of three autoantibodies which bind to a 68-70 KD polypeptide as well two other proteins known as the "A" and "C" polypeptides (33-34). These three polypeptides are seen only in association with U1RNA molecules. Anti-Sm autoantibodies bind to the B'/B doublet as well as the D protein (35-36). These polypeptides are present on U2, U4, U5 and U6RNAs as well as U1RNA. Thus, anti-Sm sera will precipitate all five URNA's and their associated polypeptides while anti-U1RNP (nRNP) sera precipitate only U1RNA and its distinctive set of proteins. These observations help explain the other earlier data which suggested that Sm and nRNP were physically associated in tissue extracts.

2.2.3 Antigen's cellular function

The extreme 5' end of this U1RNA exhibits a base sequence that is complementary to splice junctions within newly transcribed premessenger RNA (hnRNA) molecules (37). Watson-Crick base pairing permits this structure to mediate the precise excision of introns as heterogenous nuclear ribonucleoprotein particles are processed to form mature messenger RNA (38). Autoantibodies to U1RNP have been shown to be capable of directly inhibiting the splicing of hnRNA (39). The U2-6 RNP particles are also felt to play a role in hnRNA processing.

2.3 Ro/SS-A and La/SS-B

2.3.1 Historical perspective

Reichlin (40) points out that antibodies to Ro/SS-A and La/SS-B were probably first detected in 1958 in the sera of patients with Sjogren's Syndrome, employing extracts of salivary tissue as antigens (41). Later, more extensive studies (42) demonstrated two major specificities in salivary tissue designated SjD and SjT. SjD probably corresponds to the Ro/SS-A antigen as we know it today while SjT appears to be identical to La/SS-B. Precipitating antibodies to Ro were first described in SLE and Sjogren's syndrome patients by Clark et al in 1969 using human spleen extracts (43). In about 30% of these

anti-Ro positive patients, a second precipitin, designated La, was demonstrated by Mattioli and Reichlin (44). Using an EB virus transformed human B cell line (Wil-2), Alspaugh and Tan subsequently described two precipitins in Sjogren's syndrome patients which they designated SS-A and SS-B (45). These observations were unified by the studies of Alspaugh and Maddison, who showed that Ro and SS-A, on the one hand, and La and SS-B, on the other, were antigenically identical (46). Other studies have shown that another antigenic activity, designated Ha (47) was immunologically identical to the La and SS-B antigens. As with the Sm and nRNP antigen-antibody system, it was recognized quite early that patients who made anti-Ro/SS-A antibodies also frequently produced anti-La/SS-B, however the reasons for this were not clarified until much later.

2.3.2 Clinical correlations

Table 4 lists those clinical disorders in which these autoantibodies are found.

TABLE 4

CLINICAL ASSOCIATIONS OF Ro/SS-A AND La/SS-B AUTOANTIBODIES

	Anti-Ro/SS-A		Anti-La/SS-B	
Clinical Disorder	ID[1]	ELISA[2]	ID[1]	ELISA[2]
Sjogren's Syndrome				
Primary	60%	96%	20%	90%
Secondary	20%			7%
SLE (unselected)	25%		10%	
"ANA-Negative" SLE	62%		32%	
Subacute Cutaneous LE	62%	90%	25%	
SCLE/Sjogren's Overlap	100%	100%	10%	50%
Discoid LE	3%			
Neonatal LE	>90%		>50%	
Isolated Congenital Heart Block	80%			
C_2 and C_4 Defficiency	80%		<10%	
Primary Biliary Cirrhosis	Occassionally			
Chronic Active Hepatitis	Occassionally			
Normal Controls	<0.1%	12%	<0.1%	12%

1- Double immunodiffusion assay
2- Enzyme-linked immunosorbent assay

Reichlin and Harley have offered several recent comprehensive reviews of the clinical correlations of the Ro/SS-A and La/SS-B antigen-antibody systems (40,48). Most of the attention has been paid to the Ro/SS-A system since an autoantibody response to this antigen is much more common than one to La/SS-B. In addition, an autoantibody response to La/SS-B is almost invariably associated with anti-Ro/SS-A antibody production. However, some have questioned the emphasis which has been placed upon the role of Ro/SS-A autoantibody in the pathogenesis of certain clinical disorders (49).

Anti-Ro/SS-A autoantibodies were first described in Sjogren's syndrome (SS) patients and occur with the highest prevalence in this disorder. Some workers have suggested that if sensitive solid phase assays based upon purified native Ro/SS-A antigen are employed, virtually all primary SS patients produce abnormal amounts of this autoantibody (50). Others have suggested that high levels of Ro/SS-A precipitins are more often found in patients who have clinical and laboratory evidence of the extraglandular manifestations of this disorder: vasculitis, lymphadenopathy, central nervous system disease, and hematological involvement (50-51).

One-fourth of unselected SLE patients make Ro/SS-A autoantibody. It has been suggested that SLE patients who make only anti-Ro/SS-A have

a higher risk of developing nephritis compared to those who make both anti-Ro/SS-A and anti-La/SS-B (52). Anti-Ro/SS-A has been found in much higher frequencies in several LE subsets. Maddison and coworkers (53) described a group of antinuclear antibody negative LE patients who had in common a high incidence of widespread, photosensitive LE skin lesions but a relatively low frequency of renal or CNS involvement. Approximately two-thirds of these patients had anti-Ro/SS-A precipitins while one-third produced anti-La/SS-B autoantibodies. These same workers later confirmed that the majority of these patients actually did have antinuclear antibodies if a human tissue substrate was used in the ANA assay rather than the conventional rodent tissue substrates which had been used up until that time. At approximately the same time, Sontheimer et al (54) described a group of LE patients who also had a widespread, non-scarring form of LE-specific skin disease which they designated subacute cutaneous LE (SCLE). These patients also frequently produced anti-Ro/SS-A antibodies and only rarely developed severe manifestations of SLE, however, as opposed to the "ANA-Negative" SLE patients, they were usually ANA positive (55). This resulted from the fact that these patients were examined from the outset with a human tissue substrate ANA assay. Anti-Ro/SS-A have also recently been noted in patients with overlapping features of SCLE and Sjogren's syndrome (56). Later studies documented that cases of neonatal LE and isolated congenital heart block were also highly associated with maternal anti-Ro/SS-A antibody production (57-59). In addition, congenital homozygous deffeciency of the C_2 and C_4 components of complement have also been linked to the presence of anti-Ro/SS-A (60-61). Anti-Ro/SS-A precipitins are occassionally present in patients with primary biliary cirrhosis or chronic active hepatitis, however they rarely ever occurs in normal individuals (48).

