

Allergy to limpet

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Allergy to mollusk has rarely been described. The limpet, belonging to *Phylum mollusca*, is one of the most frequent mollusks in the Canary Islands, as in all warm maritime regions. We report two cases of atopic patients who developed anaphylactic reactions after ingestion of this mollusk. Type I hypersensitivity to limpet antigens was demonstrated by means of immediate skin test reactivity, specific IgE determination by RAST, and histamine release test to cooked limpet extract. The controls did not react to any of these tests. Allergic activity was only found with a cooked limpet extract; this suggests that the offending antigen/s may have been released by cooking this food.

Key words: cross-reactions; crustacea; limpet; mollusk; RAST

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Clinical manifestations of food allergy are generally the result of a type I hypersensitivity IgE-mediated mechanism. Estimates of the incidence of IgE-mediated food allergy range from 0.1 to 0.7% of the population with the male to female ratio being approximately 2:1 (3). The prevalence of reactions to specific foods depends in part on the eating habits of a given population (7). Sea food, including crustacea, are among the most frequently mentioned foods claimed to cause adverse reactions in food-sensitive subjects (1, 13). Although mollusks have frequently been referred to as inducers of adverse reactions when ingested by sensitive subjects, there has been little investigation of IgE antibody reactivity with mollusk allergens. Anaphylactic reactions to snail, mussel and oyster have been reported (9).

The limpet, belonging to *Phylum mollusca*, class Gastropoda and genus *Patella*, is one of the most frequent sea mollusks in the Canary Islands and other warm maritime regions. More than 20 different species have been described. Their structure is composed of a smooth or striated conical shell and the body,

and they adhere tightly to rocks (4). Limpets are usually consumed cooked or raw.

In the present report we described two patients with anaphylactic reactions after eating limpets. To our knowledge limpet allergy has not previously been reported.

CASE REPORTS

Case 1

The patient was a 22-year-old woman, non-smoker, with a 6-year history of perennial rhinitis and mild asthma exacerbated by contact with house dust from mattress and bedding. She also had seasonal exacerbations during March/April and September/October; she had a positive family history of atopy.

Five months previously, 60 min after eating rice with limpets she developed diffuse urticaria and angioedema, status asthmaticus and severe hypotension. She was admitted to the intensive care unit for 24 h. The patient had previously eaten limpets and after this episode

she was able to eat mussels, squid, and shrimps without any symptoms. She has not eaten limpets since the reaction. Total IgE (Enzygnost, Behringwerke, Marburg, Germany) was 260 IU/l.

Case 2

The patient was a 29-year-old man, non-smoker, with a 15-year history of perennial symptoms of rhinitis and moderate asthma; he experienced attacks of rhinitis and asthma after dust exposure. He also had a positive familial history of atopy. Four months previously, within 40–60 min of eating cooked limpet, he experienced abdominal cramps, dysphagia, diffuse erythema, dysphonia, severe bronchospasm, loss of consciousness and, finally, respiratory arrest. He was immediately admitted, to the intensive care unit where cardiopulmonary resuscitation was performed. He was discharged 48 h later. He had eaten limpets before without any symptoms and after the anaphylaxis crisis he was able to eat squid, octopus, round clam and cockle. Total IgE was 520 IU/l.

MATERIAL AND METHODS

Antigen extract for in vivo and in vitro tests

Briefly, 4 g of fresh limpet meat was removed from the shell and washed with water. The meat was triturated and two extraction procedures performed: a) 2 g of limpet meat were dissolved in 20 ml of phosphate buffered saline (PBS) (pH: 7.3) at laboratory temperature. The suspension was stirred for 60 min and centrifuged at 1000 g for 10 min. The supernatant was recovered and sterilized with a Millipore filter (pore size 0.22 µm) (Millipore Co. Bedford: MA) and a final concentration of 1:10 w/v was obtained; b) 2 g of triturated limpet meat was dissolved in 20 ml of PBS and boiled at 75°C for 15 min. The mixture was centrifuged at 1000 g for 10 min, the supernatant was recovered and passed through a Millipore filter for sterilization and a final concentration of 1:10 w/v was obtained. Ten-

fold dilutions were made for skin testing. Protein content of extract was estimated by bio-Rad and was 1 mg/ml in cooked limpet extract and 0.35 mg/ml in raw limpet extract. The same extraction procedure was done with squid, round clam and snail.

Skin test

Skin prick tests were performed in duplicate on the volar side of the forearm of each subject with serial dilutions of raw and cooked limpet extracts, squid and round clam extracts. Further, skin prick test with raw limpet was done by piercing the food with a skin prick needle and immediately puncturing the skin. Histamine (10 mg/ml) was used as control.

Prick tests were also performed with a battery of commercially available common inhalants (pollens, mites, animal dander and fungal spores) (Abello, Spain) and such foods as milk, egg, nut, fish (Bencard, Worthing, UK). Skin prick tests were carried out as well with commercial mollusk and crustacea (mussel, oyster, shrimp, lobster and crawfish) (Bencard, Worthing, UK)

The same tests were performed on five atopic subjects and five normal subjects who served as controls. All skin tests were examined after 15 min, and 6 and 24 h later. A reaction was considered positive if the wheal size induced by the allergen was $\geq$ than that produced by the positive control.

