Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F.P.)

M. D. CHAPMAN* & T. A. E. PLATTS-MILLS Division of Immunology, Clinical Research Centre, Harrow, Middlesex

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**SUMMARY**

An extract of *Dermatophagoides pteronyssinus* culture has been fractionated by chromatography on Sephadex G-100 and Pevikon block electrophoresis to obtain a partially purified allergen (F.P.). This preparation has a molecular weight of between 15-25,000 Daltons, migrates slowly on electrophoresis, and is colourless in solution. The skin-test reactivity of F.P. was comparable to that of crude *D. pteronyssinus* extract. F.P. was radio-labelled with 125I and used in an antigen-binding radioimmunoassay to measure IgG, IgA and IgE antibody (ab) to *D. pteronyssinus*. IgG, ab was detected in serum from 32/34 (94% ) mite-allergic persons, and from 10/31 (33%) non-allergic persons. IgA ab and IgE ab were found in sera from 22/34 (65% ) and 27/34 (79%) allergic persons respectively. Neither IgG nor IgE ab could be detected in sera from non-allergic persons. An excellent correlation was found between radioallergosorbent technique (RAST), using crude *D. pteronyssinus* extract and IgE-binding activity (BA) for F.P., (r = 0.94, P=0.001). The antigen-binding assay for IgE BA was as sensitive as RAST, but less sensitive than PK testing. There was a very good quantitative correlation between F.P. BA and IgE BA (r = 0.84, P=0.001). IgG BA was shown to rise in the serum of three patients treated with injections of *D. pteronyssinus* extract.

**INTRODUCTION**

The role of mites of the *Dermatophagoides* species, notably *Dermatophagoides pteronyssinus* and *D. farinae* as allergens of house dust have been well established (Voorhees, Speckama-Boezema & Speikema, 1964; Voorhees et al., 1967; Maunsell, Wraith & Cunningham, 1968; Holbrook-Stavros et al., 1970; Miyamoto et al., 1968). Mite sensitivity is thought to be of great importance in the etiology of perennial rhinitis and extrinsic asthma (Morrisson-Smith et al., 1969; McAllen, Assem & Maunsell, 1970; Frankland, 1971). In the past, crude extracts of mite culture have been used both for skin-testing and for measuring serum IgE antibodies (ab) in the radioallergosorbent technique (RAST) (Wilde, Bennich & Johannson, 1967; Semina & Wilde, 1969). RAST results have shown to correlate well with other methods of assessing IgE ab, i.e. skin-testing, PK and provocation tests (Wilde et al., 1967; Berg, Bennich & Johannson, 1971; Bennich & Johannson, 1971). In addition, IgG ab to Dermatophagoides has been demonstrated in sera from patients by radiommunoelectrophoresis (D’Souza et al., 1973; Gabriel et al., 1977).

The most sensitive method for measuring IgG ab to allergens is the double antibody or antigen-binding technique (Osler, Mulligan & Rodrigues, 1966; Newcomb & Ishizaka, 1967; Zugmeyer &

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Correspondence: M. D. Chapman, Division of Immunology, Clinical Research Centre, Harrow, Middlesex HA1 3UJ. 0003-1047/78/0101-0126/02.00 ©1978 Blackwell Scientific Publications 126
Antibody measurement to *D. pteronyssinus*

Gleich, 1973; Platts-Mills et al., 1976). Antigen-binding activity associated with IgE has also been demonstrated (Ishizaka, Ishizaka & Futamura, 1967). Recently we have reported the development of an antigen-binding technique for measuring IgE in parallel with IgG and IgA (Platts-Mills et al., 1978). However, antigen-binding techniques require purified or at least partially purified allergens (Marsh, 1975; King, 1976). As yet no purified allergen has been described for either *D. farinae* or *D. pteronyssinus*. On the other hand, several structurally similar to the major allergens of mite allergens have been made, based on the skin-test reactivity of fractions of mite extract separated by gel filtration (Miyamoto, Oshima & Ishizaka, 1969; Romagnani et al., 1972, 1976).

The present report describes the partial purification of a *D. pteronyssinus* allergen which has good skin-test reactivity and is suitable for radiolabelling. Using this allergen an antigen-binding radioimmunoassay has been developed for measuring IgG, IgA and IgE ab to *D. pteronyssinus*.

