Heterogeneity in allergy to mollusks: a clinical-immunological study in a population from the North of Spain.

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Abstract

Background: Allergy to mollusks has been the focus of fewer studies than crustaceans and is less well characterized.

Objective: To describe the clinical characteristics of patients allergic to mollusks, identifying the allergens responsible and to assess the cross-reactivity.

Methods: A prospective multicenter study including 45 patients with mollusk allergy, defined as a suggestive anamnesis and a positive skin test to the agent involved. Allergic fractions were identified using SDS-PAGE and immunoblotting. The proteins responsible were subsequently identified using mass spectrometry. ELISA inhibition studies were performed among mollusks, dust mites and crustaceans.

Results: Twenty-five (55%) of the patients were allergic to cephalopods, 14 (31%) to bivalves, and 11 (24%) to gastropods. Limpet represented the third cause of allergy, with 15% of cases. In 31 (69%) the manifestation was systemic, 10 (22%) exhibited oral allergy syndrome and 7 contact urticaria (15%).

The majority of the major allergens were found between 27 and 47 kDa. ELISA inhibition assays revealed a high degree of inhibition of cephalopods and bivalves from all the groups of mollusks, mites and crustaceans. Mass spectrometry identified tropomyosin, actin and myosin as the major allergens.

Conclusions: Cephalopods, especially squid, are the most frequently mollusks triggering allergic symptoms. It is striking the high occurrence of allergy to limpets in contrast with their low consumption. It is worth highlight the heterogeneity observed, exemplified by the gastropods. Tropomyosin appears to be responsible for the high cross-reactivity found between mollusks, mites and crustaceans. Three new mollusk allergens were also identified: actin, enolase and a putative C1q domain-containing protein.

Resumen

Antecedentes: La alergia a moluscos ha sido menos estudiada y está peor caracterizada que la alergia a crustáceos.

Objetivo: describir las características clínicas de pacientes alérgicos a moluscos, identificar los alérgenos responsables y estudiar la reactividad cruzada entre ellos.

Métodos: Estudio multicéntrico, prospectivo. Se incluyen 45 pacientes con alergia a moluscos, definida como una clínica sugestiva y prueba cutánea positiva con el molusco sospechoso. Se identificaron las bandas alergénicas mediante SDS-PAGE e inmunodetección. Las proteínas responsables se identificaron utilizando espectrometría de masas. Se realizaron ensayos de inhibición de ELISA entre moluscos, ácaros y crustáceos.

Resultados: Veinticinco (55%) de los pacientes eran alérgicos a cefalópodos, 14 (31%) a bivalvos y 11 (24%) a gasterópodos. La lapa resultó ser la tercera causa de alergia (15% de los casos). Los síntomas fueron sistémicos en 31 pacientes (69%), diez (22%) tuvieron síndrome de alergia oral y siete (15%) urticaria de contacto.

La mayoría de las bandas alergénicas estaban entre 27 y 47 kDa. Los ensayos de inhibición de ELISA mostraron un alto grado de inhibición de cefalópodos y bivalvos por parte de moluscos, ácaros y crustáceos. Mediante espectometría de masas se identificaron tropomiosina, actina y miosina como los alérgenos mayoritarios.

Conclusiones: los moluscos que con más frecuencia provocan reacciones alérgicas son los cefalópodos, especialmente el calamar. Llama la atención la elevada frecuencia de alergia a la lapa, a pesar de su bajo consumo. También hay que resaltar la heterogeneidad observada, por ejemplo en los gasterópodos. La tropomiosina parece ser responsable de la elevada reactividad cruzada encontrada entre moluscos, ácaros y crustáceos. Se han identificado tres nuevos alérgenos en los moluscos: actina, enolasa y putative C1q domain-containing protein.

Introduction

Seafood plays an important role in human nutrition and health. However, shellfish including crustaceans and mollusks are one of the most common causes of food allergy in the world. Within the group of edible mollusks, three classes can be identified: cephalopods (squid, cuttlefish, and octopus); bivalves (mussels, clams, razor fish, winkles, oysters, and scallops) and gastropods (limpets, snails, and abalone). Although the importance of molluscan shellfish allergy is becoming increasingly recognized, its prevalence is unknown. Sicherer et al, in a nationwide random telephone survey in the USA, found a self-reported prevalence of 0.4% (1). Rance et al, obtained a self-reported prevalence of 0.15% in French children (2). In Spain, Crespo et al reported that mollusks were responsible for 1.6% of food allergies in a sample of children (3).

Given the high levels of consumption of shellfish and their early introduction into the diet, a large number of studies regarding mollusk allergy have been published in south-east Asia and Japan (4).

