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Total and specific IgG4 antibody levels in atopic eczema

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SUMMARY

Total IgG4 and IgG4 antibody levels specific for 10 allergens (three inhaled and seven ingested) were measured by radioimmunoassay of sera taken from three groups of adult patients: (1) 32 cases of atopic eczema, (2) 28 cases of respiratory allergy and (3) 156 normal volunteers. In all three groups IgG4 antibody activity was mainly directed against common foods, and generally the group with atopic eczema had higher total and specific IgG4 levels than the cases of respiratory allergy, who in turn had higher titres than the normal group. There was within each group a tendency for men to have more total IgG4 than women and the difference was statistically significant among the normals. There was evidence of an IgG4 restricted response in atopic eczema because despite the group's elevated total IgG4 its total IgG4 remained within normal limits. Furthermore specific IgG4 was correlated with the corresponding specific IgE level in five of the 10 allergens examined. These results are generally consistent with the view that IgG4 levels are raised in cases of atopic eczema due to prolonged exposure to an allergen which initiated an IgE response.

Keywords atopic eczema respiratory allergy IgG4 IgE

INTRODUCTION

Cases of severe atopic eczema are usually associated with elevated circulating levels of IgE, values ranging from 5,000–50,000 units per ml being commonly observed (Juhlin *et al.*, 1969; Clendenning *et al.*, 1973). It is thought that such patients lack the factor(s) necessary to suppress the IgE response (Strannegard, 1979; Leung, Rhodes & Geha, 1981). We have demonstrated that this immunoglobulin has antibody activity against common inhalant allergens, e.g. *Dermatophagoides pteronyssinus* (Barnetson, Merrett & Ferguson, 1981), and additionally, unlike cases involving only asthma or rhinitis, there is often IgE antibody activity against common foods. Food specific antibodies of the subclass IgG4 are also frequently found in the normal population (Merrett, Burr & Merrett, 1983).

It has previously been shown that patients with atopic eczema have much higher serum IgG4 concentrations than do healthy controls, or patients with respiratory allergy alone (Shakib *et al.*, 1977; Barnetson & Merrett, 1983). This study was performed to compare the distribution of IgG4 antibody activity between common inhalant and food allergens in atopic eczema patients, normal controls and patients with atopic asthma and/or rhinitis.

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MATERIALS AND METHODS

Patients. Thirty-two patients with atopic eczema were studied. Eighteen were male and 14 female, and their ages ranged from 18 to 54 years; 10 of them also had a history of asthma and rhinitis, nine had asthma and six rhinitis. The criteria for the diagnosis of atopic eczema were as follows: (1) onset in early childhood; (2) flexural distribution of the eczema at some time during the disease; (3) presence of lichenification at some time during the disease; (4) history of asthma or allergic rhinitis; (5) family history of asthma, atopic eczema or rhinitis; (6) positive skin prick tests to common allergens (e.g. pollens, animal fur, house dust mite) and (7) serum IgE concentrations greater than 180 u/ml. Five of these criteria had to be satisfied for the diagnosis to be upheld.

Of the 32 patients with eczema, 13 gave a history of allergy to foods with swelling of the mouth and, in some cases, vomiting. Five of these gave a history of allergy to fish, four to eggs and two to both allergens: one patient had a history of allergy to cow's milk, but he had since outgrown it.

They were compared with 28 patients with atopic asthma and/or allergic rhinitis who were matched for age and sex (nine had a history of asthma and rhinitis, seven had asthma and 12 had rhinitis). Only one gave a history of food allergy, having experienced angio-oedema following ingestion of fish.

These two groups were compared with 156 adult healthy controls, 57 of whom were male and 99 female.

Venous blood was withdrawn from subjects in each of these groups, and serum stored at $-20°C$ until assayed.

Total IgG. Sufficient serum was available from 26 of the group 1 (atopic eczema) patients to estimate total IgG levels by a nephelometric assay which gave a normal adult range of 5–13 g/l.

