

Antibodies to mouse laminin in patients with systemic sclerosis (scleroderma) recognize galactosyl (α 1–3)-galactose epitopes

A. GABRIELLI, M. CANDELA*, A. M. RICCIATTI*, M. L. CANIGLIA* & J. WIESLANDER† *Max-Planck Institut für Biochemie, Martinsried bei München, Germany (On leave of absence from Istituto di Clinica Medica, Università degli Studi, Ancona, Italy), *Istituto di Clinica Medica, Università degli Studi, Ancona, Italy, and †Biocarb AB, Lund, Sweden*

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SUMMARY

Employing radioimmuno-inhibition assays with distinct oligosaccharides as inhibitors, this study demonstrates that the epitope recognized on mouse laminin by sera from patients with systemic sclerosis (scleroderma) is a terminal galactosyl (α 1–3)-galactose disaccharide. The reaction with this α -digalactose was further confirmed when the sera were tested in radioimmunoassay (RIA) binding assay and in ELISA with synthetic galactose α 1–3 galactose coupled to human serum albumin. The circulating antibody appeared restricted to the IgG class and mostly to the subclass IgG3 and IgG4. Antibodies with the same specificity can be found in patients with American cutaneous leishmaniasis and Chagas disease; however, whilst in these diseases the antibody production is triggered by antigenic determinants present on the surface of the parasites, the events eliciting their appearance in systemic sclerosis are unknown.

Keywords Gal α 1–3Gal laminin scleroderma

INTRODUCTION

Serum antibodies against basement membrane antigen laminin, purified from the Engelbroth–Holm–Swarm mouse sarcoma, have been detected in a good proportion of patients with systemic sclerosis (scleroderma) (SSc) (Gabrielli *et al.*, 1988). At present, their pathogenetic role is still unclear. Recently, however, sera from patients with American cutaneous leishmaniasis, Chagas disease and from monkeys infected with *Trypanosoma cruzi* or *Trypanosoma rhodiensis*, showing high binding to mouse laminin, have been found to recognize on this basement membrane antigen a carbohydrate epitope which appears to be a terminal galactose in α 1–3 linkage to a second molecule of galactose (Gal α 1–3Gal) (Towbin *et al.*, 1987; Avila, Rojas & Towbin, 1988). In this study we provide evidence that an identical or very similar epitope is recognized on mouse laminin by SSc sera.

PATIENTS AND METHODS

Sera

Inhibition and titration studies were performed using some of the sera with high binding capacity for mouse laminin obtained by screening sera of 56 consecutive SSc patients evaluated at Istituto di Clinica Medica, Ancona, Italy, during the period 1986–1988. The 56 patients have been part of a multicentre

clinical study undertaken to determine the prevalence and the clinical significance of anti-Gal α 1–3Gal (anti-Gal) antibodies in systemic sclerosis. The results of this study are reported elsewhere (Gabrielli *et al.*, in press).

Diagnosis of SSc was made according to accepted criteria (Subcommittee for Scleroderma Criteria, 1980).

Sera from patients with American cutaneous leishmaniasis (ACL) were kindly provided by Dr Rupert Timpl (Münich, Germany). Since they contain high levels of anti-mouse laminin and anti-Gal α 1–3Gal antibodies, they were used in some experiments for comparison with SSc sera. Age and sex matched normal volunteers, recruited from the geographical areas of the SSc and ACL patients, constituted the control group.

Blood was obtained by venepuncture, allowed to clot at room temperature for 1–3 h and centrifuged at 1500 g. The serum was removed and divided into aliquots which were stored at -70°C . Sera were thawed once only.

Antigens and inhibitors

Laminin was purified from the mouse Engelbroth–Holm–Swarm tumour (Timpl *et al.*, 1979). Various mono and oligosaccharides were obtained from BioCarb AB (Lund, Sweden) or Sigma Chemical Co. (St Louis, MO) and were either natural or synthetic products.

