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The SL autoantibody-antigen system: clinical and biochemical studies*

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SUMMARY A recently described autoantibody, SL, was found in serum from 27 patients with autoimmune disease, including 20 with systemic lupus erythematosus (SLE) where the frequency was 7%. Analysis of clinical, serological, and HLA data from 119 SLE patients showed no positive associations with anti-SL antibody apart from a higher frequency of non-infective fever. Most SL positive sera contained other precipitins, notably antibodies to Ro(SS-A) and the proliferating cell nuclear antigen, PCNA. Anti-SL IgG recognised a protein of 32 000 daltons without associated RNA. This polypeptide was distinguished from a similarly sized component of the Sm and RNP ribonucleoprotein particles by demonstrating different products of partial proteolysis. Although anti-SL antibody is of limited clinical importance, it occurs with twice the frequency of anti-Sm antibody in white patients with SLE. Preliminary studies indicate that SL and the Japanese Ki system are identical.

Key words: antinuclear antibody, autoimmunity, connective tissue disease, systemic lupus erythematosus, Sjögren's syndrome, thyroid disease, 32 kilodalton cellular protein, Sm, RNP, Ki.

Antibodies directed against saline soluble cellular antigens are found in the sera of patients with many forms of systemic autoimmune disease.[1] Although some are of diagnostic and prognostic value, most antibodies are not totally disease specific.[2] For example, antibody to the anti-Ro(SS-A) antigen occurs in a number of connective tissue disorders, though most frequently in primary sicca syndrome and systemic lupus erythematosus (SLE). Harmon *et al* have described their preliminary clinical experience with a subset of patients identified by a new precipitating antigen/antibody system designated SL (sicca lupus).[3]

In a survey of over 1000 patients with autoimmune disease, including 300 with SLE, we noted a precipitin line that was named PL-2 in our laboratory. Through exchange of serum samples PL-2 and SL were shown to be identical.[1] This report concerns a clinical study of patients with anti-SL antibody and a preliminary biochemical characterisation of the SL antigen.

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Patients and methods

Clinical and serological data on 20 patients with anti-SL antibody were reviewed. Serological data and the referring physician's diagnosis were available in a further seven cases seen elsewhere. The findings in the anti-SL positive patients who had SLE[4] were compared with data collected at the same time by chart review of 107 SLE patients who were anti-SL negative. Clinical and laboratory features were defined according to the 1982 revised criteria for the classification of SLE[4] or according to accepted clinical practice (e.g., splenomegaly means a palpable spleen). Despite varying lengths of follow up, clinical features noted at any time were recorded as positive whether or not they recurred or persisted.

Statistical significance was assessed by the χ^2 test. Because so many tests were carried out two approaches were taken to avoid attributing undue significance to high χ^2 results arising by chance.[5] Firstly, in the usual approach p values were multiplied by 36 (as 36 tests were carried out). Secondly, and less conservatively, square roots of χ^2 results were ordered and plotted against half-normal scores to establish visually whether high results had deviated from the normal distribution (Fig. 1).[6]

Antibodies to soluble cellular antigens were detected by counterimmunoelectrophoresis (CIE) using extracts of rabbit thymus and human spleen,