As is the case in crustaceans, tropomyosin appears to be the most important allergen identified to date in the three classes of mollusk (4). Another protein that has been identified is paramyosin (5). However, a considerable number of allergens remain unidentified (4).

Although the in vitro cross-reactivity between mollusks and crustaceans is extremely high (6), very frequently patients allergic to crustaceans tolerate mollusks and vice-versa. This could be due to the presence of different epitopes of tropomyosin in the two classes of shellfish as well as the presence of proteins specific to each class. The homology in the protein sequence of tropomyosin in crustaceans is 98%, and is also high between mollusks – 68% to 88% - while between crustaceans and mollusks it ranges from 56% to 68% (6-8).

Equally well known is the cross-reactivity between dust mites and crustaceans. Within the mollusks it is striking that the allergic symptoms triggered by gastropods (limpets, snails) invariable occur in patients with allergy to dust mites, which indicates the presence of common allergens (9,10).

Furthermore, within mollusks, the allergy in each patient is most frequently limited to some of the three classes described above (cephalopods, bivalves and gastropods), which suggests the presence of several proteins or at least differences in the antigenic recognition of the same.

In the literature there are no extensive series of patients with an allergy to mollusks in which immunologic analyses have also been performed. The primary aim of this study is to analyze the clinical characteristics of a group of patients in Spain who are allergic to mollusks. The secondary aims are to identify the proteins responsible, and

assess the degree of cross-reactivity among them as well as that with crustaceans and mites.

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Materials and methods

Patients

Sera from 45 patients allergic to mollusks were collected from the Hospital Universitario Central de Asturias (Asturias), Hospital de Cruces (Vizcaya), Hospital Virgen del Camino (Navarra), Hospital Universitario de Araba (Álava), Hospital Donostia (Guipúzcoa), Clínica Universidad de Navarra (Navarra), Hospital de Basurto (Vizcaya), Hospital de Mendaro (Gipúzcoa) and Hospital de Galdácano (Vizcaya). Patients were recruited during December 2013 and May 2014. Mollusk allergy was diagnosed in patients having a clear history of adverse reactions suggestive of IgE-mediated allergy after eating a mollusk (cephalopods, bivalves or gastropods), along with a positive skin prick test with the same mollusk (11) and negative skin tests to other foods or drugs took at the same time. If the allergic reaction occurred more than five years ago, the patient was included if the diagnosis was done at the time of the reaction, and the prick test to the allergenic mollusk remained positive at the time of the study. The inclusion of the patients was done consecutively among all those who met the inclusion criteria described.

Symptoms suffered during the allergic reaction were classified as: (i) systemic (urticaria and/or angioedema and/or bronchospasm and/or abdominal symptoms and/or hypotension); (ii) contact urticaria (Urticarial lesions upon the food touching the skin); (iii) oral allergy syndrome (Itching or burning sensation or swelling of the lips, mouth or pharynx, withing minutes after eating the culprit food).

The study was approved by the Ethics Committees of the respective hospitals, and written informed consent was obtained from all patients.

Mollusk, shrimp and mite extracts

Mollusk, shrimp, and mite extracts were prepared as follows: 10 gr of each raw material were homogenized in 100 ml of PBS and extracted overnight at 4° C under constant magnetic stirring. After centrifugation at 14.000 g for 45 min. at 4°C, the supernatant was dialyzed against NH_4HCO_3 0.1M, lyophilized and dissolved in PBS. For in vitro experiments, the protein concentration was adjusted to 1 mg/ml.

Skin tests

All patients underwent a prick test with *Dermatophagoides Pteronyssinus* (ALK-Abelló, Madrid. Spain) and a prick by prick test with shrimp *(Parapenaeus longirostris)* using the same material in all hospitals. Each patient reported the type of mollusk which triggered the allergic symptoms in order to administer the prick by prick test with the same agent. A skin test was considered positive if a wheal > 3 mm in diameter, greater than negative control, was observed.

Specific IgE

The ImmunoCAP FEIA 250 test (Thermo Fisher Scientific, Uppsala, Sweden) was used to determine the presence of specific IgE against the mollusk triggering the allergic reaction and against shrimp tropomyosin, rPen a 1.

