

Shrimp Allergy in Italian Adults: A Multicenter Study Showing a High Prevalence of Sensitivity to Novel High Molecular Weight Allergens

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Key Words

Food allergy · Shrimp · Allergens · Cross-reactivity · House dust mite

Abstract

Background: Shrimp is a frequent cause of food allergy worldwide. Besides tropomyosin, several allergens have been described recently. **Objective:** We investigated which allergens are involved in Italian shrimp-allergic adults. **Methods:** Sera from 116 shrimp-allergic patients selected in 14 Italian allergy centers were studied. Skin prick tests with house dust mite (HDM) as well as measurements of IgE to Pen a 1 (shrimp tropomyosin) and whole shrimp extract were performed. All sera underwent shrimp immunoblot analysis, and inhibition experiments using HDM extract as inhibitor were carried out on some Pen a 1-negative sera. **Results:** Im-

munoblots showed much variability. IgE reactivity at about 30 kDa (tropomyosin) was found in <50% of cases, and reactivity at about 67 kDa and >90 kDa was frequent. Further reactivities at 14–18, 25, 43–50, about 60 and about 80 kDa were detected. Most subjects had a history of shrimp-induced systemic symptoms irrespective of the relevant allergen protein. IgE to Pen a 1 were detected in sera from 46 (41%) patients. Skin reactivity to HDM was found in 43/61 (70%) Pen 1-negative subjects and inhibition studies showed that pre-adsorption of sera with HDM extract induced a marked weakening of the signal at >67 kDa. **Conclusions:** Several allergens other than tropomyosin are involved in shrimp allergy in adult Italian patients. Some hitherto not described high molecular weight allergens seem particularly relevant in this population and their cross-reactivity with HDM allergens makes them novel potential panallergens of invertebrates.

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Introduction

Shrimp allergy is one of the most frequent causes of food allergy worldwide, and Italy makes no exception. In recent multicenter studies carried out on an adult population in Italy, shrimp allergy represented the second cause of type 1 food allergy after plant-derived foods [1] and the second cause of food-induced anaphylaxis after lipid transfer protein [2]. Tropomyosin has been the first shrimp allergen detected [3–5] and its cross-reactive nature has led to define it as a panallergen of invertebrates [5–7]. Tropomyosin has been considered the major allergen in shrimp and molluscs and a minor allergen in other invertebrates ever since, and it has been reported that 85% of shrimp-allergic individuals show reactivity to this protein [8]. In recent years, several other shrimp allergens have been detected and characterized, including arginine kinase [9, 10], sarcoplasmic calcium-binding protein [11, 12] and myosin light chain [13], although little is known about the clinical relevance of these allergen proteins, and large population-based studies of shrimp allergy are missing. In the present study many Italian allergy centers participated in the detection and clinical characterization of shrimp-allergic subjects with the aim to investigate which are the shrimp allergens most frequently associated with clinically relevant shrimp allergy in this country.

Patients and Methods

Patients

Fourteen allergy centers scattered throughout Italy took part in this multicenter study. Participating doctors selected all shrimp-allergic outpatients seen during their routine clinical activity from the beginning of June to the end of December 2009 using the following common criteria: (1) clear-cut clinical history of allergic symptoms (including oral allergy syndrome, urticaria with or without angioedema, asthma and/or anaphylaxis) following the ingestion of shrimps and (2) positive skin test using fresh shrimp and/or commercial shrimp extract (ALK-Abello, Madrid, Spain; 1/20 w/vol) as defined by the EAACI criteria [14].

Virtually all shrimp species consumed in Italy are seawater shrimps belonging to the Penaeidae family, including *Aristeus antennatus*, *Parapenaeus longirostris*, *Parapeneopsis cornuta* and *Melicertus kerathurum*. As a consequence, in view of possible allergenic differences with freshwater shrimps [15], fresh shrimps belonging to these seawater species were employed to carry out both in vivo and in vitro tests.