Synthetic carbohydrate conjugate

Disaccharides were coupled to human serum albumin (HSA) according to described procedures (Zopf *et al.*, 1978; Kallin, Lonn & Norberg, 1986; Wieslander *et al.*, 1990). Briefly, a

Correspondence: Armando Gabrielli, Istituto di Clinica Medica, Ospedale Regionale, Torrette di Ancona, 60020 Ancona, Italy.

solution of albumin (22 mg in 3.5 ml of 0.1 M phosphate, pH 9.5) was mixed with a solution of the disaccharide *p*-isothiocyanatophenylethylglycosid (0.01 mmol) in water (1.0 ml). The pH was adjusted to 10 and the mixture stirred overnight at room temperature. The yield of coupled carbohydrate was determined by the anthron reaction. The incorporation varied from 14 to 30 mol/mol.

Radioimmunoassay

Laminin was labelled with ^{125}I to a specific activity of 8000–12 000 ct/min per nanogram by the chloramine T procedure and used at 2 ng/0.4 ml.

Direct binding analysis was performed after standard protocol (Timpl, 1982). Briefly, human sera were diluted in Dulbecco's phosphate-buffered saline (pH 7.8), containing 0.05% Tween 20 (PBS-Tween) and 100 μl of two-fold serially diluted sera were pipetted into glass tubes, in duplicate. Control tubes for determining non-specific binding received 100 μl of 0.5% normal human serum without antibody. The content of each test tube was then mixed with 100 μl of a working dilution of labelled antigen (containing 0.5 ng to provide 4000–6000 ct/min protein-bound radioactivity). After addition of 200 μl PBS-Tween, the samples were incubated for about 16 h at 4°C. Then a pretested amount of second antiserum was rapidly added to each test tube and incubation was continued for another 16–24 h at 4°C. Subsequently, precipitates were collected in a refrigerated centrifuge (4°C, 30 min, 1500 g) and the supernatant fluid carefully sucked off by aspiration on a water pump. The precipitates were washed two times with cold PBS-Tween and their radioactivity determined by counting in a well-type scintillation crystal (Beckman gamma 4000 counting system; Beckman, Fullerton, CA). The results were expressed as per cent of total antigen added bound by antibody: antigen bound (%) = $a - b/c - b \times 100$, where a = ct/min in precipitates containing first antibody, b = ct/min in precipitates containing non-immune carrier, and c = total protein-bound radioactivity.

For radioimmuno-inhibition assay (Timpl & Risteli, 1982) each test tube received 100 μl of antiserum dilution sufficient to bind 50% of the labelled antigen added later. The tubes (blank) for determining non-specific binding of labelled antigen received only normal human serum without antibody. The antiserum-containing test tubes were then mixed with varying amounts of non-labelled inhibitor dissolved in 200 μl of PBS-Tween, while the blanks and the two tubes with antibody (antigen-binding in the absence of inhibitor) received 200 μl of PBS-Tween. The mixtures were incubated for 16 h at 4°C and after addition of 100 μl of labelled antigen were further incubated for 6 h at 4°C. The addition of second antibody, the collection, washes and determination of radioactivity of the precipitates were performed as described for the binding assay. The results were calculated in the following way: inhibition of antigen binding (%) = $1 - (c - b/a - b) \times 100$, where a = ct/min in precipitates containing first antibody and only labelled antigen (antigen binding in the absence of the inhibitor), b = ct/min in precipitates containing non-immune carrier, and c = ct/min in precipitates containing first antibody and labelled and non-labelled antigen.

ELISA

For ELISA (Engvall & Perlmann, 1972), plates were coated with Gal α 1–3Gal coupled to HSA (1 $\mu\text{g}/\text{ml}$, 200 $\mu\text{l}/\text{well}$) dissolved in

0.05 M sodium carbonate, pH 9.5–9.7. The plates were extensively washed with Dulbecco's PBS (pH 7.8), containing 0.05% Tween 20 (PBS-Tween), before use. Non-specific sites on the plates were blocked by two further incubations with PBS-Tween containing 1% HSA for 60 min each. Human sera were diluted in PBS-Tween and 200 μl of two-fold serially diluted sera were added in duplicate to antigen-coated wells. After 60 min of incubation at room temperature, with rocking, the plates were again washed with PBS-Tween and 200 μl of alkaline phosphatase-conjugated swine anti-human IgG, IgA and IgM antibody (Orion, Esbo, Finland) were added to each well, at 1/100 dilution in PBS-Tween. The plates were incubated for an additional 60 min and, after thorough washing, bound reactants were detected, after 90 min of incubation, with *p*-nitrophenylphosphate. The absorbance was determined at 405 nm on a Multiskan plate reader (Titertek; Flow, Hamden, CT).

