
Occupational asthma in a seafood restaurant worker: cross-reactivity of shrimp and scallops

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Background: The case of a restaurant seafood handler with IgE-mediated occupational asthma and contact urticaria to both shrimp and scallops is presented. Independent hypersensitivity to both seafoods was demonstrated by skin testing, inhalation challenge, and immunoassays. Bronchial challenge with extracts of shrimp and scallops each produced an isolated early asthmatic response.

Objective: To investigate cross-reactivity of shrimp (phylum Arthropoda) and scallops (phylum Mollusca).

Methods: Shrimp and scallops extracts were prepared from raw seafood and seafood boiling water. Distillate was collected over boiling shrimp. Specific-IgE ELISA and immunoblot assays were accomplished for shrimp and scallops extracts inhibited by each other.

Results: SDS-PAGE of shrimp boiling water and distillate showed similar protein patterns. SDS-PAGE and immunoblot demonstrated prominent protein allergens for shrimp boiling water at 21, 26, and 35 to 38 kD; for raw shrimp at 26 and 38 kD; for scallops boiling water at 20, 35 to 39 and 42 kD; and for raw scallops at 36 to 38 and 41 kD. Significant inhibition of the 35 to 39-kD band of each shrimp and scallops extract was demonstrated on immunoblot inhibition by seafood of the opposite phylum. IgE ELISA inhibition demonstrated 17% to 28% inhibition of shrimp by scallops and scallops by shrimp.

Conclusions: Seafood allergens aerosolized during food preparation are a source of potential respiratory and contact allergens. Shrimp and scallops demonstrate significant cross-reactivity. These findings confirm that the primary cross-reactive allergen of shrimp (phylum Arthropoda) and scallops (phylum Mollusca) is the 35 to 39 kD heat-stable allergen, previously demonstrated to be muscle topomyosin in both phyla.

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INTRODUCTION

Lehrer¹ has demonstrated both clinical and laboratory evidence of significant cross-reactivity among crustaceans

(shrimp, crab, lobster, and crawfish) in Gulf Coast residents. Cross-reactivity of shrimp with mollusks is less extensively studied. We present the case of a restaurant seafood handler with IgE-mediated occupational asthma and contact urticaria to both shrimp and scallops. Using this seafood handler's hypersensitive sera in specific-IgE immunoblot inhibition assays and in enzyme-linked immunosorbent assay (ELISA) inhibition assays, we demonstrated cross-reactivity of shrimp (phylum Arthropoda) and scallops (phylum Mollusca). Immunoblot inhibition assays identified the primary cross-reactive allergen in scallops and shrimp at 35 to 39 kD, a protein previously identified in shrimp as the muscle protein tropomyosin, the major shrimp allergen.^{2–4}

METHODS

Preparation of Extracts

Extracts were prepared, lyophilized, assayed for protein content, and stored as previously described for other antigens.⁵ In brief, seafood extracts were prepared from raw, cleaned shrimp and scallops obtained from our patient's restaurant of employment. Raw seafood was extracted overnight at 4°C in distilled water at a ratio of 1:10, weight per volume. Six additional extracts were prepared to investigate the physical distribution of shrimp antigens and their modification during boiling. Separate extracts of isolated raw shrimp shell, raw shrimp meat, and cooked shrimp meat were prepared by cold extraction overnight at 4°C in the same manner as for whole raw shrimp. Two extracts at boiling temperature were prepared in a distillation apparatus. Raw shrimp at a 1:10 wt/vol in distilled water was boiled for 10 minutes, 50 mL of clear aromatic distillate was collected, and the remaining boiling water was separated and saved. A boiling water extract of scallops was similarly prepared without distillate collection. All extracts (except for the distillate) were filtered, dialyzed, lyophilized, quantitated for protein content, and stored at 4°C.

