

CASE REPORT

Cross-reactivity among shrimp, crab and scallops in a patient with a seafood allergy

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ABSTRACT

Shrimp is known to be the most common causative agent in seafood allergy. Patients with shrimp allergy often exhibit allergic symptoms to a variety of seafoods such as crabs and clams. We experienced a 14-year-old girl with shrimp allergy who developed oral swelling and pain accompanied with an uncomfortable feeling after ingestion of scallops followed by intensive exercise. Laboratory investigation showed that she had serum immunoglobulin (Ig)E molecules reacting with several kinds of crustaceans and mollusks, including shrimp, crab and scallops. Immunoblotting revealed that her serum IgE reacted with the 38 kDa bands for shrimp, crab and scallops, suggesting that tropomyosin was the major allergen. Dot-blot inhibition analysis showed a cross-reactivity among shrimp, crab and scallops. We conclude that the cross-reactivity of IgE in this patient resulted from the high homology of tropomyosins.

Key words: crab, cross-reactivity, scallop, shrimp, tropomyosin.

INTRODUCTION

Seafood allergy is common in coastal countries such as Japan and Scandinavia. Among the seafood allergens, shrimp is the most frequent culprit.¹ Moreover, patients with shrimp allergies often exhibit allergic symptoms in response to a variety of other seafoods such as crabs and clams. Patrick *et al.* reported both clinical and experimental evidence of significant cross-reactivity among crustaceans (shrimp, lobster, crab and crawfish).² In such patients, immunoglobulin (Ig)E molecules against shrimp allergens have been demonstrated to cross-react with homologous allergens from multiple seafoods. Herein, we report a case of shrimp allergy whose serum IgE reacted with both crustaceans and mollusks.

CASE REPORT

A 14-year-old girl was referred to our hospital because of oral swelling and pain accompanied with

an uncomfortable feeling after ingestion of scallops followed by intensive exercise. No systemic symptoms were noted. The symptoms faded within 2 hours without any medications. No sign of infection or history of medication intake was present before onset of the symptoms.

The patient had a 3-year history of atopic dermatitis and one of conjunctivitis since birth. From the age of 5 years, she had often had oral pain and mucosal swelling after eating shrimp. Similar episodes occurred after eating other crustaceans and mollusks such as crab, squid and octopus. There had been no symptoms with foodstuffs devoid of crustaceans and mollusks. Sometimes oral swelling appeared when she ate foods supplemented with shrimp, either raw or cooked. There was no history of fish allergy.

Laboratory investigation showed an elevated concentration of serum IgE (1072.7 IU/ml). Serum-specific IgE antibodies are summarized in Table 1. The allergen-specific IgE test revealed positive reactions to tropomyosin (shrimp), shrimp, crab, squid, octopus,

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Table 1. Food-specific IgE Levels (Ua/ml)

Seafood	Value*	Other food	Value*
Tropomyosin (shrimp)	19.2	Egg white	< 0.35
Shrimp	18.9	Milk	< 0.35
Crab	18.9	Wheat	0.56
Squid	10.5	Gluten	< 0.35
Octopus	8.99	Buckwheat	< 0.35
Scallop	4.95	Soybean	< 0.35
Clam	4.64	Cod roe	< 0.35
Mackerel	2.89		
Anisakis	2.65		
Tuna	2.65		
Oyster	1.98		
Horse mackerel	1.34		

*Food-specific IgE antibodies were measured by CAP System fluorescent-enzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden).

scallop, clam, mackerel, anisakis, tuna, oyster and horse mackerel. Strongly positive reactions were observed to extracts of shrimp and crab by skin testing using allergen extracts (Torii Pharmaceutical, Tokyo, Japan) (Fig. 1).

Immunoblotting

A piece of raw shrimp, crab or scallop was ground separately in a Petri dish with distilled water at a concentration of approximately 3 mg/ml. Aliquots of the extracts were boiled for 15 min. All the extracts were filtered, dialyzed, lyophilized, and quantitated for protein content. To identify the allergenic protein, the extracts were analyzed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immunoblotting. Ten μ g of protein lysate per sample were subjected to electrophoresis on 12.5% polyacrylamide gel. Then they were electroblotted onto a polyvinylidene difluoride (PVDF) microporous membrane (Millipore Corporation, MA, USA). After being blocked with 5% skim milk in 0.1% Tween-Tris-buffered saline (TTBS), the membrane was incubated overnight at 4°C with the patient's serum diluted 1:10 with 5% skim milk in TTBS. Afterward, the membrane was extensively washed and incubated with goat antihuman IgE antibodies conjugated with horseradish peroxidase (BioSource, Camarillo, CA, USA) at a concentration of 0.04 μ g/ml for 1 h at room temperature. Bound antibodies were reacted with enhanced chemiluminescence plus Western Blotting Detection Reagents (Amersham Biosciences, Uppsala, Sweden). The results revealed that the



Figure 1. Skin testing showed strongly positive reactions to extracts of shrimp and crab.

serum IgE of the patient reacted with similar bands of 38 kDa for shrimp, crab and scallop (Fig. 2). Boiling had no effect on the intensities of the bands.