In all tests the following controls were included: (i) pooled negative serum control; (ii) pooled positive serum control; (iii) conjugate control (i.e. instead of serum, PBS, pH 7.8, containing 1% HSA was added; (iv) substrate control (i.e. instead of serum and conjugate, PBS, pH 7.8, containing 1% HSA was added); (v) control for non-specific binding (i.e. wells treated with coating buffer without antigen). For each serum dilution the result was calculated, subtracting the mean absorbance of the non-specific binding from the absorbance obtained in antigen-coated wells. The results have been reported as absolute absorbance.

The interassay coefficients of variation of the pooled negative and pooled positive serum controls were 2.5% and 5.6%, respectively.

The determinations of the immunoglobulin class and IgG isotype of the anti-galactose antibodies were performed in a similar way, with human sera diluted 1/100 in PBS-Tween, followed by the addition of peroxidase-conjugated goat anti-human IgG, IgA and IgM (Cappel, PA) or of mouse monoclonal anti-human IgG subclasses and peroxidase-conjugated goat anti-mouse immunoglobulin. Optimal dilutions of mouse monoclonal anti-human IgG1, 2, 3, and 4 antibodies were determined on the basis of preliminary titration studies, using in ELISA assays known concentrations of human myeloma IgG1, 2, 3 and 4. The plates were developed with ABTS and the reaction read at 417 nm on a Multiskan plate reader (Titertek, Flow). The results have been expressed as absolute absorbance or as a ratio of the absorbance value of serum samples to the mean of three known negative sera.

Statistical analysis

Data have been expressed as mean $\pm$ s.e.m. Scheffe's method has been employed for multiple comparison between groups. Student's *t*-test was employed for comparison of means. All reported values are two-tailed; *P* values less than 0.05 were regarded as significant.

RESULTS

All sera tested against mouse laminin produced a clear titration curve. The titres of antisera were highest in patients with American cutaneous leishmaniasis, followed by patients with systemic sclerosis and by normal subjects (Fig. 1).

Previous studies have demonstrated that the antibodies against mouse laminin in patients with cutaneous leishmaniasis

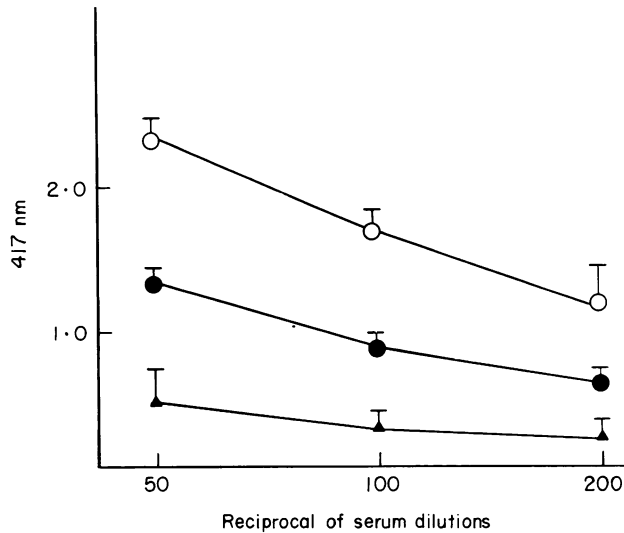


Fig. 1. Antibodies against mouse laminin in three patients with American cutaneous leishmaniasis (○), in three patients with systemic sclerosis (●) and in three normal controls (▲).

and with Chagas disease recognize on the basement membrane antigen a terminal galactosyl (α 1-3)-galactose group. In Table 1 we provide evidence that Gal α 1-3Gal is a good inhibitor of the reaction between SSc sera and mouse laminin, although less active on a molar and weight basis. This feature can be ascribed to the tighter interaction of both binding sites of the antibody with the possible multiple α -galactose epitopes of mouse laminin. No inhibition was demonstrated when Gal α 1-2Gal or Gal α 1-4Gal were employed as inhibitors.