SDS-PAGE and immunoblot analysis

SDS-PAGE was carried out according to Laemmli (12) using the Hoefer SE 600 electrophoresis system (GE Healthcare). Polyacrylamide concentrations of 14% (w/v) and 5% (w/v) were used for separating and stacking gels, respectively. 20 µg of protein extract were applied per lane. The samples were mixed with 0.1 M Tris, pH 6.8, containing 4% (w/v) SDS, 20% (w/v) glycerol, 10% (w/v) 2-β-mercaptoethanol and 0.02% (w/v) bromophenol blue. To ensure proper protein separation and visualization, the gels were stained with PageBlue Protein Staining Solution (Fermentas International, Inc, Canada) or used for immunoblotting as described below. Immunodetection was carried out as previously described (13) with the 45 individual sera from mollusk-allergic patients. Sera from five non-atopic patients were used as negative controls.

Protein identification and characterization by Mass-Spectrometry

Protein bands recognized by the sera of more than 50% of patients were selected for further study. Bands were extracted from the gel and proteins were identified by mass spectrometry (MS) using liquid chromatography-MS/MS, as previously described (13). Protein identification was performed by searching a nonredundant protein sequence database (NCBI), using the Mascot program (http://www.matrixscience.com.).

ELISA and ELISA inhibition studies

ELISA and ELISA inhibition assays were carried out as previously described (14). Briefly, 96-well flat-bottom plates were used (Immunolon 4HBX, Thermo). The plates were coated in duplicate overnight at 4°C with 10 µg of mollusk proteins per well diluted in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6). Sera from allergic patients to each mollusk were pooled: those of a specific allergy e.g. all squid-allergic sera are pooled for inhibition of coated squid extract. Sera from 5 non-atopic patients were used as negative controls. IgE reactivity was considered positive when a patient's OD (optical density) at 620 nm was two times higher than a negative control's OD at the same absorbance. All tests were performed in duplicate. For ELISA inhibition assays, a mollusk extract (10 µg) was used as the solid phase and pooled sera from allergic patients were preincubated for 4h at RT with 100 µg of the inhibitor (protein extract) per milliliter of serum.

Results

Clinical features

Forty-five patients were included in the study. The demographic data along with the clinical characteristics, the results of the skin tests performed, as well as the results of the specific IgE determinations to mollusks and shrimp tropomyosin are shown in Table 1.

The mean age of the patients was 30 years (IIC: 17-40). Twenty-four were male. The mean time elapsed since the last reaction up to the time of the study was 5 years.

The distribution by class of mollusk was the following: 25 (55%) were allergic to cephalopods; 14 (31%) to bivalves and 11 (24%) to gastropods. The number of patients by mollusk was: 18 (40%) were allergic to squid, 8 (18%) to clam, 7 (15%) to limpet, 5 (11%) to octopus and cuttlefish, 4 (9%) to mussel and snail, 3 (7%) to razor fish, and 1 (2%) to cockle (Table 2). Five patients (11%) were allergic to cephalopods and bivalves. Three patients presented with allergic reactions to several mollusks from the same group, cephalopods and gastropods.

In 31 of the 45 patients (69%) the symptoms were systemic. Ten (22%) presented oral allergy syndrome (OAS) and 7 (15%) contact urticaria. All the patients allergic to gastropods had systemic reactions. Among the bivalves the proportion was 80%, and among the cephalopods 67%.

Mite allergy

29 (64%) of the patients had associated symptoms of rhinitis and/or asthma due to dust mites with the following distribution: 11 cases (100%) of those allergic to gastropods; 13 (55%) of those allergic to cephalopods, and 5 (50%) of those allergic to bivalves.

A skin test to mites was positive in 35 patients (78%) of the series.

To be noted that the population studied comes from a geographical area with a high incidence of dust mite sensitisation.

Crustacean allergy

Nineteen (42%) of the patients were allergic to crustaceans, according to a clear history of adverse reaction suggestive of IgE-mediated allergy, along with positive skin prick test. The result by class of mollusk was: 14 (58%) of those allergic to cephalopods; 4 (40%) of those allergic to bivalves, and just 1 (9%) of those allergic to gastropods.

The total number of patients with a skin test positive to crustaceans was 30 (66%).

Specific IgE

83 % of the patients showed a specific IgE to the triggering mollusk of the allergic reaction, using a cut off point of 0.1 kUA/L. This cipher changed to 52% when the cut off point was 0.35 kUA/L.

IgE to rPen a 1 greater than 0.1 kUA/L was obtained in 51% of the whole series. This percentage became 44% when the cut off point used was 0.35 kUA/L. When the result was evaluated according to the type of mollusk and taking as reference the 0.1 kUA/L value, we obtained a positivity to rPen a 1 in 72% of the patients allergic to cephalopods, 57% in the bivalve allergic group, and 18% in the gastropod allergic group.

