Antireticulin antibody in systemic sclerosis

T Mahmud, M Peakman, G Senaldi, A McWhirter, C M Black, D Vergani

Abstract

The prevalence, immunoglobulin class, and IgG subclass of antireticulin antibody in the serum samples of 32 patients with systemic sclerosis were investigated by indirect immunofluorescence on unfixed rodent tissue. Antireticulin antibody was present in 22/32 (69%) of patients and belonged to the IgG class in 19/22 (86%), the IgA class in 13/22 (59%), and the IgM class in 6/22 (27%) of positive sera. IgG1 was the predominant subclass of IgG antireticulin antibody, occurring either alone or in association with IgG3 in 12/19 cases (63%). Thus antireticulin antibody of the IgG and IgA classes is found in most patients with systemic sclerosis. The finding of an autoantibody with reactivity for collagen-like fibres in systemic sclerosis indicates that the antibody has a potential role in the pathogenesis of the disease, and as it belongs to the IgA class this suggests that it arises in response to antigens presented to the immune system at the mucosal level.

Systemic sclerosis is a multisystem disorder of unknown aetiology characterised by abnormal deposition of collagen in the skin and internal organs, and microvascular obliteration, owing, in part, to intimal deposition of collagen.^{1,2} Circulating autoantibodies are a feature of systemic sclerosis, and include antibodies to various collagen types.³

Reticulin is a connective tissue component which stains black using a silver impregnation technique⁴ and which closely resembles type III collagen.⁵ Autoantibodies to reticulin have been described in several disorders, and derive their name from the fact that with immunofluorescence on rodent tissue they give a 'reticular' pattern which corresponds to that of reticulin fibres obtained by silver impregnation. Several variants of these antibodies have been described on the basis of subtle differences in the immunofluorescence patterns.⁶

Antireticulin antibodies have not previously been described in systemic sclerosis, but we reasoned that a disease process characterised by the production of antibodies to collagen might also result in the formation of antireticulin antibody. We therefore investigated the prevalence, titre, immunoglobulin class, and IgG subclass of antireticulin antibody in patients with systemic sclerosis.

Patients and methods

SUBJECTS

Thirty two patients (23 female, nine male, mean

age 46 years, range 17-74), all satisfying the preliminary American Rheumatism Association criteria for scleroderma, were studied.⁷ Patients were divided into two groups according to the extent of skin involvement^{7,8}: 19 had diffuse cutaneous involvement, while 13 had limited cutaneous involvement. Presence of organ involvement or other complications was also assessed. Serum samples were obtained from each patient and stored at -70°C until the time of testing.

One hundred and three healthy subjects (83 female, 20 male, mean age 32 years, range 18-48) were also studied as controls.

DETECTION OF ANTIRETICULIN ANTIBODY

Unfixed 5 µm sections of rodent liver, kidney, and stomach were used as substrate. The presence of antireticulin antibody was determined by conventional two step indirect immunofluorescence.⁹ Serum was decomplexed, diluted 1/10 in phosphate buffered saline (PBS, 0.15 mol/l, pH 7.2), and incubated with the substrate for 30 minutes. The sections were then washed in PBS for 15 minutes with two changes of buffer. To show the presence of antibody bound to the substrate fluorescein isothiocyanate (FITC) conjugated goat anti-total human immunoglobulin (Unipath) diluted 1/30 in PBS was added and the preparation was incubated for a further 30 minutes. After washing the slides were mounted in 90% glycerol in PBS and examined with a Polyvar fluorescence microscope (Reichert Jung).

CHARACTERISATION OF ANTIRETICULIN ANTIBODY *Immunoglobulin class*

The immunoglobulin class of antireticulin antibody was investigated by a similar two step indirect immunofluorescence technique using as second antibody FITC conjugated rabbit antihuman IgG, IgM, and IgA antisera (Wellcome) diluted 1/30 in PBS.