2.3.3 HLA Associations

Anti-Ro/SS-A autoantibody production has been shown to be a feature of a certain type of genetic background. Bell and Maddison first identified in unselected SLE (62) and Sontheimer et al in subacute cutaneous LE (63) a strong relationship between anti-Ro/SS-A and the HLA-DR3 phenotype. In addition, a similar association has been noted in Sjogren's syndrome (50) where a relationship between the quantity of anti-Ro/SS-A has also been found to correlate with the HLA-DR3, DQ1,2 phenotype (64). Systemic LE patients who have both anti-Ro/SS-A and anti-La/SS-B precipitins tend to be older and have the DR-3 antigen while those with anti-Ro/SS-A alone are younger and have the DR-2 phenotype (65).

2.3.4 Autoantibody pathogenicity

Anti-Ro/SS-A autoantibodies have been implicated in the pathogenesis of cardiac and cutaneous tissue injury in neonatal LE/congenital heart block as well as subacute cutaneous LE. Over 90% of the mothers of infants who develop neonatal LE (transient SCLE-like skin lesions +/- permanent heart block) have anti-Ro/SS-A precipitins in their circulation at the time of delivery while 50% have anti-La/SS-B as well (57-59). The infants usually develop skin lesions, often photosensitive, several weeks after delivery. These

lesions resolve spontaneously at about 6 months of age, at approximately the time that maternal IgG disappears from the fetal circulation. Some are born with heart block and never develop skin disease. This sequence of events has been interpreted to represent direct participation of transplacentally passaged IgG anti-Ro/SS-A antibodies in the elicitation of neonatal LE cutaneous and cardiac tissue injury, perhaps thru immunological mechanisms such as antibody dependent cell mediated cytotoxicity (ADCC) (66). This hypothesis has been extended to include subacute cutaneous LE as well. In support of this idea are the findings that: 1) Ro/SS-A antigen is expressed in the epidermis and cardiac tissue (67-68), 2) ultraviolet light exposure appears to be capable of modulating Ro/SS-A expression in epidermal keratinocytes both in vivo and in vitro in a manner which enhances anti-Ro/SS-A-keratinocyte binding (69-70), 3) anti-Ro/SS-A antibody in subacute cutaneous LE patients is predominately IgG1, a subclass which avidly fixes complement, mediates ADCC, and freely crosses the placenta (71) 4)immunoglobulins have been identified in cardiac tissue of a patient with congenital heart block. However, against this hypothesis are the observations that: 1) most SLE and Sjogren's syndrome patients, who have equally high levels of anti-Ro/SS-A, do not develop photosensitive cutaneous LE skin lesions, although a small group of Sjogrens-subacute cutaneous LE overlap patients have recently been described (56), 2) the vast majority of women who have circulating anti-Ro/SS-A at the time of delivery do not deliver infants with neonatal LE or congenital heart block, 3) there is no correspondence between the levels of circulating anti-Ro/SS-A and subacute cutaneous LE skin disease activity (73).

2.3.5 Autoantibody binding specificity

The initial immunochemical studies of Ro/SS-A and La/SS-B suggested that they were protein antigens with molecular weights of 60 KD and 50 KD respectively (primary data reviewed in reference #40). However, the initial immunoprecipitation studies by Lerner and coworkers employing internally labelled antigens, revealed that like U1RNP and Sm, these antigens were also present on particles composed of RNA and protein (74). However, these ribonucleoprotein particles were unique on several accounts. Rather than being located predominately in the nucleus, the Ro/SS-A was found to be located predominately in the cytoplasm, and where thereby named small cytoplasmic ribonucleoproteins (scRNP's). Other studies have localized Ro/SS-A to the nucleus (75). In addition, Ro/SS-A RNP particles were found to contain a totally new class of small, uridine-rich RNA, hYRNA (h-"human", Y-"cytoplasmic"). Four unique species of hYRNA have since been identified, hY1, hY3-hY5. The hY2 RNA is felt to be a breakdown product of hY1. RNAase protection studies have indicated that the 60 KD Ro/SS-A polypeptide binds to a helical region at the 3' and 5'ends of the hYRNA molecule which is stabilized by complementary base pair binding (76). The currently accepted model of the Ro/SS-A RNP particle is shown in Figure 4.

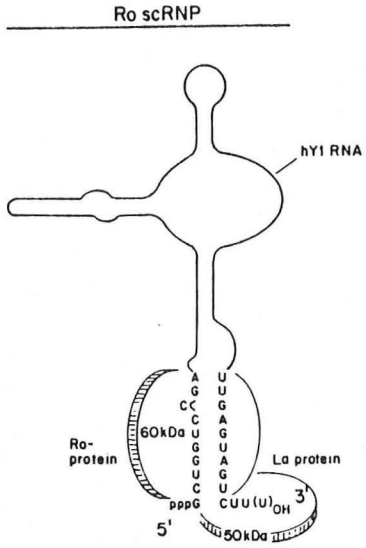

Figure 4 - Currently accepted model for the Ro/SS-A and La/SS-B antigen bearing ribonucleoprotein (from reference #19).

