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Tropomyosin or not tropomyosin, what is the relevant allergen in house dust mite and snail cross allergies?

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Summary

Since tropomyosin is cross reactive in many arthropods, it was assumed that this highly conserved protein could be responsible for cross reactions in house dust mite (HDM) allergic patients who experienced adverse reactions after crustacean and mollusc ingestion. Here we report two clinical cases where the role of tropomyosin is a matter of debate. In the first case, the clinical history, as well as the results of in vivo and in vitro investigations, are in favour of a shrimp allergy without any snail allergy in a patient sensitized to HDM. In the second, the clinical history and the cutaneous tests are in favour of an allergy to snails without any allergy to shrimps in a patient suffering from HDM allergies. The clinical presentation is different in shrimp and snail allergies. In shrimp allergy, symptoms are mainly urticaria or angio-oedema. In snail allergies, adverse reactions are especially severe asthma. Shrimp tropomyosin is a dominant allergen in crustaceans whereas has a much less prominent role in HDM sensitization. Cross reactivities between HDM and snails have been confirmed by inhibition experiments. However, tropomyosin appears to be a minor allergen or even is not involved in snail allergy. It is necessary to clarify the allergens shared between HDM and snails. The effects of HDM immunotherapy in snail allergy are questioned. Knowledge of taxonomy can contribute to more precise evaluation of cross reactivities between crustaceans and molluscs.

In 1993, Shanti et al (1) identified tropomyosin as the major shrimp allergen. One year later, Witteman et al (2) reported that tropomyosin was also a cross allergen in house dust mites (HDM). Since tropomyosin is cross reactive among many arthropods, it was assumed that this highly conserved protein could be responsible for cross reactions in HDM allergic patients who experienced allergic reactions after crustacean and mollusc ingestion. Here, we report two clinical cases where the role of tropomyosin as a cross allergen is a matter of debate.

Case report n. 1

Mrs C… E…, a 31-year-old woman was referred to our office for medical advice. She had experienced generalized urticaria occurring one hour after shrimp ingestion. She had previously frequently eaten shrimps without any adverse reactions. Eating snails never generated any allergic reactions. She had no respiratory symptoms in favour of HDM allergy.

Allergological investigations:
- Skin prick tests to common aeroallergens showed positive reactions to Dermatophagoïdes pteronyssinus (Der p) and farinae (Der f).
- Cutaneous tests were also performed with native foods from shrimp, spiny lobster, crab, mussels and snails. These tests were positive for shrimp (mean weal diameter: 8 mm, histamine control test: 4 mm), spiny lobster and crab (mean weal diameter: 6 mm). They were negative for snails (Helix pomatia sp), mussels and oysters.
- Allergen specific IgE antibodies were measured by ImmunoCAP (Phadia Lab.). Specific IgE for Dpt were 44.3 kU/l and 80.9 kU/l for shrimp (Penaeus aztecus sp). Specific IgE for shrimp recombinant (r Pen a 1) were high: 68.7 kU/l.

Conclusion: The clinical history and the results of in vivo and in vitro investigations are in favour of shrimp allergy without any snail allergy in a patient sensitized to HDM.

Case report n. 2

Mrs A… F…, a 62-year-old woman, had experienced acute rhino-conjunctivitis and severe asthma, one hour after eating snails at a Christmas dinner. For years, she had complained of per-annual symptoms: rhino-conjunctivitis and asthma in relation with HDM allergy. Desensitization with a HDM crude extract was performed over a period of 4 years, 10 years ago. Previous ingestions of crustaceans had been well tolerated.

Allergological investigations:
- Skin prick tests for a panel of common aeroallergens were only positive for HDM (Der p and Der f) with a mean weal diameter of 7 and 8 mm respectively.
- A prick test with native snails (Helix pomatia sp) was positive with a mean weal diameter of 5 mm. The prick test with native shrimp was negative.
- Specific IgE determination (ImmunoCAP, Phadia Lab.) for Der p and Der f showed positive results with values of 33.10 kU/l and 22 kU/l respectively.
- However, the values of specific IgE against snails (ImmunoCAP, Phadia Lab.) remained negative (< 0.35 kU/l) as well as specific IgE for shrimp recombinant allergen (r Pen a 1). After the severe allergic reactions to snails, the patient had eaten oysters, mussels, and scallops i.e. molluscs, without any adverse reactions.

