ANTINUCLEAR AND ANTICYTOPLASMIC ANTIBODIES

Concepts and Misconceptions

Medical Grand Rounds

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Outline

Abbreviations used in Text

ACA - anticytoplasmic antibodies

ANA - antinuclear antibodies

DM - dermatomyositis

EB - Epstein-Barr

EBNA - Epstein-Barr virus associated nuclear antigen

ENA - extractable nuclear antigen

IIF - indirect immunofluorescence

PM - polymyositis

PSS - progressive systemic sclerosis

RA - rheumatoid arthritis

RANA - rheumatoid arthritis associated nuclear antigen

SCLE - subacute cutaneous lupus erythematosus

SLE - systemic lupus erythematosus

SS - Sjogren's syndrome

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- CONCEPTS AND MISCONCEPTIONS -

INTRODUCTION

We have had to deal with the concept of circulating autoreactive antibodies now for one third of a century following the demonstration by Haserick et al (2) that Hargraves' LE cell phenomenon (3) resulted from the reaction of a gamma globulin fraction with nuclear material. During this period circulating immunoglobulins (Ig) of all classes with specificity for many different human cellular constituents have been found to occur in numerous diseases, especially systemic lupus erythematosus (SLE).

TABLE 1

Partial catalogue of autoantibodies in SLE

General class	*Example*
Antibody to blood cell elements	Anti-erythrocyte
Antibody to cytoplasmic constituents	Anti-lymphocyte
	Anti-monocyte
	Anti-PMNs
	Anti-microsome
	Anti-mitochondria
	Anti-cytoskeleton (smooth muscle actin and myosin)
Antibody to plasma proteins	Anti-γ-globulin
	Immuno-conglutinin
Antibody to nuclear components	Anti-clotting factors
	Anti-native DNA
	Anti-denatured DNA
	Anti-DNA-histone
	Anti-nRNP (UI-RNP)
	Anti-histone (H1, H2A, H2B, H3, H4)
	Anti-SM
	Anti-SS-B/La
	Anti-SS-A/Ro
	Anti-proliferating cell nuclear antigen
	Anti-Ma
	Anti-double-stranded RNA
Antibody to organ-related antigens	Anti-neuron
	Anti-thyroid

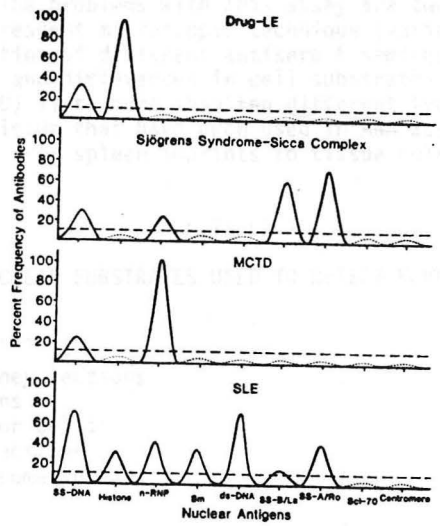
nRNP = native ribonucleoprotein.
Brackets indicate cross-reactivities currently documented.

from reference # (4).

There is little doubt that immune complexes containing antibodies to certain normally occurring cellular constitutents are capable of triggering tissue injury in different human organ systems. For example, there is a great deal of evidence to suggest that double-stranded DNA containing immune complexes participate directly in the pathogenesis of certain forms of glomerulonephritis in SLE patients. It is not so clear, however that free antinuclear antibodies (ANA) that are not bound to their antigens in the form of an immune complex are capable of injuring viable cells. There is even one school of thought which suggests that ANA's might play a physiological role by facilitating the removal of cellular breakdown products (5). It has become clear, however, that many of

these antibodies are quite specific for certain disease states and therefore can be of great value diagnostically as disease markers. In addition, the serum levels of some of these antibodies vary directly with disease activity or severity. This has led to the concept of defining subsets of complex, heterogeneous clinical diseases such as SLE based upon serological profiling (6). Patients in such serological subsets frequently share certain common clinical features as illustrated in Table 2.

Table 2



Distinctive profiles of ANAs are seen with various autoimmune diseases. **SLE** is characterized by multiplicity of ANAs occurring in different frequencies. Anti-Sm is present in 35% of SLE but in no other diseases and is a diagnostic marker. Anti-double-strand DNA is also diagnostic of SLE when in high titer. MCTD is characterized by anti-nuclear RNP without other ANAs except for anti-single-strand DNA which is present in many rheumatic diseases. Sjögren's syndrome sera contain anti-SS-A and anti-SS-B with a low frequency of anti-nRNP. Drug-induced LE is characterized by presence of antihistone antibody. The broken lines indicate relative absence of antibodies.

From ref. # (7)

It is therefore important that not only the rhumatologist, but also the general internist, internal medicine subspecialist, and the dermatologist have a sound understanding of the issues involved in ordering and interpreting the results of tests for these antibodies. This can, however, be a difficult task for several reasons - the large number of antinuclear antibodies that have recently been described, the lack of standarization of some of the assays used to detect these antibodies and some confusion in both the recent dermatologic and rheumatologic literature regarding the relationship between antinuclear and anticytoplasmic antibodies (ACA). The goal of this discussion is to make this task less burdensome for the physician by summarizing the pertinent literature that exists regarding the interpretation of the results of ANA and ACA assays and pointing out the basis for some of the present confusion that exists in this area.

