

Short communication

Skin test reactivity to natural and recombinant *Blomia* and *Dermatophagoides* spp. allergens among mite allergic patients in the UK

Background: Many asthmatics in tropical and subtropical areas have positive skin prick tests to both *Dermatophagoides* spp. and to the mite *Blomia tropicalis*. This may be due to recognition by IgE of cross-reactive allergens between the different mite species or because of sensitization to species-specific allergens. A 14-kDa *Blomia tropicalis* allergen, Blo t 5, has been cloned and shows 40% sequence homology with Der p 5. The aim of this study was to investigate reactivity to *B. tropicalis* in patients known to be sensitized to *D. pteronyssinus* and to assess allergenic activity and cross-reactivity of recombinant (r) Group 5 allergens amongst these patients, who live in the UK and who are not exposed to *B. tropicalis* in their homes.

Methods: Patients ($n = 19$) with asthma and/or rhinitis were selected based on clinical history and a positive skin prick test to *D. pteronyssinus* extract and were compared with non-allergic skin test negative controls ($n = 10$). IgE antibody responses to *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, rDer p 5 and rBlo t 5 were compared by quantitative intradermal skin testing using serial 10-fold dilutions of each allergen. End point titre was the highest dilution giving an 8×8 mm wheal at 15 min. IgE antibodies to *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, rDer p 5 and rBlo t 5 were measured using RAST, CAP and RIA, respectively.

Results: All 19 patients had positive skin tests to *D. pteronyssinus* at concentrations of 0.001 to 1 AU/ml and 10 were skin test positive to rDer p 5 at concentrations of 10^{-4} to 5 μ g/ml. Positive intradermal tests to *Blomia tropicalis* were seen in 12/19 patients at concentrations of 0.002 to 2 μ g/ml. However none of the patients had positive skin tests to rBlo t 5. Non-allergic controls were all skin test negative at the highest concentration of each allergen tested. All subjects had quantifiable specific IgE to *D. pteronyssinus*, but only two had IgE to *B. tropicalis*. IgE to Der p 5 was found in six patients, but no patients had IgE to Blo t 5.

Conclusions: This study of patients naturally exposed to *D. pteronyssinus* but not to *Blomia tropicalis*, provides evidence for IgE mediated cross-reactivity between allergens produced by both mite species. The results suggest that the Group 5 allergens of *D. pteronyssinus* and *B. tropicalis* are species-specific.

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In 1964 Voorhorst et al. discovered that mites of the genus *Dermatophagoides* (family Pyroglyphidae) are the major source of allergen in house dust (1) and sensitization to these mites is now recognized as a major cause of asthma world-wide (2). Other non-pyroglyphid mite species are also found in dust from domestic dwellings (3). In tropical and subtropical climates the mite *Blomia tropicalis* is commonly found in homes (4). Many patients with allergic disease in these areas have positive skin prick tests to *B. tropicalis* (up to 90%) (5), and sensitization to this mite is associated with asthma (6). However *D. pteronyssinus* usually coexists in homes with

high levels of *B. tropicalis* and is often numerically dominant (4). As a result asthmatics exposed to *B. tropicalis* tend also to be exposed to *D. pteronyssinus* and may have raised serum-specific IgE to both mite species (7). It is unclear whether this occurs because of recognition by IgE of cross-reactive allergens between the different mite species or because of sensitization to species-specific allergens, and this cannot be studied *in vivo* in populations exposed to both species. Cross-reactivity between *B. tropicalis* and *Dermatophagoides* species has previously been investigated *in vitro* in Cuban subjects (8) and *in vivo* in Swedish subjects (9).

Recently a major 14 kDa *B. tropicalis* allergen, Blo t 5, has been identified, cloned and sequenced (10). Blo t 5 shows 40% sequence identity with *D. pteronyssinus* allergen Der p 5 (10), and approximately 70% of atopic patients exposed to *B. tropicalis* make IgE antibody to this allergen (6). Both recombinant allergens have been shown to give positive skin test responses in patients sensitized to the respective mite species.