**MATERIALS AND METHODS**

**Allergen preparation.** *Dermatophagoides pteronyssinus* whole culture was kindly provided by Bernard (Bechworth, England). 117 g of mites was extracted in Le PBS by stirring overnight at 4°C. The extract was centrifuged at 12,000 g for 60 min, and the supernatant concentrated down to 50 ml over Amicon PM10 filter (Amicon, Lexington, Massachusetts, U.S.A.). The dark brown concentrate (Extract 1) was then dialysed for 24 hr against two changes of borate-buffered saline pH 9.0 (BBS), and sterilized through an 0.45 µm Millipore filter. 7.5 ml Extract 1 was applied to an upward flow, gravity fed, 75 x 2.5 cm Sephadex G-100 column, equilibrated in BBS at 4°C. Samples of 6.3 ml were collected at a flow rate of 10 ml/hr, and the absorbance of each sample was measured at 280 nm. The eluted samples were then pooled in six fractions designated F1-6. These fractions were then dialysed, concentrated to 4 ml BBS and centrifuged at 12,000 g for 30 min. The protein concentration of the fractions was determined by the method of Lowry et al. (1951) using bovine albumin as a standard. On the basis of skin-testing (see below), fraction F4(2/3) was used for further purification.

Preparative block electrophoresis of F4 using Pevikon G-870 (Shandon Southern Ltd., Runcorn, Cheshire) was carried out as described by Miller-Eberhard (1960). Approximately 50 mg F4 was applied to a 50 x 1 cm block of Pevikon equilibrated in 0.03 M borate buffer pH 8.4 at 4°C. Electrophoresis at 200 V and 10 mA was continued for 2 hr. The protein concentration of 10 ml eluates of 1 cm segments of the block was then determined. The eluates were pooled and concentrated on the basis of their protein content and reactivity in in-vivomodulation against rabbit anti-D. pteronyssinus serum (see Results). The PEP (fraction was fractionated on Sephadex G-100 and the single, apparently homogeneous, peak was concentrated by negative-pressure dialysis. After pooling, all fractions were stored at -70°C.

**Antiserum.** Rabbit anti-*D. pteronyssinus* serum was prepared by injecting two NZW rabbits with 1 mg of *D. pteronyssinus* Extract 1 in PCA (Difco Labs, Detroit, Michigan, U.S.A.) at fortnightly intervals. The rabbits were bled 11 days after the fifth injection, and the serum stored at -20°C. The preparation of microtiter plates, anti-IgG, anti-IgA and anti-IgE has been described previously (Platts-Mills & Ishizaka, 1973; Platts-Mills et al., 1976, 1978). The animals were raised in gnotobiotic animals, by repeated immunization with Fc fragments of IgG, IgA myeloma protein or Fc fragment of IgE. (FPS) myeloma protein (Takara, IShizaka & Ishizaka, 1973). Each antiserum was made monospecific by repeated passage over immunosorbent columns of Sepharose 2B, linked to human proteins by the conjugated bromide technique (Axen, Pestal & Erichard, 1967). Anti-IgG was absorbed with Fc, IgA and IgE. Anti-IgA was absorbed with IgG and set from an IgA-deficient patient, and anti-IgE was absorbed with IgG.

**Skin testing.** Intradermal skin testing with *D. pteronyssinus* Extract 1, Sephadex G-100 fractions, F1-6, and the Group 1 protein of *R. av stalin* patient, E.V. (March, 1977) was carried out on the arms of twenty-volunteers, who had been inoculated with the allergens involved. The preparations were sterilized through an 0.45 µm Millipore filter. Serum skin-test dilutions of allergens were made from 10 µg/ml down to 10-5 µg/ml in 0.05% human serum albumin (HSA) in plain saline. Skin tests were performed by successive intradermal injection of 0.01 ml of increasing concentrations of allergens. The diameter of the vesicle and flare was measured 20 min after injection.

**PFC testing.** PFC testing was performed with five healthy individuals (Australia antigen negative) using only two recipients, M.C. and T.F.M., whose total serum IgE was 11 mg/ml and 175 mg/ml, respectively. The sera were fractionated with saturated ammonium sulphate (SAS) and dialysed extensively against BBS. The 15-30% SAS fraction, which has been shown to be enriched for IgE (Garrus, Morgan & Bacon, 1973), was diluted from 1:10 to 1:1000 in 0.05% HSA, plain saline, and sterilized through an 0.45 µm Millipore filter. 0.05 ml aliquots of suitable dilutions were injected intradermally. After 24 hr each site was challenged with 0.05 ml of a 1:10 solution of *D. pteronyssinus* extract T, and the wheal and flare reactions recorded.

