

## Effect of house dust mite on serum complement activation and total haemolytic activity *in vitro* in normal subjects and in patients with bronchial asthma

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### Summary

House dust mite (*Dermatophagoides farinae*) has been shown to activate the complement system *in vitro* by both the classical and alternative pathways in normal serum and in two groups of (skin test positive and skin test negative) patients with bronchial asthma. The activation was dose dependent in the lower range of allergen concentrations. Skin test positive asthmatic subjects showed the maximum activation. Skin test negative patients had activation between skin test positive patients and normal subjects. However, at the higher allergen concentration normal serum and bronchial asthma patients showed similar results.

### Introduction

In a previous communication the changes in the amounts of C3 and C4 in asthma patients indicated the possible involvement of the complement system (Srivastava, Srivastava & Gupta, 1982). In order to examine the mode of involvement and activation of complement in bronchial asthma it was considered relevant to examine the direct effect of common inhalant allergens on the activation of serum complement in normal serum and asthma patients.

*In vitro* studies have demonstrated the activation of the complement cascade via the alternative pathway by organic dust (Edwards, Wanger & Seal, 1976; Marx & Flaherty, 1976), airborne rye, oat and spring wheat dusts (Olenchock *et al.*, 1978; Olenchock, Mull & Major, 1979) and cotton dust (Olenchock *et al.*, 1981). Olenchock *et al.* (1980) demonstrated the activation of both classical and alternative pathways by aqueous extracts of airborne grain dust such as barley dust, corn, oat, rye, spring wheat etc. However, all these studies were made with normal human serum (NHS) only. Moreover, the reported observations on the role of complement in asthma are scanty (Kay, Bacon & Mercer, 1974; Delaney & Kay, 1976; Arroyave *et al.*, 1977; Hutchcroft & Guz, 1978; Baur, Borsch & Becker, 1980) and most of these studies have been performed by estimating only the total loss of haemolytic activity by allergens.

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As a part of programme of investigations on the biochemical and immunological aspects of bronchial asthma, the effect of common inhalant allergen, house dust mite was studied on complement (C3) activation and total haemolytic complement activity (CH50) in serum samples from normal controls and asthma patients both skin test positive (STP) and skin test negative (STN) to this allergen.

### Materials and methods

Ethylene diamine tetra acetate, (EDTA), Ethylene glycol-bis ( $\beta$ -amino ethyl ether, *N*, *N'*-tetra acetic acid (EGTA) and agarose were obtained from Sigma, U.S.A., Monospecific rabbit anti-human C3 from Behringwerke, W. Germany.

### Allergen

House dust mite, *Dermatophagoides farinae* (1000 protein nitrogen units (PNU)/ml), was procured from Miles Laboratories Ltd, U.K.

### Patients

Seven asthma patients (five skin test positive and two skin test negative) were examined for complement (C3) activation. In addition the effect of *D. farinae* on total haemolytic activity was examined in two skin test positive patients. The studies were carried out when patients were in clinically stable phase and all drugs were withheld for 24 hr before the study. None of the patients were on sodium cromoglycate therapy. Age of the patients ranged from 15 to 44 years and duration of symptoms ranged from 4 to 15 years.

### Controls

Eight healthy subjects with no family history of asthma or any other allergic diathesis formed the control group. Their ages ranged from 22 to 42 years.

### Allergen-specific IgE (RAST)

Sera were screened for allergen-specific IgE for *D. farinae* by RAST (Pharmacia, Sweden). Skin test positive (STP) patients had RAST scores of 3+ and 4+ while skin test negative (STN) patients and controls had a RAST score of 0.

All the sera were also screened for precipitating antibodies against *D. farinae* by double diffusion technique and were demonstrated to be free of precipitating antibodies.

### Complement (C3) activation

Activation of complement was monitored by the degree of conversion of C3 to C3a (small peptide) and C3b which was assayed by two dimensional cross electrophoresis (Laurell, 1965) using 2% rabbit anti-human C3 in 1% agarose (w/v). Increasing amounts of allergen were added to 0.5 ml serum incubated at 37°C for 1 hr. Half a millilitre of serum was also differentially chelated with 0.025 ml 100 mM EDTA or 0.025 ml 100 mM EGTA before incubating with the allergen. EDTA chelates both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , thus blocking both the classical and alternative pathways. On the other hand in the presence of EGTA which binds to  $\text{Ca}^{2+}$  as strongly as EDTA but binds  $\text{Mg}^{2+}$  very weakly only the classical pathway is blocked but not the alternative pathway. Complement C3 has  $\beta_1\text{C}$  electrophoretic mobility and C3b has  $\beta_1\text{A}$  mobility.

