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Immunoglobulin E Reactivity of Recombinant Allergen Tyr p 13 from Tyrophagus putrescentiae Homologous to Fatty Acid Binding Protein

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The storage mite, Tyrophagus putrescentiae, is one of the important causes of allergic disorders. Fifteen allergenic components were demonstrated in storage mite by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, but only the group 2 allergen Tyr p 2 has been cloned and characterized. In this study, we attempted to identify and characterize new allergens from T. putrescentiae, which is a dominant species of storage mite in Korea. Expressed sequence tags were analyzed to identify possible storage mite allergens, and the cDNA sequence encoding a protein homologous to fatty acid binding protein, a mite group 13 allergen, was identified and named Tyr p 13. Its deduced amino acid sequence showed 61.1% to 85.3% identity with other mite group 13 allergens. The recombinant protein was expressed in Escherichia coli using a PET 28b vector system, and its allergenicity was investigated by enzyme-linked immunosorbent assay (ELISA). The recombinant allergen was detected in 5 of 78 (6.4%) T. putrescentiae-positive sera tested, and it inhibited 61.9% of immunoglobulin E binding to crude extract at an inhibitor concentration of 10 μg/ml by inhibition ELISA using serum from the patient who showed the strongest reaction by ELISA. In this study, a novel allergen was identified in T. putrescentiae. This allergen could be helpful for more-detailed characterizations of storage mite allergy.

The storage mite, Tyrophagus putrescentiae, is found in stored products such as dried eggs, ham, herring meal, cheese, and different kinds of nuts (17). Storage mites are the cause of occupational allergic diseases of bakers (21) and food industrial workers (2, 25) and among residents in agricultural environments (4). Koreans store uncooked rice in rice bins or chests in their homes, and thus the storage mite, T. putrescentiae, is often found on kitchen floors in urban areas (10). By crossed immunoelectrophoresis using the sera from occupationally exposed farmers, 14 different allergenic components were identified from the whole-body extract of T. putrescentiae (1). However, less is known about the molecular nature of the allergens derived from T. putrescentiae. Only the group 2 allergen Tyr p 2 has been cloned and characterized from T. putrescentiae (6), although 20 groups of mite allergens have been cloned to date (26).

cDNA library screening and phage display technology have recently been utilized to identify and clone new allergens (7). However, both of these strategies have obvious limitations, i.e., due to posttranslational modifications and improper protein folding. In a previous study, 15 allergenic components were demonstrated in T. putrescentiae by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, and a 16-kDa allergen was found to be most prevalent (52%) (18). In addition, considerable cross-reactivity between T. putrescentiae and Dermatophagoides species was demonstrated by inhibition enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis (16).

A cDNA sequence encoding a protein homologous to fatty acid binding protein, mite group 13 allergen, was identified by analyzing the expressed sequence tag data of T. putrescentiae. Mite group 13 allergens have been cloned and characterized from Blomia tropicalis (Blo t 13) (5), Acarus siro (Aca s 13) (8), and Lepidoglyphus destructor (Lep d 13) (7). The cross-reactivity of the Blo t 13 with other dust mites was also demonstrated using monoclonal antibodies (14).

In this study, we undertook to produce a recombinant fatty acid binding protein and then characterize the allergenic properties of this protein from T. putrescentiae.

MATERIALS AND METHODS

Preparation of crude extract. The allergen extract was prepared by using phosphate-buffered saline (PBS) and protease inhibitors. Thirty grams of frozen mites which had been reared as described previously (20) was homogenized in liquid nitrogen. The sample was defatted in 200 ml of a 1:1 volume of ethyl ether and ethyl acetate, and 1 mg of 1-phenyl-3-(2-thiazolyl)-2-thiourea (Sigma, St. Louis, Mo.) was added to prevent melanization. The proteins was extracted with slow overhead stirring at 4°C overnight in phosphate-buffered saline (PBS), pH 7.4, containing 6 mM 2-mercaptoethanol and a 1/1,000 volume of protease inhibitor set III (Calbiochem, San Diego, CA). The extract was then centrifuged.
TABLE 1. Expressed sequence tags matched with allergenic clones