The aim of the present study was to investigate reactivity to *B. tropicalis*, *in vivo*, using skin testing and *in vitro*, using specific IgE measurements, amongst patients known to be sensitized to *D. pteronyssinus* and living in the UK, and where *B. tropicalis* does not form part of the acarofauna (11). Reactions to *B. tropicalis* in this population are likely to represent cross-reactions rather than a true sensitization. This study also provided the opportunity to assess the allergenic activity of the recombinant Group 5 allergens of *D. pteronyssinus* and *B. tropicalis*, and to study the extent to which they showed cross-reactivity.

Material and methods

Subject selection and skin prick testing

Adult patients with allergic asthma and/or allergic rhinitis (12), sensitized to *D. pteronyssinus*, were selected from the chest clinic of Wythenshawe Hospital in South Manchester, UK. A control group with no history of allergic disease and negative skin prick tests to *D. pteronyssinus* was identified. Subjects were excluded if they were using oral steroids or antihistamines. Pregnant and breast-feeding women were excluded. Nineteen subjects were recruited (10 male), 15 of whom had asthma and four perennial rhinitis. Ten control subjects were also recruited.

To confirm allergic status, all patients underwent skin prick testing to *D. pteronyssinus* extract (10 000 AU/ml, Hollister Stier, Spokane WA, USA), *B. tropicalis* extract (1.9 mg/ml dry weight of freeze-dried material, kindly provided by E. Fernandez-Caldas), recombinant (r) Der p 5 and rBlo t 5, (5 µg/ml). Histamine dihydrochloride (10 mg/ml) was used as a positive control, normal saline as a negative control and glutathione-S-transferase (5 µg/ml) as a vector control for the recombinant allergens. Skin prick testing was performed on the volar aspect of the forearm, with tests at least 5 cm apart, and wheal size was read at 15 min and recorded as the mean of the longest and the midpoint orthogonal diameters. A mean wheal diameter 3 mm greater than the negative control was considered a positive result.

Quantitative intradermal skin test

Quantitative intradermal skin tests were performed on the other arm using serial 10-fold dilutions of each allergen; the dilutions used were 0.001 to 1 AU/ml for *D. pteronyssinus*, 0.0002 to 2 µg/ml for *B. tropicalis* and 10^{-4} to 10^{-5} µg/ml for rDer p 5 and rBlo t 5. Starting with the weakest concentration, 30 µl of the appropriate solution was injected intradermally on the marked volar aspect of the forearm using a 1-ml syringe and a 27-gauge needle. The end point titre was the weakest concentration required to cause an 8 × 8 mm wheal at 15 min, which was measured as outlined above. All tests were performed by the same investigator (AS) over a 3-month period, and were carried out singly because of the

discomfort involved in intradermal tests. Schistosome GST solution at 0.1 to 5 µg/ml was used as a vector control for the intradermal tests. All test solutions were stored at 4°C.

Measurement of IgE antibodies

Serum was collected and stored at -20°C. Total IgE (measured in kU/l) and serum-specific IgE (measured in kUA/l) to *Dermatophagoides pteronyssinus* were measured using the Pharmacia CAP System (Pharmacia-Upjohn, Piscataway, NJ). Serum-specific IgE ab to *B. tropicalis* was measured using RAST as previously described (6), one RAST unit being equivalent to ~0.1 ng of IgE. Serum-specific IgE ab to Blo t 5 and Der p 5 was measured using a radio-immuno assay (RIA) (13). Briefly, 20 µg rBlo t 5 and rDer p 5 were radiolabelled with 0.5 mCi ^{125} I, using the chloramine-T technique (specific activity, 21.5 µCi/µg and 16 µCi/µg, respectively). Sera diluted 1/2 and 1/10 were incubated with 2 ng ^{125} I-rBlo t 5 or ^{125} I-rDer p 5 (~100 000 cpm added) for 4 h at room temperature. IgE myeloma serum (P.S) diluted 1 : 200 was used as a carrier. Immune complexes were precipitated overnight at 4°C with 50 µl sheep antihuman IgE (The Binding Site, San Diego, CA), washed, and counted in a γ-counter. The assays were quantitated using control curves constructed with serial two-fold dilutions of patient's sera no. 37 (Brazil) or V.P (Charlottesville), for the rBlo t 5 and the rDer p 5 assays, respectively. Each serum was assigned 1000 units/ml of IgE antibody. The cut-off limit was the mean of the background plus two standard deviations.