Among the sera tested, individual variations were noted in the quantitative aspects of the inhibition which may be explained by the polyclonal characteristics of the sera and/or the individual variability of the immune response.

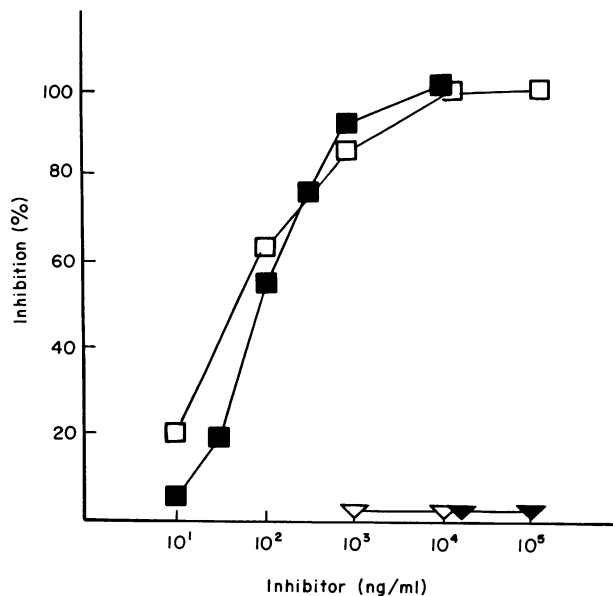


Fig. 2. Inhibition of the RIA reaction between ¹²⁵I-labelled mouse laminin and a systemic sclerosis serum by mouse laminin (□), Gal α 1-3Gal-HSA (■), Gal α 1-4Gal-BSA (▽), and HSA (▼).

Table 1. Carbohydrate specificity of the anti-mouse laminin reaction of individual SSc sera determined by radioimmunoassay*

Inhibitor	Concentration (μ M) required for 50% inhibition			
	SSc 1	SSc 2	SSc 3	SSc 4
Laminin	6×10^{-4}	15×10^{-5}	2×10^{-4}	3×10^{-5}
Gal α 1-3Gal-HSA	2×10^{-3}	2×10^{-4}	4×10^{-2}	1×10^{-4}
Gal α 1-3Gal	2.4	5.0	20	90
Gal α 1-2Gal	40	70	40	150
Gal α 1-4Gal	> 1500	> 1500	> 1500	> 1500
HSA	> 1500	> 1500	> 1500	> 1500

* Using mouse laminin as labelled antigen.

SSc, Systemic sclerosis (scleroderma); HSA, human serum albumin.

When Gal α 1-3Gal was coupled to HSA, the resulting conjugate, with approximately 14-30 epitopes per molecule, had a hapten density comparable to that estimated for mouse laminin. Therefore, it was not surprising that its inhibition capacity was stronger than the uncoupled Gal α 1-3Gal and comparable to mouse laminin in radioimmunoassay (RIA) inhibition using laminin as tracer (Table 1). The slope of the inhibition curve is illustrated in Fig. 2. Gal α 1-3Gal-HSA produced almost complete inhibition with profiles similar in steepness to that of laminin. The reaction could not be inhibited by other conjugates.