IgG subclass

The IgG subclass of antireticulin antibody was studied in a three step indirect immunofluorescence assay.¹⁰ The following murine monoclonal antibodies were used: anti-IgG1 (clone NL16), anti-IgG2 (GOM1), anti-IgG3 (ZG4), and anti-IgG4 (RJ4; all Oxoid). The specificity and reactivity of these monoclonal antibodies have been evaluated in a large World Health Organisation collaborative study.¹¹ Briefly, the decomplexed serum samples were diluted 1/10 in PBS and incubated with the substrate

Department of
Immunology, King's
College School of
Medicine and Dentistry,
Denmark Hill, London
SE5 8RX
T Mahmud
M Peakman
G Senaldi
D Vergani

Department of
Rheumatology, West
Middlesex Hospital,
Isleworth, Middlesex
A McWhirter
C M Black

Correspondence to:
Dr Vergani.

Accepted for publication
5 June 1989

Figure 1: Distribution of antireticulin antibody according to immunoglobulin class. (■) represents IgG or IgA antireticulin antibody occurring alone.

Figure 2: Prevalence of IgG subclasses of antireticulin antibody among patients with systemic sclerosis.

Figure 3: Staining pattern produced by the serum of one patient with systemic sclerosis containing antireticulin antibody as detected by indirect immunofluorescence on mouse tissue. (a) Periportal staining; (b) endothelial staining of a large renal vessel.

for 30 minutes. After washing the samples in PBS the substrate was incubated with each subclass specific monoclonal antibody diluted 1/30 in PBS. The presence of IgG subclasses of antireticulin antibody was shown by addition of FITC rabbit antimouse immunoglobulins (Dakopatts) diluted 1/30 in PBS, and incubation for 30 minutes. After washing the samples in PBS autoantibody patterns were examined as above.

COMPARISON OF ANTIRETICULIN AND ANTIPROCOLLAGEN TYPE III ANTIBODY STAINING PATTERNS

To explore the possibility that the pattern observed with antireticulin antibody is a result of anticollagen type III reactivity we compared the staining given by antireticulin antibody with that obtained using an antibody which detects collagen type III. This antibody was raised in rabbit and is directed to the amino terminal propeptide of type III procollagen. The amino terminal propeptide is retained on mature type III collagen fibrils.¹² This reagent was used in the first layer of a two step indirect immunofluorescence technique and the staining pattern visualised with FITC conjugated swine anti-rabbit (Dakopatts) and examined as above.

Control experiments were performed using PBS in place of the test serum or reagent in the first step of both the two and three step indirect immunofluorescence techniques.

STATISTICAL ANALYSIS

The distribution of antireticulin antibody according to different clinical characteristics was analysed by Fisher's exact test.

Results

Negative results were obtained in the 103 healthy subjects and when test serum was omitted as the first step of the immunofluorescence.

Antireticulin antibody was found in 22/32 (69%) patients, with titres ranging from 1/10 to 1/80. There was no association between the presence and titre of antireticulin antibody and the degree of skin or organ involvement.

Antireticulin antibody of the IgG class was found in 19/22 (86%) positive patients, of the IgA class in 13/22 (59%), and of the IgM class in 6/22 (27%) (fig 1). Antireticulin antibody was confined to the IgG class in six patients and to the IgA class in two; IgM was always found in association with at least one other isotype. There was no significant association between the degree of skin or organ involvement and the presence of antireticulin antibody of any immunoglobulin class.

In the 19 patients with antireticulin antibody of the IgG class, the IgG1 subclass was found in 16. In seven cases IgG1 was the only isotype found, while it was associated with IgG3 in five cases (fig 2). In the remaining four, IgG1 was associated with IgG2, IgG3, and IgG4 (one case); IgG2 and IgG3 (one case); IgG2 and IgG4 (one case); and IgG4 (one case). Of the three

Figure 4: Staining pattern produced by antibody directed against the amino terminal propeptide on collagen type III on a large vessel in kidney tissue. There is a characteristic cotton wool appearance perivascularly and intermittent peritubular staining.

patients negative for the IgG1 isotype, antireticulin antibody belonged to the IgG2 and IgG3 isotypes in one patient, to IgG2 and IgG4 in the second, and to IgG4 in the third. There was no significant association between the degree of skin or organ involvement and the presence of antireticulin antibody of any IgG subclass.

The immunofluorescence pattern of the antireticulin antibody characteristic of systemic sclerosis is known as antireticulin type 2⁶: this stains the periphery of portal tracts in the liver (fig 3a) and the endothelial surfaces of large blood vessels in the kidney, leaving the tubules unstained (fig 3b). The antibody used to detect collagen type III also stains the periphery of the portal tract but, in contrast with antireticulin antibody type 2, it stains the perimeter of tubules in an intermittent fashion and the perivascular aspects of large vessels with a characteristic cotton wool appearance (fig 4).

