

Inactivation of haemolytic complement by house dust allergen in the serum of children with atopic diseases

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Summary

Purified house dust allergen has been employed for screening the susceptibility to inactivation of haemolytic complement in the blood serum of atopic children and control subjects. Fluid phase complement in the control group of children was more sensitive to allergen-induced inactivation than observed in a normal adult population. Though the mean complement sensitivity indices in all groups of patients were below the value for the control group, there was considerable statistical overlap. The serum complement sensitivities were in no way related to the clinical manifestations. The results of the complement test were not correlated to the total IgE levels, the RAST scores nor the skin reactions with house dust allergen.

Some evidence for the *in vivo* involvement of the complement system in childhood atopic allergy was provided: the mean C₃ proactivator level was significantly lower in atopic children, than in the control group; the mean C₄ level in children with bronchial asthma and in children with atopic dermatitis was significantly depressed.

A significant positive correlation between the serum C₄ levels and allergen-complement sensitivities in children with both bronchial asthma and atopic dermatitis was observed.

Introduction

It has been shown that human haemolytic complement is readily inactivated by several of the common inhalant allergens, especially those of the house dust group (Berrens & Van Rijswijk-Verbeek, 1973; Berrens, 1974). Furthermore, complement in the sera of normal adults often is less sensitive to inactivation by allergens than is observed with the sera of atopic subjects. In a recent study, the mean complement sensitivity index to house dust allergen in normal adults was established at 18.8 µg (s.d. 29.8), against a mean value of 5.4 µg (s.d. 13.8) in adult atopic patients (Berrens, 1975). Though the atopic sera therefore displayed an average tendency of higher complement susceptibility, the wide range of standard deviations precluded a practical distinction of atopic

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sera from those of normals or of patients with various forms of non-atopic skin or chest conditions. The present study was undertaken to investigate the possible relationship between complement sensitivity to house dust *in vitro* and the clinical symptomatology in groups of children. In order to assess the possible *in vivo* involvement of the complement system in atopic allergic reactions, the serum levels of the individual components C₄ and C₃ proactivator (C₃PA) were also determined.

Using photoinactivated allergens, the diminishing capacity of house dust allergen to inactivate human haemolytic complement was found to run parallel to impressive reduction of the allergenic skin-reactivity in atopic patients (Berrens, Henocq & Radermecker, 1973).

A further argument for the possible relationship between the properties of activity in skin tests and *in vitro* complement inactivation was provided by Berrens & Guikers (1975), who demonstrated a close parallelism between these properties in allergenic fractions obtained from house dust extracts during purification. In view of these results, it was hypothesized that complement might be involved in the mechanism of weal and flare skin reactions to house dust allergen. Extrapolating to human serum, the present investigation included an attempt to establish a possible relation between the skin-reactivity of atopic children to house dust allergen and the sensitivity of haemolytic complement in their sera to inactivation by the same allergen.

Patients and methods

Patients

The clinical investigations involved eighty-six children attending the out-patient department of paediatrics; they were grouped into four different classes according to the clinical features: (I) bronchial asthma (twenty-one); (II) bronchial asthma combined with atopic dermatitis (twenty-seven); (III) atopic dermatitis (fourteen); (IV) controls with a negative history of allergy (twenty-four).

Each child was submitted to a standard program of history taking, physical examination, laboratory investigation and skin testing. If indicated, chest and sinus X-ray pictures, nasal smears and bacterial and parasitic cultures were examined. The age ranged from 2 years to adulthood.

All medication, including antihistaminics, sympathicomimetics and/or disodium cromoglycate were withdrawn 1 week before the allergy tests. Children who received oral corticosteroids were excluded. One patient had undergone a grass pollen immunotherapy course the preceding year, and one patient had received two intradermal injections of house dust.

Sera

Venous blood samples were obtained, in the autumn of 1973; the blood was allowed to clot in glass tubes. After centrifugation, the fresh sera were aliquoted into 0.5 ml polythene vials and stored at -70°C until use.

Allergen

House dust allergen, fraction E (batch 72E), was isolated from a large pool of house dust as described (Berrens & Young, 1961).

Skin tests

Intradermal tests were performed on the volar surface of the arm, using five-fold

sequential dilutions of purified house dust allergen. The skin reactions were read by visual appraisal after 20 min. Skin reactivity was expressed in the highest dilution still given a detectable positive reaction.