Radioimmunoassays. Radiolabelled anti-IgG4 was used to quantify both total and specific IgG4 antibodies (Merrett *et al.*, 1983). Immunosorbent purified polyclonal anti-IgG4 (Dutch Red Cross, Amsterdam) was labelled monthly with ^{125}I to a specific radioactivity of approximately 15μCi/μg by the chloramine-T technique (Greenwood, Hunter & Glover, 1963). Serum was taken from a myelomatous patient and the IgG4 fraction (18 mg/ml) precipitated by 18% sodium sulphate was linked to CNBr activated microcrystalline cellulose (Sigma Chemical Co. Ltd) as previously described (Merrett *et al.*, 1983). An excess of these particles bound greater than 90% of radiolabelled anti-IgG4. In the assay procedures Phadebas RAST buffer (Pharmacia Diagnostics) was used as a diluent for (1) tracer, diluted so that 100 μl added to each assay tube produced approximately 100,000 ct/min; (2) myeloma derived IgG4 –cellulose particles and (3) heat-inactivated fetal bovine serum (FBS).

Total serum IgG4. Each test serum was diluted 200-fold with 0·9% saline and then 50 μl used to compete with 100 μl of IgG4–cellulose particles for binding to 100 μl of ^{125}I-anti-IgG4. The incubation was stopped after 1 h by centrifuging and washing the particles four times with 0·9% saline; radioactivity associated with particles was determined and test sera were quantified by reference to standards' calibration curve which ranged from 0·06 to 7·36 g IgG4/l. Another reference serum (Dutch Red Cross) diluted in 50% FBS so as to yield concentrations of 0·06, 0·12, 0·24 and 0·46 g IgG4/l was used for quality control, assays were rejected if the overall co-efficient of variation for these controls exceeded 13%. When known amounts of IgG4 were added to an IgG4 containing serum the recoveries ranged from 88–110% of those expected.

Specific IgG4 antibody. The allergens tested included: timothy grass pollen, *D. pteronyssinus*, cat epithelium, egg white, cow's milk, codfish, wheat flour, peanut, brazil nut and crab. Test sera were diluted 10-fold with 50% FBS and 50 μl was added to a Phadebas RAST allergen disc (Pharmacia Diagnostics); after incubating for 1 h the discs were washed free from excess serum and incubated overnight with 100 μl of ^{125}I-anti-IgG4. Binding of test sera (Bi) were related to milk positive reference serum which bound 15% of ^{125}I-anti-IgG4 (Bref) in the assay, and IgG4 levels expressed as (Bi/Bref) × 100 the reference serum was arbitrarily assigned a value of 100 ku/l. Non-specific binding ^{125}I-anti-IgG4 was allowed for in the calculations and by using a hypogammaglobulinaemic serum was found to vary from 0·8 to 1·2% according to the allergen tested.

IgE measurements. Total serum IgE measured by a fast double antibody solid phase technique

(Merrett & Merrett, 1978) and specific IgE antibodies were estimated by Phadebas IgE RAST (Pharmacia GB Ltd) using a modification of the manufacturer's procedure (John & Merrett, 1979).

Statistical analyses. Frequency distributions were calculated from log transformed data and significant differences between groups were tested using non-parametric statistics.

RESULTS

Total serum IgG4

Group 1 patients (atopic eczema) had significantly higher IgG4 concentrations than those in group 2 (asthma/rhinitis) and the 'normals' of group 2. Geometric means were respectively 0·73, 0·35 and 0·38 g/l, values which were about half the corresponding arithmetic means because of the skewed frequency distributions.

The data were analysed according to sex and statistical comparisons made within and between groups (Fig. 1). Within groups men tended to have higher IgG4 levels than women, but this was only significant in the largest group (normals). No group differences in IgG4 levels were found among the women but there was one significant group difference among the men, those in group 1 (atopic eczema) had higher levels than those in group 2 (asthma/rhinitis).

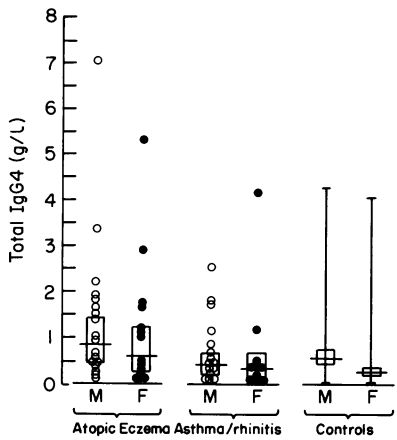

Fig. 1. Total serum IgG4 concentrations in 32 cases of atopic eczema (18 male and 14 female), 28 cases of asthma and/or rhinitis (17 male and 11 female) and 156 healthy controls (57 male and 99 female). The geometric means (——) are enclosed in blocks (□) which represent the 95% confidence intervals for differences in the means. In controls the ranges are indicated (I).

