

The atopic syndrome: *in vitro* immunological characteristics of clinically defined subgroups of atopic subjects

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Summary

Total IgE, selected specific IgE antibody levels, C2 and yeast opsonization were measured in four clinically defined subgroups of atopic patients who had previously been HLA-typed. Each group was characterized by a distinctive mean total IgE level and profile of specific IgE responses. All groups gave comparable levels of IgE antibodies to Timothy grass pollen but in patients initially presenting with infantile eczema and who subsequently developed asthma and/or hay fever, IgE antibodies to bovine milk proteins, egg ovalbumin and cat dander were frequently observed whereas in patients initially presenting with hay fever later in life these antibodies were usually absent. Computer assisted analysis failed to show any association between IgE antibody responses and (a) the HLA phenotypes A1,B8 and A3,B7 (b) C2 levels and (c) the yeast opsonization index.

Introduction

In an attempt to dissect genetic factors in atopy we have previously defined clinical groups consisting of eczema alone, eczema complicated by asthma and/or hay fever, hay fever with asthma and hay fever alone (Turner *et al.*, 1977). This clinical distinction is important because the onset of symptoms in the subgroups is quite different. Eczema in children classically starts early in life, often within the first few months, whereas hay fever often starts in the second or third decades. The clinical history and prognosis of these subgroups is also different, but they are all included under the atopic 'umbrella'.

We have now looked at serological and other markers in these four subgroups of the atopic syndrome in order to define more precisely their *in vitro* characteristics.

Materials and methods

Patients

The patients, who fall into two main groups, have been described in our previous paper (Turner *et al.*, 1977). Forty patients from a paediatric dermatology clinic (mean age 8.2 years), presented with atopic eczema before 2 years of age; twenty-six of them subsequently developed other manifestations of allergy (asthma and/or hay fever). Forty patients from an allergy clinic (mean age 23.7 years) presented with hay fever (seasonal allergic rhinitis with a positive skin test to grass pollen); nineteen subsequently developed asthma but none gave a history of eczema. Thus four subgroups were defined: (1) atopic eczema uncomplicated by asthma or rhinitis; (2) eczema associated with asthma and/or rhinitis; (3) hay fever and asthma but no eczema; and (4) hay fever alone.

An allergic history was obtained from each patient and skin prick tests to 18 common antigens were done (Turner *et al.*, 1977). A sample of blood was obtained and used as a source of lymphocytes for tissue typing and serum for other investigations. Serum samples were aliquoted for opsonization, complement and IgE antibody studies and stored at -70°C until required.

Serum IgE

IgE was measured by a modification of the double antibody procedure (Nye *et al.*, 1975) using rabbit anti-human IgE (Hoechst Pharmaceuticals, England) in the first stage and donkey anti-rabbit serum (Wellcome Reagents Ltd.) in the second stage; results were expressed as international units per millilitre using a commercial calibrated standard (Pharmacia).

IgE antibodies

Specific IgE antibodies were measured to the following: extracts of Timothy grass, *Dermatophagoides pteronyssinus*, cat dander, bovine β -lactoglobulin and ovalbumin. Allergens were coupled to micro-crystalline cellulose particles (Sigma). A 1 mg/ml suspension (0.5 ml) of cellulose particles with bound antigen was mixed with 50 μl of serum and incubated at room temperature for 16 hr with continuous mixing. Following five successive washes with physiological saline containing 1% Tween-20, 100 μl of ^{125}I -labelled specific rabbit anti-human IgE (Fce-immunosorbent purified) were added. The contents of the tubes were incubated with agitation at room temperature overnight, the supernatants removed and the particles washed five times with physiological saline containing 1% Tween-20. Residual radioactivity was counted in a γ -counter and results expressed as a percentage of the total radioactive counts which became bound. All sera were analysed in a single batch and appropriate positive and negative (cord serum) controls were included with each assay. Binding levels greater than 1.6 times the mean cord serum value for each allergen were regarded as positive.

HLA tissue typing

This was performed as previously described (Turner *et al.*, 1977).

