

Immune reactivity to *Der p* I and *Der p* II in house dust mite sensitive patients attending paediatric and adult allergy clinics

R. M. O'BRIEN and W. R. THOMAS*

The University of Melbourne, Department of Medicine, Western Hospital Footscray, Victoria and

*The Western Australian Research Institute for Child Health, Perth, Australia

Summary

A comparison was made of immune responses to house dust mite allergens in symptomatic patients from paediatric and adult allergy clinics. IgE-specific immune reactivity to *Dermatophagoides pteronyssinus* was examined by Western blotting and, for *Der p* I and *Der p* II, by radioimmune dot-blot using purified allergens. Nineteen mite-sensitive children (mean age 9 years) and 26 adults (mean age 31 years) were compared. Positive IgE responses by dot-blot were found to *Der p* I and *Der p* II in 79% of children, whereas reactivity was only present in 23% and 19% respectively of adults, and densitometry indicated a weaker response. In children, Western blotting indicated that the majority of the serologic reactivity was directed to *Der p* II (17/19, 89%) or a 100 kDa fraction whereas in adults, reactivities were generally directed to other fractions with only 15/26 (58%) recognizing *Der p* II. Consistent with results of others, a fraction corresponding to *Der p* I was poorly detected on Western analysis despite positive dot-blot. To determine whether the relative lack of IgE serological immune reactivity in adults was associated with a similar lack of cellular recognition, T-cell proliferation studies were performed, also using purified allergens. Interestingly, these revealed that cellular responsiveness, without serological reactivity, was present in 29% of subjects to *Der p* I and 50% to *Der p* II. Proliferative responses were evident in all individuals with specific IgE.

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Introduction

Immune responses to the house dust mite (HDM) are important in the pathogenesis of extrinsic asthma, allergic rhinitis and atopic dermatitis. Although multiple IgE-binding allergens have been identified in extracts of house dust mite, *Der p* I and *Der p* II are generally considered to be the major allergens with serological recognition often reported to be present in about 80% of mite allergic subjects [1–3]. Moreover, responses to *Der p* I can often comprise more than a half of the total anti-HDM IgE reactivity [1,2]. Nevertheless, the published frequency of recognition of these two

major allergens does differ considerably and in recent reports, IgE reactivity to *Der p* I varied from 46% [4], 69% [5] and 73% [6] and to *Der p* II from 27% [6], 54% [4] to 81% [5].

There are a number of potential reasons for this variation, including the study of patients from different environments and of differing ages with varied clinical manifestations. Also, a variety of assay systems have been utilized. With regard to the clinical manifestations, Thompson *et al.* found that patients with asthma generally recognized *Der p* I and those with eczema more commonly reacted to *Der p* II [5]. In support of this, in an earlier publication, we showed that T-cell reactivity to *Der p* I was seen more commonly in asthmatics [7]. Calkhoven *et al.*, however, did not find a difference in IgE reactivity correlating with symptoms, but interest-

Correspondence: R. M. O'Brien, The University of Melbourne, Department of Medicine, Western Hospital Footscray, Victoria 3011, Australia.

Table 1. Characteristics of the children and adults in this study

	Children (≤ 15 -years-old)	Adults (≥ 16 -years-old)
Number	19	26
Mean age	8.6 years	31 years
Rhinitis	47%	88%
Asthma	58%	42%
Eczema	21%	19%

ingly showed that the proportion of specific IgE reactivity to *Der p* I and *Der p* II vs the total *Dermatophagoides pteronyssinus* reactivity increased from only 25% (approximately) in infants to 35% in young children and to 50% in adults [8].

Our studies were likewise aimed at investigating the frequency of recognition of *Der p* I, *Der p* II and other allergens from the HDM in both children and adults using Western immunoblotting and a radioimmune dot-blot of the native antigen. Additionally, to gain an insight into cellular immune responses, we also measured T-cell reactivity to *Der p* I and *Der p* II in both seropositive and seronegative individuals. The results show a far greater reactivity to the major allergens in patients attending a children's clinic.