Complement inactivation tests were carried out as described in detail elsewhere (Berrens, 1975). Briefly, 15 μ l serum samples, diluted 1:30, were incubated for 30 min at 37°C with purified house dust allergen 72E in the range of 0.5–3.0 μ g. Residual complement was then evaluated by adding sheep erythrocytes optimally sensitized with rabbit amboceptor. Complement sensitivity was expressed in the amount of allergen, in μ g, required for complete loss of haemolytic activity of the serum sample, as described (Berrens, 1975).

Complement factors C4 and C3 proactivator were determined by single radial immunodiffusion using commercial antibody-containing agarose plates and a standard serum as prescribed by the manufacturer (Behringwerke, Germany).

Total serum IgE was determined by radioimmunoassay as described by Aalberse *et al.* (1974).

Allergen-specific IgE was determined by radioallergosorbent test (RAST), using house dust allergen 72E coupled to CNBr-activated Sepharose 4B (Pharmacia) as the insoluble phase. The RAST was performed with the modifications proposed by Aalberse (1974).

Results

(1) Complement-sensitivities

Haemolytic complement in the sera of nearly all patients proved quite sensitive to inactivation by purified house dust allergen (Fig. 1). The mean complement sensitivity index in sera of the children of the control group was 6.0 μ g, i.e. significantly below the

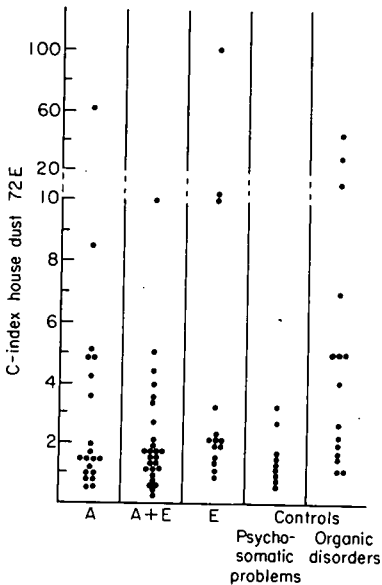


Fig. 1. Complement sensitivity index to house dust 72E, in μ g required for complete C consumption in 20 μ l serum samples, in the sera of patients with atopic diseases and in non-allergic controls. A: asthma; A + E: asthma + eczema; E: eczema.

Table 1. Complement sensitivity index to house dust allergen 72E in normal and atopic children

Diagnosis	No. of children	Mean C-index 72E (μ g)	s.d.	Range
I Asthma	20	2.3	2.1	0.5-8.5
II Asthma + atopic dermatitis	27	2.1	2.0	0.3-10
III Atopic dermatitis	13	3.2	3.2	0.9-11
IV Normal	24	6.0	9.6	0.5-42

mean value of 18.8 μ g on record for a normal adult population (Berrens, 1975). The control group comprised ten non-allergic children with psychosomatic problems and fourteen children with organic disorders. The sera of children with psychosomatic problems displayed high complement sensitivity and could not be distinguished in this respect from the sera of the groups with atopic diseases (Fig. 1).

Although high complement-sensitivity was observed in the sera of children with atopic diseases, only the mean complement index of group II (asthma combined with atopic dermatitis) was significantly different from the mean value established for the control group (Table 1). There was no correlation between the complement sensitivity of sera to house dust allergen 72E and the skin reactions of the patients to the same allergen (Fig. 2).

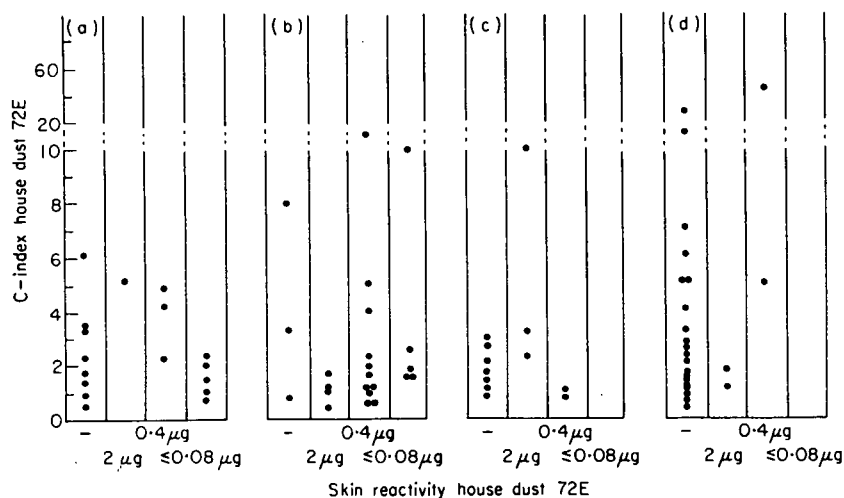


Fig. 2. Relationship between complement sensitivity index and skin reactivity to house dust 72E in normal children and patients with atopic diseases. Each point represents one patient (serum). Skin reactivity established by end-point titration for minimal positive skin reaction. Using 72E solutions at 2 μ g/ml, 0.4 μ g/ml and 0.08 μ g/ml, respectively; 0.1 ml was injected intradermally. (a) Asthma; (b) asthma + eczema; (c) eczema; (d) controls.