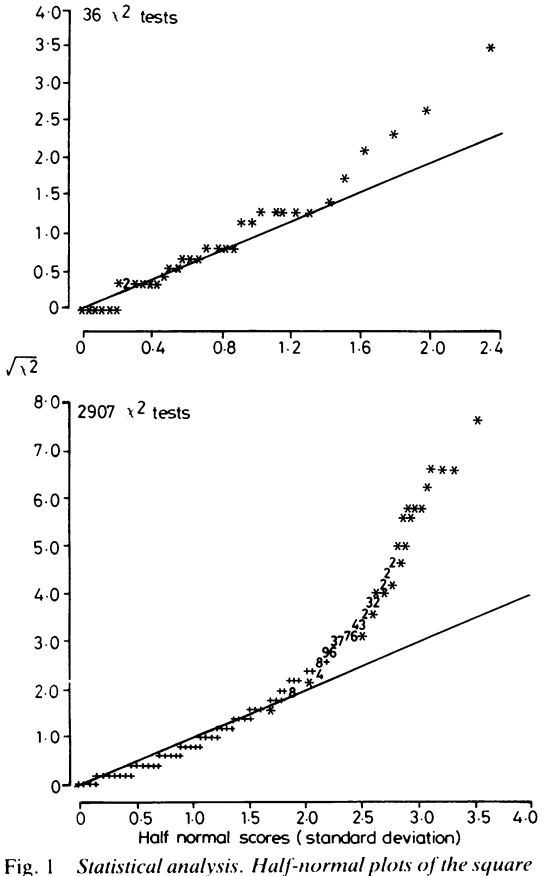

Fig. 1 *Statistical analysis. Half-normal plots of the square roots of χ^2 values obtained in the present study (upper panel) and in a more extensive intercomparison of 88 variables in the same group of SLE patients (lower panel).*[7] *$\sqrt{\chi^2}$ follows the normal distribution (straight line with slope of 1·0) until higher values emerge. The diverging points indicate χ^2 test results that are less likely to have arisen by chance through multiple testing. Symbols used: *=one datum point; 2–9=two to nine points in proximity; +=10 or more datum points in proximity.*

and precipitin systems were identified by comparison with reference sera in adjacent wells.[1 8] Antinuclear antibodies were detected by indirect immunofluorescence on rat liver frozen sections and in some cases on the HEp_2 cell substrate as well.[9] Rheumatoid factor was measured by the slide latex test (Ortho Diagnostics, High Wycombe, UK). HLA-A, B, and DR typing of 87 SLE patients, including six with anti-SL antibody, was performed with a standard microcytotoxicity assay.[10]

For immunoprecipitations mediated by *Staphylococcus aureus* protein A, IgG preparations and HeLa cell extracts labelled with [^{35}S]methionine, [^{32}P]phosphate, or [^{3}H]leucine were employed as described in detail elsewhere.[11 12] Immunoprecipitates were analysed by gel electrophoresis[13] and autoradiography or photofluorography. Immunoblotting with HeLa cell extracts was carried out according to Towbin *et al.*[14]

For partial proteolysis immunoprecipitated protein was eluted from the dried gel after photofluorography by boiling in a solution containing 0·5% sodium dodecyl sulphate (SDS), 10 mM 2-mercaptoethanol, and 10 mM trometamol hydrochloride (TRIS-HCl) pH 7·4. Aliquots of the proteins to be compared, containing approximately equal radioactivity, were incubated with varying quantities (0–100 ng) of protease from *S aureus* strain V8.[15] After digestion for 15 min at 37°C the products were resolved by electrophoresis in 20% SDS-polyacrylamide gels.[13]

Results

CLINICAL AND SEROLOGICAL CORRELATION

Over a three year period anti-SL antibody was identified in the serum of 27 patients. Twenty four were female and the age range was 13–70 years (mean 31). Table 1 shows the diagnoses made and the frequency of anti-SL antibody in patients with these conditions. The highest prevalence was in SLE

Table 1 *Frequency of anti-SL antibody according to diagnosis*

Diagnosis	*Frequency*	*%*
Systemic lupus erythematosus	20/300	6·7
Mixed connective tissue disease	1*/50	2·0
Rheumatoid arthritis	2†/70	2·9
Primary sicca syndrome	2/60	3·3
Primary biliary cirrhosis	1/135	0·7
Idiopathic thrombocytopenic purpura	1‡/110	0·9

*This patient had arthritis, malaise, severe Raynaud's phenomenon, and anti-RNP antibody with normal DNA binding.[15]
†Includes one out of eight patients with rheumatoid vasculitis (skin ulceration or neuropathy, or both).
‡This patient also had thyrotoxicosis.

