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Clinical significance of anti-RNP and anti-Sm autoantibodies as determined by immunoblotting and immunoprecipitation in sera from patients with connective tissue diseases

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SUMMARY

Antibodies to Sm and RNP antigens have been detected by immunoblotting and immunoprecipitation of small nuclear ribonucleoproteins in 168 sera from patients with connective tissue diseases previously characterized by immunodiffusion. Anti-RNP and anti-Sm antibodies immunoprecipitated U1 and U1–U6 snRNA respectively. By immunoblotting anti-Sm reacted with B-B′ and D polypeptides and we have distinguished two types of anti-RNP sera: 1) 'full spectrum' anti-RNP sera reacted with the 68 kD, A, C and B-B′ polypeptides; 2) 'partially reactive' anti-RNP sera reacted with various combinations of these polypeptides but not the four of them. A strong specificity of anti-Sm antibodies for systemic lupus erythematosus (SLE) was found with all three methods but immunoblotting was more sensitive and detected anti-Sm in 76% of SLE sera. Sera containing a high titer of 'full spectrum' anti-RNP without anti-Sm activity were only detected in mixed connective tissue disease (MCTD) whereas anti-68 kD antibodies alone seemed to be less specific. This strong association between 'full spectrum' anti-RNP antibodies and MCTD supports the hypothesis that MCTD is a distinct clinical entity associated with a specific serologic marker.

Keywords anti-RNP antibodies anti-Sm antibodies immunoblotting mixed connective tissue diseases

INTRODUCTION

Antibodies reacting with various cellular constituents are detected in sera from many patients with connective tissue diseases. Remarkable associations between specificities of antinuclear antibodies (ANA) and clinical expression of these connective tissue disorders have been described (Tan, 1982). Anti-Sm antibodies seemed to be highly specific for systemic lupus erythematosus (SLE) (Notman, Kurata & Tan, 1975; Sharp *et al.*, 1976) while anti-RNP antibodies have been associated with mixed connective tissue disease (MCTD) (Sharp *et al.*, 1972) but have also been found in SLE and less frequently in other rheumatic diseases (Tan, 1982; Munves & Schur, 1983). Anti-Sm antibodies are almost always accompanied by anti-RNP antibodies whereas anti-RNP antibodies are frequently detected in the absence of anti-Sm (Reeves *et al.*, 1985). Most of the clinical studies have employed unpurified and uncharacterized extractable nuclear antigens and classical tests: double immunodiffusion (ID), counter immunoelectrophoresis, haemagglutination. Anti-RNP and anti-Sm specificities were determined by enzyme sensitivity and by lines of identity with reference sera in immunodiffusion. Sm antigen is resistant to ribonuclease and trypsin whereas RNP antigen is sensitive to both enzymes. These techniques did not always clearly identify different specificities and detected only soluble antigens.

Biochemical studies have identified Sm and RNP antigens as small nuclear RNA-protein complexes (snRNPs) that contain one or several small nuclear RNA species termed U1, U2, U4, U5 and U6 and a set of six to nine proteins (Lerner & Steitz, 1979; Kinlaw, Robertson & Berget, 1983; Pettersson *et al.*, 1984; Brunel, Sri-Widada & Jeanteur, 1985). Immunoblotting and immunoprecipitation methods allow us to respectively detect the specific polypeptides and snRNPs with which the sera are reacting (Habets *et al.*, 1983; Williamson, Pennebaker & Boyle, 1983; Pettersson *et al.*, 1986; Williams *et al.*, 1986). Anti-RNP antibodies immunoprecipitate snRNPs containing the U1 snRNA while anti-Sm antibodies recognize snRNPs containing U1, U2, U5 and U4–U6 snRNAs. By immunoblotting, anti-Sm antibodies react with B-B′ (28–29 kD), and D (16 kD) polypeptides and anti-RNP sera react with 68 kD, A (33 kD) and C (22 kD) peptides. Recent studies have shown that anti-

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RNP sera can also react with the B-B′ doublet (Habets *et al.*, 1985; Van Venrooij & Habets, 1986; Pettersson *et al.*, 1986; Combe *et al.*, 1987).