Specific-IgE ELISA Inhibition

Specific-IgE ELISA inhibition assays were accomplished as previously described for other antigens.⁵ In brief, 96-well plates were coated with 300 µg/mL of one of the four extracts: raw shrimp, shrimp boiling water, raw scallops, or scallops boiling water. Our patient's serum was pre-incubated overnight with 1, 10, or 100 µg/mL of each of the four seafood extracts; with similar concentrations of rye grass extract, or without an inhibitor. Each se-

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rum sample was then assayed for specific-IgE against each of the four seafood extracts using a monoclonal assay for human IgE.

Specific-IgE Immunoblots and Immunoblot Inhibition

Specific-IgE immunoblotting and immunoblot inhibition was accomplished as previously described for other antigens.⁵ The proteins of each extract were separated by molecular weight using the sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) technique. Gels were stained with Commassie Blue to reveal protein bands. Immunoblots were prepared by electrophoretic transfer of proteins from SDS-PAGE gels to nitrocellulose membranes. Each nitrocellulose membrane, containing proteins of a particular seafood extract, was cut into 4 to 5 mm-wide strips. These strips then were overlaid with patient sera and the seafood allergens which bound patient IgE were identified by anti-human IgE monoclonal antibody detection. Immunoblot inhibition studies were carried out in a similar fashion, but with patient serum preincubated with 100 $\mu\text{g/mL}$ of each of the four seafood extracts (shrimp or scallops, raw or boiling water), 100 $\mu\text{g/mL}$ of rye grass extract, or without an inhibitor. Control immunoblots were performed during each experiment using a non-atopic human sera.

THE CASE

The patient was a previously non-atopic, non-asthmatic 22-year-old black female who developed contact urticaria and occupational asthma during the initial 9 months of employment in the kitchen of a local seafood restaurant. The patient had no prior history of urticaria, asthma, food allergy, eczema, or allergic rhinitis. She was in good health, did not smoke, and ingested shrimp, scallops, and other seafood without adverse reaction. After the first 3 months of preparing raw seafoods before cooking, she noted linear urticaria of the hands and forearms whenever she handled raw shrimp or scallops. Using gloves prevented the urticaria.

As much as 50% of her daily work-time was spent within 1 to 2 meters

of the large seafood boiling pots within the kitchen. After 9 months of employment, she developed wheezing and shortness of breath on workdays, beginning shortly after the seafood cooking pots had begun to boil each morning. She never wheezed on days away from work. Between 9 and 12 months, she presented three times to the emergency department with wheezing and was treated for her acute symptoms.

Her physical examination on a non-workday was unremarkable, except for hyperpigmented linear excoriations on her forearms. Prick skin tests were positive to shrimp, crab, lobster, and oyster; but negative to a panel of 43 aeroallergens as well as halibut, salmon, cod, sole, milk, and peanut. An ice cube test was negative. Total IgE was 163 IU/mL. Commercial RAST was positive (class 3 or 4) to shrimp, crab, and lobster; but negative (class 0, 0/1) to egg, milk, wheat, soy, cod, and tuna. Baseline spirometry were normal on non-workdays. Histamine challenge was positive at 0.1 mg/mL, which provoked a 23% decrease in FEV_1 .

Titration prick skin tests to both shrimp and scallops extracts were positive at 1 μg of protein/mL for each boiling water extract and 10 μg of protein/mL for each raw seafood extract. Parallel skin testing of two non-atopic controls was negative, while one of two atopic controls had a positive skin test to shrimp boiling water at 1000 μg of protein/mL. Progressive antigen inhalation challenges to shrimp and scallops boiling water extracts were accomplished on separate days employing 1, 10, and 100 μg of protein/mL extracts. Spirometry was accomplished 2 minutes after each sequential tidal inhalation from an acorn nebulizer for 1, 2, and 2 minutes. The patient's FEV_1 decreased by more than 20% at 100 μg of protein/mL after a total of 3 minutes of shrimp and after 1 minute of scallops. After reversal to baseline with beta agonists, subsequent pulmonary functions at 2 and 6 hours remained normal after each challenge. An atopic control similarly challenged demonstrated no change in pulmonary

functions to 10,000 μg of protein/mL. The patient's challenge with saline control was negative.