**125I labelling of Fc.** 125I labelling was carried out using the Chloramine-T technique in a modification of the method of Kline- man & Taylor (1966). To 40 µg Fc, 20 µl of 2.5 µC 125I phosphorus buffer pH 7.5, 1.4 x 106 c/mm and 50 µl of Chloramine T. The reaction was stopped by the addition of sodium metabisulphite and 3 min later the reaction mixture was diluted with 2 ml of 0.15% BSA containing 50 µl 1% sodium metabisulphite. After dialysis against three changes of BBS the
RESULTS

The elution profile for Sephadex G-100 fractionation of Dermatophilus pneumoniae Extract I showed two peaks at 280 nm. A small peak eluted with the void volume, and a very broad peak eluted over the 35-65,000 Daltons molecular weight range, indicated by the positions of BSA and cytochrome c markers (arrows in Fig. 1). The fractions were pooled and tested by quantitative intradermal skin-testing; maximal skin-sensitivity was shown with fractions 3 and 4. In general, the skin-reactivity of these fractions was ten- to one-hundred-fold greater than that of the other fractions in each of the six individuals tested. While each of the fractions were coupled to RAST discs 8/6 sera from allergic persons showed maximal binding towards fractions 4 (see Materials and Methods, Fig. 4). There was no significant binding to any fraction by sera from non-allergic persons. Separation of fraction I by Perkin block electrophoresis showed two protein peaks (Fig. 2). The major peak, F^1, migrated faster than albumin, was dark brown in colour, and by immunodiffusion showed no reactivity with a rabbit antiserum to Extract I of D. pneumoniae. The smaller peak, F^2, showed only a faint brown colour, migrated slowly, and was strongly reactive on immunodiffusion (Fig. 2). This fraction, F^2, was passed through Sephadex G-190 and eluted with a molecular weight of between 15-25,000 Daltons. This recomhanomorphated material was used for skin-testing and radiolabeling and is hereafter referred to as F^2. Radiolabeled F^2 was used in a double antibody antigen-binding assay to measure specific IgG, IgA and IgE ab to D. pneumoniae. The effect of the amount of antigen added to this assay was investigated over the range 0.5-5 µg F^2 added/tube. Five µg 125I F^2, F^A, and radiolabelled fraction of this solution were made. One hundred µl of each dilution of antigen was used to assay IgG and IgE ab in six sera diluted 1:125. The results in Fig. 3a and 3b show that
**Antibody measurement to D. peroniiensis**

**Fig. 1.** Fractionation of *Doreomygialis peroniiensis* Extract 7 on Sephadex G-200. Arrows indicate the positions of BSA (MW 66,000) and cytochrome c (MW 12,300) markers. For each fraction skin-test sensitivity was assayed by quantitative intradermal skin-testing. Each fraction was also coated on cellulose discs and used for RAST. Typical results are shown on a semi-quantitative basis (+ to ++ + +).

**Fig. 2.** Protein block electrophoresis of Sephadex G-200 Fraction 4. Arrow indicates the position at which 30 mg IgG was applied to block.
maximum differences between allergic and non-allergic sera were achieved by adding 0.5-5 ng radiolabelled antigen for both IgG and IgE. With increasing quantities of antigen (over 50 ng P, tube) although the percentage radioactivity bound by non-allergic sera decreased, the absolute levels of radioactivity (counts/100 sec, Fig. 3a and 3b) increased dramatically. This resulted in a progressive loss of sensitivity because the sensitivity of the assay depends on the absolute background radioactivity with serum from non-allergic persons. Assays for IgG, IgA and IgE BA were carried out routinely using 7 ng Tm, P, tube. The other details of this assay were similar to those used previously for antigen E and Rye E (see Materials and Methods). Typical detection curves for IgG and IgE BA are shown in Fig. 4. The IgE BA curve could be superimposed on those for IgG BA, and therefore all quantitative results were obtained by extrapolation from a single IgG BA control curve.