Antibodies to La/SS-B precipitate a protease-sensitive 50 KD polypeptide which is associated with a number of different type of small RNA molecules, including the adenovirus (VAI and VAII) and EB virus (EBER 1 and EBER 2) encoded RNA's. The common structural feature of these RNA's which appears to bind them to La/SS-B is an oligouridylate stretch found at the 3' end of all polymerase III transcripts (77). This has led to the hypothesis that all RNA polymerase III transcripts are at least temporarily associated with the La/SS-B protein. Since hYRNA is itself a RNA polymerase III transcript (78), the La/SS-B protein is at least temporarily associated with Ro/SS-A RNP particles. The presence of both Ro/SS-A and La/SS-B antigenic proteins on the same particle could help explain the frequent concurrence of anti-Ro/SS-A and La/SS-B antibody production by the same patient, if such a response were antigen driven.

Recent progress has been made in further characterizing the La/SS-B protein. This protein has 2 autoantigenic epitope-bearing domains ("X" - 28 KD and "Y" - 23 KD) (79). La/SS-B cDNAs have been isolated from lambda gt11 expression library (80). These tools have allowed the identification of an autoantigenic epitope near the carboxy terminus. Another group has isolated a 1.4 kilobase La/SS-B cDNA which codes for an expressed peptide which appears to bear much if not all the autoantigenic activity of native La/SS-B (81).

The 60 KD Ro/SS-A protein has been difficult to characterize for several reasons: relatively few copies of this protein are expressed per cell and it has been very difficult to seperate Ro/SS-A antigenic activity from other antigens, particularly La/SS-B. This physical association of the Ro/SS-A and La/SS-B polypeptides with the same RNA molecule now makes this easier to understand. Lieu et al have developed a technique for purifying Ro/SS-A from Wil-2 cells, an EB transformed human B cell line (82). Figure 5 illustrates the model of

this polypeptide which has very recently been deduced from biochemical, immunological and molecular genetic studies of this material carried out by Drs. T-S Lieu and Dan McCauliffe in our laboratory in collaboration with Dr. Don Capra and coworkers (83-84).

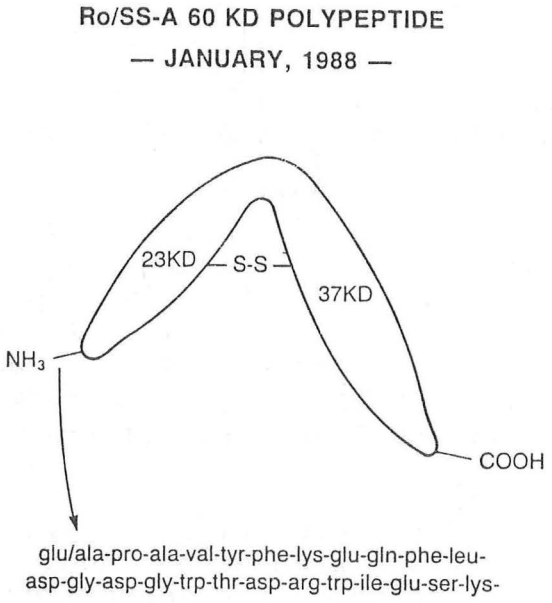

Figure 5 - Author's concept of the structure of the 60 kilodalton, native, human Ro/SS-A polypeptide.

Staph. aureus V-8 protease digestion studies have revealed that the native 60 KD Ro/SS-A polypeptide consists of two disulfide-linked domains which have molecular masses of 23 KD and 37 KD. The 23 KD domain contains the amino terminus of the native polypeptide (83). The 24 amino acid sequence of the amino terminus has been determined by microsequencing techniques to be: **glu/ala-pro-ala-val-tyr-phe-lys-glu-gln-phe-leu-asp-gly-asp-gly- trp-thr-asp-arg-trp-ile-glu-ser-lys-** (85). A synthetic peptide corresponding to amino acid residue 7-24 has been shown to contain a major autoantigenic epitope (84) (see Table 5).

Table 5

ANTIGENIC ACTIVITY OF Ro/SS-A AMINO TERMINUS SYNTHETIC PEPTIDE 7-24[1]

Clinical Disorder	# Sera Studied	% Positive in Ro/SS-A SP 7-24 ELISA	
		All Sera	Ro/SS-A Precipitin Positive Sera
Sjogren's Syndrome	41	36%	71%
Subacute Cutaneous LE	56	38%	68%
Neonatal LE Mothers	10	50%	55%
Congenital Heart Block	5	20%	20%

1- Data from reference #84

A 1.4 kilobase cDNA has been isolated using several synthetic nucleotides which correspond to the deduced amino acid sequence of the native polypeptide. This cDNA, which has been shown to have sequence correspondence to the native polypeptide, binds to a single band of genomic DNA by Southern blot (83).

2.3.6 Autoantigen's cellular function

The normal cellular function of the Ro/SS-A RNP is currently unknown. Some have suggested that it could be involved in translation since certain studies have localized it predominately to the cytoplasm. Any functions attributed to this particle must be compatible with the following observations: 1) relatively few copies of this particle are expressed per cell, 2) levels of tissue expression can vary greatly between species and between different organs within the same species (75) 3) its subcellular localization might be linked to the cell cycle. The role played by the Ro/SS-A ribonucleoprotein particle in cell biology is probably not as primal as the one assumed by U1RNP, since the degree of evolutionary conservation of Ro/SS-A does not approach that of U1RNP.

It has been suggested that the La/SS-B protein might participate in RNA synthesis as a cofactor of RNA polymerase III. It has also been speculated that this protein could be involved in the transport or maturation of RNA polymerase III transcripts. This is compatible with the observation that La/SS-B antigen is primarily a component of the nucleus.