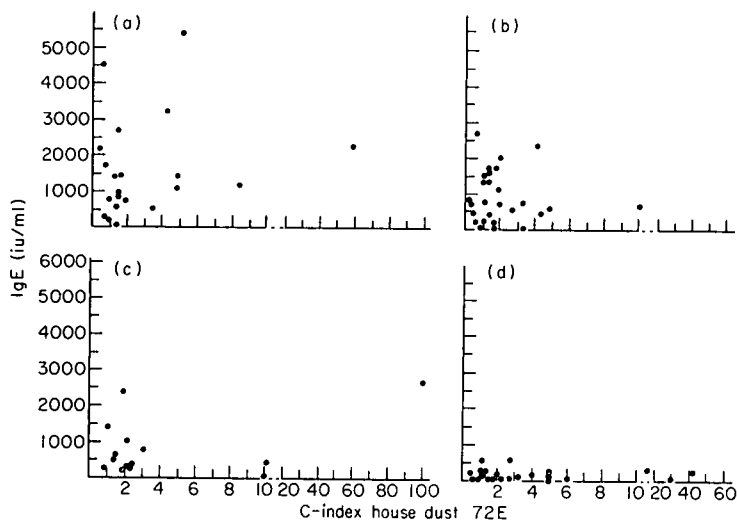


Fig. 3. Relationship between complement sensitivity index to house dust 72E and total serum IgE level in normal children and in patients with atopic diseases. (a) Asthma; (b) asthma + eczema; (c) eczema; (d) controls.

The complement sensitivity indices were neither correlated to the total serum IgE levels (Fig. 3), nor to the house dust specific IgE antibody levels (RAST-scores, Fig. 4). Similarly, there existed no apparent relationship between the complement sensitivity of the serum and the extent or nature of the clinical manifestations of the atopic condition in our patients.

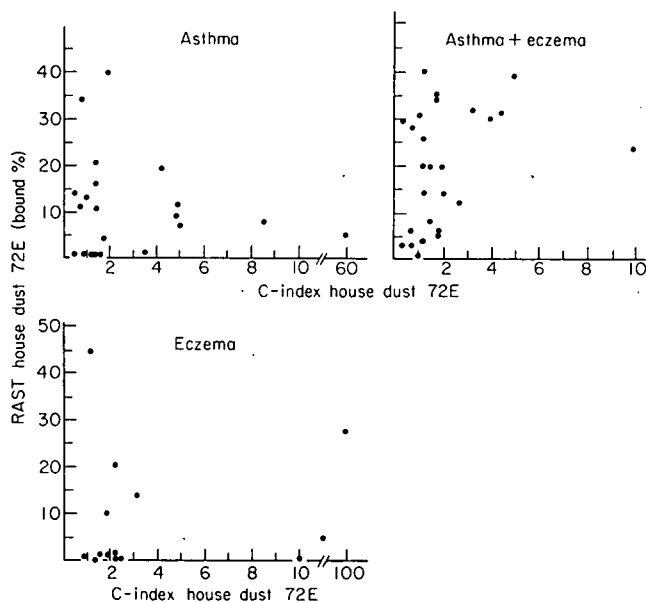


Fig. 4. Relationship between complement sensitivity index and RAST values to house dust 72E in children with atopic diseases. RAST values expressed in percentage of the added radioactivity counts fixed to the sepharose beads.

Table 2. Serum C4 concentrations in normal and atopic children

Diagnosis	No. of children	Mean C4 level (mg/100 ml)	s.d.	Range
I Asthma	21	25	9.8	10-47 ($P < 0.005$)
II Asthma + atopic dermatitis	27	28	10.4	16-62 ($P > 0.10$)
III Atopic dermatitis	14	24	6.5	12-38 ($P < 0.005$)
IV Normal	24	35	13	11-60

Table 3. Serum C3PA concentrations in normal and atopic children

Diagnosis	No. of children	Mean C3PA level (mg/100 ml)	s.d.	Range
I Asthma	21	21	7	10-36 ($P < 0.05$)
II Asthma + atopic dermatitis	27	22	6	11-36 ($P < 0.05$)
III Atopic dermatitis	14	21	5	14-34 ($P < 0.025$)
IV Normal	24	26	9	13-40

(2) Levels of complement components C4 and C3PA

As shown in Table 2 the mean level of complement component C4 in the serum of children of group I (asthma) and III (atopic dermatitis) was significantly depressed relative to the control group. However, the C4 levels varied widely in all groups.