Statistical analysis

Total IgE and mite specific IgE results were log-normally distributed, therefore geometric means and 95% confidence interval are presented.

Results

In vitro analysis of IgE responses

Of the 19 subjects recruited with a positive skin prick test to *D. pteronyssinus*, all had detectable IgE antibodies to *D. pteronyssinus* by CAP (GM 21.9 kUA/l, 95% CI 7.7–62.6). The majority of these patients (15/19) had CAP scores > 3, equivalent to > 3.5 kUA/l. Total IgE was elevated in 13 subjects (GM 244 kU/l, 95% CI 114–521). By antigen binding RIA, 6/19 patients had detectable IgE ab to Der p 5 (range 656–31 300 cpm bound) as compared to negative controls (< 300 cpm bound).

Although the patients made strong IgE responses to *D. pteronyssinus*, serum IgE antibodies to *B. tropicalis* were largely undetectable by RAST and only 2/19 patients showed a response (40 and 64 RAST units/ml, respectively). None of the patients had detectable IgE ab to Blo t 5 by RIA.

In vivo studies – quantitative intradermal skin test using natural and recombinant allergens

Patients were selected for the study based on a positive skin prick test (> 3 mm wheal) to *D. pteronyssinus* (10 000 AU/ml). Seven patients (37%) had a positive skin

Table 1. Skin prick and intradermal test results and specific IgE to *B. tropicalis* and *D. pteronyssinus* and total IgE

Patient number	SPT <i>D. pter</i> mean wheal diameter (mm)	QIS <i>D. pter</i> (AU/ml)	QIS rDer p 5 (mg/ml)	SPT <i>B. trop</i> mean wheal diameter (mm)	QIS <i>B. trop</i> (µg/ml)
1	7.5	10 ⁻³	10 ⁻⁴	5	2 × 10 ⁻³
2	5.5	10 ⁻³	10 ⁻⁴	3.5	2 × 10 ⁻²
3	5.5	10 ⁻³	0.1	0	2 × 10 ⁻²
4	8	10 ⁻³	> 5	9	2 × 10 ⁻²
5	4.5	10 ⁻³	> 5	4	0.2
6	12	10 ⁻³	> 5	1.5	0.2
7	5.5	10 ⁻³	> 5	0	> 2
8	6	10 ⁻²	> 5	5.5	2 × 10 ⁻³
9	6	10 ⁻²	10 ⁻⁴	3	2 × 10 ⁻²
10	5	10 ⁻²	10 ⁻³	2	2
11	4.5	10 ⁻²	10 ⁻²	2	2
12	5.5	10 ⁻²	10 ⁻²	1.5	> 2
13	5.5	10 ⁻²	> 5	0	> 2
14	4	10 ⁻²	0.1	0	> 2
15	5.5	10 ⁻²	> 5	0	> 2
16	5.5	0.1	5	3.5	0.2
17	4	0.1	> 5	2	> 2
18	3.5	0.1	0.1	2	> 2
19	4	1	> 5	2	2

QIS, quantitative intradermal skin tests; SPT, skin prick tests; md, missing data.

prick test to *B. tropicalis* extract (used as 20 µg/ml). As expected, all subjects had a positive intradermal skin test to 0.01–0.001 AU/ml *D. pteronyssinus*, as did 12 subjects to 0.02–2.0 µg/ml *B. tropicalis* (Table 1).

Intradermal skin tests using the recombinant allergens showed 7/19 patients (37%) gave positive reactions to rDer p 5 at 10⁻⁴ to 10⁻² µg/ml, and a further three had a positive reaction at 0.1 µg/ml. However none of the patients gave positive skin tests to rBlo t 5. Schistosome GST, used as a vector control, did not induce any nonspecific reactions when used at 5 µg/ml in intradermal tests.

