Anti-IgE autoantibodies in systemic sclerosis (scleroderma)

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SUMMARY An enzyme immunoassay was used to determine the prevalence of anti-IgE autoantibodies in 66 patients with systemic sclerosis (scleroderma) stratified according to extent and duration of disease. Serum IgG anti-IgE antibodies were detected in 14 (21%) patients and IgM anti-IgE antibodies in nine (14%) patients. The overall prevalence of IgG or IgM isotypes was 21/66 (32%). Anti-IgE autoantibodies were not found in six patients with undifferentiated connective tissue disease or two patients with eosinophilic fasciitis. Attempts to demonstrate histamine release from basophils in vitro by using serum samples containing high titre anti-IgE antibody were unsuccessful. By multivariate analysis the presence of anti-IgE antibody was not associated with duration of systemic sclerosis; extent of scleroderma; specific visceral features, including heart, lung, renal, and gastrointestinal involvement; or mortality.

Recent studies have focused attention on the potential role of mast cells in the pathogenesis of systemic sclerosis (scleroderma) and other similar fibrosing syndromes.¹ An increased density of dermal mast cells has been described in patients with scleroderma,² the toxic oil syndrome,³ graft versus host disease,⁴ and in the tight skin mouse model of scleroderma.⁵ Furthermore, the dermal mast cells of tight skin mice show massive degranulation,⁶ and treatment with disodium cromoglycate (an inhibitor of mast cell degranulation) has been observed to produce a marked decrease in skin fibrosis.⁷

Mast cells and circulating basophils may release their inflammatory mediators when activated by lymphocyte products or histamine releasing factors secreted from neighbouring mononuclear cells⁸ also present in the dermis of patients with scleroderma.⁹ After degranulation mast cell granules can be internalised by fibroblasts, and this interaction may then promote local fibrosis.¹⁰ Anti-IgE autoantibodies, previously reported in systemic lupus erythematosus¹¹ and other autoimmune and atopic

disorders,^{12–15} may represent an additional mechanism for activation of mast cells and their subsequent interaction with fibroblasts in scleroderma. The current study sought to determine the prevalence of anti-IgE autoantibodies in a group of patients with scleroderma stratified according to the extent and duration of disease as well as their potential functional activity as determined by an in vitro basophil release assay.

Patients and methods

PATIENTS

Sixty six patients fulfilled preliminary American Rheumatism Association criteria for classification as definite systemic sclerosis¹⁶ and were selected to reflect a balanced stratification of disease extent and duration. Classification as generalised scleroderma required the presence of truncal skin thickening, whereas limited scleroderma described those patients with skin involvement limited to the hands, forearms, face, and feet. Onset of systemic sclerosis was dated from the first non-Raynaud's clinical manifestation, with three years' duration chosen as the divider between those patients with early and those with late systemic sclerosis.¹⁷ In addition, we studied six patients with undifferentiated connective

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tissue disease (defined as having at least four of the following six features: late age of onset of Raynaud's phenomenon, finger oedema or sclerodactyly, or both, abnormal nail fold capillaroscopic findings, serum anticentromere antibody with a titre $\geq 1/160$, and laboratory evidence of impaired maximal digital arterial flow or of in vivo platelet activation¹⁸) and two patients with recently diagnosed eosinophilic fasciitis.

All patients underwent a detailed clinical and laboratory assessment as described by the scleroderma criteria cooperative study,¹⁶ which included chest radiograph, 12 lead electrocardiogram, cine-oesophagography, pulmonary function testing, plasma renin activity, 24 hour urine collection for protein excretion and creatinine clearance, and a detailed serological profile. Serum samples were collected and stored at -70°C until needed for assay.

Data were entered on the Medlog clinical data management system, and this system was used for statistical analysis. Fisher's exact and paired *t* tests were used for univariate analyses and linear regression of least squares or stepwise linear regression model procedures for multivariate analysis. Mean antibody titres among patient subgroups were compared using Student's *t* distribution for two means (unpaired).

ENZYME IMMUNOASSAY

Anti-IgE antibodies for both IgG and IgM isotypes were determined by an enzyme linked immunosorbent assay (ELISA) as described by Nawata *et al.*¹⁵ with minor modifications. Multiwell polystyrene plates (Dynatech Inc, Alexander, VA) were coated with myeloma IgE at 1 $\mu\text{g}/\text{well}$ in bicarbonate buffer pH 9.6. Further blocking of any remaining reactive sites to reduce background was not necessary and, in fact, created false positive reactivity directed against the blocking reagents—for example, bovine serum albumin. The background in these unblocked plates remained less than 0.01 at optical density 405 nm. The plates were stored at 4°C and the assay was performed by diluting serum in phosphate buffered saline-Tween. The serum was diluted at 1:10 and 1:100 for the IgG anti-IgE and IgM anti-IgE ELISAs respectively and allowed to incubate on the plates overnight at 4°C . (These dilutions were chosen as the approximate mean along the linear slope of serum concentrations versus optical density plotted in semilogarithmic form.) The plates were then extensively washed with phosphate buffered saline. Alkaline phosphatase labelled goat antihuman IgG or IgM (Fc specific, Cappel Laboratories, Malverne, PA) at 1:1000 dilution was added for two hours and allowed to react with *p*-nitrophenyl

