Antinuclear antibodies in the relatives and spouses of patients with systemic sclerosis

P J MADDISON,¹ R P SKINNER,¹ R S PEREIRA,² CAROL M BLACK,²
B M ANSELL,³ MALCOLM I V JAYSON,⁴ N R ROWELL,⁵ AND K I WELSH⁶

From the ¹Royal National Hospital for Rheumatic Diseases, Bath; the ²Department of Rheumatology, West Middlesex University Hospital, Isleworth; the ³Clinical Research Centre, Harrow; the ⁴Rheumatic Diseases Centre, University of Manchester, Hope Hospital, Salford; the ⁵Leeds General Infirmary, Leeds; and the ⁶Department of Tissue Typing, Guy's Hospital Medical School, London

SUMMARY The families of 65 patients with systemic sclerosis were examined clinically and serum samples from each subject were tested for antinuclear antibodies (ANA) by immunofluorescence on HEp₂ cells and for precipitating antibodies to soluble cellular antigens including Scl-70.

Of 217 blood relatives, 58 (27%) had ANA (42 speckled, 13 nucleolar, one centromere, two homogeneous); 22 (10%) had precipitins, one anti-Scl-70, one anti-PM-Scl, one anti-nRNP, two anti-Ro(SSA), the remainder unidentified). Family members tended to share ANA patterns. Of 38 spouses, nine (24%) had ANA (all speckled) and two showed unidentified precipitins. This compares with an incidence of ANA and precipitins in a control population of 8% and 1% respectively. Antibodies were more common in female than male relatives (particularly in mothers and sisters of probands). Twenty one of the 58 family members with ANA had clinical features of connective tissue disease; the remainder were asymptomatic.

The presence of genetic factors influencing autoimmunity is suggested by the incidence of autoantibodies in first degree relatives. Similar observations in spouses, however, indicate that environmental factors may also have a role in these immune abnormalities.

Systemic sclerosis (SS) is a chronic disease characterised by prominent vascular involvement and progressive fibrosis of various organ systems of the body. The aetiology of SS is unknown, but the disease is characterised by a variety of abnormal serological and cellular immune reactions.¹ The presence of antinuclear antibodies (ANA) is particularly common, and when cultured cells are used as the substrate for immunofluorescence tests ANA are found in over 90% of cases.² Certain antibodies are particularly characteristic of SS, such as anti-nucleolar and anticentromere antibodies and antibodies to the soluble nuclear antigen Scl-70.^{2–5}

Systemic sclerosis and related connective tissue diseases in family members have been reported,^{6–20} and in the majority of such kindreds affected members have been first degree relatives of the

proband. In contrast with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), familial cases of SS are uncommon. Nevertheless, there are previous reports of immunological abnormalities in asymptomatic relatives of patients with SS, though the frequency of the findings varies widely in different series.^{20–23} Furthermore, the demonstration by several groups that certain HLA haplotypes are associated with the disease^{24–27} also suggests that genetic factors might be involved in the development or the expression of SS, or both.

Given the opportunity to study large numbers of patients and family members through the collaborating centres of the UK Systemic Sclerosis Study Group, particularly in the light of increased knowledge of the serological abnormalities in SS, it was the aim of this study to determine the frequency and specificity of ANA in the sera of relatives of patients with SS.

Patients and methods

Sixty five patients with SS and their blood relatives

Accepted for publication 21 April 1986.

Correspondence to Dr P J Maddison, Consultant Rheumatologist, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL.

and spouses were investigated as part of a multicentre family study of systemic sclerosis organised by the UK Systemic Sclerosis Study Group. Patients fulfilled the American Rheumatism Association criteria for SS²⁸ and were selected only on the basis of having sufficient blood relatives to allow genetic haplotype assignment.

Serum samples were collected at the time of examination of the subject and stored at -70°C until testing. They were examined without knowledge of the clinical data, together with serum from healthy blood donor controls.

IMMUNOFLUORESCENCE

Antinuclear antibodies were examined on a substrate of HEP₂ cells fixed in acetone using serum diluted in phosphate buffered saline and fluorescein isothiocyanate conjugated goat antihuman polyvalent immunoglobulins (Sigma). The presence of immunofluorescence at a serum dilution of 1:40 was considered to be positive.

IMMUNODIFFUSION

Precipitating antibodies to soluble nuclear and cytoplasmic antigens were detected by the Ouchterlony technique as described previously.² Reference serum standards were used to detect antibodies to nRNP, Sm, Ro(SSA), La(SSB), Scl-70, Jo-I, and PM-Scl.