The purpose of this study was to analyse the clinical significance of anti-RNP and anti-Sm when detected by immunoblotting and immunoprecipitation techniques in the sera of 168 patients with various connective tissue diseases.

MATERIALS AND METHODS

Patients

Sera from 168 patients with ANA (titre greater than 1 : 64) and a positive reaction by ID were selected. Most patients had a flare-up of their disease and were seen in the department of Immuno-Rheumatology of Saint-Eloi Hospital in Montpellier. Some were seen in other departments in Montpellier (see acknowledgments). Patients with SLE, rheumatoid arthritis (RA) and Sjögren syndrome (SS) satisfied the American Rheumatism Association criteria for these diseases (Ropes, Bennet & Cobb, 1958; Tan *et al.*, 1982; Fox *et al.*, 1986). Patients with polymyositis (PM) were diagnosed according to Bohan *et al.* (1977). MCTD patients had typical clinical features (Raynaud's phenomenon, polyarthralgia or polyarthritis, swollen hands, often myositis and eventually systemic features) and high titres of anti-RNP antibodies, confirmed by ID (Sharp *et al.*, 1972; Alarcon-Segovia, 1976). The disease of some patients could not be classified into one of these categories and was therefore referred to as undifferentiated connective tissue syndrome (UCTS). Sera obtained from 40 normal healthy blood donors were used as controls.

Serologic evaluation

The routine techniques used for the detection of ANA were the same for all patients and included determination of ANA in serial serum dilutions by indirect immunofluorescence on rat liver section (Harmard, Cannat & Seligmann, 1964), detection of anti-DNA antibodies by Farr's assay (Holian *et al.*, 1975) and identification of antibodies to extractable nuclear antigen (ENA) by ID using the Ouchterlony's technique (Rucheton *et al.*, 1985).

Cell growth, labelling and hn-RNP preparation

HeLa cells (clone S3) were grown in spinner culture to a density of approximately 5×10^5 cells/ml. Cellular extracts (HnRNP) were prepared according to Brunel & Lelay (1979). For immunoprecipitation technique, HeLa cells were labelled for 36 h with 5 μCi/ml of $H_3{}^{32}PO_4$ in the presence of 0·05 mM phosphate.

Immunoprecipitation technique

See Sri-Widada *et al.* (1986) but, briefly, 5 μl of patients' sera were added to 1 mg of protein A-sepharose CL-4B (Pharmacia) preswollen in buffer A (50 mM Tris-HCl pH 8·0, 100 mM NaCl, 0·1%, NP 40) and were allowed to react for 2 h at 4°C with gentle stirring. After five washes in buffer B (10 mM Tris-HCl pH 7·4, 100 mM NaCl, 1·5 mM $MgCl_2$), 50 μl of ^{32}P-labelled cellular extract corresponding to about 10×10^6 cells were added to 50 μl of antibody-bound protein-A-sepharose and incubated with gentle stirring for at least 4 h at 4°C. After three washes in buffer C (10 mM Tris-HCl pH 7·2, 500 mM LiCl, 0·5% NP 40) bound RNAs were extracted by the proteinase K/phenol method and separated by electrophoresis on a 12·5% polyacrylamide gel in Tris-borate-EDTA buffer containing 8 M urea which was then autoradiographed.

Immunoblotting

The immunoblotting method has already been described in detail (Rucheton *et al.*, 1985). Nuclear extracts from Hela S3 cells were used as antigen source and subjected to 17·5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli & Favre (1973). Proteins were then electro-transferred onto nitrocellulose sheets (BA 83, Schleicher and Schull). Then, after saturation, strips were cut out and incubated (4 h, 20°C) with diluted patients' sera (1 : 5, 1 : 50 and 1 : 500 in PBS). After four washes strips were incubated with ^{125}I-radiolabelled protein A (10–20,000 ct/min/cm^2), washed six times and autoradiographed for 1 to 6 days.

