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Original article

Shellfish hypersensitivity and specific IgE detection

Background: Hypersensitivity reactions in relation to shellfish ingestion are among the most frequent food allergy reactions in the adult. We report the study carried out on three patients, two of them with a history of hypersensitivity manifestations after the ingestion of various shellfish and the third one with a history of inhalational hypersensitivity reactions during the preparation –boiling– of sea crabs (Spanish: "nécora"). **Methods:** A clinical questionnaire was administered and prick tests performed with a panel of airborne allergens and shellfish. The total serum IgE levels were quantitated, and SDS-PAGE and IgE immunodetection was carried out with both raw and cooked shrimp, Norway lobster, sea crab (two species) and prawn antigens. **Results:** The skin tests were positive in all three patients with some of the shellfish tested. Multiple allergens with varying molecular weights were detected with the sera of two of the patients, but with greater intensity for those with molecular weights below 20 kDa and with the boiled extracts; in the third patient only three low-molecular weight (ca. 14 kDa) allergens were detected in the boiled crab extract, with no further allergen recognition in any other of the shellfish studied. **Conclusions:** The diagnosis of shellfish hypersensitivity must always be carried out using both raw and boiled extracts, and it must be complemented with specific IgE immunodetection studies.

Key words: Hypersensitivity. Shellfish. Immunodetection. IgE.

Hipersensibilidad a crustáceos y detección de IgE específica

Fundamento: Las reacciones de hipersensibilidad relacionadas con la ingestión de marisco constituyen una de las alergias alimentarias más frecuentes en adultos. Se presenta el estudio realizado a tres pacientes, dos de ellos con historia de hipersensibilidad tras la ingesta de distintos crustáceos y el tercero con historia de hipersensibilidad por vía inhalatoria durante la cocción de nécora. **Métodos:** Se realizaron un cuestionario clínico y *prick test* frente a una batería de neumoaergenos y crustáceos. Se determinó la IgE total y se realizó SDS-PAGE e inmunodetección de IgE con antígenos de gamba, cigala, nécora, buey de mar y langostino, tanto crudos como cocidos. **Resultados:** Las pruebas cutáneas fueron positivas en los tres pacientes con algunos de los distintos crustáceos. Se detectaron múltiples alérgenos de distintos pesos moleculares, aunque con mayor intensidad para pesos moleculares inferiores a 20 kDa y con los extractos hervidos en dos de ellos; en el tercer paciente sólo se detectaron tres alérgenos de bajo peso molecular, aproximadamente de 14 kDa, en la nécora cocida, y no se reconocieron otros alérgenos en

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los otros crustáceos testados. **Conclusiones:** El diagnóstico de hipersensibilidad a crustáceos debe hacerse siempre con extractos tanto crudos como hervidos y completarse con estudios inmunológicos de detección de IgE específica.

Palabras clave: Hipersensibilidad. Crustáceos. Inmunodetección. IgE.

Hypersensitivity reactions after the ingestion of shellfish represent one of the more frequent food allergies in adults¹. Most cases involve allergy to multiple shellfish, such as lobster, crabs and shrimp/prawns. The affected individuals may develop urticaria, angioedema, asthma and anaphylaxis²⁻⁵. Hoffmann et al.⁶ demonstrated the presence of both thermo-stable and thermo-labile antigens in shrimp. The thermo-stable antigen was identified, and had a molecular weight of ca. 28 kDa. Recent studies have identified the shrimp allergen as being a muscular protein, tropomyosin⁷⁻⁹. Hefle et al.¹⁰, in a study carried out on sixteen shellfish manipulators and two cooks using specific IgE detection, found a number of crab allergens with molecular weights ranging between 25 and 45 kDa; two of the patients' sera only recognised antigenic proteins with ca. 14 kDa molecular weight, and a further two sera recognised allergens with 97 kDa molecular weight. Leung et al.¹¹ characterised the major allergen of the *Charybdis ferox* crab, *Cha f 1*, which is a tropomyosin with a molecular weight of ca. 34 kDa. In the course of the last Symposium of the Spanish Society of Allergy and Clinical Immunology the case was presented of a patient reporting symptoms of bronchial asthma in association to the manipulation of and with the inhalation of cooking (boiling) particles of Norway lobster. The specific IgE detection disclosed one single 30.2 kDa band with the Norway lobster extract, and one further protein (ca. 46 kDa) with the shrimp, mussel, barnacle and snail extracts. According to the authors¹², none of these proteins was tropomyosin. We now report three patients with clinical symptoms in association to various shellfish and with varying specific IgE recognition patterns.

