
Seafood hypersensitivity in mite sensitized individuals: is tropomyosin the only responsible allergen?

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Background: Seafood allergy has been related to mite sensitization, mainly mediated by the muscle protein tropomyosin.

Objectives: To determine the correlation between seafood hypersensitivity and mite sensitization (*Dermatophagoides pteronyssinus* and *Chortoglyphus arcuatus*, a highly prevalent storage mite in Spain) and to investigate the implication of tropomyosin in cross-reactivity.

Methods: Patients from Northwest Spain were divided into 2 groups. The mite-seafood group contained 30 allergic mite individuals with a clinical history of food hypersensitivity. The mite group contained 40 individuals with positive skin prick test results to *D pteronyssinus* and *C arcuatus* but negative seafood test results. Specific IgE (sIgE) to whole mite and shrimp extracts, mite tropomyosin (rDer p 10), and shrimp tropomyosin (rPen a 1) were determined in each serum sample. Allergenic profiles were analyzed by immunoblot. Cross-reactivity studies were investigated by enzyme-linked immunosorbent assay and immunoblot inhibition studies.

Results: In the mite-seafood group, 71% of patients had positive sIgE results to shrimp and 55% of them to shrimp tropomyosin. A strong correlation was found between sIgE to shrimp tropomyosin and mite tropomyosin. Positive correlation was observed between sIgE to shrimp tropomyosin and severity of symptoms. In the mite group, none of the 20% of patients with sIgE to shrimp tested positive to shrimp tropomyosin. In the immunoblot inhibition experiment, the shrimp extract was totally inhibited by mite extract. These data suggest that primary sensitization is related to mite sensitization.

Conclusion: Tropomyosin does not seem to be the main allergen involved in mite-seafood sensitization in mite sensitized individuals. High levels of sIgE to tropomyosin seem to be related to severity of symptoms.

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INTRODUCTION

The prevalence of seafood allergy is usually high when consumption of seafood plays a greater part in the diet of an observed community. Previous studies have demonstrated that the major allergen responsible for ingestion-related allergic reactions due to crustacean is the muscle protein tropomyosin.¹ However, other characterized allergens, including a 40-kDa arginine kinase,² a 20-kDa protein identified as a myosin light chain,³ and recently a sarcoplasmatic calcium-binding protein, described in black tiger shrimp⁴ and white shrimp,⁵ can be responsible for allergic sensitization to seafood.

On the other hand, seafood sensitization has also been related to mite sensitization,⁶ mainly mediated by the highly conserved muscle protein tropomyosin. This allergen has been identified as the responsible allergen for cross-reactivity between seafood and mites. The molecular basis for this

cross-reactivity was reviewed by Ayuso et al,⁶ and it is based on the amino acid sequence homology (81%) between mite tropomyosin (Der p 10) and shrimp tropomyosin (Pen a 1). This cross-reactivity has been proposed as the origin of the sensitization to shrimp tropomyosin after mite immunotherapy,⁷ although this de novo sensitization has been questioned in posterior studies.^{8,9} However, group 20 mite allergens (arginine-kinase, Der p 20), also present in decapod crustaceans, have recently been considered as new allergens involved in cross-reactivity between seafood and mites. Group 20 mite allergens have been proposed as probable new pan-allergens,¹⁰ showing a 78% amino acid sequence homology with Pen m 2. Recently, a new 20-kDa allergen present in shrimp and house dust mites (HDMs) has also been suggested as responsible for cross-reactivity between crustaceans and HDMs.¹¹ The authors speculate that this allergen could correspond to the sarcoplasmatic calcium-binding protein and myosin light chain allergens.