RESULTS

By immunoblotting assay, we distinguished four different types of sera: 1) sera reacting with 68 kD, A, C and B-B′ proteins were considered to contain 'full spectrum' anti-RNP antibodies; 2) sera reacting with one or more but not all these proteins were called 'partially reactive' anti-RNP sera; 3) sera that showed reactivity with B-B′ and D peptides were considered to contain anti-Sm antibodies; 4) some sera contained both anti-Sm and 'full spectrum' anti-RNP antibodies. The presence of anti-RNP or anti-Sm was detected in 79 of the 168 sera tested. The other 89 sera reacted with none of the six proteins (68 kD, A, B-B′, C, D) and were not selected for this study. Except for the presence of an occasion weak reaction with one peptide, sera from the normal control group were negative in immunoblotting assay using a 1 : 5 dilution.

According to most of the authors, we considered that sera with anti-RNP antibodies immunoprecipitated U1 snRNA and that sera with anti-Sm antibodies immunoprecipitated U1, U2, U4, U5 and U6 snRNA. The diagnoses of the 79 patients were as follows: 46 had SLE, 22 had MCTD, three had UCTS, three had SS, two had RA, one had PM and two had autoimmune cirrhosis (AIC).

Immunodiffusion and RNA immunoprecipitation findings

Results are summarized in Tables 1, 2, 3, and 4. Among the 79 positive sera by immunoblotting, ID detected anti-RNP and anti-Sm antibodies in 48 and in 24 of them respectively. Anti-RNP were found in sera from 22 MCTD patients (45·8%), in sera from 20 SLE patients (41·6%) and in six sera with other diagnoses (12·5%). Anti-Sm were detected mainly in SLE patients (87%) and in one SS and in two UCTS patients.

The immunoprecipitates of 43 sera contained only U1 snRNP. Three sera reacted with both U1 and U2 snRNP and 16 immunoprecipitated U1–U6 snRNPs. Other antibodies reactive with YscRNAs (SSA, SSB), 5,8sRNAs and tRNAs were detected in 33 sera. Anti-(U1)RNP antibodies occurred in 21 SLE patients (48·8%), in 17 MCTD patients (39·5%) and also in sera of five other patients (two SS, one UCTS, one PM, one RA). The presence of antibodies against U1 and U2 snRNA was detected in two MCTD patients and in one AIC. Thirteen SLE sera (81·3%), two MCTD and one UCTS contained anti-U1-U6 snRNA. Anti-SSA antibodies were found in six SLE and in two

Table 1. Diagnosis and serologic data on patients with 'full spectrum' anti-RNP without anti-Sm by immunoblotting

