

Discrepancies between *in vitro* and *in vivo* tests for house dust mite allergy: is domestic exposure a better predictor than sensitization?

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Summary

We subjected seven asthmatic children to two bronchial allergen challenges, first with an extract from the house dust mite *Dermatophagoides pteronyssinus* (*Der p*) and then *Dermatophagoides farinae* (*Der f*), or vice versa. All children had elevated specific serum IgE to both species as well as reactions by crossed radioimmuno/electrophoresis (CRIE) to both group I and II allergens from both species. Immunoabsorption and subsequent analysis by CRIE showed a considerable concentration of serum IgE with specificity for epitopes common to the two species of house dust mite. Home dust sampling established that all children were exposed to *Der f* and only two to *Der p*. On bronchial provocation tests, all responded to *Der f* with an immediate reaction and five with a late reaction, only three of seven showed an immediate response to *Der p*, with four of the seven showing a late reaction. Our data could indicate that the local allergic immune reaction in the respiratory tract is sustained by ongoing exposure, and may thus have a different species specificity than the response reflected in the serum. In conclusion, our data indicates a lack of association between *in vitro* and *in vivo* tests for house dust mite allergy, which supports the continuing need for monitoring current clinical sensitization by allergen provocation tests and by measuring domestic exposure to the corresponding allergen. Extended studies are needed to support our findings.

Clinical and Experimental Allergy, Vol. 24, pp. 946–948. Submitted 4 March 1994; revised 25 May 1994; accepted 6 June 1994.

Introduction

We observed the case of an 11-year-old boy who suffered from bronchial asthma from early childhood. Because of a strong sensitization to *Dermatophagoides pteronyssinus* (*Der p*) and *Dermatophagoides farinae* (*Der f*), and prior to possible immunotherapy, he underwent bronchial allergen challenge with *Der p*. Unexpectedly, he showed no bronchoconstrictor response up to the highest dose tested (100 000 SQU *Der p*). Investigation of dust from the boy's bed mattress revealed significant domestic exposure to *Der f* I but no measurable exposure to *Der p* I. He was then challenged intrabronchially with *Der f* and showed clear early (EAR) and late (LAR) asthmatic reactions.

This led us to investigate other asthmatic children with

a predominant domestic exposure to *Der f* I, to establish whether sensitization could be measured *in vitro* to both house dust mites, whereas clinical reaction could only be measured to the exposed one.

Materials and methods

We investigated seven children (all boys) aged 10–14 years (mean 11.5 years), suffering from mild to moderate asthma as defined by the American Thoracic Society [1]. Patients were selected from a previous study of 32 house dust mite sensitive asthmatic children [2] with regard to significant domestic exposure to *Der f*.

After baseline lung function testing (inhalation of the diluent) a titrated provocation was undertaken using a jet nebulizer (Pari-boy, Medanz, Starnberg, Germany). Biologically standardized allergen extracts of *Der p* and

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Derf from 10 to 100 000 SQU were used (ALK, Copenhagen, Denmark). Each child underwent bronchial allergen challenges with each allergen in turn. Times between the two challenges ranged from 6 to 282 days (mean 85 days). Each concentration (10, 100, 1000, 10 000 and 100 000 SQU) was inhaled for 5 min. FEV₁ from flow-volume-curves was measured directly and at 3 min after inhaling the allergen.

Provocative concentration of allergen that induces a 20% drop in the flow expiratory volume in 1S (PC20 FEV₁) determined the EAR, while a 24-h peak flow protocol detected a possible LAR (defined as typical clinical symptoms 6–10 h after allergen challenge and a drop of peak flow of at least 20% from best value after provocation). Lung function tests were performed using a body-plethysmograph (E. Jaeger, Würzburg, Germany).

Specific IgE was determined by using a solid phase immunoassay, the 'Pharmacia CAP System' (Kabi-Pharmacia, Uppsala, Sweden) [3]. Crossed radioimmuno/electrophoresis (CRIE) (ALK, Copenhagen, Denmark) was performed as described by Weeke *et al.* [4], solid phase IgE absorptions were performed as described by Lind, Ingeman and Brouvez [5].

House dust mite exposure was determined from dust samples extracted from the children's mattresses (vacuum cleaning for 10 min, sampling area 1.8–2.0 m²) using a sandwich ELISA with monoclonal non-cross-reacting antibodies to *Der p* I and polyclonal monospecific antibodies to *Der f* I (ALK, Copenhagen, Denmark) as described by Lind [6]. Significant exposure to an allergen was defined as a concentration of > 2.0 µg/g dust, as proposed by Lau [7].