Using a peak flow meter at work, the patient recorded an average 21% drop in peak flow several hours after starting work, with recovery by late evening. Initiation of treatment with cromolyn sodium administered four times a day resulted in good control of symptoms. Ultimately, the patient terminated her employment in the seafood restaurant and experience complete remission of her bronchospasm and urticaria. Seven weeks after terminating employment, the patient tolerated a 10-fold greater concentration (1 mg/mL) in the pulmonary histamine challenge.

In both shrimp and scallops specific-IgE ELISA assays, the patient's sera produced a 50 to 100 times greater optical density than nonatopic controls and a 4 to 20 times greater optical density than an atopic pool control (unselected for seafood hypersensitivity).

RESULTS

Specific-IgE Immunoblots and Immunoblot Inhibition

The proteins of the shrimp and scallops raw extracts as well as both boiling water extracts are displayed in the SDS-PAGE of Figure 1. The prominent protein staining bands of shrimp boiling water are seen at 21, 26, and 35 to 38 kD; for raw shrimp at 26, 38, and 40 kD; for scallops boiling water at 35 to 39 and 42 kD; and for raw scallops at 22, 36 to 38 and 41 kD. A major protein staining bands is present at 35 to 39 kD in each extract.

The SDS-PAGE of the boiling water, shrimp distillate, and cooked meat extracts showed similar protein patterns (Fig 2). The SDS-PAGE of the whole raw shrimp, raw meat, and shell extracts were qualitatively similar; however, the shell extract contained little total protein, probably representing shrimp meat adherent to the shells. Not surprisingly, no shell-specific antigens were detected by SDS-PAGE or immunoblot.

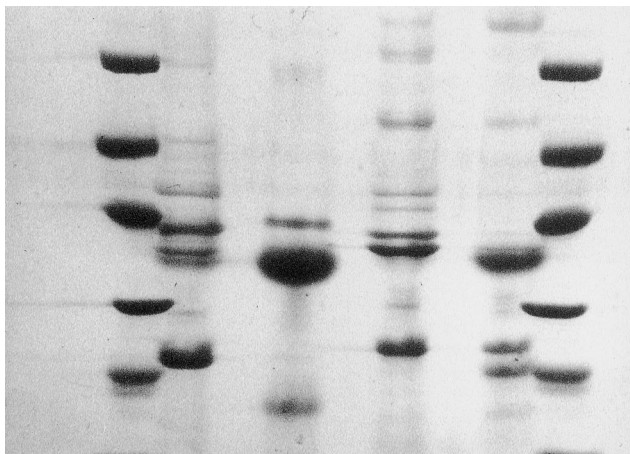


Figure 1. SDS-PAGE of scallops and shrimp extracts. Sample lanes: 1. raw scallops, 2. scallops boiling water, 3. raw whole shrimp, and 4. shrimp boiling water. Controls (top to bottom): 94 kD, 67 kD, 43 kD, 30 kD, 20.1 kD, and 14.4 kD. Each shrimp and scallops extract contains a major protein staining band at 35 to 39 kD.