The amounts of residual C3 and C3b formed were calculated by planimetry. Percentage of C3b formed was calculated in comparison to the total. Total haemolytic complement activity (CH50) estimation was based on the method of Mayer (1961), and was estimated before the treatment of the serum with allergen and after treating the serum with increasing amounts of allergen at 37°C for 1 hr. The percentage decrease in CH50 was calculated in comparison to CH50 values obtained with untreated serum. The studies were made on fresh serum.

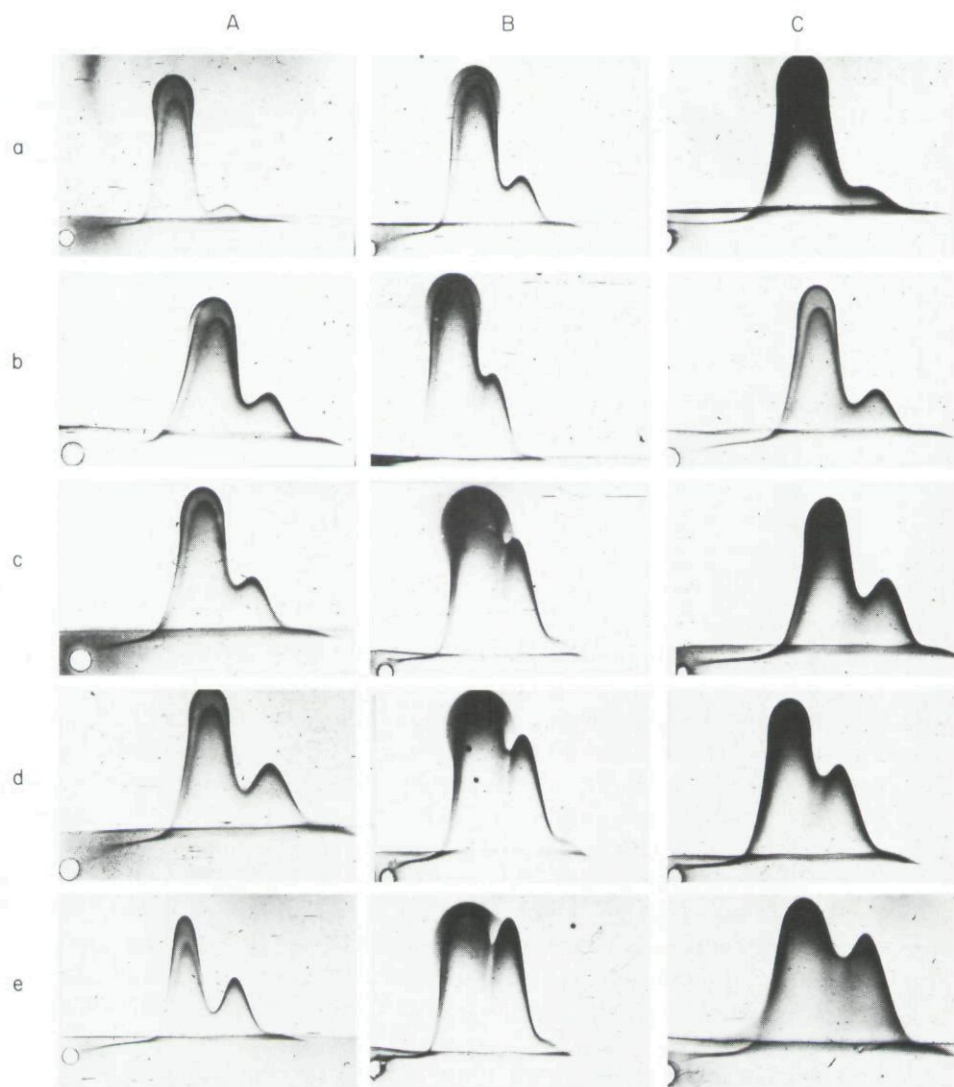


**Fig. 1.** Cross electrophoretic pattern of C3 from normal human serum (A), serum of skin test positive (STP) asthmatic (B) and serum of skin test negative (STN) asthmatic (C) treated with increasing amounts of allergen, *D. farinae*. a, Untreated serum; b, serum + allergen (1  $\mu$ l); c, serum + allergen (2  $\mu$ l); d, serum + allergen (4  $\mu$ l); e, serum + allergen (10  $\mu$ l). In each frame the left arc is C3 and right arc is C3b.

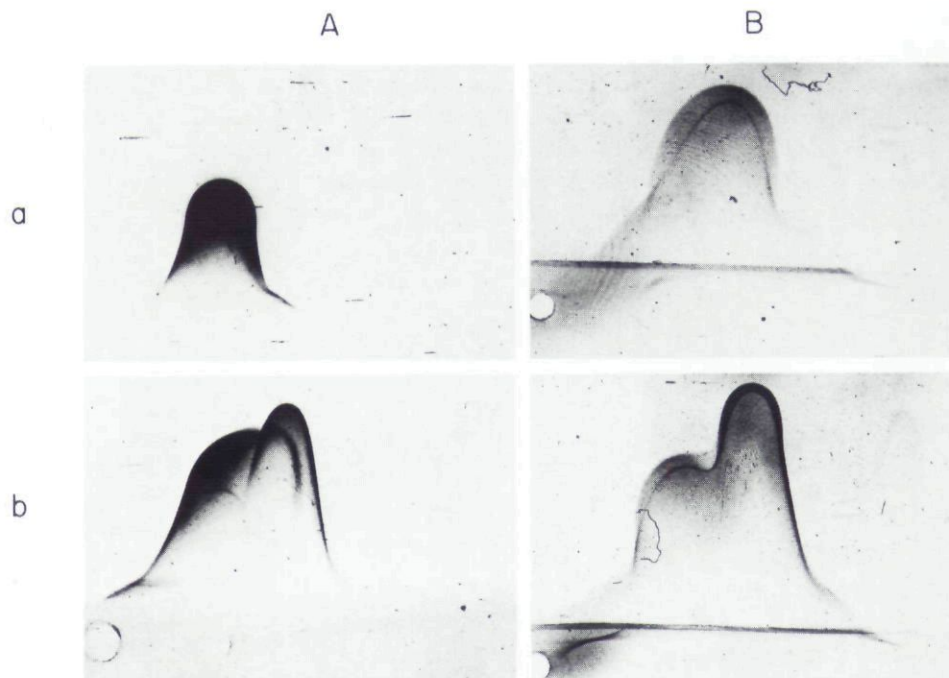


Fig. 2. Cross electrophoretic pattern of C3. A, serum from normal control; B, serum from patient with asthma (STP); a, serum + allergen, *D. farinae* (20  $\mu$ l) + 0.025 ml 100 mM EDTA; b, serum + allergen, *D. farinae* (20  $\mu$ l) + 0.025 ml 100 mM EGTA.

## Results

### Complement activation

**Normal serum.** Amounts of 1, 2, 4, 10, 20, 50, 80 and 100  $\mu$ l of *D. farinae* were tested separately with NHS. Serum was also chelated with EDTA and EGTA before treating with 20  $\mu$ l *D. farinae*. Dose-dependent activation of complement, i.e. conversion of C3 to C3b was observed (Fig. 1) and activation occurred via the alternative pathway (Fig. 2). The percentage of C3b formed increased sharply when 1, 2, 4 and 10  $\mu$ l allergen were used but tended to plateau after addition of 20, 50, 80 and 100  $\mu$ l allergen. Figure 3 shows the average values obtained in normal serum and STP and STN asthmatics.

**Skin test positive (STP) patients.** Amounts of 1, 2, 4, 10, 20, 50, 80 and 100  $\mu$ l *D. farinae* were tested separately with the sera of STP patients. Increased conversion of C3 to C3b was again evident as allergen concentration increased (Fig. 1). This conversion also was via alternate pathway (Fig. 2). As before the degree of activation of C3 tended to slow down at 20  $\mu$ l allergen and did not change much when 50, 80, 100  $\mu$ l allergen were used.

