Original Article

Molecular Diagnosis of Shrimp Allergy: Efficiency of Several Allergens to Predict Clinical Reactivity

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What is already known about this topic? Tropomyosin is considered responsible for most shrimp allergenic activity and for the cross-reactivity among crustaceans and other arthropods and other invertebrates. Other crustacean allergens have been identified, but their clinical relevance and role in cross-reactivity has not been fully defined.

What does this article add to our knowledge? Among subjects who report shrimp-allergic symptoms, sensitization to tropomyosin and sarcoplasmic calcium-binding protein is associated with clinical reactivity to shrimp. Arginine kinase and hemocyanin appear to be cross-reacting allergens between shrimp and arthropods, but still some arginine-kinase epitopes may be clinically significant. Myosin light chain may also be associated with clinical reactivity to shrimp.

How does this study impact current management guidelines? Detection of specific IgE to these allergenic components and some of their epitopes could provide more specificity for shrimp allergy diagnosis.

BACKGROUND: The diagnosis of shellfish allergy remains a challenge for clinicians. Several shellfish allergens have been characterized and their IgE epitopes identified. However, the clinical relevance of this sensitization is still not clear.

OBJECTIVE: The objective of this study was to identify allergens and epitopes associated with clinical reactivity to shrimp.

METHODS: Shrimp-sensitized subjects were recruited and grouped based on the history of shrimp-allergic reactions and challenge outcome. IgE reactivity to recombinant crustacean allergens, and IgE and IgG4 reactivity to peptides were determined. Subjects sensitized to dust mites and/or cockroach without shrimp sensitization or reported allergic reactions, as well as nonatopic individuals, were used as controls.

RESULTS: A total of 86 subjects were recruited with a skin prick test to shrimp; 74 reported shrimp-allergic reactions, 58 were allergic (38 positive double-blind placebo-controlled food challenge and 20 recent anaphylaxis), and 16 were tolerant. All subjects without a history of reactions had negative challenges. The individuals with a positive challenge more frequently recognized tropomyosin and sarcoplasmic calcium-binding proteins than those found tolerant by the challenge. Especially a sarcoplasmic-calcium-binding-protein positive test is very likely to result in a positive challenge, though the frequency of recognition is low. Subjects with dust mite and/or cockroach allergy not sensitized to shrimp recognized arginine kinase and hemocyanin. Several epitopes of these allergens may be important in predicting clinical reactivity.

CONCLUSION: Tropomyosin and sarcoplasmic-calcium-binding-protein sensitization is associated with clinical reactivity to shrimp. Myosin light chain testing may help in the diagnosis of clinical reactivity. Arginine kinase and hemocyanin appear to be cross-reacting allergens between shrimp and arthropods. Detection of IgE to these allergens and some of their epitopes may be better diagnostic tools in the routine workup of shrimp allergy. © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;■:■-■)

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Abbreviations used
AK- Arginine kinase
AUC- Area under the curve
DBPCFC- Double-blind placebo-controlled food challenge
FABP- Fatty-acid-binding protein
FDR- False discovery rate
HDM- House dust mite
LH+- Positive likelihood ratio
LH−- Negative likelihood ratio
MLC- Myosin light chain
SCP- Sarcoplasmic calcium-binding protein
SE- Sensitivity
sIgE- Specific IgE
SP- Specificity
SPT- Skin prick test

Key words: *Component-resolved diagnosis; Shellfish allergy; Epitope; Microarray; DBPCFC; Tropomyosin; Arginine kinase; Myosin light chain; Sarcoplasmic calcium-binding protein; Hemocyanin; Troponin C; Fatty-acid-binding protein*

Shellfish allergy is a common long-lasting disorder, with Crustacea species, especially shrimp, being the most frequent offender in children and adults. Reported prevalence rates range from 1.3% to 5.2% depending on the country and dietary habits. Shellfish allergy is often associated with severe reactions, including life-threatening anaphylaxis. Despite the high prevalence and severity of reactions, there is still no cure and accurate diagnosis, and strict avoidance is the only recommendation.[1-5]

Clinical history, skin prick test (SPT), and serum-specific IgE (sIgE) are aids in diagnosis. The SPT and sIgE test identify sensitization, but do not establish clinical relevance; thus, a double-blind placebo-controlled food challenge (DBPCFC) remains the gold standard, despite its potential risk.[6] Component-resolved diagnosis and epitope mapping have been applied to a wide range of allergens to elucidate distinct sensitization profiles that more accurately reflect clinical reactivity, avoiding the need for a challenge.[7,8]

Tropomyosin is considered to be responsible for most shrimp-allergic reactions.[9] Nevertheless, other shrimp proteins have been characterized (arginine kinase [AK],[10-12] myosin light chain [MLC],[13] sarcoplasmic calcium-binding protein [SCP],[14,15] hemocyanin,[16-18] troponin C,[17,19] and triosephosphate isomerase[19]) and novel potential allergens identified (fatty-acid-binding protein [FABP],[17] alpha-actinin,[20] beta-actin,[20] and ubiquitin[20]).