Thus, the synthetic antigen (i.e. Gal α 1-3Gal-HSA) appeared to be able to replace the natural antigen (i.e. mouse laminin) and this was further confirmed by RIA inhibition experiments with labelled Gal α 1-3Gal-HSA (Table 2). Low

Table 2. Carbohydrate-conjugate and oligosaccharide inhibition of the reaction between individual systemic sclerosis sera (SSc1-SSc3) and labelled Gal α 1-3Gal-HSA conjugate*

Inhibitor	Concentration (μ M) required for 50% inhibition		
	SSc 1	SSc 2	SSc 3
Laminin	5×10^{-5}	5×10^{-5}	6×10^{-5}
Gal α 1-3Gal-HSA	6×10^{-5}	5×10^{-4}	2×10^{-4}
Gal α 1-2Gal-HSA	> 0.15	> 0.15	> 0.15
Gal α 1-4Gal-BSA	> 0.15	> 0.15	> 0.15
Gal α 1-6Glc-BSA	> 0.15	> 0.15	12×10^{-3}
Gal α 1-3Gal	3.2	0.5	0.4
Gal α 1-3(Fuc α 1-2)Gal	> 68	> 68	> 68
Gal α 1-4Gal	> 1500	> 1500	> 1500
Gal α 1-6Gal	> 910	700	8.0
α -methylgalactose	> 20×10^3	18×10^3	> 200
β -methylgalactose	> 20×10^3	> 20×10^3	> 20×10^3
Galactose	> 11×10^3	4000	< 200
HSA	> 1500	> 1500	> 1500
BSA	> 1500	> 1500	> 1500

*Determined by RIA inhibition.

HSA, human serum albumin; BSA, bovine serum albumin; SSc, systemic sclerosis (scleroderma).

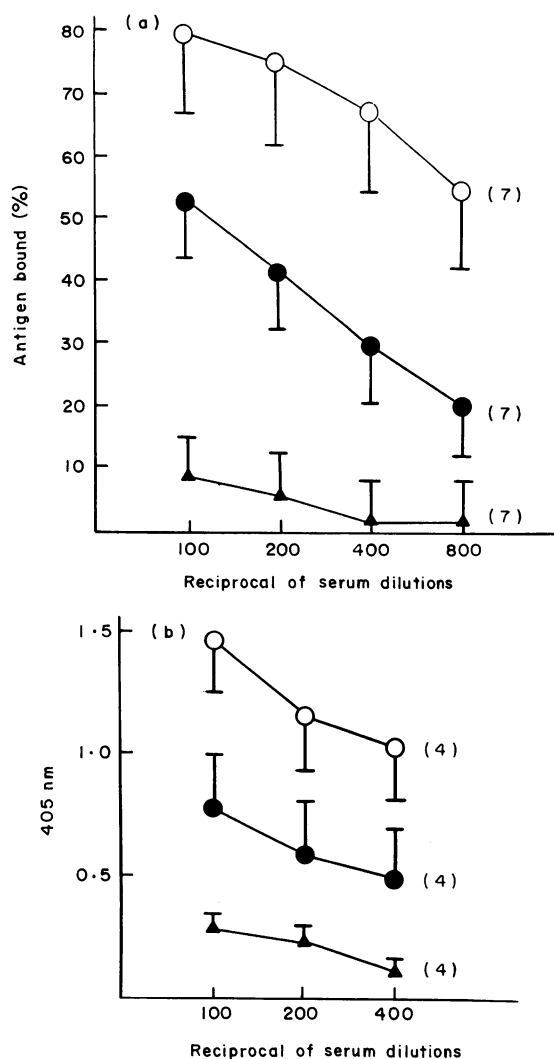


Fig. 3. Comparison of RIA (a) and ELISA binding (b) of Gal α 1-3Gal-HSA by sera of patients with American cutaneous leishmaniasis (O), systemic sclerosis (●) and of normal subjects (▲). Numbers in parentheses indicate number of subjects studied.

concentrations of the unlabelled Gal α 1-3Gal-HSA can inhibit the reaction; this did not occur when other α -linked digalactose conjugates were employed. A weaker inhibition was observed with varying the position of the linkage of the two galactose molecules or with the change of the terminal galactose with glucose.

Substitution of an additional fucose residue, such as that occurring in B blood group substance, Gal α 1-3, (Fuc α 1-2)Gal, reduces the affinity for the antibody and implies that the penultimate galactose is also recognized by the antibody. Generally, conjugated epitopes behaved better than the uncoupled antigens, probably because of a higher number of available epitopes and/or higher binding affinity.

Thus, the data given in this study support the conclusion that Gal α 1-3Gal is or is part of an epitope recognized on mouse laminin by SSc sera.