Galicia is a region located in Northwest Spain. It is characterized by high exposure to mites, mainly the HDM *Dermatophagoides pteronyssinus* and the storage mite *Chortoglyphus arcuatus*.¹² The percentage of allergic patients sensitized to mites is very high, and it is usual to find patients allergic to mites and with allergic symptoms, mainly oral allergy syndrome but also anaphylaxis, after ingestion of

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seafood. The objectives of the study were to determine the correlation between sensitization to mites (*D pteronyssinus* and *C arcuatus*) and food hypersensitivity to crustaceans and to evaluate the cross-reactivity between shrimp and these mites.

METHODS

Patient Population

The patient population consisted of 70 patients residing in Northwest Spain (Lugo, Galicia) with allergic respiratory symptoms (rhinoconjunctivitis and/or asthma) and with a positive skin prick test result to mites. All of them were clinically studied about seafood allergy and skin prick tested with biologically standardized (10 HEP/mL) *D pteronyssinus* and *C arcuatus*¹³ mite extracts (Laboratorios LETI, Madrid, Spain) and with 2 shrimp extracts (raw and boiled) at 5 mg/mL. The patient population was divided into 2 groups. The mite-seafood group (MS group) consisted of 30 patients (13 females and 17 males; mean [SD] age, 31.4 [15.3] years). All of them had positive skin prick test results to *D pteronyssinus* and *C arcuatus* and a clinical history of food hypersensitivity after seafood (shrimp) ingestion. According to their clinical history and using the grading system proposed by Brown,¹⁴ 12 patients (40%) reported oral allergy syndrome after seafood ingestion, and 18 patients (60%) reported anaphylactic reactions. Eight (26.7%) of them experienced a mild reaction, 7 (23.3%) a moderate reaction, and 3 (10%) a severe reaction. The mite group (M group) consisted of 40 control individuals (19 females and 21 males; mean [SD] age, 31.6 [15.3] years). These patients had positive skin prick test results to *D pteronyssinus* and *C arcuatus* but negative results to shrimp extract, and they did not show allergic symptoms after seafood ingestion.

Wheal sizes were measured by papulometry using the software PC Draft connected to Wacom Graphic tablet and Graphire 2 pencil (Wacom Company Ltd, Tokyo, Japan). The results were expressed in millimeters squared and were considered positive when the reaction was 7 mm² or greater.

A total of 40 serum samples from the M group and 28 from the MS group were obtained and frozen at -20°C. A pool of sera was prepared mixing 16 serum samples from the MS group individuals with detectable (>0.35 kU/L) specific IgE (sIgE) against shrimp and *C arcuatus*. The serum pool was aliquoted and stored at -20°C.

This study was approved by the ethics committee of the hospital, and written informed consent was obtained from all the participants.

Extract Preparation

Shrimp (*Penaeus longirostris*, one of the most commercialized shrimp species in Spain) were bought at a local market and used to prepare raw and boiled extracts. Briefly, whole raw shrimp or shrimp previously boiled in 0.01M phosphate-buffered saline (PBS) for 15 minutes were individually placed in a blender for 3 minutes in 0.01M PBS until the material was totally homogenized. Subsequently, both raw

and boiled extracts were kept under continuous magnetic stirring for 4 hours at 4°C, centrifuged, dialyzed overnight against bidistilled water in 3.5-kDa membranes, sterile filtered, and freeze-dried. The protein content was measured by the Lowry-Biuret method (BioRad Laboratories, Hercules, California).

sIgE Determination

sIgE to *D pteronyssinus*, nDer p 1, rDer p 2, rDer p 10 (mite tropomyosin), shrimp, and recombinant shrimp tropomyosin (rPen a 1) were measured using the UniCAP system (Phadia, Uppsala, Sweden). Specific IgE to *C arcuatus* was determined using the manufactured extract with the UniCAP system. Briefly, *C arcuatus* extract was biotin labeled (Roche Diagnostics, Indianapolis, Indiana) and incubated with streptavidin ImmunoCAPs. After washing, sIgE was measured following the manufacturer's instructions (UniCAPs100; Phadia).