METHODS OF DETECTION

Indirect Immunofluorescence. The most common assay that is currently used to detect ANA is a modification (8) of the indirect immunofluorescence (IIF) method that was originally described by Coons in 1950 (9). In this technique serum to be tested is applied to a nucleated cell substrate that has been fixed on a glass slide. Any ANA that is present in the serum will bind to the substrate nuclei and can be visualized by the addition of a fluorochrome conjugated anti human Ig antisera. The problems with this assay are two-fold: those that are inherent to any fluorescent microscopic technique (variations in effeciency of fluorochrome conjugation of different antisera & semi-quantitative nature due to subjective endpoint) and differences in cell substrates used. One recent comprehensive review (10) lists more than ten different types of human and non-human cells and cellular tissue that have been used in ANA assays by different laboratories ranging from spleen imprints to tissue culture cell lines (See Table 3).

TABLE 3

NUCLEAR SUBSTRATES USED TO DETECT HUMAN ANA'S

Non-Human	Human
Rodent liver & kidney sections	Tumor cell lines
Calf thymus sections	KB
Rodent ascites tumor cells	Hep-2
Bovine epidermal sections	Spleen imprints
Drosophilia chromosome spreads	Epidermal sections
	Kidney sections
	Thyroid sections
	Endometrial cells
	Leukocytes
	Tumor sections
	Chromosome spreads

Adapted from ref. # (10).

It is a poorly appreciated fact that there can be both qualitative and quantitative differences between such substrates. For example the nuclei of murine liver cells appear to either lack or contain very low concentrations of certain antigens that are present in the nuclei of human spleen cells and human tumor cell lines (11). Also, certain antigens present in rat kidney tissue cell nuclei are much easier to detect in the nuclei of human KB tumor cells (12). Species differences alone can account for significant differences in the expression of certain antigens in tissues from the same organ (See Table 4).

Table 4

Reciprocal titer and pattern of ANA-IF on kidney substrates with the 8 anti-SS-A containing sera*

Serum	Mouse	Rat	Chicken	Hamster	Rabbit	Human	Dog	Guinea pig
1	0	0	0	0	0	64 DS/FS	64 DS/FS	256 DS/FS
2	0	0	16 FS	4 FS	0	16 DS/FS	16 DS/FS	16 DS/FS
3	0	0	0	0	16 FS/DS†	64 DS/FS	>256 DS/FS	256 DS/FS
4	0	0	0	0	0	256 DS/FS	>256 DS/FS	>256 DS/FS
5	4 FS	4 FS	0	4 FS	0	16 DS/FS	64 DS/FS	16 DS/FS
6	4 FS	4 FS	0	4 FS	0	256 DS/FS	256 DS/FS	64 DS/FS
7	16 FS	4 FS	16 FS	16 FS	4 FS/DS†	256 DS/FS	64 DS/FS	64 DS/FS
8	16 FS	16 FS	16 FS	16 FS	16 FS/DS†	>256 DS/FS	256 DS/FS	>256 DS/FS

* ANA-IF = antinuclear antibody–immunofluorescence; DS = discrete speckles; FS = fine speckles.
† IF confined exclusively to periglomerular cells.

From ref. # (13).

Because of these issues an ever increasing number of clinical labs are adopting one or another of the commercially available human epithelial cell lines as their ANA substrates (there is very little difference between the different lines as ANA substrates) (14). In 1980 a College of American Pathologists survery revealed that approximately 50% of the responding labs across the country were using rodent tissue as their ANA substrate. A similar survey in late 1983 revealed that only about 20% of the labs were still using rodent tissue, while the remaining 80% were using epithelial cell lines. It is our understanding that all of the clinical labs in the Dallas area are currently using human epithelial cell line ANA substrates. Parkland Memorial Hospital began using the KB tumor cell substrate (Electronucleonics) in 1976 and just this past year has switched to the Hep-2 cell line (Ortho). It also appears that different fixation methods can significantly alter the nuclear antigenic profile (10). Also, some relevant nuclear antigens are cell-cycle dependent (15). Thus, it is not suprising that different results can be obtained when the same serum is assayed on different substrates (16). Therefore, as long as different nuclear substrates that have been fixed in different ways continue to be used by different laboratories across the country this will not be a standarized test and results obtained by different laboratories will not be fully comparable.

Another poorly appreciated point regarding the ANA assay is that due to it's marked sensitivity it will detect very low levels of ANA that can be found in normal individuals who are free of any evidence of autoimmune disease. In addition, the aging process alone predisposes individuals to developing ANA's that are not accompanied by clinical evidence of autoimmune disease (17). Significant ANA titers can be found in 15 - 20% of healthy populations that are older than 65 years (see Table 5).

Table 5

Autoantibodies and circulating immune complexes in healthy elderly subjects

Assay	Old subjects		Young Controls	
	positive/total tested	% positive	positive/total tested	% positive
Antinuclear Ab	50/278	18	4/98	4
Rheumatoid factor	38/278	14	4/98	4
Lymphocytotoxic Ab	27/278	10	3/93	3
Circulating immune complexes	43/197	22	5/100	5

Positive sera were defined as follows: for antinuclear Ab a titre of $\geq 1:10$, for rheumatoid factor at titre of $\geq 1:20$, for lymphocytotoxic Ab > 30% killing in > 50% of samples of peripheral blood lymphocytes from 11 normal donors, and for circulating immune complexes > 2 s.d. above the mean of values of a panel of normal control sera.

From ref. #(17).