Discussion

This study shows that antireticulin antibody is present in most patients with systemic sclerosis, and of particular interest is the comparatively high prevalence of IgA class antireticulin antibody.

We are not aware of previous reports describing antireticulin antibody in patients with systemic sclerosis and this may be owing, in part, to the variety of different techniques which can be used to investigate autoantibodies in the connective tissue disorders. Whereas the detection of antireticulin antibody requires the use of immunofluorescence on a substrate consisting of tissues from several organs, the study of autoimmune serology in systemic sclerosis has often focused on the fluorescence patterns of antinuclear antibodies,¹³⁻¹⁷ which entails the use of cell lines such as HEP₂ as substrate. As these are single cell preparations they do not allow the detection of connective tissue autoantibodies such as antireticulin.

The explanation for the high prevalence of antireticulin antibody, particularly of the IgA class, in systemic sclerosis is not known. This autoantibody is characteristically found in a variety of gastrointestinal disorders with putative immune pathogenesises, such as Crohn's disease and coeliac disease.^{18,19} In these disorders the antibody also often belongs to the IgA class, and in childhood coeliac disease IgA antireticulin antibody has been found to be of diagnostic value²⁰ and to reflect disease activity.²¹ As IgA is the main immunoglobulin class found in secretions and has a role in mucosal immunity it is suggested that the inciting stimulus for IgA antireticulin production occurs at mucosal sites. This raises the intriguing possibility that there is an abnormal immune response to antigens presented to the immune system at the mucosal level in systemic sclerosis, resulting in the production of an IgA autoantibody.

An additional finding in this study is the relative IgG1 subclass restriction of the antireticulin antibodies. Subclass restriction of autoantibodies has also been described in other connective tissue diseases. Anti-double-stranded DNA antibodies, for example, are predominantly of the IgG1 and IgG3 subclasses in systemic lupus erythematosus.²² In lupus the subclass restriction of autoantibodies has been ascribed to abnormalities of immune regulation,²² and a similar mechanism may prevail in systemic sclerosis.

The precise biochemical and structural nature of reticulin is not known, although a body of opinion holds that it is similar to type III collagen.⁵ We compared the staining patterns of antireticulin antibodies and antibodies to the amino terminal propeptide of procollagen type III which is retained on mature type III collagen.¹² The patterns observed with antireticulin and this antibody in the portal tracts were similar, suggesting that they are directed against antigens which codistribute. In contrast, however, the differences seen in the staining in the kidney and large vessels indicate that the antigenic specificities of antireticulin antibody in systemic sclerosis and anticollagen type III are distinct.

In summary, this study reports the prevalence of antireticulin antibody, particularly of the IgA class, in patients with systemic sclerosis. The reactivity of antireticulin for collagen-like fibres suggests that the antibody arises as part of the disease process which leads to systemic sclerosis, while the fact that it belongs to the IgA class implies that it is elicited in response to antigens presented at the mucosal level. This might be a primary event with direct pathogenic relevance or a secondary, reactive phenomenon in keeping with the putative role of autoantibodies in the clearance of autoantigens.

We thank Dr J Risteli and Dr L Risteli for supplying the anticollagen antibody used in this study. Dr G Senaldi is supported by Fondazione Anna Villa Rusconi, Varese, Italy. This work was supported by the Raynaud's Association Trust.

1 LeRoy E C. Scleroderma (systemic sclerosis). In: Kelley W N, Harris E D Jr, Ruddy S, Sledge C B, eds. *Textbook of rheumatology*. 2nd ed. Philadelphia: Saunders, 1985: 1183-205.

