Clinical and autoantibody correlations in Orientals with systemic lupus erythematosus

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SUMMARY Serum samples from 94 patients with systemic lupus erythematosus (SLE) from a medical unit in Singapore were analysed for autoantibodies of 10 different specificities. The prevalence of antibodies to the following antigens was as follows: double stranded (ds) DNA (43%), histone (81%), Sm (26%), nuclear ribonuclear protein (nRNP) (32%), SS-A(Ro) (63%), SS-B(La) (12%), SL/Ki (9%), ribosomal RNP (rRNP) (16%), p70/p80 (5%), proliferating cell nuclear antigen (PCNA) (3%). Except for a higher prevalence of anti-SS-A(Ro), other autoantibodies were within the range reported from Western countries, indicating a high uniformity of autoantibody profiles in SLE in different countries. Patients with neuropsychiatric manifestations showed a higher plurality of antibodies per patient than patients without neuropsychiatric symptoms, 4.22 v 2.77. Patients with anti-Sm were more likely to have active lupus disease. There was no increased prevalence or specific type of autoantibody in those with renal manifestations.

Key words: antinuclear antibodies, central nervous system disease.

Systemic lupus erythematosus (SLE) is manifested by a polyclonal autoantibody response. In this disease antibodies to native DNA, histones, Sm, nuclear RNP, SS-A(Ro), and SS-B(La) are all present. Most patients with SLE have three or more autoantibodies in the serum. Certain antinuclear antibodies such as native DNA and Sm are highly specific for SLE and show good correlation with the disease. The titre of various antinuclear antibodies has been used to follow the response to treatment and the fluctuations of disease activity. This has been clearly shown for antibodies to native DNA in SLE.¹

The purpose of this study was to employ newer diagnostic assays to determine the prevalence of defined autoantibodies in a large and carefully evaluated group of patients with SLE in a hospital in Singapore. The data were used to compare our findings with published data in Western countries and to determine the relations between autoantibodies and clinical features such as disease activity and neuropsychiatric symptoms.

Patients and methods

PATIENT SELECTION

Ninety four patients (86 women, eight men) with a diagnosis of SLE were studied. Eighty three patients were Chinese, seven Malays, and four Indians. The patients met the revised criteria of the American Rheumatism Association for SLE.²

All patients attending the lupus clinic of Tan Tock Seng Hospital, Singapore had a detailed clinical and laboratory assessment. Clinical data were obtained by review of hospital records. The following tests were done during the course of study: complete blood count, Westergren sedimentation rate, Rose-Waaler test for rheumatoid factor, total haemolytic complement (CH₅₀), and urine analysis. Patients were reviewed for particular symptoms, signs, and laboratory abnormalities of SLE. Renal biopsies were performed in 15 patients.

Disease activity as defined by Urowitz *et al* was used.³ The presence of any two of the following seven features or groups of features constituted active disease: (a) arthritis; (b) laboratory abnormalities—namely, positive LE cell preparation, white cell count <4×10⁹/l, decreased CH₅₀ or C3, or increased DNA antibody; (c) rash or mucous

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membrane ulcers or alopecia; (d) pleuritis or pericarditis; (e) seizures, psychosis, organic brain syndrome, or lupus headaches; (f) vasculitis; (g) haematuria.

Serum samples of the patients were studied for the presence of antinuclear antibodies of 10 different specificities.

IMMUNOLOGICAL ASSAYS FOR DEFINED ANTINUCLEAR ANTIBODIES

Fluorescent antinuclear antibody

Serum samples were tested for fluorescent antinuclear antibody using a HEP₂ cell substrate and a polyvalent conjugate. There were interpreted using a Leitz Ortholux II microscope with a 50× water immersion objective. Sera were considered positive if they gave a 1+ reaction (scale of 1+ to 4+) at a 1:40 dilution.

Immunodiffusion

Serum samples were tested for antibodies to Sm, nRNP, SS-A(Ro), SS-B(La), rRNP, SL/Ki, PCNA, and Ku (p70/p80) by double immunodiffusion using 8 mm wells, 4 mm apart in a gel of 0.5% agarose (Seakem ME, FMC Corp), 1.5% polyethylene glycol 8000, 0.1% sodium azide.⁴ The volume of serum used was 100 µl. The antigens used included a phosphate buffered saline soluble extract of calf thymus acetone powder and a whole cell sonicate of Wil-2 cells. Serum samples were tested at several dilutions next to standard prototypes.