Ideally, each laboratory should calibrate its technique by assaying a large number of normal control sera in order to determine what serum ANA titer is clearly abnormal. In general, the first significantly abnormal serum titer is that which is found in less than 5% of normal control sera. This titer might vary 10 fold on the basis of substrate differences alone (12). The first clearly abnormal ANA titer with a human tumor cell line substrate such as Hep-2 is 1:160 (14). The frequency with which ANA's are seen in different disease states is therefore dependent on the ANA substrate used. For example, ANA's could be detected in about 50 - 75% of progressive systemic sclerosis (PSS) patients with rodent tissue substrates (18), while greater than 95% of PSS patients are found to be ANA positive on tissue culture cell lines (19).

Immunodiffusion. Antibodies to the saline extractable nuclear antigens (ENA) are determined either by simple Ouchterlony double immunodiffusion in agarose as originally described by Holman et al in 1959 (20) or by a more sensitive immunodiffusion modification, counterimmunoelectrophoresis (CIE). Serum to be tested is placed in one well that has been cut in the agarose and the relevant solubilized nuclear antigenic material is placed in a similar nearby well. Time is then allowed for diffusion from the wells into the agarose to take place. If the serum contains ANA with specificity for antigens present in the nuclear extract a grossly visible precipitin line will develop between the two wells. The binding specificities of these antibodies can then be determined by testing for lines of identity with monospecific reference sera of known reactivity. This reaction can be produced more efficiently if a current is applied between the wells as in the CIE modification. Immunodiffusion techniques are relatively insensitive and can demonstrate only those ANA that have the ability to produce a precipitin reaction in agarose with their respective antigens. This is a feature of only certain types of immunoglobulins. These techniques, however, have the advantage of not requiring fluorescence microscopy equipment nor requiring subjective interpretation of fluorescence patterns. A passive hemagglutination assay is presently used in some labs to detect several of the ENA antibodies that were originally defined

by immunodiffusion. This assay is more sensitive than immunodiffusion; however, it is technically a more difficult procedure and does not allow for the precise identification of antibodies like the immunodiffusion technique does. ENA antibodies are currently deteced at PMH by double immunodiffusion using a calf thymus extract which contains Ro/SS-A antigen in addition to a number of other specificities. The progress that has been made recently in purifying the various extractable nuclear antigens has allowed the development of sensitive, solid phase radioimmuno- and immunohistochemical assays that should greatly enhance our ability to quantitate the levels of ENA antibodies in the future (21).

INDIRECT IMMUNOFLUORESCENCE - DEFINED ANA'S

ANA's of different antigenic specificity will produce different patterns of nuclear fluorescence depending upon the distribution of the antigens within the nucleus. If a serum contains two or more different types of ANA as is frequently the case then a combination of fluoresence patterns will result. Several classification systems for nuclear immunofluorescence patterns have been devised (See Table 6).

TABLE 6

SYSTEMS FOR CLASSIFYING NUCLEAR IMMUNOFLUORESCENCE PATTERNS PRODUCED BY ANTINUCLEAR ANTIBODIES

Traditional Clinical Laboratory Classification: (Substrate frequently not specified but until recently has often been rodent tissue)

- Homogeneous (diffuse)
- Peripheral (rim, shaggy)
- Nucleolar
- Speckled

Burnham's Simplified Classification: (Based on human spleen cell imprints)

- Nonparticulate
  1. Peripheral
  2. Homogeneous
  3. Leukocyte specific
- Particulate
  1. Nucleolar
  2. Speckled (true speckles, anticentromere)
  3. Other particulates

Personally Preferred Classification: (Based on human tumor cells such as Hep-2)

- Continuous
  - Homogeneous
  - Peripheral
- Discontinuous
  - Nucleolar
  - Discrete speckled (centromeric)
  - Particulate

From ref.# (1).

Rheumatologists and clinical labs in general have commonly recognized four main ANA fluorescence patterns in the past: homogeneous, peripheral, nucleolar, and speckled. However, some investigators such as Thomas Burnham have long maintained that a greater number of clinically relevant, distinct patterns of nuclear fluorescence can be recognized. More recent work by Eng Tan and others has begun to support Burnham's view (22).

Burnham has recognized two major categories of nuclear fluorescence patterns: particulate and nonparticulate (23). His system differs in two ways: his recognition of a leukocyte specific pattern and his designation of the true speckled pattern as a distinct entity. His recognition of the leukocyte specific pattern is based on the fact that his substrate - human spleen imprints - consists of a number of different cell types including polymorphonuclear leukocytes. Such leukocytes are not present in most other ANA substrates. Therefore, this pattern that was originally reported by Faber and Elling (24)

has only rarely been commented upon by others. His recognition in 1968 of the discrete (true) speckled pattern as a distinct entity seen predominately in those patients with acrosclerotic scleroderma (CREST syndrome) or progressive systemic sclerosis with diffuse scleroderma (25) has more recently been confirmed by others (22,26). These workers have gone on to show that sera which produce discrete speckled fluorescence on interphase nuclei will produce centromeric kinetochore fluorescence on cells in metaphase. Recent studies have shown that anti-centromere antibodies are present in 50-60% of patients with the CREST syndrome (acrosclerotic) varient of progressive systemic sclerosis and in 10-15% of patients with the diffuse form of this disease (27). The clinical correlations of anticentromere antibodies are listed in Table 7.