- 2 Perlsh J S, Bashey R I, Stephens R E, Fleischmajer R. Connective tissue synthesis by cultured scleroderma fibroblasts. I. In vitro collagen synthesis by normal and scleroderma dermal fibroblasts. *Arthritis Rheum* 1976; **19**: 891-901.
- 3 Mackel A M, DeLustro F, Harper F E, LeRoy E C. Antibodies to collagen in scleroderma. *Arthritis Rheum* 1982; **25**: 522-31.
- 4 Valdrighi L, Vidol B C. Silver impregnation: collagenic and reticular fibres and the ground substance. *Annales d'Histochimie* 1971; **16**: 29-40.
- 5 Seyer J M, Kang A H. Structural Proteins: collagen, elastin and fibronectin. In: Kelly W N, Harris E D Jr, Ruddy S, Sledge C B, eds. *Textbook of rheumatology*. 2nd ed. Philadelphia: Saunders, 1985: 211-37.
- 6 Rizzetto M, Doniach D. Types of "reticulin" antibodies detected in human sera by immunofluorescence. *J Clin Pathol* 1973; **26**: 841-51.
- 7 Subcommittee for scleroderma criteria of the American Rheumatology Association diagnostic and therapeutic criteria committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; **23**: 581-90.
- 8 LeRoy E C, Black C M, Krieg T, Medsger T, Rowell N, Wollheim F. Scleroderma (systemic sclerosis): classification, subsets, and pathogenesis. *J Rheumatol* 1988; **15**: 202-5.
- 9 Johnson G D, Holborow E J, Dorling J. Immunofluorescence and immunoenzyme techniques. In: Weir D M, ed. *Handbook of experimental immunology*. Vol 2. Oxford: Blackwell Scientific, 1978: 15.1-30.
- 10 Peakman M, Lobo-Yeo A, Mieli-Vergani G, Davies E T, Mowat A P, Vergani D. Characterisation of anti-liver kidney microsomal antibody in childhood autoimmune chronic active hepatitis: evidence for IgG1 subclass restriction, polyclonality and non cross-reactivity with hepatocyte surface antigens. *Clin Exp Immunol* 1987; **69**: 543-9.
- 11 Jefferis R, Reimer C B, Skvarile F, et al. Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of an IUIS/WHO collaborative study. *Immunol Lett* 1985; **10**: 223-52.
- 12 Fleischmajer R, Timpl R, Tuderman L, et al. Ultrastructural identification of extension aminopeptides of type I and III collagens in human skin. *Proc Natl Acad Sci USA* 1981; **78**: 7360-4.
- 13 Rothfield N F, Rodnan G P. Serum antinuclear antibodies in progressive systemic sclerosis (scleroderma). *Arthritis Rheum* 1968; **11**: 607-17.
- 14 Tan E M, Rodnan G P, Garcia I, Moroi Y, Fritzler M J, Peebles C. Diversity of antinuclear antibodies in progressive systemic sclerosis: anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum* 1980; **23**: 617-25.
- 15 Bernstein R M, Steigerwald J C, Tan E M. Association of antinuclear and antinucleolar antibodies in progressive systemic sclerosis. *Clin Exp Immunol* 1982; **48**: 43-51.
- 16 Moroi Y, Peebles C, Fritzler M, Steigerwald J C, Tan E M. Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc Natl Acad Sci USA* 1980; **77**: 1627-31.
- 17 Catoggio L J, Bernstein R M, Black C M, Hughes G R V, Maddison P J. Serological markers in progressive systemic sclerosis: clinical correlation. *Ann Rheum Dis* 1983; **42**: 23-7.
- 18 Alp M H, Wright R. Autoantibodies to reticulin in patients with idiopathic steatorrhea, coeliac disease, and Crohn's disease, and their relation to immunoglobulins and dietary antibodies. *Lancet* 1971; **ii**: 682-5.
- 19 Seah P P, Fry L, Holborow E J, et al. Antireticulin antibody: incidence and diagnostic significance. *Gut* 1973; **14**: 311-5.
- 20 Maki M, Hallstrom O, Vesikari T, Visakorpi J K. Evaluation of a serum IgA-class reticulin antibody test for the detection of childhood coeliac disease. *J Pediatr* 1984; **105**: 901-5.
- 21 Zouali M, Jefferis R, Eyquem A. IgG subclass distribution of autoantibodies to DNA and to nuclear ribonucleoproteins in autoimmune diseases. *Immunology* 1984; **51**: 595-600.
- 22 Eisenberg R A, Dyer K, Graven S Y, Fuller C R, Yount W J. Subclass restriction and polyclonality of systemic lupus erythematosus marker anti-Sm. *J Clin Invest* 1985; **75**: 1270-7.