Non-allergic controls

All had negative skin prick tests and intradermal tests to all allergens tested, and had no detectable specific IgE. Total IgE was below 30 kU/l in all but one case.

Discussion

This study provides *in vivo* evidence of partial cross-reactivity between allergens of *B. tropicalis* and *D. pteronyssinus* in mite allergic subjects not naturally exposed to *B. tropicalis*. The results suggest that allergens other than those belonging to Group 5 are responsible for the partial cross-reactivity. None of the subjects had lived in areas where *B. tropicalis* forms part of the acarofauna, but some subjects had visited such areas for short holidays. Some patients had a positive skin prick test to

B. tropicalis, but no detectable IgE to this mite. This is probably because they were reacting to *B. tropicalis* allergens not present in the RAST column.

Immunoabsorption studies using sera from Brazilian children with a known high RAST to *B. tropicalis* and *D. pteronyssinus* showed that by absorbing sera with whole body extracts of *D. pteronyssinus* it was possible to remove 36% of the IgE to *B. tropicalis* as measured by RAST (8). These results suggested that approximately 65% of the IgE to *B. tropicalis* was directed against species-specific allergens. Furthermore, crossed radioimmunoelectrophoresis and immunoblotting studies have shown that *B. tropicalis* contains multiple allergens but only two cross-reactive allergens between *B. tropicalis* and *D. pteronyssinus* (14–18). Monoclonal antibody-based ELISA results suggested that *B. tropicalis* does not produce antigens cross-reactive with *Dermatophagoides* spp. Group 1 and 2 allergens (8). These earlier studies have provided *in vitro* evidence that *B. tropicalis* produces multiple major allergens. Some of these allergens are able to cross-react with human IgE produced to other domestic mites, but most *B. tropicalis* allergens are species specific. We have found *in vivo* evidence of cross-reactive allergens between the mite species.

The prevalence of sensitization to Der p 5 was similar in our study to that found by other investigators (19). Serum collected from other asthmatics living in Poole, UK and in Virginia USA who are not naturally exposed to *B. tropicalis* contained low levels of specific IgE to Blo t 5 using the technique (10). Most of the subjects had serum-specific IgE to Der p 5, whereas the levels of IgE ab to Blo t 5, where quantifiable, tended to be much lower (10).

Recombinant Blo t 5 did not induce a positive skin test reaction in any of our patients, even when administered intradermally at high concentrations, nor was the specific IgE antibody detectable in the serum. However, rBlo t 5 has been shown previously to have strong skin test reactivity when tested on populations exposed to *B. tropicalis* (10), suggesting that our results on UK patients were truly negative. Furthermore, antigen binding radioimmunoassay in serum from populations of asthmatics who are naturally exposed to *B. tropicalis* have identified that ~45% have serum-specific IgE to Blo t 5, in most cases in the absence of IgE to Der p 5, suggesting that Blo t 5 is an important allergen (10).

A *B. tropicalis* antigen (Bt-M) has been identified by cloning from a *B. tropicalis* cDNA library which appears to share allergenic epitopes with natural allergens of 11–13 kDa, 14 kDa and 16 kDa. In addition, Bt-M shows 78% sequence identity to the C-terminal half of Blo t 5, suggesting that it may be a partial clone or an isoform (20). The function of the Group 5 allergens remains to be elucidated. Our results using recombinant Der p 5 for skin testing are similar to those recently reported by Lynch et al. on a population of Venezuelan patients with asthma (21). Skin tests using rDer p 5 were

well tolerated and, unlike the Venezuelan study, we did not observe skin test reactions to glutathione-S-transferase among adult UK patients. Our study used thrombin-cleaved recombinant allergens that had been purified from GST, as compared to GST fusion proteins used by Lynch et al. (21).

In conclusion, this study provides *in vivo* evidence of the presence of allergens in *B. tropicalis* which cross-react with IgE antibodies to allergens of *D. pteronyssinus*, and that the IgE response to group 5 allergens is species specific. These results suggest that, in areas where

B. tropicalis forms part of the acarofauna, sensitization to this mite is an independent risk factor for asthma and should be included in the evaluation of immediate hypersensitivity in patients who are exposed in their homes.

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