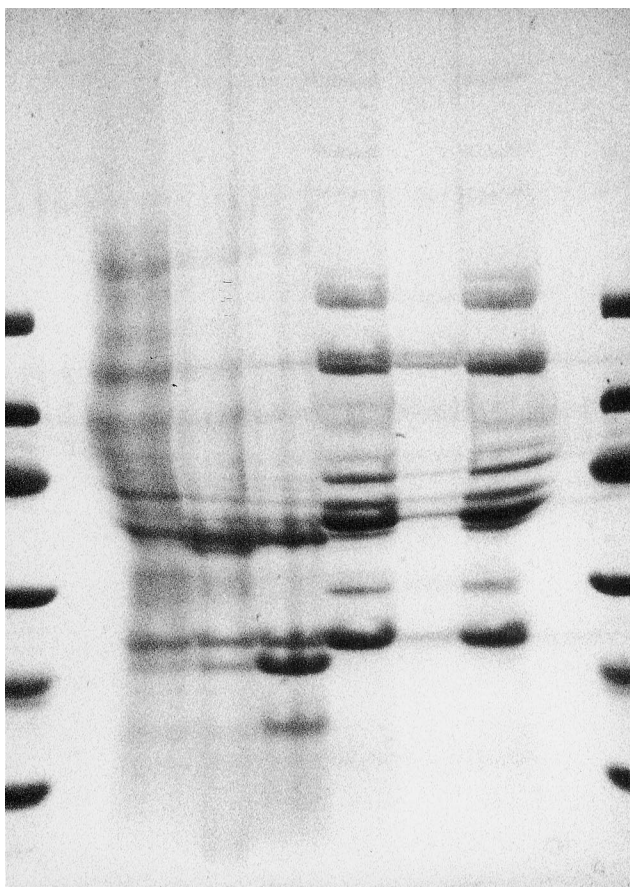


Figure 2. SDS-PAGE of shrimp extracts. Sample lanes: 1. distillate collected above boiling shrimp, 2. boiling water, 3. cooked shrimp meat, 4. raw shrimp meat, 5. raw shrimp shell, and 6. raw whole shrimp. Controls (top to bottom): 94 kD, 67 kD, 43 kD, 30 kD, 20.1 kD, and 14.4 kD. The distillate contains the same proteins as the other heat extracted preparations (2 and 3).

The shrimp immunoblot and immunoblot inhibitions using our seafood restaurant worker's sera are shown in Figure 3. The uninhibited immunoblot demonstrates shrimp boiling water allergens at 19, 21, 26, 30, and 35 to 38 kD while raw shrimp allergens are seen at 26 and 38 kD. The immunoblot of each extract was completely inhibited by itself and by the alternate shrimp extract (data not shown).

Both shrimp immunoblots inhibited by scallops boiling water and raw scallops extract were inhibited more by the scallops boiling water extract than by the raw scallops. For both shrimp immunoblots the 35 to 39 kD bands demonstrated significant inhibition by scallops and lesser inhibition of the 21, 26 kD, and other shrimp bands. The rye grass controls demonstrated no inhibition.

The scallop's immunoblot and immunoblot inhibitions are shown in Figure 4. The uninhibited immunoblot demonstrates scallops boiling water allergens at 20, 35 to 39, and 42 kD while raw scallops allergens are seen at 36 to 38, 41, and 94 kD as well as several high molecular weight bands. The immunoblot of each extract was completely inhibited by itself and by the alternate scallops extract (data not shown).

Both scallop's immunoblots were inhibited to a similar degree by shrimp boiling water and raw shrimp extracts. Both shrimp boiling water and raw shrimp extracts inhibited the 35 to 39 kD bands of each scallops immunoblot. Of secondary note, the raw scallops 36 to 38 kD band was slightly less inhibited by shrimp than was the scallops boiling water 35 to 39 kD band. The rye grass controls demonstrated no inhibition.

Specific-IgE ELISA Inhibition

At inhibitor concentrations of 100 μ g/mL, the shrimp boiling water extract ELISA demonstrated complete inhibition by both shrimp extracts, while scallops boiling water inhibited the shrimp ELISA by 17% and raw scallops and rye did not inhibit the ELISA (Table 1). The raw shrimp ELISA

demonstrated similar complete inhibition by both shrimp extracts. Raw scallops and scallops boiling water extracts inhibited the raw shrimp ELISA by 11% and 22%, respectively. These differential inhibitions by scallops were in agreement with the immunoblot result, in which shrimp inhibition by scallops boiling water extract was greater than inhibition by raw scallops. Inhibition by the smaller 10 and 1 $\mu\text{g/mL}$ concentrations of each inhibitor produced a typical inhibition curve with lesser degrees of inhibition (data not shown).