2.4 Others

Precipitating autoantibodies to the Ki antigen have been reported to occur in 10% of SLE patients, often in association with anti-Sm autoantibodies (86). Immunoprecipitation studies have shown that such antibodies bring down two proteins that are not associated with RNA (87). These proteins were found to bind to DNA and be highly conserved. The Ki system apperas to be immunologically identical to the Ku and p70/p80 systems. Anti-SL (PL-2) antibodies have been reported in 6% of SLE patients and rarely in other disorders (88). Immunobloting reveals reactivity with a 32 KD protein (89). Ribosomal RNP (rRNP) autoantibody occur predominately in SLE (90) and have been shown to be reactive with three phosphorylated "P" proteins (P0, P1, P2) present in ribosomal RNP particles (91-92). These autoantibodies have been suggested to be a marker for CNS lupus (92). A precipitating antibody that is reactive with a nuclear antigen which is expressed only in cells undergoing active proliferation has been observed in a small percentage of patients with active SLE (93). This antibody to proliferating cell nuclear antigen (PCNA) was later shown to react specifically with cyclin, a 36 KD cell cycle-specific protein (94-95). During blast transformation, early expression of PCNA/cyclin occurs in the nucleolus preceeding DNA synthesis. During DNA synthesis itself, PCNA/cyclin is expressed predominately in the nucleoplasm (96). PCNA/cyclin has recently been shown to be identical to an auxillary protein of DNA polymerase delta (97) and has been implicated in nucleotide excision DNA repair as well as proliferation (98).

3.0 PROGRESSIVE SYSTEMIC SCLEROSIS AUTOANTIGENS

PSS also patients produce autoantibodies against several intracellular antigens (see Table 6).

Table 6

PROGRESSIVE SYSTEMIC SCLEROSIS INTRACELLULAR AUTOANTIGENS

Antigen Name	Autoantibody Frequency IF[1]	ID[2]	SPA/RIA[3]	Disease Specificity	Binidng Specificity	Antigen's Function
				(CREST Syndrome)		
Centromeric	70%		96%	high	CENP-A, -B, -C proteins	Microtubule organization at centromeric kinetochore during mitosis
				(PSS with Diffuse Scleroderma)		
Scl-70	20%			high	Topoisomerase I	DNA supercoiling
Poly(A)-polymerase	50%			low	nuclear Poly(A)-polymerase	RNA polyadenylation
RNA polymerase I				high	RNA polymerase I	ribosomal RNA transcription

[1]- indirect immunofluorescence assay
[2]- double immunodiffusion assay
[3]- solid phase immunoassay (ELISA) or radioimmunoassay

3.1 Centromere/kinetochore antigens

3.1.1 Historical perspective and clinical correlations

Anti-centromere autoantibodies were first recognized in 1968 by Burnham due to the distinctive antinuclear antibody immunofluorescence pattern - the discrete (true) speckled pattern - which sera that contained this autoantibody specificity produced (99). His initial work indicated that patients with acrosclerotic scleroderma (CREST syndrome) more often produced this particular ANA pattern. Subsequently, workers showed that CREST syndrome patients sera reacted with the centromeric kinetochore during metaphase (100-101) (see Figure 6).

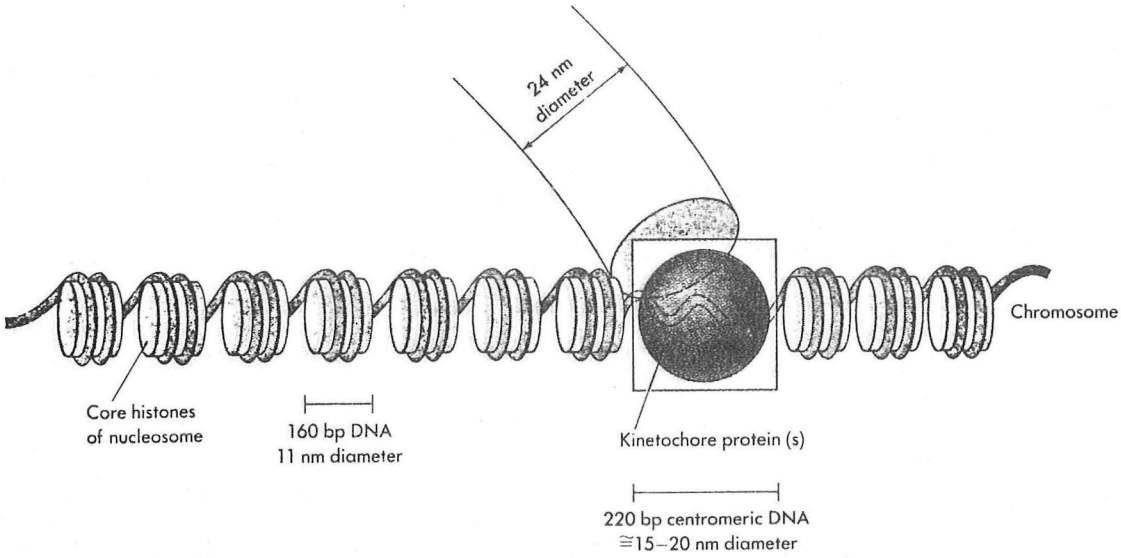

Figure 6 - Proteins in the centromeric kinetochore are the antigenic targets of anti-centromere antibodies seen in the CREST syndrome variety of progressive systemic sclerosis (from reference # 170).

Further work indicated that 50-60% of patients with the CREST syndrome and 10-15% of patients with a diffuse form of PSS produced this autoantibody specificity (102). In addition, anti-centromere antibodies have been reported to occur in approximately 30% of patients with primary biliary cirrhosis (103).