The clinical history of shrimp allergy recorded by individual clinicians was discussed with the coordinator before final approval and inclusion of all patients in the study. These criteria led to the final inclusion of 116 shrimp-allergic patients (56 male, 60 female; mean age 38.0 years, range 12–73 years).

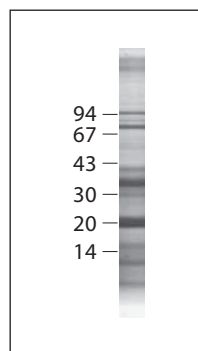


Fig. 1. SDS-PAGE electrophoresis of the shrimp extract used to perform in vitro assays. Multiple protein bands are visible at different molecular weights.

Since many of the participating centers were not sufficiently acquainted with emergency practice or did not have proper facilities to manage systemic allergic reactions, in view of the severity of many of the reported allergies, confirmative oral challenges (either blinded or open) were not performed.

Patients provided informed consent to blood collection; properly coded sampled sera were kept at -20°C until in vitro tests were carried out. Since the study was carried out with diagnostic purposes on patients who presented spontaneously in the clinics for clinical evaluation of their shrimp allergy, no permission by a central ethical committee was required, and the study was approved by the local review boards.

In vivo and in vitro Tests

Within their routine evaluation, most patients underwent skin prick test (SPT) with house dust mite (HDM) extract. Participating centers were left free to perform these skin tests employing the commercial HDM extracts usually employed (ALK-Abello in 8 centers; Lofarma, Milan, Italy in 4 centers; Allergopharma, Reinbeck, Germany in 1 center; Stallergenes, Anthony, France in 1 center).

In 42 patients, IgE to whole shrimp extract were measured by ImmunoCAP (Phadia, Uppsala, Sweden); IgE levels exceeding 0.1 kU/l were considered positive.

Detection of IgE to Pen a 1

IgE to recombinant shrimp tropomyosin, Pen a 1, were measured by ImmunoCAP (Phadia) in sera from all study patients; this analysis was carried out at one participating center (Porde-none). Levels >0.1 kU/l were considered positive.

Immunoblot Analysis

All patients' sera underwent immunoblot analysis at Lofarma Laboratories (Milan, Italy). Raw shrimps were homogenized and extracted (5%) in 0.1 M phosphate-buffered saline (PBS), pH 7.4, under shaking for 2 h at 4°C . The protein content, measured after Bradford [16], was 1.2 mg/ml. The extract was divided into aliquots and stored freeze-dried at -20°C until use. An SDS PAGE of the shrimp extract obtained is shown in figure 1.

Immunoblots were carried out under reducing conditions. The extract was mixed with LDS sample buffer (Nupage Bis-Tris;

Table 1. Clinical presentation of shrimp allergy in 116 Italian adults and main serological findings

	IgE to whole shrimp			IgE to Pen a 1			
	n	median ¹	mean ²	Pen a 1+	median ¹	mean ²	Pen a 1–
OAS	21	1.32 (0.18–36.4)	9.4 (13.3)	8	0.76 (0.18–36.4)	15.5 (24.0)	12 (60%)
Urticaria (total)	75	2.47 (0.1–33.2)	5.52 (7.7)	28	2.09 (0.17–56.2)	7.7 (13.2)	46 (62%)
Rhinitis/asthma	5	8.65 (3.9–68.4)	22.4 (30.9)	4	2.92 (0.77–51.1)	14.4 (24.5)	–
Anaphylaxis	15	3.8 (2.95–53.9)	14.8 (22.0)	7	1.19 (0.58–30.9)	5.5 (11.2)	8 (53%)

OAS = Oral allergy syndrome. IgE levels are expressed as kU/l. The group urticaria includes subjects with urticaria only (n = 55), urticaria + oral allergy syndrome (n = 13) and urticaria + respiratory symptoms (n = 6). These subgroups did not differ significantly (data not shown). Medians and means of IgE to Pen a 1 are related to Pen a 1-positive patients only.