Table 2 *Clinical, haematological, and serological features*

	Overall anti-SL positive (%)	*SLE patients*		
		Anti-SL positive (%)	*Anti-SL negative (white patients) (%)*	χ^2
Rash	60 (12/20)	73 (11/15)	79 (84/106)	0·3
Photosensitivity	37 (7/19)	36 (5/14)	54 (52/96)	1·7
Alopecia	45 (9/20)	53 (8/15)	70 (73/105)	1·6
Mouth ulcers	28 (5/18)	23 (3/13)	29 (29/100)	0.2
Raynaud's phenomenon	37 (7/19)	36 (5/14)	52 (54/103)	1·4
Digital ischaemia vasculitis	37 (7/19)	36 (5/14)	34 (36/107)	0·0
Arthritis	65 (13/20)	73 (11/15)	64 (69/107)	0·5
Myositis	0 (0/20)	0 (0/15)	11 (12/105)	1·9
Pleurisy	40 (8/20)	47 (7/15)	51 (54/105)	0·1
Pericarditis	25 (5/20)	33 (5/15)	29 (30/103)	0·1
Abnormal pulmonary function	75 (6/8)	75 (6/8)	60 (48/80)	0·7
Renal (proteinuria casts)	30 (6/20)	40 (6/15)	52 (56/107)	0·8
Hypertension (≥140/90)	10 (2/20)	13 (2/15)	42 (45/106)	4·7*
Fever (non-infective)	75 (15/20)	87 (13/15)	37 (40/107)	13·0*
Sicca syndrome	35 (7/20)	27 (4/15)	31 (27/86)	0·1
Psychosis	0 (0/20)	0 (0/15)	24 (24/101)	0·5
Seizures	5 (1/20)	7 (1/15)	15 (16/105)	0·8
Thrombosis (venous or arterial)	25 (5/20)	27 (4/15)	24 (24/101)	0·1
Stroke	10 (2/20)	13 (2/15)	13 (14/105)	0·0
Abortion	5 (1/19)	7 (1/15)	21 (15/72)	1·7
Splenomegaly	15 (3/20)	20 (3/15)	14 (15/104)	0·3
Lymphadenopathy	25 (5/20)	27 (4/15)	24 (25/104)	0·1
Raised IgG (>15 g/l)	24 (4/17)	21 (3/14)	60 (58/97)	7.3*
Low C3 (<60% normal pool)	39 (7/18)	47 (7/15)	49 (49/100)	0·0
Anaemia (<10 g/dl)	50 (10/20)	60 (9/15)	59 (63/107)	0·0
Leucopenia ($<4\times10^9$/l)	35 (7/20)	47 (7/15)	48 (50/105)	0·0
Thrombocytopenia ($<100\times10^9$/l)	40 (8/20)	47 (7/15)	36 (38/107)	0·7
Steroid therapy	65 (13/20)	80 (12/15)	93 (100/107)	3·2
Cytotoxic therapy	40 (8/20)	40 (6/15)	60 (64/106)	2·2
Antimalarial therapy	55 (11/20)	60 (9/15)	64 (68/106)	0·1
Antinuclear antibodies	100 (27/27)	100 (20/20)	91 (91/100)	1·9
Rheumatoid factor	37 (7/19)	25 (3/12)	23 (24/106)	0·0
Anti-Ro(SS-A)	48 (13/27)	45 (9/20)	31 (33/106)	1·5
Anti-RNP	11 (3/27)	15 (3/20)	22 (23/106)	0·5
Anti-Sm	0 (0/27)	0 (0/20)	4 (4/106)	0·1
Anti-PCNA	11 (3/27)	15 (3/20)	3 (3/106)	5·5*

*Possible associations with anti-SL antibody in SLE: fever—p=0·0003, p corrected (p_c)=0·011, relative risk 10·9; absence of hypertension—p=0·03, p_c=1·1; absence of raised IgG—p=0·007, p_c=0·25; anti-PCNA antibody—p=0·02, p_c=0·7.

(6·7%). In the other conditions the frequencies are rough estimates at best. Table 2 summarises the clinical and haematological data available in 20 cases (15 with SLE and one each with mixed connective tissue disease, primary sicca syndrome, rheumatoid synovitis, rheumatoid vasculitis, and idiopathic thrombocytopenic purpura with thyrotoxicosis), together with serological data on all cases. No clinical feature was particularly associated with anti-SL antibody in the SLE patients except for the more frequent record of fever (above 38°C, 100·4°F) in the absence of overt infection. Sicca syndrome, assessed by Schirmer's test or lip biopsy, was not increased in patients with SL antibody; hypertension and hypergammaglobulinaemia were less common. Two of six SL positive SLE patients carried HLA-DR3, not dissimilar to the 48% frequency in a further 81 white SLE patients who were anti-SL negative.