In conclusion: The clinical history and the cutaneous tests are in favour of an allergy to snails without any allergy to shrimps, in a patient suffering from house dust allergy.

Comments

Diagnosis procedures

In the absence of inhibition experiments, we were not able to distinguish a cross allergy from a parallel sensitization to shrimp and Der p in the first case, to snail and Der p in the second. In the second case, the results of the in vivo and in vitro tests were discordant: specific IgEs for snail were negative and cutaneous tests were positive for native snail. The discrepancies observed between cutaneous and serological tests for snail could be explained by the different sources used for the cutaneous tests (Helix pomatia sp) and serological tests (Helix aspersa sp).
Obviously, results also depend on the quality of the extracts used for in vivo and in vitro tests. These data outlined once more that in the diagnosis of food allergy, it is preferable to use native foods for cutaneous tests. They also point out that the availability of recombinant allergens such as r Pen a 1, an excellent marker of sensitization to crustacean allergens, facilitates the diagnostic approach. We did not perform any oral provocation tests (either open challenge tests or DBPCFC), due to the fact that the anamnesis was unequivocal and that the severe observed reactions made this unwise for safety reasons.
Finally, the two observations confirm that a careful anamnesis is of first interest. In shrimp allergy, symptoms vary from restricted oral reactions to severe systemic reactions, most individuals reporting urticaria or angio-oedema (3). In gastropod allergy, in more than 80% of the cases reported, the shock organ was the bronchial tree and severe asthma symptoms occurred. When dealing with HDM patients, the question: "Have you experienced any reactions when eating crustaceans or molluscs?" must be raised.

Shrimp and HDM allergy

Many case reports have described patients with combined shrimp and HDM allergy (4). As in our first observation tropomyosin seems to be the main allergenic protein involved in shrimp- HDM cross reactivity. Inhibition tests (RAST, ELISA, EAST… ImmunoCAP, Immunoblot inhibitions) have shown a cross sensitization between HDM and crustaceans. Immunoblot has revealed a stable protein allergen located at 34 to 38 kDa common to crustaceans and HDM allergens. This allergen was identified as tropomyosin. Shrimp tropomyosin has been cloned and recombinant tropomyosin is available for diagnosis tests. Tropomyosins are present in all eukaryotic cells where

they are associated with the thin filament in muscle and microfilament in many non muscle cells. Together with actin and myosin, tropomyosin plays a role not only in the contractile activity of these cells but also in the regulation of cell morphology and motility. Due to these vital functions, tropomyosin is a highly conserved protein throughout the evolution with a large distribution among invertebrates. For these reasons, tropomyosin was considered as a pan-allergen (5).

Each tropomyosin polypeptide is an alpha-helix; two parallel alpha-helical tropomyosin molecules form a coiled-coil structure (6). Several tropomyosin isoforms have been found in different species (12 in the rat for instance), in different tissues and cell varieties (7). Shrimp recombinant tropomyosin has been studied extensively. Eight B epitopes have been identified in 5 different parts of the molecule, especially in N and C terminal regions, equally distributed every 42 amino-acid intervals (8, 9). For years, tropomyosin was described as the unique relevant allergen among crustaceans. Recently another shrimp allergen has been identified: Pen m 2, an arginine-kinase, a minor allergen responsible for 27% of sensitizations in a group of 18 crustacean allergic patients |10). Two other shrimp allergens have been discovered, a myosin light chain: Lit v 2 (11) and a sarcoplasmic calcium binding protein of the black tiger shrimp Penaeus monodon (12).

Numerous studies have demonstrated that tropomyosin was an important allergen in crustaceans such as spiny lobster (Panulirus stimpsoni: Pan s 1), lobster (Homarus americanus: Hom a 1) (13, 14), crab (Charyabdis feriatus: Cha f 1) (15), crawfish, molluscs such as squid (Todarodes pacificus: Tod p 1) (16), snails (Turbo cornutus: Tur c 1) (17) and oyster (Crassotrea gigas: Cra g 1) (18). Tropomyosin is also present in house dust and storage mites such as Dermatophagoïdes pteronyssinus (Der p 10), Dermatophagoïdes farinae (Der f 10), Lepidoglyphus destructor (Lep d 10), Blomia tropicalis (Blo t 10) (19, 20). Among the insecta class, tropomyosin was identified among cockroaches (21, 22): Blatella germanica (r Bla g 7) and Periplaneta Americana (rPer a 7); among the diptera order: flies and chironomids (23, 24), among the Thysanura order: silver fish (r Lep s 1) (25) and even in nematodes (Anisakis simplex, Ascaris…) and trematodes.

