

M.F. MARTÍN-MUÑOZ¹, A. DIAZ-PERALES², J. CANNABAL¹, S. QUIRCE¹

Anaphylaxis to hidden potato allergens in a peach and egg allergic boy

¹Allergy Department, Hospital La Paz Instituto de Investigación (IdiPaz), Madrid, Spain

²Center for Plant Biotechnology and Genomic (UPM-INIA), Madrid, Spain

KEY WORDS

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Corresponding author

María Flora Martín-Muñoz
Hospital La Paz
Paseo de la Castellana 261, 28046,
Madrid, Spain
Phone: +34 62 649 3341
E-mail: fmartinmz@gmail.com

Summary

More than 170 foods have been identified as being potentially allergenic. However, a minority of these foods cause the majority of reactions. Sweets are frequently implicated in allergic reactions in children with cow's milk, egg, nuts or fruits allergy, and they are the most relevant foods investigated as responsible allergens.

We report an anaphylactic reaction to candies in an egg and peach allergic boy. We performed a study to identify responsible allergens for the reaction. We investigated hidden egg and peach allergens in the candies, but they were not found. Finally, the causative allergen resulted to be a vegetable protein from potato peel. We diagnosed a new allergy in our patient and Sol t 4 was identified as the responsible allergen of the anaphylactic reaction.

We conclude that responsible allergens should always be studied and identified in whatever allergic reaction in order to prevent new reactions.

Introduction

Sweets are frequently implicated in children's allergic reactions with cow's milk, egg, nuts or fruits allergy, and those are the most relevant foods that have been investigated as the responsible allergens.

We reported an anaphylactic reaction to candies in an egg and peach allergic boy. A study was performed to identify potential allergens present in candies and their origins. The causative allergen was identified as a vegetable protein, used as a thickener ingredient in the manufacture of one of the candies. It was a protein not related to any previously diagnosed allergy in our patient. Finally, we identified the Sol t 4, a peel potato protein, as the responsible allergen of the anaphylactic reaction.

We conclude that, in whatever allergic reaction, the responsible allergens should always be studied and identified in order to prevent new reactions.

Case report

A 21-month-old boy developed, ten minutes after ingesting some candies (with trade names "Lolipop", "Fresa besito" and "Nube fresa"), perioral urticaria with lip edema, abdominal pain, vomiting and generalized urticaria. The reaction subsided in 3 hours with H1 antihistamine and corticosteroids treatment. At that time, he was tolerating cow's milk, boiled egg, meat, fish, cereals and vegetables including boiled potato, legumes (lentils, soy, beans, chickpeas, peanuts) and other fruits including kiwi. We discharged as cofactors exercise, infections or drugs. Some days later, he developed pruritus and perioral hives immediately after ingesting soya, green peas or lentils. Previously, at twelve months, we had studied the patient because of immediate generalized urticaria after ingesting egg and perioral urticaria coinciding with peach ingestion, and the baby was diagnosed with egg and peach allergy. He had mild atopic dermatitis but he had

not had bronchospasm episodes, and his family had no history of allergic disease.

We performed a study to identify potential allergens present in candies and their sources.

Materials and methods

All candies implicated in the reaction were investigated. Their labels were studied to ascertain the ingredients, and the original components were supplied by the manufacturer.

Skin prick by prick tests (SPPT) with an aqueous solution of each candy were performed on the patient and on 10 control children (5 with egg and 5 with peach allergy) to investigate hidden egg or peach allergens. Then we completed SPPT on the patient with natural components of candies which resulted positives (peel, and raw and boiled pulp potato).

Skin prick tests (SPT) were performed with a panel of food allergens including cow's milk and white egg proteins, peach, (rPru p 3) and (rPru p 4), potato, legumes and nuts, using commercial extracts and histamine and saline solution as positive and negative controls (ALK-Abelló laboratories, Madrid, Spain); and with aqueous extract of each one of the ingredients (10 mg/ml) of the candies showing a positive SPPT response. Skin tests were considered positive if average diameter was equal or greater than histamine diameter. Total and specific IgE were assessed by ImmunoCAP and microarrays (ISAC IgE) (Thermo Fisher, Uppsala, Sweden).

IgE-immunoblot and immunoblot inhibition experiments were carried out to investigate the responsible allergens. Samples (10 mg of peel potato extract and 2 mg of Pru p 3) were separated by SDS-PAGE and replica gels were electro-transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking, the membranes were incubated overnight with patient's serum (1:3 dilution), and with polyclonal rabbit antibodies produced against Pru p 3 (peach LTP; dilution 1:1000). Detection of IgE-binding components was achieved by means of enhanced chemiluminescence, according to the manufacturer's instructions (Amersham Biosciences, Little Chalfont, UK). The inhibition assays were performed incubating Pru p 3 (5 µg/mL) with patient's serum 3 h at room temperature, previously to immunoblot.

The identification of the peptide was performed by peptide-mass fingerprinting.

Results

Information on the labels of the candies ("Lolipop", "Fresa besito" and "Nube fresa") ingested by the patient within one hour before the reaction, did not include egg or peach ingredients. The patient showed positive SPPT to "Lolipop" (7 mm mean diameter) and negative to "Fresa besito" and "Nube fresa". The control subjects showed negative SPPT to the three candies.