TABLE 7

COMPARISON BETWEEN SCLERODERMA PATIENTS WITH ANTICENTROMERE AND WITH OTHER ANTINUCLEAR ANTIBODIES

	Anti-centromere (21)	Other antibodies (55)	Significance χ^2 test
Mean age at diagnosis (years) ± SD	46 ± 13.6	43.8 ± 13.4	N.S.
Mean duration of diseases (years) ± SD	15.5 ± 9.2	7.5 ± 7.0	$p < 0.001$
Females (%)	100	67	$p < 0.003$
Calcinosis (%)	71	36	$p < 0.006$
Absence of diffuse scleroderma (%)	90	42	$p < 0.001$
Telangiectasia (%)	90	56	$p < 0.004$
Renal involvement (%)	0	18	$p < 0.04$
Mortality (1972–1980) (%)	5	23	$p < 0.06$
Raynaud's syndrome (%)	100	93	N.S.
Esophageal involvement (%)	90	96	N.S.
Small bowel involvement (%)	33	47	N.S.
Pulmonary involvement (%)	43	55	N.S.
Digital ulceration (%)	62	44	N.S.
Rheumatoid factor (titer ≥ 160) (%)	52	11	$p < 0.001$

From ref.# (7).

In addition, anti-centromere antibodies have been reported to occur in 29% of one series of 48 patients with primary bilary cirrhosis (28).

We have used Burnham's approach to formulate a classification system for the patterns that are seen with the substrate that we have found most useful - the Hep-2 tumor cell line. This transformed cell line was derived from a human laryngeal squamous cell cancer and is presently available commercially in the form of tumor cell monolayers grown on glass slides that have been fixed with either acetone or ethanol. This ANA substrate like others such as the KB tumor cell line offers the advantages of being of human origin and providing cells at different stages of the cell cycle that have large nuclei with multiple nucleoli. Table 8 summarizes the antigenic specificities and clinical correlations of the antibodies that are responsible for the five distinct immunofluorescence patterns that can be seen with this substrate.

TABLE 8

ANTIGENIC SPECIFICITY AND CLINICAL CORRELATIONS OF ANTINUCLEAR ANTIBODY IMMUNOFLUORESCENCE PATTERNS SEEN WITH THE HEP-2 TUMOR CELL SUBSTRATE

ANA IF Patterns	Antigenic Specificity of Antibodies Responsible for patterns	Clinical Correlations
Continuous		
1. Homogeneous	Deoxyribonucleoprotein, Histones	Low titer (≤ 1:1280)- Nonspecific (Drug induced, many chronic inflammatory diseases and entire spectrum of connective diseases). High titer (> 1:1280) - SLE and Occasionally drug induced LE
2. Peripheral	Double stranded DNA, rarely histone	Any titer-relatively specific for SLE, occasionally drug induced LE. High titer (> 1:160) - SLE with active nephritis
Discontinuous		
3. Nucleolar	4-6 S Nucleolar RNA	Progressive systemic sclerosis Occasionally SLE
4. Discrete (true) speckled (Centromeric)	Inner and outer plates of kinetochore (? DNA-histone complex).	CREST syndrome, Progressive systemic sclerosis, primary bilary cirrhosis
5. Particulate	Numerous saline extractable nuclear proteins (i.e., nRNP, Sm, La/SS-B, Ro/SS-A)	Low titer (<1:1280)- nonspecific High titer (> 1:1280) mixed connective tissue disease, SLE, Sjogrens syndrome

Adapted from ref.# (1) & (7)

We have categorized the patterns as to those in which the nuclear fluorescence is continuous throughout the entire nucleus (homogeneous) or around the periphery (peripheral) and those in which the fluorescence is discontinuous [nucleolar, discrete speckles (centromeric) and particulate] (1). The relatively disease-specific patterns are peripheral (SLE, rarely drug induced LE), nucleolar (PSS, rarely SLE)) and discrete speckles (centromeric) (acrosclerotic scleroderma, PSS) (29). The remaining two patterns, homogeneous and particulate are not at all disease specific, particularly when present in low titers. Since the morphologically distinct discrete speckles (centromeric) pattern has recently been confirmed to have a strong disease association (22,26)

we feel that it is justifiable to consider this as a separate, distinct pattern. This pattern along with the particulate patterns have been lumped together in the past under the "speckled" designation. It is very likely that one or more of the different configurations of nuclear fluorescence that are currently lumped together under the "particulate" designation will be found to have distinct clinical correlations in the future. Preliminary studies have indicated that one of the particulate patterns (large speckle-like threads) is produced either directly by SS-A/Ro antibodies or by other nuclear reactive non-precipitating antibodies that are present in SS-A/Ro antibody containing sera (30).

IMMUNODIFFUSION - DEFINED ANA'S.