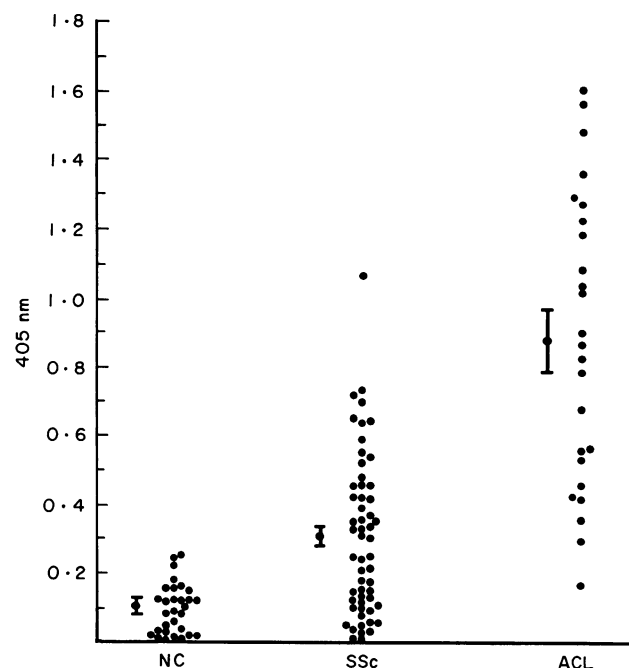


Fig. 4. Distribution of anti-Gal α 1-3Gal antibodies in 30 normal controls (NC), in 56 patients with systemic sclerosis (SSc) and in 25 patients with American cutaneous leishmaniasis (ACL). Sera were diluted 1/100 in PBS-Tween with 1% HSA before the ELISA assay was performed as described in the Patients and Methods section. Bars show s.e.m.

Table 3. Distribution of Gal α 1-3Gal antibodies among serum immunoglobulin classes in patients with systemic sclerosis and in controls*

Groups	No. of subjects	417 nm†		
		IgG	IgA	IgM
Systemic sclerosis	56	0.370 $\pm$ 0.05‡	0.035 $\pm$ 0.05	0.030 $\pm$ 0.04
Controls	30	0.163 $\pm$ 0.06	0.048 $\pm$ 0.02	0.045 $\pm$ 0.01

* Sera employed were diluted 1/100 in PBS-Tween.

† Mean $\pm$ s.e.m. The results are expressed as absolute absorbance.

‡ $P < 0.001$ versus normal controls by Student's *t*-test.

Previous methods for the detection of humoral immunity to Gal α 1-3Gal structures employed complex antigens with very likely different epitope distribution and structures (Galili *et al.*, 1984, 1985, 1987a, 1987b; Towbin *et al.*, 1987; Avila *et al.*, 1988; Fujiwara *et al.*, 1988; Klein *et al.*, 1989). In order to make the assay independent of the carrier moieties, new assays were recently developed with Gal α 1-3Gal coupled to HSA as antigen, which enabled us to assess the level of anti-Gal antibodies. Figure 3a shows a titration curve of reactivity of selected sera from patients with ACL, with SSc and normal controls in a RIA binding assay, indicating that the antibody in the serum accounted for the observed reactivity. The detection of these antibodies was more sensitive with the synthetic

Table 4. IgG subclasses of antibodies against Gal α 1-3Gal in patients with systemic sclerosis and in normal controls

Groups	No. of patients	417 nm*			
		IgG1	IgG2	IgG3	IgG4
Systemic sclerosis	56	1.15 $\pm$ 0.20	1.28 $\pm$ 0.15	1.77 $\pm$ 0.14†	1.79 $\pm$ 0.08†
Controls	30	1.02 $\pm$ 0.10	1.00 $\pm$ 0.30	1.03 $\pm$ 0.04	0.98 $\pm$ 0.05

* Mean $\pm$ s.e.m.† $P < 0.05$ versus normal controls by Student's *t*-test.

conjugate than with mouse laminin, which produced a lower binding profile. A similar sensitivity was obtained when the Gal α 1-3Gal was employed in an immunoenzymatic assay (Fig. 3b).

