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Specific oral tolerance induction for food. A systematic review

Detection of a novel 20 kDa shrimp allergen showing cross-reactivity to house dust mites

Epinephrine autoinjector prescription in food-allergic adults: symptom-based only or allergen-based also? An italian multi-centre study

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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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Key words

Tropomyosin, house dust mites, snails, shrimps, allergen cross reactivities.

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

Table 2a - Taxonomic relationship of main species where cross reactivity with shrimp tropomyosin was shown or suspected. Modified from Reese et al. (5) and fro m De Witt et al. (42)

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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Specific oral tolerance induction for food. A systematic review

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Key words
Food allergy, oral desensitization

Summary
Background: *Specific oral tolerance induction (SOTI) is a new therapeutic approach in the treatment of persistent food allergy.* ***Objective:*** *The purpose of this article is to systematically review the literature in order to identify, appraise, and synthesize the evidence about SOTI efficacy and safety. Methods: A comprehensive search for citations was conducted on May 2, 2009 using MEDLINE via PubMed. Randomized controlled trials (RCT's) including subjects of any age were considered. All these studies were assessed, discussed in details and evaluated for quality by authors in a standardized independent way.* ***Results:*** *15 clinical trials were found. Of these, six trials met the inclusion criteria: three were open label RCT, three were double blind placebo controlled RCT. Two were conducted using sublingual immunotherapy, four using oral desensitization. Overall, the methodological quality of the studies was sufficient. The mean Jadad score of the studies was 3,33 (range = 2-5). Main characteristics and results of the studies were showed and discussed.* ***Conclusions:*** *SOTI seems to be a possible approach to accelerate the development of tolerance in children affected by food allergy. However, other studies are needed to clarify which is the best treatment and protocol to follow in order to reduce the adverse events and to increase the percentage of success, before thinking that SOTI might be part of the clinical practice.*

Background

Until few years ago, the treatment of food allergies consisted in avoiding the ingestion of food responsible of specific symptoms (elimination diet), in recognizing early symptoms of an allergic reaction in cases of accidental ingestion, and in starting the appropriate emergency therapy. Food allergies' natural history showed that they generally tend to heal spontaneously with time, but tolerance seems to occur faster in cow's milk or egg allergy, and later, or sometimes never, in fish or peanut allergy.
Recently, some studies have demonstrated that food allergies' natural history seems to be less favourable, even for those food allergies considered to have a good prognosis. In a prospective population study (6205 newborns enrolled) Saarinen showed that more than 15% of the 118 children with IgE mediated cow's milk protein allergy (CMPA) did not tolerate milk at the age of 8.6 years (1). More recently, Skripak has carried out a retrospective study on 807 selected children affected by CMPA, demonstrating that tolerance may occur even later: only 42% of children tolerated milk at the age of 8 years, and 79% at the age of 16 years (2). Therefore food allergies persist in some children, and to keep a special diet may become heavier and heavier, with significant psychological and nutritional implications. In clinical practice following an elimination diet over the years

is almost impossible: most of children can occasionally and inadvertently intake food they are allergic to, sometimes going through unexpected and serious reactions. Moreover some foods commonly responsible of food allergies, such as egg and milk, are frequently found in small amounts in food trade, and they are not always declared.
The dogma that a strict elimination diet is the only way to develop tolerance has been recently put in doubt (3). Some studies have demonstrated that recurrence of peanut allergy was more probable in those subjects who broke off the peanut intake after they got tolerance, than in those who continued assuming peanut more regularly (4); this finding suggests that, instead of the strict elimination diet, the continuous administration of the food can facilitate the development and maintenance of tolerance.
Thereby a return of interest in the practice of food desensitization has come out. Subcutaneous desensitization has already been tried several years ago, but it was soon abandoned after the results of Oppenheimer (5) and Nelson (6). In these studies frequent and severe desensitization side effects were shown: this treatment was able to significantly reduce sensitization to peanuts (5 out of 6 treated subjects vs none of the control subjects), but continuing the administration of the therapy became impossible in half of the treated subjects because of recurrences of systemic reactions. In all treated patients administration of epinephrine was needed during the induction phase, and in five out of six of them also in the maintenance phase: the treated subjects received on average 7.7 doses of epinephrine, one of them even received 39 doses!
On the contrary, specific oral tolerance induction (SOTI), proposed and carried out since about 20 years ago (7), seems to be weighted by fewer side effects and therefore is now put under new interest.
SOTI, oral desensitization and oral/sublingual immunotherapy are likewise used by several authors to define this treatment.
However, according with the WHO Position paper, allergen immunotherapy consists in the administration of gradually increasing quantities of an allergen vaccine to an allergic subject, reaching a dose which is effective in ameliorating the symptoms associated with subsequent exposure to the causative allergen (8). On the contrary, allergen desensitization consists in the continuous administration of incremental doses of an allergen or allergenic substance, reaching a total dose needed for drug treatment or food nutrition.
These 2 treatments could differ from each other. In fact they seem to subtend different immunologic mechanism; for example oral desensitization done with drugs does not induce a long-lasting immunological tolerance, probably because it produces an IgE block more than a real change of the immune response (9).
The purpose of this article is to systematically review the literature in order to identify, appraise, and synthesize the evidence of SOTI efficacy and safety, underlieng the possible different approaches.
Throughout this article, the terminus specific oral tolerance induction (SOTI) was used for consistency.

Methods

Search strategy

A comprehensive search for citations was conducted on May 2, 2009 using MEDLINE via PubMed. To reduce the risk of losing relevant studies, searches were not restricted by language of publication, publication type, or study design. Index terms for "oral desensitization and food allergy", "immunotherapy and food allergy" and "specific oral tolerance induction and food allergy" were used.
We have extended our search for relevant studies looking through:
- the Cochrane Controlled Trials Register
- the references of some reviews published on this topic (10, 11)
- the references of the clinical studies identified as relevant
- hand searching of the last two-year indexes of: Allergy, Annals of Allergy Asthma and Immunology, Clinical and Experimental Allergy, Pediatric Allergy and Immunology, The Journal of Allergy and Clinical Immunology, Archives of Disease in Childhood, Pediatrics, The Journal of Pediatrics

Randomized controlled trials (RCT's) on subjects of any age were included. All these studies were assessed, discussed in details and evaluated for quality by the authors of this review in a standardized independent way.
Given the few data on this topic available in literature, we have also included a brief report about all clinical trials found, even if not randomized and controlled.

Exclusion criteria

Studies published only as abstracts were excluded. Moreover, other studies were excluded if drop out during fol-

low up was 20% or more of randomised patients (12) or if the subjects included in the study was lower than 10.

Methodological quality of the included studies

The methodological quality of the included studies was evaluated according to the criteria given by the Evidence-Based Medicine Working Group (12). In each study the following items were analysed: the randomisation process; the efficacy of randomisation (through the analysis of the RCT table where authors summarize patients general characteristics about sex, economic status, age et al.); sample size calculation; definition of end points; drop out or lost during follow up; compliance; intention to treat analysis; placebo concealement; run in. Then the Jadad score was calculated for each study (13).

Results

The search with the term "oral desensitization and food allergy" revealed 82 articles, the search for "immunotherapy and food allergy" gave 917 articles, and the other one for "specific oral tolerance induction and food allergy" gave 54 articles. No other studies were found throughout the other search.
We found 15 clinical trials. Of these, six trials met the inclusion criteria: three were open label RCT, three were double blind placebo controlled RCT. Two were conducted using sublingual immunotherapy (SI), four using oral desensitization (OD) (Tab. 1).
Overall, the methodological quality of the studies was sufficient. All studies had a drop out lower than 20% of randomised patients. Only 1 study (14) achieved the maximum Jadad score; the mean Jadad score of the studies was 3,33 (range = 2-5) (Tab. 2).
A quantitative evaluation was not possible because outcomes and results were described according to different criteria. Only qualitative analysis was performed.
Eight studies were excluded because they were open trials with (15) or without (16-21) a control group, or cases series (22). One RCT was excluded because only 13 children were enrolled, and only six of them were randomized to a double blind desensitization to milk (23). Main characteristics and results of the studied excluded are showed in table 3.