Patient	Diagnosis	ANA* IF titre	Anti-dsDNA† (units/ml)	Anti-ENA by ID‡	RNA immuno-precipitation pattern	Immunoblot pattern (titre)					
						68 kD	A	B-B′	C	D	other peptides
1	MCTD	1:256	–	RNP	U1	+++	+++	+++	+++	–	–
2	MCTD	1:1024	–	RNP	U1	+++	+++	+++	+++	–	–
3	MCTD	1:4100	–	RNP	U1+U2	+++	+++	+++	+++	–	–
4	MCTD	1:4100	156	RNP	U1	+++	+++	+++	+++	–	–
5	MCTD	1:16400	–	RNP	U1+SSA	++	++	++	++	–	+
6	MCTD	1:16400	69	RNP	U1	+++	++	++	++	–	–
7	MCTD	1:1024	–	RNP	−trna	+	+	+	+	–	–
8	MCTD	1:16400	–	RNP	U1+SSB	+++	+++	+++	++	–	+
9	MCTD	1:256	–	RNP	U1	++	++	++	++	–	+
10	MCTD	1:16400	102	RNP	U1	+++	+++	+++	+++	–	–
11	MCTD	1:4100	–	RNP	U1	+++	++	++	++	–	+
12	MCTD	1:256	–	RNP	U1	+++	+++	++	+++	–	+
13	MCTD	1:4100	–	RNP	U1+SSA	+++	+++	+++	+++	–	+
14	MCTD	1:4100	–	RNP	U1	+++	+++	+++	+++	–	–
15	MCTD	1:1024	–	RNP	U1	+	+	+	+	–	–
16	MCTD	1:256	–	RNP	U1+SSB+5·8s	++	++	++	++	–	–
17	MCTD	1:1024	–	RNP	U1	+++	+++	++	++	–	–
18	MCTD	1:256	72	RNP	U1	+++	++	++	++	–	–
19	MCTD	1:16400	80	RNP	U1+SSB	+++	++	++	+++	–	–
20	SLE	1:256	195	RNP	U1	+	+	+	+	–	–
21	SLE	1:64	73	Sm	–	+	+	+	+	–	–
22	SLE	1:16400	136	RNP	–	+	+	+	+	–	+
23	SLE	1:4100	366	RNP	U1+SSB	+	+	+	+	–	–
24	SLE	1:16400	800	RNP	U1+SSB	++	++	++	++	–	–

* Immunofluorescence (IF) titre of antinuclear antibodies (ANA).
† Positive values are 35 units/ml by Farr's assay.
‡ Antibodies to extractable nuclear antigen (ENA) detected by immunodiffusion (ID).
+++ when titre ⩾1:500; ++ when titre ⩾1:50; + when titre ⩾ 1:5.
MCTD mixed connective tissue disease, SLE systemic lupus erythematosus.

Table 2. Diagnosis and serologic data on patients with anti-Sm and 'full spectrum' anti-RNP by immunoblotting

Patient	Diagnosis	ANA IF titre	Anti-dsDNA (units/ml)	Anti-ENA by ID	RNA immuno-precipitation pattern	Immunoblot pattern (titre)					
						68 kD	A	B-B′	C	D	other peptides
25	MCTD	1:1024	–	RNP	U1+U2	+++	+++	+++	+++	+	–
26	MCTD	1:16400	59	RNP	U1-U6	++	++	++	++	+	–
27	SLE	1:4100	–	RNP	U1	+++	+++	++	+++	++	–
28	SLE	1:1024	55	Sm	U1-U6+SSA	+++	+++	+++	+++	+++	+
29	SLE	1:256	144	Sm	U1	+	+	++	+	++	–
30	SLE	1:64	720	Sm	U1-U6	++	++	+++	++	+++	–
31	SLE	1:4100	330	RNP	U1+SSA	++	++	+++	++	+++	+
32	SLE	1:4100	880	Sm	U1-U6	+++	++	+++	+++	+++	+
33	SLE	1:1024	950	Sm	U1+SSB	+	+	+++	+	+++	+
34	SLE	1:256	164	Sm	U1-U6	+	+	+++	+	+++	–
35	SLE	1:16400	–	RNP+Sm	U1-U6	+++	+++	+++	+++	+++	–
36	SLE	1:16400	900	RNP+Sm	U1	+++	++	++	++	+	–
37	SLE	1:4100	1000	RNP	U1	+	+	+	+	+	–
38	UCTS	1:4100	41	Sm	U1-U6+SSA	+++	+++	+++	+++	+	+
39	PM	1:64	–	RNP	U1	+	+	+	+	+	–
40	SS	1:1024	–	Sm	U1+SSB	++	+	++	+	++	+

UCTS undifferentiated connective tissue syndrome, PM polymyositis, SS Sjögren syndrome.

