

## Short communication

# Reactivity to recombinant house-dust-mite allergens in asthma and rhinitis in a tropical environment

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**Background** House-dust mites contain components that are allergenic in mite-sensitive patients, and a number of these have been produced in recombinant form.

**Methods** In the present study, we evaluated by skin prick testing the positivity to native Der p 2 and recombinant Der p 2, Der p 5, and Der p 7 allergens of *Dermatophagoides pteronyssinus* in patients with rhinitis, asthma, or a combination of these diseases, who were positive to whole-mite extract.

**Results** In all patients, the positivity to both native and recombinant Der p 2 was high. In patients with either rhinitis or asthma, the reactivity to Der p 5 and 7 was significantly lower than to Der p 2. However, in patients with combined disease, the positivity to the minor allergens was almost as high as that to Der p 2.

**Conclusions** These results raise the question of whether patients with combined allergic rhinitis and asthma, when compared to those with either of these diseases alone, are predisposed to react to a wider range of mite allergens, or, inversely, whether patients who respond to the minor allergens are more susceptible to suffering the combined disease.

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**Key words:** asthma; *Dermatophagoides*; house-dust mite; recombinant allergens; rhinitis.

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Many components of house-dust mites react to IgE antibody in the serum of allergic patients, and a number of broad groups of allergens have been identified (1). The cDNA corresponding to many of these allergens has been cloned and expressed in *Escherichia coli* (2), and we have demonstrated the biologic activity of the recombinant Der p 2, Der p 5, and Der p 7 allergens of *Dermatophagoides pteronyssinus*, both *in vitro* and *in vivo* (3–7). In the present study we compared the positivity to these recombinant allergens in patients with asthma, rhinitis, or a combination of these diseases in the tropical environment of Caracas, Venezuela (lat. 10°N), where house-dust mite is the dominant aeroallergen (8).

## Material and methods

A group of 150 patients were selected from the allergy clinic of the Institute of Biomedicine,

Caracas, Venezuela, on the basis of their clear clinical history of respiratory symptoms upon exposure to house dust, and their positivity in prick skin tests to extracts of *Dermatophagoides*. The mean age of these patients was 25±12 years (range 7–54 years), and 57% were female. The clinical evaluation included personal and family history, physical examination, nasopharyngeal exploration, skin testing with various environmental allergens, determination of peripheral blood and nasal mucus eosinophilia, and total and specific IgE measurement. Of these patients, 71 (47%) were classified, according to the criteria of the International Consensus Report (9), as suffering rhinitis only. A further 37 (25%) presented symptoms of asthma, as defined by the guidelines of the WHO/NIH Global Initiative for Asthma (10), without significant rhinitis. The remaining 42 (28%) reported combined asthma and rhinitis. We obtained the permission of the ethical committee of the Institute

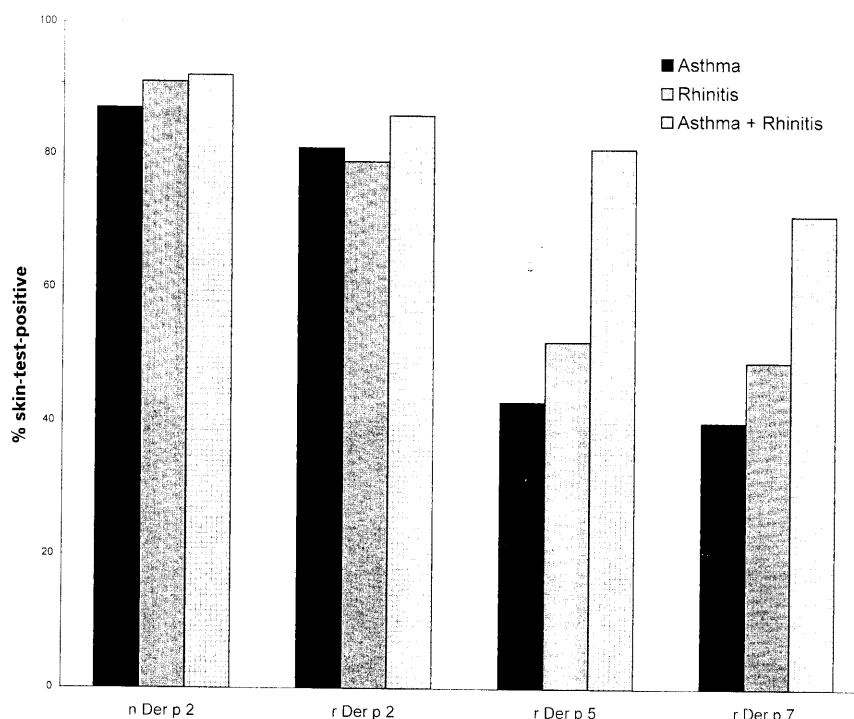


Fig. 1. Percentages of three patient groups (asthma alone, rhinitis alone, asthma plus rhinitis) who were skin-test-positive to Der p 2 in either its native (nDer p 2) or recombinant (rDer p 2) forms, and to rDer p 5 or rDer p 7. Age (mean  $\pm$  SD years) and sex distribution of groups: asthma: 33 $\pm$ 16 years, 57% female; rhinitis: 25 $\pm$ 11 years, 53% female; asthma plus rhinitis: 21 $\pm$ 10 years, 61% female.