Enzyme linked immunosorbent assay (ELISA)

Serum samples were tested for antibodies to native DNA and histone by ELISA according to the method of Rubin.⁵ In addition, the presence of antibodies to Sm, nRNP, SS-B(La), and PCNA, which were detected by immunodiffusion, were confirmed by ELISA. In contrast with DNA and histones, which can be obtained in highly purified form commercially, Sm, nRNP, SS-B(La), and PCNA cannot, and the preparation of these antigens was performed according to methods previously described from this laboratory.⁶⁻⁹ These antigens were used to coat wells of ELISA plates at the following concentrations: 'free' Sm 6 µg/ml, Sm/RNP complex 5 µg/ml, SS-B(La) 5 µg/ml, and PCNA 1.3 µg/ml.

Immunoprecipitation

Immunoprecipitation was performed as described previously.¹⁰ Briefly, monolayer Hela cells were grown in 150×25 mm dishes (Falcon 3025) and labelled for 16 hours with [³⁵S]methionine in methionine free Dulbecco's minimal essential medium at a concentration of 3.7 MBq/ml. Cells were rinsed briefly with phosphate buffered saline

and 1.5 ml lysis buffer containing 150 mM sodium chloride and 10 mM trometamol (TRIS)-HCl pH 7.2. Nonidet P-40 (NP-40) (0.5%) was added to each plate and cells removed with scraping and pooled. Cells were allowed to remain for 10 minutes on ice for lysis to continue. The supernatant obtained by centrifugation at 10 000 g for 10 minutes at 4°C was stored at -70°C and used for antigen. The antigen was precleared before use by mixing 100 µl of a 10% protein-A-Sepharose bead suspension, prepared by adding one vial of beads (Pharmacia) to 50 ml NET-2 buffer (50 mM trometamol-HCl pH 7.4, 150 mM sodium chloride, 5 mM edetic acid), 0.1% sodium dodecyl sulphate (SDS), 0.5% sodium deoxycholate, 0.5% NP-40, 0.02% sodium azide containing 2 mg/ml bovine serum albumin, to each 1 ml of extract, rotating at 4°C for 20 minutes, and centrifuging at 10 000 g for 10 minutes with the subsequent removal of the supernatant for use in the assay. Patient samples were prepared by adding 10 µl of sera to 500 µl NET-2 and 100 µl protein-A-Sepharose beads and rotating at 4°C for one hour. Samples were washed twice with NET-2, resuspended in 500 µl NET-2, and 30 µl ³⁵S extract added. Samples were rotated for one hour at 4°C, washed six times with NET-2, and the beads resuspended twice in 15 µl of Laemmli sample buffer (0.0625 M trometamol-HCl buffer pH 6.8, 2.3% SDS, 5% β-mercaptoethanol, 10% glycerol). Standard SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli¹¹ with a 17.5% separating gel and a 5% stacking gel. The gels were processed for autoradiography with enhancement with 2,5-diphenyloxazole. Immunoprecipitation was used to identify antibodies to ribosomal RNP, SL/Ki, and Ku (p70/p80).

Results

CLINICAL

Table 1 shows the clinical manifestations. Fifty two

Table 1 *Clinical and laboratory features in 94 patients with SLE at the time of serological analysis*

<i>Clinical/laboratory features</i>	
Women:men (Nos)	86:8
Race (Chinese:Malay:Indian) (Nos)	83:7:4
Mean age in years (range)	29.6 (13-63)
Mean duration of illness in years (range)	6.14 (6-21)
Active:inactive disease (Nos)	52:42
Active CNS* disease (%)	9
Active renal disease (%)†	24

*CNS=central nervous system.

†Renal disease was indicated by one or more of the following: red blood cells >5 per high power field, casts in urine sediment, proteinuria >500 mg/24 hours, deteriorating creatinine clearance.

patients had active disease (Table 2). Although renal disease was particularly common, the clinical spectrum included rash, arthritis, serositis, cutaneous vasculitis, and neuropsychiatric disease.

The patients who fulfilled the revised criteria for the classification of SLE had four or more criteria (Table 3). The prevalence of various manifestations

was found to be similar for Chinese, Japanese, and American patients except for differences in the prevalence of malar rash, Raynaud's phenomenon, arthritis, psychoses, pleurisy, leucopenia, thrombocytopenia, and DNA antibody (Table 4).