Histamine release test

This was carried out using an automated fluorometric method according to Siraganian (12), with the modifications previously described (10), with four different concentrations (1:5, 1:25, 1:125, 1:625) of all extracts used in the skin prick tests. Results $> 10\%$ release were considered positive.

Specific IgE by RAST

Nitrocellulose paper disks (Hoeffer Scientific Instruments, US) were coupled with commer-

cial crustacea and mollusk extracts (mussel, oyster, shrimp, lobster, crawfish). *Dermatophagoides pteronyssinus* and our cooked extracts (limpet, squid, snail) by incubation 24 h at room temperature. The disks were washed three times in PBS 0.1% Tween 20 (PBS-Tw). To facilitate the desorption, 1% bovine serum albumin (BSA) in PBS was added and the disks incubated for 1 h at room temperature. They were then stored in PBS at room temperature until use.

For the test, 50 µl of serum were added to tubes containing antigen-coupled disk, incubated 3 h at room temperature and washed three times with PBS-Tw to remove unreacted serum. 50 µl of I-labeled anti-IgE (Pharmacia Diagnostic, Sweden) were added and incubated 18 h at room temperature. The washing steps were repeated and tubes counted on a gamma counter. All RAST were performed in duplicate. Finally, a semiquantitative RAST score (0 to 4) based on the commercial reference system (Pharmacia Diagnostic, Sweden) was used for interpretation of the results.

The control group consisted of a pool of sera from five mite-allergic patients, a pool of sera from five crustacea- (shrimp)- sensitive subjects and a pool of sera from five non-atopic subjects.

RAST inhibitions

RAST inhibition experiments were performed essentially as previously described by Yman et

al. (14), using 10 and 20 µl of each allergen extract and BSA. The allergen extracts: limpet, mussel, squid, round clam, oyster, snail, shrimp, lobster, crawfish, and *D. pteronyssinus*, were incubated with 30 µl of patient serum for 2 h in duplicate at room temperature.

RESULTS

Fifteen minutes after skin prick testing with the 10 w/v cooked limpet extract, patients 1 and 2 exhibited a 16 by 14 mm and 12 by 9 mm wheal, respectively, with erythema response. No reactions were evident after 6 and 24 h. Prick test with raw limpet extract, raw and cooked squid extracts and round clam extract were negative. Also, skin prick test with raw limpet was negative. In contrast the five atopic and five normal subjects did not react to cooked limpet extract. Both patients had positive skin tests for house dust mite and did not react to pollen, fungal spore and other food allergen, including Bencard crustacea and mollusk extracts.

The histamine release test was strongly positive for cooked limpet extract in both patients (Case 1: 32%, 55%, 80% and 86% respectively; Case 2: 16%, 25%, 43% and 50% respectively). A dose-related histamine release was detected with cooked round clam and snail extracts and commercial shrimp extract. Histamine release with raw extract (limpet, squid, snail and round clam) and commercial crustacea and mollusk (lobster, crawfish, oys-

Table 1.

Results of RAST in two limpet-sensitive patients

Allergen	Case 1 (PRU/l)	Case 2 (PRU/ml)
Cooked limpet	3.4	1.75
Squid	< 0.35	< 0.35
Round clam	< 0.35	< 0.35
Snail	< 0.35	< 0.35
Mussel	< 0.35	< 0.35
Oyster	< 0.35	< 0.35
Shrimp	< 0.35	< 0.35
Lobster	< 0.35	< 0.35
Crawfish	< 0.35	< 0.35
<i>D. pteronyssinus</i>	> 17.5	> 17.5

Table 2.

Results of RAST-inhibitions in two limpet-sensitive patients

RAST	% inhibition	% inhibition
	Case 1	Case 2
Limpet	92.7	80
Round clam	64	56
Snail	70.8	60
Oyster	59.9	67
Mussel	0	13
Squid	8	3.6
Shrimp	74	61.5
Lobster	60.6	53.8
Crawfish	31.8	61.9
<i>D. pteronyssinus</i>	91.9	81.7

ter, mussel) was not detected in our two patients.

Specific anti-limpet IgE were found in both patients (RAST class 2 and 3). All pools of control sera had a score 0. The RAST reactivity of limpet-sensitive patients to limpet, mollusks, crustacea and *D. pteronyssinus* is illustrated in Table 1.

RAST inhibition studies were performed with limpet, other mollusks (mussel, snail, squid, oyster, round clam), crustacea (shrimp, lobster, crawfish) and *D. pteronyssinus* on two limpet-sensitive subjects. Table 2 shows results of RAST inhibition in our two patients. It was found that the limpet and *D. pteronyssinus* extracts inhibited the anti-limpet IgE antibodies. The other extracts inhibited limpet uptake much less than the limpet and *D. pteronyssinus* extracts. These results indicate that there is some cross-reactivity between limpet and *D. pteronyssinus*.