IMMUNOGENETIC STUDIES

HLA-A, B, C, DR typing was performed by a standard microlymphocytotoxicity method.²⁹

Gm allotyping was carried out by red cell agglutination inhibition, using human group O erythrocytes sensitised with anti-rhesus antiserum as indicator cells, except for Gm23, for which a modified method involving direct coupling of myeloma IgG to the erythrocytes was used.³⁰

COMPLEMENT ALLOTYPES

C4 alleles were established by high voltage electrophoresis of desialated plasma according to the method of Bruun-Petersen *et al*³¹ using both immunofixation and functional testing of electrophoresed samples.

Bf alleles were determined by immunofixation with electrophoresed untreated plasma by a modification of the method of Alper *et al*,³² in which the same tris-glycine buffers were used except for the addition of 0.4 g/l calcium lactate in the tank buffer and 0.2 g/l calcium lactate in the gel buffer. The running times for the C4 and Bf electrophoreses were 2.5 h and 1.5 h respectively. The demonstration of a null allele at either the C4A or C4B locus was designated C4Q0.

Results

OCCURRENCE OF ANTINUCLEAR ANTIBODIES

The family members of 65 probands with SS were studied. These included 217 blood relatives and 38 spouses. The demographic features of these subjects are summarised in Table 1.

With HEP₂ cells as substrate ANA were detected in 93% of sera from probands, 27% from blood relatives, and in 24% from spouses (Fig. 1). This compares with 8% in the healthy controls. Fig. 2 shows that the ANA titres were highest in the patients, intermediate in the relatives, and generally

Table 1 Demographic features of 65 probands with SS, 217 blood relatives, and 38 spouses

Subjects studied	Age in years (mean and range)	Nos female
Patients with systemic sclerosis (n=65): 34% with diffuse scleroderma 40% with acrosclerosis 26% with CREST*	43 (13-73)	58
Relatives (n=217): 31 mothers 45 sisters 34 daughters 107 other relatives	63 (37-81) 38 (6-69) 27 (9-51) 38 (4-82)	4
Spouses (n=38)	49 (29-73)	3

*CREST=calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia.

Fig. 1 Frequency of ANA in patients, relatives, spouses, and controls using HEP₂ cells.

Fig. 2 ANA titres in the ANA positive serum samples.

low in the ANA positive spouses and healthy controls. Table 2 summarises the patterns of immunofluorescence in the ANA positive sera. Antibodies characteristically found in systemic sclerosis were identified in the sera of blood relatives, especially antinucleolar antibodies, which were present in 22%. In one case anticentromere antibodies were present. Although ANA were more commonly found in spouses of patients than in healthy controls, they were generally of low titre and showed a non-specific speckled pattern of fluorescence.

The frequency and specificity of antibodies to soluble non-histone tissue antigens are summarised

Table 2 Patterns of nuclear immunofluorescence on HEP₂ cells observed in patients with SS, relatives, and spouses and in ANA positive healthy controls

Pattern	Probands (n=60)	Relatives (n=58)	Spouses (n=9)	Controls (n=4)
Diffuse speckled	29 (48%)	42 (72%)	9	2
Nucleolar	18 (30%)	13 (22%)		
Centromere	12 (20%)	1		
Homogeneous	1	2		2

in Table 3. Antibodies were detected by immunodiffusion in 57% of the probands, 10% of the blood relatives, and 5% of the spouses, which compares with the incidence of 1% in the healthy controls. These antibodies were detected to a variety of cellular constituents in the tissue extracts, and in many cases they did not correspond to the reference sera and still remain uncharacterised. As expected, antibodies to Scl-70 represented the most commonly identified precipitin system in the probands and were present in 17% of cases. In two instances antibodies with a high degree of specificity for systemic sclerosis were detected in relatives, anti-

Table 3 Frequency of precipitating antibodies to soluble cellular antigens in patients with SS, relatives, spouses, and healthy controls using immunodiffusion

	Total (%)	Anti- Scl-70 (%)	Anti- PM-Scl (%)	Anti- nRNP (%)	Anti- Ro(SSA) (%)	Unidentified antibodies (%)
Probands	57	17	6	3	2	29
Relatives	10	0.5	0.5	0.5	1	7.5
Spouses	5					5
Controls	1					1

Scl-70 in one case who also developed SS, and antibodies to the PM-Scl system³³ in the other.

