

IgE antibodies to *D. pteronyssinus* in atopic patients

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Accepted for publication 24 February 1988

SUMMARY

An aqueous house dust mite extract was separated by SDS-PAGE and transferred to nitrocellulose membranes by Western Blotting. Two major allergens, *Der pI* and *Der pII* associated with the mite faeces and body, respectively, were identified on the protein blot. The blot was then probed with atopic sera and the bound antibodies were labelled with ^{125}I anti-IgE and visualized by autoradiography. Using this technique it is shown that serum samples from eczema patients contain a higher proportion of anti-mite body IgE antibodies, whereas those from asthma patients contain a higher proportion of antibodies against the major mite faecal allergen *Der pI*. Minor mite body allergens are also shown to be important in eczematous patients. The role of mite allergens in atopic conditions is discussed, along with the significance of the findings presented here.

INTRODUCTION

The association between house dust allergen, asthma and *Dermatophagoides pteronyssinus* has long been recognized. It is clear that house dust mite extracts contain a complex array of allergens (Lind & Lowenstein, 1983; Tovey & Baldo, 1984; Krilis *et al.*, 1984). The major allergen from *D. pteronyssinus*, at least in asthma, is present in the mite faecal particles (Chapman & Platts-Mills, 1980). It has a molecular weight of 25,000. This allergen has been isolated and purified by a number of groups (Chapman & Platts-Mills, 1980; Lind, 1985; Stewart, 1982) and is internationally recognized as *Der pI*. Another major *D. pteronyssinus* allergen, *Der pII*, has been isolated from mite body extracts. It differs chemically from *Der pI* and has a molecular weight of 15,000. Such body allergens have also been implicated as possible causative agents of asthma and eczema (Platts-Mills *et al.*, 1983; August, 1984). It has been shown that, in eczema, the hypersensitivity reaction against the body allergens is greater than that in the case of asthma. It was also suggested that sensitization in eczema may occur through the skin rather than by inhalation or ingestion (Carswell & Thompson, 1986). This is hypothesized as the mite body, being much larger (270 μm length) than the faecal particle (20 μm diameter) (Tovey, Chapman & Platts-Mills, 1981), is not likely to become airborne. The faecal particles are of comparable dimensions to grass pollen which can act as an aeroallergen (Chapman, Tovey & Platts-Mills, 1982).

The present study was designed to examine this hypothesis further. SDS-PAGE was used to separate the mite proteins. These were then transferred to nitrocellulose paper by the

relatively new technique of electroblotting. The separated protein components can be fixed to the membrane in such a way that they retain many of their biological properties and are available for immunological characterization. We have applied this technique using different antibodies to define 'major' allergen bands, and with sera from house dust mite-sensitive patients we have examined the concentration and IgE-binding pattern in atopic subjects diagnosed primarily with asthma or eczema. These were a different series of patients to those from which we derived our initial hypothesis (Carswell & Thompson, 1986).

MATERIALS AND METHODS

House dust mite extract

Mites were grown in pure culture, on sterilized extract of bovine liver powder (Oxoid Ltd) as described previously (Carswell & Thompson, 1987). Equal volumes of whole mite culture and phosphate-buffered saline (PBS), pH 7.4, were mixed on a rotor at room temperature for 24 hr. The supernatant was decanted into Eppendorf tubes, and then clarified by centrifugation in a microcentrifuge for 6 min. The supernatant was then drawn off and frozen at -20° until required. As a control for substrate interference, the growth media was extracted for 24 hr alongside the whole mite culture. The culture extract contained 320 $\mu\text{g}/\text{ml}$ of protein.

Antisera

Murine monoclonal antibodies. Mouse monoclonal antibody to mite allergen *Der pI* was kindly provided by Dr P. Lind, University of Copenhagen, Denmark. Anti-DPX monoclonal (*Der pII*) was supplied by Dr R. Aalberse, University of Amsterdam, The Netherlands.

Atopic sera. Serum samples were collected from 16 atopic subjects attending the asthma and eczema clinics at Bristol

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Children's Hospital (aged 4–12 years, mean 7.5; 1:1 male:female). The severity of the clinical symptoms was ascertained from the patients' medical records. The group of eight asthma patients had a clinical history of mite-sensitive asthma with little or no dermatological involvement. Similarly the eight eczema patients were diagnosed as having moderate to severe dermatitis, with little or no respiratory problems. These patients were not the patients tested in our previous publication (Carswell & Thompson, 1986).