While tropomyosin is a most dominant allergen in shrimp and other crustaceans, with a prevalence of sensitization varying from 72 to 100%, it has a less prominent role in sensitization to HDM where allergenicity is dominated by other components (Tab. 1). Except in one study (28) tropomyosin appears to be a minor allergen among HDM and storage mite allergic patients.

HDM and snail allergy

Cross reactivities between HDM and snails were suspected as soon as 1992 (30, 31) especially in regions where snail consumption was not unusual: France, Italy, Spain,

Table 1 - Prevalence of tropomyosin (T) sensitization in different countries

Allergen sources	Tropomyosin (T)	(T) sensitization prevalence	Number of patients	Countries	References
House dust mites	r Der p 10	9-18%	243	Europe	Weghofer (26)
	r Der p 10	5.6%	71	Spain	Asturias (27)
	r Der f 10	3%	31	Japan	Aki (28)
	n Der f 10	80%	31	Japan	Aki (28)
Storage mites	r Blo t 10	29%	93	Singapore	Yi (8)
	r Lep d 10	13%	136	Sweden	Saarne (20)
Cockroaches	r Bla g 7	16.2%	37	Corea	Jeong (22)
	r Per a 7	41.4%	29	Spain	Asturias (21)
Silverfish	r Lep s 1	21%	42	Italy	Bartella (25)
Chironimids	r Chi k 10	81%	21	Corea	Jeong (23)
Anisakis simplex	r Ani s 3	13%	62	Spain	Pascual (29)
Helix aspersa	r Hel as 1	18%	n.a.	Spain	Asturias (27)

n.a.: data not available

Portugal… Epidemiological studies have revealed the existence of a significant link between sensitization to HDM and snail allergens (32). Inhibition experiments (30, 33-36) have confirmed the cross reactivity between snails and HDM allergens. However the role of tropomyosin in these cross-allergies is questioned. De Maat-Bleeker et al. (35) have reported a case of cross reactivity in an allergic HDM patient presenting a severe reaction after eating snails for the first time. Immunoblot studies excluded the role of tropomyosin. Similar results were published by van Ree et al. (37): In the sera obtained from 28 allergic patients to HDM and snails, tropomyosin was only recognized by 2 sera; moreover the sera were those of two patients concomitantly allergic to shrimps.

Guilloux et al. (36) have reported in vitro studies concerning the cross reactivity between terrestrial snails (Helix sp) and house dust mites (Dpt). These authors confirmed the previous data and suggested several candidate cross reacting allergens between snails and Dpt: Der p 4, which has an amylase function, Der p 5 and Der p 7. Hemocyanin, an important component of hemolymph which, in invertebrates, is the equivalent of blood in vertebrates, was also a potential candidate.

In limpet allergy, a mollusc belonging to the gastropoda class, found along sea shores, Azofra and Lombardero (38) showed by immunoblotting several allergic fractions with a wide molecular weight range (15-250 kDa). Dpt extract inhibited the IgE binding to a 75 kDa protein which might be related to Der p 4 amylase. A thorough study of the allergen repertoire of Helix aspersa, the brown snail, was performed by Martins et al. (39). In 44 patients sensitized to snails, immunoelectrophoresis (IEF) and SDS-Page permitted the identification of 20 allergens; among them a protein with a molecular weight superior to 200 kDa. Hel a RAST was inhibited by the Dpt extracts to a much greater extent (76%) than Der p RAST by Hel a (5.6%). This is in favour of a primary sensitization by mite allergens in the case of the snail-HDM syndrome, as previously demonstrated (35-37).

According to Asturias et al. (27) the prevalence of sensitization to snail tropomyosin in snail allergic patients is only 18%. Moreover B epitopes of C-terminal region of Tur c 1, the tropomyosins of the snail Turbo cornutus are different from those identified in Pen a 1 (17). Furthermore, snail allergy without sensitization to mites was described by Caiado et al. (40) ; immunological investigations eliminated Der p and tropomyosin sensitization. In immunoblotting the IgE of their patient recognized two bands at 55 kDa and 95 kDa. This does not exclude the former idea that tropomyosin is a major allergen in crustaceans and a minor one in some molluscs..

Taking into account all the publications we have referred to, it seems that no single allergen is responsible for cross reactivity between HDM and snails.