Yeast opsonization

Yeast opsonization was measured as previously described (Soothill & Harvey, 1976) on seventy-seven patients' sera and results expressed as an opsonization index (mean number of yeast/polymorph—Y/P).

C2 activity

C2 activity was measured in seventy-seven patients' sera as previously described (Turner *et al.*, 1978).

Statistical analyses

Statistical analyses were performed at the University of London Computer Centre using the Statistical Package for the Social Sciences (SPSS) program.

Results

Total serum IgE levels

The total serum IgE levels of atopic individuals classified into one of the four subgroups are shown in Fig. 1.

The log means for each group (indicated on the figure) were as follows: patients with uncomplicated atopic eczema, 750 i.u./ml; patients with eczema associated with asthma and/or hay fever, 3952 i.u./ml; patients with hay fever and asthma, 222 i.u./ml and patients with hay fever alone, 126 i.u./ml. Two of the patients with complicated eczema had levels in excess of 80,000 i.u./ml (i.e. > 192,000 ng/ml assuming 1 i.u. = 2.4 ng (Bazaraal & Hamburger, 1972).

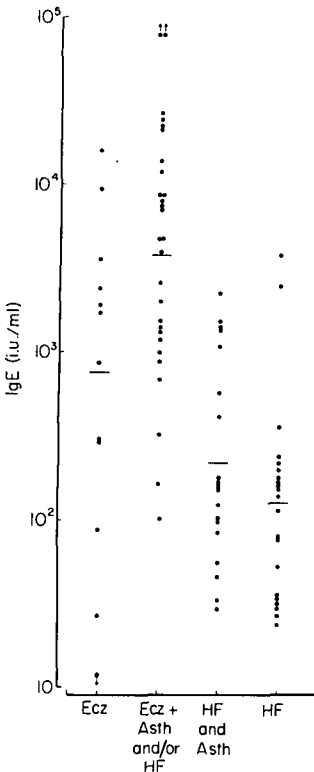


Fig. 1. Total serum IgE levels in four clinical subgroups of atopic patients. Abbreviations: Ecz, uncomplicated atopic eczema; Ecz + Asth and/or HF, eczema associated with asthma and/or hay fever; HF and Asth, hay fever with asthma; HF, hay fever alone. Horizontal bars indicate log mean IgE levels.

Specific IgE to eight allergens

The levels of specific IgE antibody to eight allergens as measured by RAST in the four clinical subgroups of atopic patients are shown in Fig. 2.

When considering all the patients together, the inhalant allergens Timothy grass and *D. pteronyssinus* were the best indicators of the state of atopy. Thus sixty-four out of sixty-nine (93%) patients were positive to Timothy grass and fifty-eight out of seventy-one (82%) were positive to *D. pteronyssinus*. However, using another presumed inhalant allergen, namely cat dander, only one patient from the two hay fever groups was positive whereas approximately $\frac{1}{4}$ to $\frac{1}{3}$ of eczema patients were cat-positive. The latter findings closely resembled the results with ingested allergens. No hay fever