¹ Figures in parentheses are ranges. ² Figures in parentheses are SD.

Novex, Prodotti Gianni, Milan, Italy) and 5% B-mercaptoethanol. The samples were then denaturated by heating at 100°C for 10 min.

Electrophoresis of shrimp extract (25 µg/lane) was carried out in a 10% polyacrilamide precast gel (Nupage Bis-Tris; Novex, Prodotti Gianni) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane according to Towbin et al. [17]. The membrane was saturated with 0.1 mol/l Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with sera (dilution 1:1.5 in saturation buffer). After 3 washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:4,000 in saturation buffer; Biospecific, Emeryville, Calif., USA) and using an ECL Western blotting kit (Amersham, Milan, Italy) as substrate.

Immunoblot inhibition experiments using HDM extract as inhibitor were carried out on sera from 12 patients showing IgE reactivity at a molecular weight other than that of tropomyosin (see the Results section). To this end, HDM extract was prepared as follows.

Dried *Dermatophagoides pteronyssinus* mites were obtained from CSL (Melbourne, Australia). Mites were extracted (4% wt/vol extract) in 0.1 mol/l PBS, pH 7.4 shaking overnight at 4°C. The extract was centrifuged at 2,000 g for 30 min, the supernatant was filtered through a 0.45-µm filter (Whatman, Florham Park, N.J., USA) and stored at –20°C. Protein content was 1.2 mg/ml as determined by the Bradford method.

In immunoblot inhibition experiments the shrimp immunoblot was repeated using sera (1:2 dilution) pre-adsorbed with 600 µg of HDM extract or with 600 µg of bovine serum albumin (BSA) for 4 h at room temperature.

Direct Elisa with HDM Extract

IgE to HDM extract (obtained as described above) were measured in sera from some subjects by direct ELISA using a serum pool from nonatopic individuals as normal control. In these experiments, 2 µg/100 µl of coating buffer (15 mmol/l Na₂CO₃ and 35 mmol/l NaHCO₃, pH 9.6) of HDM extract per well were used in 96-well microtiter plates (Maxisorp Nunc, Roskilde, Denmark) for the coating phase. After washing with 0.1 mol/l PBS, pH 7.4 and 0.05% Tween 20 (Sigma, Milan, Italy), wells were saturated with 2% BSA in PBS (dilution buffer) for 2 h at room temperature.

Subsequently, after further washing 100 µl of sera from normal subjects and patients (diluted 1:3 in dilution buffer) were added to the wells and incubated for 2 h at room temperature. Wells were washed, and bound specific IgE was detected by adding a peroxidase-conjugated anti-human IgE from goat (diluted 1:1,500; Biospecific); developing colorimetric reaction was induced by using tetramethyl-benzidine/H₂O₂ as the substrate.

The enzyme reaction was stopped after 20 min by the addition of 1 mol/l HCl. Absorbance values were read at 450 nm by spectrophotometer. IgE levels were expressed as optical density (OD) × 1,000. Based on the mean + 2 SD of IgE levels found in normal control sera, values <300 OD were considered negative.

Statistical Methods

Specific IgE levels for shrimp and Pen a 1 in the different subgroups of patients were compared by nonparametric means using Mann-Whitney's U test. Probability values <5% were considered statistically significant.

Results

Clinical Presentation of Shrimp Allergy

The clinical presentation of shrimp allergy in the 116 study subjects is shown in table 1. The majority of patients reported systemic symptoms following the ingestion of crustaceans (15 patients experienced anaphylaxis and 75 urticaria/angioedema); 21 patients reported immediate oral itching as the only shrimp-induced symptom, whereas a minority of patients (n = 5) reported uniquely immediate respiratory symptoms following the ingestion of shrimp.