SDS and Immunoblot

The protein profile of raw shrimp and *C arcuatus* extracts were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Shrimp and *C arcuatus* extracts were run in SDS-PAGE gels with 2.67% C, 15% T acrylamide as described by Laemmli.¹⁵ A total of 100 µg of protein was loaded onto the gels and Coomassie stained.

Allergenic profiles of shrimp and *C arcuatus* extracts were studied by immunoblot, using individual serum samples. Briefly, after electrophoresis, gel was electrotransferred to a P-immobilon membrane (Millipore, Bedford, Massachusetts) and dried at room temperature. Thereafter, membranes were incubated overnight with individual serum samples (1:2 dilution) and developed using antihuman IgE (Ingenasa, Madrid, Spain) labeled with horseradish peroxidase. The reaction was visualized by chemiluminescence.

ELISA Inhibition

Plastic microtiter plates (Immulon IV; Dynex Technologies, Chantilly, Virginia) were coated with *C arcuatus* extract (10 µg/mL of protein) and incubated overnight. Several dilutions were made from raw shrimp and *C arcuatus* extract. Each dilution was incubated with the serum pool for 2 hours at room temperature. Afterward, 100 µL was transferred to the shrimp-coated plate and incubated overnight. After washing, 100 µL of peroxidase-labeled anti-human IgE (Ingenasa) was added and allowed to stand for 30 minutes at room temperature. After washing, the plates were developed for 30 minutes and stopped with 1N sulfuric acid.

Immunoblot Inhibition Experiments

Immunoblot inhibition experiments were performed using raw shrimp and *C arcuatus* extracts in solid phase. A total of 100 µg of proteins of each extract was transferred to a nitrocellulose membrane following the procedure previously described. The serum pool was incubated beforehand for 2 hours with *C arcuatus* (in case of shrimp in solid phase) or raw shrimp extract (500 µg/mL) (in case of *C arcuatus* in

solid phase) and then added to the membranes and incubated overnight. After washing, membranes were incubated with anti-human IgE peroxidase (Ingenasa), developed with luminol solution (Immun-Star HRP Chemiluminescent Kit; BioRad), and detected using chemiluminescence (ChemiDoc XRS; BioRad).

Statistical Analysis

The statistical analysis consisted of descriptive and nonparametric statistics. Wheal sizes and sIgE were compared between both groups of patients using the Mann-Whitney test. Differences in each group of patients were calculated using the Mann-Whitney test, Wilcoxon signed rank test, and multiple comparisons for analysis of variance on ranks (Dunn test). Proportions were analyzed using contingency tables, and the Fisher exact test was conducted. $P < .05$ was considered statistically significant.

RESULTS

Comparison Between Groups of Patients

Skin prick tests. Table 1 lists the percentage of individuals with positive skin prick test results and the wheal sizes obtained with the different extracts. Wheal sizes with *C arcuatus* were higher in the MS group than in the M group; the opposite happened with the *D pteronyssinus* extract. Statistically significant differences were not observed in any group.

In the MS group, wheal sizes induced by mites were larger than those induced by shrimp. After comparing shrimp extracts, raw extract elicited larger wheals than those induced by boiled extracts but without significant difference. There was a significant correlation between wheal size induced by raw and boiled shrimp ($P < .001$).

In this group there was an increase in the percentage of patients with positive skin prick test results to shrimp with the severity of symptoms; 75% of the individuals with mild symptoms, 85.7% of those with moderate symptoms, and 100% with severe symptoms had positive skin prick test results to shrimp extract.