Table 3. Diagnosis and serologic data on patients with anti-Sm without 'full spectrum' anti-RNP by immunoblotting

Patient	Diagnosis	ANA IF titre	Anti-dsDNA (units/ml)	Anti-ENA by ID	RNA immuno-precipitation pattern	Immunoblot pattern (titre)					
						68 kD	A	B-B′	C	D	other peptides
41	SLE	1:256	9000	Sm	U1	−	++	++	−	++	++
42	SLE	1:256	66	RNP	U1	−	+	++	+	++	−
43	SLE	1:64	1160	Sm	U1-U6	+	+	+++	−	+++	++
44	SLE	1:1024	84	SNP	5·8s	−	−	+	−	+	++
45	SLE	1:64	2480	Sm	U1-U6+SSA	−	−	+++	−	+++	++
46	SLE	1:256	1220	RNP	5·8s	−	−	++	−	++	−
47	SLE	1:64	8400	Sm	5·8s	−	+	++	−	++	−
48	SLE	1:4100	156	SNP	U1+5·8s	−	++	+++	+	+++	+
49	SLE	1:1024	−	RNP	U1	+	−	+	−	+	+
50	SLE	1:256	62	RNP	SSA	+	+	+	−	+	−
51	SLE	1:16400	12400	Sm	U1-U6	−	−	+++	−	+++	++
52	SLE	1:16400	110	Sm	U1-U6	+	+	+++	−	+++	−
53	SLE	1:1026	125	RNP	U1	−	+	+	−	+	+
54	SLE	1:1084	13000	Sm	5·8s	−	−	+++	−	+++	++
55	SLE	1:1024	81	Sm	U1-U6+SSA	−	−	+++	−	+++	−
56	SLE	1:1024	41	Sm	U1-U6+SSA	+	+	+++	−	+++	−
57	SLE	1:256	216	SNP	U1	+	−	+++	−	+++	++
58	SLE	1:256	100	SNP	U1+SSB+5·8s	−	−	++	−	++	+++
59	SLE	1:1024	480	RNP	U1-U6+SSB	−	−	+++	−	+++	−
60	SLE	1:1024	8600	Sm	SSB+5·8s	−	−	+	−	+	−
61	SLE	1:256	3560	SNP	U1+SSB	−	−	++	−	++	+++
62	SLE	1:1024	1216	Sm	SSB	−	−	+	−	+	+
63	SLE	1:64	63	SNP	SSB	−	−	+	−	+	−
64	SLE	1:4100	4560	Sm	U1-U6	−	−	+++	−	+++	++
65	SS	1:4100	−	SNP	U1+SSB	−	−	+++	−	+++	+++
66	UCTS	1:256	−	RNP+Sm	SSB	−	−	+++	−	+++	++

SNP soluble nuclear protein (resistant to RNAse and sensitive to trypsin).

Table 4. Diagnosis and serologic data on patients with 'partially reactive' anti-RNP by immunoblotting

Patient	Diagnosis	ANA IF titre	Anti-dsDNA (units/ml)	Anti-ENA by ID	RNA immuno-precipitation pattern	Immunoblot pattern (titre)					
						68 kD	A	B-B′	C	D	other peptides
67	UCTS	1:64	−	RNP	U1	−	−	++	−	−	+
68	RA	1:64	100	RNP	U1	+	+	−	−	−	−
69	RA	1:256	−	SNP	5·8s	−	+++	−	−	−	+++
70	MCTD	1:16400	−	RNP	U1-U6	+	+	+	−	−	+
71	SLE	1:1024	−	RNP	U1	−	−	+	−	−	+++
72	SLE	1:256	200	RNP	−	−	−	+++	−	−	+++
73	SLE	1:1024	400	RNP	U1+SSB	−	+	+	+	−	+
74	SLE	1:256	62	RNP	−	+	−	+	−	−	−
75	SLE	1:256	320	RNP	U1	+++	+++	−	+++	−	−
76	SLE	1:256	248	SNP	U1	−	+	−	+	−	+
77	SS	1:4100	−	SNP	SSB	−	+	+	−	−	+
78	AIC	1:256	−	RNP	U1+U2	−	+	−	+	−	+++
79	AIC	1:1024	−	RNP	−	−	++	++	++	−	−