Only in the sera of patients with asthma combined with atopic dermatitis (group II) was found a significant positive correlation between the complement-sensitivities to house dust and the serum C4 levels (Spearman rank correlation, $P < 0.01$).

The mean serum levels of C3 proactivator (factor B) have been listed in Table 3. In all groups of patients the mean values were significantly below the control. Individual values again varied within a wide range.

Discussion

Haemolytic complement in the serum of almost every patient in this investigation proved to be very sensitive to inactivation by purified house dust allergen. The mean C-inactivation index in these patients, 2.3 (asthma, group I), 2.1 (asthma and atopic

dermatitis, group II) and 3.2 (atopic dermatitis, group III) was below the mean value of 5.4 recorded for an adult atopic population (Berrens, 1975). Fluid phase complement in the control group of children was similarly more sensitive to allergen-induced inactivation (mean index 6.0) than observed in normal adults (mean value 18.8). Particularly striking was the high C-sensitivity in the control group of the sera of children with psychosomatic problems, which could not be distinguished in this respect from the atopic group. It is well known that psychosomatic factors play a part in atopic disease. The question whether the high C-sensitivities to allergen *in vitro* in both groups of children can at all be referred to a common psychosomatic substrate requires further investigation.

Though the mean complement sensitivity indices in all groups of patients were below the value for the control group, there was considerable statistical overlap. The difference between the means was significant only between the values found for group II (asthma combined with atopic dermatitis) in relation to the control group.

No correlation was found between the sensitivity of haemolytic C to allergen-induced inactivation in serum, and the patient's reactivity to intradermal injection of the same allergen. Results to be described in a following paper similarly showed lack of quantitative correlation between the level of house dust specific IgE in the serum (RAST scores) and the intensity of the skin test response. If, as has been suggested both a non-immunological (complement-mediated) and an immunological (IgE-mediated) mechanism underly allergic reactions to house dust in atopic subjects, good correlation with the skin reaction can only be obtained if the relative contribution of each is known (Berrens, 1974).

As shown in Fig. 4, no relationship was apparent between house dust specific IgE levels in serum and the house dust sensitivity of haemolytic complement.

It must be pointed out, however, that the combination of relatively resistant complement and elevated specific IgE rarely occurred. This suggests the possibility that an easily activatable complement system is a prerequisite for the ready production of specific IgE upon allergen exposure.

As expected from the lack of correlation with the skin test response, the serum complement sensitivities were in no way related to the patients' clinical condition or to the extent or nature of the clinical manifestations. The difference between the C-reactivities of the sera of individual patients was very small; moreover, high C-reactivity was often observed in the sera of control subjects. In confirmation of studies in adults, therefore, it must be concluded that the complement-allergen reaction in its present form fails to provide a practical distinction of the sera of allergic and non-allergic children (Berrens, 1975).

The results of this investigation provide some evidence for the *in vivo* involvement of the complement system in childhood atopic allergy. The mean serum level of C3 Proactivator was significantly lower in the three groups of patients than found in the control group. Furthermore, the serum concentration of the C4 component was statistically below that of the control group in the bronchial asthma children (Group I) and in the patients with atopic dermatitis (Group III). There was a significant positive correlation between the C4 levels and the allergen-complement sensitivities in the group of children with both bronchial asthma and atopic dermatitis.

The depressed C4 and C3PA levels are not based on a (partial) genetic deficiency, because most children had normal or elevated C4 levels when examined some months before the start of treatment. This finding is in agreement with the results of Kay

et al. (1974), who detected high levels of C4 in a group of children with asthmatic symptoms of short duration, but significantly lowered C4 levels in children with persistent asthma. Our own groups of patients were heterogeneous with regard to clinical symptomatology: some children had been clinically well for quite some time, others had constant (mild) symptoms during the month before obtaining the blood sample. If complement is involved in the allergy process, the level of individual components established may depend on the moment chosen for blood sampling.

Since these serum levels reflect the balance between consumption and biosynthesis in a dynamic system, the singular determination of C components in individual patients may have little interpretative value.

Acknowledgments

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