Specific IgE. Table 2 gives the mean level and percentage of patients with detectable sIgE (>0.35 kU/L) in both groups. Levels of sIgE to *D pteronyssinus*, Der p 1, Der p 2, and *C arcuatus* were clearly higher in the M group compared with the MS group, with statistically significant differences in the case of *D pteronyssinus* ($P = .005$), Der p 1 ($P = .008$), and

Der p 2 ($P = .007$). In both group of patients, sIgE levels to Der p 2 were statistically significant higher than to Der p 1 (MS group: $P = .003$; M group: $P = .002$). In the M group, a small percentage of patients had detectable sIgE to shrimp, with a mean value statistically lower ($P < .001$) than that obtained in the MS group. Levels of sIgE to shrimp and mite tropomyosin, Der p 10, were only detected in the group of MS patients. sIgE levels to Der p 10 were lower than those obtained in Der p 1 and Der p 2, although the difference was only significant with Der p 2 ($P = .02$). However, it is noteworthy that 13% of the patients have sIgE to Der p 10 but not to Der p 1 or Der p 2. In the MS group, 55% of the patients with sIgE to shrimp had sIgE to shrimp tropomyosin too. A high correlation between shrimp and mite tropomyosin ($P < .001$) was detected. Regarding differences in sIgE levels to *D pteronyssinus* and *C arcuatus* between patients with or without sIgE to rPen a 1, no significant differences were observed ($P > .05$).

Figure 1 shows the sIgE levels to shrimp and shrimp tropomyosin in the MS patients, sorted according to severity of symptoms after ingestion of shrimp. There was a significant difference among sIgE to rPen a 1 in the different groups ($P = .03$), with the level of sIgE to rPen a 1 being statistically higher ($P < .05$) in patients with severe reactions than in those with oral allergy syndrome or mild reactions after seafood ingestion. No significant differences were observed with sIgE to shrimp or mites.

Cross-reactivity Between *C arcuatus* and Shrimp

Antigenic profiles of *C arcuatus* and shrimp extracts. Protein profile of *C arcuatus* and shrimp extracts, analyzed by SDS, and matching between both extracts evidenced the presence of common proteins with the same molecular weight in both extracts (Fig 2). The individual immunoblots (Fig 3) clearly showed a higher and more diverse recognition pattern to *C arcuatus* than to shrimp extract.

ELISA inhibition experiments. The results of the ELISA inhibition experiments showed that the amount necessary to reach the 50% inhibition point using *C arcuatus* extract in the solid phase was $0.237 \mu\text{g}$ for *C arcuatus*, whereas the shrimp extract did not inhibited mite extract, suggesting a lack of cross-reactivity between *C arcuatus* and shrimp, using the serum samples of patients sensitized to both.

Immunoblot inhibition experiments. Figure 4 shows the immunoblot inhibition experiments using *C arcuatus* or

Table 1. Patients With Positive Skin Prick Test Results to Mite and Shrimp Extracts

Extract	Patient population (MS) (n = 30)		Control population (M) (n = 40)	
	Mite and seafood positive, %	Wheal size, mean (SD), mm ²	Mite positive and seafood negative, %	Wheal size, mean (SD), mm ²
Raw shrimp extract	80.0	55.8 (51.6)		
Mite extracts				
<i>Dermatophagoides pteronyssinus</i>	100	125.0 (103.3)	100	143.5 (90.5)
<i>Chortoglyphus arcuatus</i>	100	101.3 (82.9)	100	69.5 (42.5)

Table 2. Patients With Detectable sIgE (>0.35 kU/L) and sIgE Levels

	Patient population (MS) (n = 28)		Control population (M) (n = 40)	
	Mite and seafood positive, %	sIgE level, kU/L	Mite positive and seafood negative, %	sIgE level, mean (SD), kU/L
Shrimp	71.4	5 (12.4)	20	0.2 (0.4)
Shrimp tropomyosin (rPen a 1)	39.3	3.9 (10.3)	0	
<i>Dermatophagoides pteronyssinus</i>	89.3	20.9 (31.8)	100	32.2 (27.8)
<i>Chortoglyphus arcuatus</i>	71.4	3.8 (4.5)	92.5	6.2 (6.7)
Mite tropomyosin (Der p 10)	32 ^a	1.6 (3.9)	0	
Der p 1	39.1 ^a	5.6 (12.1)	77.5	11.8 (17)
Der p 2	47.8 ^a	10.9 (20.2)	85	18.7 (20.7)

Abbreviation: sIgE, specific IgE.