Materials and methods

Patients

Adult patients, defined as 16 years old or older, and children, defined as 15 years old or younger, were recruited from Allergy Clinics at the Royal Melbourne Hospital and Western Hospital Footscray in Melbourne and Princess Margaret Hospital, Perth, Australia. Adults and children were all symptomatic and had a positive skin-prick test using a *D. pteronyssinus* extract (Miles Inc./Hollister-Stier, Elkhart, IN, USA). Radioallergo-sorbent tests (RASTs) to the house dust mite were performed in the majority of patients and were all positive. Clinical features are presented in Table 1. Eczema and asthma were present in a similar percentage of individuals whereas rhinitis was more prevalent in adults. About a half of the patients were referred directly from community-based general practitioners, the others coming from other hospital clinics. In general, the patients studied would represent the more severe end of the clinical-disease spectrum.

IgE assays

IgE reactivity was determined both by Western blotting

and by specific dot-blot analysis. Serum samples were stored at -80°C and, if transfer between Institutes was required, this was always performed with the serum frozen on dry ice.

For Western blotting, proteins from a HDM extract were separated by 8–18% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein bands were transferred to nitrocellulose filters.

Nitrocellulose strips were then incubated with the serum from individual patients and bound IgE detected using an ^{125}I -radiolabelled mouse monoclonal anti-human IgE [9]. *Der p* I was characterized by a band at approximately 25 kDa and *Der p* II at approximately 14 kDa, described previously by Chua *et al.* [9] and Baldo *et al.* [10].

Dot blotting was performed by dotting samples of 5 mcg of affinity purified *Der p* I or *Der p* II onto nitrocellulose, incubating with the individual sera and then detecting bound IgE with the radiolabelled mouse anti-human IgE. Affinity purification was as reported previously [9] using monoclonal antibody provided by M. D. Chapman and T. A. E. Platts-Mills, Charlottesville, VA, USA. Quantitation of intensity was performed by densitometry using a GT400 scanner (Seiko-Epson, Nagano, Japan) and expressed in Gray scale units (GSU) [11].

Cellular responses

T-cell reactivity was determined in 7-day proliferation assays using peripheral blood mononuclear cells and affinity purified *Der p* I and *Der p* II proteins as previously described [7]. The cultures (in triplicate) were set up at 10^6 cells per ml in the 200 μl wells of U bottom trays in RPMI-1640 medium containing 10% human AB serum and were pulsed with [^3H] Thymidine (0.5 μl) for the final 16 h. Incorporation was measured in a liquid scintillation counter. As the background (medium alone) counts varied considerably, SI results are presented as a stimulation index (SI). A positive result for an SI is only presented if it is equal or greater than 3.0 and the mean of the stimulated cultures is significantly different from that of the medium-alone wells ($P < 0.05$).

Results

IgE-specific serologic reactivity

IgE reactivity to fractions of dust mite proteins, separated by SDS-PAGE, was determined for all the children and adults by Western blotting. Figures 1a and b show representative autoradiographs from both groups and complete data is summarized in Tables 2 and 3. The Mr 14K fraction characteristic of the *Der p* II protein [9] was the major reactivity, both quantitatively and qualitatively.

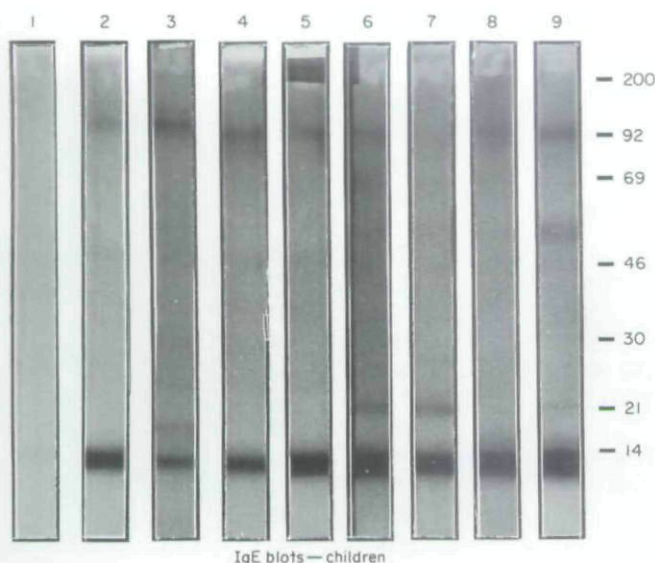


Fig. 1. Western blot analysis of the IgE binding pattern to proteins from a house dust mite extract separated by SDS-PAGE. The sera are from eight representative children and correspond as follows: Lane 2, patient no 4; Lane 3, 19; Lane 4, 5; Lane 5, 6; Lane 6, 14; Lane 7, 8; Lane 8, 11; Lane 9, 7. Lane 1 is serum from an IgE myeloma patient and is at 1000 IU/ml.