Radiolabelling. Monoclonal anti-*Der pI* and monoclonal anti-*Der pII* were radiolabelled with ^{125}I by the Chloramine-T-Technique (Klinman & Taylor, 1969). Ninety percent of the activity could be removed by precipitation with trichloroacetic acid (TCA) (Sigma) from both preparations. ^{125}I anti-IgE antisera were kindly provided by Pharmacia Ltd (Uppsala, Sweden).

Gel electrophoresis. Two-hundred microlitres aliquots of the house dust mite extract were boiled for 2 min with 100 μl of sample buffer containing mercapto-ethanol and then separated by SDS-PAGE (Laemmli, 1970) in a Biorad mini protean II cell using a 15% running gel and a 5% stacking gel, running at 330 V for 30 min. Low molecular weight markers from LKB Ltd (Pharmacia LKB Ltd, Milton Keynes, Bucks) were used to determine the molecular weight of the house dust mite proteins. After electrophoresis, a strip was cut from the gel and stained with Coomassie brilliant blue R250 (Sigma) (Diezel, Kopperschlager & Hoffman, 1972). The remainder of the gel was equilibrated for 30 min in Tris/glycine transfer blot buffer to allow protein renaturation and removal of the electrophoretic buffer from the gel prior to Western blotting.

Electrophoretic transfer of proteins to nitrocellulose. Protein bands were transferred to nitrocellulose (trans-blot 0.45 micrometers, Biorad Ltd) using a modification (Sutton, Wrigley & Baldo, 1985) of the method of Towbin (Towbin, Staehelin & Gordon, 1979) using a Biorad trans-blot cell according to the manufacturer's instructions for 3 hr at 70 V. During this procedure, the contents of the cell were stirred continuously to assist cooling of the transfer buffer (0.025 M Tris, 0.192 M glycine, pH 8.3, 20% v/v methanol). The residual gel was stained with Coomassie brilliant blue to confirm transfer. A 1-cm strip of nitrocellulose was cut post-transfer and its proteins stained with amido-black (0.15% in water, methanol, acetic acid, 65:25:10) for 5 min. The remainder of the blot was washed for 1 hr in 10% methanol in PBS with rocking, followed by immersion in 0.1% Tween 20 (Sigma) in PBS at 4° to block unbound membrane sites (Batteiger, Newhall & Jones, 1982). All nitrocellulose membranes were air-dried and stored at 4°.

Immunological detection. The radiolabelled monoclonal antibodies against *Der pI* and *Der pII* allergens were diluted with 1% BSA in PBS to a specific activity of approximately 50,000 c.p.m./ml. One-centimetre wide nitrocellulose strips were incubated with these solutions for 24 hr. This directly detected the protein bands corresponding to the *Der pI* and *Der pII* allergen bands. Further nitrocellulose strips, to which the mite proteins had been transferred, were incubated with atopic sera (diluted 1 in 5 with 0.1% sodium azide in PBS) for 48 hr on a shaker at room temperature. The membrane strips were then washed in 0.5% Tween 20 PBS for approximately 1 min and incubated with ^{125}I -anti-human IgE for a further 24-hr period on the shaker. After extensive washing (1 hr) with 0.5% Tween 20 PBS, the nitrocellulose strips were air-dried at room temperature and

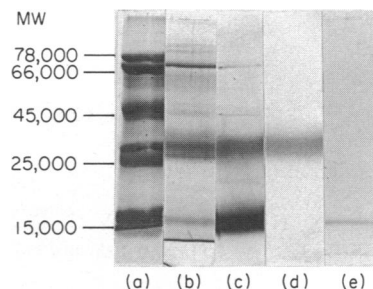


Figure 1. SDS-PAGE and autoradiographic analysis of the 24-hr house dust mite extract. Track (a) MW markers stained with Coomassie brilliant blue; (b) 24-hour mite culture stained with Coomassie brilliant blue; (c) house dust mite electroblot probed with pooled atopic sera, IgE-binding bands visualized by autoradiography; (d) anti-*Der pI* on house dust mite blot; (e) anti-*Der pII* on house dust mite blot.

then loaded into a Kenex cassette with high speed X intensifying screens (both from Genetic Research Instrumentation Ltd), together with photographic film (Fuji RX Safety, Japan). The autoradiographs were developed and scanned using a Biorad video densitometer to semi-quantify the level of IgE binding (Tovey & Baldo, 1987). Densitometric scanning of autoradiographs allows the semi-quantitative comparison of IgE binding to different regions of the same nitrocellulose strip or to the same region of strips incubated with the different sera. Fetal cord serum was the negative control.