^a Performed in 25 patients.

shrimp extract in solid phase. Only bands with molecular weights lower than 21 kDa were inhibited using *C arcuatus* in solid phase. However, inhibition experiment between *C arcuatus* and shrimp, using shrimp extract in the solid phase, showed a practically total inhibition. Only a weak recognition of a 25 kDa was detected.

DISCUSSION

The relationship between shrimp and HDM allergy is well known. Tropomyosin is the usual suspect protein in mite-crustacean cross-reactivity because it is present in both groups with a high sequence homology.⁶ However, although tropomyosin is an important allergen in crustaceans, it is considered as a minor allergen in HDMs and storage mites.¹⁶ The existence of mite sensitized individuals who do not recognize mite tropomyosin but with adverse reactions after crustacean ingestion suggests the existence of other responsible allergens. Besides, there is little information about the

role of storage mites in these symptoms, although the lack of cross-reactivity between shrimp and storage mites has been described.¹⁷

In the present study, we compared the different sensitization profiles between 2 groups of individuals sensitized to the most abundant mites in Northwest Spain, *D pteronyssinus* and *C arcuatus*. Patients from one of the groups experienced allergic symptoms after seafood ingestion. By contrast, the other group did not experience adverse reactions after crustacean ingestion. Besides, cross-reactivity between *C arcuatus* and shrimp, using serum of patients sensitized and with sIgE to both of them, was also determined. It is worth mentioning that, although no *C arcuatus* allergens have been noted by the International Union of Immunological Societies, the *C arcuatus* tropomyosin has been cloned, and it presents a high sequence homology (94%) with Der p 10.¹⁸

More than 70% of patients with symptoms after crustacean ingestion had sIgE to shrimp, and 55% of them had sIgE to

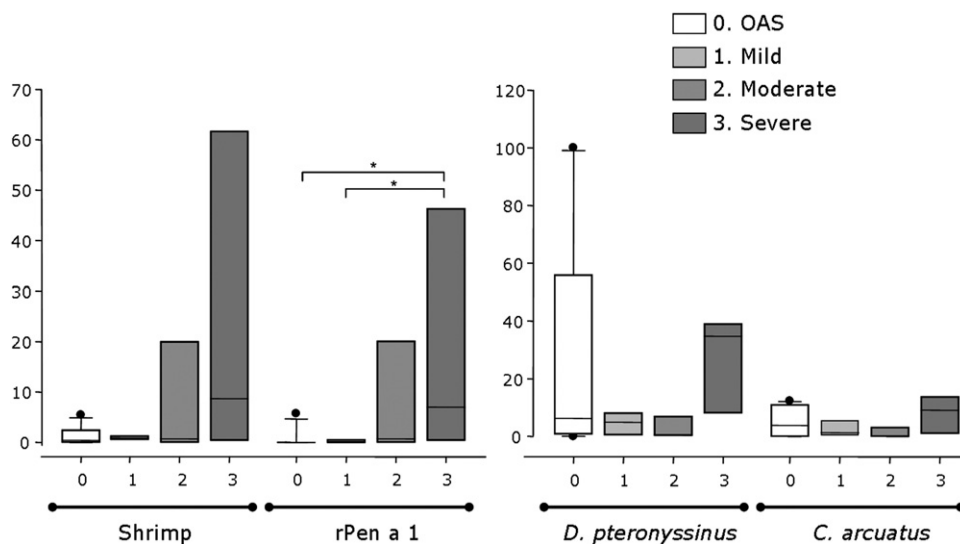


Figure 1. Box plot of specific IgE (ImmunoCAP) against shrimp, tropomyosin (rPen a 1), *Dermatophagoides pteronyssinus*, and *Chortoglyphus arcuatus* in patients in the mite-seafood group sorted according to severity of symptoms after ingestion of shrimp.