RA rheumatoid arthritis, AIC autoimmune cirrhosis

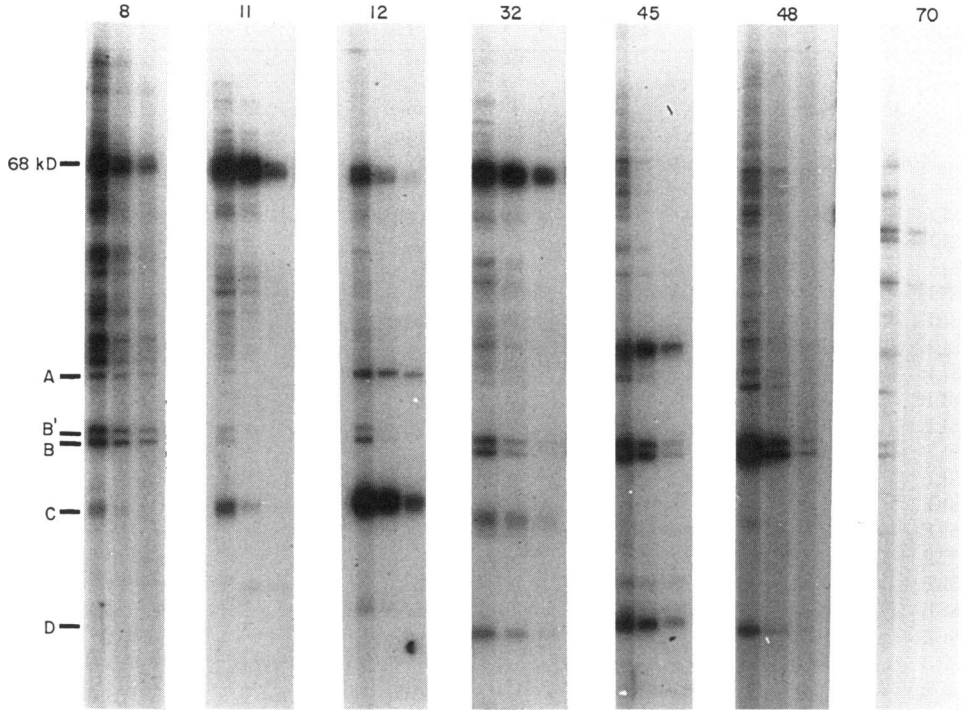

Fig. 1. Immunoblot analysis of sera with 'full spectrum' anti-RNP antibodies (8, 11, 12, 32), anti-Sm antibodies (32, 45, 48) and 'partially reactive' anti-RNP antibodies (70). Hela S3 cells were electrophoresed on 17·5% sodium dodecyl sulphate-polyacrylamide gels and transferred onto nitrocellulose. Each blot was incubated with three dilutions (1 : 5, 1 : 50, 1 : 500) of patient's sera.

MCTD patients and anti-SSB were present in ten SLE patients (58·8%), in three MCTD, in one UCTS and in the three SS patients studied.

Clinical significance of 'full spectrum' anti-RNP antibodies as determined by immunoblotting

Results of immunoblot analysis are shown in Fig. 1. Forty sera contained 'full spectrum' anti-RNP antibodies (Tables 1 and 2). The presence of these antibodies without anti-Sm antibodies seemed highly characteristic of MCTD (Table 1). A high titre ($\geqslant 1:50$) of antibodies reacting with the five peptides (68 kD, A, C, B-B′) without anti-Sm activity was present in 18 sera, 17 from MCTD patients (94·5%) and one from an SLE patient (a titre higher than 1 : 500 was only detected in MCTD patients). A weak reaction with other peptides was rarely associated.