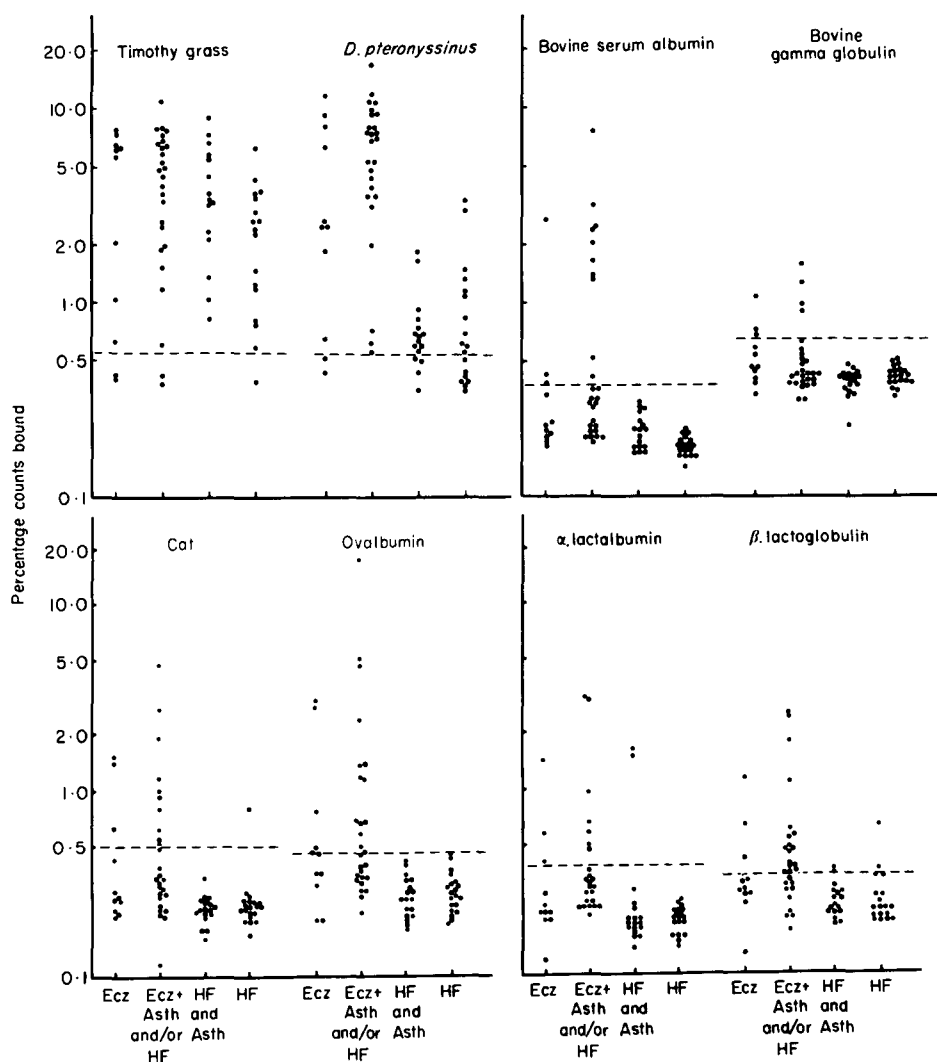


Fig. 2. Specific IgE to eight allergens measured by RAST in the four clinical subgroups of atopic patients (see legend to Fig. 1 for abbreviations). Horizontal dashed lines indicate the laboratory threshold for significant binding appropriate for each allergen (i.g. 1.6 times cord serum binding).

patient was positive to ovalbumin, or to the milk proteins, bovine serum albumin, bovine gamma globulin or α -lactalbumin. Only four such patients were positive to β -lactoglobulin and three of these only weakly so. Higher levels of IgE antibodies to ingested allergens were found in the complicated eczema patients with β -lactoglobulin and ovalbumin giving the highest number of positive reactions (63 and 52% respectively).

Four individuals with complicated eczema (including both patients with a serum IgE of $>80\,000$ i.u./ml) were positive to all the antigens except bovine gamma globulin. In the remaining patients there were no obvious patterns of reactivity.

Relationship between total and specific serum IgE

We have examined our data for specific IgE antibody levels to eight antigens for evidence of associations with the total serum IgE levels of the individual patients. The Pearson correlation tests for each of the four patient subgroups are given in Table 1. This shows that for patients with complicated eczema there are significant correlations between antibody levels and total IgE for each of the antigens studied. For patients with pure eczema antibody levels to Timothy grass, *D. pteronyssinus*, cat dander and bovine gamma globulin correlated positively with serum IgE. For patients with hay fever and asthma antibody to Timothy grass was significantly correlated with total IgE level but, surprisingly, this was not found to be so for patients with hay fever alone. We believe this arises from a distortion of the distribution pattern by the inclusion of one

Table 1. Pearson correlation tests between total serum IgE and specific IgE antibody levels in four patient groups