MATERIAL AND METHODS

Clinical cases

Case 1. A 26-year-old male, who since ten years ago works as a fishmonger and shellfish boiler. Three years

ago, after eating boiled prawns, he developed labial oedema with palatine and lingual pruritus. The same clinical manifestations have again occurred with lobster, shrimp and sea crab (*Portunus puber L.*), all of them boiled. The patient tolerates grilled shrimp and Norway lobster; he has never eaten grilled prawn, nor has he eaten Mexican prawn, spider crab, ox-crab, common sea crabs, barnacles, mussels, clams, razor shell mussels, cockles or oysters. He tolerates without problems both boiled and grilled octopus, squid and cuttlefish.

Case 2. A 38-year-old male, working as a restaurant waiter. Some 20 years ago, after eating spider crab and ox-crab, he developed a generalised erythematous rash with coincident feeling of chest tightness. The clinical manifestations remitted after receiving corticosteroid and antihistamine therapy at an Emergency Service. He reports palatine and pharyngeal pruritus and a feeling of mild labial oedema after eating spiny lobster, Maine lobster, Norway lobster, prawns, shrimp and sea crabs (*Portunus puber L.*); he tolerates mollusks.

Case 3. A 25-year-old female with a personal history of pollen allergy treated with immunotherapy. Since about six years ago she works at a company that cooks (boils) various shellfish. While at work she manipulates octopus, shrimp, Norway lobster and sea crabs (*P. puber* and others). Since about two years ago, and in coincidence with the months when *P. puber* is boiled at her workplace (September-October), she reports dyspnoea, wheezing and unproductive cough when she enters her workplace, with nighttime exacerbations. She also reports urticarial lesions on the skin areas contacting the broth of the crab-boiling cauldrons. She is asymptomatic for the remainder of the year. She tolerates the ingestion of cooked shrimp and prawns, and has never eaten spiny, Maine or Norway lobster, Atlantic shrimp, Mexican prawn, spider crab, ox-crab or other sea crabs. She fully tolerates all sorts of fish.

Allergens

For the skin tests, extracts were prepared from the raw flesh of sea crabs (*Portunus puber* and another species), prawn and ox-crab; the flesh was minced, extracted in PBS and then centrifuged at 4,500 g for 15 minutes. The supernatant was separated and sterilised by filtration through a Millipore filter. The final protein concentration was 1 mg/ml.

For the *in vitro* assays, further extracts were prepared from the same shellfish, both raw and boiled. The flesh was minced, extracted in PBS and then centrifuged

at 4,500 g for 15 minutes. The supernatant was then again centrifuged at 16,000 g for 10 minutes. The final protein concentration in these extracts was 1 mg/ml.

Skin tests

Skin tests were performed by the prick procedure with a standard commercial airborne allergen panel (*Der-matophagoides* mix, moulds mix, grass mix, tree pollen mix, shrub pollen mix) and with a cockroach extract (ALK-Abelló, Madrid, Spain). The tests were read (immediate reaction) after 15 minutes; the results were classed as positive when the greater diameter of the wheal was grater than 3 mm.

SDS-PAGE

Sodium dodecylsulfate-polyacrylamide gel electrophoresis of all the extracts prepared using a 16% polyacrylamide gel as separator¹³.

Specific IgE antibody detection

The detection of the IgE antibodies was carried out after electrophoresis and transfer to a nitrocellulose membrane, as previously described by Moneo et al.¹⁴. The nitrocellulose membranes were then incubated with the patients' sera and then with a monoclonal anti-IgE antibody. After a final incubation with an alkaline phosphatase-labelled murine anti-immunoglobulin antiserum, the membranes were developed with BCIP-NBT¹⁴.