Immunoblot analysis

Cephalopods. In 14 of the 18 patients allergic to squid immunoblot analysis revealed IgE-binding bands (fig. 1A), especially those of 22, 24, 38 and 100 kDa of apparent molecular weight, which were seen in more than 50% of the patients. The 3 cases of allergy to cuttlefish showed IgE-binding bands with an apparent molecular weight of 38 and 40 kDa (fig. 1B).

Bivalves. IgE-binding bands were found in all the patients allergic to clams. The bands appearing in more than 50% of patients corresponded to 20, 27 and 36 kDa (fig. 2A). In the case of razor fish, the 3 patients presented bands most frequently of 42, 44 and 45 kDa (fig. 2B). Only in 1 patient allergic to mussels was an IgE-binding band found corresponding to 40 kDa (fig. 2C).

Gastropods. IgE-binding bands were found in all the patients allergic to limpets. These corresponded to 46 and 47 kDa (fig. 3).

In the patients allergic to octopus and snails no IgE-binding bands were found for any of the extracts (data not shown). Control immunoblot assays with the pooled serum from non atopic patients did not show any IgE-binding bands.

Protein identification by MS

Using MS, we identified several IgE-binding proteins: tropomyosin, actin, myosin and enolase (Table 3). The proteins most frequently detected, in more than 50% of patients, by specific IgE against each mollusk were: tropomyosin and myosin in squid; tropomyosin in cuttlefish; tropomyosin and actin in clams; actin in razor fish and in limpets; and a putative C1q domain-containing protein in one patient allergic to mussels.

ELISA inhibition

The inhibition assays are shown in Fig 4. Squid was widely inhibited by the other cephalopod, cuttlefish. It was also inhibited by the other mollusks, mites and crustaceans. Cuttlefish and clams were similarly inhibited by the remaining mollusks as well as by mites and crustaceans. Limpets were inhibited only by mite extract.

Discussion

Few studies on allergy to mollusks have been published and they include a small number of cases. To the best of our knowledge, our study is the largest published to date with the clinical characterization and identification of allergens.

In our series the most widely represented age group was that of young adults, with a mean age of 30 years, as has been the case in other studies (10, 15-19). Cephalopods were the mollusk class most frequently involved, and in this group squid in particular. This is consistent with other publications (15, 20, 21). Bivalves were the second most frequent group in spite of the fact that this type of mollusk has the highest levels of consumption in Spain (22) and probably in the world (7). In the literature these represent the class which most infrequently causes allergic reactions (7). We found no cases of allergy to oysters or scallops. Gastropods were the group least frequently involved with a predominance of limpets. It is striking that this mollusk was the culprit in 15% of our series, given the low levels of consumption in Spain, although this may be related to the constant association of this mollusk with allergy to dust mites which has a high prevalence in the regions we studied. However, this group is the main trigger in Southeast Asia (18, 23).

Only 15% of patients were allergic to several classes of mollusks: cephalopods or bivalves and 7% to several mollusks from the same group: cephalopods or gastropods. However, the inhibition assays revealed important cross-reactivity between cephalopods and bivalves (Fig. 4). The reason for this difference may be that patients who have had an allergic reaction to one mollusk subsequently tend to avoid new exposures to any other mollusk. The case of gastropods is different as these are not inhibited by other groups of mollusk which explains why we found no patients allergic to them and also to cephalopods or bivalves.

It is worth highlighting that although most of our patients (69%) experienced systemic reactions, one third only had OAS and/or contact urticaria. In the literature systemic reactions have been reported but no reference has been made to other types of clinical manifestation. Interestingly, all the patients with allergy to gastropods showed systemic symptoms but this was not so for bivalves and cephalopods. Other studies have also reported this finding (10, 16, 19). These patients are almost always asthmatics allergic to dust mites, who frequently develop serious bronchospasm or anaphylaxis immediately after eating the gastropod, as occurred in our cases.

64% of our series had rhinitis and/or asthma due to mites, although almost 80% were sensitized to the same. In line with this, we found mites to inhibit the allergenicity of all the mollusk groups (Fig. 4). This link has been reported in most studies although sometimes it is difficult to appropriately separate the group with allergy to mollusks from those also allergic to crustaceans (15, 18).