tively, in the children, being present in 17/19 (89%). Reactivity to an Mr 100K fraction was seen in 44% (8/18) of the children whereas a band probably attributable to the *Der p I* protein, at 25–27 kDa [5], was only present faintly in 4/18. The children recognized an average of 3.05 ± 1.5 (SD) bands, range 1–5. In adults, the *Der p II* reactivity was only detected in 15/26 and the Mr 90–100K band was only present in 6/26 with 7/26 showing 24–26K reactivity. The positive adults recognized an average of 3.35 ± 1.9 bands, range 1–7.

It is evident from the Western blotting that in children, most of the reactivity is directed to the *Der p II* protein with a lesser amount to the 100 K band and only a small amount to other proteins. In adults, however, reactivity is less intense, but when present it is spread over a number of different Mr bands, with only a relatively small proportion directed to the *Der p II* fraction. As previous researchers had shown that certain protein bands, including *Der p I*, do not transfer well on Western analysis [10], radioimmune dot-blots were performed using affinity purified *Der p I* and *Der p II*. Intensity of reactivity was determined by densitometry and results are also presented in Tables 2 and 3. For *Der p I*, 15/19 (79%) of children were positive with a mean level of positives of 2098 GSU, whereas for the adults, 6/26 (23%) were positive with a mean of 1173 GSU. For *Der p II*, 15/19 (79%) children were positive, mean 3208 GSU, and 5/26 (19%) adults, mean 2358 GSU of positives.

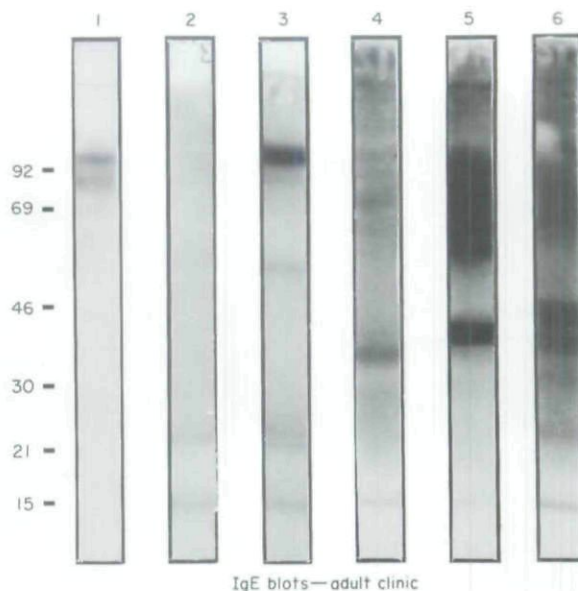


Fig. 2. Western blot analysis of the IgE binding pattern to proteins from a house dust mite extract separated by SDS-PAGE. The sera are from six representative adult patients and correspond as follows: Lane 1, patient 8; Lane 2, 18; Lane 3, 17; Lane 4, 24; Lane 5, 7; Lane 6, 1.

T-cell proliferative responses to Der p I and Der p II

To determine if the lack of detectable IgE reactivity to *Der p I* and *Der p II* in many of the adults was accompanied by a lack of T-cell recognition of either allergen, proliferation assays were set up and results are presented in Table 4, along with the result from the serological determinations. Twelve patients were studied of whom seven were reactive to *Der p I* and eight to *Der p II*. Of the five who showed specific IgE reactivity to *Der p I*, all had T-cell recognition. A further two had T-cell reactivity without detectable IgE. Of the four who produced specific IgE to *Der p II*, all had T-cell recognition, a further four had T-cell reactivity alone. The T-cell reactivity was generally greater in those individuals with specific IgE than those without. For *Der p I*, the mean SI for those with and without specific IgE were 12.6 and 4.6 respectively, for *Der p II*, the equivalent means being 21.5 and 10.6.