ANTINUCLEAR ANTIBODIES IN FAMILY MEMBERS
As shown in Table 4 ANA were more commonly found in female than in male relatives ($\chi^2=5.26$; $p<0.02$) and were most commonly found in the mothers and sisters of the probands ($\chi^2=8.53$; $p<0.005$). In female relatives the frequency of ANA

was uninfluenced by age (Fig. 3), but in men the highest frequency was found in relatives older than 60 years.

In 23 of the 35 families where there were ANA positive cases among relatives the ANA specificity was shared among the family members. Two examples are shown in Fig. 4. In family A father and daughter shared antibodies to Scl-70 and both had SS with diffuse scleroderma. This was the only example of a multiple case family seen in this study. In family B two relatives and the proband had antinucleolar antibodies. In the remaining families probands and ANA positive relatives showed different ANA specificities, and two examples are illustrated in Fig. 5.

Family members were screened clinically at the time the blood samples were taken. Details of this clinical examination will be reported in detail elsewhere. Table 5 shows that ANA positive

Table 4 Frequency of ANA in relatives of patients with SS

Relation	Positive ANA (%)
Mother (n=31)	39
Sister (n=45)	38
Daughter (n=34)	20
Other female (n=4)	25
Male (n=103)	21

Table 5 Features of connective tissue disease in relatives of patients with SS

	Number with diagnosed CTD	Number with symptoms of CTD	Total
Relatives with positive ANA (n=58)	4*	17	21 (36%)
Relatives with negative ANA (n=159)	2†	18	20 (12%)

*Two with RA; one with SLE; one with progressive systemic sclerosis. †Two with RA.

Fig. 3 Frequency of ANA in male and female relatives of different age groups. Those with positive ANA are indicated by the cross hatching.

relatives more commonly had either a full blown connective tissue disease or clinical features of a connective tissue disease (CTD), predominantly arthralgias or arthritis and Raynaud's phenomenon.

ANTINUCLEAR ANTIBODIES IN SPOUSES
ANA were detected in the serum of nine of the 38 spouses. All were men with a mean age of 49. Information concerning the duration of marriage was available in 26 cases, and in the great majority

marriage took place before the onset of disease in the proband and the couple had lived together throughout the course of the disease. In this respect there were no obvious differences between those spouses who had ANA (six cases) and those who did not (20 cases). Information of this sort was not obtained from the other 12 cases.

IMMUNOGENETIC ASSOCIATIONS
Twenty one patients and 120 blood relatives have so

Fig. 4 Two families showing concordance of ANA specificity among family members.

Fig. 5 Two families in which family members show different ANA specificities.

Table 6 Association of ANA in first degree relatives with HLA-DR5 and C4Q0

Subjects	No with DR5 or C4Q0, or both	p Value
ANA positive relatives (n=36)	34 (94%)	<0.01
ANA negative relatives (n=84)	56 (67%)	

far been fully HLA typed. The results will be reported in detail elsewhere. ANA positivity was shown to have a significant association with C4Q0 and DR5 (Table 6). Of 36 relatives with a positive ANA, 34 were either DR5 or had a null allele at the C4 locus. When compared with the incidence of these markers in the ANA negative relatives (56 out of 84) this result is significant at the $p < 0.01$ level.

Discussion

Although families with multiple cases of systemic sclerosis are uncommon, genetic factors have been implicated in this disease by a number of studies of immunogenetic markers in patients with SS.²⁴⁻²⁷ There appears to be an association with the HLA haplotype A1,B8,DR3 and more particularly with the linked complement allele C4A*Q0 (unpublished data). Furthermore, the HLA antigens DR1 and DR5 seem to be associated with the CREST variant of the disease.^{27 34}

In this study an increased frequency of ANA was shown in the sera from relatives of patients with SS. This finding supports the concept that a familial background of immunological abnormalities may contribute to the development of SS. Fennell *et al* were the first to describe this observation,²² which has now been repeated on a number of occasions,^{9 19 20 23} though in most reports numbers of individuals studied have been small or families selected with multiple SS cases. Using a sensitive ANA test with HEp₂ cells as substrate we found the frequency of ANA in relatives to be 27%. Furthermore we also took the opportunity to study ANA specificity. Antinuclear antibodies, characteristically found in high titre in patients with SS, were quite commonly found in blood relatives but usually in moderate or low titres. Other autoantibodies with a high degree of specificity for SS were only rarely found in family members. The only relative with anti-Scl-70 in fact developed SS with diffuse scleroderma, but the two relatives with anticentromere antibodies and anti-PM-Scl respectively are as yet asymptomatic. Since it is our impression from this

study that these antibodies are closely linked to the development of SS, time will tell whether or not these two relatives will develop symptoms.