phosphate (100 $\mu\text{g}/\text{well}$). The optical density (OD) at 405 nm was determined at 30 and 60 minutes on a Dynatech ELISA reader. The relative quantity of bound IgG or IgM was calculated using standard amounts of coupled purified IgG (Miles Laboratories, Naperville, IL) or IgM (Calbiochem, Summer-ville, NJ) ranging from 10 ng to 1 μg . The data are expressed as a ratio as follows:

$$\frac{\text{OD } 405 \text{ nm (sample)}}{\text{OD } 405 \text{ nm (standard)}} \times \text{ng of standard}$$

Positivity was defined as a value greater than two standard deviations from a control group of 22 healthy, non-atopic individuals. The specificity of this assay has been previously confirmed.¹¹

SERUM IgE CONCENTRATIONS

Total serum IgE concentrations were measured by a radioimmunosorbent (PRIST) assay (Pharmacia Diagnostics, Piscataway, NJ).

BASOPHIL STUDIES

Leucocytes were separated from whole blood obtained from non-atopic healthy volunteers and from selected patients with raised levels of anti-IgE antibodies after informed consent using dextran sedimentation (0.03% dextran, 0.3% glucose, 0.15 M NaCl) by the method of Lichtenstein and Osler.¹⁹ The leucocytes were washed in HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) containing 0.03% human serum albumin and aliquots were incubated at 37°C for 30 minutes with serum containing anti-IgE antibodies in HEPES buffer with 1.5 mM CaCl_2 and 2.0 mM MgCl_2 . The histamine released was measured in duplicate samples by the double isotopic radioenzyme assay of Beaven *et al.*²⁰ Commercially obtained goat antihuman IgE (Kirkegaard and Perry Laboratories Inc, Gaithersburg, Maryland) was used as a positive control during each basophil release assay and consistently produced histamine release at dilutions of both 1:100 and 1:1000.

Results

Figure 1 shows the distribution of anti-IgE autoantibodies by isotype for patients and controls. The overall prevalence of IgG or IgM anti-IgE was 21 out of 66 patients (32%). Of these 66 patients, 14 (21%) had IgG anti-IgE and nine (14%) had IgM anti-IgE. Two patients had both IgG and IgM isotypes. Anti-IgE antibodies were not found in the sera from six patients with undifferentiated connective tissue disease or two patients with eosinophilic fasciitis. Table 1 shows the prevalence of anti-IgE antibodies by isotype for each clinical subset of

Fig. 1 Anti-IgE autoantibodies by IgG and IgM isotype versus non-atopic controls. *IgG versus controls $p<0.02$; *IgM versus controls $p=NS$. SS=systemic sclerosis.

Table 1 Number (%) of anti-IgE autoantibodies in systemic sclerosis

	EG* (n=18)	EL* (n=12)	LG* (n=15)	LL* (n=21)
IgG	4 (22)	3 (25)	1 (7)	6 (29)
IgM	2 (11)	1 (8)	4 (27)	2 (9)
Total	6 (33)	3† (25)	5 (33)	7† (33)

*EG=early generalised; EL=early limited; LG=late generalised; and LL=late limited systemic sclerosis.

†One patient had both IgG and IgM isotypes.

disease. There were no significant differences in the prevalence of anti-IgE antibody of either isotype between patients with early or late and limited or generalised involvement. Furthermore, when the levels of IgG and IgM anti-IgE were compared among the various subsets of patients examined there was no difference between patients with early limited and late limited disease, or between patients with early generalised and late generalised disease. When the total number of patients with generalised disease (early and late) was compared with the total number with limited disease (early and late) the mean antibody titre for IgG was significantly higher in patients with limited disease ($p<0.05$). The difference between these groups for IgM was not statistically significant.

There was a significant correlation between IgM anti-IgE and total serum IgE concentrations ($R=0.4106$; $p<0.01$), but no relation between IgG anti-IgE and total serum IgE concentrations ($p=NS$). In addition, the total mean serum IgE concentration in patients was not abnormal. We were unable to demonstrate the ability of these autoantibodies to degranulate circulating basophils. Normal donor basophils were incubated with varying concentrations of sera containing high titre anti-IgE of both IgG and IgM isotypes in vitro, but multiple attempts to induce the release of measurable histamine failed. Furthermore, heating of reactive sera, which may increase the amount of free antibody available,¹² also had no significant effect on in vitro histamine release from basophils.