Description of the results of each clinical study

Sublingual immunotherapy

Enrique (24) enrolled 29 allergic adults to hazelnut. After randomization, a sublingual solution containing the major

Table 1 - Main characteristics of the studies enclosed in the analysis

Author	Treatment	Design	Age	Cases (n.)	Controls (n.)	Food	Level of Evidence
Enrique, 2005	Sublingual Immunotherapy	RCT-DB	Adults	12	11	Hazelnut	1b-
Fernandez Rivas, 2009	Sublingual Immunotherapy	RCT-DB	Adults	37	19	Peach (Pru p-3)	1b-
Morisset, 2007	Oral desensitization	RCT	Children (mean age 2.2 yrs and 3.5 yrs for milk and egg, respectively)	27 (milk) and 49 (egg)	30 (milk) and 35 (egg)	Milk/egg	1b-
Staden, 2007	Oral desensitization	RCT	Children (mean age 2.5 yrs)	25	20	Milk / egg	1b-
Longo, 2008	Oral desensitization	RCT	Children (mean age 7.9 yrs)	30	30	Milk	1b-
Skripak, 2008	Oral desensitization	RCT-DB	Children (mean age 10 yrs)	13	7	Milk	1b-

Table 2 - Methodological quality of the studies according to the Jadad score

Jadad score	Enrique	Fernandez-Rivas	Morisset	Staden	Longo	Skripak
Is the study described as randomized?	1	1	1	1	1	1
Was the randomization method appropriate?	0	1	1	0	1	0
Was the study described as double blind?	1	1	0	0	0	1
Is the blindness method described and appropriate?	1	1	0	0	0	0
Is there a description of the lost at follow-up and of the excluded subjects?	1	1	1	1	1	1
Remove one point if the method used to generate the randomization sequence was not appropriate	0	0	0	0	0	0
Remove one point if the study was described as double blind but the method used was not appropriate	0	0	0	0	0	0
Overall Jadad score	4	5	3	2	3	3
Mean Jadad score	**3,33**					

Table 3 - Main characteristics and results of the studies excluded from analysis

Author	Treatment	Design	Age	Cases (n.)	Controls (n.)	Food	Adverse effect	Failure (%)
De Boissieu, 2006	SI	Open	Children (over 6 yrs)	8	-	Milk	12,5	50
Wuthrich, 1996	OD	Open	Adult	16	-	Milk	?	25
Patriarca, 2003	OD	Open controlled	Children and adult (3-55 years)	59	16	Milk (29), egg (15) fish (11) other foods (6)	67,8	16,7
Longo, 2004	OD	Open	Children (mean age 6.8 yrs)	30	-	Milk	100	10
Meglio, 2004	OD	Open	Children (median age 6 yrs)	21	-	Milk	62	14,2
Buchanan, 2007	OD	Open	Children (14-84 months)	7	-	Egg	100	43
Zapatero, 2008	OD	Open	Children (mean age 5 yrs)	18	-	Milk	68,5	11,4
Staden, 2008	OD	Open	Children (3-14 yrs)	9	-	Milk	100	33,3
Caminiti, 2009	OD	RCT (in a subgroup)	Children (mean age 8 years)	3 (+ 7 in open)	3	Milk	80	20

antigen of hazelnut or a placebo was double-blinded administered. The protocol provided for taking 1 drop of the solution, which was to be retained in the mouth at least 3 minutes and then spat out; the number of drops was increased every 15 minutes up to 10 drops per day. The drops contained increasing concentrations of the standardized hazelnut solution, up to 2.6 mg of hazelnut. The highest drops' dose was reached after 4 days, then the patient was discharged and continued the therapy at home taking 5 drops per day. The follow-up consisted in medical visits to be performed every 3 weeks for 3 months. Then an oral food challenge and the dosage of specific IgE level were performed. The aim of this study was to evaluate the possibility of reaching tolerance to hazelnut, and to describe the changes in the maximum tolerated dose by performing a double blind placebo controlled food challenge (DBPCFC) before and 8-12 weeks after treatment. Six out of the 29 subjects enrolled refused to participate. Of the remaining 23, 12 were randomized to the active group and 11 to the placebo group. One patient of the treated group dropped out at the beginning of the study. At the end of the treatment plan, 5/11 (45.4%) of patients vs 1/11 (9%) of controls tolerated an amount of 20 gr. of hazelnut (about 15-20 hazelnut). The average amount tolerated increased from 2.3 gr. to 11.3 gr. in the treated group, while it increased from 3.5 gr. to 4.1 gr. in the placebo group. Three systemic reactions (in the 0.2% of the 1466 doses administered) were described during treatment: one facial urticaria in the placebo group and two urticaria manifestations in 1 patient of the treated group. Local reactions, such as oral pruritus, were described in 109/1466 (7.4%) doses.

Fernandez Rivas (14) enrolled a group of adults with peach allergy, immediate reaction and positive SPT or specific IgE. The diagnosis was made after a positive DBPCFC, which was considered positive after the first clinical sign or after 3 consecutive doses in which an unequivocal oral allergy syndrome was shown. The cumulative dose of Pru p 3 given during DBPCFC was 3249 mcg, corresponding to 200 g of pit-less unpeeled peach.

Of 76 screened patients, 52 were enrolled and randomized in a 2:1 proportion to the group of SI (37 patients) or the control group (19 patients). The immunotherapy schedule comprises a build-up phase of two week in the hospital and a home maintenance phase of six months. During the first phase the treatment was administered sublingually (sublingual-swallow technique) starting with 0,22 mcg of Pru p3 in the first day, increased to 50 mcg in the fifth day. During the home maintenance phase a dose of 10 mcg/die of Pru p3 was administered three days a week. After 6 months the DBPCFC and the allergy-tests were repeated. Forty-nine patients completed the trial, 33/39 of the SI group and 16/19 of the placebo group. One subject was unable to take the dose of 10 mcg, and carried out a maintenance with the dose of 2 mcg. In the placebo group no differences in doses that could determine local or systemic reactions were observed, while doses able to determine local reactions or systemic reactions in the SI group increased of 9 and 3 times respectively. About safety, reactions occurred after the administration of 1356 out of 1480 doses administered. Systemic reactions occurred in 16 cases, none was severe.

Oral desensitization

The study of **Morisset** included a population of 150 children, 60 with cow's milk proteins allergy (CMPA) and 90 with egg allergy (25). The diagnosis of food allergy was made on the basis of the presence of sensitization, Skin Prick Test (SPT) or specific IgE, and confirmed by a positive result to the placebo controlled oral challenge. Only subjects reactive to >60 ml of cow's milk or >965 mg of white raw egg were enrolled to exclude the most sensitive patients. After 6 months of desensitization, SPT or specific IgE and the oral challenge were performed again in order to assess tolerance. The protocol provided a slow administration of cow's milk, starting with 1 ml on the first day, increasing to 20 ml the 1 week, then to 50 ml the second week, to 100 ml the third, to 250 ml the sixth. A similar dose increasing protocol was used for those children with egg allergy.

Among the children with CMPA, 3/27 (11,1%) had to stop OD because of clinical reactions, while the remaining 24/27 (89.9%) tolerated up to 200 ml of cow's milk; in the control group, 12/30 (40%) were still allergic ($P < 0.05$), and 7/12 reached lower cumulative reactive doses than that used in the first DBPCFC, and there were more severe symptoms. The drop out was 10%.

Among children with egg allergy, 15/49 (30.6%) had to stop OD because of clinical reactions, while the remaining 34/49 (69.4%) tolerated up to 4 gr. of yolk and 4 gr. of albumen every other day. In the control group 17/35 (48.6%) of the children were still allergic ($P = 0.1$) and 9/17 had a positive challenge test to lower doses of egg and more severe symptoms. The drop out was 6.6%.