DISCUSSION

Our two patients showed severe respiratory and systemic symptoms after ingestion of limpets. The positive skin prick tests, the antigen-induced histamine release from peripheral blood basophils, and the detection of specific IgE against cooked limpet extract using a RAST procedure, strongly suggest that both patients developed a type I hypersensitivity reaction to limpet antigen. Surprisingly, the onset of symptoms did not occur, as is usual, immediately after ingestion of the suspected agent, but approx. 40–60 min elapsed. This suggests that the antigenic penetration in both cases was via the gastrointestinal route.

We did not consider a specific oral challenge because the patients' clinical manifestations had been life-threatening and *in vitro* and *in vivo* tests supported the diagnosis of an IgE-mediated limpet reaction.

We found no serological or skin IgE mediated reaction against limpet in the controls. It is noteworthy that we only found allergic activity with cooked limpet extract. This suggests that the offending antigen/s may have been released by cooking the food.

In our study, antigen specificity could be demonstrated by achieving a cpm inhibition of > 90%. We did not achieve a significant inhibition with extracts from mollusk phylogenetically close to limpet (mussel, squid). However, employing other mollusks (oyster, round clam) as well as some crustacea (shrimp, lobster, crawfish) we obtained an intermediate level of inhibition. These results indicate that the mentioned foods have common antigenic epitopes, which is an unexpected finding since mollusk and crustacea belong to different Phylum, although this fact has been previously reported by other workers (5, 6, 11). Again, important inhibition was achieved with *Dermaphagoides pteronyssinus*, both our patients being sensitized to this arthropod frequently found in house dust. At present we have no explanation for this. Perhaps both Phylum conserve primitive molecular entities that could explain these common antigenic structures; or maybe through ingestion of some sea mite (limpets, feed by filtration) they incorporate some allergens present in these arachnids. It is noticeable that, recently, cross-reactivity between chironomids, crustacea, mollusk and parasitic mites has been demonstrated, suggesting a partial immunological identity between them (2, 8). Our results agree with this theory.

In conclusion the limpet, a favorite seafood in our region, can induce IgE-mediated reactions in sensitized subjects and must be included among the potential allergens. It is possible that the allergen/s responsible for the symptoms in our patients have common antigenic epitopes with some mollusks or arthropods. We are investigating this at present.

REFERENCES

- 1 Daul G, Morgan A, Waring N-P, McCants L, Hughes J, Lerher S. Immunologic evaluation of shrimp-allergic individuals. *J Allergy Clin Immunol* 80, 716–722, 1987.
- 2 Eriksson NE, Ryden B, Jonsson P. Hypersensitivity to larvae of chironomids (non-biting midges). *Allergy* 44, 305–313, 1989.
- 3 Farrell MK. Food allergy. In Lawlor G, Fischer T (eds): *Manual of allergy and immunology*, pp 272–283. 2nd ed. Little Brown, Boston/Toronto, 1988.

- 4 Gomez-Rodriguez JE. Estudios de las especies del Genero *Patella* presentes en el litoral de la Isla de la Palma. Tesina, La Laguna, 1976.
- 5 Lehrer SB, McCants ML. Reactivity of IgE antibodies with crustacea and oyster allergens. Evidence for common antigenic structures. *J Allergy Clin Immunol* 80, 133-139, 1987.
- 6 Lehrer SB. The complex nature of food antigens: studies of cross-reaction crustacea allergens. *Ann Allergy* 57, 267-271, 1986.
- 7 Kettelhut BV, Metcalfe AD. Adverse reactions to foods. In Middleton E, Reed C, Ellis E, Adkinson F, Yunginger J (eds): *Allergy: Principles and practice*, pp. 1481-1502. 2nd ed. CV Mosby, St. Louis, 1988.
- 8 Koshie VI, Kagen SL, Aalberse RC. Cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca. *J Allergy Clin Immunol* 84, 174-183, 1989.
- 9 Moneret-Vautrin A, Andrieu CL. Immunopathologie de l'allergie alimentaire et fausses allergies alimentaires, pp. 55. Masson Ed, Paris, 1983.
- 10 Moneo I, Bontello A, Hinojosa M, Alcover R. Nuevos avances en la determinación de histamina automatizada. *Allergol Immunopathol Suppl* VIII, 311-315, 1980.
- 11 Sachs M, O'Connell E. Cross-reactivity of foods: mechanisms and clinical significance. *Ann Allergy* 61, 36-40, 1988.
- 12 Siraganian RP. An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Ann Biochem* 57, 383-394, 1974.
- 13 Waring NP, Daul CB, de shazo RA, McCants ML, Lehrer SB. Hypersensitivity reaction to infested crustacea: clinical evaluation and diagnostic studies in shrimp-sensitive individuals. *J Allergy Clin Immunol* 76, 440-445, 1985.
- 14 Yman I, Ponterius G, Brandt R. RAST-based allergen assay methods. *Dev Biol Stand* 29, 151-165, 1975.

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