3.1.2 Antibody binding specificity

Several groups initially identified a family of polypeptides which appear to have reactivity with anti-centromere antibody (104-105). Subsequently, Earnshaw and Rothfield identified by immunoblotting, with a purified chromosome preparation as the source of antigen, a family of three antigens localized to the centromere: CENP-A (17,000 KD), CENP-B (80 KD) and CENP-C (140 KD) (106). This same group later demonstrated reactivity with CENP-B in all 39 anti-centromere antibody positive patient sera and failed to observe reactivity with this polypeptide in any anti-centromere antibody negative sera tested. Other workers (107) have also noted a high frequency of reactivity of anti-centromere antibody containing sera with a simily sized polypeptide. The CENP-B autoantigen has since been molecularly cloned. A radioimmunoassay based on cloned CENP-B protein has demonstrated that sera from greater 96% of patients with anti-centromere antibody recognized this cloned antigen (108). The function of these proteins appears to relate to the binding of microtubules to the kinetochore during mitosis. Anti-centromere antibodies have been shown to be capable of specifically inhibiting *in vitro* the organization of microtubules at the kinetochore (105).

3.2 Scl-70

3.2.1 Historical perspective and clinical correlations

Shero et al (109) have comprehensively reviewed the issues relating to anti-Scl-70 autoantibodies. They point out that this precipitating autoantibody was first described by Douvas et al (110) to react with a 70 KD protein present in calf thymus extract. Subsequent reports showed that anti-Scl-70 was highly specific for this disease, occurring in approximately 15-20% of patients with progressive systemic sclerosis with diffuse scleroderma (102,111-112). This autoantibody was also seen on occasion in patients with these CREST syndrome variant of PSS.

3.2.2 Antibody binding specificity

Immunoblotting studies with anti-Scl-70 sera showed reactivity with a 100 KD protein (113-114). It was concluded that the 70 KD molecule represents a proteolytic breakdown product of the 100 KD antigen-bearing protein. Because of the similarities between the two size classes of Scl-70 and DNA topoisomerase I, anti-Scl-70 positive sera were tested in a solid phase radioimmunoassay against bovine topoisomerase I. The anti-Scl-70 sera were noted to react strongly to topoisomerase I in this assay (114). Further studies by these workers indicated that anti-Scl-70 autoantibodies were able to absorb topoisomerase I activity from crude extracts of the enzyme. Another laboratory has recently reported similar findings (115).

Topoisomerase I was originally isolated as a nicking-closing enzyme which is known to modify and control the topological state of DNA by introducing transient, single stranded cuts into the duplex DNA, thereby catalyzing the relaxation of supercoiled DNA (116-117) (see Figure 7).

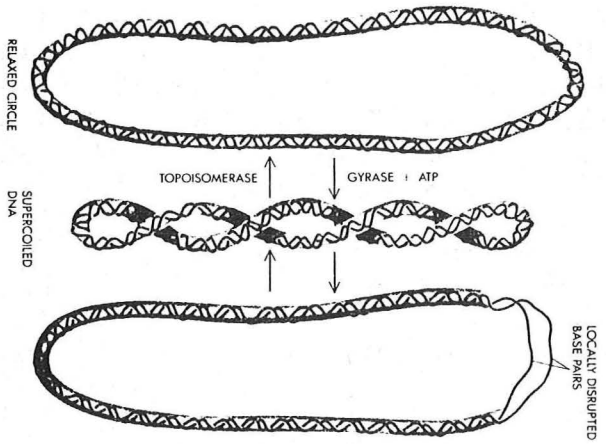

Figure 7 - Function of Topoisomerase I, the target of progressive systemic sclerosis-associated anti-Scl-70 autoantibodies (from reference #168).

Topoisomerase I activity has been implicated in replication, recombination and transcription (data reviewed in reference #118). The

reason for the presence of anti-topoisomerase I autoantibody in the sera of patients with scleroderma is currently unknown. It is projected that a sensitive radioimmunoassay for antibodies to topoisomerase I could lead to early detection of the more severe form of this disease at a time where it might be more amenable to pharmacological modulation.

3.3 Others

Reimer et al have recently reported the presence of autoantibody to RNA polymerase I, a constituent of the nucleolus, in the sera of PSS patients with diffuse scleroderma (119). This autoantibody was not seen in other connective tissue diseases or normal controls. In addition, antibodies to nuclear poly(A) polymerases have also been reported in PSS sera (120). This specificity was present in 5 of the 10 PSS sera examined, however, they were also present in SLE, Sjogren's syndrome and rheumatoid sera with equal or higher frequencies. These antibodies were shown to inhibit poly(A) polymerase activity.

4.0 DERMATOMYOSITIS/POLYMYOSITIS AUTOANTIGENS

Although seen in a lower frequency than with SLE and PSS, antibodies to a number of intracellular autoantigens also develop in DM/PM patients (Table 7).

Table 7

DERMATOMYOSITIS/POLYMYOSITIS INTRACELLULAR AUTOANTIGENS[1]

Antigen Name	Autoantibody Frequency ID[2]	Frequency ELISA[3]	Disease Specificity	Binding Specificity	Antigen's Function
Jo-1	18%		high	histidyl-tRNA synthetase	protein synth.
U1RNP	13%		low	ribonucleoprotein	RNA processing
PM-Scl	8%		high	nucleolar protein	?
Mi-2	8%	20%	high	nuclear protein	?
Ro/SS-A	7%		low	ribonucleoprotein	?
La/SS-B	3%		low	ribonucleoprotein	RNA transport
PL-7	3%		high	threonyl-tRNA synthetase	protein synth.
PL-12	3%		high	alanyl-tRNA synthetase	protein synth.
Ku	Rare			DNA binding protein	? DNA repair
SRP	Rare			signal recognition peptide	protein synth.
Fer	Rare			other tRNA associated antigens	protein synth.
Mas	Rare			other tRNA associated antigens	protein synth.