Most (80%) of the anti-SL positive sera contained other precipitating antibodies, notably those to Ro and PCNA. Antinuclear antibody was detected by indirect immunofluorescence in every case. Where the pattern was recorded it was speckled in 14 and homogeneous in five, emphasising the polyspecificity of the sera tested; cytoplasmic and nucleolar staining was uncommon.

SL ANTIGEN

In all 16 cases tested by protein A facilitated

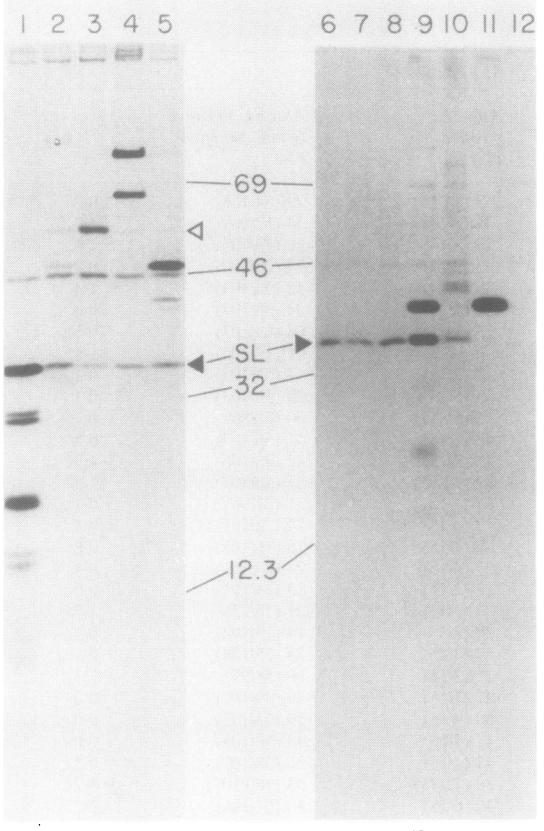

Fig. 2 *Immunoprecipitation of SL antigen. ^{35}S Labelled proteins precipitated by autoantibodies were resolved by 15% SDS-polyacrylamide gel electrophoresis and photofluorography: (1) RNP; (2) Ro, La, SL; (3) Ro, SL; (4) PL-9 (Ku), Ro, SL; (5) Ro, La (with breakdown product), SL; (6–8) SL; (9) PCNA, SL, RNP; (10) SL, other; (11) PCNA; (12) control. The positions of molecular weight markers (kilodaltons) are indicated; the carbonic anhydrase marker (32 kilodaltons) falls below the calibration curve from which the molecular weight of the SL polypeptide was measured. An open arrowhead indicates the Ro antigen. (Actin is precipitated non-specifically, near the 46 kilodalton marker.)*

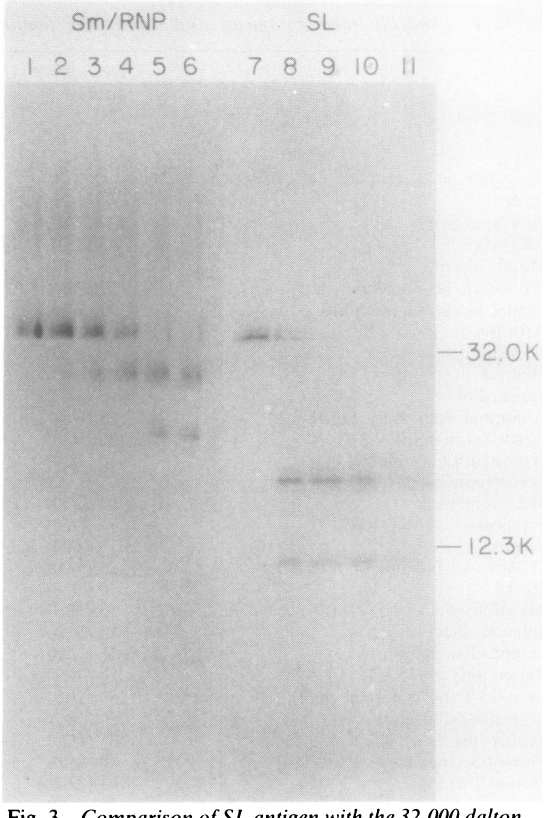

Fig. 3 *Comparison of SL antigen with the 32 000 dalton component of the Sm and RNP particles. ^{35}S Labelled peptides released by partial proteolysis are shown on a 20% SDS-polyacrylamide gel. The Sm/RNP polypeptides (lanes 1–6) and the SL protein (lanes 7–11) were digested by amounts of* S aureus *V8 protease as follows: none (lanes 1, 7), 0·3 ng (lane 2), 1 ng (lanes 3,8), 3 ng (lanes 4,9), 10 ng (lanes 5,10), 100 ng (lanes 6, 11).*

