

Short communication

Limpet anaphylaxis: cross-reactivity between limpet and house-dust mite *Dermatophagoides pteronyssinus*

Background: Limpet is a mollusc that is frequently found along the shores of warm seas. Few allergic reactions to limpet have been described, and cross-reactivity with house-dust mites has not been established.

Methods: We report five patients with anaphylactic reactions due to limpet ingestion. A limpet extract was prepared and skin prick tests (SPT), radioallergosorbent assays (RAST), sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and IgE-immunoblotting were performed. In order to evaluate cross-reactivity, an immunoblotting inhibition assay with *Dermatophagoides pteronyssinus* was also done.

Results: All patients were asthmatics sensitized to house-dust mites. In each case, severe bronchospasm was evidenced, and three required mechanical ventilation. Positive SPT with limpet extract was found in the four patients tested. RAST showed specific IgE on each patient's sera. Immunoblotting showed several allergenic fractions with a wide molecular weight range (15–250 kDa). *D. pteronyssinus* inhibited IgE-binding molecules above 50 kDa in the limpet extract, particularly one molecule of 75 kDa.

Conclusions: We describe five patients with IgE-mediated limpet allergy. Severe bronchospasm was the most important symptom. Cross-reactivity with *D. pteronyssinus* was demonstrated.

J. Azofra¹, M. Lombardero²

¹Unidad de Alergia, Hospital Central de Asturias, Oviedo; ²Departamento de Investigación, ALK-Abelló, Madrid

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J. Azofra
Unidad de Alergia
Hospital Central de Asturias
Dr Bellmunt s/n. 33006 Oviedo
Spain

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Shellfish is one of the most frequent causes of "IgE-mediated food allergy"—according to the terminology from the EAACI nomenclature position statement (1)—in adults on the north and north-western coasts of Spain. Crustacea are responsible in most cases, although allergy to mollusc is also frequently found, usually associated with allergy to crustacea.

The limpet is a mollusc belonging to the Gastropoda class, order Archaeogastropoda. It has a worldwide distribution, and is abundant along the northern coast of Spain, although its consumption is limited.

Allergy to limpet, genus *Patella*, has been described (2–6). In the four cases reported in Japan, the etiologic agent was a variety of limpet, grand keyhole limpet, which belongs to a different genus, *Fissurellidae* (2).

We report five cases of anaphylactic reaction due to limpet ingestion, evidencing specific IgE against this mollusc by *in vivo* and *in vitro* methods. We describe cross-reactivity with house-dust mite (*Dermatophagoides pteronyssinus*).

Material and methods

Five subjects, two women and three men, whose ages ranged from 23 to 47 years, were included in the study. Each patient

suffered from anaphylactic reaction after eating cooked limpets. All patients tolerated crustaceans as well as other molluscs afterwards, although they were not exposed any longer to the gastropod class.

A limpet extract was prepared by mixing 2 g of ground limpet meat with 20 ml of phosphate-buffered saline (PBS), and heated for 15 min at 75°C in order to reproduce the conditions in which limpets are consumed. It was centrifuged at 1000 g for 10 min, and the supernatant was recovered and sterilized by passing through a Millipore filter (size 0.22 µg) (3). The protein concentration of the extract was 5 mg/ml (Sigma protein assay). A 2.5 mg/ml protein concentration was used for skin testing. The same extract was used for the "*in vitro*" tests: radioallergosorbent assay (RAST), sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and IgE-immunoblotting.

Specific IgE was determined by RAST. Paper disks were activated with BrCN and coupled with *D. pteronyssinus* and limpet extracts. Sensitized disks were incubated overnight with the patient's serum and, after washing, with ~100 000 c.p.m. of ¹²⁵I-mouse antihuman IgE monoclonal antibody (mAb) HE-2. *Lolium* disks and 4 dilutions of a grass-specific serum pool, calibrated against the Phadebas RAST system (Pharmacia, Uppsala, Sweden), were used as reference.

Immunoblotting was carried out with the individual serum and the pool of sera from the five patients. In order to study possible cross-reactivity between limpet and house-dust mite, the pool of sera was preincubated with a *D. pteronyssinus* extract, and a new immunoblotting with the limpet extract was obtained afterwards.