Scallop's inhibition experiments mirrored the shrimp results. At 100 $\mu\text{g/mL}$, scallops extracts inhibited each other by 92% to 99%. Raw shrimp inhibited the scallops boiling water ELISA by 19% and the raw scallops ELISA by 23%, while shrimp boiling water inhibited the scallops ELISA by 26% and 28%, respectively. Rye inhibited neither assay.

Overall each seafood inhibited itself almost completely, while the seafood of the other phylum produced up to 28% inhibition of the opposite seafood. The results suggest an overall cross-reactivity between boiled shrimp and boiled scallops in the range of 17% to 28%.

DISCUSSION

Patel and Cockcroft⁶ reported the case of a chef who developed allergy to ingested crustaceas, followed 7 years later by onset of occupational asthma when baking lobster. Lobster extract bronchial challenge produced an isolated early asthmatic response. Lemi re et al.⁷ reported the case of an atopic fishmonger who developed contact urticaria, allergic rhinitis, occupational asthma, and ultimately oral/gastrointestinal allergy to lobster and shrimp prepared in her shop. Lobster and shrimp extract bronchial challenges produced isolated early asthmatic responses. Nagano et al.⁸ have reported isolated shrimp contact urticaria. Desjardins et al.⁹ reported occupational asthma to shrimp in 2% and to clam in 4% of seafood processing workers.

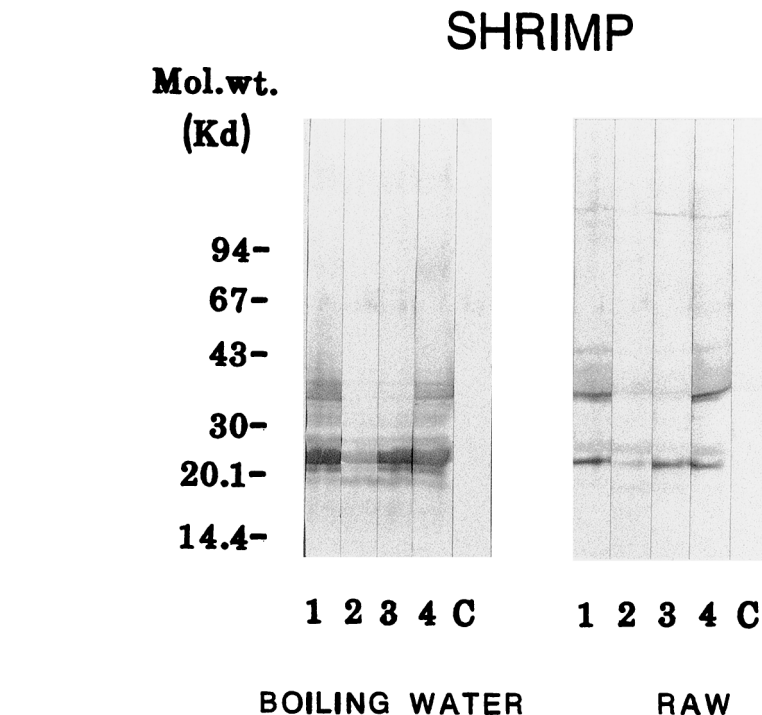


Figure 3. Shrimp s-IgE immunoblot inhibition. Serum from the food handler with shrimp and scallops occupational asthma. Sample lanes: 1. uninhibited, 2. inhibited by scallops boiling water, 3. inhibited by raw scallops, 4. inhibited by rye grass, and 5. control immunoblot with non-atopic human serum. The 35- to 39-kD bands in both raw and shrimp boiling water extracts were inhibited by the scallops extracts. The inhibition of the 21 and 26-kD shrimp bands differed for the two scallops extracts.