Immunoblot Analysis

Shrimp immunoblot assay scored positive with sera from 62 patients and negative in 54 cases. In positive cases the IgE reactivity pattern showed much variability, with a number of different combinations of reactive

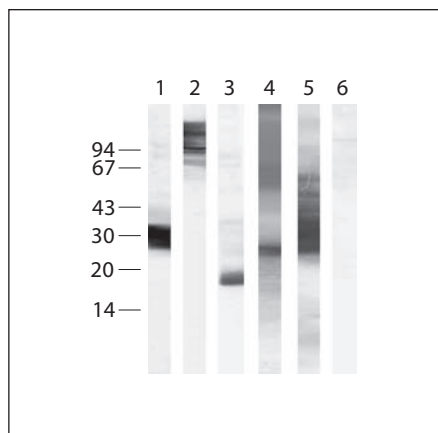


Fig. 2. Shrimp immunoblot of some index sera showing the large array of shrimp allergens recognized by allergic patients. Lane 1: exclusive IgE reactivity at about 30 kDa. Lane 2: IgE reactivity to different high molecular weight proteins in the absence of recognition of low molecular weight allergens. Lane 3: a serum reacting almost exclusively against a protein showing a molecular weight <20 kDa. Lane 4: IgE reactivity against an allergen whose molecular weight ranges between 20 and 30 kDa. Lane 5: multiple IgE reactivity against proteins at different molecular weight ranging from 25 to 60 kDa. Lane 6: weak, single IgE reactivity to a protein showing an apparent molecular weight >90 kDa.

bands. A majority of sera recognized a band at about 30 kDa ($n = 40$) but IgE reactivity at higher molecular weights, at about 67 and >90 kDa, was also frequently observed (in 26 and 34 cases, respectively). Further IgE reactivity was detected at 14–18 kDa ($n = 11$), 25 kDa ($n = 4$), between 43 and 50 kDa ($n = 11$), at about 60 kDa ($n = 13$) and at about 80 kDa ($n = 1$). Some immunoblot assays carried out with representative sera showing reactivity to the different protein bands mentioned above are shown in figure 2.

Detection of IgE to rPen a 1

IgE to Pen a 1 were measured in 113/116 patients, and were detected in sera from 46 (41%) patients. In 37/46 cases the positive finding was associated with a clear-cut IgE reactivity at about 30 kDa on immunoblot assay ($p < 0.001$). In 2 cases Pen a 1 reactivity was associated with IgE reactivity at a molecular weight other than about 30 kDa, whereas in the remaining 7 cases the immunoblot scored negative. On the other hand, 2 sera showing IgE reactivity against a protein band at about 30 kDa on immunoblot did not show any IgE reactivity to rPen a 1. Of 67 sera that did not show any IgE reactivity to rPen a 1, 21 (31%) scored positive on immunoblot and 46 were negative. In 16 of the latter, IgE specific for whole shrimp ex-

tract were measured, scoring positive in 12 cases. With the exception of the few patients reporting uniquely respiratory symptoms following the ingestion of shrimps, the lack of IgE reactivity to rPen a 1 was uniformly distributed in the different subgroups of patients with local and/or different systemic symptoms (table 1).

Association between Clinical Presentation and Pattern of IgE Reactivity

The clinical presentation of shrimp allergy was analyzed also in view of individual IgE reactivity to different allergens on both immunoblot and ImmunoCAP. As a whole, the majority of monosensitized subjects (that is, patients showing a single reactive band on immunoblot) experienced systemic symptoms (urticaria/angioedema or anaphylaxis) irrespective of the molecular mass of the relevant allergen protein, although most cases of isolated respiratory symptoms occurred in tropomyosin-sensitized subjects.

Of 21 patients reporting oral allergy syndrome as the only shrimp-induced symptom, 10 scored negative both on immunoblot and CAP, 8 (38%) were rPen a 1 reactive and 2 were rPen a 1 negative but showed reactivity to other allergens on immunoblot (in both cases at high molecular weight).