Staden enrolled 45 children with cow's milk and egg allergy (26). The diagnosis of food allergy was made on the basis of the presence of sensitization (SPT or specific

IgE) and confirmed by a positive result to DBPCFC. Children were randomized in two groups, one received OD (25 children, 14 allergic to cow's milk, 11 to egg), and one received placebo (20 children, 10 allergic to cow's milk, 10 to egg). All children were reassessed by DBPCFC after 18-24 months of treatment. Moreover, children who underwent OD were reassessed after a period of secondary elimination diet of 2 months, in order to evaluate the persistence of tolerance. The OD was performed at home with lyophilized milk or egg, with starting doses of 0.02 mg of milk proteins and 0.006 mg of egg proteins; the doses were then slowly (in about two months) increased up to 8250 mg of milk (250 ml) or 2800 mg of egg (half of an egg). Then the patient continued to assume a minimum of 100 ml of milk or around $^1/_2$ an egg. At the end of the study (after an average of 21 months), 16/25 (64%) children tolerated milk: of these, 9 (36%) tolerated a free diet, 4 (16%) tolerated only low doses of milk, and 3 (12%) had new reactions after the secondary elimination diet, while 9 (36%) continued to be allergic. In the control group 7/20 (35%) developed tolerance. All children treated with OD didn't have side effects during the study. Twenty-one children showed mild symptoms of allergy, 4 had more severe symptoms and required the administration of steroids and antihistamines.

In **Longo's** study (27) 97 children with CMPA and history of severe allergic reactions and of specific IgE levels > 85 KU/l were selected. CMPA was diagnosed by DBPCFC performed at the beginning and at the end of the study. Sixty children were then enrolled and randomized to OD (30 children) and placebo (30 children). Children started treatment in the hospital were they were admitted for 10 days: here very diluted and gradually increased doses of cow's milk were administered, up to achieve the administration of 20 ml of milk. Then the treatment was continued at home, and doses were increased of 1 ml every other day to reach the maximum dose of 150 ml. At the end of the first 10 days, 9/30 (30%) reached the dose of 20 ml, while the remaining reached lower doses of milk because of frequent allergic reactions, which obliged them to change or stop the protocol. After 1 year, 11/30 (36%) children achieved tolerance for 150 ml and a free diet, 16/30 (54%) tolerated lower doses of cow's milk (between 5 and 150 ml), while 3/30 (10%) had to stop OD. All controls did not tolerate milk at the DBPCFC performed after 12 months. All children virtually showed reactions during OD. During the first 10 days in the hospital 4 (13.3%) children required the administration of IM epinephrine and 18 children aerosolised epinephrine. During the protocol phase performed at home 4 children required epinephrine. 20% children of the control group had clinical reactions during the study: all of them were mild.

Skripak is the author of the only DB-RCT with milk enrolling 20 children with CMPA (28). Children with a history of anaphylaxis or severe-persistent asthma or who had required intubation were excluded. The diagnosis of CMPA was made by DBPCFC at the beginning and at the end of the study. After recruitment 2/3 (n. 13) of children were randomized to the OD and 1/3 (n. 7) to placebo. The treatment began with the dose of 0.4 mg of milk protein with daily increases up to 50 mg (1.5 ml); the increases were made every 1-2 weeks in order to reach the dose of 500 mg (15 ml). Then this dose (15 ml) was continued for other 13 weeks. After 23 weeks DBPCFC was again performed. Those children who tolerated after treatment less than 2540 mg (about 70 ml) were again put on diet. The median maximum dose tolerated before the OD was 40 mg (1.2 ml) in both groups (OD group and placebo group), and it increased significantly up to 5100 mg (150 ml) in the OD group. At the end of the study 4/13 (30.7%) of the OD group were able to take the full dose of 8140 mg (245 ml) of milk: two children had a mild reaction and 2 did not have any reaction. 6/13 (46.1%) children of the OD group tolerated doses above 70 ml, but less than 150 ml; 3/13 (23%) did not tolerate doses of 70 ml, whereas all patients in the placebo group reacted at 1.2 ml.

Concerning the safety of the study, the median frequency of reactions was 35% in the treated group and 1% in the placebo group: most reactions were local, 8% interested the low respiratory tract and in 4 cases epinephrine was needed.

Discussion

Up to now four RCTs on OD and two on SI are available. These studies are somewhat different because of the population enrolled (children or adults, severe allergies or mild allergies), the food causing allergy (milk, egg, peach, hazelnut) the protocol (rush, slow, rush phase followed by a slow phase), the way of SOTI administration (oral, or sublingual-swallow or sublingual-spit), and food doses administered (maximal -i.e. the regular intake-, sub-maximal -very less than the regular intake-). Moreover, put all together, these studies include only about 200 subjects. Therefore, to draw precise conclusions is rather difficult.

We can say that 4 are the events that can happen after performing a SOTI program:

Table 4 - Main outcome of RCT's of oral desensitization for milk.

Author	Age	Popolation	Tolerance	Partial tolerance	Non responder	Tolerance in controls
Morisset, 2007	(mean age 2.2 yr)	Less sensitive patients	24/27 (89.9%)		3/27 (11.1%)	18/30 (60%)
Longo, 2008	(mean age 7.9 yrs)	Only severe cow's milk allergy	11/30 (46%)	16/30 (54%)	3/30 (10%)	0/30 (0%)
Skripak, 2008	(mean age) 10 yrs	Excluding severe Cow's milk allergy	4/13 (30,7%) (> 250 ml)	6/13 (46,1%) (> 70 ma < 250 ml)	3/13 (23%) (< 70 ml)	0/7 (0%)

a) to reach a full tolerance, or tolerance to regular intake of the food. Most of the studies have showed that both SI and OD can accelerate the development of complete tolerance, with respect to the elimination diet. If we consider only the RCTs on cow's milk (Tab. 4), this goal seems reached at any age. Even if SOTI is more effective in the first years of life, it is probably more useful over the age of 5-6 years, when spontaneous tolerance happens more rarely. Besides, SOTI appears to be independent from the severity of cow milk allergy;
b) to reach a partial tolerance, or tolerance to lower amount of food than the regular intake. All the studies were consonant in demonstrating that both SI and OD increases the average amount of food allergen tolerated. This result should be considered important, as far as it would allow to safely intake food containing traces of allergen;
c) to reach a transient tolerance, which might disappear without a regular intake of the food. This event was first described by Rolink-Werninghouse (29) and was then confirmed by Staden. We remind that other factors, such as physical exercise, can similarly make disappear tolerance, although transiently (30);
d) to failure desensitization: SOTI must be stopped because of severe and/or repeated allergic reactions. This eventuality seems to occur only in OD studies, in about 10-20% of cases of OD for Cow's Milk Allergy.
It must be underlined that not all children successfully treated with SOTI continue to take milk over the years. Meglio (31) has reported the results obtained after a 5 years follow-up of 20 previously enrolled (17): the rate of children who still resulted tolerant to milk lowered from 85% to 70% because some children stopped taking milk after a rebound of symptoms.
With regard to safety, all studies have reported the occurrence of adverse events during SOTI, in variable percentages from 45,4% to 100%: these events are probably related to the severity of the allergies, SOTI treatment, the protocol used and the food given. Severe reactions and epinephrine administration are reported in variable percentage from 0% in SI studies, to 30,7% in OD studies, conducted both with maximal and sub-maximal protocol. Subjects unable to complete SOTI due to repeated and often severe allergic reactions vary from 0% in SI studies vs 10% to 36% OD studies (Tab. 5).
In conclusion, SOTI seems to be a possible approach to accelerate tolerance development in children affected from food allergy. However, other studies are needed to clarify which is the best treatment and protocol to follow in order to reduce the adverse events and to increase the percentage of success, before thinking that SOTI might be part of the clinical practice.
It must be stressed that in most of the studies the initial phase have been performed in hospital and that all treatment protocols have been performed in highly supervised research settings. Mortality rate for food anaphylaxis is a relatively rare event, which is estimated approximately in 1/154 (32) - 1/675 (33) episodes, and which seems to occur even if appropriate therapy has been performed. Therefore, given that so far - also considering the open studies- only few hundreds of children have been treated with SOTI, we agree with the recommendation of limiting the spread of such therapy, limitating it to selected allergologic centres (34).