<table>
<thead>
<tr>
<th>Allergens</th>
<th>Biochemical identity</th>
<th>Clones (n = 22)</th>
<th>EST no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr p 13</td>
<td>Fatty acid binding protein</td>
<td>8</td>
<td>21, 41, 58, 366, 433, 445, 472, 479, 485</td>
</tr>
<tr>
<td>Tyr p 1</td>
<td>Cysteine protease</td>
<td>4</td>
<td>28, 115, 161, 242</td>
</tr>
<tr>
<td>Tyr p 3</td>
<td>Serine protease</td>
<td>2</td>
<td>186, 485</td>
</tr>
<tr>
<td>Tyr p 8</td>
<td>Glutathione-S-transferase</td>
<td>2</td>
<td>185, 513</td>
</tr>
<tr>
<td>Tyr p 10</td>
<td>Tropomyosin</td>
<td>1</td>
<td>360</td>
</tr>
<tr>
<td>Tyr p 20</td>
<td>Arginine kinase</td>
<td>1</td>
<td>259, 59</td>
</tr>
<tr>
<td></td>
<td>α-Tubulin</td>
<td>1</td>
<td>22, 89, 606</td>
</tr>
</tbody>
</table>

* Full-length sequence.

Skin prick test. A total of 432 pediatric subjects (male:female ratio = 223:209, average age, 8 ± 3 years, mean ± 1 standard deviation) was enrolled in this study; 358 children suffered from asthma, 325 from allergic rhinitis, 17 from atopic dermatitis, and six children from urticaria. By skin prick testing, 69.5% had positive responses to D. pteronyssinus, 57.8% to D. farinae, 34.2% to B. germanica, 22.3% to P. americana, 16.3% to cat dander, 15.7% to Alternaria, 12.8% to dog hair, 12.7% to T. putrescentiae, 6.0% to Penicillium, 5.3% to oak, 4.4% to ragweed, and 4.2% to alder.

Among these, 55 children (male:female ratio = 26:29) showed positive reactions to T. putrescentiae. The mean total IgE level of these 55 children was 708.03 ± 522.79 (ranging from 11.2 to 2,960) IU/ml, and the mean eosinophil count was 493.07 ± 264.56/mm³ (ranging from 82 to 1,430). Twenty-four adult patients (9 patients suffering from allergic asthma, 6 from urticaria, 3 from allergic rhinitis, 3 both from allergic asthma and allergic rhinitis, 2 from severe coughing, and 1 from atopic dermatitis) with a mean total IgE of 501.33 ± 645.82 (ranging from 13.9 to 4,530) IU/ml and a mean eosinophil count of 449.01 ± 283.13/mm³ (ranging from 130 to 1,120) also showed a positive reaction to T. putrescentiae.

Analysis of expressed sequence tag database. A total of 382 readable amino acid encoding sequences were produced from 446 clones in the of T. putrescentiae cDNA library. A BLASTX search of 22 clones showed high homology to the previously known allergens. Seven different groups of mite allergens were identified, and three clones showed significant homology to cockroach group 6 allergen (Table 1).

Homology to fatty acid binding proteins. Eight clones out of 446 analyzed were found to be homologous to fatty acid binding protein, and 6 clones contained the full-length open reading frame. The open reading frame was found to be 643 nucleotides in length (Fig. 1) and included a 5’ noncoding region of 59 bp, a 3’ untranslated region of 184 bp, and an open reading frame of 396 bp. This open reading frame codes for a 132 amino acid protein with a calculated molecular mass of 14.57 kDa and an isoelectric point of 7.22. A cytosolic fatty acid binding protein signature at amino acid residues 5 to 22 was detected using the PROSITE program (http://www.isb-sib.ch/annoucne/). The protein also contains a potential N-glycosyl-
ation site at position 5 to 7, and a putative polyadenylation signal was found between nucleotide residues 542 and 548.

The amino acid sequence of the putative fatty acid binding protein from *T. putrescentiae* shows 85.3% identity with *Aca s* 13 from *A. siro*, 62.3% identity with *Blo t* 13 from *B. tropicalis*, and 61.1% identity with *Lep d* 13 from *L. destructor* (Fig. 2).