Of 15 patients with a history of shrimp-induced anaphylaxis, 7 (47%) reacted to rPen a 1, 4 did not show any IgE reactivity both on immunoblot and on ImmunoCAP, and 4 reacted to allergens other than Pen a 1 on immunoblot (with a molecular weight of >60 kDa in 3 cases, and a molecular weight of 18 kDa in 1 case). Altogether, none of the detected allergens was associated with a specific clinical presentation of shrimp allergy.

The median Pen a 1 IgE levels found in the different subgroups of patients are shown in table 1. Although patients reporting oral allergy syndrome showed a lower median level than subjects with a history of systemic symptoms, no significant difference between the subgroups was found. Similarly, within patients with a history of systemic reactions the higher severity of allergy (that is, anaphylaxis versus urticaria) was not associated with higher levels of IgE to rPen a 1 (table 1).

Skin Reactivity to HDM in Pen a 1-Sensitive and Pen a 1-Negative Subjects

SPT with commercial extract of HDM were carried out in 45 Pen a 1 reactors. Skin tests scored positive in 37/45 (82%) subjects. Of the 8 HDM-negative subjects, 5 were from the only center that used Stallergenes HDM extract (notably, 4 of these patients showed rPen a 1 IgE

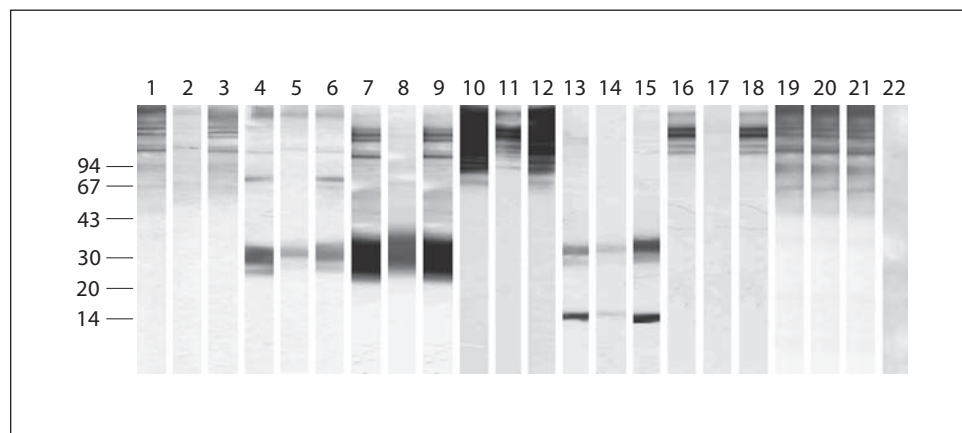


Fig. 3. Some examples of shrimp immunoblot inhibition experiments using HDM as inhibitor. Each of the 7 sera tested appears in 3 consecutive lanes [uninhibited (lanes 1, 4, 7, 10, 13, 16, 19), inhibited with HDM (lanes 2, 5, 8, 11, 14, 17, 20) and inhibited with bovine serum albumin (lanes 3, 6, 9, 12, 15, 18, 21)]. Lane 22 is a normal control serum. Pre-absorption of sera with BSA does not inhibit IgE reactivity to shrimp. Pre-adsorption with HDM causes a marked weakening of reactivity at >94 kDa (lane 1 vs.

lane 2), the disappearance of 67 kDa reactivity (lanes 4–5), a marked weakening of the signal at about 30 kDa and the disappearance at >94 kDa (lanes 7 to 8), a marked weakening at >94 kDa (lanes 10–11), a marked weakening at 14 and 30 kDa (lanes 13–14), the total disappearance of IgE reactivity at high molecular weight (lanes 16–17), and has no effect on serum from another patient (lanes 19–20).

levels in excess of 10 kU/l), whereas the remaining 3 were scattered in 3 different centers and showed weak Pen a 1 reactivity on UniCAP (0.22, 0.28 and 0.93 kU/l).