Table 5 - Adverse effect and failure of RCT studies

Author	Treatment	Protocol	Adverse effect (%)	Systemic reactions	IM epinephrine administration	Unable to complete OD protocol
Enrique, 2005	Sublingual Immunotherapy (Sublingual/split)	Rush phase followed by slow phase	45,4	0.2% (3 reactions/ 1466 doses)	0	0% (20 g of hazelnut)
Fernanez Rivas 2009	Sublingual Immunotherapy	Rush phase followed by slow phase	100	16 systemic reactions in 5/37 (13.5%) patients	0	0 (0%)
Morisset, 2007	Oral desensitization	Slow	?	0 (0%)	NS	3/27 (11,1%)
Staden U, 2007	Oral desensitization	Slow	100	4/25 (16%) of children had moderate side effect	0	9/25 (36%)
Longo, 2008	Oral desensitization	Rush phase followed by slow phase	100	NS	5	3 (10%) (16.6%)
Skripak, 2008	Oral desensitization (sub-maximal)	Slow	35% (median frequency for total reactions in each partecipant)	17,7 (Median frequency of 1% of 177 doses per partecipant administered)	4/13 (30.7%)	0 (0%)

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Detection of a novel 20 kDa shrimp allergen showing cross-reactivity to house dust mites

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Key words

Tropomyosin, 20 kDa allergen, crustacean shellfish allergy, immunoblotting, cross-reactivity

Summary

Background: *Allergy to crustacean shellfish is one of the most common IgE-mediated food allergies, and tropomyosin has been identified as the major allergen. However, not all subjects affected by this allergy are IgE-positive to tropomyosin.* ***Aims:*** *To evaluate whether sera of patients with shrimp allergy but negative for tropomyosin react to other allergen(s); and to evaluate the role such allergen(s) may play in cross-reactivity between crustaceans and house dust mites (HDMs).* ***Methods:*** *Three different pools of sera-one from subjects with shellfish allergy and HDMs positivity, but negative for recombinant and native tropomyosin (rPen a 1 and nPen m 1) (Pool 2); a second from subjects with tropomyosin and HDMs positivity (Pool 1); and the last from subjects allergic only to HDMs (Pool 3) were submitted to immunoblotting. Subsequently, a 20 kDa protein- enriched fraction of shrimp extract was used at two different concentrations (10 and 100 μg/mL) to pre-absorb the Pool 2 serum and to evaluate, by ELISA assay, the level of inhibition on shrimp and HDMs-coated wells, respectively.* ***Results:*** *The Pool 2 serum showed IgE reactivity against a 20 kDa component. Its pre-absorption with an enriched fraction of 20 kDa protein caused an inhibition of 56% in IgE binding to shrimp extract at a concentration of 100 μg/mL, and of 14% and 35% to HDMs extract at concentrations of 10 and 100 μg/mL, respectively, as measured by ELISA assay.* ***Conclusions:*** *The 20 kDa component seems to be a new crustacean allergen and it could play a role in cross-reactivity with HDMs.*

Abbreviations:
HDM, house dust mites; ELISA, enzyme-linked immunosorbent assay; SPT, skin prick tests

Introduction

Allergy to crustacean shellfish (shrimp, crab, lobster) is one of the most common IgE-mediated food allergies and is often associated with severe reactions. Tropomyosin, a highly conserved and heat-stable myofibrillar protein of 35-38 kDa has been identified as the major allergen from crustaceans (1). Moreover, many studies have suggested that tropomyosin is also present in house dust mites (HDMs) (2), cockroach (3), squid (4), and other molluscs (5) and it may be responsible for cross-reactivity among different shellfish, between cockroach and HDMs, and between crustaceans and HDMs. For this reason, the tropomyosin molecule can be considered a pan-allergen of invertebrates (6).

In the last few years, *in vitro* assays for detection of specific IgE against recombinant tropomyosin from *Penaeus*

aztecus (rPen a 1) or against native purified tropomyosin from *Penaeus monodon* (nPen m 1) have been developed, and are increasingly used for molecular diagnosis of shellfish allergy in clinical practice. However, in our experience, about 20% of subjects with HDMs sensitisation as confirmed by *in vitro* and *in vivo* assays and showing allergic symptoms after crustacean ingestion resulted IgE negative to rPen a 1 or nPen m 1. This observation suggests that other components might play a role in the cross-reactivity between crustaceans and HDMs. The aim of our study was, therefore, to evaluate whether the sera of these patients were able to recognize allergen(s) other than tropomyosin, and whether such allergen(s) play(s) a role as a cross-reactive allergen between crustaceans and HDMs.

Material and methods

Sera of 21 patients with both SPTs (ALK Abellò, Madrid, Spain) and IgE (Phadia, ImmunoCAP, Uppsala, Sweden) positivity for HDMs and shrimp extract were also tested for IgE against recombinant (rPen a 1, Phadia, ImmunoCAP) and natural (nPen m 1, DPC, Immulite 2000, Siemens, Erlangen, Germany) tropomyosins. Five of the sera scored negative for both these types of tropomyosins; they were pooled (Pool 2) and tested by immunoblotting (IB) in comparison with two other pools: a pool of five sera selected from the tropomyosin IgE-positive patients (Pool 1), and a pool of five sera from patients who were IgE-positive only for HDMs (Pool 3).
Of the five patients from Pool 2 with crustacean allergy but negative for tropomyosins, two presented an oral allergic syndrome (OAS) as the clinical manifestation, while one presented both OAS and rhinitis and two presented urticaria-angioedema. Unlike patients with positivity for tropomyosins (Pool 1), none presented asthma or anaphalaxis. The limited number of subjects, however, does not allow for defining significant differences in the clinical presentation of the two populations.

Preparation of crude shrimp extract

Peeled shrimps were homogenized and submitted to an aqueous extraction in 0.1M phosphate-buffered saline, pH 7.4 (PBS) by shaking for 16 hours at 4 °C. The suspension was centrifuged at 3000 g for 30 minutes at 4°C and corresponding supernatant was filtered through a 0.45-μm membrane. Protein content was 3.2 mg/ml as measured according to Bradford (7) by the Bio Rad method (BioRad, Milan, Italy).

Purification of tropomyosin and of a 20 kDa component from shrimp

Peeled shrimps were snap-frozen and ground in a mortar. 5g of the resulting powder were added to 50 ml of extraction buffer (1 M KCl and 0.5 mM DTT, pH 7.0). The mixture was left for 16 hours at room temperature. After centrifugation at 5000*g* for 15 minutes, the supernatant was cooled to 4°C and its pH adjusted to 4.6 with HCl 1M, leaving the sample under stirring for 30 minutes until a precipitate (representing the tropomyosin-enriched fraction) was obtained. The precipitate was then dissolved in extraction buffer, and both the precipitate and the supernatant (representing the 20 kDa-enriched component) were dialyzed against PBS before use.

Immunoblotting (IB) and IB-inhibition

The three different pool samples were first checked on shrimp extract by IB under reducing conditions according to Towbin (8). IB and IB inhibition experiments were performed as previously described (9). Briefly, shrimp extract was mixed with LDS sample buffer (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) and 5% β-mercaptoethanol. The sample was heated at 100°C for 5 minutes before being submitted to electrophoresis run (25 μg/lane) in a 10% polyacrilamide precast gel (Nupage Bis-Tris) at 180 mA for 1 hour. The resolved proteins were transferred onto a nitrocellulose membrane and left to rest for 1 hour. The membrane was then saturated with 0.1 mol/L Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 hours at 4°C with 700 μl of the serum pool and 500 μl of saturation buffer. After three consecutive washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies goat serum (Biospacific, Emeryville, CA) diluted to 1:3500 in saturation buffer, using an ECL western blotting kit (Amersham, Milan, Italy). In inhibition studies, pool 1 was pre-absorbed with 100 μg of an enriched fraction of tropomyosin obtained as previously described.

ELISA inhibition assay

ELISA inhibition assays were performed as previously described (10). For the coating phase, two micrograms/100 μl (coating buffer: 15 mmol/L Na_2CO_3 and 35 mmol/L

$NaHCO_3$, pH 9.6) of mite extract or 2μg/100 μL of shrimp extract were used per well of 96-microtitre plates (Maxisorp Nunc, Roskilde, Denmark). After washings, wells were saturated with 2% bovine serum albumin (BSA) in PBS for 2 hours at room temperature, and then washed again before being dried until use. In parallel, for pre-absorption experiments, 100 μL of Pool 2 were added to tubes containing one of the following: for inhibition of IgE response to shrimp extract, 100 μL of 20 kDa enriched fraction (100 μg/mL), as inhibitor, or 100 μL of PBS, as control; and for inhibition of IgE response to HDMs, 100 μL of 20 kDa-enriched fraction, at two different concentrations (10 and 100 μg/ml); 5 μg of HDMs extract, 5 μg of an unrelated extract (Grass), as inhibitors, or 100 μL of PBS, as control. Pre-absorption was prolonged for 2 hours at room temperature. A 100-μL of sample from each tube was collected and added to the corresponding well and incubated for 2 more hours. After washings, specific IgE was detected by a peroxidase-conjugated anti-human IgE from goat (diluited 1:1500) (Biospacific) and the absorbance values were read spectrophotometrically at 450 nm. The percentage of inhibition was calculated on the basis of the absorbance value of the corresponding control.