Discussion

Our results show that most children presenting to an allergy clinic with HDM sensitivity had major serological reactivity to *Der p I* and *Der p II*. In adults, though, there was a much lower frequency of recognition of these allergens with a greater reactivity on immunoblotting to other bands. Although our data do not indicate a reason for this difference, there would seem to be two

Table 2. IgE reactivity to *Dermatophagoides pteronyssinus* allergens in children

Patient	Age (years)	Symptoms*	Western Immunoblot (Mr)#	Dot-blot	
				<i>Der p I</i>	<i>Der p II</i>
1	2	C	26, 20, 14	< 200	< 200 ^x
2	3	A	90, 61	1016	1510
3	3	A	58, 25, 14	913	2117
4	4	A	100, 14	4903	4304
5	5	A	100, 84, 14	887	2175
6	5	A, E	100, 14	5238	5867
7	6	A	100, 52, 23, 14	1380	2469
8	7	R	100, 20, 14	2974	4813
9	9	A, E	Not done	2783	3129
10	10	E	94, 53, 44, 36, 26, 14	2888	3532
11	10	R	100, 14	2974	4813
12	10	R	130, 56, 45, 14	< 200	< 200
13	11	A	58, 37, 27, 24, 14	864	2102
14	11	R	100, 20, 14	1239	2747
15	13	A, C, R	55, 14	pos +	pos +
16	14	A, R	120, 60, 45, 14	< 200	< 200
17	15	R	14	< 200	< 200
18	15	E	58, 56, 23, 14	303	1516
19	15	A, C, R	100, 22, 18, 14	1010	3814

* C. Conjunctivitis. A. Asthma. E. Eczema. R. Rhinitis.

+ Densitometry was not performed for these positive dot-blots.

Bands present on Western analysis and their Mr in K.

^x Densitometry expressed as GSU. Readings of less than 200 are not visible and are less than the mean 2 SD of a negative (ovalbumin) control.

likely explanations. The first is that individuals with allergic responses to *Der p I* and *Der p II* presenting to hospital allergy clinics earlier in life could be those with a heightened clinical response resulting in earlier and more severe symptoms. Those individuals without response to these allergens could have a slower development of disease and a later referral for allergic assessment. Alternatively, hypersensitivity responses may evolve during childhood and early adult life with *Der p I* and *Der p II* being prominent early but declining with continuing exposure and other allergens becoming more important. Longitudinal studies would be required to clearly distinguish between these two possibilities.

Certainly there are adults with strong reactivities to *Der p I* and *Der p II* and, in fact, five of the 26 adults presented here had significant reactivities to both allergens, albeit at a lower level generally than in the children. It is important to note that the adult patients were individuals presenting with mite allergy for the first time and not established allergy patients diagnosed as children. We have also studied a separate group of seven adult laboratory-staff

volunteers (data not shown) who had a life-long history of severe allergic disease. These adults had specific *Der p I* and *Der p II* IgE levels in the same range as the children presented here. Although this does suggest that antibodies to *Der p I* and *Der p II* are markers of more severe allergic disease, it does not exclude the possibility of immune reactivity altering during life with those individuals who improve losing their reactivity to the major allergens. Allergic individuals who improve would not, of course, have been selected as volunteers.

Our findings do highlight the added information obtained if more than one method is used for serological determinations of specific IgE titres. For *Der p I*, the molecules does not transfer well on Western immunoblotting and is more accurately detected by radioimmune dot-blot [10]. As is evident from Tables 2 and 3, a band consistent with the *Der p I* molecule, at 25–27 kDa, was only present in four of the children and three of the adults. In contrast, *Der p II* is enhanced by Western blotting, suggesting that critical linear determinants are not exposed in the 'native' configuration [12]. In children,