The level of reactivity of SSc sera fell between normal controls and ACL patients even when all patients were analysed (Fig. 4). The mean $\pm$ s.e.m. absorbance value of sera from patients with SSc (0.315 $\pm$ 0.03) was statistically greater ($P < 0.05$) than that of sera from normal controls (0.101 $\pm$ 0.01) but lower ($P < 0.05$) than in the ACL group (0.886 $\pm$ 0.08).

No correlation was found between the level of anti-Gal α 1-3Gal antibodies and the total serum IgG.

Tables 3 and 4 show the immunoglobulin class distribution and the IgG subclasses of anti-Gal antibodies in the control group and in the SSc patients. The antibody activity was exclusively restricted to the IgG class, with no reactivity in IgM and IgA antibody; instead, the analysis of the IgG subclass of the antibody revealed a predominance of IgG3 and IgG4. Furthermore, using a 2 s.d. above the mean of normals as cut off for abnormal levels of IgG subclass antibodies, it is noteworthy that of 21 patients reacting with Gal α 1-3Gal, 13 (69%) had a reaction restricted to one subclass, mostly IgG3, six (28.5%) to two and only two patients (9.5%) had IgG anti-Gal antibodies belonging to three subclasses.

DISCUSSION

Vascular abnormalities are a consistent finding in the clinical history of systemic sclerosis (Fleischmajer *et al.*, 1976). Several reports have suggested a possible immunologically mediated vessel damage (Fleischmajer, Perlsh & West, 1977; Fleischmajer *et al.*, 1978; Lapenas, Rodnan & Cavallo, 1978; Penning *et al.*, 1984; Huffstutter, DeLustro & LeRoy, 1985; Evans, Cashman & Walport, 1987; Marks *et al.*, 1988) which seemed further supported by the demonstration, in SSc patients, of circulating antibodies against basement membrane antigen type IV collagen and laminin (Mackel *et al.*, 1982; Gabrielli *et al.*, 1988).

However, these later observations have been made employing, as reference antigens, constituents of mouse origin which may contain antigenic determinants not normally expressed in normal human basement membrane components. This is the case with Gal α 1-3Gal sequences which are widely distributed in

animal tissues, including basement membranes, whereas no such epitopes have been demonstrated in normal human structures.

The results of our study, showing that the specificity recognized on mouse laminin by sera of SSc patients is a Gal α 1-3Gal epitope, are important because they indicate that antibodies reacting with mouse laminin should not be considered the equivalent of anti-human basement membrane antibodies.

Since the Gal α 1-3Gal epitope is contained in distinct mouse basement membrane proteins (Towbin *et al.*, 1987), it is likely also that the antibodies against mouse type IV collagen detected in SSc sera by Mackel *et al.* (1982) are antibodies against Gal α 1-3Gal.

Thus, in order to analyse the presence of immunity against basement membrane components, future studies should involve the use, as reagents, of human basement membrane antigens.

As for the assessment of the serum levels of anti-Gal antibodies, the new RIA and ELISA assays, based on synthetic Gal α 1-3Gal-HSA, appeared very sensitive and less time consuming than previous techniques (Galili *et al.*, 1984, 1985, 1987a, 1987b; Avila, Rojas & Rieber, 1984; Avila *et al.*, 1986, 1987; Towbin *et al.*, 1987; Avila *et al.*, 1988; Klein *et al.*, 1989) and allowed us to demonstrate anti-Gal antibodies in a good proportion of SSc patients. This percentage (55.3%) is quite similar to that (48%) which reacted with mouse laminin in another group of patients with systemic sclerosis (Gabrielli *et al.*, 1988), further suggesting that the Gal α 1-3Gal sequences are the target of SSc sera. It is also likely that the same specificity is recognized by sera of the subgroup of patients with primary Raynaud's phenomenon and antibodies against mouse basement membrane antigens (Gabrielli *et al.*, 1988).