SPT with commercial extract of HDM were performed in 62/67 Pen a 1-negative subjects. Interestingly, 43/62 (69%) patients showed HDM hypersensitivity. This proportion largely exceeded that of a matched control population of subjects sensitized to lipid transfer protein allergen to plant-derived foods [1/57 (2%); $p < 0.001$]. The sera from 28 of these 43 HDM+/Pen a 1-negative subjects scored negative on immunoblot, whereas the remaining 15 subjects reacted to proteins at 67 kDa ($n = 9$), 94 kDa ($n = 8$), 18–25 kDa ($n = 4$) and 30 kDa ($n = 1$). In view of the observed association between IgE reactivity to shrimp allergens other than tropomyosin and skin reactivity to HDM, some immunoblot inhibition experiments were carried out (fig. 3). Pre-adsorption of sera with HDM extract induced a marked weakening of the signal at >94 kDa in one patient, and the total disappearance of shrimp reactivity between 67 and 94 kDa in another patient. In contrast, no inhibition was observed with sera from two other patients. Altogether, these results suggest cross-reactivity between shrimp and HDM allergens other than tropomyosin. Interestingly, the inhibition exerted by HDM on IgE reactivity to shrimp was directly related to the strength of IgE reactivity to HDM of individual sera on direct ELISA assay (table 2).

SPT to Commercial Shrimp Extract

A total of 100 patients underwent SPT with the commercial shrimp extract used at their respective allergy centers, scoring positive or negative in 81 and 19 cases, respectively (the latter were positive on prick-prick with fresh shrimp only, as stated in the Patients and Methods section).

Both SPT with commercial shrimp extract and measurement of IgE to whole shrimp were performed in 36 patients. The two tests were in keeping in 30 cases (29 positive and 1 negative), whereas in 6 cases they were discordant: ImmunoCAP was positive and SPT negative in 5 cases (IgE levels found were 20, 5.7, 5.21, 3.63 and 0.65 kU/l), whereas in 1 case the SPT was positive and UniCAP negative.

In contrast, immunoblot analysis proved less sensitive than SPT as it scored positive in 28 of 81 SPT-positive patients.

Among patients negative on SPT with commercial shrimp extract, only 2/18 were rPen a 1 reactors and immunoblot analysis was negative in 18/19 cases, the only positive serum showing IgE reactivity at 60 and 94 kDa.

IgE to Whole Shrimp Extract

IgE to whole shrimp extract were measured in sera from 43 patients and scored positive (>0.1 kU/l) in 40 cases. The median IgE level in the whole population stud-

Table 2. IgE reactivity to HDM in a direct ELISA of some sera employed to perform immunoblot inhibition studies

Patient No.	Corresponding lanes in figure 3	OD
28	7–9	3,315
20	16–18	2,041
73	19–21	735
74	4–6	3,423
113	1–3	3,377

OD = Optical density $\times 1,000$. Based on the mean + 2 SD of IgE levels found in normal control, sera values <300 OD are considered negative. The level of IgE reactivity to mite is related to the inhibition of shrimp reactivity (fig. 3).

ied was 3.63 kU/l (range <0.1–68.4 kU/l), and the median levels found in the different subgroups of patients are shown in table 1. Although patients reporting oral allergy syndrome showed a lower median level of IgE to whole shrimp than subjects with a history of systemic symptoms, and the highest median IgE levels were observed in those with a history of anaphylaxis or respiratory symptoms, the difference between the single subgroups did not reach statistical significance (table 1). Also the difference between subjects with oral allergy syndrome and all patients with a history of systemic symptoms was not statistically significant.