[1]- adapted from reference #162.
[2]- indirect immunodiffusion assay
[3]- enzyme-linked immunosorbent assay

4.1 Jo-1.

4.1.1 Historical perspective and clinical correlations

Anti-Jo-1 was first defined in 1980 by Nishikai and Reichlin (121), and has been found in approximately 20% of patients in a number of studies on genetically different populations (122-125). Walker et al recently found a higher frequency (44.5% for all myositis and 65% of active patients) (126). There is very high myositis specificity for this antibody. It is quite uncommon in DM in most studies, except that of Walker et al (126), and rare in children. Even in its most common group, adult PM, anti-Jo-1 antibody is usually found in less than half of patients (30-40%). It has not been reported in myositis associated with malignancy. Anti-Jo-1 may be seen in patients with overlap syndromes, most commonly with Sjogren's syndrome (122).

The subgroup of clinical features associated with anti-Jo-1 has been further defined. There is a strong association of anti-Jo-1 with interstitial lung disease (ILD), now documented in at least 4 patient populations. Yoshida et al (125), in Japan, found ILD by x-ray in all 9 patients with this antibody, but in only 22% of those without the antibody. Bernstein et al (127) found anti-Jo-1 in 68% of patients with both myositis and ILD, but in only 7.5% with myositis alone, among 72 British myositis patients studies. Anti-Jo-1 was also found in 2 patients among 62 who had ILD without myositis. Hochberg et al (128)

found ILD in 50% of anti-Jo-1 patients (in the U.S.), and recently Walker et al found anti-Jo-1 to be associated with ILD in Australian patients (126). The clinical course of some patients with the syndrome of myositis and lung disease may be dominated by the lung disease (129), which can be severe and even fatal. Bernstein has suggested that there are other clinical features associated with the Jo-1 antibody, possibly constituting a clinical syndrome (130). Arthritis, sicca syndrome, Raynaud's phenomenon, sclerodactyly, and lung disease without the DM rash, were felt to be characteristic of these patients. Anti-Jo-1 is also linked with HLA-DR antigens. Arnett et al (131) found that 64% of 11 anti-Jo-1 patients also had DR3, vs. 22% of 36 anti-Jo-1-negative patients ($p>0.05$). All anti-Jo-1 positive patients had either DR3, DR6, or both ($p>0.01$). This may reflect direct control of antibody production by D-region immune response genes as has been suggested for anti-Ro/SS-A and anti-La/SS-B (132). Whether the association is primarily with anti-Jo-1 or with the associated clinical syndrome has not yet been determined (130).

4.1.2 Autoantibody binding specificity

The Jo-1 antigen is the enzyme histidyl-tRNA synthetase (133), which attaches histidine to its cognate tRNA, activating it for incorporation into nascent polypeptide chains (see Figure 8).

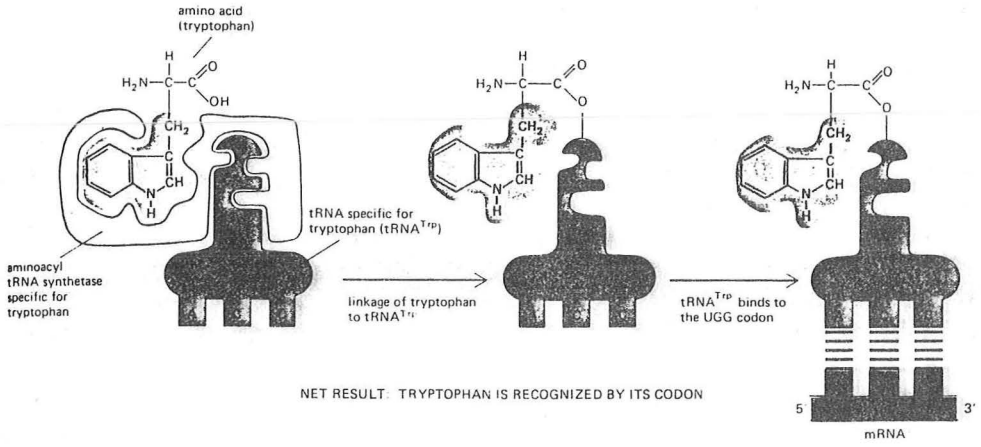

Figure 8 - Function of the aminoacyl tRNA synthetase specific for tryptophan. Histidyl tRNA synthetase is the myositis-related Jo-1 autoantigen (from reference #169).

This was discovered after Hardin et al (134), using immunoprecipitation, found that a specific type of tRNA was contained in the antigen recognized by anti-Jo-1 sera. This tRNA was sequenced by Rosa et al (135), and found to be the tRNA for histidine. The antigen itself, however, was known to be a protein, as confirmed by Rosa et al. Since histidyl-tRNA synthetase has relatively high affinity for its substrates and can be present as a complex with its specific tRNA in vivo, it was a good candidate for the antigenic

protein. Mathews and Bernstein (133) proved this by showing that anti-Jo-1 IgG blocks the reaction catalyzed by the enzyme in vitro and that the inhibition was specific for histidine and for Jo-1 antibody. The identity of this antigen as histidyl-tRNA synthetase has been independently and directly confirmed by showing that purified Jo-1 antigen had synthetase enzymatic activity (136).

