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Characterization of Allergenic Properties of German Cockroach Tropomyosin Using Recombinant Proteins

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Cockroach infestation may sensitize and elicit allergic responses to genetically predisposed individuals. Invertebrate tropomyosins are a frequent cause of allergy and highly cross-reactive in nature. For the initial characterization of German cockroach tropomyosin (Bla g 7), cDNA cloning, expression of recombinant protein, and investigation of its allergenicity were performed. German cockroach tropomyosin was cloned by reverse transcriptase polymerase chain reaction (RT-PCR) using degenerate primers designed on the basis of known tropomyosins. The cloned Bla g 7 shared up to 98.5% amino acid sequence identity with other allergenic tropomyosins. The cloned cDNA was over-expressed in *Escherichia coli* and purified by affinity chromatography using Ni-nitrilotriacetic acid (NTA) resin. The allergenicity of the recombinant tropomyosin was examined by enzyme-linked immunosorbent assay (ELISA). ELISA showed a recombinant Bla g 7 sensitization rate of 16.2% to German cockroach allergic sera. Recombinant Bla g 7 was able to inhibit 32.4% of the specific IgE binding to cockroach extract. Tropomyosin represents a minor allergen in cockroach extracts. The allergenicity of the recombinant tropomyosins derived from various arthropods are not coherent. To gain a deeper insight into the allergenicity, the IgE-binding reactivities of native and recombinant German cockroach tropomyosins (E. coli-expressed and Pichia-expressed) were compared. Native tropomyosin was purified by ammonium sulfate fractionation, hydroxyapatite column chromatography, and electroelution. Recombinant tropomyosin was expressed in *Pichia pastoris* in an attempt to produce the recombinant protein which is comparable to natural counterpart in allergenicity. The allergenicity of native and recombinant tropomyosins was compared by ELISA inhibition study. Native German cockroach tropomyosin showed 17.6% IgE binding reactivity from German cockroach sensitized sera. Recombinant tropomyosin was produced without fusion protein and the N-terminus was blocked as a native counterpart. Its IgE binding reactivity was comparable to that of native tropomyosin over the concentration range of 1 to 1000 ng/mL in the ELISA inhibition test. Recombinant tropomyosin expressed in *P. pastoris* showed better allergenicity than that expressed in *E. coli*. Besides the structural differences of native and recombinant proteins, other factors may also influence on the various IgE reactivities of tropomyosins. Diverse amino acid sequences have been described for individual allergens from various sources. The effects of such sequence diversity need to be considered with respect to IgE binding reactivity of an allergen. Two-dimensional (2-D) gel electrophoresis and immunoblot analysis using mouse anti-recombinant Bla g 7 serum were performed to investigate isoforms at the protein level. RT-PCR was applied to examine the sequence diversity. Eleven different variants of deduced amino acid sequences were identified. German cockroach tropomyosin has only minor sequence variations, which do not seem to affect the allergenicity significantly. These results support the molecular basis underlying the cross-reactivity of arthropods tropomyosins. Recombinant fragments were also generated by PCR and IgE-binding epitopes were assessed by ELISA. The sera of seven patients' revealed heterogeneous IgE-binding responses. This study demonstrates multiple IgE-binding epitope regions in a single molecule. A two-site ELISA was developed using monoclonal antibodies (mAbs) raised against recombinant German cockroach tropomyosin, and was applied to assess the invertebrate tropomyosin level in house dust samples. The detection limit of the developed two-site ELISA was determined to be about 8 ng/mL for recombinant German cockroach tropomyosin and 1 μg/mL for German cockroach whole body extract. Tropomyosin was detected in three samples (24.20 ± 32.11 μg/g) from nine bedding samples (33.3%) and in only one (6.80 μg/g) of 13 kitchen dust samples (7.7%). These data support the low rate of sensitization to cockroach tropomyosin in Korean respiratory allergy patients. The results of the present study led us to conclude that the most important determinant of the sensitization rate and allergenicity of German cockroach tropomyosin is not the structural characteristics or amino acid sequence variations, but the environmental factors such as distribution in house dust. In summary, tropomyosin is found to be a minor allergen in German cockroach. The low rate of sensitization is not by structural differences from native protein or by its amino acid sequence variations. The low level of tropomyosin in house dust may explain the low rate of exposure, sensitization and allergenicity. The key elements affecting the production of IgE antibodies and the onset of allergic disorders, are thought to be the route of exposure, the allergen dose, age at sensitization, and the genes regulating host immune responses, rather than the intrinsic properties of a given allergen. The clinical relevance of the Bla g 7 remains to be evaluated in vivo by large-scale study. Tropomyosin could be a good molecular model for the investigation of factors affecting the sensitization and the onset of allergic disease. It is believed that the results of the present study could facilitate the development of diagnostic and immunotherapeutic strategies based on the recombinant proteins obtained during the course of this study. In addition, the developed two-site ELISA could aid allergen standardization, allergen avoidance procedures, the assessment of environmental allergen exposure, and the further characterization of tropomyosin.