Specific IgG, IgA and IgE ab to D. pteronyssinus were measured by antigen-binding assay in the serum of thirty-five persons who had positive prick tests with D. pteronyssinus extract (Benzoars Ltd.) (Fig. 5). Sera were also studied from thirty-one non-allergic persons with negative skin tests. The results show that IgE BA was detectable in 32/34 sera from prick-test-positive persons, IgG BA was detectable in 22/34 and 32/34 sera contained IgA BA. In sera from prick-test-negative persons IgE and IgA BA were not detectable, but in 10/31 sera IgG BA could be detected. However, the levels of IgG BA were much lower than those in most prick test-positive persons (Fig. 5). Twenty-eight of the prick-test-positive persons had perennial rhinitis suggestive of house dust sensitivity, and the remaining six persons had rhinitis, but their symptoms did not seem to be related to house dust. In sera from these two 'asymptomatic' persons IgE BA and IgA BA were not detectable, but 4/6 contained low levels of IgG BA. In Table 3 the clinical symptoms, intradermal skin-test reactivity and serum ab levels of fifteen prick-test-positive volunteers are shown. The results of intradermal skin-testing with D. pteronyssinus Extract 1 and P, showed that all fourteen persons tested were sensitive to both preparations. In general, the minimum quantity of P, necessary to give a positive skin-test was about one-fifth the quantity of Extract 1. To check the specificity of the skin-tests non-allergic persons were skin-tested with 10 µg P, none of these five gave a positive reaction. In addition, two persons with grass-pollen hay fever who gave positive skin-tests of 10 µg Rye E failed to react to 10 µg P, Using the IgE-rich fractions of five sera from skin-test-positive persons, there was a good correlation between PK titre tested with D. pteronyssinus.
Extract I and IgE BA for F₅P₃ (Table 1). PK tests were completely negative using two sera from skin-test-negative persons, and sera from the two persons with grass-pollen hay fever described above.

IgE ab was measured by both antigen-binding assay and RAST in thirty-three of the sera from skin-test-positive persons. An excellent quantitative correlation between the results of the two methods was found by linear regression analysis ($r = 0.94, P < 0.001$, Fig. 6). Analysis of the results in Fig. 5 shows an excellent correlation between IgE BA and IgG BA ($r = 0.87, P < 0.001$). The mean ratio of IgG BA/IgE BA was 7.4:1. We also found a good correlation between IgE BA and total IgE ($r = 0.63, P < 0.001$), and a modest correlation between IgG BA and total IgE ($r = 0.28, P < 0.001$). IgG BA was assayed in pre- and post-treatment sera from three patients who had received at least six graded injections of aqueous
Table 1. Antibody levels in canine allergic subjects measured by antigen-specific skin testing, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA).

<table>
<thead>
<tr>
<th>Subj</th>
<th>Species/strain</th>
<th>Anti-Canine IgE (IgG)</th>
<th>RAST</th>
<th>IgE RA</th>
<th>IgG RA</th>
<th>IgA RA</th>
<th>P.E.</th>
</tr>
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<tr>
<td>JS (17)</td>
<td>R. hist. ser.</td>
<td>0.001</td>
<td>0.001</td>
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<tr>
<td>M.A. (14)</td>
<td>R. hist. ser.</td>
<td>0.001</td>
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<td>R.G. (10)</td>
<td>R. hist. ser.</td>
<td>0.001</td>
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<td>M.K. (10)</td>
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<tr>
<td>S.M. (10)</td>
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<td>D.T.G.L. (10)</td>
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<td>P.M.D. (10)</td>
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<td>S.S. (10)</td>
<td>R. hist. ser.</td>
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| * Other allergens given in parentheses and positive allergens are indicated in parentheses. Group = general population. 

Note: Antibody levels were determined by skin testing, ELISA, and RIA. The levels of IgE, IgG, and IgA antibodies were measured and compared among the subjects.
D. pteronyssinus extract between January and July. The levels of IgG BA rose from 41, 204 and 750 units/ml in pre-treatment sera, to 562, 2600 and 8000 units/ml in post-treatment sera, respectively.