4.2 PL-7, PL-12.

Mathews et al (137) and others (138) have identified a few myositis patients who have antibodies to threonyl-tRNA synthetase, the enzyme that performs for threonine the same function that the Jo-1 enzyme performs for histidine (but these sera had no anti-Jo-1). The antibody, named anti-PL-7, occurs in 3-4% of myositis patients (124). Antibodies to alanyl-tRNA synthetase (anti-PL-12) have also been observed in about 3% of myositis patients (139). Both anti-PL-7 and anti-PL-12 have been seen in rare patients without recognized myositis. In contrast to sera with antibodies to threonyl-tRNA synthetase and histidyl-tRNA synthetase, in which only antibodies to the enzyme itself have been found, sera with antibodies to the alanyl-tRNA synthetase enzyme have also contained antibodies to alanine tRNA. These antibodies do not react with other tRNAs (139). The antibodies to the synthetase and to the tRNA can be separated, and evidence suggests that antibody to the enzyme may react with the tRNA binding site. Bunn et al (139) postulated that one antibody was the anti-idiotype of the other. Bernstein et al (124) have found anti-PL-7 and anti-PL-12 to be associated with the same clinical subgroup as that of anti-Jo-1, particularly with a high incidence of ILD, Raynaud's phenomenon, and arthritis. Only a few patients have been reported, but most have had ILD. The finding of antibodies to a functional class of enzymes (with each patient selecting only one) associated with a similar clinical syndrome is unique among connective tissue diseases.

4.3 Mi-2.

4.3.1 Historical perspective and clinical correlations

In 1976, Reichlin and Mattioli (140) described an antibody in the serum of a DM patient (Mi) directed at an unknown antigen in calf thymus extract. Using a modification of complement fixation, the same antibody was found in 60% of sera from other DM/PM patients but not among controls with muscular dystrophy or other connective tissue diseases (without myositis). This was the first antibody associated with myositis without overlap syndromes. Mi serum reacted with two different antigens, labelled Mi-1 and Mi-2. Anti-Mi-1 was rarely found in other sera, even by sensitive methods, and was not specific for myositis (141). Anti-Mi-2 was most likely the original Mi antibody, although it was detected in lower frequency (142). It has thus far been seen only in DM/PM. It is found in about 8% of DM/PM patients using the ELISA technique. It is almost exclusively found in DM (as opposed to PM), where its frequency approaches 20% (by ELISA) (142). It is the only autoantibody associated only with DM, although others, such as anti-PM-Scl, are sometimes found in DM. It is most frequent in adult DM, but is found in occasional patients with juvenile DM and DM

with malignancy. The finding of the antibody in at least a few patients in all DM subgroups may be significant in understanding the relationship between different forms of DM.

4.3.2 Autoantibody binding specificity

Mi-2 antigen is a nuclear protein that shows 2 bands on polyacrylamide gels (142), but little else is known of its nature or function.

4.4 PM-Scl.

4.4.1 Historical perspective and clinical correlations

In 1977, Wolfe et al (143) described a precipitating antibody that was found in 61% of DM/PM patients, which they labeled anti-PM-1. Many of these patients had myositis-scleroderma overlap. Subsequent studies have shown that more than one antibody was being detected. The unique specificity, labeled PM-Scl, was still associated with myositis/scleroderma overlap (123). A number of antibodies other than anti-PM/Scl are found in patients with this syndrome, including antibodies to U1RNP, U2RNP (144), Ku, Ro/SS-A, Jo-1 and others. Anti-PM-Scl was found in only 12% of 77 such patients in one study (145). It may also be seen in patients with DM/PM without overlap, and occasionally in scleroderma without evidence of myositis. In some cases, the myositis is present initially and resolves or responds to treatment while the scleroderma persists (130). Anti-PM-Scl has been associated with interstitial lung disease (130), which could be a part of the overlapping scleroderma.

4.4.2 Autoantibody binding specificity

Although the function of the PM-Scl antigen is unknown, it appears to be a nucleolar protein (146-147) that may have a pre-ribosomal origin (its expression is inhibited by drugs that suppress rRNA transcription) (147). The fact that this nucleolar myositis antigen is associated with scleroderma overlap is interesting in view of the general association of scleroderma with anti-nucleolar antibodies (148). Bernstein et al (62) found the antigen to contain three polypeptides of 26-36 KD, while Reimer et al found it to be a particle of 11 polypeptides including a 90KD antigenic component and no RNA (147,149).

4.5 Anti-Ku.

4.5.1 Historical perspective and clinical correlations

Anti-Ku, described by Mimori et al in 1982 (150), was originally found in 9 patients from Japan, at least 7 of whom had overlap syndromes involving myositis, particularly with scleroderma. It is less common in U.S. or British myositis patients (as opposed to anti-PM-Scl, which is less common in Japanese patients). It also occurs in SLE (151).

4.5.2 Autoantibody binding specificity

The structure of the Ku antigen has been studied in detail (87,152), but the function is not as clear as it is for Jo-1. Ku is a non-histone DNA-binding protein with 2 polypeptide chains of 70 and 80 kD, both of which are usually antigenic. It appears to bind to inter-nucleosomal segments of DNA, and can bind *in vitro* to free ends of ds-DNA segments, possibly indicating a role in DNA repair.

4.6 Others.

Other anti-cytoplasmic antibodies are found in myositis. A few myositis sera have autoantibodies that are directed at tRNA-associated antigens (the sera immunoprecipitate tRNA) (127), but no other anti-synthetases have been detected. The *Fer* antibody is directed at tRNA associated protein(s), and precipitates an array of different tRNAs (153). The *Mas* antibody appears to be directed at a form of tRNA itself (124,153). Other myositis sera inhibit translation of pre-formed mRNA *in vitro*, indicating that the antigen is involved in protein synthesis (154,155). Antibody to the small cytoplasmic ribonucleoprotein *Ro/SS-A*, sometimes accompanied by *anti-La/SS-B*, has been found in myositis patients, usually in about 7-8% (122,124), although a remarkably high frequency was found in one study (38%)(156). It is usually, but not always, found in patients with overlapping features of Sjogren's syndrome or SLE. An interesting antibody recently reported in myositis patients (157-158) is directed at the 54 KD protein of the *signal-recognition particle*, a cytoplasmic ribonucleoprotein particle which helps transport newly translated secretory proteins across the endoplasmic reticulum. The 68 KD protein of this particle is believed to be the target of anti-"Alu" antibodies (159), that have been found in a few patients with myositis, although they are also found in other conditions (160).