The increased frequency of ANA in female relatives may be linked to the female preponderance in SS and other CTD. A role for constitutional factors in influencing the development of autoimmune abnormalities and in the disease itself is strengthened by the preliminary findings of HLA analysis in this family study.

The demonstration of familial aggregation of clinical and serological features of CTD helps to support the concept of genetic influences but in no way excludes the importance of environmental factors. In SS the influence of environmental factors is quite unknown, with the exception of scleroderma-like disorders induced by vinyl chloride³⁵ and contaminated rape seed oil.³⁶ A recent report of PSS in husband and wife³⁷ reopens the question of the relative contribution of genetic and environmental factors in this disease. Our observation of an increased frequency of ANA in spouses, albeit of low titre, suggests that environmental factors may indeed have a role in this autoimmune response. It must be noted that the controls for this study were blood donor controls and details of age and sex are not available. It is probable that the controls are younger than the spouses (mean age 49) and although it is unlikely that this accounts for the increased frequency of positive ANA tests, this possibility needs to be considered. Similar findings are well known in non-consanguineous relatives of patients with SLE, who show an increased frequency of ANA, antibodies to polynucleotides, and antilymphocytotoxic antibodies,³⁸⁻⁴⁰ suggesting the possibility of a transmissible agent. Further prospective studies of spouses of patients with SS may furnish clues to the presence of a similarly transmissible agent in this disease.

This study was funded by a grant from the Arthritis and Rheumatism Council. We are grateful to Drs Denman, Frank, and Dieppe for allowing us to include their patients.

References

- 1 Haynes D C, Gershwin M E. The immunopathology of progressive systemic sclerosis. *Semin Arthritis Rheum* 1982; 11: 331-51.
- 2 Catoggio L J, Bernstein R M, Black C M, Hughes G R V, Maddison P J. Serological markers in progressive systemic sclerosis: clinical correlations. *Ann Rheum Dis* 1983; 42: 23-7.
- 3 Tan E M, Rodnan G P, Garcia I, Moroi Y, Fritzler M, Peebles C. Diversity of antinuclear antibodies in progressive systemic sclerosis. *Arthritis Rheum* 1980; 23: 617-25.
- 4 Tuffanelli D L, McKeon F, Kleinsmith A M, Burnham T K, Kirschner M. Anti-centromere and anti-centriole antibodies in the scleroderma spectrum. *Arch Dermatol* 1983; 119: 560-5.