Results

Immunoblot analysis of Pool 1 serum showed strong reactivity against components of the shrimp extract, ranging between 30 and 43 kDa (Fig. 1, line 1). In particular, a component of about 38 kDa, corresponding to tropomyosin, was recognized as shown by the almost complete disappearance of such reactivity when the serum pool was pre-incubated with 100 μg of tropomyosin-enriched fraction (Fig. 2, lane 2). In contrast, subjects with positivity for HDMs and shrimp extract, but negative for rPen a 1 and nPen m 1 (pool 2), showed IgE reactivity mainly against the 20 kDa component (Fig. 1, lane 3). The preincubation of this serum pool with the 20 kDa protein-enriched fraction, at a concentration of 100 μg/mL, caused an inhibition of 56% of IgE binding to shrimp extract, as shown by ELISA inhibition tests (Fig. 3, column 1). Even if the inhibition of IgE binding resulted incomplete, probably because of an insufficient amount of inhibitor, our experiments indicate that the 20 kDa component could be a new shrimp allergen. In addition, IgE binding to HDM extract of the same pool after preincubation with two different concentrations of en-

Figure 1 - *Lane 1*, SDS-PAGE of shrimp extract; *Lane 2*, IgE-reactivity of pool 1; *Lane 3*, IgE-reactivity of pool 2; *Lane 4*, IgE-reactivity of pool 3; *Lane 5*, IgE-reactivity of non atopic serum

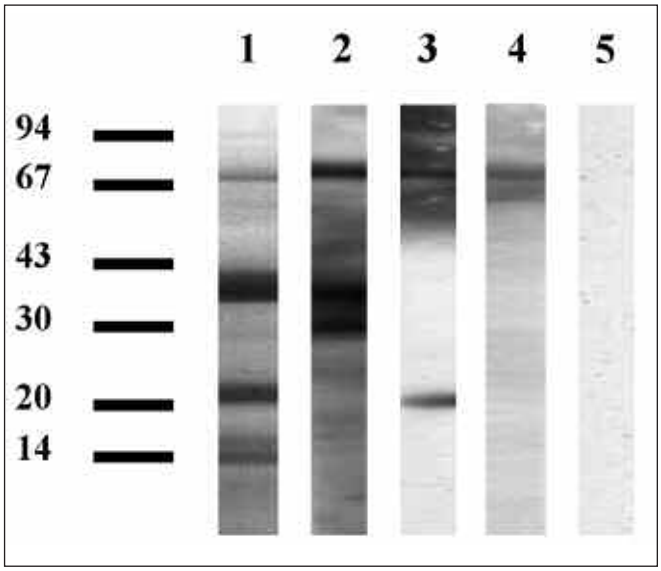


Figure 2 - *Lane 1*, IgE-reactivity of pool 1 on shrimp extract; *Lane 2*, inhibition with 100 μg of a fraction enriched of tropomyosin

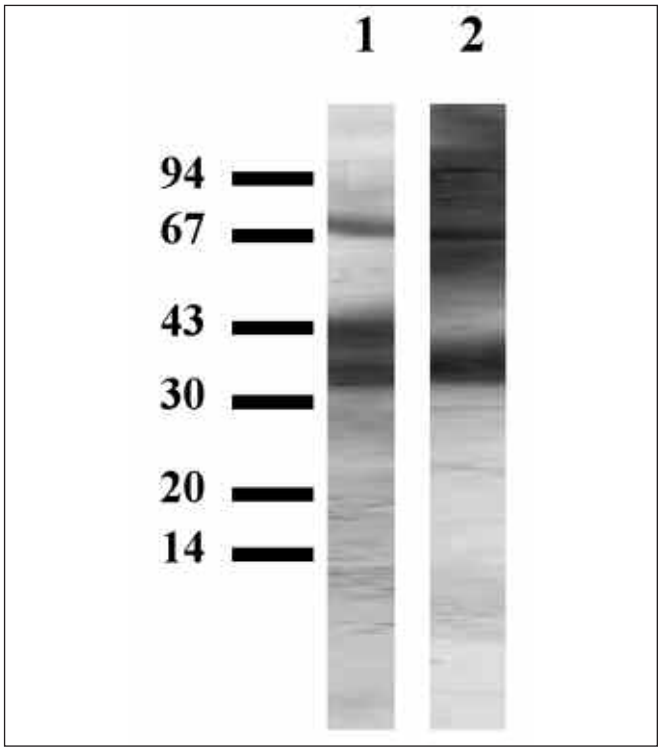
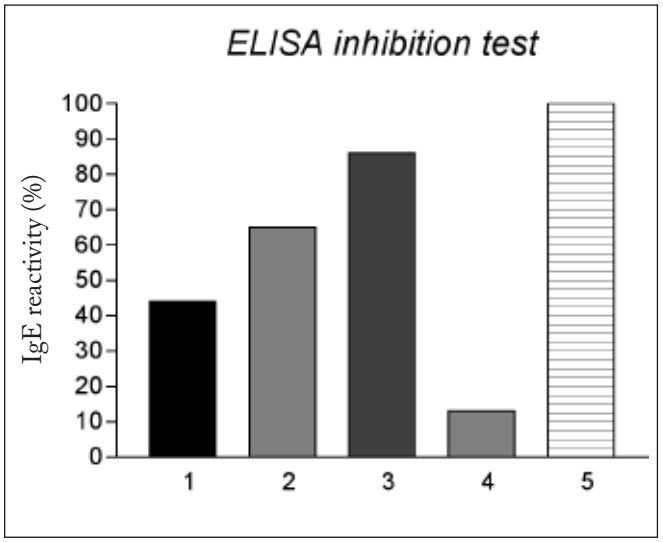


Figure 3 - IgE reactivity to shrimp extract (1) after pre-absorption of pool 2 with a 20 kDa enriched fraction (100 μg/mL). IgE reactivity to HDMs extract after pre-absorption of pool 2 with a 20 kDa enriched fraction at (2) 100 μg/mL and at (3) 10 μg/mL concentrations; (4) with 5 μg of HDMs extract and (5) with 5 μg of an unrelated Grass extract



riched fraction of 20 kDa protein (10 μg/mL and 100 μg/mL) was partially inhibited in a dose-dependent manner (14% and 35%, respectively), suggesting that this allergen is also present in HDM extract.

Discussion

In this study we demonstrated that cross-reactivity between HDMs and crustaceans might also be due to the presence of a 20 kDa component of shrimp extract. Such a component seems involved as a cross-reacting molecule only in a subset of patients with crustacean allergy. The results are very similar to those reported in the recent paper of Shiomi *et al*. (11). In their study, 8 out of 16 sera from crustacean-allergic patients showed reactivity against a 20 kDa allergen, identified as a sarcoplasmic calcium-binding protein (SCP), and probably limited to shrimp and crayfish. More recently, Ayuso *et al.* (12), on 21 out of 38 sera of patients with immediate allergic reaction to shrimp, showed an IgE binding to a 20 kDa shrimp component that they identified as a myosin light chain (MLC) called Lit v 3.0101. They also demonstrated that the amino acid sequence of MLC is 66% similar to cockroach MLC of *Blatella germanica* (Bla g 8). On the basis of the molecular weight deduced by our IB experiments, we could speculate that SCP, Lit v 3.0101 and our 20 kDa component might be the same molecule. More studies on 20 kDa component at the level of amino acid sequence must to be performed to confirm this possibility.