Inhibition assays

Specific-IgE inhibition assays were performed using a dot-blotting test. A PVDF microporous membrane was spotted with 2 μ g of extracted proteins (boiled shrimp, crab and scallop), blocked with 5% skim milk in TTBS, and incubated with the patient's serum. Before incubation with the membrane, 40 μ l of the serum were pre-incubated at 37°C for 2 h with the extracted proteins serially diluted from 333 to 0.03 μ g/ml with 5% skim milk in TTBS. After washing with TTBS, the membrane was incubated with goat antihuman IgE antibodies conjugated with horseradish peroxidase for 1 h at room temperature.

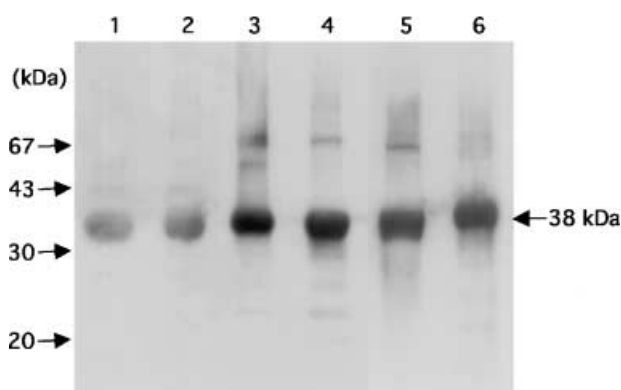


Figure 2. Immunoblot analyses using extracts from scallop, shrimp and crab. Lane 1, raw scallop; lane 2, boiled scallop; lane 3, raw shrimp; lane 4, boiled shrimp; lane 5, raw crab; lane 6, boiled crab.

The inhibition of the specific IgE binding to extracted proteins was visualized using ECL plus Western Blotting Detection Reagents. The resulting light was detected with autoradiography film (Hyperfilm ECL, Amersham Biosciences). After scanning the film, the spot intensities were measured using the Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA).

The inhibition was expressed as a percentage change from the patient's serum treated with Tris-buffered saline. The incubation of patient's serum and shrimp extracts caused dose-dependent inhibitions in IgE binding to extracted shrimp and crab proteins. Both of the IgE bindings were completely inhibited by extracted shrimp proteins at the concentration of 333 $\mu\text{g/ml}$. A similar result was obtained with extracted crab proteins (Fig. 3a,b). However, IgE binding to extracted scallop proteins was only partially inhibited even at the highest concentration: 60% by shrimp and 35% by crab, although almost 100% inhibition was obtained by scallop itself at 333 $\mu\text{g/ml}$ extracted proteins (Fig. 3c). Scallop extracts inhibited IgE binding to extracted proteins from shrimp or crab by only about 20% even at the highest concentration (Fig. 3a,b).

DISCUSSION

Herein, we report a case of shrimp allergy exhibiting a cross-reactivity with crab and scallop. Immunoblot analysis with the patient's serum detected a major band of 38 kDa in every extract, indicating that tropomyosin is the common and major antigen.^{2,3} This is supported by the highest score of serum-specific