- 5 Douvas A S, Achten M, Tan E M. Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. *J Biol Chem* 1979; **254**: 10514-22.
- 6 Blanchard R E, Speed E M. Scleroderma: periodontal membrane manifestations in two brothers. *Periodontics* 1965; **3**: 77-80.
- 7 McAndrew G M, Barnes E G. Familial scleroderma. *Ann Phys Med* 1965; **8**: 128-31.
- 8 Burge K M, Perry H O, Stickler G B. Familial scleroderma. *Arch Dermatol* 1969; **99**: 681-4.
- 9 Tuffanelli D L. Scleroderma, immunologic and genetic diseases in three families. *Dermatologica* 1969; **138**: 93-100.
- 10 Rendall J R, McKenzie A W. Familial scleroderma. *Br J Dermatol* 1974; **91**: 517-22.
- 11 Greger R E. Familial progressive systemic scleroderma. *Arch Dermatol* 1975; **111**: 81-5.
- 12 Freyha R A, Tabbara K F, Geha R S. Familial CREST syndrome with sicca complex. *J Rheumatol* 1977; **4**: 53-8.
- 13 Gray R G, Altman R D. Progressive systemic sclerosis in a family: case report of a mother and son and review of the literature. *Arthritis Rheum* 1977; **20**: 35-41.
- 14 Sasaki S, Yoshino H. Systemic scleroderma in mother and daughter. *Arch Dermatol* 1977; **113**: 378-9.
- 15 Strosberg J M, Peck B, Harris E D. Scleroderma with intestinal involvement: fatal in two of a kindred. *J Rheumatol* 1977; **4**: 46-52.
- 16 Taj M, Ahmad A. Familial localised scleroderma (morphoea). *Arch Dermatol* 1977; **113**: 1132-3.
- 17 Mund D J, Greenwald R A. The CREST syndrome variant of scleroderma in a mother-daughter pair. *J Rheumatol* 1978; **5**: 307-10.
- 18 Sheldon W B, Lurie D P, Maricq H R, *et al.* Three siblings with scleroderma (systemic sclerosis) and two with Raynaud's phenomenon from a single kindred. *Arthritis Rheum* 1981; **24**: 668-76.
- 19 Soppi E, Lehtonen A, Toivanen A. Familial progressive systemic sclerosis (scleroderma): immunological analysis of two patients and six siblings from a single kindred. *Clin Exp Immunol* 1982; **50**: 275-82.
- 20 Takehara K, Moroi Y, Ishibashi Y. Antinuclear antibodies in the relatives of patients with systemic sclerosis. *Br J Dermatol* 1985; **112**: 23-33.
- 21 Corcos J M, Robbins W C, Rogoff B, Heimer R. Some serum protein abnormalities in patients with progressive systemic sclerosis and their relatives. *Arthritis Rheum* 1961; **4**: 107.
- 22 Fennell R H Jr, MacLachlan M J, Rodnan G P. The occurrence of antinuclear factors in the sera of relatives of patients with systemic rheumatic disease. *Arthritis Rheum* 1962; **5**: 296.
- 23 Rothfield N F, Rodnan G P. Serum antinuclear antibodies in systemic sclerosis (scleroderma). *Arthritis Rheum* 1968; **11**: 607-17.
- 24 Clements P J, Opelz G, Terasaki P I. Association of HLA antigen A9 with progressive systemic sclerosis (scleroderma). *Tissue Antigens* 1978; **2**: 357.
- 25 Gladman D D, Keystone E C, Baron M, Lee P, Cane D, Mervert H. Increased frequency of DR5 in scleroderma. *Arthritis Rheum* 1981; **24**: 854-6.
- 26 Kallenberg C G M, van der Voort-Beelen J M, D'Anaro J, The T H. Scleroderma: increased frequency of B8/DR3 in scleroderma and association of the haplotype with impaired cellular immune response. *Clin Exp Immunol* 1981; **43**: 478-85.
- 27 Black M B, Welsh K I, Maddison P J, Jayson M I V, Bernstein R M. HLA antigens, autoantibodies and clinical subsets in scleroderma. *Br J Rheumatol* 1984; **23**: 267-71.
- 28 Subcommittee for Scleroderma Criteria of the American Rheumatism Association. Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; **23**: 581-90.
- 29 Welsh K I, Batchelor J R. In: Weir D M, ed. *Handbook of experimental immunology*. Oxford: Blackwell Scientific, 1978: Chap. 35.
- 30 Van Logem E. In: Weir D M, ed. *Handbook of experimental immunology*. Oxford: Blackwell Scientific, 1978: Chap. 35.
- 31 Bruun-Petersen G, Lamm L U, Sorensen I J, Buskjaer L, Mortensen J P. Family studies of complement C4 and HLA in man. *Hum Genet* 1981; **58**: 260-7.
- 32 Alper C A, Boenisch T, Watson L. Genetic polymorphism in human glycine-rich beta-glycoprotein. *J Exp Med* 1972; **135**: 68-80.
- 33 Reichlin M, Maddison P J, Targoff I, *et al.* Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes. *J Clin Immunol* 1984; **4**: 40-4.
- 34 Lynch C J, Singh G, Whiteside T C, Rodnan G P, Medsger T A, Rabin B S. Histocompatibility antigens in progressive systemic sclerosis. *J Clin Immunol* 1982; **2**: 314-20.
- 35 Lilis R, Anderson H, Nicholson W J, Daum S, Fischbein AS, Selikoff I J. The prevalence of disease among vinyl chloride and polyvinyl chloride workers. *Ann NY Acad Sci* 1975; **246**: 22-41.
- 36 Tabuenca J M. Toxic allergic syndrome caused by ingestion of rape seed oil denatured with aniline. *Lancet* 1981; **ii**: 567-8.
- 37 Christy W C, Rodnan G P. Conjugal progressive systemic sclerosis (scleroderma); report of the disease in husband and wife. *Arthritis Rheum* 1984; **27**: 1180-2.
- 38 Cleland L G, Bell D A, Williams M, Saurino B C. Familial lupus: family studies of HLA and serologic findings. *Arthritis Rheum* 1978; **21**: 183-91.
- 39 De Horatius R J, Pillarisetty R, Messner R P, Talal N. Antinuclear acid antibodies in systemic lupus erythematosus patients and their families. *J Clin Invest* 1975; **56**: 1149-54.
- 40 Folomeeva O, Nasonova V A, Alekberova A S, Talal N, Williams R C. Comparative studies of antilymphocyte, antipoly-nucleotide and antiviral antibodies among families of patients with systemic lupus erythematosus. *Arthritis Rheum* 1978; **21**: 23-7.