RESULTS

SDS-PAGE and transfer of separated mite components to nitrocellulose

The capacity of polyacrylamide gel electrophoresis to separate components of a mite extract is demonstrated in Fig. 1, where at least 20 different protein bands can be seen on staining with Coomassie brilliant blue. After electrophoretic transfer to nitrocellulose and probing with pooled sera from 10 mite allergic subjects, at least nine separate IgE-binding bands can be distinguished. Two of these stained intensely, having molecular weights of approximately 25,000 and 15,000. Probing of the nitrocellulose membrane with monoclonal anti-*Der pI* and monoclonal anti-*Der pII* showed that these two allergens were identified at the same position as the two intensely staining bands observed with the pooled atopic sera (Fig. 1). When the group of 16 atopic sera were examined individually, at least nine separate IgE-binding bands were detected. These bands corresponded to those found with the pooled sera. Seven of the proteins had molecular weights of less than 60,000. Figure 2 illustrates the use of the nitrocellulose blot technique for the screening of IgE antibody patterns in panels of eczematous and asthmatic patients. The eczema patients had a greater concentration of IgE antibodies to the *Der pII* allergen and the other minor body allergens than did the asthma patients. Table 1 shows the relative intensities of the autoradiographic bands when measured with the scanning densitometer. The *Der pI* faecal allergen band also stained less intensely in the eczematous group and was only detected in three out of eight eczema patients but in all eight asthmatics. *Der pII* was detected in all eczema patients but only in five of the asthma patients. The bands detected in the former were much more intense on the whole than those seen in the latter cases. The minor body allergens were only detected when testing eczematous sera.

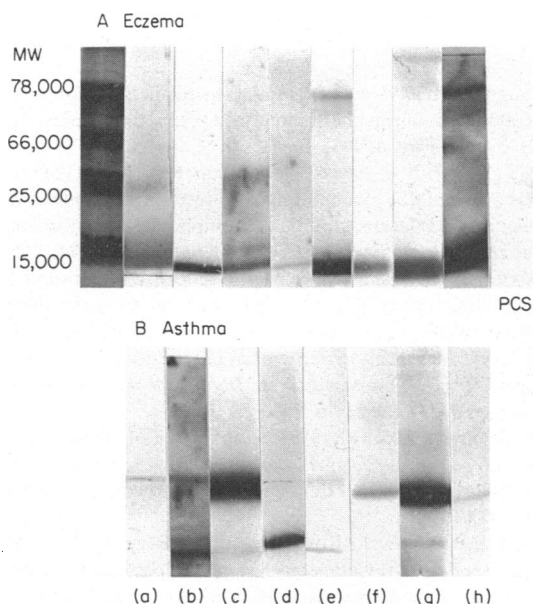


Figure 2. IgE-binding bands visualized by autoradiography in eczema and asthma patients. (A) eczema patients; (B) asthma patients; MW, molecular weight markers; PCS, placental cord sera, negative control; a-h, patient identification.

Table 1. The relative intensities of the IgE-binding bands in asthma and eczema patients when analysed with the scanning densitometer

IgE-binding bands (% absorbance)						
Patients	<i>Der pI</i> 25,000 MW	<i>Der pII</i> 15,000 MW	Other			
			1 78	2 60	5 55	9 17
Eczema group						
(a)	15	45				
(b)	0	35				
(c)	24	32	9		8	12
(d)	8	14				
(e)	0	52		12	5	
(f)	0	41	10		6	
(g)	0	63	8	5		
(h)	10	72	5	22	5	
Asthma group						
(a)	5	0				
(b)	25	35				
(c)	74	7				
(d)	4	36				
(e)	10	12				
(f)	24	0				
(g)	68	15	4	5	5	
(h)	12	0				