Allergen	Patient group			
	Eczema	Eczema, asthma and hay fever	Asthma and hay fever	Hay fever
Timothy grass	$r=0.92$ $P=0.00004$	$r=0.74$ $P=0.00001$	$r=0.79$ $P=0.0004$	$r=0.41$ n.s.
<i>D. pteronyssinus</i>	$r=0.80$ $P=0.002$	$r=0.67$ $P=0.00018$	$r=0.24$ n.s.	$r=0.55$ $P=0.01$
Cat dander	$r=0.61$ $P=0.04$	$r=0.67$ $P=0.0001$	$r=0.38$ n.s.	$r=-0.02$ n.s.
α -lactalbumin	$r=0.159$ n.s.	$r=0.63$ $P=0.0006$	$r=0.40$ n.s.	$r=-0.05$ n.s.
β -lactoglobulin	$r=0.49$ n.s.	$r=0.47$ $P=0.01$	$r=0.42$ n.s.	$r=0.22$ n.s.
Bovine serum albumin	$r=0.26$ n.s.	$r=0.60$ $P=0.0009$	$r=0.12$ n.s.	$r=0.12$ n.s.
Bovine gamma globulin	$r=0.65$ $P=0.029$	$r=0.58$ $P=0.001$	$r=0.11$ n.s.	$r=0.11$ n.s.
Ovalbumin	$r=0.58$ n.s.	$r=0.61$ $P=0.0007$	$r=0.26$ n.s.	$r=-0.08$ n.s.

individual who was skin-test positive but RAST-negative and subsequently considered to be predominantly mite-sensitive.

The complicated eczema group exemplify two distinct types of relationship between total and specific IgE levels. With Timothy grass allergen virtually all the patients with a total IgE greater than 100 i.u./ml were considered to be above the threshold for positive binding, and the correlation coefficient of 0.74 was highly significant ($P=0.00001$). In contrast, with cat dander allergen the correlation coefficient was 0.67, also significant at $P=0.0001$, but many individuals were seen to have high levels of total IgE (one greater than 20 000 i.u./ml), and low binding to the allergen.

Association of HLA haplotypes with total and specific IgE antibody levels

An increased frequency of the two haplotypes HLA-A1,B8 and HLA-A3,B7 has been previously noted by us in the complicated eczema (A1,B8) and hay fever with asthma (A3,B7) subgroups (Turner *et al.*, 1977). With one exception in this study no significant association was found between the presence of either haplotype and levels of specific or total IgE. However, three patients with pure hay fever and with the tissue type HLA-A3,B7 had significantly higher levels of IgE antibodies to *D. pteronyssinus* than similar patients without A3,B7. However, since associations were sought between three common haplotypes (A1,B8, A3,B7 and A2,B12) and nine serological measurements (twenty-seven comparisons) one positive association would be expected by chance alone.

Yeast opsonization

We have previously observed a high frequency of defective yeast opsonization in these patients (Turner *et al.*, 1978). Thus 26% of all eczema patients and 29% of all hay fever patients had opsonization indices below 2.5 yeasts/polymorph (the lower limit of normal in this assay). This gave mean values for two of the subgroups which were at the low end of the normal range (2.96 for eczema and 2.81 for hay fever with asthma). Pure hay fever patients gave a mean Y/P value of 3.24 and the complicated eczema patients a value of 3.45.

There was no significant correlation between the yeast opsonization index and total IgE levels, specific IgE antibodies to the eight antigens, C2 levels or the presence of any of the three common HLA haplotypes A1,B8, A2,B12 and A3,B7.

C2 component of complement

Low C2 levels were observed in a number of these patients (Turner *et al.*, 1978) and, as with yeast opsonization, we have, in this study, sought associations with other serological parameters. The mean C2 for each subgroup (expressed as percentage of a normal serum) were as follows: 101% for pure eczema, 81% for hay fever with asthma and 79% for hay fever alone.