immunoprecipitation anti-SL IgG recognised a [^{35}S]-methionine labelled polypeptide of 32 000 daltons (Fig. 2), that was also detected by Western immunoblotting. There was no RNA associated with this protein, and the antigen was not phosphate labelled (data not shown). As expected from the serological studies several IgG samples also precipitated the 55 000 dalton Ro antigen,[1 16] 46 000 dalton La,[1 11] or 35 000 dalton PCNA.[1 17]

As seen in Fig. 1 the SL protein has a mobility similar to that of a component of the Sm and RNP antigens.[1] To discover whether or not these polypeptides were identical they were eluted from the gel and subjected to partial proteolysis. As shown in Fig. 3 the peptides released from the SL antigen are clearly different from those derived from the Sm/RNP component, and we conclude that the two proteins are distinct.

Discussion

Our studies show anti-SL to be a fairly uncommon autoantibody directed at a protein of 32 000 daltons that does not appear to be associated with RNA. The antibody was found chiefly in SLE, with a frequency of about 7%, and less often in primary sicca syndrome, mixed connective tissue disease,[18] primary biliary cirrhosis, rheumatoid arthritis, and rheumatoid vasculitis, as well as in a case of

idiopathic thrombocytopenic purpura with thyrotoxicosis.

This experience parallels that of Harmon and her colleagues, who identified anti-SL antibody in a similar spectrum of disease, including SLE, sicca syndrome, autoimmune thyroid disease, and a case of rheumatoid lung disease.[3] On the other hand, we could not confirm a trend to increasing cutaneous, pulmonary, or haematological abnormalities, and, despite the name 'sicca lupus' (SL), features of the sicca syndrome were found in only 35% of cases.

Most anti-SL sera contained other precipitins, particularly antibodies to Ro and PCNA. The well known associations of anti-La with anti-Ro and of anti-Sm with anti-RNP are mirrored by relations between the antigens,[1] but as yet there is no sign of a connection between SL, PCNA, and Ro. We did note a similarity in size between SL and a component of the Sm and RNP complexes, and showed by peptide comparison that these proteins differ; this excludes the possibility that the SL antigen is a free component or precursor of a (U)RNP particle.

The results of immunoblotting and immunoprecipitation are complementary. The blotting study shows that anti-SL binds directly to the 32 000 dalton protein, while the immunoprecipitations show that this protein is not part of a ribonucleoprotein or protein-protein complex (at least, not one that is soluble and stable). Further immunoprecipitations with cell extracts labelled with [^{32}P]-phosphate and [^{3}H]leucine confirmed that no other proteins are immunoprecipitated. This is an important control because some proteins such as the 68K RNP antigenic polypeptide detected by immunoblotting are not labelled with methionine.[1]

Anti-Sm antibody was an infrequent finding in our patients with SLE. At one time there was a practice of equating all ribonuclease resistant precipitin lines with Sm, but now several distinct precipitin systems involving protein antigens can be distinguished;[1] we identify these antibodies by comparison with reference sera. Another factor influencing the frequency of anti-Sm may be the different racial mix in British and North American clinics.[19] We have observed that anti-Sm antibody is up to 10 times more common in black and Chinese than in white SLE patients;[1] almost all the patients in the present study were white. Anti-SL antibody seems to show no such racial preponderance: one of our patients was Chinese, one black, two Indian, two Greek, and the remaining 21 were English—much in line with our overall patient population. The number of precipitin systems in SLE is quite limited.[1] SL antibody is identical with the Ki system reported in Japanese patients with SLE[20] and the antigens have the same molecular weight (T Mimori and RMB, unpublished data). A relation between SL and the Su system[21] also seems likely and is being explored. Further studies are required to elucidate the cellular function of SL antigen and to show how in some patients this protein becomes the target of an autoimmune response.

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