SEROLOGICAL FINDINGS

Sixty per cent of patients had three or more autoantibodies (Figs 1 and 2). The average number of antibodies per patient was 2.9. The most prevalent antinuclear antibodies found were those to histones. History of ingestion of hydralazine and procainamide before the diagnosis of SLE was not obtained in any patient. Antibodies to SS-A(Ro) were found in 63% of patients. This figure is higher than a Western series, which quotes between 20 and 30% in the lupus population.^{12 13} Patients with central nervous system (CNS) disease had a greater number of

Table 2 Lupus activity criteria

Criteria according to Urowitz <i>et al</i> ³	No of patients
Arthritis	17
Rash	19
Mucous membrane ulcers	6
Alopecia	3
Pleuritis/pericarditis	8/4
Seizures	2
Psychosis	7
Organic brain syndrome	1
Vasculitis	5
Haematuria	3
Proteinuria	24
LE cell	1
Total white cell count $<4 \times 10^9/l$	13
Decreased CH ₅₀ or C3	47
Increased dsDNA	43

Table 3 Prevalence of manifestations in 94 Singapore patients using the 1982 American Rheumatism Association revised criteria for systemic lupus erythematosus²

Criterion	Singapore patients No (%)
Malar rash	55/94 (58)
Discoid rash	10/94 (11)
Alopecia	48/94 (51)
Photosensitivity	15/94 (16)
Oral ulcers	18/94 (19)
Raynaud's phenomenon	10/94 (11)
Arthritis	63/94 (67)
Proteinuria	
>0.5 g/day	35/94 (37)
<3.5 g/day	25/94 (27)
Granular casts	39/94 (41)
Dementia	0/94 (0)
Seizure	14/94 (15)
Coma	1/94 (1)
Psychosis	21/94 (22)
Focal neurological disorder	6/94 (6)
Pleurisy	6/94 (6)
Pericarditis	7/94 (7)
Haemolytic anaemia	12/94 (13)
Leucopenia	53/94 (56)
Lymphopenia	32/94 (34)
Thrombocytopenia	42/94 (45)
LE cells	61/89 (69)
Fluorescent antinuclear antibody	91/94 (97)
DNA antibody	43/94 (46)
Sm antibody	25/94 (26)
False positive serological test for syphilis	11/55 (20)
Hypocomplementaemia	81/94 (86)

Table 4 Differences in prevalence of certain manifestations in the Singapore patient group, the Japanese group, and the American Rheumatism Association (ARA) patient group. Values are Nos (%)

Criterion	Singapore (n=94)	Japanese ²² (n=285)	ARA ²² (n=177)
Malar rash	55/94 (58)	219/285 (77)	101/177 (57)
Raynaud's phenomenon	10/94 (11)	135/284 (48)	51/176 (29)
Arthritis, non-erosive	63/94 (67)	228/285 (80)	152/177 (86)
Pleurisy	6/94 (6)	51/284 (18)	92/177 (52)
CNS (psychosis)*	21/94 (22)	41/285 (14)	22/176 (13)
Thrombocytopenia*	42/94 (45)	68/282 (24)	37/177 (21)
Leucopenia	53/94 (56)	191/285 (67)	82/177 (46)
DNA antibody	42/94 (45)	244/272 (90)	113/168 (67)

*Items CNS/psychosis and thrombocytopenia were added to those studied in reference 22 because significant differences were noted in the Singapore population.

Fig. 1 Number of antibodies detected in 94 patients.

Fig. 2 Autoantibody profile in 94 patients with SLE.

antibodies per patient (4.22) than the total population (2.91) and patients without CNS symptoms (2.77). This was reflected in antibodies to dsDNA (88%), histones (100%), Sm (77%), nRNP (66%), SS-A(Ro) (44%), and rRNP (22%) but not in antibodies to SS-B(La), SL/Ki, and p70/p80 (Fig. 3, Table 5). This differs from the observation of Bonfa *et al*, who reported a lower prevalence of anti-DNA and anti-Sm in their 18 patients with lupus psychosis who were positive for antiribosomal P protein antibodies (Table 6).¹⁴ The CNS disease in our patients was marked by psychosis in six, seizures and focal neurological deficits in three. The male patients with SLE did not differ from the women in clinical manifestations or in severity of disease.

Fig. 3 Antibody profile in nine patients with active central nervous system involvement.

Renal disease was present in 60 patients. Antibodies to SS-A(Ro) were present in 37% with and 26% without renal disease ($p < 0.15$) (Table 7). No correlation could be detected between the severity of proteinuria (> 3.5 g protein loss being regarded as severe), renal histology, and the number of antibodies present. Although there was a concomitant rise in dsDNA in patients with renal disease, the difference was not significant compared with those without renal disease ($p < 0.1$). There was no

Table 5 Immunological correlates in patients with active central nervous system (CNS) disease

Antibody specificity	Prevalence (%)	
	Active CNS disease	Total population
dsDNA	88	43
Sm	77	26
nRNP	66	32
rRNP	22	16
p70/p80	11	5
Histone	100	81
Total patients with active CNS disease = 9/94 (10%)		
Antibody plurality per patient (CNS) = 4.22		
Antibody plurality per patient (total population) = 2.91		
Antibody plurality per patient (without CNS symptoms) = 2.77		

Table 6 Serological features in patients with central nervous system (CNS) lupus

Antibody	No (%) of patients	
	New York* (n=18)	Singapore† (n=6)
Anti-DNA	8 (44)	5 (83)
Anti-Ro	9 (50)	1 (17)
Anti-La	2 (11)	0 (0)
Anti-Sm	6 (33)	5 (83)
Anti-RNP	6 (33)	2 (33)

*Patients had lupus psychosis and were positive for antiribosomal P protein antibodies.¹⁴

†Six of nine patients with active CNS disease had psychosis (present study).