Recently, elevated anti-Gal antibodies have been detected in seemingly unrelated disorders, such as inner ear disease (Klein *et al.*, 1989), Chagas disease, ACL (Towbin *et al.*, 1987; Avila *et al.*, 1988), and here, systemic sclerosis. However, whereas in patients with trypanosoma and leishmania infections the antibodies are triggered by the Gal α 1-3Gal or similar antigenic determinants present on glycolipids or glycoproteins of the parasites (Szarfman *et al.*, 1982; Avila, Rojas & Galili, 1989), the stimuli evoking their production in systemic sclerosis and their role in the pathogenic process of the disorder remain unknown.

Normal subjects, as shown here also, possess a natural antibody against the Gal α 1-3Gal epitope although at a lower titre (Galili *et al.*, 1984, 1985). This may be stimulated by antigens of the diet and/or of bacterial strains of the intestinal flora expressing α -galactosyl epitopes (i.e. *Escherichia coli* and

Klebsiella pneumoniae) (Galili *et al.*, 1988). It cannot be ruled out that the same is happening in patients with SSc, in whom intestinal involvement with malabsorption and bacterial overgrowth is reported as a frequent event (Myers, 1979). Alternatively, other low grade infections, more or less related to the etiology of the disease, could be regarded as the triggering event of the antibody, considering that the α -galactosyl epitopes seem to be widely expressed on infectious agents, being present, among others on *E. coli*, *klebsiella* (Galili *et al.*, 1988) protozoa (Avila *et al.*, 1989), and salmonella (Luderitz, Simmons & Westphal, 1965).

Another question still unanswered is the role of anti-Gal antibodies. They have been considered involved in removal of altered erythrocytes (Galili *et al.*, 1984, 1985; Galili, Clark & Shohet, 1986) and in the recognition of tumour cells (Towbin *et al.*, 1987; Castronovo *et al.*, 1989) since evidence for the presence of oligosaccharides terminating with Gal α 1-3Gal has been found in senescent erythrocytes and in cancer cells.

Their implication in the pathogenesis of connective tissue diseases and in autoimmunity in general is less defined. Galili (1989) has demonstrated that species (humans and Old World monkeys) with circulating, natural anti-Gal antibodies do not show in tissues α -galactosyl epitopes, because of suppression of α 1-3 galactosyltransferase gene. He suggested that any mechanism activating this gene would induce an autoimmune process through the reaction of anti-Gal antibodies and newly formed α -galactosyl epitopes. Whether this mechanism takes place in systemic sclerosis is difficult to say.

Employing affinity-purified anti-Gal α 1-3Gal antibodies and an immunohistochemical technique, we failed to detect these epitopes in unfixed and acetone fixed cryostat sections of skin biopsies of three patients with SSc (unpublished data). However, it cannot be excluded that more sensitive techniques may be more successful and due to their central role in the pathogenesis of SSc, fibroblasts should be the first cells to be explored for abnormal glycosylation.

In any case, the tendency of patients with early onset SSc to have high levels of anti-Gal antibodies (Gabrielli *et al.*, in press) implies that they may be part of an early event in the clinical history of the disease. Furthermore, their presence in patients with other unrelated fibrotic disorders such as idiopathic pulmonary fibrosis or idiopathic myofibrosis may suggest a relationship with the process of fibrosis (Gabrielli *et al.*, 1991).

The predominance of IgG antibodies to Gal α 1-3Gal is at variance with what has been found in patients with parasitic disease, where the reaction against Gal α 1-3Gal epitopes was mainly distributed among IgG and IgM (Avila *et al.*, 1988). However, it remains to be proved whether this finding is relevant for the understanding of the pathogenesis of systemic sclerosis.

Finally, of interest appears the preferential production of IgG3 and IgG4, since others have found that IgG antibodies to polysaccharides consist predominantly of IgG2 (Yount *et al.*, 1968) and that, anyhow, in autoimmune diseases IgG autoantibodies are usually represented among all subclasses in a normal distribution (Schur, Monroe & Rothfield, 1972). Since IgG3 is the most potent complement-activating IgG subclass in humans, the presence of IgG3 could be consistent with the concept that humoral immunity to the galactose may be relevant for the development and/or the maintenance of the inflammatory process.

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