Thus, there are a number different cytoplasmic proteins, many of them associated with tRNA or protein synthesis, that become the targets of autoantibodies in myositis. Antibodies to each particular protein are quite uncommon, but there appears to be a predilection for such antibodies not seen in other connective tissue diseases (137). This interesting finding may be of fundamental significance to etiology. However, the other myositis-associated antibodies are not directed at cytoplasmic or tRNA related antigens; Mi-2, Ku and U1RNP are nuclear antigens, and PM-Scl is nucleolar.

Antibodies to the cytoskeleton are found in sera from myositis patients, but they are commonly found in a large number of conditions, including most other connective tissue diseases as well as other autoimmune diseases, patients with recent viral infections, and normals. Senecal *et al* (161) found antibodies to intermediate filaments microfilaments, or both, in almost all of their patients with myositis or myositis-scleroderma overlap. Despite the wide distribution of these antibodies, these investigators felt that they were more closely associated with DM/PM than any of the other connective tissue diseases.

The foregoing discussion of myositis-related autoantigens was excerpted from reference #162.

5.0 IMPLICATIONS REGARDING THE MECHANISM(S) OF AUTOANTIBODY PRODUCTION

A number of hypotheses have been offered to explain the large number of autoantibodies which are produced in the rheumatic disorders (Table 8).

Table 8

THEORIES OF HUMAN AUTOANTIBODY PRODUCTION

Infection (Virus) or Chemical - Induced
Polyclonal B cell activation
Molecular mimicry / Epitope-specific crossreactivity
Viral antibody-induced anti-idiotypic "autoantibodies"
"Self" Ia antigen alteration
Genetically - Induced
B cell mutations
High immune responder genetic background
Loss of Tolerance
Disordered regulation of autoantigen-driven "physiological" autoimmune response by CD5 (Lyt-1, Leu-1) B cells

Several workers have pointed out that some of these theories have become less tenable in the light of our current understanding of the fine molecular specificity of the rheumatic disease autoantibodies (1, 19,108). The possibilities of molecular mimmicry / epitope-specific cross reactivity, chance B cell mutation and virus-induced anti-idiotypic "autoantibody" production are all biased toward an autoimmune response to a single epitope. However, the current data suggest that rheumatic disease associated autoimmune responses are often directed at multiple epitopes on the same particle or molecular configuration.

Craft and Hardin (19) point out that in a given patient only a few different autoantibodies are generally found and a subset of "predominant" antibodies stand out because they occur in a very high proportion of patients and are often found in very high titers. These

"predominant" autoantibodies can often be grouped in linked sets. U1RNP and Sm as well as Ro/SS-A and La/SS-B autoantibodies would be examples of two such linked sets. Moreover, antibodies within each set occur in ordered heirarchies. Some patients produce antibodies to U1RNP or Ro/SS-A alone while others produce both U1RNP and Sm or Ro/SS-A and La/SS-B autoantibodies. These authors suggest that these observations could be explained by the possibility that individual nucleoprotein particles simply act as direct immunogens to trigger each set of autoantibodies. For example, the U1RNP particle could trigger anti-U1RNP and Sm autoantibody production since it bears both U1RNP and Sm peptide determinants. The same could be true for the Ro/SS-A RNP particle since it is at least temporarily associated with the La/SS-B antigenic protein. Several other lines of investigation support this idea. When normal mice are immunized with isolated human U1RNP particles, antibodies are obtained that recognize the same epitopes as those bound by patient sera (163). In addition, in vitro stimulation of SLE patient B cells with Sm antigen results in production of anti-Sm antibodies (164). Cloned autoantigens such as CENP-B have also been shown to be immunogenic in mice and rabbits (108). Another bit of circumstantial evidence is the observation that anti-histone autoantibodies react to the relatively exposed epitopes on the histone octamer of the nucleosome. However, the internal epitopes of this structure are equally immunogenic when animals are challenged with purified histones (20-21). This would suggest that histone autoanitbodies might arise as a result of intact nucleosome autoimmunization.

The hypothesis of molecular mimicry has received some support from recent observations related to myositis associated autoantigens. In dermatomyositis/polymyositis, interest has centered on enteroviruses, a group of small RNA viruses of the picornavirus group, as an etiologic agent. These viruses have muscle tropism, and are capable of inducing acute and chronic inflammatory disease in muscle. An interesting theory has been suggested specifically for the development of Jo-1 antibodies after infection with picornaviruses, based on specific viral/antigen interaction. Many plant viruses have tRNA-like structures on their genomes, which can be charged with specific amino acids as if they were tRNA's (165). Picornaviruses share certain similarities of structure with plant viruses, and two are reported to accept amino acids, one of which is Mengovirus, which can accept only histidine (166). This implies a specific interaction with the required enzyme, histidyl-tRNA synthetase. There may be similar tRNA-like structures on picornaviruses capable of inducing human myositis. This could lead to autoantibodies by formation of a stable complex of enzyme and virus (similar to that of enzyme and tRNA) which becomes antigenic (133). Alternatively, the viral RNA could induce antibodies which in turn induce anti-idiotypic antibodies (167). Since both the anti-viral antibody and histidyl-tRNA synthetase react with the virus, their structures may be similar, and the anti-idiotype to the anti-viral antibody may react with the synthetase. The finding of independent, non-cross-reacting antibodies in different patients to functionally analogous enzymes (different synthetases) and related proteins, suggests that the antigens are being targeted because of their function, as in a mechanism such as a specific viral/antigen

interaction. Even if it is not by the proposed interaction, picornaviruses, with an RNA genome that serves as a mRNA, interact directly with the translational apparatus and this may somehow promote the formation of autoantibodies to the proteins involved.

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