Identification of *Blomia tropicalis* Allergen Blo t 5 by cDNA Cloning

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Key Words

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Introduction

Mites of the genus *Blomia*, including *Blomia tropicalis* and *Blomia kulagini*, are an important cause of IgE antibody responses among asthmatic patients in tropical and subtropical areas of the world. Exposure to *B. tropicalis* has been documented in houses from Hong Kong, Brazil, Venezuela, Colombia, Taiwan, India, Spain, Egypt and other countries [1-3]. In the United States, *B. tropicalis* has been identified in significant numbers in homes from Tampa Fla., New Orleans, La., Memphis, Tenn., Galveston, Tex., Delray Beach and San Diego, Calif. [4]. There has been increasing interest in the antigenic cross-reactivity between *Blomia* and *Dermatophagoides* spp., since exposure to both mite species is very common in such environments. *Blomia* spp. are the source of multiple allergens, the majority of which are species specific, as judged by results of serologic analysis and skin testing [3-6]. However, at present no purified *Blomia* allergens have been reported. We have used molecular cloning techniques to further identify, characterize and sequence *B. tropicalis* allergens. Expression of the recombinant allergens will allow studies on the immune responses to *B. tropicalis*, and development of methods for assessing risk levels of exposure to this mite.

Methods and Results

IgE antibody responses to *B. tropicalis* were studied in a panel of sera of asthmatic patients from Brazil, Puerto Rico and Florida (n = 83), and compared to patients with asthma or atopic dermatitis from Charlottesville, Va., and England (n = 56), who were allergic to *Dermatophagoides pteronyssinus* but unlikely to have been exposed to *B. tropicalis*. In the first

group, 83% of *B. tropicalis* radioallergosorbent test (RAST)-positive patients also had a positive RAST to *D. pteronyssinus*. The ratio *D. pteronyssinus*/*B. tropicalis* RAST was > 10 in only 22% of the patients, probably reflecting exposure to both mite species. In contrast, although 50% of the Virginia/England *D. pteronyssinus* RAST-positive subjects also had positive RAST to *B. tropicalis*, the ratio *D. pteronyssinus*/*B. tropicalis* RAST was > 10 in 68% of the patients and this difference was significant ($p < 0.001$). Our results are in keeping with previous studies and suggest that only 20-30% of *B. tropicalis* allergens may be shared with *D. pteronyssinus*. A cDNA library was prepared from *B. tropicalis* mRNA and screened with human IgE antibodies. A representative 522-bp cDNA clone was sequenced, containing a 432-bp open reading frame. The predicted amino acid sequence

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showed approximately 40% sequence homology to Der p 5, a 14-kd *D. pteronyssinus* allergen [7]. Plaque immunoassay results revealed that 70% of *B. tropicalis* RAST-positive asthmatic patients had IgE to the cloned protein. The *B. tropicalis* allergen has been designated as Bio 15. To obtain purified recombinant allergen, Bio t 5 cDNA was subcloned into the pGEX-4T1 vector and expressed in *Escherichia coli* as a fusion protein with glutathione S-transferase. Following thrombin cleavage, the *B. tropicalis* allergen was released and purified over glutathione agarose. Results of an antigen-binding RIA showed that 12/12 *B. tropicalis* RAST-positive selected patients had IgE and IgG antibodies to recombinant Bio 15, whereas nonallergic controls showed no binding to this allergen.

Discussion

B. tropicalis allergen Der p 5; however, no sequence homology with other proteins has been identified and the function of these proteins remains unknown. IgE to Der p 5 has been reported to be present in 50% > of *D. pteronyssinus*-allergic patients [7], whereas IgE to Bio 15 appears to occur in a greater proportion of *B. tropicalis*-allergic patients. This observation suggests that Bio 15 may be a more important allergen in *B. tropicalis* than the homologous molecule in *D. pteronyssinus*. Further studies using recombinant Bio t 5 and Der p 5 are underway in our laboratory to examine in detail the anti-genic cross-reactivity between these two proteins.

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