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42% of our series were allergic to crustaceans, with a high frequency in the group of those allergic to cephalopods (58%), and in contrast only one case among those allergic to gastropods (9%). This association between allergy to crustaceans and mollusks has been widely recognized in the literature (24). The degree of skin sensitization to both has been reported to be as high as 90% (18), although in our study it was only 66%. Similarly, in our inhibition assays cephalopods were widely inhibited by crustaceans (Fig. 4). In spite of this, 40% of the patients allergic to cephalopods and 60% of those allergic to bivalves tolerated crustaceans. However, in gastropods the results were more homogeneous: we found no inhibition by crustaceans (Fig.4); only 9% had skin tests positive to crustaceans and 100% tolerated crustaceans, a finding reported in previous studies (10, 16). We believe therefore that the management of patients allergic to mollusks should be individualized and we are against the widely held view that all types of shellfish should be avoided (6, 9, 15), so that diets are not unnecessarily restricted. As part of this management and if a recent tolerance to a specific mollusk is not known, we consider that an oral food challenge should be performed.

It is worth highlighting that immunoblot analyses did not reveal any IgE-binding bands for octopus and snails, either under reducing or non-reducing conditions. We have no explanation for this since as all these patients, as did the rest of the series, presented an unequivocal allergic reaction and specific IgE against these mollusks was found using skin tests. A possible explanation could be the low level of specific IgE obtained against both mollusks. Nevertheless in the case of other mollusks we observed IgE fixing bands in the immunoblotting despite very low levels of specific IgE or even undetectable ones.

Tropomyosin is the most widely reported allergen in mollusks (4), and has been identified in cephalopods (25, 26), bivalves (27, 28), and gastropods (29). It should be noted that in some of these studies the sera used came from patients allergic to crustaceans. In our series, the protein was identified in squid, cuttlefish and clams. We observed a directly proportional relationship between the specific IgE levels to shrimp tropomyosin and the intensity of the immunoblotting bands identified in the MS as tropomyosin.

Tropomyosin is also the allergen supposedly responsible for the cross-reactivity between mollusks, and between mollusks and crustaceans, and between both of these and dust mites, a reactivity which reached high levels in in vitro studies (4). Consistent with this, in our series we found that most of the patients allergic to several classes of mollusks were also allergic to those in which tropomyosin was identified as the allergen (squid, cuttlefish and clam). Furthermore, in the inhibition assays these mollusks inhibit one another and are in turn inhibited by mites and crustaceans, both of which contain tropomyosin as an allergen (Fig. 4). The presence of different epitopes in each of these groups, and in particular in each mollusk could be the cause of the variability we observed.

The homology in the protein sequence of tropomyosin in crustaceans is 98%, and is also high between mollusks – 68% to 88% - while between crustaceans and mollusks it ranges from 56% to 68% (6,8). When we compared the immunoblotting patterns of patients only allergic to mollusks to those allergic to mollusks and crustaceans, no differences were found. This runs counter to the notion of the presence of mollusk-specific allergens which could explain the existence of these two groups of patients. However, there are also isolated reports of allergy to mollusks, with tolerance to crustaceans, in which tropomyosin does not appear to be implicated (30-34).

Together with tropomyosin, the only allergen identified at the molecular level in mollusks was myosin, with a molecular weight of 100 kDa, the well-known major allergen in abalone (5). Again it must be noted that the sera employed in the study came from patients allergic to crustaceans with no documented allergic reaction to mollusks. In our series we characterized three new allergens in mollusks: actin as the major allergen in razor fish and limpet with a molecular weight of 45 kDa, enolase with a weight of 50 kDa in razor fish, and a putative C1q domain-containing protein of 42 kDa in mussel.

In our series the gastropods, both clinically and in vitro, showed very high cross-reactivity with dust mites, and absence of the same with other mollusks and crustaceans. The fact that we did not identify tropomyosin as an allergen in limpets would argue against a role for this protein in the cross-reactivity between gastropods and mites, a possibility that has been raised in other studies (9, 10, 35, 36). Alpha-actin was reported as a new crustacean allergen by Abdel Rahman et al. (37), and has just been recognized by Gámez et al. as being linked to mite-crustacean cross-reactivity (38). Given our identification of actin in limpets, the possibility exists that it might also play a role in the cross-reactivity between mites and gastropods.

We are well aware that our study does not include the gold standard of food allergy diagnosis, the double blind placebo controlled food challenge (DBPCFC), which is its main limitation. However, the prospective design which confers great reliability to the data collected, plus the fact that the patients selected had an unequivocal history of IgE-mediated processes confirmed by skin prick tests means that the diagnosis of allergy to mollusks was made with very high levels of accuracy. Furthermore, analysis of the articles published on mollusk allergy shows that the diagnoses were based on the same criteria as those used in our study, without the inclusion of the DBPCFC (10, 15-18, 20, 39).