Moreover, we observed that pre-incubation of Pool 2 with an enriched fraction of 20 kDa component inhibited the IgE binding to both shrimp and HDM extracts, although inhibition was less for HDM than for shrimp (35% vs 56%, fig.3). Our observations confirm, however, the presence of the 20 kDa protein in HDMs, and might explain how all patients allergic to crustaceans and positive for 20 kDa protein – similar to findings reported in the study by Ayuso *et al.* – also present sensitization to HDMs.

In conclusion, we identified a new allergen correlated with crustacean allergy and HDM cross-reactivity. Since some patients are positive only for this allergen, it is important to add it to the component-resolved diagnosis methods for shellfish allergy to avoid the loss of some positivities.

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Epinephrine autoinjector prescription in food-allergic adults: symptom-based only or allergen-based also? An italian multi-centre study

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Key words

Food allergy, epinephrine, anaphylaxis

Summary

Background: *Epinephrine is the treatment of choice for acute food-allergic reactions but existing guidelines state that it should be prescribed uniquely to patients who already experienced at least one food-induced anaphylactic episode.* ***Objective:*** *We investigated whether in Italy epinephrine auto-injector is prescribed uniquely following the existing guidelines only, or is allergen-informed as well (i.e., based on the potential risk associated with sensitization to certain food allergens), and hence preventive.* ***Methods:*** *1110 adult patients (mean age 31 years; M/F 391/719) with food allergy seen at 19 allergy outpatient clinics were studied. Patients with a history of probable anaphylaxis were identified. Subjects were classified as having primary (type 1) and/or secondary (type 2) food allergy and were divided into several subgroups based on the offending allergen/food. Epinephrine prescriptions were recorded and analyzed both as a whole and by sensitizing allergen.* ***Results:*** *Epinephrine was prescribed to 138/1100 (13%) patients with a significant difference between subjects with type-1 and type-2 food allergy (132/522 [25%] vs 6/629 [1%]; p< 0.001). The epinephrine group included most patients with a history of anaphylaxis (55/62 [89%]) or emergency department visits 106/138 (77%). In some specific subsets, namely fish-, tree nuts-, and lipid trasfer protein (LTP)-allergic patients, epinephrine was prescribed to*

patients without a history of systemic allergic reactions. ***Conclusions****: Italian allergy specialists prescribe epinephrine auto-injectors both on the basis of clinical history of severe reactions and on a critical analysis of the hazard associated with the relevant protein allergens, which suggests a good knowledge of allergens as well as acquaintance with the guidelines for prescription of emergency medication.*

Introduction

Foods are unquestionably one of the main causes of anaphylaxis worldwide (1). As a rule, unless there is sensitivity to labile plant food allergens, food-allergic patients are recommended the strict avoidance of the ingestion of potentially offending food(s). However, allergen avoidance is often difficult due to several reasons. Some allergen proteins are widespread and cross-reacting, which poses the patient at risk of allergic reactions following the ingestion of foods that are completely different from the offending one and hence considered harmless. Further, contamination of safe foods may occur by the use of kitchen utensils both at home and at public places such as restaurants or bars (2). Finally, the presence of a certain food is not always clearly specified on product labels or on restaurant menus. All these situations pose a considerable risk of accidental exposure to the offending allergen (3).

Epinephrine administration remains the milestone of treatment of acute allergic reactions (4), and food-allergic individuals at risk of anaphylactic reactions should be always prescribed an epinephrine auto-injector and given proper instructions about its correct use (5). These patients should always carry the device since it has been shown that allergic reactions may occur at sites considered as safe such as home, school, workplaces, and hospitals (6).

Data about the implementation of existing guidelines about epinephrine prescription in peripheral non-academic outpatients clinics are very few in medical literature. A recent study performed in the Netherlands showed that prescription of emergency medication did not fully reflect the potential severity of adverse reactions in patients allergic to plant-derived foods (7).

In Italy, during the last 2 years, epinephrine auto-injector has become free of cost for allergic patients and completely reimbursed by the NHS if the drug is prescribed by specialist allergologists working in public hospitals and outpatient clinics on the basis of a defined diagnosis of food allergy. This implies that epinephrine should be prescribed uniquely to food-allergic patients who already experienced at least one anaphylactic episode but not to subjects sensitized to potentially harmful allergens reporting reactions other than anaphylaxis, or without a clinical history of adverse reactions to foods. In other words, the Italian NHS presently guarantees a preventive treatment uniquely in a proportion of the population potentially at risk. The present study aimed to investigate in a multi-center survey whether epinephrine auto-injector prescription in Italy follows the existing guidelines only (i.e. if only patients with a history of food-induced anaphylaxis are prescribed the drug) or it is also allergen-informed preventive (i.e. based on a risk assessment depending on the chemical/physical characteristics of sensitizing allergens).

Patients and methods

Patients

1110 (4%) patients older than 12 years (mean age 31 years [range 12-79]; M/F 391/719] diagnosed as having IgE-mediated food allergy out of 25813 subjects first visited at 19 allergy outpatient clinics scattered throughout Italy from January 1st to December 31st, 2007 were included in this study.

Food allergy was diagnosed only in the presence of an unequivocal history of adverse reactions occurring some minutes up to 2 hours after the ingestion of the offending food(s) confirmed by a clear-cut positive SPT and/or by elevated circulating food-specific IgE. Clinical symptoms suggesting food allergy included oral allergy syndrome (defined as the rapid onset of itching of the oral mucosa with or without angioedema of lips and tongue)(8), acute generalized urticaria with or without angioedema (9), and/or anaphylaxis (10).

Definition of anaphylaxis

The doctors of participating centres reviewed the medical recordings of patients reporting suspect anaphylaxis. Following previously published clinical criteria (11) an ana-

phylactic reaction was considered highly likely when any of the following 3 criteria were fulfilled:

1. Acute onset of an illness involving skin, mucosal tissue, or both plus at least 1 of the following: a) respiratory compromise; b) reduced blood pressure or associated symptoms of end-organ dysfunction (collapse, syncope, incontinence).
2. Rapid onset after exposure to a likely allergen for that patient of 2 or more of the following: a) Involvement of skin or mucosal tissue; b) respiratory compromise; c) reduced BP or associated symptoms; d) persistent gastrointestinal symptoms.
3. Systolic BP < 90 mmHg or > 30% decrease from baseline BP after the ingestion of a known allergen for that patient.

In-vivo and in-vitro tests

Hypersensitivity to food allergens was detected by commercial food extracts (ALK-Abello, Spain). The series tested in all patients with suspect food allergy in all participating centres included egg white, egg yolk, cow's milk, shrimp, codfish, wheat, maize, soybean, peanut, sunflower seed, bean, walnut, hazelnut, tomato, carrot, orange, peach, celery, almond, sesame seed, kiwi, and banana.

In the case of suspect allergy to foods not included into this series, commercial extracts from the same or other companies (where available) and/or fresh foods were used for skin testing. Anisakis simplex SPT (ALK-Abello) was tested in patients reporting systemic allergic symptoms following the ingestion of raw or underdone fish and scoring negative on SPT with fish extract. Fresh foods were tested by the prick-prick technique. All SPT were carried out on the volar side of the forearm using disposable prick lancets (ALK-Abello). SPT with saline and histamine 10 mg/ml were used as negative and positive control, respectively. Readings were taken at 15 minutes; wheals with a mean diameter > 3 mm were considered positive (12).

In some centres hypersensitivity was confirmed also by specific IgE measurements (Uni-CAP, Phadia Sweden). In these cases specific IgE levels > 0.35 kU/l were regarded as positive.

Although a recent study on patients sensitised to stable food allergens, namely lipid transfer protein, showed that double-blind, placebo-controlled food challenges (DBPCFC) can be carried out quite safely (13), in view of the severity of reported allergic reactions and of the limited acquaintance of many of the participants with oral food challenges, due to the fear of possibly severe adverse reactions, diagnosis of food-induced anaphylaxis was not confirmed by DBPCFC.