There was no correlation between the C2 level and total IgE levels or with specific IgE antibodies to the eight antigens. A weak association between C2 level and the HLA haplotype A1,B8 was observed for patients with complicated eczema. Ten of these patients had the A1,B8 haplotype and their C2 level was significantly higher than eighteen other similar patients who lacked this haplotype ($P=0.038$). A similar, but

probably chance, association between high C2 level and possession of the A3,B7 antigens was also observed in two individuals from this subgroup.

Skin tests

The skin test results obtained with eighteen common antigens are summarized in Table 2. Using such a range of antigens (basically five pollen extracts, two mites, three moulds, five mammalian and avian extracts and three food antigens) patients in both hay fever subgroups were positive in approximately 23% of the tests whereas patients with eczema were positive in only 12–16% of the tests. However, we may have under-estimated the number of positive skin tests in these patients because of the difficulties in the interpretation of such tests in severely eczematous skin.

Table 2. Frequency of positive skin tests in four patient subgroups to eighteen common antigens*

Patient group	No. of individuals tested	Observed/possible no. of positive skin tests per group	Percentage positive skin tests per group	Mean no. of positive skin tests per individual
Eczema	12	20/216	12.0	2.2
Eczema with asthma and/or hay fever	28	84/504	16.7	3.0
Hay fever and asthma	19	78/342	22.9	4.1
Hay fever	21	83/378	22.2	3.9

* Mixed grasses, Timothy grass, silver birch, plane, nettle feathers, *D. farinae*, *D. pteronyssinus*, cat dander, dog dander, horse dander, horse serum, egg yolk, egg white, milk, *Cladosporium*, *alternaria* and *A. fumigatus*.

With a more restricted range of antigens (corresponding broadly to the antigens used in the specific IgE antibody assays) patients in all subgroups were positive to 30–37% of the tests although the hay fever patients were again positive more frequently than the eczema patients (see Table 3). However, this was not the case with egg white and milk where the eczema patients gave more positive reactions. Whereas six out of seven eczema patients with a positive skin test to egg and/or milk had a positive RAST result this was not the case with the hay fever patients. In four such patients with five positive skin tests to egg and milk antigens the corresponding RAST results were negative. Moreover seventeen out of twenty-eight complicated eczema patients with a positive RAST to egg or milk were skin test negative to these antigens so that the overall correlations between skin test and RAST results was very poor. In contrast only seven individuals out of seventy-one from all four subgroups gave discordant results between skin test and RAST for Timothy grass antigen.

Table 3. Frequency of positive skin tests in four patient subgroups using five selected antigens*

Patient group	No. of individuals tested	Observed/possible no. of positive skin tests per group	Percentage positive skin tests per group	Mean no. of positive skin tests per individual
Eczema	12	18/60	30	1.5
Eczema with asthma and/or hay fever	28	45/140	32	1.6
Hay fever and asthma	19	35/95	37	1.8
Hay fever	21	37/105	35	1.7

* Mixed grasses, *D. pteronyssinus*, cat dander, egg white and milk.

Discussion

An association between serum IgE levels and atopic disease was established (Johansson, 1967). Subsequent studies (Berg & Johansson, 1969; Kumar *et al.*, 1971) have shown that different atopic syndromes may be broadly distinguished on the basis of IgE level. In general, patients with hay fever have levels in the range 20–150 i.u./ml, patients with asthma >200 i.u./ml and those with eczema have the highest levels, frequently >500 i.u./ml. The present study confirms these generalizations and in addition suggests that the highest levels are found in patients who initially presented with infantile eczema and subsequently developed asthma; the mean level for such individuals being four fold greater than that for patients with eczema alone. In contrast, our other groups of patients who developed asthma after initially presenting with allergic rhinitis had much lower serum IgE levels. Of course the asthma in such individuals was probably pollen induced and none of these patients had at any stage suffered from eczema. Despite the clear subgroup distinctions when mean serum IgE is considered the groups overlap to a considerable extent. Apart from the extremely elevated levels observed in some eczema patients an individual IgE value is rarely diagnostic of one particular subgroup.