The past decade has witnessed the identification of a number of antigenically distinct small protein-RNA complexes that can easily be extracted from nuclear or cytoplasmic subcellular fractions due to their marked solubility in saline. Patients with certain autoimmune diseases develop circulating autoantibodies to one or a combination of these saline extractable nuclear or cytoplasmic antigens. It was pointed out in a comprehensive review in 1981 that at least twenty different ENA antibody systems had been described (31). These antibodies are usually seen in patients who have high titer immunofluorescence - defined ANA's that produce a particulate pattern of fluorescence. Since many of these antibodies were originally identified by separate investigators over a relatively short period of time, no uniform system of nomenclature was used to designate them. Some were named after the initials of the first patient in whom they were identified (e.g., Sm, Ro, La, Ma, Jo-1, Mi-2, Mo, Ha, Su, Ku). Others were named according to their associated disease state (e.g., ScL-70, SS-A, SS-B, PM-1, RANA, RAP) while still others were named according to the chemical characteristics of the antigen [e.g.,nRNP (nuclear ribonucleoprotein), PCNA (proliferating cell nuclear antigen)]. Adding to the confusion is the fact that different investigators often used nuclear preparations from different tissues or cell lines to prepare their saline extracts (e.g., calf thymus, rabbit thymus, human liver, human spleen, Wil 2 human lymphoblastoid cell line). As a result several ENA antibodies that were originally felt to have distinct reactivities have more recently been shown to produce lines of identity in immunodiffusion and therefore possess the same antigenic specificity. For example, it has been shown that anti-Ro and anti-SS-A antibodies have identical antigenic specificities (32). The same study also demonstrated that anti-La, anti-SS-B and anti-Ha antibodies also had identical reactivities. Likewise, anti-RNP and anti-Mo antibodies appear to be identical (33). The antigenic specificity and disease correlations of the various ENA antibodies are listed in Table 9.

TABLE 9

CLINICAL CORRELATIONS OF PRECIPITATING ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS

Disease Categories	Associated Clinical Features
Lupus Erythematosus	
anti-Sm	Mild SLE (?)
anti-Sm + nRNP(Mo)	Mild SLE without nephritis
anti-MA	SLE with nephritis and cutaneous disease
anti-Ro/SS-A (? SjD)	Subacute cutaneous LE skin lesions, Sjögrens syndrome, neonatal LE, C_2 deficiency, SLE.
anti-La/SS-B,(Ha) (? SjT)	Sjögrens syndrome, SLE
anti-PCNA	Severe SLE with nephritis
Scleroderma	
anti-Scl-70	PSS (CREST syndrome & diffuse scleroderma) with sclerodermatous lung disease
Mixed Connective Tissue Disease	
anti-nRNP (Mo)	Raynaud's phenomenon, esophageal and pulmonary disease, discoid LE, alopecia, swollen hands and fingers. Low incidence of renal disease.
Dermatomyositis	
anti-Mi-2	Cutaneous & muscular manifestations of dermatomyositis
Polymyositis	
anti-PM-1	PM, PM/PSS overlap
anti-Jo-1 (Su)	PM, PM with interstitial lung disease.
anti-Ku	PM overlapping with SLE and/or PSS
Rheumatoid Arthritis	
anti-RANA (RAP, SS-C)	Rheumatoid arthritis, Sjogren's syndrome

Adapted from ref.# (1)

"Anticytoplasmic" Antibodies. Precipitating antibodies to the saline soluble antigens, Ro and La, have been generally referred to as the "anticytoplasmic antibodies" since they were originally identified in immunodiffusion with saline solubilized extracts of cytoplasmic fractions of human spleen cells (34) and calf thymus cells (35) respectively. As was previously mentioned, antibodies to the Ro and La antigens have been shown to be immunologically equivalent to antibodies to the SS-A and SS-B antigens respectively. The SS-A and SS-B antigens, however, were originally shown to occur in the nuclei rather than cytoplasm of Wil/2 lymphoblastoid cells. The La/SS-B antigen has subsequently been shown to occur in the nucleus as well as the cytoplasm of other cell types. It has also been suggested that the Ro antigen might also be represented in the nucleus during some phases of the cell cycle (30). Recent studies in our own laboratory as well as those by Harmon et al. (13) have demonstrated that the Ro/SS-A antigen occurs predominantly in the nucleus of cultured human tumor cells such as the Hep-2 cells line. Therefore, antibodies to the Ro/SS-A and La/SS-B antigens should not be considered to be exclusively anticytoplasmic antibodies, and can be fairly categorized as ENA antibodies. The original characterization of the Ro antigen suggested that it was an acidic cytoplasmic glycoprotein with a molecular weight in the 100,000-150,000 range that was resistant to both deoxyribonuclease and ribonuclease (34). However, the more recent molecular biology studies of Lerner and coworkers have shown that anti-Ro and anti-La antibodies bind antigens that are present on a small set of discrete particles composed of RNA and protein (36) that are present in saline extracts of cytoplasmic fractions. These small "cytoplasmic" ribonucleoproteins are different from the small nuclear ribonuculeoproteins that the same workers have shown to bind anti-Sm and anti-n-RNP antibodies from systemic lupus erythematosus sera. One of the small nuclear ribonucleoproteins that bears both the n-RNP and Sm determinants appears to be involved in the nuclear splicing of messenger RNA precursors (37). The cellular roles for the Ro and La containing small "cytoplasmic" ribonucleoproteins are at present, however, unknown. Thus, any potential role that anti-Ro and anti-La antibodies might play in altering the normal physiology of cells has yet to be determined.

Clinical Correlations of Ro/SS-A and La/SS-B Antibodies. Antibodies to the Ro/SS-A and La/SS-B antigens occur predominantly in association with Sjogren's syndrome and diffuse forms of lupus erythematosus (38) (39). Anti-La/SS-B almost always occur in parallel with anti-Ro/SS-A, however, anti-Ro/SS-A not infrequently occur in the absence of anti-La/SS-B. Table 10 shows the frequencies with which anti-Ro/SS-A antibodies are associated with different disease states.