The percentage of total IgG in subclass IgG4 (Fig. 2) was determined in 26 of the patients with atopic eczema and found to be above normal (i.e. over 3% of total IgG) in 19 cases (73·1%); in one case IgG4 represented more than half of the total IgG. Total IgG levels were within normal limits (i.e. 5–13 g/l) in 20 cases (77%), and in the remaining six IgG concentrations ranged from 13·3 to 15·0 g/l.

Allergen specific IgG4 antibodies

Analysis of combined male and female data. Geometric mean values for the allergens tested were normally 6–13 ku/l, but cow's milk (16·5 ku/l) and egg white 30·2 ku/l) were notable exceptions. Usually IgG4 antibody concentrations were significantly higher in group 1 than group 2, which in turn were higher than group 3 (Fig. 3). Interesting results against this trend involved cow's milk (groups 1 and 2 both similarly higher than group 3), timothy grass pollen (no differences between groups) and wheat flour (group 2 values were normal yet significantly less than group 1).

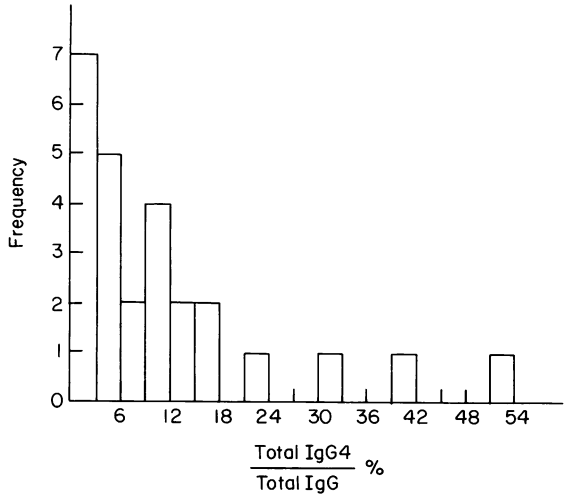

Fig. 2. Histogram which summarises the frequency with which the proportion of total IgG which is subclass IgG4 varies from 0–55% in 26 cases of atopic eczema.

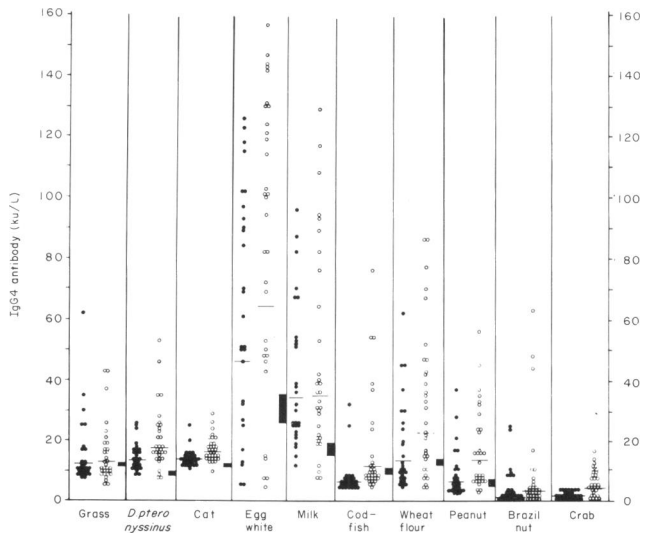

Fig. 3. Scattergrams indicating the distribution of IgG4 antibodies to three inhaled and seven ingested allergens in eczema (○) and asthma/rhinitis (●). Geometric means are indicated (——) and the confidence with which these means are different. Scattergrams indicating the distribution of IgG4 antibodies to three inhaled and seven ingested allergens in 32 cases of atopic eczema and 28 cases of asthma/rhinitis. Geometric means are indicated (——) and the confidence with which these differences between these means and normal can be judged from the 95% confidence intervals (■) found when analysing data from 156 normal controls.