RESULTS

In vivo tests

The skin tests with the standard airborne allergen panel were negative in Cases 1 and 2; Case 3 had positive results for the grass pollen mix. The prick test with cockroach extract was positive only in Case 1. The results achieved with the various shellfish tested are presented in Table I.

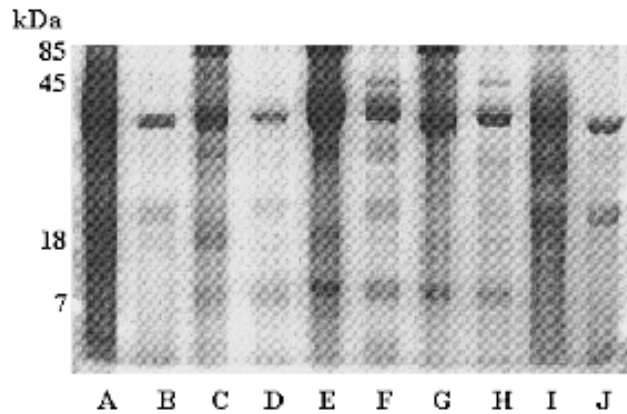


Fig. 1. SDS-PAGE. Lane A: raw shrimp; lane B: boiled shrimp; lane C: raw Norway lobster; lane D: boiled Norway lobster; lane E: raw sea crab (*Portunus puber*); lane F: boiled sea crab (*P. puber*); lane G: raw ox-crab (*Cancer pagurus*); lane H: boiled ox-crab; lane I: raw prawn; lane J: boiled prawn.

In vitro tests

Specific IgE (Allergocoat 6): The specific anti-crab IgE was assessed only in Case 3, with negative results (<0.35 AU/ml).

SDS-PAGE: Multiple protein bands were observed in all lanes, with smaller numbers of bands in the lanes carrying the boiled extracts (Fig. 1). There was in all lanes one major band corresponding to a ca. 39 kDa protein. In lanes E to H there was an intensely staining band with a molecular weight of ca. 14 kDa, which was not visible in the other lanes.

Detection of specific IgE antibodies: There was considerable heterogeneity in the antigen recognition with the three patients' sera. The IgE binding was more intense in the lanes corresponding to the boiled extracts (Figs. 2 to 4). There was IgE recognition of antigens with molecular weights ranging from ca. 14 kDa to greater than 100 kDa, with more marked IgE detection for antigens with molecular weights below 20 kDa.

In one of the cases (Fig. 3) there was only a single intense IgE-binding band in the lane corresponding to the boiled crab (*P. puber*) extract at molecular weights below 20 kDa.

Table I. Results of the skin tests with shellfish extracts and of the total serum IgE quantitation in the three patients studied.

Case	Prick test <i>Portunus puber</i> (mm)	Prick test Prawn (mm)	Prick test <i>Cancer pagurus</i> (mm)	Prick test Sea crab (mm)	IgE serum IgE (UI/ml)
1	5 x 5	Negative	Negative	Negative	50
2	10 x 5	8 x 5	6 x 5	5 x 5	694
3	10 x 8	Negative	Negative	10 x 5	25

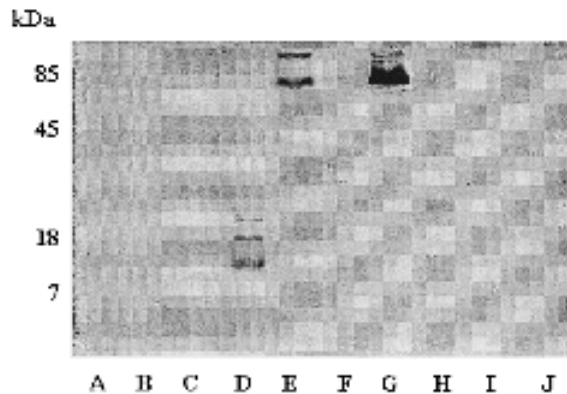


Fig. 2. Specific IgE detection, Case 1. The order and contents of the lanes is the same as in Fig. 1.