Comparison of degrees of activation of complement, i.e. conversion of C3 to C3b in STP asthmatics and normal serum when 1, 2, 4 and 10  $\mu$ l allergen were used, revealed the presence of correspondingly higher peaks in STP asthmatics as compared to normal serum at all concentrations of allergen stated above (Figs 1 and 3). But at high allergen concentration (50, 80 and 100  $\mu$ l) the degree of activation in normal serum and

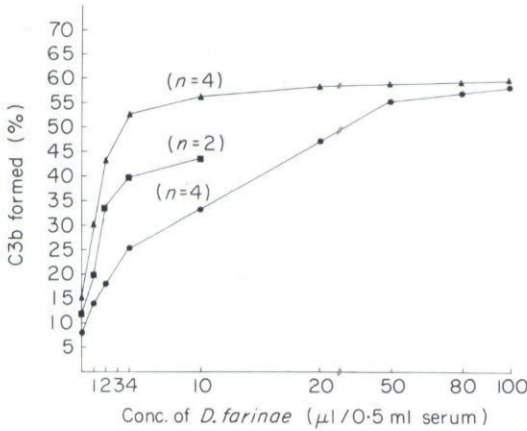


Fig. 3. Relationship between concentration of *D. farinae* and percentage of C3b formed in skin test positive (STP) asthmatic ( $\blacktriangle$ — $\blacktriangle$ ), skin test negative (STN) asthmatic ( $\blacksquare$ — $\blacksquare$ ) and normal human serum ( $\bullet$ — $\bullet$ ).

STP asthmatics was similar (Fig. 3). Thus at a given low allergen concentration the magnitude of complement activation was more in STP asthmatics than normal serum.

*Skin test negative (STN) patients.* Dose dependent conversion of C3 to C3b via the alternative pathway was observed when 1, 2, 4, and 10  $\mu\text{l}$  *D. farinae* were added to the serum samples of STN patients (Fig. 1).

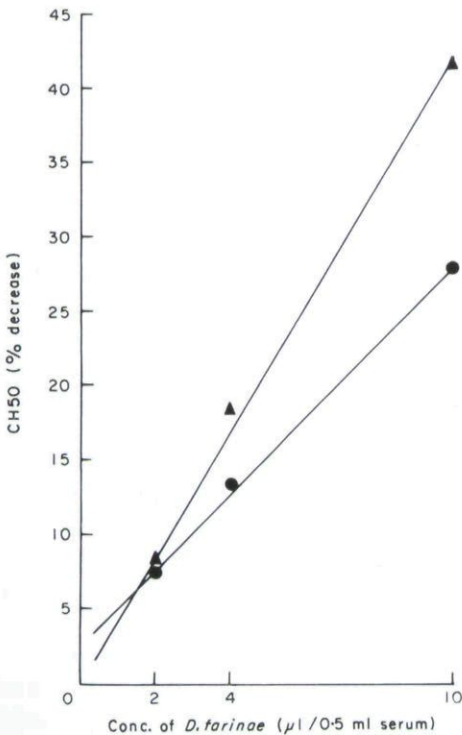


Fig. 4. Effect of *D. farinae* on percentage decrease in CH50 in skin test positive (STP) asthmatic ( $\blacktriangle$ — $\blacktriangle$ ) and normal human sera ( $\bullet$ — $\bullet$ ). Each point represents the mean value obtained from two experiments.

Comparison of C3b peaks in cross electrophoretic pattern (Fig. 1) and percentage of C3b formed (Fig. 3) after addition of 1, 2, 4 and 10  $\mu$ l allergen demonstrates that at a given low allergen concentration, the degree of complement activation in STN asthma patients was less than STP patients but more than normal serum and followed the pattern: STP asthmatics > STN; asthmatics > normal serum. However, at the higher allergen concentration (50, 80 and 100  $\mu$ l) normals and asthmatics behaved more or less in a similar fashion.

#### *Effect of D. farinae on total haemolytic complement activity (CH50)*

Treatment of the serum with *D. farinae* decreased the CH50 both in normal serum and skin test positive patients, when 2, 4 and 10  $\mu$ l allergen were added. A linear relationship was obtained between percentage decrease in CH50 and the allergen concentration in both the groups (Fig. 4).