In summary, our analysis of an extensive series of patients allergic to mollusks, shows that in our setting squid was the mollusk most frequently involved and highlights the high frequency of allergy to limpets in contrast with the minimum levels of consumption of this gastropod. Although in the literature allergic reactions to mollusks are always systemic, we highlight the absence of such reactions in one third of our patients. Further, we identified as new allergens in mollusks actin, enolase and a putative C1q

domain-containing protein. In those mollusks in which tropomyosin is detected as an allergen, this protein is responsible for the high cross-reactivity between mollusks, mites and crustaceans. The heterogeneity among the mollusks, as exemplified by the gastropods, should also be noted. Finally, the management of patients allergic to mollusks should be individualized, similar to the developing approach to tree nuts, so that diets are not unnecessarily restricted.

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FIGURE LEGENDS

Fig. 1. IgE-binding bands in mollusk extract by immunoblotting. Panel A: Squid. Panel B: cuttlefish. Lane number represents serum patient number. Lane (–) represents a negative control of serum pool from non-atopic patients. MW: molecular weight markers (kDa).

Fig. 2. IgE-binding bands in mollusk extract by immunoblotting. Panel A: Clam. Panel B: razor fish. Panel C: mussel. Lane number represents serum patient number. Lane (-) represents a negative control of pooled serum from non-atopic patients. MW: molecular weight markers (kDa).

Fig. 3. IgE-binding bands in limpet extract by immunoblotting. Lane number represents serum patient number. Lane (-) represents a negative control of pooled serum from non-atopic patients. MW: molecular weight markers

Fig. 4. ELISA inhibition assay. A mollusk extract (10 µg) was used as solid phase and pooled serum was preincubated with other mollusk extracts, mite extract (*D. pteronyssinus*) and shrimp extract (Penaeus indicus). All tests were performed in duplicate.

Table 1. Clinical features of mollusk-allergic patients. *Mollusk:* S: squid. O: octopus. Cu: cuttlefish. M: mussel. Rf: razor fish. C: clam. Co: cockle. L: limpet. Sn: snail (Land). *Symptom:* OAS: Oral Allergy Syndrome. S: Systemic symptoms. CU: Contact Urticaria. *Specific IgE:* kUA/L. [1]ImmnunoCAP performed with the triggering mollusk. If there were several mollusks involved, the first one indicated as the responsible for the most severe reaction was chosen. *ND:* Not done.

Patient	Age	Mollusk	Mollusk symptoms	Mite allergy	Mite skin test	Crustacean allergy	Shrimp skin test	Specific IgE to mollusk[1]	Specific IgE to rPen a 1
1	35	S,C	OAS	-	+	+	+	0.85	20.7
2	7	S,C	OAS	+	+	+	+	2.13	26.5
3	27	S	S	+	+	+	+	0.06	2.98
4	47	S	S	+	+	+	+	0.03	0
5	37	S	CU	+	+	-	-	0	2.19
6	35	S,O,Cu,M,C,Co	S	+	+	+	+	1.51	11.4
7	6	S, Cu	CU	-	+	-	+	0.31	2.14
8	14	S	OAS	+	+	+	+	0.08	0.1
9	12	S	CU	+	+	-	+	0.36	3.81
10	47	S	S	-	-	+	+	0	0.23
11	17	S	OAS,CU	+	+	-	-	0.37	0
12	70	S	S	-	-	+	+	0	0.85
13	25	S	OAS	+	+	+	+	0	0
14	18	S	CU	+	+	+	+	0.06	6.13
15	25	S	S,CU	-	-	+	+	7.34	1.5
16	20	S	S	-	+	-	+	0.03	4.72
17	16	S	OAS	-	+	+	+	0.32	8.76
18	18	S	OAS	+	+	-	+	0.67	0
19	34	O	S	-	-	-	+	0.03	0
20	28	O	S	-	-	-	-	0	0
21	40	O	CU	-	+	-	-	0.25	0
22	30	Cu	S	-	-	-	+	ND	0.61
23	23	Cu	S	+	+	+	+	ND	3.59
24	15	Cu,C	S	+	+	+	+	ND	13.8
25	17	M	S	-	-	-	-	0	0
26	40	M	S	-	-	-	+	1.49	0
27	20	M	OAS	+	+	+	+	0.41	3.23
28	17	C,O	S	+	+	+	+	0.24	1.04
29	49	C	S	+	+	+	+	3.09	13.4
30	40	C	S	+	+	+	+	1.6	4.15
31	33	C	S	-	-	-	+	0.56	0
32	26	R	S	+	+	-	-	ND	0.02
33	14	R	OAS,CU	-	+	-	-	ND	0
34	78	R	S	-	-	-	+	ND	0
35	33	L	S	+	+	-	-	0.16	0
36	60	L	S	+	+	-	-	8.9	0.11
37	34	L	S	+	+	-	-	0.06	0
38	32	L	S	+	+	-	-	0.31	0
39	23	L,Sn	S	+	+	-	-	0.13	0
40	13	L	S	+	+	-	-	0.13	0
41	46	L	S	+	+	-	+	0.91	0.05
42	43	Sn	S	+	+	-	+	0.01	0
43	31	Sn	S	+	+	+	+	0.33	0.32
44	59	Sn	OAS,S	+	+	-	-	0.36	0.02
45	55	Sn	S	+	+	-	-	0.01	0