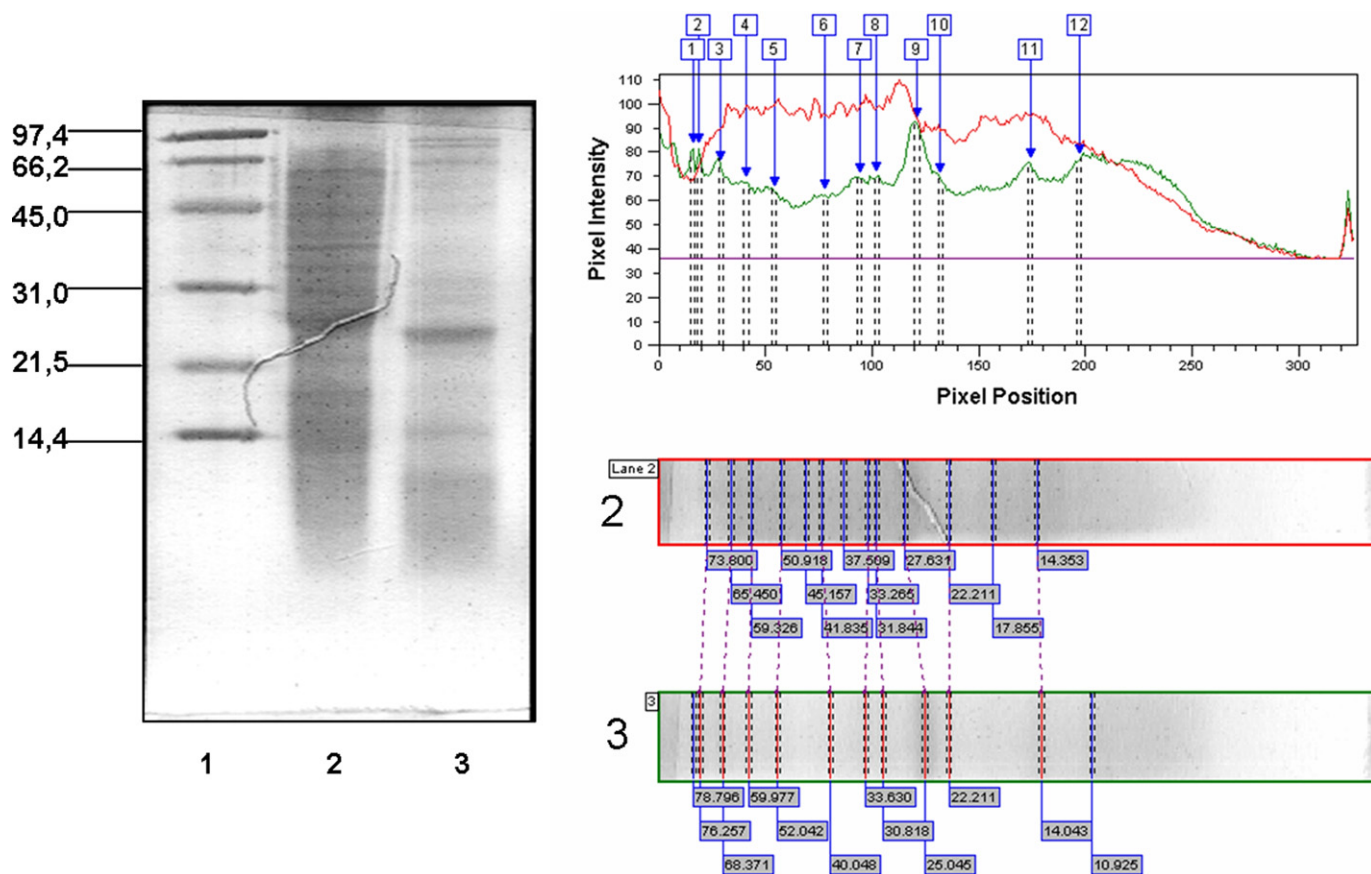


Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and matching of *Chortoglyphus arcuatus* and shrimp extract. Lane 1, standard; lane 2, *C. arcuatus*; lane 3, raw shrimp.

tropomyosin. This percentage is lower than those described in previous published articles,^{19,20} in which 70% to 80% of patients with shrimp allergy had sIgE to shrimp tropomyosin. In the control group, a small percentage of individuals had detectable sIgE to shrimp, although these values were low. No sIgE to raw tropomyosin was observed in this group.