Taxonomy and cross reactivities

Taxonomy knowledge can contribute to a better interpretation of cross reactivities. Cross reactive allergens, especially highly conserved proteins throughout evolution with a major cellular function, can be present in unrelated zoological or botanical families. On the other hand, taxonomic proximity favours cross reactions, a typical example being provided by the homologous allergens in Der p and Der f.

The terms of shellfish or sea foods used to name both crustaceans and molluscs may be a factor of confusion. Crustaceans and gastropods are taxonomically unrelated: crustaceans belong to the phylum arthropoda whereas gastropods belong to the phylum mollusca. Three classes involved in respiratory and food allergy belong to the phylum arthropoda: arachnida, crustacea and insecta. In the three classes, tropomyosin has been identified as a cross allergen. In the phylum mollusca, three classes are also present: gastropoda, lamellibranchia and cephalopoda. Table 2 a and b show the taxonomic relationship of species where cross reactivity with HDM was shown or suspected as well as the amino sequence identity between shrimp tropomyosin and tropomyosins from different organisms. The more distant the species are, the more the amino-sequence identity with tropomyosin will be reduced (41).

Treatment

Outside prescription in crustacean allergy of self-injectable epinephrine, two therapeutic approaches are available for the clinician allergologist: desensitization and avoidance.

Desensitization: The beneficial or detrimental effect of house dust mite immunotherapy in snail or shrimp allergy is still controversial: In our reported observation of snail allergy, the patient had been desensitized to house dust mite 10 years ago. Peroni et al (43) reported a snail anaphylactic reaction in a 12 year old girl who received HDM immunotherapy. Obviously, no decisive conclu-

Table 2a – Taxonomic relationship of main species where cross reactivity with shrimp tropomyosin was shown or suspected. Modified from Reese et al. (5) and from De Witt et al. (42), Mol Nutr Food Res 2004; 48: 370-379

Phylum	Class	Order	Family	Species	Current denomination	Allergen	Degree of sequence identity with shrimp tropomyosin
Arthropoda	Crustacea	Decapoda	Crangonidae	Penaeus aztecus	Brown shrimp	Pen a 1	99%
				Penaeus monodon		Pen m 1	
				Penaeus indicus		Pen i 1	
				Metapenaeus ensis		Met e 1	
				Metapenaeus indicus	Indian shrimp	Met i 1	
			Homaridae	Homarus americanus	Lobster	Hom a 1	98%
			Palinuridae	Panulirus stimpsoni	Spiny lobster	Pan s 1	
				Panulirus homarus		Pan h 1	
			Cancridae	Charybdis feriatus	Crab	Cha f 1	92%
				Procambarus clarkia	Crawfish	n.a.	
	Arachnida	Sarcoptiformes	Pyroglyphidae	Dermatophagoïdes pteronyssinus	House dust mites	Der p 10	81%
				Dermatophagoïdes farinae		Der f 10	
			Glycyphagidae	Lepidoglyphus destructor	Storage mites	Lep d 10	81%
				Blomia tropicalis		Blo t 10	
	Insecta	Blattaria	Blattidae	Blatella germanica	Cockroaches	Bla g 7	82%
				Periplaneta americana		Per a 7	
		Thysanura		Lepisma saccharina	Silverfish	Lep s 1	67%
		Diptera	Chironomidae	Chironomus thummi thummi	Chironomids	Chi t 1	78 %
				Chironomus plumosus			

n.a.: data not available

sions can be drawn from isolated observations. None of the five patients suffering from limpet allergy described by Azofra and Lombardero (38) had received immunotherapy whereas in the study of Carrillo et al. (34) five out of six patients with anaphylaxis to limpet were desensitized with HDM extract. Pajno et al. (44) observed in four children allergic to HDM and snails, 8 to 25 months after the onset of HDM immunotherapy, anaphylactic reactions following accidental snail ingestion. Van Ree et al. (45) studied 17 sera of HDM allergic patients receiving HDM immunotherapy. At the beginning of immunotherapy, 13/17 had positive RAST for snails. RAST for shrimp were positive in 3/17. 14 to 20 months later, the IgE response against snails showed a significant increase whereas IgE responses for Der p 1 and Der p 2 were not increased. The 3 patients with initial positive RAST to shrimp were the only patients who had clinical symptoms after eating shrimps.

