Autoantibodies in childhood scleroderma

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SUMMARY The clinical presentation of scleroderma in childhood is even more varied than in adult life. This study of 19 children shows that antinuclear antibodies (ANA) detected on the HEp₂ cell substrate are just as common as in the adult disorders. All seven children with diffuse scleroderma or acrosclerosis were ANA positive, as were eight of the 12 with more localised disease. Antinucleolar antibodies were particularly frequent (58% overall), but anticentromere antibody was not observed. Enzyme-linked immunosorbent assays (ELISAs) for antibodies to collagen types I-V, in contrast to adult scleroderma, gave normal results in all childhood cases.

Key words: systemic sclerosis, children, antinuclear antibodies, antinucleolar antibodies, anticentromere antibody, anticollagen antibodies.

Antinuclear antibodies in considerable titre were described in four of nine cases of childhood scleroderma reported in 1976;¹ although low titres were found in the remaining five, none had antibodies to DNA or extractable nuclear antigens or low complements. In contrast to the findings of Hanson *et al.*² we did not find rheumatoid factor in our children with local scleroderma.

Antibodies to collagen types I and IV have been described in adult scleroderma,³ and other collagen antibodies have been shown in adults with rheumatic diseases⁴⁻⁶ and with a variety of non-rheumatic disorders.⁷⁻⁹ Some previous assays of apparent collagen antibody have been criticised on the grounds that fibronectin (cold insoluble globulin) could affect binding.¹⁰ Assays were therefore performed at room temperature in our studies, and fibronectin levels were measured independently by radial immunodiffusion.

The present report concerns a study of autoantibodies in children with a variety of the different scleroderma syndromes seen in childhood.

Patients and methods

Nineteen children were studied. Three had diffuse adult-type cutaneous scleroderma with Raynaud's

phenomenon; all three had serious contractures, in addition to which one had visceral involvement (kidney and heart) and another had begun as mixed connective tissue disease with antibody to the nuclear RNP antigen at that time before progressing to severe scleroderma with calcinosis. Four patients had acrosclerosis with calcinosis and Raynaud's phenomenon. In the remaining 12 children scleroderma was limited to sclerodactyly (one patient), linear morphea 'en coup de sabre' (four patients), multiple morphea (two patients), a single patch of morphea (one patient), and multiple cutaneous nodules consisting of fibrinoid material,¹ leading to skin tightness of hands and feet (four patients).

ANTINUCLEAR ANTIBODIES

Antinuclear antibodies (ANA) were examined on the HEp₂ cell substrate (Antibodies Inc, Davis, CA), with serum diluted 1:40 in phosphate-buffered saline and fluorescein isothiocyanate conjugated sheep antihuman IgG (Wellcome Laboratories, Beckenham, Kent). Nuclear, nucleolar, cytoplasmic, and mitotic cell staining was recorded with brightness on a 1-4+ scale. Under similar conditions 15% of 60 healthy adult control sera were ANA positive. The methods and variety of staining patterns revealed have been described previously.¹¹

COLLAGEN ANTIBODIES

Serum antibodies to collagens were measured by an ELISA technique. Flat-bottom microtitre plates

Accepted for publication 21 February 1985.

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which was finely speckled in nine, diffusely grainy in two, homogeneous in one, dots (probably in these cases micronucleoli) in two, and coarsely speckled in one. Eleven sera (58%) showed nucleolar staining, which was clumpy in two (associated with the micronucleolar staining in both cases), speckled in two, and homogeneous in seven (associated with fine speckled nuclear staining in eight and the diffusely grainy pattern in one). Overall, 11 sera gave staining of brightness $>2+$, four gave $1+$ staining, and four were negative. Only one serum gave cytoplasmic staining, and none contained anticentromere antibody.

A combination of antinuclear and antinucleolar antibodies was observed in all seven patients with either diffuse scleroderma or acrosclerosis and in three of the four patients with nodules (Table 1). Three of the four patients with linear morphea were ANA positive (only one with antinucleolar antibody), as were both patients with multiple morphea. The ANA negative patients thus comprised one of four patients with nodules, one of four with linear scleroderma, the one patient with isolated sclerodactyly, and the one case with a single patch of morphea. All patients with diffuse scleroderma or acrosclerosis and two-thirds of the patients with localised scleroderma were ANA positive.

COLLAGEN ANTIBODIES

It can be seen from Table 2 that there were no significant differences between the measured collagen antibody levels in the three groups tested. The number of samples with values more than one SD higher than the mean was similar for each group of subjects, and further analysis showed that there was no evidence that individual patients differed from age-matched normal controls.

FIBRONECTIN

The fibronectin concentration in each serum sample was compared with the collagen antibodies. No correlation was detected (Table 3).