Table 2. Percentage of allergy to each mollusk.

Mollusk	%
Cephalopods	55
Squidd	40
Cuttlefish	11
Octopus	11
Bivalves	31
Clam	18
Mussel	9
Razor fish	7
Cockle	2
Gastropods	24
Limpet	15
Snail	9

Table 3. Protein identification by MS

Mollusk	Apparent molecular weight	Identification	% Recognition
Clam	20	Tropomyosin	50
	23	Beta-actin	75
	24	Beta-actin	50
	27	Actin	72
	30	Actin	50
	31	Beta-actin	50
	34	Actin	75
	36	Tropomyosin	75
Squid	22	Tropomyosin	50
	24	Tropomyosin	50
	35	Tropomyosin	22
	38	Actin	22
	40	Actin	78
	55	Myosin	22
	60	Myosin	11
	100	Myosin	50
Razor fish	40	Actin	100
	42	Actin	67
	44	Actin	67
	45	Actin	100
	50	Enolase	33
Cuttlefish	38	Tropomyosin	100
	40	Tropomyosin	100

Limpet	46	Actin	86
	47	Actin	67
Mussel	42	Putative C1q domain containing protein	33

FIGURA 1.

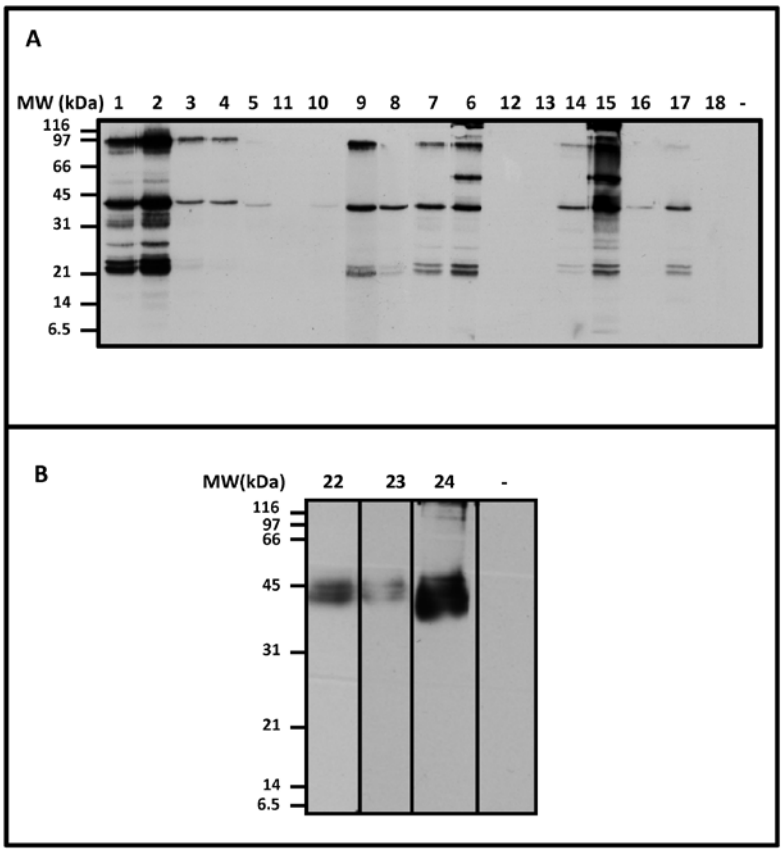

FIGURA 2.

