Antinuclear antibodies in patients with scleroderma (systemic sclerosis) and in their blood relatives and spouses

A J Barnett, L J McNeilage

Abstract

Objective—To test the postulate that there is a higher prevalence of antinuclear antibodies (ANAs) in serum samples from blood relatives and from spouses of patients with scleroderma than in control samples, and that this provides evidence for both genetic and environmental factors influencing autoimmunity in scleroderma.

Method—Testing for ANAs was performed on 58 patients with scleroderma, 30 of their spouses, 74 first degree relatives, and 66 control subjects broadly age matched to the patients, their spouses, and about half of the relatives (siblings and parents).

Results—On the basis of an ANA titre of >40 as positive, 12 (18%) of the controls, 55 (95%) of the patients, one (3%) of the spouses and five (7%) of the relatives would be classified as positive. Thirty seven (64%) of the patients had defined specificities (ACA, Scl 70, U1 (RNP)) but none of the controls, spouses, or relatives had antibodies of these specificities.

Conclusion—These findings give no support to the postulate that environmental or genetic factors contribute to the ANAs in scleroderma.

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Scleroderma (systemic sclerosis) is an uncommon condition of obscure aetiology characterised clinically by symmetrical stiffness of the skin, Raynaud's phenomenon, and involvement of various internal organs. Over 90% of patients with scleroderma have antinuclear antibodies (ANAs) of various types in their serum¹ and, although it is not clear whether their presence is an initial feature of the disease or whether they develop secondarily, certain specificities, namely anti-centromere, antitopoisomerase (Scl 70), and antinucleolar antibodies are characteristic of scleroderma.² Rare examples of a familial occurrence of the condition have been reported^{3–7} and there have been reports of HLA associations,^{8–14} suggesting a genetic predisposition. The development of scleroderma or scleroderma-like conditions, however, in association with various toxic agents including silica,^{15–17} silicon,¹⁸ vinyl chloride,^{19–21} industrial solvents,^{22–23} and rape seed oil,²⁴ have suggested the possibility of environmental factors as a cause of the condition.

Maddison *et al*²⁵ examined the prevalence of ANA in patients with scleroderma, their blood relatives, and spouses. They found a higher prevalence of ANA both in the blood relatives (27%) and in the spouses (24%) compared with controls (8%) and suggested that these findings support the notion that both genetic and environmental factors influence autoimmunity in scleroderma. The present study was undertaken to investigate this idea in another population of patients with scleroderma.

Patients and methods

PATIENTS

The patients with scleroderma were under the care of one of us (AJB) or were members of the Scleroderma Foundation of Victoria. They had symmetrical skin stiffness, Raynaud's phenomenon, and some evidence of systemic involvement. They were classified according to a scheme previously described²⁶ based on the extent of the skin stiffness; type 1 disease indicates involvement of the skin of the fingers only, type 2 involvement of the arms and face but excluding the trunk, and type 3 extensive involvement including the trunk. The patients were asked to bring one or more first degree blood relatives (offspring, parents, siblings) and, if possible, a spouse. The relatives and spouses were all interviewed with the aim of detecting any evidence of scleroderma or connective tissue disease and their hands and face were inspected. Control serum samples (total 65) were obtained from patients in hospital (31) and a healthy population (34) broadly matched with the scleroderma patients by age. The healthy controls were obtained partly from volunteer receptionists in doctors' rooms and partly from volunteer relatives of patients in a repatriation hospital. They were questioned to exclude the presence of any autoimmune disease. The hospital controls were obtained from patients attending a haematology clinic for routine blood tests. Their notes were inspected to exclude any subjects with autoimmune disease such as diabetes or thyroid disease, infections, or autoimmune cytopenias including thrombocytopenia. Only two patients were blood relatives (first cousins). About one quarter of the relatives were siblings, one quarter parents, and one half children of the patients. Five only had experienced Raynaud's phenomenon; one of these had nailfold capillary changes

Alfred Hospital,
Melbourne, Australia
A J Barnett

Walter and Eliza Hall
Institute of Medical
Research,
Melbourne, Australia
L J McNeilage

Correspondence to:
Dr A J Barnett,
517 St Kilda Road,
Melbourne 3004,
Australia.

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suggestive of scleroderma. None had the skin changes characteristic of scleroderma. Several complained of joint aches and pains but these were no more frequent than in the general population and none had features of connective tissue disease. Among the spouses, husbands predominated over wives in the ratio of about 3 to 1, the reverse of the sex incidence in patients with scleroderma. None had Raynaud's phenomenon or features of connective tissue disease. The table shows the age distribution and sex of the subjects and the type of the scleroderma.

METHODS

Blood samples were taken by venepuncture, centrifuged, and the serum samples coded and frozen on the same day. The coded samples were stored at -20°C until tested. The ANA tests were performed without knowledge of clinical data or subject group.

Autoantibodies to nuclear and cytoplasmic antigens were detected by immunofluorescence with the human epithelial cell line HEp2 (Immunoconcepts, Sacramento, California). Serum was titrated in quadrupling dilutions from 1/40 to 1/640. The staining pattern was determined for all positive samples. Those positive by immunofluorescence were tested for antibodies to the extractable nuclear antigens topoisomerase 1 (Scl 70), U1 (RNP), Sm, Ro (SS-A), La (SS-B), Jo-1, Mi 2, and Ku by counter-immunoelectrophoresis (CIEP) with rabbit thymus (Pel Freeze Biologicals, Rogers, Arkansas) and human spleen as sources of antigens and appropriate reference serum samples for specificity.