of Biomedicine, which was ratified by the Academic Council of the Medical Faculty of the Central University of Venezuela, and by the National Council for Scientific and Technological Investigation, to perform skin prick tests with native Der p 2 and recombinant Der p 2, Der p 5, and Der p 7. The informed consent of the patients or their legal representatives was also obtained. The native Der p 2 was isolated from spent mite culture medium (Commonwealth Serum Laboratories, Melbourne, Australia) by affinity chromatography using a specific anti-Der p 2 monoclonal antibody kindly provided by Dr M. D. Chapman, University of Virginia, USA. The recombinant allergens were expressed in *E. coli* as fusion proteins with glutathione-S-transferase (GST), and purified by affinity chromatography (11). The homogeneity of these preparations was established by SDS-PAGE and Western blot (3-5). The skin tests were performed with allergen concentrations that started at 0.1  $\mu$ g/ml, and increased in 10-fold steps until positive immediate reactions occurred ( $\geq$ 3 mm wheal diameter), up to a maximum of 100  $\mu$ g/ml. The patients were also prick tested with the GST fusion partner of the recombinant allergens, and extracts of a variety of inhalent allergens, including common molds, insects, mammal epithelia, pollens, and an extract of the most common

intestinal parasite in the study population, *Ascaris lumbricoides*. These extracts were used at concentrations of 1500 PNU/ml, and the diluent (50% glycerol saline, 0.4% phenol) was used as a negative control. Histamine (1%) served as a positive control.

## Results

The results of these tests are presented in Figs. 1 and 2, where it can be seen that the positivity of the three patient groups to Der p 2 was uniformly high, and little different between the native and recombinant forms. However, in the patients with either asthma or rhinitis, the positivity to Der p 5 and 7 was significantly ( $P<0.01$ ) lower than to Der p 2 (Fig. 1). This confirms previous studies indicating that Der p 2 and Der p 1 are generally the allergens of most significance for mite-sensitive patients, and that Der p 5 and 7, although making an important contribution to the allergenicity of these mites, have a lower level of positivity (2-7). In contrast, when the patients with combined asthma and rhinitis were considered (Fig. 1), the reactivity to Der p 5 and 7 was substantially higher ( $P<0.01$ ), with the positivity approaching that to Der p 2. There were no significant differences in the positivity to the GST fusion partner, or to other

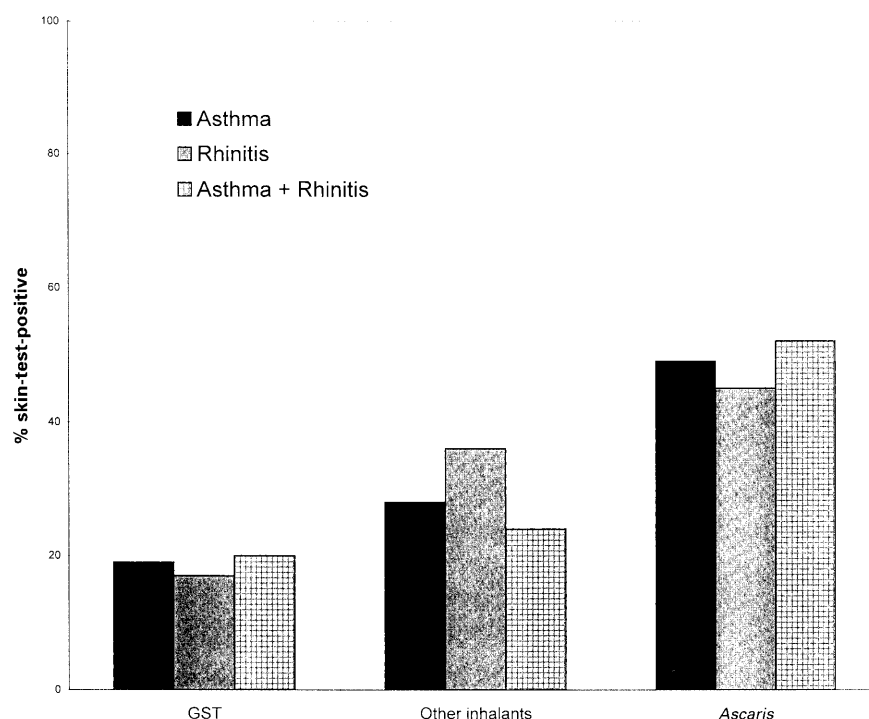


Fig. 2. Percentages of three patient groups who were skin-test-positive to GST fusion partner of recombinant allergens, other common, non-house-dust, inhalent allergens (combined positivity is presented), and to *Ascaris lumbricoides* extract.

common allergens, between the three patient groups (Fig. 2).

## Discussion

These results indicate that, while in patients with either asthma or rhinitis Der p 5 and 7 are less important allergens than Der p 2, this is not the case for those with combined asthma and rhinitis. This higher reactivity does not seem to be related to the severity of the allergic disease, as there were no evident differences in the symptom scores between the patient groups (results not presented). In addition, we did not detect any significant correlation between the reactivity to the recombinant allergens and other aspects of the patients, such as family history, active or passive exposure to tobacco smoke, etc. Previous studies have indicated that children are more reactive to Der p 2 allergen than adults (12), but we found no association in the present study between age and the occurrence of combined disease, or positivity to Der p 5 and 7.

These observations raise the question as to what factors determine whether a given patient suffers allergic disease of the upper or lower respiratory tract, or both of these together. Future studies could be directed toward determining whether the higher reactivity to the minor mite allergens found in the patients with both asthma and rhinitis is a

cause-and-effect relationship. Thus, patients who, for some reason, are strong responders to a wider variety of mite allergens may be more likely to suffer combined disease. Alternatively, some patients may be predisposed to suffer both diseases, and this could be accompanied by reactivity to a wider range of mite allergens.

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