Clinical significance of 'partially reactive' anti-RNP antibodies as determined by immunoblotting

Various combinations of reactions with the 68 kD, A, C or B-B′ peptides (without the D peptide) were found in 13 patients: six with SLE, one with MCTD, two with RA, one with UCTS, one with SS and two with A1C. Several of these sera reacted with B-B′ doublet but never with D-peptide (Table 4).

Clinical significance of anti-Sm antibodies as determined by immunoblotting

Forty-two patients' sera contained antibodies, reacting with the B-B′ doublet and D peptide (Tables 2 and 3 and Fig. 1). Thirty-five of the 46 SLE patients (76%) had anti-Sm antibodies in their sera. These antibodies occurred mainly in SLE (83·3%), and also in two patients with MCTD, two with UCTS, two with SS, and one with PM. In addition, anti-Sm antibodies seemed more characteristic of SLE when they occurred without 'full spectrum' anti-RNP antibodies (Table 3). Sera with anti-Sm antibodies reacted usually with other peptides (characterized or not) and only 5 (12%) anti-Sm sera were pure.

Table 5. Diagnosis in 50 patients whose sera contained anti-68 kD antibodies

Diagnosis	Number of patients	Anti-68 kD activity (titre)		
		$\geqslant 1:500$	$\geqslant 1:50$	$\geqslant 1:5$
MCTD	22	15	4	3
SLE	46	6	3	15
UCTS	3	1	–	–
SS	3	–	1	–
RA	2	–	–	1
PM	1	–	–	1

Clinical significance of the recognition of a 68 kD polypeptide

Fifty sera reacted with the 68 kD protein (Table 5). All MCTD sera but also 24 SLE (52%) sera recognized the 68 kD protein. However, a high level ($\geqslant 1:500$) of anti-68 kD activity was mainly found in MCTD sera (68·2%) and was almost always (21 of 22) contained by sera with 'full spectrum' anti-RNP activity. Five of six SLE sera that contained a high level of anti-68 kD, also had an anti-Sm activity.

DISCUSSION

The purpose of this study was to determine the diagnostic specificity of the presence of anti-RNP and anti-Sm antibodies,

as detected by immunoprecipitation and immunoblotting in patients with connective tissue diseases. We have screened a chart of 168 patients with ANA and anti-ENA as determined by Ouchterlony's method. From this group, we selected 79 patients with antibodies to 68 kD, A, B-B′, C or D polypeptides by immunoblotting assay. Immunoprecipitation of snRNP was also performed. The principal findings of this study are that i) similar clinical correlations were obtained using ID and immunoprecipitation; ii) according to immunoblotting peptide reactivity, four different types of sera could be distinguished: sera with 'full spectrum' anti-RNP antibodies, sera with 'partially reactive' anti-RNP antibodies, sera with anti-Sm antibodies and sera with both anti-Sm and 'full spectrum' anti-RNP antibodies; iii) sera containing a high titre of 'full spectrum' anti-RNP antibodies without anti-Sm were only found in MCTD patients; iv) the 68 kD peptide was mainly detected in MCTD sera but also in 52% of SLE sera; v) anti-Sm antibodies were more frequently detected in SLE sera using immunoblotting technique than other methods and were found to be highly specific for SLE.

Anti-RNP antibodies detected by immunoprecipitation and ID were found to be associated with MCTD (39·5% and 45·8%) and SLE (48·8% and 41·6%) and less frequently with other rheumatic diseases, and were not disease-specific. The frequency of occurrence of anti-RNP is in agreement with previous studies (Sharp *et al.*, 1976; Scopelitis, Biundo & Alspaugh, 1980; Tan, 1982; Williamson, Pennebaker & Boyle, 1983). The presence of antibodies that immunoprecipited U1 and U2 snRNP was detected in 2 MCTD and one A1C. A similar pattern has already been reported in MCTD (Habets *et al.*, 1985; Pettersson *et al.*, 1986) in psoriasis and Raynaud's phenomenon (Reeves *et al.*, 1986) in scleroderma-polymyositis overlap syndrome (Mimori *et al.*, 1984) and does not seem to be of diagnostic value.