Table 2 ELISA results expressed as mean $OD_{410\text{ nm}}$ * with 1 SD

		Native collagen type					Denatured collagen type				
		I	II	III	IV	V	I	II	III	IV	V
Juvenile (19) scleroderma	Mean	0.91	0.86	0.91	1.44	0.51	0.97	0.57	0.72	0.63	0.82
	SD	0.30	0.26	0.33	0.58	0.14	0.32	0.29	0.21	0.19	0.59
Juvenile (20) normal	Mean	0.91	0.88	0.95	1.34	0.58	1.01	0.64	0.79	0.57	0.73
	SD	0.29	0.38	0.34	0.45	0.26	0.34	0.46	0.39	0.22	0.26
Adult (20) normal	Mean	0.90	0.82	0.99	1.26	0.55	0.97	0.50	0.71	0.72	0.63
	SD	0.33	0.28	0.33	0.45	0.18	0.40	0.23	0.26	0.29	0.24

* $OD_{410\text{ nm}}$ =optical density at 410 nm.

Table 3 Correlation coefficients of serum fibronectin concentrations measured by immunodiffusion, with collagen antibodies measured by ELISA $OD_{410\text{ nm}}$ *

Collagen type	Native	Denatured
I	-0.008	-0.006
II	0.25	0.25
III	0.05	0.01
IV	0.31	0.007
V	0.29	0.002

* $OD_{410\text{ nm}}$ =optical density at 410 nm.

Discussion

Antinuclear antibodies are an uncommon finding in childhood, and only 10% of over 1400 patients seen in one paediatric immunology/rheumatology clinic were ANA positive (titre ≥ 20 on rat tissue).¹² Most of those ANA were associated with rheumatic disease, chiefly systemic lupus erythematosus and juvenile arthritis.

Sera from 96% of adults with systemic sclerosis and 73% with morphea were ANA positive when tested on a tissue culture cell substrate.^{11 13 14} Scleroderma is a rare condition in childhood. The present study shows that the prevalence of antinuclear and antinucleolar antibodies is as high as in adult scleroderma. Thus all seven children with diffuse scleroderma or acrosclerosis and eight (67%) of the 12 with localised scleroderma had ANA.

Anticentromere antibody is a common finding in adult CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia),^{11 13 15-17} yet this antibody was not found in any of the children's sera, though several had calcinosis, Raynaud's phenomenon, and acrosclerosis. A larger study might reveal anticentromere antibody in childhood. Alternatively, it may be that CREST syndrome is a purely adult disease, evolving as it usually does from a long prodrome of Raynaud's phenomenon.

Anti-Scl-70 antibody (recognised by immunodiffusion) is a marker for systemic sclerosis found in up to 20% of adult cases.^{11 13 16 17} Although we did not look for anti-Scl-70 directly in this study, we did note in two patients the diffusely grainy ANA pattern that is often associated with that antibody.¹¹

Antinucleolar antibodies have been reported in 43% of adults with systemic sclerosis and 41% with localised scleroderma, frequencies much higher than in other systemic rheumatic diseases.^{11 14 16} Among the children with scleroderma the prevalence of these antibodies was particularly high, including all seven patients with diffuse disease or acrosclerosis, and 33% of those with localised involvement. We have also observed antinucleolar antibody in two out of three patients with sclerodermatous graft-versus-host disease but not at all among patients with the scleroderma-like reaction to vinyl chloride (RMB, unpublished data). The link between scleroderma and the nucleolus remains obscure but could be of pathogenetic or aetiological significance. Within the cells nucleoli form around the DNA of the short arms or 'satellites' of the acrocentric D and G group chromosomes at their nucleolar organiser regions. One of the cytogenetic abnormalities found frequently in leucocytes from patients with scleroderma is increased 'satellite association' or clumping together of D and G group chromosomes in metaphase,¹⁸ and it is intriguing to consider whether antinucleolar antibodies might be involved in this process. Indeed, the speckled pattern antinucleolar antibody binds to a nucleolar organiser region protein (RNA polymerase I).¹⁹ On present evidence it seems unlikely that autoantibodies generally enter cells to cause damage directly; rather it may be that whatever agent causes the satellite associations also renders the nucleolus immunogenic.

Fibronectin in clotted blood samples has been reported to be a source of error in measuring collagen antibodies.^{10 20} Our study shows that there is no correlation between serum fibronectin and antibodies measured to collagen types I to V; using a two-tailed test of the probability that such a link existed, a correlation coefficient of >0.444 would be needed even at the 5% level, and this is not achieved (Table 3).

There is no apparent difference in antibody levels to any of the collagen types tested when comparison is made with age-matched or adult controls. In particular no cases in this study had significantly raised antibodies to native or denatured collagen type II. Collagen antibodies may not have pathogenic significance in the childhood disease.

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