Data analysed according to sex. Within the groups significant differences between the sexes were found only in group 3 (normals) where men had higher concentrations of grass pollen and wheat flour IgG4, but women had the higher levels against cat and peanut.

Comparison of the men in each group showed that group 1 had significantly greater results than group 3 for all allergens except grass pollen and codfish. Group 2 men tended to have IgG4 levels intermediate between group 1 and 3, and the same trend was observed among women, but the differences were less significant than between the corresponding male groups. Men outnumbered women in groups 1 and 2.

Correlation between total IgG4 and IgG4 antibodies

Atopic eczema. Inspection of scattergrams (eg. Fig. 4) led us to doubt calculated Spearman rank correlation co-efficients which indicated greater than chance associations ($P < 0{\cdot}05$) between total IgG4 and all specific IgG4 antibodies except cow's milk ($r = 0{\cdot}15$) and codfish ($r = 0{\cdot}31$). However when specific IgG4 results were compared for sera with total IgG4 concentrations below and above 1 g/l Wilcoxon Rank sum tests supported positive correlations for grass pollen ($P < 0{\cdot}05$), *D. pteronyssinus* ($P < 0{\cdot}04$), peanut ($P < 0{\cdot}03$), brazil nut ($P < 0{\cdot}001$) and crab ($P < 0{\cdot}001$). However there were several examples of exceptions even for these allergens (Table 1).

Asthma/rhinitis. The range of total IgG4 levels was too restricted to allow similar statistical analyses, 22 patients of the 28 had total IgG4 concentrations below 1 g/l.

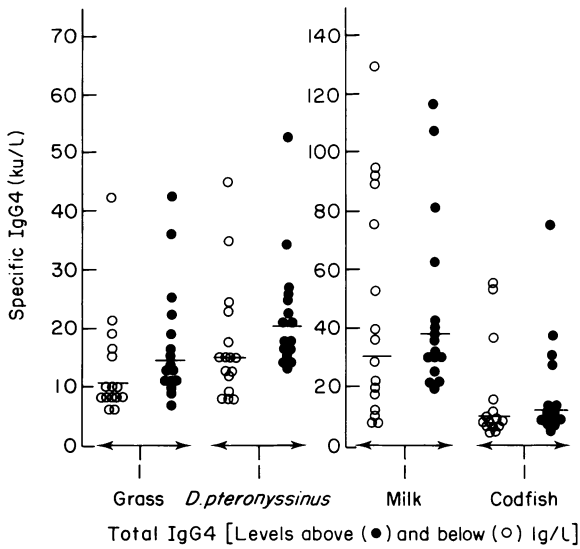

Fig. 4. Scattergrams correlating total and specific IgG4 levels in sera from 32 atopic eczema patients. Half of the patients had total IgG4 concentrations of at least 1 g/l and this was significantly correlated with specific IgG4 against timothy grass pollen ($P < 0{\cdot}05$) and *D. pteronyssinus* ($P < 0{\cdot}05$), but neither against cow's milk nor codfish allergens. Geometric means are indicated (——).

Table 1. A selection of strongly positive specific IgG4 results from atopic eczema patients with both high and normal total IgG4 concentrations

Patient number	Total IgG4 (g/l)	Specific IgG4 (ku/l)									
		Grass	Mite	Cat	Egg	Milk	Fish	Wheat	Peanut	Brazil nut	Crab
3	7·02	14	52	17	101	116	11	43	33	43	11
7	2·19	19	27	19	71	40	75	69	17	10	10
14	2·86	22	23	20	144	30	28	35	29	7	13
15	3·35	25	15	18	114	41	30	85	36	16	10
24	5·30	11	18	18	46	32	5	8	9	4	14
2	0·22	21	23	20	124	129	15	26	8	5	8
10	0·48	10	12	28	129	76	11	46	7	2	5
11	0·42	15	45	14	14	18	8	7	5	1	3
20	0·43	42	24	14	118	94	9	23	31	4	5
22	0·63	17	15	16	121	39	53	67	45	48	8

Correlations between IgG4 and IgE

Representative scattergrams of grass pollen, *D. pteronyssinus*, cow's milk and codfish allergens are shown for eczema patients (Fig. 5). These scattergrams suggested that if the IgE antibody response was sufficiently large then there was also a significant IgG4 response. Results for the two inhaled allergens were also similar in group 2 (asthma/rhinitis) patients.