The specific IgE antibody results obtained in this study confirm, in part, some recently published data on the value of RAST in food allergic patients (Wraith *et al.*, 1979). These authors found that the people most likely to have food-specific IgE antibodies were under 30 years of age and presented with a combination of asthmatic and eczematous symptoms.

Furthermore their patients usually had a total IgE level of >300 i.u./ml and gave a positive 'atopy screen' by RAST. We would concur with these views although our range of food allergens was much smaller than that used by Wraith *et al.* (1979). IgE antibodies to the milk protein β -lactoglobulin and egg ovalbumin were frequently observed in our patients with complicated eczema but rarely or not at all in hay fever patients. Bovine serum albumin and α -lactalbumin were less useful screening reagents and bovine gamma globulin was the least reactive.

The overall correlation between skin tests and milk/egg RAST results in our study was very poor. We observed many (17/28) patients with complicated eczema in whom specific IgE antibodies were demonstrable by RAST but the corresponding skin tests were negative. Galant *et al.* (1973) has made similar observations in a group of food sensitive patients. Our forty eczema patients were not selected for food sensitivity but several (14/40) gave a positive clinical history of egg and milk intolerance. Ten of these fourteen were found to have a positive RAST to egg and/or milk but seventeen other individuals with a positive food RAST gave no clinical history of egg or milk intolerance. This lack of association between demonstrable IgE antibodies to milk and/or egg and any apparent clinical (i.e. gastrointestinal) symptoms has been the subject of previous comment (Foucard, 1973; Schur, Hyde & Wypych, 1974; Dannaeus *et al.*, 1977). The mechanisms whereby IgE antibodies or complexes of such antibodies may manifest the skin lesions characteristic of eczema (if indeed they play any such role) are still the subject of much speculation (Brostoff *et al.*, 1979; Paganelli *et al.*, 1979).

We observed a significant positive correlation between the total serum IgE and specific IgE antibody levels for our patients with complicated eczema but whereas almost all patients with a high total IgE were found to have high levels of specific antibody to Timothy grass extract this was not the case for IgE antibody to cat dander and the various food proteins. In a number of such patients very high levels of serum IgE were observed in association with a negative RAST result. We believe this is an indication of the specificity of the assay.

A major aim of the present investigation was to search for possible associations between the patients' tissue type and the observed levels of specific IgE antibody to various allergens. We had previously shown that the presumed tissue type haplotypes A1,B8 and A3,B7 were increased in two of our subgroups, namely the complicated eczema and hay fever with asthma groups (Turner *et al.* 1977). Marsh *et al.* (1977) have clearly shown similar tissue type associations in North American patients with clinical sensitivity to the ragweed antigen Ra3 (A2,B12 association) and the rye grass antigen, Rye 1 (A1,B8 association). Interestingly, however, these associations were much more marked in those individuals with low total serum IgE levels. We did not observe in our patients any association between either the HLA-A1,B8 or the HLA-A3,B7 phenotypes and total serum IgE level but our patient numbers were much smaller than those studied by March *et al.* (1977). Similarly, there was no association between the levels of specific IgE antibody and the presence of one or other of these two phenotypes.

Thus we were unable to extend to IgE responses the observations by Eddleston (1977) in which the HLA-A1,B8 phenotype was found to be frequently associated with high titres of non-reaginic antibodies to various viruses.

We also found no correlation between either the yeast opsonization indices or the C2 levels of our patients and any of the other serological or cell surface parameters measured in this study. We have, however, previously reported a weak association of C2 deficiency with BW40 in our patients (Turner *et al.*, 1978), which is in agreement with the findings of Gibson *et al.* (1976). In contrast to these authors we did not find an association of heterozygous C2 deficiency with excess of the A10 or B18 antigens.

Although we have failed to identify any associations between IgE antibody responses in atopic patients and the common HLA phenotypes, C2 level or yeast opsonization index, we believe that there may well be weak associations which are readily masked in a disease syndrome which is well recognized to have a multifactorial

aetiology and to be under polygenic control. Further studies with more sensitive techniques may well be required to identify such associations.

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