Our patient developed contact urticaria and occupational asthma to both shrimp and scallops encountered while working in a seafood restaurant kitchen. Similar to the two case reports above, she demonstrated an isolated early asthmatic response upon bronchial challenge with the offending seafoods (shrimp and scallops). Unlike the two cases noted above, she never manifested allergy to ingestion of either seafood. Nonatopic and a nonsmoker before her occupational exposure, our patient was an exception to the report of McSharry et al.¹⁰ that nonatopic nonsmoking seafood handlers are unlikely to become sensitized to shrimp. Preparing seafood while working near boiling vats of seafood, our patient most likely was exposed to high concentrations of shrimp and scallops allergen aerosols.⁹ Similar to the previous demonstration by Lehrer et al.¹¹ we found that that shrimp boiling water and boiled

shrimp meat have similar allergenic activity. Our examination of shrimp allergens in boiling water distillates demonstrated that a full complement of allergens is aerosolized above boiling seafood. Water-soluble antigens of cooking shrimp are aerosolized in approximate proportion to their presence in the boiling water. Nebulization of boiling water extracts should have closely reproduced our seafood restaurant worker's occupational exposure during bronchial challenge.

The occupational nature of her asthma was demonstrated by decreased peak flow measurements at work, as well as by (1) the resolution of symptoms on non-workdays, (2) positive inhalation challenges to shrimp and scallops, and (3) resolution of asthma and decreased nonspecific bronchial hyper-sensitivity following job termination. Protection from direct contact with seafood at work by the use of gloves

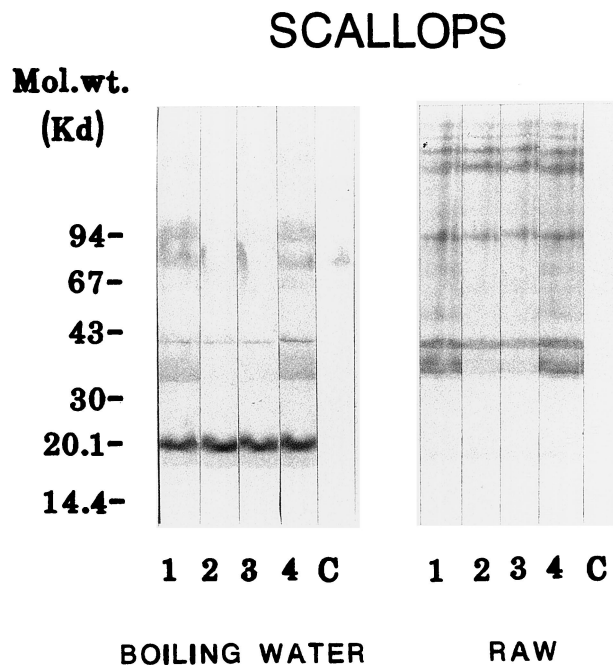


Figure 4. Scallops s-IgE immunoblot inhibition. Serum from the food handler with shrimp and scallops occupational asthma. Sample lanes: 1. uninhibited, 2. inhibited by shrimp boiling water, 3. inhibited by raw shrimp, 4. inhibited by rye grass, and 5. control immunoblot with non-atopic human serum. The 35- to 39-kD bands in both raw and scallops boiling water extracts were inhibited by the shrimp extracts.

Table 1. ELISA inhibition by 100 μ g of protein/mL inhibitor concentrations

Inhibitor	ELISA assay			
	Shrimp Boiling Water	Shrimp Raw	Scallops Boiling Water	Scallops Raw
Shrimp boiling water	100%	100%	26%	28%
Shrimp raw	100%	100%	19%	23%
Scallops boiling water	17%	22%	99%	96%
Scallops raw	0%	11%	92%	96%

eliminated her urticaria but did nothing to protect against the development of asthma. Her asthma symptoms responded promptly to treatment with cromolyn sodium and ultimately to an occupational change.