TABLE 10

CLINICAL ASSOCIATIONS OF Ro/SS-A ANTIBODIES

Sjogren's Syndrome	60%
Subacute Cutaneous LE	75%
"ANA-Negative" SLE	65%
Neonatal LE	100%
C_2 deficiency	75%
SLE	25%

Anti-Ro/SS-A are seen more commonly in those Sjogren's syndrome patients who have extraglandular disease (vasculitis, purpura, and lymphadenopathy), hematological abnormalities and serological abnormalities (40), as can be seen in Table 11.

TABLE 11

Table 4. Clinical and Laboratory Features in Patients with Anti-Ro(SS-A) Antibodies

Features	Patients with Anti-Ro(SS-A)		
	Positive ($n=33$)	Negative ($n=42$)	p Value
	n	*n*	
Clinical			
Vasculitis	24	4	0.0005
Salivary gland enlargement	26	15	0.0005
Lymphadenopathy	19	4	0.0005
Purpura	12	1	0.0005
Hematologic*			
Anemia	19	6	0.0005
Leukopenia	14	3	0.001
Thrombocytopenia	7	1	0.025
Serologic†			
Hyperglobulinemia	21	8	0.0005
Rheumatoid factor	29	17	0.0005
Antinuclear antibody	3	10	0.0005
Cryoglobulinemia	7/13	0/22	0.001
Hypocomplementemia	11	2	0.005

From ref. # (40).

Anti-Ro antibodies have been shown to occur in high frequency in three groups of patients that have prominent cutaneous abnormalities. We have found anti-Ro antibodies in 63% of patients who have subacute cutaneous lupus erythematosus (SCLE) skin lesions (41). The cumulative frequency of these antibodies in SCLE patients is even higher (75%) (42). This antibody was present in an even higher frequency in those patients who had the annular type of SCLE lesions. These figures represent significant elevations above the reported frequency of anti-Ro antibodies (26%) in large, unselected systemic LE populations (43). We have also found that 74% of our SCLE patients had significant levels of antinuclear antibodies when assayed on a human tumor cell line substrate (KB cells). Many of the patients described by Provost, Maddison and coworkers (11,44) as having "antinuclear antibody negative" systemic lupus erythematosus have a widespread, photosensitivie form of histologically specific cutaneous LE that in many ways appears to be similar to what we have described as SCLE. Like our SCLE patients about one-half of the "antinuclear antibody negative" systemic LE patients had evidence of a widespread multisystem disease, but as a group had a low incidence of significant nephritis or neuropsychiatric disease (11). Also like our SCLE patients, the "antinuclear antibody negative" systemic lupus erythematosus patients had a high frequency (62%) of antibodies to the "cytoplasmic" antigen, Ro (11). Thus, the main difference between these two groups of patients has been the fact that by definition none of the "antinuclear antibody negative" systemic lupus erythematosus patients had detectable serum antibodies that bound to mouse liver cell nuclei; whereas, 74%

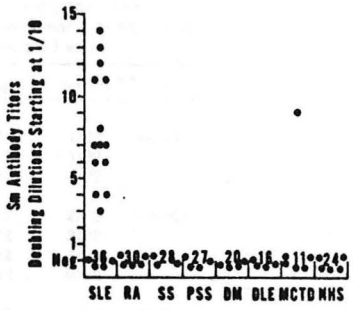
of our SCLE patients had antibodies which bound to human tumor cell line nuclei in significant titer (1:160 or greater). As it turns out, this is not such a great difference after all, considering the fact that 66% of the "antinuclear antibody negative" systemic lupus erythematosus patients were in fact antinuclear antibody positive when their sera were further tested with the human KB tumor cell substrate (11). In addition, Provost and coworkers have recently reported that 16 of 27 (60%) anti-Ro antibody containing sera that had originally been ANA negative on mouse liver substrate became ANA positive when assayed on human spleen cells (45). We have also shown that Ro/SS-A antibody containing SCLE sera frequently is ANA negative on mouse kidney sections, but ANA positive on Hep-2 cells (46). It is now clear that rodent cell nuclei either lack certain relevant antigens that are present in human nuclei or have these antigens present in concentrations too low to be detected by indirect immunofluorescence. ANA substrate variability appears to be the basis of the "ANA negative" systemic LE phenomenon that has been widely publicized over the past 8 years. The clinical and serologic features of the SCLE and "ANA negative" SLE patient are strikingly similar considering the different criteria that were used to select these two groups of patients.

Anti-Ro (SS-A) and La (SS-B) antibodies have also been found to occur in neonatal LE patients. Some infants born to mothers with systemic lupus erythematosus develop transient forms of histologically specific cutaneous lupus erythematosus on light exposed areas at about 4-6 weeks of age. The clinical descriptions of this often annular eruption are very close to what we have seen in adults with annular SCLE. However, some infants as well have developed scarring cutaneous LE lesions that would best be classified as discoid LE. At least three different groups have now reported finding anti-Ro (SS-A) or anti-La (SS-B) antibodies in the sera of neonatal lupus erythematosus infants and their mothers (47-49). In the infants these antibodies have disappeared at about the same time that the skin lesions have subsided -- usually by six months of age. The antibodies, however, have been shown to persist in the circulation of the mothers. These observations have been interpreted to suggest that transplacental passage of IgG anti-Ro/SS-A or anti-La/SS-B antibodies from the mothers to the affected infants has occurred and that these maternal antibodies while present in the infant in some way triggered the expression of the cutaneous lupus erythematosus lesions. This interesting hypothesis suggests a humoral or immune complex mediated etiology for the cutaneous lupus erythematosus lesions that occur in these infants which, however, is contrary to a large body of indirect evidence which points more toward a cell mediated pathogenesis of cutaneous lupus erythematosus (50). Anti-SS-A/Ro antibodies have also recently been shown to occur in C2 deficient patients that have systemic and cutaneous LE (51, 52).