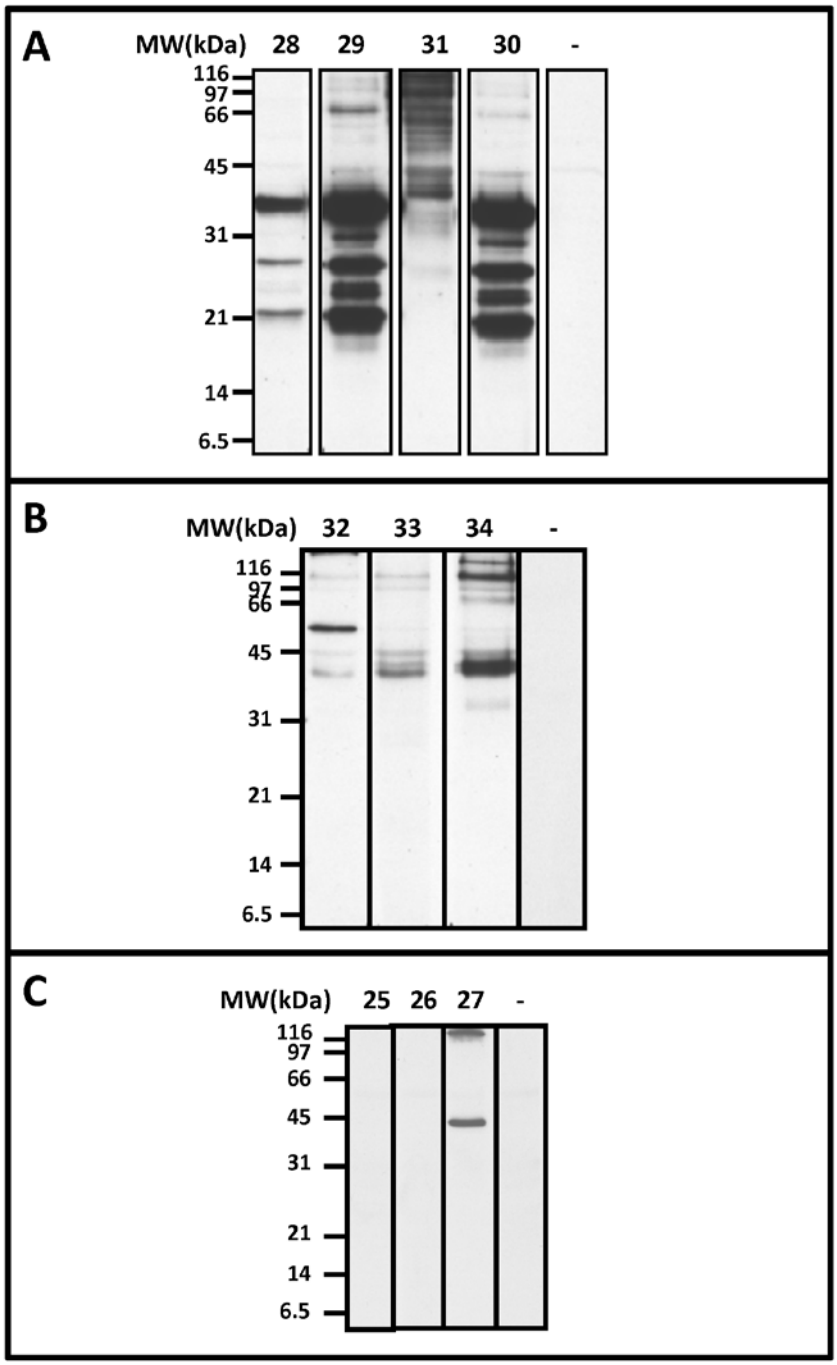

FIGURA 3.

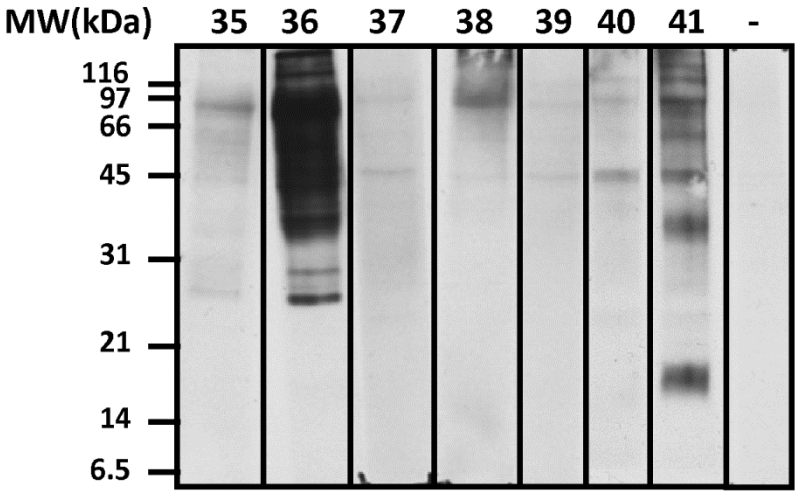

FIGURA 4.