Classification of patients sensitised to plant food allergens

In view of the extremely large variety of plant-derived foods possibly involved in allergic reactions, in order to uniform the recording of clinical data by participating centres, patients with plant-food allergy were distinguished in two main groups:

1. Type 1 (Primary) food allergy. This category included the following subgroups of patients with primary sensitisation to plant-derived foods.

a) Lipid transfer protein (LTP). This group included all patients allergic to LTP irrespective of the offending food(s). LTP hypersensitivity was diagnosed in the presence of a positive SPT with commercial peach extract (ALK-Abello, Spain). Previous studies showed that this peach extract virtually contains only LTP at a concentration of 30 μg/ml, and that a positive SPT with this extract may be used as a clinical marker of sensitization to this protein (14, 15) with only minor exceptions (16). Offending foods for LTP-allergic patients included all *Rosaceae* (apple, pear, peach, cherry, plum, apricot, medlar, almond, strawberry), tree nuts, maize, rice, beer, and grapes (17).

b) Tree nuts. This group included all patients allergic to tree nuts (including hazelnut, walnut, Brazil nut, pine nut, almond, pistachio, chestnut, and cashew) but not to LTP. Diagnosis was based on a positive SPT with commercial extract (when available) or with fresh offending nut in the absence of skin reactivity to both commercial peach extract and birch pollen extract. Previous studies showed that commercial walnut extract contains only stable allergens and can therefore be used as a means to rule out sensitisation to labile allergens homologous to pollen proteins (15).

c) Seeds. Patients allergic to one or more seeds (such as sesame, sunflower, poppy, or other seeds) but not sensitised to tree nuts were included in this group.

d) Legumes. This group included subjects allergic to one or more legumes including peanut, bean, string bean, pea, chickpea, lupine, and lentil.

e) Cereals: This subgroup included patients with clinical allergy to cereals (wheat, barley, maize, rice, rye) not sensitised to LTP, as shown by negative SPT with commercial peach extract.

f) Kiwi: this category included subjects with single kiwi allergy.
g) Allergy to single vegetable foods. This category included all remaining plant-derived foods that caused isolated allergic reactions in single individuals in the absence of birch pollen hypersensitivity..

Type 2 (Secondary) food allergy:
This category included patients with plant-food allergy caused by cross-reactivity to a primary sensitizer, and included the following subgroups:
a) Pollen-food allergy syndrome: This subgroup included patients either mono-sensitised to birch pollen (Bet v 1) or showing sensitization to all seasonal airborne allergens (and, hence possibly sensitised to Profilin). Since both Bet v 1-homologous proteins and profilin are heat- and pepsin-labile allergens, a pollen-food allergy syndrome was diagnosed if patients reported good tolerance of the offending foods if these were cooked or otherwise processed, and/or in the presence of positive SPT with fresh offending foods but negative SPT with commercial extract of the same foods.
b) Latex-fruit allergy syndrome. Patients primarily sensitised to natural rubber latex with a history of allergy to foods known as being potentially cross-reacting, such as chestnut, avocado, kiwi, papaya, and banana.
c) Mugwort-celery- spice syndrome. Patients primarily sensitised to mugwort with a history of allergy to potentially cross-reacting vegetable such as celery, fennel, anise, bell pepper, and other spices.

Patients sensitised to non-plant foods were grouped by allergen. For instance, patients allergic to shrimp, squid, octopus or shellfish were considered as possibly sensitised to tropomyosin and grouped together (group "shrimp"); similarly, those allergic to different fishes were grouped together, as were those allergic to different meats, and so on.

Study approval and informed consent

Since this observational study was carried out on patients spontaneously presenting at the different centres for routine evaluation and epinephrine was prescribed based uniquely on the basis of doctors' experience, no institutional review board was needed. As all other subjects attending allergy clinics in Italy, study patients gave an informed oral consent to the use of their data in an anonymous form for study purposes.

Statistics

Proportions were compared by chi-square test with Yates' correction. Means were compared by two-tailed Student's t-test. Probability levels < 5% were considered statistically significant.

Results

The overall prevalence and the clinical features of the different types of food allergy, along with the rate of epinephrine auto-injector prescription, are shown in table 1. A total of 522 patients had a type-1 food allergy; in these patients fruits and vegetables represented by far the most frequently offending foods (393/522; 75%). Among animal-derived foods, shrimp was the most frequently offender. Notably, the large majority of patients with type 1 food allergy had a clinical history of systemic symptoms following exposure to offending food, the only exception being kiwi, which induced local symptoms in a majority of cases.
Type-2 food allergy was diagnosed in 629 cases. The pollen-food allergy syndrome represented the most frequent type-2 food allergy (98% of cases), whereas both the latex-fruit allergy syndrome and the mugwort-celery-spice syndrome were very uncommon. The large majority of those with pollen-food allergy syndrome had only mild local symptoms and reported systemic symptoms only in a very little proportion of cases (3%), whereas both the latex-fruit allergy syndrome and the mugwort-celery-spice syndrome were frequently associated with systemic symptoms.
Fifty-one patients showed a type 1 + 2 food allergy due to co-sensitization to pollen related food allergens and to primary food allergens (plant-derived foods in most cases) following the criteria adopted in this study. Not surprisingly, most of these cases were observed in the northern part of the country where birch pollen allergy is rather common. These subjects were analyzed as they had a primary (type-1) food allergy only.
Epinephrine auto-injectors were prescribed to 138/1100 (13%) patients (M/F 55/83; mean age 31.4 years, range 12-72 years) with a statistically significant difference between subjects with type 1 and type 2 food allergy (132/522 [25%] vs 6/629 [1%], respectively; $p< 0.001$). The epinephrine group included the large majority of patients with a history of food-induced anaphylaxis (55/62 [89%], table 1). The 7 subjects that were not prescribed epinephrine despite a clinical history of anaphylaxis in-

Table 3 - Offending foods, prevalence of systemic reactions (other than anaphylaxis) and of anaphylaxis, and rate of epinephrine auto-injector prescription in 1110 food-allergic Italian adults

		Clinical history		Auto-injector prescription			Prescriptions	
Allergen	No.	U/A	Anaphylaxis	Total	Anaphylaxis	ER	Missing	Exceeding
Fish	22	18 (82%)	1 (5%)	6 (27%)	1	2	0	4
Shrimp	68	61 (90%)	10 (15%)	14 (31%)	8	14	2	0
Milk	13	9 (69%)	1 (8%)	4 (31%)	1	2	0	2
Egg	17	13 (76%)	1 (6%)	2 (12%)	1	1	0	1
Meat	4	3 (75%)	1 (25%)	1 (25%)	1	1	0	0
Snail	2	2 (100%)	0	0 (0%)			0	0
Anisakis	3	3 (100%)	1 (33%)	1 (33%)	1	1	0	0
Wheat	11	8 (73%)	3 (27%)	7 (64%)	3	6	0	1
LTP (incl. Rosaceae, nuts, maize, etc).	216	130 (60%)	19 (9%)	45 (21%)	19	32	0	13
Sesame/sunflower seed	6	6 (100%)	2 (33%)	4 (67%)	2	3	0	1
Peanut	19	16 (84%)	1 (5%)	5 (26%)	1	4	0	1
Tree nuts	65	52 (80%)	9 (14%)	25 (38%)	9	19	0	6
Kiwi	23	7 (30%)	0	0 (0%)			0	0
Brazil Nut	1	1 (100%)	1 (100%)	1 (100%)	1	1	0	0
Soybean	9	5 (56%)	0	0 (0%)			0	0
Legumes	9	7 (78%)	4 (44%)	5 (55%)	2	5	2	0
Pineapple	3	2 (67%)	0	0 (0%)			0	0
Avocado	1	1 (100%)	1 (100%)	1 (100%)	1	1	0	0
Pine nut	12	10 (84%)	0	4 (33%)		4	0	0
Fig	1	1 (100%)	0	1 (100%)			0	1
Eggplant	2	0 (0%)	0	0 (0%)			0	0
Buckwheat	4	3 (75%)	1 (25%)	1 (25%)	1	1	0	0
Spinach	2	2 (100%)	1 (50%)	1 (50%)	1	1	0	0
Mango	1	1 (100%)	0	0 (0%)			0	0
Boletus mushroom	1	0 (0%)	0	0 (0%)			0	0
Tomato	2	1 (50%)	1 (50%)	2 (100%)	1	2	0	0
Watermelon	3	1 (33%)	0	2 (66%)	0	1	0	1
Fennel	1	0 (0%)	0	0 (0%)			0	0
Garlic	1	0 (0%)	0	0 (0%)			0	0
Type 2 food allergies	629	39 (6%)	4 (<1 %)	6 (1%)	1	5	3	1

U/A: urticaria with or without angioedema

Missing prescriptions: Patients with a clinical history of anaphylaxis that were not prescribed epinephrine.