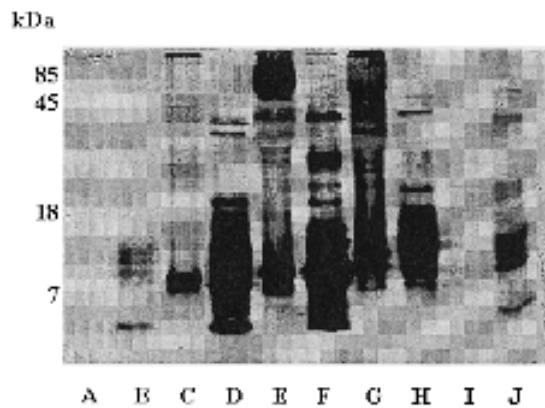


Fig. 3. Specific IgE detection, Case 2. The order and contents of the lanes is the same as in Fig. 1.

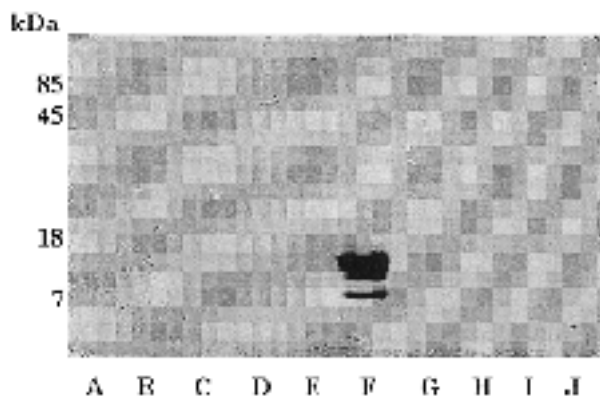


Fig. 4. Specific IgE detection, Case 3. The order and contents of the lanes is the same as in Fig. 1.

DISCUSSION

Sensitisation to shellfish may induce quite varied clinical manifestations, ranging from urticaria, through an-

gioedema or asthma, to anaphylaxis^{1-5,12}, and the antigen entry portal may be both digestive and inhalational. In the present series, two patients had cutaneous symptoms after the ingestion of a number of shellfish, and the third one evidenced episodes of asthma in relation to the inhalation of sea crab-boiling vapours at her workplace.

The SDS-PAGE study disclosed one common protein band in all lanes, which might correspond to a protein similar in molecular weight to tropomyosin. Leung *et al.*¹¹ identified a 34-kDa protein, which was termed *Cha f 1*, as the major allergen in the *Charybdis feriatus* crab, and demonstrated its similarity to shrimp tropomyosin. A number of studies stress this protein as an important panallergen in cross-sensitisation to crustaceans^{3,4,6-8,15}.

However, this could not be demonstrated in our patients: only one of them evidenced IgE binding to this protein, but also with more intense recognition of other proteins below 20 kDa in molecular weight (Fig. 2). The other patients evidenced considerable heterogeneity in the IgE binding, suggesting the possibility that other major allergens may exist in crustaceans, different from tropomyosin and with molecular weights in the order of 14 kDa, which is where IgE recognition occurred in most cases.

Differences were also observed in the IgE detection for raw and boiled extracts: stronger IgE binding was observed in the lanes carrying the boiled extracts, even though the protein content was greater in the raw extracts. This suggests the possibility that the increased temperature (boiling) might induce a conformational change in the proteins, causing them to express occult epitopes responsible for the new antigen recognition.

A particularly interesting observation is that of Case 3 (Fig. 3), in which no specific IgE could be detected; the immunodetection test, however, disclosed intense and highly specific IgE binding in the lane carrying the boiled crab (*P. puber*) extract in the molecular weight range of ca. 14 kDa or lower. Clinically, the patient evidenced contact urticaria, which might have been due to the presence of these proteins in the crab boiling broth, and asthma episodes that might have been induced by aerosolisation of the antigen.

In the present series, none of the patients' sera recognised tropomyosin as the major allergen; however, IgE binding occurred to other allergens with ca. 14 kDa molecular weights. Further studies are required, with larger patient series, in order to ascertain whether these low-molecular weight antigens might represent major

allergens. The diagnosis of shellfish hypersensitivity should always be established using both raw and cooked extracts, as a difference in recognition has been demonstrated between them.

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