We selected 24 independent variables for multivariate analysis based on suggested associations with anti-IgE antibodies, including patient age, sex, total skin score, clinical estimates of skin oedema, skin biopsy specimen weights, history of pruritus, presence of digital ischaemic ulcerations, tendon friction rubs, serum anticentromere antibody, serum creatinine, thyroid function profiles, presence of ventricular arrhythmia on 24 hour ambulatory electrocardiography, pulmonary involvement including reduced vital capacity or diffusing capacity, or both, chest radiographic evidence of interstitial fibrosis,

Table 2 Number (%) of anti-IgE autoantibodies by organ system involved

	IgG (n=14)	IgM (n=9)	IgG or IgM (n=21)
Heart	7 (50)	3 (33)	10 (48)
Lung	9 (64)	4 (44)	13 (62)
Kidney	2 (14)	1 (11)	3 (14)
Gastrointestinal	13 (93)	7 (78)	20 (95)

cine-oesophagography, plasma renin activity, clinical evidence of sicca complex, capillaroscopic classification, treatment with either corticosteroid or H₂ blockers, total white blood cell count, haemoglobin concentration, and mortality. We found no significant associations of anti-IgE antibody of either isotype with any of these features of systemic sclerosis. Table 2 presents the distribution of anti-IgE antibodies according to major features of visceral involvement.

Discussion

Mast cells are found throughout connective tissues in a predominantly perivascular location. They possess high affinity IgE receptors, and cross linking of membrane bound IgE leads to cellular activation and release of multiple inflammatory mediators from intracytoplasmic granules. An increased number of dermal mast cells have been noted in a number of pathological fibrosing conditions, including systemic sclerosis,² graft versus host disease,⁴ and the toxic oil syndrome,³ which resembles systemic sclerosis. Furthermore, the tight skin mouse model, which has been compared with human scleroderma, has also been shown to have an abundance of dermal mast cells,⁶ and when these mice are treated with an inhibitor of mast cell degranulation (disodium cromoglycate) there is a decrease in the amount of fibrosis.⁷

Whether or not tissue mast cells participate in the development of tissue fibrosis and by what precise mechanism remains uncertain. Nevertheless, in addition to being found at sites of collagen accumulation a number of observations have been made that corroborate their potential role in the pathogenesis of scleroderma. Mast cell degranulation in vitro is associated with fibroblast stimulation and collagen production.^{21 22} Furthermore, mast cell granules are internalised by fibroblasts,¹⁰ demonstrating that an interaction between the two cell types may exist.

Although mast cell degranulation usually occurs via antigen cross linking of two IgE membrane bound molecules, another potential mechanism for

release is the aggregation of IgE receptors through the interaction of autoantibodies directed against membrane bound IgE. The inability to demonstrate histamine release after exposure of basophils in vitro to sera containing these autoantibodies raises questions about the significance of their presence. Several factors may play a part in the functional activity of anti-IgE antibodies. Differences in the responsiveness of mast cells and basophils to various secretagogues have been noted²³ and may explain why basophils could not be activated in our study. Recent evidence in some patients with systemic lupus erythematosus¹¹ showed that the IgG fraction from sera containing high titre anti-IgE antibody can degranulate cutaneous mast cells in situ even though most systemic lupus erythematosus sera cannot degranulate circulating basophils in vitro. Furthermore, the avidity or concentration of these autoantibodies in the circulation may be too low to promote membrane aggregation of IgE. Finally, it may be that the epitope on the IgE molecule reactive with anti-IgE antibodies is not accessible after binding to cell membranes.

In view of the possible role of the mast cell in fibrosis and scleroderma and evidence that anti-IgE autoantibodies occur in other connective tissue and atopic diseases¹¹⁻¹⁵ the current study sought to evaluate the prevalence and possible clinical correlates of these antibodies in various subsets of systemic sclerosis. We found that approximately one third of patients have anti-IgE autoantibodies irrespective of the extent or duration of clinical disease. It is of interest that patients with limited disease appear to have higher mean anti-IgE titres than patients with generalised disease ($p<0.05$), but the clinical significance of this finding is at present undefined.

In our patients the number of individuals with IgM anti-IgE autoantibodies was not significantly different from the number in the control population (Fig. 1). In addition, there was a significant correlation between IgM anti-IgE autoantibodies and total serum IgE concentrations. It is of interest in this regard that Williams *et al* have previously described the existence of IgM anti-IgE autoantibodies that arise naturally.²⁴ Furthermore, IgM anti-IgE antibodies appear to have restricted specificity, which is directed against a non-idiotypic epitope in the F(ab)₂ fragment, whereas IgG anti-IgE appears to be reactive with the Fc fragment.²⁵

Although we were unable to identify any specific clinical or serological feature associated with the presence of anti-IgE autoantibodies, a role in disease pathogenesis is not excluded. We are currently engaged in a study of a mast cell stabilising agent (ketotifen) to elucidate further the potential

for mast cell modulation of the clinical course in scleroderma.

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