While our patient's dual sensitivity to shrimp and scallops might have reflected a primary sensitivity to one seafood with secondary limited cross-reactivity between shrimp and scallops, instead our patient demonstrated inde-

pendent, broad IgE hypersensitivity to multiple allergens in both seafoods by immunoblot. Independent hypersensitivity was corroborated by similar intensities of response to both shrimp and scallops skin testing, inhalation challenge, and specific-IgE ELISA; as well as by similar degrees of reciprocal inhibition on shrimp and scallops IgE ELISA and immunoblot. Our patient's dual sensitivity provided an opportunity to investigate the extent to which

shrimp and scallops allergens are cross-reactive.

Shrimp allergens have been extensively investigated. In 1981, Hoffman et al.¹² isolated two shrimp allergens by gel filtration: a 38-kD, heat stable, self-agglutinating major allergen and a 21-kD minor allergen which existed primarily as a 45-kD dimer. Nagpal et al.¹³ later described a heat-stable 34-kD shrimp antigen obtained by ion-exchange chromatography. Hoffman's 38-kD and Nagpal's 34-kD major allergens isolated by different physical methods, as well as our major cross-reactive shrimp allergen at 35 to 38 kD on SDS-PAGE, most probably represent the same major shrimp allergen which has been identified as the muscle protein tropomyosin.²⁻⁴ Our 21-kD shrimp allergen probably corresponds to Hoffman's minor allergen of the same molecular weight.

Lehrer¹ described the extensive allergen cross-reactivity among several crustaceans using CIE, CRIE, CLIE, and RAST inhibition techniques. Compared with our description of six boiling water shrimp allergens by immunoblot, Lehrer described seven shrimp allergens on CRIE, of which five cross-react with crawfish, three with lobster, and one with crab.

Possible allergenic cross-reactivity of crustaceans outside their own order, class, and phylum has been suggested in several reports. Erickson et al.¹⁴ reported cross-reactivity between chironomids (non-biting midges) and shrimp in chironomid sensitive patients. Koshte et al.¹⁵ reported limited cross-reactivity of caddis fly with shrimp in caddis fly sensitive patients. Using RAST inhibition, Lehrer et al.¹⁶ reported oyster cross-reactivity in shrimp sensitive individuals, none of whom demonstrated independent oyster sensitivity. More recent DNA sequence analysis by Reese et al.¹⁷ demonstrated variable sequence homology of shrimp tropomyosin, the major shrimp allergen, with other arthropods, helminths and vertebrates. Additional cross-reactivities of shrimp tropomyosin with other tropomyosins have been shown, including squid,¹⁸

snail, and mite.¹⁹ Leung et al.²⁰ in 1996 used sera of shrimp-sensitive patients to demonstrate cross-reactive binding to the 38-kD tropomyosin of mollusks, including scallops.

Our case history patient being strongly and independently sensitive to both shrimp and scallops gave us a unique opportunity to address serologic cross-reactivity of two phyla, shrimp in the phylum Arthropoda and scallops in the phylum Mollusca. Overall, our inhibition experiment by ELISA and immunoblot inhibitions demonstrated up to 28% bidirectional cross-reactivity between shrimp and scallops. The major cross-reactive antigens were the heat-stable 35 to 39-kD proteins, most probably representing the respective tropomyosins of shrimp and scallops. The additional finding of less extensive inhibition of 21 and 26-kD shrimp proteins by scallops supports the existence of more than one cross-reactive allergen between these species.

In conclusion, boiling shrimp in open containers produces aerosols that contain all the antigens found in cooked shrimp and shrimp boiling water. Such aerosols are a source of potential respiratory and contact allergens, especially for those who prepare and cook seafood. Although present in differing concentrations, the allergens in cold and heat extracted cooked shrimp preparations were similar. SDS-PAGE and immunoblot identified multiple allergens in shrimp and scallops extracts (raw and boiling water). These findings confirm that the primary cross-reactive allergen of shrimp (phylum Arthropoda) and scallops (phylum Mollusca) is the 35 to 39-kD heat-stable allergen, previously demonstrated to be muscle tropomyosin in both phyla.

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