Exceeding prescriptions: Patients without a history of anaphylaxis or ER assistance that were prescribed epinephrine.

Type 2 food allergies include subjects with pollen food allergy syndrome, latex-fruit allergy syndrome, and mugwort-celery spice syndrome.

The last 3 columns show the number of patients prescribed epinephrine (column 4), and how many of those prescribed epinephrine had a clinical history of anaphylaxis (column 5) and/or a history of Emergency Department visits (column 6).

cluded 2 shrimp-allergic patients, 2 subjects allergic to legumes, and 3 patients with type-2 food allergy (table 1). The rate of epinephrine prescription in patients with a history of anaphylaxis differed significantly between patients with type-1 or type-2 food allergy (54/58 [93%] vs 1/4 [25%]; $p < 0.001$).

The analysis of data showed that another main criterion adopted by participating doctors to prescribe epinephrine was a history of emergency department visit due to food-induced systemic reactions (including anaphylaxis or urticaria/angioedema with or without respiratory symptoms). In fact, 106/138 (77%) subjects who were pre-

scribed epinephrine auto-injector had sought for care at the ER (table 1). Interestingly, in this case no difference between patients with type-1 or type-2 food allergy was observed (101/132 [77%] vs 5/6 [83%], respectively; p= NS).

Within the different subgroups with type-1 food allergy including > 5 individuals, epinephrine prescriptions ranged between 12% (egg) and 67% (sesame seed, sunflower seed) with most frequent prescriptions occurring in patients allergic to wheat or legumes (table 1). Interestingly, although patients were mostly prescribed epinephrine in the light of a clinical history of anaphylaxis and/or emergency department visits, in some specific subsets epinephrine prescriptions in excess (i.e., in subject without a history of severe allergic reactions) were observed. This was particularly common in patients allergic to fish (4/6 [67%] prescriptions in excess), to tree nuts (6/25 [24%]), and especially to LTP (13/45 [29%]).

In patients with type 2 food allergy epinephrine was rarely prescribed (4/629; < 1%); of 6 patients prescribed the drug, 1 had a mugwort-celery-spice syndrome, 1 a latex-fruit-allergy syndrome, and 4 a pollen-food allergy syndrome. In 5 cases prescriptions followed an emergency department visit, although 3/4 patients diagnosed as having had an anaphylactic episode were not prescribed epinephrine (table 1).

Discussion

Although several surveys of epinephrine prescription appeared recently in the medical literature (18-20), this is probably one of the first studies analysing epinephrine auto-injector prescription in food allergy not only as a whole, but also by sensitising allergen. The virtual lack of peanut allergy in Italy (21), which represents the major cause of fatal or near-fatal anaphylaxis in Anglo-Saxon as well as in some European and Asian countries (4,7,20,22), clearly produces a change in the epinephrine prescription patterns and leads to consider other subsets of food-allergic patients. In this sense, allergy to lipid transfer protein, which is the most relevant cause of primary food allergy in Italy (21), as well as the main cause of food-induced anaphylaxis (23), represents an interesting model. Only about 20% of LTP–allergic patients were prescribed epinephrine auto injector, a proportion that is inferior to that of patients with other types of food allergy. However, LTP-allergic patients may experience an array of clinical conditions ranging from a life-lasting oral allergy syndrome to anaphylaxis, and this is the most likely reason why the majority of patients sensitised to this allergen were not prescribed epinephrine. In this subgroup most prescriptions were symptom-based (i.e., based on a history of severe clinical symptoms, as suggested by the emergency department visits). However, interestingly, in about 30% of cases epinephrine prescriptions were allergen-based (i.e., patients were prescribed auto-injectors because they were sensitised to a potentially harmful allergen, although the did not yet experience any severe allergic reaction). A similar trend was observed in patients sensitised to foods that are more frequently associated with systemic reactions, such as milk, wheat, shrimp, seeds, tree nuts, peanut, and fish. In these subgroups, along with an overall high (symptom-based) rate of epinephrine prescription, a proportion of patients were prescribed epinephrine auto-injectors with an exclusively preventive intent. This might depend on the fact that this study was based on specialized allergy clinics where doctors are acquainted with the guidelines for prescription of emergency medication and show a good knowledge of the chemical/physical characteristics of the various allergen proteins and, consequently, a higher consciousness of the potential risks associated with sensitisation to certain foods (24).

By comparing this study with a similar Dutch survey (7), it appears that in the Netherlands epinephrine prescriptions were on the whole limited and seemingly biased by the impact of food allergy on patient's quality of life, which is negative for patients and unrelated to both the allergen involved and the severity of the allergic reaction. The present Italian survey seems to reveal a more careful and critical analysis of the potential role of allergen proteins involved in allergic reactions by participating doctors and, hence, an improved appropriateness of the prescription of epinephrine.

In fact, the rates of symptom-based epinephrine prescriptions in patients with type-1 and type-2 food allergy were very similar, which is in keeping with studies showing that even allergens involved in pollen-food allergy syndrome, and hence presumptively pepsin-sensitive, may in some cases induce severe reactions (25).

In conclusion, this study shows that, along with the obvious symptom-based epinephrine prescription (as recommended by most guidelines as well as by Italian national drug regulatory organisms) a new, allergen-based, trend in epinephrine prescription is slowly emerging. This type of prescription is not based on clinical history but on the potential harmfulness of sensitizing allergen, and hence

points to prevent severe allergic reactions in sensitized patients that did not experience systemic reactions yet. It is possible that such way of prescribing will grow-up as far as an increasing number of recombinant allergen proteins, including many food allergens, are becoming available for in-vitro diagnosis of allergic diseases leading to a more refined component-resolved diagnosis and to a better definition of the pathogenic role of the various allergen proteins (26). In this sense the allergy specialist remains the only professional able to integrate clinical experience and knowledge of the characteristics of the allergens.

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News

Allergy Vaccinations Reduce Children's Health Care Costs by One-Third

ARLINGTON HEIGHTS, Ill. – Allergy immunotherapy, generally referred to as allergy vaccinations or shots, reduce total health care costs in children with allergic rhinitis (hay fever) by one-third, and prescription costs by 16 percent, according to a study published this month in Annals of Allergy, Asthma & Immunology, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

"This large-scale, comparative effectiveness study of health outcomes clearly demonstrates the benefits of allergen immunotherapy for children with allergic rhinitis," said Cheryl Hankin, Ph.D., president and chief scientific officer of BioMedEcon, and lead author of the study. "Findings are even more impressive, considering the results were based on 'real world' healthcare delivery, rather than on treatment provided within a tightly controlled clinical trial."

The 10-year U.S. retrospective study is the first to show significant health care cost reductions in as early as three months and continued decreases over an 18-month period. The study compared Florida Medicaid claims data of 2,770 children with allergic rhinitis who received allergen immunotherapy to a matched control group of over 11,000 affected children who did not receive such treatment.

"This is great news, not only for families who will experience fewer out-of-pocket expenses for allergy medications, but also for the ever increasing national health care crisis," said Linda S. Cox, M.D., immediate past chair of the ACAAI Immunotherapy and Diagnostic Committee and study co-author. "Because of the serious medical and economic consequences of childhood allergic rhinitis, early diagnosis and aggressive treatment need to be our priority."

Allergic rhinitis is the third most common chronic disease in U.S. children, affecting up to 40 percent of the population. Each year, allergic rhinitis accounts for two million missed school days and $2.3 million in health care costs for children younger than 12 years.

Allergen immunotherapy is the only treatment shown to decrease the risk of allergic rhinitis developing into asthma or other allergies.

"We are missing an opportunity to significantly improve health care outcomes and reduce costs when allergen immunotherapy treatment is not considered," said ACAAI President Sami Bahna, M.D., Dr.P.H. "We must be sure primary care physicians have the information they need to identify appropriate patients for referral and evaluation by an allergist."

Parents and others can take the ACAAI-sponsored Asthma and Allergy Relief Self-Test which reviews symptoms, identifies suffering and provides plans for relief at www.AllergyAndAsthmaRelief.org.