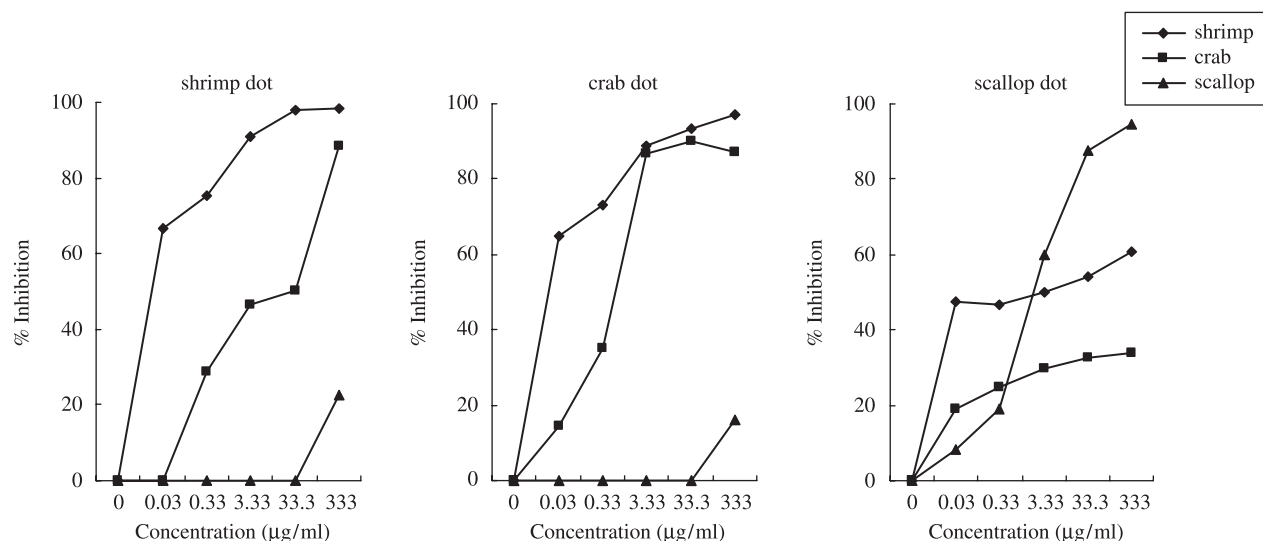


Figure 3. Inhibitions of immunoglobulin (Ig)E binding to extracted proteins from shrimp (◆), crab (■) and scallop (▲). (a) Two μg of shrimp extract was spotted and incubated with the patient's serum treated with serially diluted shrimp extract, crab extract and scallop extract; (b) 2 μg of crab extract was spotted and incubated with the patient's serum treated with serially diluted shrimp extract, crab extract and scallop extract; (c) 2 μg of scallop extract was spotted and incubated with the patient's serum treated with serially diluted shrimp extract, crab extract and scallop extract.

IgE antibodies to shrimp tropomyosin. The specific IgE inhibition analyses with the patient's serum demonstrated a marked cross-reactivity between shrimp and crab, paralleling the great homology in the amino acid sequences of shrimp and crab tropomyosins (91.7%).⁴ The IgE binding to extracted scallop proteins was remarkably inhibited by shrimp or crab extracts, indicating a cross-reactivity among shrimp, crab and scallops. However, the inhibition percentage with scallop extracts was found to be lower in the IgE binding to the shrimp extracts and the crab extracts. This may be attributed to the lesser homology in the amino acid sequence of scallop tropomyosin with the shrimp and crab tropomyosins. The homologies are 61.6% between shrimp and scallop and 61.7% between crab and scallop.^{4,5} In this patient, scallop-induced allergic symptoms were exhibited only when the patient did intensive exercise after ingestion of scallops. The scallop-specific IgE level is lower than those for shrimp and crab; thus, the symptoms may have been induced by a mechanism of food-dependent exercise-induced anaphylaxis. Goetz *et al.* reported a similar cross-reactivity between shrimp and scallop in a patient with occupational asthma using a specific IgE inhibition test and an immunoblotting test.⁶ Therefore, it appears that individuals allergic to shrimp or crab are at risk for reacting to mollusks, because these seafoods share common antigenic epitopes in tropomyosin, even though their homologies are not so high.

Only the IgE binding to scallop protein was inhibited up to 60% by the shrimp extracts and up to 35% by the crab extracts. This partial inhibition may be due to the presence of IgE antibodies reacting only to scallop-specific epitopes of the tropomyosin in the patient.

The patient also exhibited an oral allergy syndrome after eating squid and octopus, but no symptoms after ingestion of fish. Her specific IgE scores to squid and octopus were 10.5 and 8.99, respectively,

much higher than those of fishes such as mackerel and tuna (2.89 and 2.65, respectively). These scores are well correlated with the amino acid sequence homology between those seafood tropomyosins and shrimp tropomyosin. The amino acid sequence homology of squid tropomyosin and shrimp tropomyosin is 70.3%, but that of tuna tropomyosin and shrimp tropomyosin is only 57.2%.⁴

In conclusion, we demonstrated marked cross-reactivities of IgE among shrimp, crab and scallop extracts in the patient with seafood allergy, which may be a result of the high homology of their tropomyosins. Further examination of IgE-binding epitopes is necessary in order to substantiate this speculation.

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