Results

All five patients were from the area around Oviedo in Spain. They all had house-dust mite-related asthma. None of them had received mite immunotherapy previously. Symptoms started between 10 and 90 min after limpet ingestion. Each patient suffered from anaphylactic reaction. All had cutaneous symptoms as well as bronchospasm. Three had rhinitis and one had dysphonia. The most important manifestation was bronchospasm, appearing in three of the patients and requiring mechanical ventilation. The limpet skin prick test (SPT) was positive in four patients tested (one patient refused). Ten asthmatic subjects sensitized to house-dust mites (*D. pteronyssinus*) served as controls, showing a negative prick test. Specific IgE antilimpet (RAST) was evidenced in all the five sera tested (Table 1).

It is worth noting that two patients described allergic reactions (dyspnea and eyelid edema) after eating terrestrial snails.

The IgE-immunoblotting with the individual sera (Fig. 1) and the serum pool (Fig. 2, lane 1) revealed several allergenic bands in the limpet extract.

In the inhibition assay, a *D. pteronyssinus* extract inhibited several IgE-binding molecules in the limpet above 50 kDa, remarkably a 75-kDa fraction (Fig. 2, lane 4).

Discussion

Although IgE-mediated allergic reactions to shellfish are frequent, few reports describe limpets as causative agents, probably related to their limited consumption. However, it seems to be true even in regions where limpets are eaten regularly, as in the Canary Islands.

It is noteworthy that all five of our patients, as well as most cases reported in the literature, were sensitized to house-dust mites (*D. pteronyssinus*). This prompted us to evaluate the presence of cross-reactivity between limpet and *D. pteronyssinus*, demonstrating common IgE-binding epitopes. Our cases are included in the context of

cross-reactivity between invertebrates. House-dust mite (phylum *Arthropoda*; subphylum *Chelicerata*) allergens have been proposed to cross-react with allergens from other invertebrates, including crustaceans (phylum *Arthropoda*; subphylum *Antennata*) and molluscs

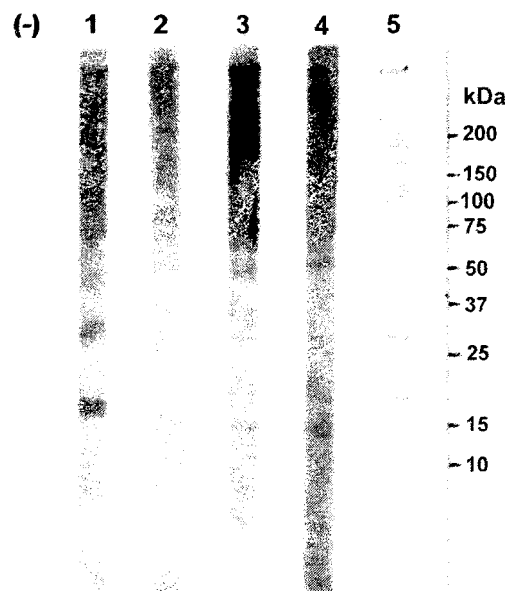


Figure 1. Limpet IgE immunoblotting. Limpet extract was run in sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE; 10–20% Novex tricine gels). After transferring the limpet proteins to nitrocellulose membranes, these were incubated with the sera from patients and then with a mAb anti-human IgE. Detection of IgE-binding proteins was carried out by chemiluminescence (ECL, Amersham, UK). Lane numbers correspond to patient numbers. (–) Negative control.

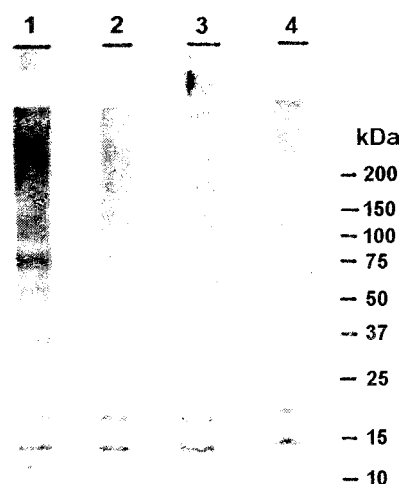


Figure 2. IgE immunoblotting inhibition. Lane 1: pool of sera from patients allergic to limpet. Lane 2: preincubation with limpet extract (0.1 mg/ml). Lane 3: preincubation with limpet extract (0.5 mg/ml). Lane 4: preincubation with *Dermatophagoides pteronyssinus* extract (0.3 mg/ml).