Table 7 Immunological correlates in renal disease

	Prevalence (%) of antibody to:					
	DNA	Histone	Sm	nRNP	SS-A	SS-B
Renal disease	27	48	15	18	37	5
Non-renal disease	20	35	12	15	26	6

Total patients with history of renal disease = 60/94 (64%)

Active disease (systemic/renal) at time of sampling = 24/60 (40%)

Inactive disease (systemic/renal) at time of sampling = 36/60 (60%)

increased prevalence of rash or thrombocytopenia in patients with anti-SS-A(Ro) as observed by some.^{15 16}

When the prevalence of other precipitins in patients with SLE with anti-SS-A(Ro) was compared with that of the entire group, there were no appreciable differences. dsDNA was present in 53% of patients with anti-SS-A(Ro), histones in 90%, nRNP in 33%, SS-B(La) in 22%, and Sm in 25%.

Discussion

Our study shows that the prevalence of antinuclear antibodies in Oriental patients was within the range reported from Western countries¹⁷; the exception being a higher prevalence of antibodies to SS-A(Ro). This indicates a high uniformity of antibody profile in SLE in different populations.

Anti-SS-A(Ro), often accompanied by anti-SS-B(La) is found in SLE with a prevalence varying between 20 and 30%.¹⁸ These antibodies are found in up to 70% of patients with primary Sjögren's syndrome and in the sicca complex associated with SLE.¹⁹ The increased prevalence of this autoantibody in our patients with SLE may indicate the presence of subclinical coexisting Sjögren's syndrome or the likelihood of the future development of sicca symptoms in this subset of patients.

Neuropsychiatric manifestations occurred in 21% of patients at some time in the course of the disease. There is a tendency for these manifestations to occur early, especially within the first two years of diagnosis.²⁰ The higher number of antibodies in these patients may reflect a greater severity of disease. CNS disease is the second commonest cause of death among our patients with SLE.²¹

Anti-Sm antibody occurred in 26% of patients and was more prevalent in those with active disease (36% in active versus 11% in inactive disease). This figure is comparable with the Japanese value²² but much lower in white patients with SLE.¹⁸ The association of anti-Sm with anti-RNP is well known and may be related to the association of these antigens in small nuclear ribonucleoprotein particles.

The prevalence of antibodies to rRNP was 16%. This is marginally higher than the reported occurrence of 12% in patients with SLE.^{14 23} No distinctive clinical features were recognised in our patients who were anti-rRNP positive. Anti-rRNP antibodies may be a marker for SLE, being found almost exclusively in SLE, but the clinical significance of these antibodies remains to be determined.

Anti-SL/Ki, p70/p80, and PCNA are uncommon antibodies, and no particular clinical correlation was found with these antibodies. We could not confirm any trend towards increasing cutaneous, haematological abnormalities, or sicca symptoms.

Pertinent to the discussion of the pattern of disease involvement in SLE are selection bias and referral patterns. These two variables could account for the minor differences observed in our patients. Our patients attended a referral centre for autoimmune diseases and their disease tended to be severe.

We conclude that there is similarity of autoantibody profile among patients with SLE in different races and countries, despite differences in severity of disease and mortality in different SLE populations.^{24 25} The higher prevalence of anti-SS-A(Ro) in our population deserves further study to determine the possible involvement of genetic factors in this situation.

There have been few comprehensive studies of this nature in which a large cohort of patients with SLE has been analysed for the prevalence of autoantibodies using recently developed sensitive assays such as ELISA, immunoprecipitation of isotope labelled antigens, and Western blotting. An interesting feature emerging from this study is that the prevalence of autoantibodies is approximately similar to previously published data, suggesting that there is no pool of patients with low levels of autoantibodies. Also, this study calls attention again to the fact that SLE is a disease characterised by the simultaneous presence of multiple autoantibodies (2.9 per patient), a feature reported many years ago¹⁷ but not appreciated when attempts are made to associate subsets of clinical features with single autoantibodies. It would surely be difficult to associate a clinical picture such as CNS involvement with a single autoantibody when the average number of autoantibodies in such patients is 4.2. The observed multiclonality of autoimmune responses in SLE is a phenomenon whose elucidation should add insights into the nature of the autoimmune process.

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