DISCUSSION

Several groups have analysed mite extract by immunoblotting and have each described varying numbers of IgE-binding proteins in their respective systems (Tovey & Baldo, 1985; Geissler *et al.*, 1986). Tovey & Baldo (1985) have reported over 20 IgE-binding mite proteins. In this report, we were only able to identify nine mite fractions on the nitrocellulose membranes that bound atopic sera. Two of these bands were identified as the major house dust mite allergens *Der pI* and *Der pII* by direct labelling with monoclonal antibodies. The divergencies between reports reflects the differences in the techniques employed: the mite extracts used and the immune sera utilized (adult, child or asthmatic, eczematous, etc.). We have applied the electroblotting technique using monoclonal antibodies and atopic patient sera to characterize house dust mite extracts and to identify the different IgE-binding proteins to which children with atopic eczema and asthma are sensitive. Pooled atopic serum samples were first used to detect as many allergenic components as possible (Fig. 1). In addition to the two major allergens previously described, other fainter bands representing minor body allergens were also detected. Using individual serum samples, the prevalent allergen seemed to be *Der pII* and this was detected in almost every sera tested, whereas *Der pI* was less prevalent.

This is in contrast with the previous findings of Chapman *et al.* (1983); utilizing an antigen binding assay they found IgE antibodies against *Der pI* in the sera of 46/69 atopic dermatitis patients. This anomaly may reflect the differences in the populations studied. In this report children are under examination whereas Chapman was looking at an adult population, which is more likely to be sensitized to a wider range of allergens.

Eczematous sera stained more body allergen bands and less faecal allergen bands on the autoradiographs than did the sera from asthmatic patients (Fig. 2; Table 1). The two groups of autoradiographic strips illustrated in Fig. 2 confirm this distinct difference between the eczematous and asthmatic patients. Five of the eczematous cases had bands other than those of the (two) major allergens. Although these bands are, therefore, defined as 'minor' allergens or infrequent sensitizing agents, they may represent important allergens in those sensitized individuals.

These results show a difference in the house dust mite allergens involved in the causation of asthma and eczema; this was first suggested by Carswell & Thompson (1986) using only two allergen sources. The mechanism they suggested for this was due to a difference in the route of allergen penetration. While inhalation of the faecal particle may occur to a small extent (Tovey *et al.*, 1981), it is quite unlikely that the large (270 μ m length) body will be inhaled. Eczematous patients have a reduced skin thickness in exacerbated areas (Reitamo *et al.*, 1986) and the body allergens may penetrate such skin more readily. The presence of considerable levels of IgE specific for the mite body may be a consequence of sensitization through the skin.

Whether such sensitization produces the disease or its exacerbation has not been established here. Delayed reactions histologically identical to eczema may occur on application of high concentrations of allergen (birch pollen and house dust mite) to the skin for 48 hr (Reitamo *et al.*, 1986). House dust mite allergen has also been shown to penetrate gently abraded skin comparable with the situation in eczema (Mitchell *et al.*,

1984). Recently, Desager, Van Bever & Stevens (1987) reported that contact with house dust mite allergens apparently produced an exacerbation of dermatitis on uninvolved skin.

In asthma where sensitization is more commonly to the faecal (*Der pI*) allergen, sensitization may be by inhalation. Indeed, multiple authors have shown that *Der pI* is in the atmosphere and Tovey *et al.* (1981) have shown that about 4% of this allergen is of a size likely to penetrate the airways. Asthma patients also have IgE antibodies against *Der pII*. How this allergen enters the body in such patients is uncertain. It is too large to be inhaled whole, but fragmentation of the mite body may produce smaller fragments that could be inhaled. It is unlikely that the swallowing of mites will cause sensitization to the body allergen since only 0.1% of ingested protein is absorbed antigenically intact (Lachman, 1977). With the use of a scanning densitometer it is possible to semi-quantify this method to confirm the results of our previous findings using independent panels of atopic sera.

ACKNOWLEDGMENTS

The authors wish to thank Miss H. Coatsworth for preparing the manuscript. We gratefully acknowledge support from the Asthma Research Council.

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