Results

The table shows that 39% of the controls were negative for ANAs, and 42% had ANAs of low

titre (40) and 18% (all but two in the 60–89 age group) had ANAs of >160 . By contrast, most (70%) of the spouses were negative for ANAs, with 27% having an ANA titre of 40 and only 3% (one patient) with a higher titre (160). The patterns of ANAs detected in the controls, spouses, and first degree relatives were speckled, nucleolar, and homogeneous. In general the first degree relatives resembled the spouses: 65% were ANA negative, 28% had ANAs of low titre (40) and 7% had ANAs of titre 160. All 58 patients were positive for ANAs with most (95%) having a titre of >160 , and only 5% a titre of 40. Anticentromere antibodies (ACAs) were detected in 48% of the patients, antitopoisomerase 1 (Scl 70) in 14%, and anti-U1 (RNP) in 2%. None of the controls, spouses, or first degree relatives had ACA, antitopoisomerase 1, or anti-U1 (RNP) antibodies.

Discussion

The data showed that the serum samples of our control subjects had a high prevalence of positive ANAs, particularly at the low titre of 40, suggesting that positivity at this titre is not clinically significant (figure). As would be expected the patients showed a high prevalence of positive ANAs, particularly at the higher titres and 95% were positive at a titre of >160 .

By contrast the spouses showed a lower prevalence of positive ANAs than controls at all titres, indicating that the present study provides no evidence that environmental factors contributed to the production of autoantibodies found in patients with scleroderma. This study does not, however, rule out the possibility of a contribution of environmental factors operating in the patients before marriage.

The prevalence of ANAs in the first degree relatives was similar to that in the spouses, again lower than in the control subjects. It is

Autoantibody results for various types of subjects

Age	No	F	M	ANA titre				Defined specificity		
				Negative	40	160	640	ACA	Scl 70*	U1 (RNP)
Controls:										
40–59	19	7	12	10	7	1	1	None detected		
60–79	38	13	25	13	15	6	4			
80–89	9	3	6	3	6	—	—			
Total	66	23	43	26 (39)	28 (42)	7 (18)	5			
Patients:										
20–39	7	4	3	—	—	1	6	1	3	1
40–59	16	14	2	—	1	1	14	8	2	—
60–79	33	29	4	—	2	2	29	17	3	—
80–89	2	2	—	—	—	—	2	2	—	—
Total	58	49	9	—	3 (5)	4 (95)	51	28 (48)	8 (14)	1 (2)
Spouses:										
20–39	3	2	1	2	1	—	—	None detected		
40–59	7	—	7	5	2	—	—			
60–79	20	3	17	14	5	1	—			
Total	30	5	25	21 (70)	8 (27)	1 (3)	—			
Relatives:										
0–19	1	1	—	1	—	—	—	None detected		
20–39	38	18	20	26	11	1	—			
40–59	14	9	5	11	3	—	—			
60–79	17	10	7	9	5	3	—			
80–89	4	4	—	1	2	1	—			
Total	74	42	32	48 (65)	21 (28)	5 (7)	—			

*Scl 70=Antitopoisomerase 1; percentage of total number in group (160 and 640 groups combined) in parentheses.

Titres of antinuclear antibodies (ANAs) in control subjects, patients with scleroderma, their spouses, and first degree relatives. The dashed horizontal line indicates suggested level of clinical significance.

relevant that the disease specific autoantibodies (anticentromere and antitopoisomerase I) were confined to the patients with scleroderma and did not occur in any of their family members who would have shared similar HLA and immunoglobulin lines. Unfortunately there are no HLA data in this paper. We can only say that we could find no evidence of a genetic factor in our study. A group of relatives of this size (74) would be expected to contain a significant number with considerable HLA concordance and it would be expected that any important genetic factors would be detected. The relatives fall into two broad groups: a younger group of under 40 years, mainly children of the patients and, a group aged 40 and older, mainly siblings of the patients. The control subjects were broadly age matched with the siblings only. When the data from this group of relatives was compared with the controls, however, the relatives still failed to show any excess of positivity of ANAs. Thus the present study did not find any evidence for genetic influences contributing to autoantibodies in patients with scleroderma.

The control group showed a high prevalence (60%) of ANAs compared with other studies²⁷ including that of Maddison *et al* (8%).²⁵ Most (70%) of the positive results were of low titre (40). The reason for this high prevalence of ANAs in our controls is not clear. Age may be a factor. All were over 40 and 70% over 60. The age of the healthy blood donor controls used by Maddison *et al*²⁵ was not stated; however it would seem probable that they were a considerably younger group. The prevalence of autoantibodies generally has been found to rise with age (particularly in women),²⁷ but it is unlikely that age alone would account for the high prevalence of ANAs in our control subjects. Half of the control subjects were patients in hospital (but without evidence of connective tissue disease) suggesting that ill health may be an influencing factor. The prevalence of ANAs in the patients in hospital was the same as for patients not in hospital.

Other possible variables that are difficult to assess include technique, geography, and race. The technique for detection of ANAs is now standardised worldwide. Our subjects were white people from the city of Melbourne, Australia.

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