Table 1. Clinical data, skin prick test and specific IgE of the patients

Patient characteristics			<i>D. pteronyssinus</i>		Limpet			Snail
Number	Age	Sex	Prick (mm)	CAP (kU/l)	Symptoms	Prick (mm)	RAST (kU/l)	Symptoms
1	46	M	11 × 7	>17.5	B A D	7 × 5	0.46	D
2	28	M	20 × 13	>17.5	B U	5 × 3	0.40	–
3	34	M	10 × 6	>17.5	B R U	6 × 4	0.56	–
4	27	F	14 × 10	>17.5	B R U A	5 × 4	0.51	A
5	23	F	20 × 14	>17.5	B R	ND	0.39	–

B: bronchospasm; A: angioedema; R: rhinitis; U: urticaria; D: dysphonia; ND: not done; *D. pteronyssinus*, *Dermatophagoides pteronyssinus*.

(phylum *Mollusca*). Tropomyosin (40 kDa) seems to be the protein involved in house-dust mite–crustacean cross-reactivity (7).

Gastropoda is a mollusc class, which includes snail (terrestrial and marine) and limpet. Clinically important cross-reactivity between house-dust mites and snails has been confirmed (6, 8). Most often, more than one allergen is involved. House-dust mite allergens responsible for the cross-reactivity with snail may include Der p 4, Der p 5, Der p 7, and hemocyanin (8). Tropomyosin does not seem to be involved. Inhibition tests show that the IgE reactivity to snails is generally inhibited by extract from house-dust mites, whereas IgE reactivity to house-dust mite is not significantly inhibited by snail extracts. Such results imply that house-dust mite is usually the sensitizing agent.

According to these comments, cross-reactivity between limpet, house-dust mite and snail is very plausible. Van Ree et al. described 28 patients with combined allergy to snail and house-dust mite (6). As well as in our cases, severe asthmatic symptoms were observed. Some patients also reported asthmatic reactions after ingesting limpets. A close correlation was observed between the snail and limpet RAST. García de la Cuesta et al. also described a severe bronchospasm in a group of 10 patients allergic to snails. Two of them had asthmatic reactions after limpet ingestion (9). In our cases, two patients described allergy to snails.

In the study by Carrillo et al. (3), an inhibitory effect of the *D. pteronyssinus* CAP with limpet extract could not be established, although, as discussed by the authors, this could not be completely excluded because of the high specific IgE levels to *D. pteronyssinus* (CAP class 6). A limpet RAST inhibition study with *D. pteronyssinus* could not be performed. In fact, in a previous study, the same authors showed that this mite inhibited the RAST of a limpet extract in two patients (10).

As noted, several allergens have been described as responsible for house-dust mite–mollusc cross-reactivity.

The 75 kDa protein described in our cases might be related to Der p 4 amylase (60 kDa).

A common characteristic in the literature is the severity of bronchospasm. Two patients in the report by Carrillo et al. (3), one in Morikawa's article (2), and three in ours required mechanical ventilation. It is known that underlying asthma appears to be a particular risk factor for severe reactions in food-allergic patients (11). All of our patients, as well as most of those described in the literature (2, 3, 5) had anaphylaxis, which means that limpet should be added to the list of very dangerous food allergens.

A link between allergen immunotherapy with *D. pteronyssinus* and development of food reactions to molluscs and crustacea, or worsening of pre-existing snail allergy, has been reported (3, 12, 13). However none of our patients had received immunotherapy with *D. pteronyssinus*, weakening the role of this type of treatment as a predisposing factor in mollusc allergy.

All our patients tolerated other molluscs and crustaceans. The same fact was observed by Carrillo et al. (3). This "selectivity" contrasts with the usual multiple sensitization described in shellfish allergy, among crustaceans, and between crustaceans and molluscs. However, our patients were advised to avoid molluscs of the gastropod class because of the possibility of cross-reactivity with limpet.

In summary, limpet may induce anaphylaxis in asthmatics sensitized to house-dust mites. The bronchospasm characteristic of the anaphylactic reaction may be severe, even requiring mechanical ventilation. Cross-reactivity between limpet and house-dust mite has been described.

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