Clinical Correlations of Anti-Sm and anti-nRNP antibodies.

Antibodies to Sm and nRNP antigens were originally identified in the sera of SLE patients (53, 54). Further studies revealed that anti-Sm antibodies were seen almost exclusively in SLE (see Table 12).

TABLE 12



From ref. # (55).

The specificity of anti-Sm antibodies for SLE is reflected by the fact that their presence now constitutes one of the 11 ARA Revised Criteria for the classification of SLE (56). However anti-Sm antibodies are not a sensitive index of SLE in that only about 30% of SLE patients possess these antibodies (55,57). Some workers have found that SLE patients who have anti-Sm and anti-DNA antibodies together more frequently have cutaneous vasculitis, pleuropulmonary, and cardiac manifestations than do those SLE patients who have only anti-DNA antibody (57). (See Table 13). The relationship between anti-Sm and LE nephritis continues to be in dispute with some workers claiming a higher incidence of renal disease (58) and others claiming milder histopathological types of glomerular disease (57) and milder systemic disease overall (59). Anti-Sm antibodies do not appear to fluctuate significantly with changes in systemic disease activity (60). SLE patients who have anti-Sm antibody frequently have anti-nRNP antibody as well (33).

Anti-nRNP antibodies are seen in a number of different connective tissue diseases states (see Table 14) and occassionally in completely normal individuals (61).

TABLE 13

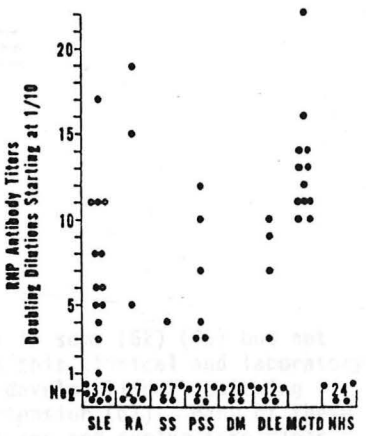
Frequency of Cutaneous (Other Than Erythema), Pleuropulmonary, and Cardiac Manifestations in Groups I and II

	Group I *	Group II **	p
Cutaneous			
No. of patients	6/12	1/22	<0.01
Vascular purpura	3/12	1/22	
Skin necroses	3/12	0/22	
Pleuropulmonary			
No. of patients	9/12	2/22	<0.001
Pleuritis	4/12	2/22	
Lupus pneumonia	4/12	1/22	
Pulmonary hemorrhages	2/12	0/22	
Cardiac			
No. of patients	8/12	4/22	<0.01
Pericarditis	5/12	4/22	
Myocarditis	2/12	0/22	
Endocarditis	1/12	0/22	
Heart block	1/12	0/22	

* Group 1 - anti-DNA + anti-Sm
** Group 2 - anti-DNA only

From ref. # (57).

TABLE 14



From ref. # (55)

High titers of anti-nRNP antibodies in the absence of anti-Sm antibodies are frequently seen in patients who have overlapping features of progressive systemic sclerosis, SLE and polymyositis. Sharp and coworkers described a group of such patients in 1972 and designated this clinical and laboratory constellation as mixed connective tissue disease (MCTD)(62). Since its description 12 years ago the concept of mixed connective tissue disease has generated a great deal of debate among rheumatologists, with some claiming that it should be thought of as a seperate disease entitiy and others claiming that it represents but one clinical stage of a disease process such as SLE or PSS. Whichever bias one prefers the following points can be made regarding patients who have high titer (greater than 1:10,000 by hemagglutination) anti-nRNP antibodies in the absence of anti-Sm antibody. Such patients frequently have Raynaud's phenomenon, esophageal dysmotility, characteristically swollen hands and a non-deforming polyarthritis as part of their presenting clinical illness. High titer particulate pattern ANA's and marked hypergammaglobulinemia in addition to anti-nRNP antibodies are frequently seen laboratory abnormalities in these patients. (63,64) (Sharp, 1982). See Table 15.

TABLE 15

Most common characteristics of 308 patients with high titers of nRNP antibody only*

Characteristic	% Positive
Positive fluorescent ANA	95
Polyarthralgia	90
Raynaud's phenomenon	81
Hypergammaglobulinemia	74
Polyarthritis	74
Swollen hands	73
Positive rheumatoid agglutinins	60
Esophageal hypomotility	58
Sclerodactyly	50
Myositis	50

From ref. # (63)

Renal disease is infrequently seen in such patients in some (62) (65) but not all (66) series. The course taken by patients with this clinical and laboratory constellation does not appear to be uniform. Some develop life threatening pulmonary interstitial fobrosis and pulmonary hypertension (67). Many of these patients with time lose their "mixed" clinical features and evolve into clear cut progressive systemic sclerosis (68). It has been suggested that the pulmonary and esophageal manifestations of MCTD are as a rule more responsive to systemic corticosteroid therapy than is the case in progressive systemic sclerosis (67). Anti-nRNP antibody titers do not fluctuate greatly with changes in disease activity of MCTD (69).