#### **Discussion**

Examination of C3 activation by two dimensional electrophoresis revealed the conversion C3 by house dust mite, *D. farinae* to the active fragment C3b in a dose-dependent manner, in lower ranges of concentrations in NHS as well as in sera obtained from patients with bronchial asthma. But the degree of activation by the same allergen concentration was highest in skin test positive (STP) patients and lowest in normal serum. Skin test negative (STN) patients occupied a position in between these two. Differential chelation of serum with EDTA and EGTA clearly showed that the activation was via the alternative pathway. House dust mite was also shown to consume haemolytic complement (CH50) *in vitro* in a dose-dependent manner thus indicating the involvement of classical pathway both in normal serum and asthma patients.

It is evident from the results of the present study that house dust mite activates both the classical and alternative pathways of complement *in vitro*. The results of these *in vitro* studies of complement activation, may be related to *in vivo* conditions after inhalation of allergens. Anaphylatoxins, C3a and C5a the breakdown products of C3 and C5 respectively, formed as a result of complement activation by the allergens, may lead to inflammatory changes as well as the spasm of bronchial smooth muscle. Since the complement system of skin test positive (STP) patients is highly sensitive to activation by allergens even in the lowest concentration this may be the reason for such persons having repeated asthma attacks throughout the year. Relative low sensitivity of complement system in STN and even lower sensitivity in normals could provide some protection to such persons against the allergens present in the atmosphere. It is perhaps that only when allergen concentration increases beyond a certain level in the environment e.g. high pollen counts during pollination season or heavy atmospheric smog, that these people may suffer from asthmatic attacks.

Complement activation as observed *in vitro* was not a function of the surface properties of the particles since these studies were performed with particle-free extracts. The mechanism by which classical pathway activation occurs by this allergen is not clear. Serum obtained from normal subjects as well as asthmatics was demonstrated by Ouchterlony technique to be without detectable precipitating antibodies to *D. farinae*. House dust mite, *D. pteronyssinus* and *Aspergillus fumigatus* were also shown to activate the alternative pathway of the complement system (in a dose-dependent manner) in both normals and bronchial asthma patients (unpubl. data).

The observation reported in this communication concerning the complement system (Haemolytic complement) of asthma patients being more sensitive to *D. farinae*, a common inhalant allergen, is in agreement with the findings of Berrens and Rijswijk-Verbeek (1973), Berrens (1974, 1975) and Weemaes, Stoop and Berrens (1977). These workers reported that human haemolytic complement is readily inactivated (consumed) by several common inhalant allergens especially those of house dust group. Complement in serum of normal adult is often less sensitive to inactivation by allergen than is observed with the serum of atopic subjects.

Berrens (1975), in addition, calculated the mean complement sensitivity index to house dust allergens in normal adults as 18.8  $\mu$ g against a mean value of 5.4  $\mu$ g in adult atopic patients, thereby suggesting that atopic serum displayed a tendency for higher complement susceptibility.

In a recent study, Weemaes *et al.* (1977) used purified house dust allergens to study the inactivation of haemolytic complement in the serum of atopic children and control subjects. Children were grouped into three different groups according to clinical features: Group I, patients with asthma; Group II, patients with asthma and atopic dermatitis; and Group III, patients with atopic dermatitis. The mean complement inactivation index in these groups of patients was 2.3, 2.1 and 3.2  $\mu$ g respectively against 6.0  $\mu$ g in control group of children. Haemolytic complement in the serum of almost every patient proved to be very sensitive to inactivation by purified house dust allergen.

The results reported in the present communication indicate that although complement activation via both the classical and alternate pathways occurs by house dust mite in patients with bronchial asthma as well as normal subjects, but the variation in the degree of activation and dose dependency indicate an increased sensitivity of asthma patients to such allergen.

Genetic factors might also play some role. Our preliminary results show a high frequency of C3<sup>F</sup> gene in bronchial asthma samples over normal controls. Although this increase was not statistically significant it does indicate that there may be involvement of certain genetic factors which might make certain individuals more prone to bronchial asthma.

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