Large series have been published by Meglio (46) and Asero (47). Meglio et al. (46) observed a significantly higher prevalence of snail sensitization evaluated by skin prick tests in 101 mite allergic children who had never

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Phylum	Class	Order	Family	Species	Current denomination	Allergen	Degree of sequence identity with shrimp tropomyosin
Mollusca	Gastropoda	Pulmonata	Helicidae	Helix pomatia Helix aspersa Eobamia vermiculata	Terrestrial snails	Hela TM Hela as 1	61%
		Archeogastropoda	Patellidae	Turbo cornutus Patella vulgate	Marine snails (Limpet)	Tur c 1	57%
	Lamellibranchia (or Bivalvia)	Anisomyaria	Fissurelidae	Mizuyopecten yessoensis	Scallop	n.a.	62%
			Mytilidae	Mytilus edulis	Mussel	My t e	57%
			Ostreidae	Ostrea edulis Crassostrea gigas	Oyster	Cra g 1	65%
	Cephalopoda	Octopoda	Octopodidae	Octopus vulgaris			
		Decapoda	Loliginidae	Todares pacificus	Squid	Tod p	72-75%
				Loligo vulgaris	Cuttlefish		

n.a.: data not available

undergone immunotherapy than in 82 mite allergic children who underwent HDM immunotherapy. This study was criticized by Antonicelli et al. (48) mainly for the reasons that the content of the extracts used for immunotherapy was unknown. Asero (47) studied 70 HDM allergic patients. 31 underwent a 3 year mite subcutaneous immunotherapy and 39 served as controls. No mite allergic patient was sensitized to tropomyosin at the beginning of the study and after at least 3 years, none of them was sensitized to tropomyosin. Moreover, among the 31 patients receiving HDM immunotherapy, shrimp ingestion in open oral challenges was well tolerated. This elegant study demonstrates a lack of de novo sensitization to shrimp tropomyosin, although sensitization to other snail allergens was not investigated.

The contradictory results observed could be due to the different qualities of the HDM extracts used for immunotherapy, especially their content of minor allergens such as Der p 10. In the future, immunotherapy with defined molecular allergens responsible for house dust mite sensitization could avoid injections of snail allergens. Epidemiological studies have shown that the main allergens recognized by house dust mite allergic patients are Der p 1 and Der p 2 but also Der p 4 and Der p 8 (26). A mixture of these molecular allergens could be the solution for eliminating a risk of house dust mite food syndrome in HDM immunotherapy. Results also depend on the criteria used to select patients, their serological repertoires and the IgE affinities. Considering the severity of allergic reactions to snails, it is necessary to warn HDM allergic patients about a risk of occurrence of associated snail allergy, and to recommend snail avoidance to patients undergoing HDM immunotherapy, even if such snail allergy only occurs in a low percentage of patients.

Avoidance measures: Avoidance measures are the basic means of managing food allergies. Food avoidance seems theoretically easier than aeroallergens avoidance. Never-

theless, recommending food avoidance remains difficult: patients sensitized to a particular food may in fact tolerate this food, since sensitization is not always accompanied with clinical relevance. Moreover, allergic reactions can occur in the future only, even to previously well tolerated foods.

In crustacean allergy, a comprehensive list of the crustaceans where shrimp is assumed to be cross reactive must be delivered to the patients; concerning gastropods, the minor role of tropomyosin in snail allergy makes it possible, in our opinion, to eat them.

In gastropod allergy, recommended avoidance measures are different. Terrestrial and marine gastropods such as snails and limpets must be imperatively avoided. The exclusion of crustaceans does not seem necessary if specific IgEs for r Pen a 1 are negative. In our second observation, the patient had eaten oysters, scallops and mussels without any symptoms. Identical data were shown by Azofra and Lombardero (38), by de la Cuesta et al. (49) in food allergy to gastropods. All their patients tolerated the ingestion of cephalopods and Bivalvia, which belong to other phylogenic lines. Skin tests to squids, prawns, lobsters and clams were negative.

Conclusion

In conclusion, snail allergy appears as a specific entity. The HDM-snail syndrome is different from the HDM-shrimp syndrome in clinical presentations as in immunological findings. Knowledge of taxonomy is important not only to clarify cross reactive allergies between crustaceans and molluscs, but also to propose avoidance measures. To answer the question that gave the paper its title, tropomyosin is unlikely to be the relevant allergen in HDM and snail cross-allergies. Further researches are necessary in order to identify the specific allergens of Dermatophagoïdes responsible for the HDM-snail syndrome.

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