Patients who have high titer anti-Sm or anti-nRNP antibodies can frequently be shown to have nuclear deposits of IgG in the cells of the epidermis in biopsies of clinically normal appearing skin (70-71) (Gilliam, 1977). This phenomenon has been turned "epidermal nuclear staining" and has been felt by some workers to represent penetration of antibody through the plasma and nuclear membranes of viable epidermal cells followed by binding to nuclear antigens (72-75). Others (76) including those in our own laboratory (77) believe that this is an in vitro artifact that occurs during the normal processing of the tissue as a result of the large amount of unbound extravascular IgG that is always present in the dermis of hypergammaglobulinemic patients.

ENA Antibodies Associated with Polymyositis (PM)/Dermatomyositis (DM). Four different ENA antibody systems have been described in patients with PM/DM. Three of these antibodies (anti-PM-1, anti-Jo-1, and anti-Ku) are associated predominantly with PM, while the fourth (anti-Mi-2) appears to be a serological marker for DM. Wolfe et al., in 1977 (78) described the first of these precipitating antibody systems which they designated as anti-PM-1. This antibody system was present in 64% (9/14) of their PM patients and in 87% (7/8) of their PM/scleroderma overlap patients. Nishikai and Reichlin (79) next described a precipitin system designated as Jo-1 which they found to be present in 31% of their PM patients. A follow-up study involving Japanese patients has confirmed that anti-Jo-1 antibodies are seen predominantly in PM patients (80). These same workers also noted that interstitial pulmonary fibrosis was present in all of their anti-Jo-1 positive PM patients, suggesting that this precipitating antibody system might be a serological marker for a subset of PM patients who have or are at risk for developing interstitial pulmonary fibrosis.

The anti-Su antibody system also described by these workers has been shown to be identical to the anti-Jo-1 system (80). The anti-Ku antibody system initially reported by Mimori et al. (81) was found in about 1/2 of PM/PSS overlap patients and 1/3 of PM/PSS/SLE patients. Nishikai and Reichlin (82) also described in 1980 a precipitin system in two DM sera that they called anti-Mi-2 that was not present in PM, SLE, scleroderma and RA sera. Further studies using an ELISA with purified Mi-2 antigen have confiemd the specificity of this antibody system for DM, finding it in approximately 20% of the adult DM sera tested (83).

Significance of anti-Scl-70 in Progressive Systemic Sclerosis. Douvas et al. first reported in 1979 the presence of a precipitating antibody system in the sera of PSS patients (84) which they named anti-Scl-70. Later studies confirmed the specificity of this system for PSS, but found that its presence did not distinguish between diffuse scleroderma and the CREST syndrome (85-86). Only about 20% of PSS patients have anti-Scl-70 antibodies. However, this anti-Scl-70 positive subgroup appears to have a high frequency of sclerodermatous lung involvement (27). Table 16 lists the clinical associations of anti-Scl-70 antibodies.

TABLE 16

Clinical associations of ACA and anti-Scl-70

	ACA		*Anti-Scl-70*	
	+	−	+	−
Number of patients	22	53	16	59
Age (years: mean and range)	56 (34–73)	50 (27–73)	50 (27–68)	51 (29–73)
Disease duration (years: median and range)	17 (1–37)	8·5 (1–43)	9 (2–43)	11 (1–37)
Diffuse skin involvement (%)	9	51 (p<0·02)	62	32
CREST (%)	73	28 (p<0·01)	25	47
Arthritis (%)	14	37 (p<0·05)	50	25
Myositis (%)	3	15	31	5
Oesophageal (%)	69	67	75	66
Pulmonary (%)	34	54 (p<0·05)	75	39 (p<0·05)
Renal (%)	9	20	19	17
Cardiac (%)	11	19	31	12
Sjögren's (%)	16	12	19	7

ACA = Anticentromere antibodies.

From ref. # (27)

Clinical Correlations of Other ENA Antibody Systems. A number of other infrequently occurring precipitating antibody systems have been described. In 1978 Miyachi et al (87) described a saline extractable nuclear antigen present in actively proliferating cells but not in resting cells which they designated as proliferating cell nuclear antigen (PCNA). Precipitating antibodies to this antigen occur in only about 2-4% of SLE sera. Preliminary studies have indicated that the SLE patients who have this antibody have a higher incidence of lymphoproliferation (87) and diffuse proliferative glomerulonephritis (88). Although it is not often present, this antibody appears to be quite specific for SLE. Another precipitating antibody system known as anti-MA was described by Winn et al in 1979 (89). This antibody was present in only 20% of the SLE patients studied but was found to be very specific for SLE. Those SLE patients who had this antibody were said to have more severe disease (recalcitrant skin disease, serious renal disease, hypertension, hepatosplenomegaly, lymphadenopathy, and neurologic disorders) than did SLE patients with Sm

or native DNA antibodies. Miyawaki et al (90) have described two other precipitating antibody systems, anti-Mu and anti-TM that are found infrequently in several of the connective tissue diseases (DM, SLE, MCTD, RA). Based on the limited data available, the presence of these two antibodies does not seem to correlate with any specific clinical feature of these diseases.

In 1976 Alspaugh et al (91) (92) described a precipitating antibody to a rheumatoid arthritis associated nuclear antigen (RANA) that is present only in cells that have been transformed with the EB virus (93). This antibody has also been referred to as the rheumatoid arthritis associated precipitin (RAP). Anti-RANA antibodies are present in over 90% of RA patients and in between 6 to 25% of other connective tissue disease patients and normal controls (92, 94-96. The presence of anti-RANA antibody is most RA patients and the close relationship between RANA and the EB virus associated nuclear antigen (EBNA) has led to the suggestion that the EB virus might in some way be related to the pathogenesis of RA.

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