According to our results, sIgE to tropomyosin is a marker of the severity of allergic symptoms after seafood ingestion because the level of sIgE to rPen a 1 was higher in patients with severe reactions than in those with oral allergy syndrome or mild reactions. Regarding the sIgE levels to mites, statistically significant differences were observed between the 2 groups, with higher levels of sIgE to *D. pteronyssinus*, Der p 1, and Der p 2 in control patients (M group) and with sIgE to Der p 10 detected only in patients (30%) with symptoms after crustacean ingestion. A noteworthy finding is that 13% of patients had sIgE to mite tropomyosin but not to Der p 1/Der p 2. Besides, differences in sIgE to the different mite allergens into each group were found, with dominance of sIgE to Der p 2. Similar results were observed in mite allergic patients residing in Northwest Spain.²¹ These results seem to confirm the importance of sensitization to tropomyosin in

patients allergic to mites and crustaceans, related to the severity of symptoms. However, it is also remarkable that a high percentage of mite sensitized patients who have symptoms after shellfish ingestion did not show detectable sIgE to tropomyosin and they did not recognize this allergen in the immunoblot.

Cross-reactivity was investigated by ELISA and immunoblot inhibition experiments. These studies suggest a lack of cross-reactivity between *C. arcuatus* and shrimp, when a pool of serum from patients sensitized to both allergens were used. This finding has been observed in previous studies,¹⁷ in which despite the fact that tropomyosin is found to be a common allergen in mites, there is no cross-reactivity between mites (including *C. arcuatus*) and shrimp. A possible explanation is related to the different tropomyosin concentrations in mite and shrimp extract, with tropomyosin being the dominant protein in shrimp extract because crustaceans are almost entirely muscle, whereas mites have little muscle. In addition, a multivariate statistical analysis of IgE antibody serum concentrations of a high number of sensitized individuals to 89 allergen extracts showed the reactivity to crustaceans was clustered with cockroach but not with mites.²²

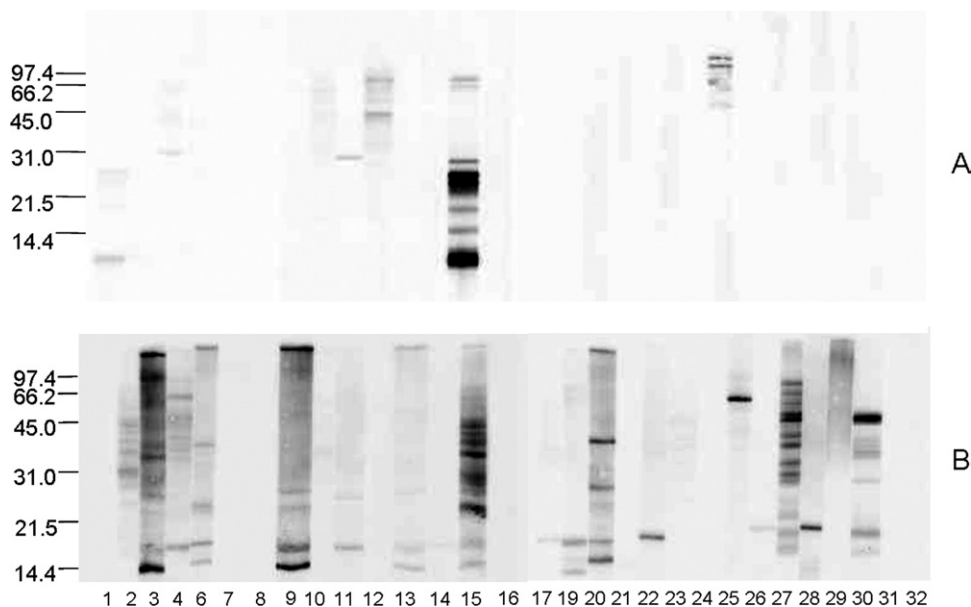


Figure 3. Immunoblot using individual patient's serum. A, Raw shrimp extract; B, *Chortoglyphus arcuatus* extract.