Table 3. IgE reactivity to *Dermatophagoides pteronyssinus* allergens in adults

Patient	Age	Symptoms*	Western Immunoblot (Mr)#	Dot-blot	
				Der p I	Der p II
					x
1	17	R	91, 61, 56, 41, 30, 24, 14	<200	<200
2	18	A, R	55, 50, 28, 25, 14	<200	<200
3	18	R, U	89	<200	<200
4	19	R	Negative	<200	<200
5	21	A, C, E, R	14	<200	<200
6	21	C, R	Negative	<200	<200
7	21	A, R	91, 60-70, 56, 43, 14	<200	<200
8	24	A, C, E, R	100, 89	1409	<200
9	25	R, U	27, 24, 21.5, 14	<200	<200
10	26	A, R	36	<200	<200
11	26	A, E, R	Negative	<200	<200
12	27	C, R	14	1576	3289
13	28	C, R	54, 30, 24, 14	<200	<200
14	29	C, E, R	28, 21.5, 14	<200	<200
15	30	C, R	28, 26, 14	<200	<200
16	31	R	Negative	<200	<200
17	33	R	100, 48, 28, 24, 19, 14	487	1135
18	37	A, E, R	21.5, 14	1080	3489
19	38	A, R	44	<200	<200
20	40	A, R	90, 55, 21.5, 14	789	520
21	42	R	Negative	<200	<200
22	43	A, U	Negative	<200	<200
23	44	A	14	<200	<200
24	45	R	92, 69, 39, 31, 14	1700	3360
25	46	R	Negative	<200	<200
26	62	A	24, 21.5, 14	<200	<200

* C. Conjunctivitis. A. Asthma. E. Eczema. R. Rhinitis.

Bands present on Western analysis and their Mr in K.

x Densitometry expressed as GSU. Readings of less than 200 are not visible and are less than the mean 2 SD of a negative (ovalbumin) control.

virtually all (17/18) showed an appropriate band at 14 kDa on Western blotting, however, some (4/19) were not positive by dot-blot. In the less intensely reacting adult patients, 15/26 were positive by Western analysis whereas only 5/26 were positive by dot-blot. Importantly, all those positive by Western were also positive by dot-blot.

Somewhat in opposition to our own study, Calkhoven *et al.* published data in 1992 showing that newly generated IgE antibodies to HDM in children had activity directed to components apart from Der p I and Der p II [8]. In fact, our study is not strictly comparable and, in general, they were investigating much younger children, from a mean age of 17 months to a mean of 3 years and 9 months. Also, their study was mainly directed to looking at the contribution of specific antibody in quantitative

rather than absolute terms. Nevertheless, in nine of the patients they studied where longitudinal measurements of levels of specific IgE antibodies to Der p I and Der p II were presented, all had reactivity to both allergens at some stage. Furthermore, comparing their later-age children (approximately 4-years-old) with their adults, the percentage where the combined Der p I and Der p II contribution to total anti-mite IgE response exceeded 20% decreased from 78 to 73%.

Studies of Der p I and Der p II specific T-cell reactivity were undertaken in 10 of the adults and two of the older children to compare with the serological responses. A specific aim was to determine if the absence of detectable IgE recognition was associated with a similar lack of T-cell response. There was often a good correlation

Table 4. Correlation between T-cell reactivity and serological recognition of *Der p* I and *Der p* II allergens in HDM sensitive individuals

Patient	Age	<i>Der p</i> I		<i>Der p</i> II	
		T-cell reactivity (SI)	Serological recognition IgE dot-blot	T-cell reactivity	Serological recognition
17	15	4.7	< 200	8.5	< 200
19	15	10	1010	53	3814
2	18	4.5	< 200	—	< 200
5	21	—	< 200	22.5	< 200
8	24	5.8	1409	—	< 200
10	26	—	< 200	3.6	< 200
11	26	—	< 200	—	< 200
12	27	37	1576	11	3289
17	33	5.4	487	16	1135
18	37	5	1080	6	3489
19	38	—	< 200	—	< 200
22	43	—	< 200	8	< 200

between the two. Taking *Der p* I and *Der p* II collectively, there were nine positive serological reactivities and all of these showed significant T-cell proliferation. Of the 15 with negative serological reactivity, only six showed proliferation which, with one exception, tended to be at the lower end of the range. Whether these allergen specific T-cells are involved in disease pathogenesis in the absence of specific IgE is not addressed by our studies. Published data does suggest that T-cells can be directly involved in chronic allergic inflammation, principally via the production of lymphokines acting particularly on eosinophils [13].

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