DISCUSSION

The results of gel filtration of mite allergens reported here suggest a molecular weight of between 15-25,000 Daltons, and are similar to those reported by previous workers (Miyamoto et al., 1969; Romagnani et al., 1972, 1976; Holmford-Stevenson, 1972). Attempts to further purify the allergens in mite extracts have previously been unsuccessful. Neither isoelectric-focusing (Ricci, Biliotti & Romagnani, 1975), chromatography on DEAE-Sephadex (Romagnani et al., 1970) or affinity chromatography on lectins (Maldo & Uhlhoehrke, 1977) have yielded an allergen with skin-test reactivity comparable to that of the crude extract. The electrophoresis of mite extract in Polyvon reported here has given very good yields of allergenic material. The large peak of rapidly migrating material, F3P2, was not found to react with rabbit antisera. This highly coloured material was assumed to be made up predominantly of the culture medium in which Dermatophagoides pteronyssinus was grown. The culture medium was described by the manufacturer as an animal protein of non-dermal origin which was only very weakly immunogenic in rabbits. The slowly migrating fraction F3P1 was compared with crude mite extract by skin-testing, reactivity with rabbit antisera, and by the properties which radiolabelled with 121I. There was an excellent correlation between the two preparations when compared by quantitative intradermal skin-testing (Table 1). It was disappointing that the increased skin-reactivity of F3P1 was only about five-fold. However, our estimates of the protein concentration of crude mite extract are only approximately because of the complex mixture of the proteins present. When tested by immunodiffusion and Laurell electrophoresis F3P1 gave one major line, and two faint lines. By contrast, D. pteronyssinus Extract 1 gave six lines on immunodiffusion, and at least fifteen on Laurell electrophoresis (data not shown). F3P1 could be radiolabelled with 121I approximately three times more efficiently than Extract 1. Furthermore, using serum from patients who had been treated with mite extract a large proportion (68%) of the radioactivity in 121I-labelled F3P2 could be bound by IgG at. The properties of F3P1 were as good as those we have obtained in antigen-binding assays with other inhalant allergens, e.g. antigen E and Rye 1 (Platts-Mills
The antigen-binding radioimmunoassay used here was arranged to give maximum sensitivity. This was made possible by using small amounts of radiolabelled F.P., (2 ng/tube). Results obtained with larger quantities of antigen have given very similar quantitative relationships both between classes and between different sera (data not shown). However, using higher quantities of antigen the ab levels in many of the untreated sera would not have been detectable. Because we can quantitate IgG BA in sera from untreated patients the assay is suitable for measuring the IgG ab responses to desensitizing injections. This will allow a direct evaluation of the relationship between clinical effects and the IgG ab response produced by injections of mite extract (M. Chapman, T. A. E. Platt-Mills, Sister M. Gabriell, H. C. Ng, W. G. L. Allen, E. E. Hill & A. J. Nunn; also M. W. Turner, F. F. Soothill, J. F. Price, M. Chapman, T. A. E. Platt-Mills, E. Hey & J. F. Mowbray—both manuscripts in preparation). The sensitivity of the assay for IgE BA was very similar to that of RAST, but approximately five-fold less sensitive than PK testing. The quantitative relationship between IgG, IgA and IgE BA to D. pteronyssinus in serum from untreated patients was similar to that reported previously for antibodies to Rye I in serum from patients with hay fever (Platt-Mills et al., 1978). Thus in most cases IgG BA IgE BA IgA BA and IgE BA was never found in the absence of IgG BA. The finding that a significant proportion of non-allergic persons had IgG BA to D. pteronyssinus in the absence of IgE or IgA BA, was not surprising, since similar results have been described for both Antigen II and Rye I (Levine, Steinberg & Fortino, 1972; Black et al., 1976). A previous report using a modified RAST technique suggested that IgA ab to D. farinae were present at higher levels in sera of non-allergic individuals than in sera of allergic individuals (Stokes, Tayye & Turner, 1974). Our results show that IgA BA was present in serum from the majority of allergic persons (22/38) but was not detectable in serum from non-allergic persons. It seems possible that the previous results represent variations in the IgA ab RAST background, because several groups have found that non-specific binding of human IgA, and IgG, to RAST beads is frequently too high to allow sensitive assays (Johansson et al., 1974; Soothill et al., 1976). Although transient low levels of serum IgA have been shown to precede atopic disease in infancy (Taylor et al., 1973; Soothill et al., 1976), there is no evidence from the present investigations to suggest that poor IgA responses are involved in the aetiology of allergic illnesses in early childhood and adult life (Taylor, 1974). This may indicate that transient, low levels of serum IgA in infancy are merely a marker for some other mechanism which is related to the onset of allergy (Soothill et al., 1976). The data reported here support the view that allergic patients make an immune response to inhalant allergens which includes IgG, IgA and IgE ab. By contrast, non-allergic persons make a small IgG ab response or a detectable ab response at all. The very good correlation found between IgG BA and IgE BA (r = 0.84, P < 0.001) adds further support to the view that the factors that control the occurrence of inhalant allergy are acting over antibody responses in general, and not over IgE ab alone.

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