Identification of Allergens from the Serrated Mud Crab (Scylla serrata) by IgE Immunoblotting

Chintana Phawong 1*, Supranee Fooanant 2, Suriyan Tunkijjanukij 3,
Chatchai Tayapiwattana 1, Pattama Ekpo 4

1* Department of Clinical Immunology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand; 2 Department of Otolaryngology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200 Thailand; 3 Department of Marine Science, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok 10900, Thailand; 4 Department of Immunology, Faculty of Medicine at Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok, 10700, Thailand. E-mail address: chintana@mail.ams.cmu.ac.th

Abstract: Crab sensitivity is one of the most common seafood allergies. However, there has been no data available on their allergenic components from common crab species consumed as seafood in Thailand. This study attempts to identify allergenic components of crab extract from the serrated mud crab (Scylla serrata) by IgE immunoblotting. Allergenic components from crab were extracted with extraction buffer and detected by IgE immunoblotting with sera from 15 patients with skin prick test positive to crab allergen. Eight IgE-binding bands, ranging in molecular weight from 23 to 86 kDa, were identified as allergens of the serrated mud crab. Fifty three percents (8/15) of crab allergic patients demonstrated IgE-binding to the component with molecular weight of about 79 kDa and was considered to be one of its major allergen.
Methodology: 1. Sera. Sera from 15 patients with skin prick test positive to crab allergen were collected at the Allergy Clinic of Maharaj Hospital, Department of Otolaryngology, Faculty of Medicine, Chiang Mai University, Chiang Mai, between October 2002 and January 2003. Sera from 5 normal volunteers without any previous history of seafood allergy and skin prick test negative to crab allergen were used as negative control. 2. Allergen preparation. Allergen extraction procedure was a modification of the Crespo et al (1). Serrated mud crab (S. serrata) was boiled for 1 h in distilled water. The meat of the cooked crab was removed and blended in extraction buffer. After stirring overnight at 4° C, the extract was centrifuged. The supernatant fluid was collected, centrifuged and kept at -20 ° C until use. 3. SDS-PAGE and IgE immunoblotting. SDS-PAGE was performed according to Laemmli (2) and the separated proteins were transferred onto a nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% non-fat milk in 0.05% PBST for 1 h, washed five times with PBST and incubated with serum (1:2) for 2 h at room temperature. After washing, biotinylated anti-human IgE (epsilon chain specific) (1:200) was added and incubated at room temperature for 1 h. The nitrocellulose membrane was again washed, horseradish peroxidase-streptavidin (1:200) was added and incubated at room temperature for 1 h. After an additional washing, the blot was developed with substrate solution, stopped reaction and then air-dried.

Results, Discussion and Conclusion: Protein components of crude extract from crab were separated by SDS-PAGE, and allergenic components were identified with IgE antibodies in immunoblotting. The Coomassie brilliant blue R250 stained protein profile of the crude extract of S. serrata showed at least 6 different components with molecular weights ranging from 15 to about 100 kDa were the predominant components had molecular weights of about 15-18, 20-23, 27-30, 37-51 and 68-78 kDa. For IgE immunoblot analysis, serum samples from 15 patients who are allergic to crab and 5 negative controls were used. Fourteen of 15 allergic patients showed IgE-binding bands in immunoblotting and revealed 8 allergenic components, ranging in molecular weights from 23 to 86 kDa. The component with a molecular weight of about 79 kDa showed the highest frequency of IgE-binding, and was recognized by 53% (8/15) of the sera tested. Thus, it was considered to be a major allergen of crab. Other components showed IgE-binding frequencies less than 30%. For negative control showed IgE-binding activity with crab extract with molecular weights of more than 120 kDa and was not observed in the molecular weights ranging from 15 to 100 kDa. In conclusion, the present study identified a 79 kDa as a major allergen of the serrated mud crab (S. serrata) by IgE immunoblotting. Further study will be needed to determine the amino acid composition and sequence of this allergen as well as the study on the cross-reactivity among different kinds of seafood and arthropod.

References

Keywords: seafood allergy, allergen, Scylla serrata, crab, IgE, Immunoblotting