Results

The data of all patients is shown in Table 1. For one

patient (F.H.) the mattress could not be investigated for allergen content since it was brand new at time of provocation. All other asthmatic children showed a significant domestic exposure (> 2.0 µg/g dust) to *Der f* I on their mattresses, only two of these to *Der p* I as well. Specific IgE was found to *Der p* as well as to *Der f* in corresponding levels (Table 1).

The six children who were domestically exposed to *Der f* (and the child whose exposure could not be measured) all developed an EAR to *Der f*; five children showed a dual asthmatic response (Table 1). Three children presented an EAR to *Der p*, two of these children showed a significant exposure to *Der p* I on their mattresses.

CRIE determines allergen specific IgE in a semi-quantitative way, and all patients tested exhibited significant amounts of IgE to both species of house dust mite. The IgE observed by CRIE demonstrated that the patients generally had reactions to the major allergens of both species (non-absorbed). One patient had negligible reactivity with the group II allergen of *Der p* and two had only background reactivity with the group II of *Der f*. Most patients exhibited reactions to several other allergens (data not shown), but since the major allergens (I and II) account for the majority of the house dust-mite specific IgE [8], only these are mentioned.

Immunoabsorption with extract of *Der p* removed almost all of the IgE reaction to the two homologous major allergens as well as the majority of IgE to the major allergens of *Der f*. Absorption with extract of *Der f* almost totally eliminated the homologous IgE reaction and led to a significant reduction in the heterologous IgE reaction. Taken together, these results demonstrate a considerable concentration of serum IgE with specificity for epitopes common to the two species of house dust mite. The patients exhibited more species-specific reactivity with *Der p* than with *Der f*.

Table 1. Laboratory data of the patients

Patient	Total-IgE (kU/l)	Specific IgE <i>Der p</i> / <i>Der f</i> (CAP-class)	EAR/LAR <i>Der p</i>	EAR/LAR <i>Der f</i>	Exposure <i>Der p</i> I/ <i>Der f</i> I (µg/g dust)	PC20 FEV ₁ <i>Der p</i> / <i>Der f</i> (SQU)
F.H.	956	6/5	-/+	+/+	No data	> 100 000/10 000
S.K.	978	6/6	+/+	+/-	2.0/12.5	100/100
G.D.	308	4/4	+/-	+/-	0.3/3.3	100 000/10 000
D.S.	224	4/4	+/+	+/+	6.5/42.0	100 000/1000
T.L.	207	4/4	-/+	+/+	0.3/1.0	> 100 000/1000
B.I.	816	5/5	-/-	+/+	0.0/4.0	> 100 000/1000
F.F.	331	4/4	-/-	+/+	0.2/13.9	> 100 000/1000

Legend: > 100 000: no bronchoconstrictor response up to the highest dose tested (100 000 SQU).

Discussion

It is generally accepted that there is crossreactivity between *Der p* and *Der f*. Serum IgE to both species of house dust mite was demonstrated in our children by CAP methodology as well as by CRIE. All patients exhibited serum IgE to both species and furthermore, as shown by CRIE, almost all patients reacted to both group I and II allergens. Immunoabsorption and subsequent analysis by CRIE showed that the house dust mite specific IgE contained a major element of reactivity with epitopes shared by the two species.

These observations could indicate sensitization to both species, however, with a preponderance of IgE specific for *Der p* as indicated by IgE directed to epitopes specific for this species. Alternatively, the pattern observed in the absorption experiments could reflect differences in the affinity of IgE for the epitopes presented on the allergens of the two species.

The patients who had used their mattresses for some time were exposed primarily to *Der f* with only two being significantly exposed to *Der p* as well.

The bronchial allergen provocation tests indicated that the prevalent mite species rather than the species specificity of the serum determines the clinical pattern of the ongoing mite allergy. One patient (D.S.), however, was heavily exposed to *Der p* but exhibited only a weak EAR ($> 100\,000$ SQU) to this species upon provocation. His mattress was also very heavily infested with *Der f* (Table 1).

It does not seem possible that our observations could be explained by unreliability of the bronchial allergen challenge procedure. Time intervals between the two provocations ranged from 6 to 282 days (mean 85 days); therefore bronchial hyperreactivity from the first provocation was not likely to influence the second challenge. Furthermore, baseline FEV₁ did not differ at the first and second provocation (89 vs 89% predicted).

The results obtained could indicate that the local allergic immune response in the respiratory tract is maintained by ongoing exposure. It may thus have a different specificity with regard to the two mite species than the response reflected in the serum.

Our data indicates a lack of association between *in vitro* and *in vivo* tests for house dust mite allergy, which supports the continuing need for proving current clinical sensitization by allergen provocation tests and by measuring domestic exposure to the corresponding allergen.

Since the number of patients is rather small, observed frequency of positive reactions using the two mite species might be due to chance; however, the differences in allergen concentration (PC₂₀ FEV₁) do seem to reinforce our observations. Further studies are needed to support our findings.

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