Atopic eczema. No significant correlation was found between total IgG4 and total IgE ($r = 0{\cdot}22$), but greater than chance ($P < 0{\cdot}05$) rank correlation co-efficients were found for IgG4 and IgE antibodies directed against grass ($r = 0{\cdot}54$), *D. pteronyssinus* ($r = 0{\cdot}39$, cow's milk ($r = 0{\cdot}41$), wheat flour ($r = 0{\cdot}44$) and brazil nut ($r = 0{\cdot}39$). Wilcoxon rank sum tests also found significant differences in IgG4 antibody levels between sera with score '0' IgE RASTs and those with higher scores for grass ($P < 0{\cdot}005$) milk ($P < 0{\cdot}04$) and wheat ($P < 0{\cdot}02$). There was a trend to lower levels which did not reach significance for *D. pteronyssinus*, cat, codfish, peanut and brazil nut were probably due to insufficient numbers of IgE positive sera, because when results for group 1 and group 2 patients were analysed together differences for all these allergens were significant at the 5% level.

Asthma/rhinitis. Although there was no significant correlation between total IgG4 and total IgE ($r = 0{\cdot}04$), greater than chance rank correlation coefficients were found for the specific IgG4 and IgE against the inhaled allergens: grass ($r = 0{\cdot}73$), *D. pteronyssinus* ($r = 0{\cdot}52$) and cat ($r = 0{\cdot}54$). The majority of IgE RAST scores to food were '0' so correlations were not attempted. Rank sum tests also produced significant differences in IgG4 responses to grass ($P < 0{\cdot}001$) and *D. pteronyssinus* ($P < 0{\cdot}05$) depending whether the corresponding IgE scores were high or low. The result for cat also approached significance ($P < 0{\cdot}09$) although only three sera had IgE RAST scores of '4' to cat.

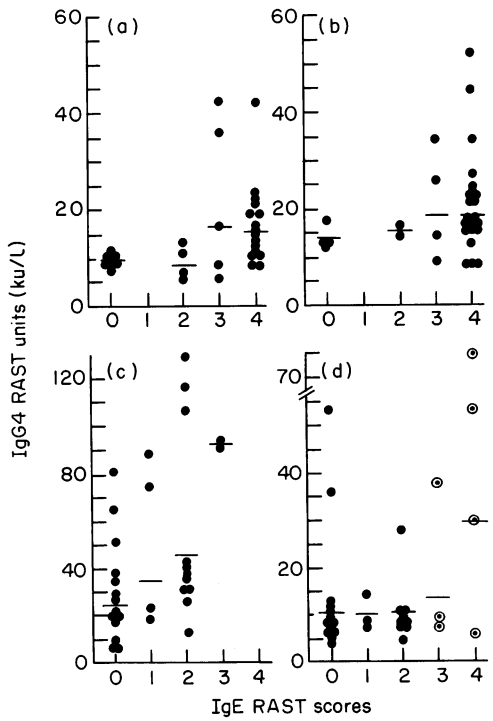

Fig. 5. Sera from 32 atopic eczema patients were examined for correlations between circulating specific IgG4 and IgE antibodies to the same allergenic extract. Sera with IgE RAST scores of '0' were associated with lower specific IgG4 concentrations than were those with IgE RAST scores of '4' ('3' in the case of cow's milk) as follows: (a) timothy grass pollen ($P = 0{\cdot}005$); (b) *D. pteronyssinus* ($P < 0{\cdot}1$); (c) cow's milk ($P < 0{\cdot}05$) and (d) codfish ($P > 0{\cdot}1$). Seven patients with immediate angio-oedema after eating fish are circled (◉).