The immunoblotting results are consistent with those obtained previously. However, we distinguished sera designated as 'full spectrum' anti-RNP, reacting with the 68 kD, A, C and B-B′ peptides and sera designated as 'partially reactive' anti-RNP, reacting with various combinations of these peptides. 'Full spectrum' anti-RNP antibodies were detected in 21 of 22 MCTD sera. A high titre ($\geqslant 1:50$) of these antibodies without anti-Sm activity was detected in one SLE and 17 MCTD (94·5%) patients, and a titre higher than 1 : 500 was only found in MCTD. Then, we suggested that the presence of antibodies that react with 68 kD, A, C and B-B′ polypeptides could be considered as a highly specific serologic marker for MCTD. Anti-dsDNA was present at a low level in six patients and a weak anti-Sm activity was only detected in two MCTD patients (Table 2). These data, as well as a distinct genetic background that has been suggested (Sharp, 1982), supported the hypothesis that MCTD is a distinct clinical entity associated with a specific serologic marker.

Several authors have suggested that antibodies to the 68 kD peptide might be a more specific marker for MCTD than other anti-RNP antibodies (Kinlaw, Robberson & Berget, 1983; Habets *et al.*, 1985; Petersson *et al.*, 1986). Pettersson *et al.* (1986) found anti-68 kD antibodies in 26 of 34 patients with MCTD and in one of 13 patients with SLE using sera diluted 1 : 250 and suggested that these antibodies could be regarded as a highly specific serologic marker for MCTD. Habets *et al.* (1983) detected anti-68 kD antibodies in 95% of MCTD sera but also in 21% of anti-RNP positive SLE sera (sera diluted, 1 : 50). Using a quantitative immunoblotting they have also shown that the 68 kD antigen seemed to be a triplet of bands recognized by MCTD sera as well as by the monoclonal anti 68–70 kD antibody and they mainly found high levels of anti-68 kD in MCTD (Habets *et al.*, 1985). Our results are consistent with these data and we detected anti-68 kD antibodies in all MCTD sera that we have tested. We also mainly found a high level of these antibodies in MCTD sera (68·2%). However, we detected anti-68 kD in 24 of 46 SLE patients and in several cases at a high level. These discrepancies could be explained by the fact that we have analysed a larger number of SLE patients than other investigators. According to our data 'full spectrum' anti-RNP antibodies seem to be a more specific marker for MCTD than anti-68 kD antibodies.

Anti-Sm immunoblot reactivity was directed against the B-B′ and D peptides and in most cases was associated with anti-RNP or undetermined peptides pattern according to earlier reports (Tan, 1982; Reeves *et al.*, 1985; Pettersson *et al.*, 1986). Anti-Sm antibodies detected by immunoblotting, immunoprecipitation or ID were strongly associated with the diagnosis of SLE (81·3–87%) and this specificity is in agreement with previous studies (Notman, Kurata & Tan, 1975; Sharp *et al.*, 1976; Munves & Schur, 1983; Williamson, Pennebaker & Boyle, 1983). Anti-Sm antibodies are usually detected in 20 to 40% of patients with SLE (Tan, 1982; Munves & Schur, 1983; Scopelitis, Biundo & Alspaugh, 1980; Barada *et al.*, 1981). We found a similar result with immunoprecipitation and ID but immunoblotting showed an anti-Sm activity in 76% of SLE sera. These data suggested that immunoblotting assay is extremely sensitive and might be a great help in the management of connective tissue diseases.

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