IgE to both whole shrimp extract and to rPen a 1 were measured in 40 patients (table 3). Discrepancies between the two assays were observed in 16/40 cases, in keeping with sera reactivity to allergens other than r Pen a 1 seen on immunoblot. The immunoblot analysis scored negative in 19/40; in most cases negative results were associated with low levels of IgE to Pen a 1 or to whole shrimp, thus confirming that immunoblot is less sensitive than specific IgE measurements by ELISA.

Discussion

The present clinical and serological study performed on more than 100 shrimp-allergic subjects seen in 14 Italian allergy centers produced a number of unexpected results that are worth discussing.

First, less than half the patients seemingly reacted to tropomyosin, which is commonly considered the major shrimp allergen. Thus, the prevalence of tropomyosin hypersensitivity in this population is well below that reported in previous studies [8]. The possibility that some

Table 3. Levels of IgE to whole shrimp extract, and to r Pen a 1 and immunoblot assay results in 40 shrimp-allergic patients

Shrimp	Pen a 1	Immunoblot
0.18	0	negative
12.80	3.92	14 kDa, about 30 kDa, and between 43 and 67 kDa
68.40	51.1	about 30 kDa
1.71	0.63	<20 kDa, about 30 kDa and 50 kDa
53.90	30.9	25 kDa and smear between 30 and 97 kDa
3.80	0.75	about 30 kDa
0.53	0.28	about 30 kDa and 94 kDa
9.95	0.04	18 kDa
1.08	0	negative
0.60	0	negative
4.50	0.77	about 30 kDa
0.41	0.03	negative
0.71	0	negative
1.61	0.07	negative
12.00	3.92	>30 kDa and weakly at 67 kDa
0.95	0	negative
1.69	0	weak at 67 kDa
1.21	0.05	negative
3.63	0.93	negative
3.40	1.13	about 30 kDa
2.95	1.19	about 30 kDa, and between 67 and >94 kDa
13.20	0.75	20 kDa, 30 kDa and from 40 kDa onwards
20.00	0.14	negative
0	0	negative
14.80	0.55	about 30 kDa, 60 kDa and 80 kDa
1.82	0	60 kDa and 94 kDa
5.84	0.35	negative
0	0	negative
3.12	0	20 kDa
9.39	4.67	about 30 kDa
0.65	0	negative
7.88	0	about 30 kDa
5.21	0.05	negative
0.54	0	negative
0.48	0	negative
5.54	0.18	negative
20.50	0	smear from 94 kDa onwards
8.05	0	smear from 67 kDa onwards
0	0	negative
33.20	14.9	about 30 kDa

The left column shows IgE levels to whole shrimp extract; levels are expressed in kU/l (positive if >0.1). The middle column shows IgE levels to rPen a 1 (positive if > 0.1 kU/l). The right column shows the findings on immunoblot.

tropomyosin reactors were lost cannot be ruled out; however, this seems unlikely as the detection of IgE to rPen a 1 is probably the most sensitive means presently available to detect tropomyosin allergy, and reactivity at about 30 kDa on immunoblot was seldom observed in Pen a 1-neg-

ative subjects. The possibility that, as an allergen, rPen a 1 is less sensitive than homologous proteins from different sources, such as rPen m 1 or rDer p 10, due to little epitopic differences, is very unlikely as the differences in aminoacid sequences are minimal. In effect, in a preliminary (unpublished) study, two of us (R.A. and D.V.) measured IgE to several recombinant tropomyosins (Pen a 1, Pen i 1, Pen m 1, Der p 10, Bla g 8, Ani s 3) by a commercial ISAC microarray in sera from 7 shrimp-allergic patients, and all sera but one (that did not react to Pen i 1) reacted to all homologous allergens.