DISCUSSION

Total serum IgG4 levels above the normal range have been reported in atopic eczema (Shakib *et al.*, 1977; Barnetson & Merrett, 1983) and now we are reporting intermediate concentrations in cases of asthma and rhinitis (Fig. 1). It is interesting to note that although half the patients with atopic eczema have total IgG4 concentrations over 1 g/l total IgG concentrations have been unaffected. Despite the increased concentrations of subclass IgG4, total IgG levels mostly remained within anticipated normal limits. This argues in favour of a shift in antibody production from at least one other subclass to IgG4. Such a shift, from IgG1 to IgG4, has been found in several patients hyposensitized with grass pollen, house dust mite and bee venom, and in non-allergic bee keepers (Aalberse, van der Gaag & van Leeuwen, 1983). It seems possible that an IgG4 restricted response in atopic eczema is a natural defence mechanism to reduce the effects of complement-dependent antibodies.

In a community survey, it was found that more IgG4 antibody activity was directed towards common foods, especially egg and cow's milk (Merrett *et al.*, 1983) than to inhaled allergens. Atopic individuals also have more IgG4 antibody directed against foods than inhaled allergens (Fig. 3). The increased amounts of IgG4 antibody in atopic eczema are in line with total IgG4 levels and there was, not unexpectedly, evidence for positive correlations between total and specific IgG4 levels, especially for grass, house dust mite, peanut, brazil nut and crab antigens. Some antigens almost produced a significant correlation at the 5% level, notably egg, codfish and wheat flour, and with increased numbers of patients, a more significant correlation between specific and total IgG4 might be found.

Total serum IgE is often markedly raised in cases of atopic eczema, and levels in excess of 10,000 ku/l are not uncommon in adults. In contrast to IgG4, most of the IgE antibody activity is directed against inhaled allergens, although IgE antibody titres were often higher than normals or patients with respiratory allergies (Barnetson *et al.*, 1981). Nevertheless it is interesting to note in atopic eczema significant correlations between specific IgE and IgG4 antibodies against grass pollen, cow's milk and wheat flour. Because relatively few individuals have high IgE antibody levels to a variety of foods the data from atopic eczema and respiratory atopics were pooled, whence the correlations between IgE and IgG4 antibodies were extended to other inhaled (*D. pteronyssinus* and cat) and ingested allergens (codfish and peanut). These correlations were in accord with results from at least two other studies: (1) 14 asthmatics selected for their ability to respond to antigen challenge with immediate, isolated late or dual reactions all had IgE antibodies directed against the challenge antigen and in seven cases specific IgG4 antibodies were also detected (Lee *et al.*, 1983); (2) using antigen binding assays Platts-Mills *et al.* (1976) usually found IgG antibodies to pollens only when IgE specific to the same pollens could be identified. These results suggest that our IgG4 RAST technique is measuring antibodies to the same antigens (i.e. allergens) as IgE RAST, but conclusive data on this point is not yet available.

Although there is no evidence to support the idea that units of IgG4 antibody are quantitatively the same for each antigen, it is noticeable that the highest IgG4 antibody levels were measured against the foods most difficult to avoid, eggs and milk. However, although this supports the concept of a restricted subclass IgG4 response after prolonged exposure to antigen the codfish results are anomalous. High codfish specific IgG4 levels were found in four of seven patients who have scrupulously avoided eating fish since childhood because of its association with angio-oedema. These patients were the only ones with IgE RAST scores of 3 and 4 to codfish so we presume that this prolonged IgE response is a result of minimal exposure to fish allergen (eg. by inhalation) which may also maintain the IgG4 levels in some cases.

Another similarity between IgE and IgG4 was the finding of a sex difference. There is a tendency for men to have higher total IgE levels than women, significantly so among adult UK asthmatics (Burr *et al.*, 1975), normals over 70 years old (Merrett *et al.*, 1980) and non-atopic smokers (Pantin & Merrett, 1982). In the current study total IgG4 levels also tended to be higher among men than women, and this was significant for the numerically largest group of normals.

In conclusion, it seems to us unlikely that a raised IgG4 level in atopic eczema is a cause of the

disease, rather it is a monitor of prolonged exposure to an allergen which may have initiated an IgE response. Its role in reducing the effects of complement-dependent antibodies might be construed as being either a natural defence mechanism or an interference in the normal immune processes for expelling antigen. Further research to differentiate these alternatives is necessary.

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