The clinical relevance of sensitization to some of them still needs to be fully determined.[1,2] The relevance of tropomyosin sensitization has been studied in subjects with house dust mite (HDM) allergy sensitized to shrimp, finding tropomyosin-sIgE more efficient to diagnose shrimp allergy than the whole shrimp extract.[21,22] The use of the 6 allergens of the North Sea shrimp seemed to improve the diagnosis and treatment of shrimp allergy in a European cohort.[19] Recently, AK and SCP have been found to be minor allergens but clinically relevant in an Italian study.[18]

Cross-reactivity issues also limit correct diagnosis.[1,2,6] Tropomyosin sensitization accounts for the extensive cross-reactivity observed among crustaceans and other arthropods and other invertebrates.[1,2,23,24] However, the role of cross-reactivity with the other allergens has not been fully defined. Some HDM allergens potentially cross-react to homologous allergens in crustaceans,[25] such as HDM-AK (Der p 20),[26] and high molecular weight allergens in both shrimp and HDM, such as hemocyanin.[18,27] Recently, ubiquitin, alpha-actinin, and AK shrimp allergens have been found to be responsible for mite-seafood cross-reactivity.[20] Also cockroach Bla g 8 shows 62.6% of similarity and 48.5% of identity to shrimp MLC.[13,28]

The IgE epitopes of 4 shrimp allergens (Lit v 1-4) were identified.[29] Later, some of those epitopes were identified as clinically relevant in a pilot study.[30]

Hereby we sought to determine whether sensitization to certain allergens and their epitopes correlated more closely with clinical reactivity.

METHODS

Patient selection

Subjects were recruited from the Allergy Clinic at the Mount Sinai Medical Center, New York, USA; the Division of Clinical Immunology and Allergy, University of São Paulo School of Medicine, São Paulo, Brazil; the Allergy Clinic of Hospital Infantil Universitario Niño Jesús and Allergy Department of Fundación Jimenez-Diaz, Madrid, Spain. The inclusion criterion was a positive shrimp SPT, including subjects with shrimp-allergy-related symptoms and also subjects with respiratory allergy, eczema, or food allergies that were skin-prick tested for shrimp-sIgE because it is included in the routine diagnosis panel. DBPCFCs were performed in all individuals except those who reported an anaphylactic episode within the previous 3 months. Subjects were grouped based on whether histories of immediate shrimp-allergic reactions were reported and challenge results. Two control groups were included, one with subjects allergic to HDM and/or cockroach with no sensitization to shrimp or reported histories of allergic reactions after ingestion, and another one with nonatopic individuals. The respective hospital local ethical committees approved the study and informed consent was obtained.

Specific IgE detection and challenge

Serum sIgE to shrimp (f24, a mixture species: *Pandalus borealis, Penaeus monodon, Metapenaeopsis barbata*, and *Metapenaeus joyneri*), HDM (d1: *Dermatophagoides pteronyssinus,* DP), and cockroach (i6: *Blatella germanica*) was measured by ImmunoCAP (ThermoFisher Scientific, Sweden) (cutoff: >0.35 kU_A/L). SPT commercial extracts had to be the ones available in each hospital: shrimp (Spain: Leti, Spain; USA: Alk-Abelló, Spain; Brazil: Hollister-Stier Laboratories, USA), DP, and cockroach (Spain and USA: Alk-Abelló, Spain; Brazil: IPI-ASAC, Spain). Tests were performed according to the European guidelines.[31]

Briefly, DBPCFCs were performed with increasing doses of ground boiled shrimp obtained from a local market (500 mg to a maximum of 8 g) or placebo (lactose) in a chocolate pudding.[21,22,30] Nobody was lactose intolerant or allergic to any pudding components. If the DBPCFC was negative or inconclusive, an open oral challenge receiving a total of 12 cooked shrimps (equivalent to 30 g) was performed. Responses were considered positive if objective signs of allergy were observed.

Purification of recombinant proteins

Ten recombinant (r) proteins were evaluated. TM (Lit v 1), AK (Lit v 2), MLC (Lit v 3), SCP isoform alpha (SCP-alpha, Lit v 4), hemocyanin (Hemo, GenBank Acc. HQ709161), and FABP