In our study, in a population sensitized and exposed to high levels of mites and with a clinical history of food hypersensitivity after crustacean ingestion, the results (higher wheal size and sIgE levels to mites than those to shrimp extracts) suggest that sensitization to mites is the primary cause of

allergy, with food hypersensitivity being a consequence of cross-reactivity. Similar findings have been described in other places with high exposure to mites, such as Martinique.²³ This fact is confirmed by the allergenic profile of our patients because more than half of the patients had a high and diverse recognition pattern in the mite extract, whereas only a few patients recognize shrimp proteins. Besides, the immunoblot inhibition experiment showed an almost complete inhibition of the shrimp extract by the mite, whereas only mite proteins lower than 21 kDa were inhibited by the crustacean.

However, in the case of the patients sensitized to tropomyosin, the primary sensitization to this protein by mites is not so clear. The theory that the sensitization to shrimp tropomyosin could be due to respiratory allergens, such as mites and/or cockroaches, has been confirmed in non-crustacean-eating patients, such as orthodox Jews.²⁴ However, in our case the primary sensitization to tropomyosin could happen through ingestion of seafood better than through respiratory sensitization to mites. The higher sIgE levels to shrimp tropomyosin than to the mite tropomyosin, detected in some patients, seems to substantiate this supposition.

In summary, and according to our results, tropomyosin does not seem to be the major allergen involved in mite-seafood sensitization in mite sensitized individuals with mild symptoms, mainly oral allergy syndrome, after crustacean ingestion. The implication of other allergens should be further analyzed in detail. However, when mite allergic patients have severe reactions to crustacean intake, tropomyosin can be considered as an important allergen. Regarding the role of the prevalent storage mite *C arcuatus* in cross-reactivity with shrimp, we consider that their importance is clearly lower

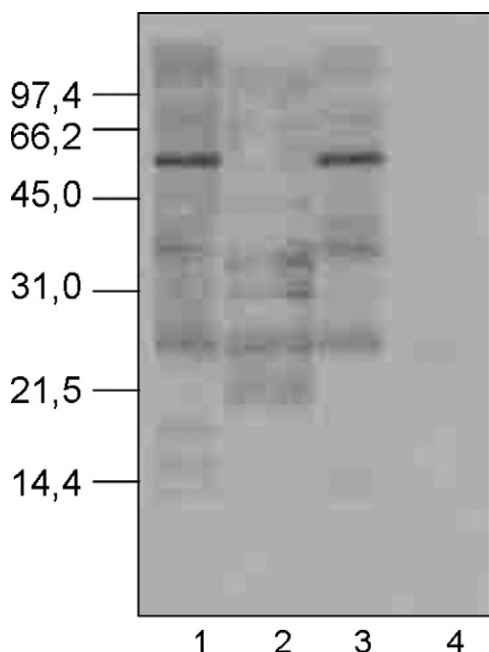


Figure 4. Immunoblot inhibition. Lane 1, Solid-phase *Chortoglyphus arcuatus* (not inhibited); lane 2, solid-phase raw shrimp extract (not inhibited); lane 3, solid-phase *C arcuatus* (inhibited with raw shrimp extract); lane 4, solid-phase raw shrimp extract (inhibited with *C arcuatus*).

than those observed with *D pteronyssinus*. Further studies are needed to clarify the implication of other allergens in the seafood-mite syndrome.

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