Another interesting aspect regards IgE reactivity to shrimp allergens other than tropomyosin. Recently, several new shrimp allergens have been detected, including arginine kinase (Pen m 2, Lit v 2; 40 kDa) [9, 10], sarcoplasmic calcium-binding protein (Lit v 4; 20 kDa) [11, 12] and myosin light chain (Lit v 3; 20 kDa) [13]. By comparing the molecular weight of these allergens with those recognized by our patients on immunoblot analyses, it is possible to conclude that a proportion of our Pen a 1-negative patients was most probably reactive to AK, SCB or MLC. However, these were a minority, and this is one main finding of the present multicenter study. This is in keeping with the observation that some of the shrimp allergens mentioned above, namely sarcoplasmic calcium-binding protein and myosin light chain, seem to play a more relevant role in the pediatric population [14, 15], whereas all our patients were adults. However, in other adult populations the prevalence of sensitization to myosin light chain, arginine kinase or sarcoplasmic calcium-binding proteins largely exceeded that found in our patients [18]. It seems therefore possible to conclude that in Italian shrimp-allergic adults the relevance of the allergens mentioned above, namely AK, SCB and MLC, is limited, and most patients react against hitherto not described shrimp allergens showing higher molecular weights.

Similarly to Pen a 1-positive patients, our Pen a 1-negative patients showed a very high prevalence of sensitivity to HDM on SPT, which suggests that invertebrates probably also share allergen proteins other than tropomyosin. This observation confirms recent findings that some of the many HDM allergens described so far [19] are potentially cross-reacting to homologous allergens in crustaceans. One of these is arginine kinase (Der p 20) [20]; moreover, the cross-reactivity between shrimp myosin light chain, which also shows a high similarity to the cockroach MLC, Bla g 8, [13], and a HDM allergen has been recently observed by some of us [21]. The observation of cross-reactivity between high molecular weight

allergens in shrimp and HDM is a novel one. Some mite allergens show molecular weights that are very close to those of the high molecular weight proteins recognized in shrimp by a large number of our patients. One of these is paramyosin (Der p 11), a protein involved in muscle contraction in invertebrates that shows a molecular weight of about 90 kDa. Further, a pair of chitinases (Der p 15 and Der p 18) show a molecular weight of about 60 kDa, and in its heavily glycosylated form, Der p 15 may show an apparent molecular mass of >95 kDa [19]. All these proteins are reportedly recognized by a significant proportion of humans allergic to HDM [19]. Our inhibition studies showed cross-reactivity between shrimp and mites allergens with molecular weights >60 kDa that seem to correspond to some of the proteins mentioned above.

We did not carry out confirmative oral challenges in our patients, but it is rather unlikely that this may have somehow biased the study population because all had a clear-cut history of shrimp allergy and positive SPT, and previous studies showed that in shrimp-allergic patients a positive SPT is strongly associated with a positive challenge [22]. In 47 patients the culprit allergen could not be identified as their sera did not react to rPen a 1 and scored negative on immunoblot analysis as well. Nonetheless, 14/17 showed IgE reactivity to whole shrimp extract on ImmunoCAP, leaving 33 patients in whom the diagnosis of shrimp allergy was supported by clinical history and positive skin tests with either fresh shrimp and/or commercial extract only. As most of these 33 patients had experienced systemic reactions following the ingestion of shrimp [4 had a history of anaphylaxis, 24 of urticaria (associated with respiratory symptoms in 6 cases), and only 5 of oral allergy syndrome], it seems unlikely that they were not allergic to shrimp. A reduced sensitivity of in vitro tests in shrimp-allergic patients sensitized to allergens other than Pen a 1 seems a more likely explanation for these findings [23].

In conclusion, this study shows that Italian adult shrimp-allergic subjects react to a large spectrum of allergens, that tropomyosin is the relevant allergen in a minority of patients and that a large proportion of subjects react to hitherto not identified, high molecular weight allergen proteins that are probably homologous to HDM allergens. The negative results obtained with some commercial shrimp extracts for SPT suggest that the use of fresh material by the prick-prick test is warranted to exclude shrimp allergy.

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