Due to its high homology with mite group 13 allergens and in accord with the allergen nomenclature (13), it was named *Tyr p* 13.

**Expression and IgE binding of recombinant *Tyr p* 13.** Recombinant *Tyr p* 13 with a short N-terminal fusion protein which includes 6-histidine was expressed in *E. coli* and metal affinity purified (Fig. 3). The yield of recombinant *Tyr p* 13 was 5.432 mg/liter bacterial culture as determined by Bradford assay. The recombinant allergen was detected by IgE antibodies in 5 of the 78 (6.41%) *T. putrescentiae*-positive sera tested (Fig. 4).

**Inhibition of IgE reactivity by the recombinant protein.** This inhibition study was performed using the serum from the patient who showed the strongest IgE reactivity to the recombinant allergen. The crude extract inhibited a maximum IgE reactivity by 95.7%, whereas the recombinant allergen inhibited it by 61.9% at an inhibition concentration of 10 μg/ml (Fig. 5).

**DISCUSSION**

Fatty acid binding proteins has been described as a putative vaccine candidate for some parasitosis-inducing species such as *Fasciola hepatica* (22), *Schistosoma japonica* (3), and *Schistosoma mansoni* (24). The first fatty acid binding protein reported as an allergen was the ABA-1 of *Ascaris suum* (23). It was also found that a small number of low-molecular-weight proteins which share the similarity with fatty acid binding proteins are highly up-regulated in psoriatic skin (15). Even though they bear no significant homology with ABA-1, several proteins homologous to fatty acid binding proteins from dust mites have been known to be allergenic (group 13). However,
the frequencies of IgE binding to these allergens are low, suggesting that they are minor allergens, e.g., 11% (5 of 45) to Blo t 13 from *Blomia tropicalis* (5), 23% (3 of 13) to Aca s 13 from *Acarus siro* (8), and 13% (6 of 45) to Lep d 13 from *Lepidoglyphus destructor* (7). However, recombinant Blo t 13 inhibited 62% of IgE binding to crude extract according to the radioallergosorbent inhibition test using one patient’s serum, which suggests that it may be clinically significant for some patients. In the present study, a 6.41% (5 of 78) IgE binding frequency to recombinant Tyr p 13 was observed and recombinant Tyr p 13 at 10 μg/ml inhibited 61.9% of IgE binding to the crude extract by inhibition ELISA using the serum from the patient who showed the strongest reaction to recombinant Tyr p 13. However, more biochemical analysis is necessary because N-glycosylation of the allergen may have affected its allergenicity. Furthermore, patients from rural area or bakers who are more likely to be sensitized to storage mite allergens may show different allergic responses to this molecule.

Until now, the diagnosis of allergic disorders has mainly been based on crude extracts. Allergen extracts have several limitations such as batch-to-batch variations and difficulty in standardization. Moreover, false-positive reactions may occur because of the nonallergenic compounds. In addition, skin prick testing using *T. putrescentiae* commercial allergen extract is not believed to be reliable for the detection of measurable levels of IgE antibodies (11). Thus, the cloning and genetic engineering of the allergens offers many advantages, and they are expected to replace crude extracts for diagnosis and treatment in the near future (12).

In a study using a panel of seven purified natural or recombinant allergens, mite-allergic patients who were polysensitized to various minor allergens, which included highly cross-reactive allergens and storage mite allergens, could be discriminated from those who were mainly sensitized to major allergens (e.g., Der p 1 and Der p 2) (19). This is important, as atopic individuals polysensitized to many minor allergens are less likely to benefit from immunotherapy based on crude allergen extracts. Therefore it is necessary to characterize minor allergens in more detail, especially with respect to allergenicity and cross-reactivity. Detailed characterization of the minor allergens may lead us to the more-beneficial immunotherapeutic approaches.
More clones are being analyzed in an effort to characterize *T. putrescentiae* allergens in detail, and possible allergens identified by expressed sequence tag analysis are now being investigated. Moreover, the clinical relevance of Tyr p 13 remains to be evaluated in vivo. It is hoped that the use of recombinant Tyr p 13 in combination with other allergens could improve the diagnostic specificity.

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**REFERENCES**

AUTHOR’S CORRECTION

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