"Lolipop" label declared as ingredients: sugar, glucose syrup, vegetable fat, maltodextrin, water, aromas, vegetable protein, starch, soy lecithin, E-330 (citric acid) and E-120 (cochineal red). The SPT to each of these components (10 mg/ml) resulted positive only to the vegetable protein (mean diameter 11 mm). The source of vegetable protein was identified by the manufacturer as potato peel proteins. Results of skin tests, food specific IgE and tolerance are shown on the **table**.

The patient's serum recognized Pru p 3 and a low molecular weight IgE-binding band in the potato peel extract. To identify the nature of this band, inhibition assays were conducted. Pru p 3 was capable of self-inhibition but did not inhibit the IgE-binding reactivity of the potato peel band. The IgE-binding potato band wasn't recognized by polyclonal rabbit antibodies against Pru p 3 (**figure 1**). Finally this potato allergen was identified as Sol t 4, a protease inhibitor belonging to the family of Kunitz-type soybean trypsin inhibitors.

When the study was concluded, the patient was tolerating cooked potatoes, and symptoms with legumes (soya, green peas, lentils and chickpeas) had disappeared. However, he was on a kiwi, walnut and peanut free diet. The patient's mother didn't accept a challenge to prove tolerance of patient to these foods.

Figure 1 - Results of IgE-immunoblot and immunoblot inhibition experiments:

- Line 1 vegetable protein of "Lolipop" (peel potato) and line 2 Pru p 3 separated by SDS-PAGE and stained with Coomassie.
- Replicas of lines 1 and 2 immunodetected with patient's serum (Immunoblot; dilution 1:3).
- Replicas of lines 1 and 2 immunodetected with patient's serum preincubated with Pru p 3.
- Replicas of lines 1 and 2 immunodetected with anti LPT antibodies.

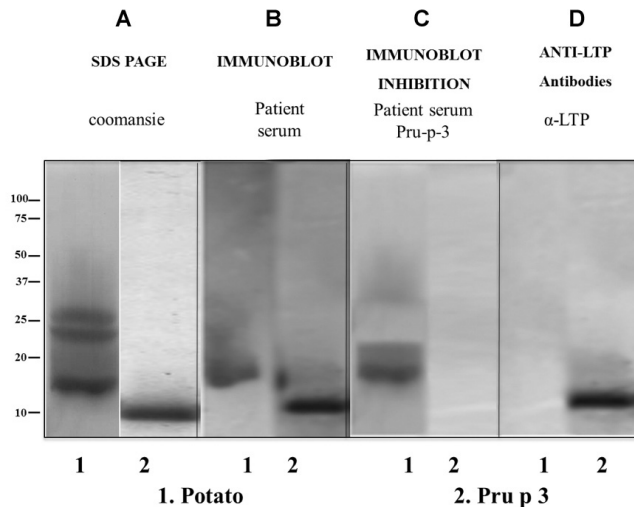


Table 1 - Results of allergic study and tolerance to different foods and latex. Results of skin test (prick and prick by prick) and specific IgE (Cap and Microarrays) and tolerance at time of the study. Tolerance: Yes, No or NI (not introduced in the patient diet).

	Prick by Prick		Prick		Specific IgE		Tolerance
	Mean diameter (mm)		Cap U/L		Microarrays ISU		
"Lolipop" candy	7						
"Fresa besito" candy	0						
"Nube fresa" candy	0						
White egg			5.3	0.49	0.0		Yes (boiled egg)
Ovalbumin			6	0.5	0.0		
Ovomucoid			0	0.01			Yes
Cow's milk			0	0.01			NI
Sesame			0	0.02			NI
Mustard			0	0.01			NI
Peanut			0	0.12			NI
Walnut			0	0.04			Yes
Latex			0	0.01			
Peel potato protein			11				No
Potato				6.12			
Peel	7						
Boiled pulp	5						Yes
Raw pulp	10						
Tomato				0.62			Yes
peel	10	6					
pulp	0						
Peach			8	13.2			No
Pru p 4			0	0.01			
Pru p 1				0.00	2.9		
LTP rPru p 3			6.5	15.10			
nsLTP rAra h 9				0.5	0.5		
rCor a 8				0.0	0-01		
nJug r 3				1.1	1.1		
nArt v 3				0.0			
nOle e 7				0.0			
rPla a 3				0.3			
Peas			3	0.52			No
Lentil			4	0.65			No
Soya			3	0.40			No
rGly m 4				0.00	0.0		
nGly m 5				0.07	0.0		
nGly m6				0.04			
Kiwi			8	5.53			Yes
n Act d1					2.7		
n Act d 2					0.3		
nAct d 5					0.0		
r Act d 8					0.0		
D pteronyssinus							
Der p 1			0		0.0		
Der f 1			0		0.0		

Discussion

Food allergens in food supplements and sweets have been implicated as elicitors of anaphylactic reactions in allergic children, and hidden components are sometimes identified as causal allergens (1,2). Our patient developed an anaphylactic reaction immediately after eating candies. He was allergic to egg and peach, and showed a positive SPPT to "Lolipop" candy. Although these components were not declared in the candy labels, they could be hidden allergens in some sweets. However, the negative results of SPPT with "Lolipop" on egg or peach allergic control subjects ruled out this possibility. Finally, the vegetal protein component in "Lolipop" (protein from peel potato) was confirmed as the responsible allergen. The proved tolerance to cooked peeled potato by our patient and his intense sensitization to Pru p3, made us think about the possibility of a lipid transfer protein (LTP) from potato as the responsible allergen. However, IgE in the patient's serum recognized a band of approximately 15 kDa in the vegetable protein, which were neither inhibited by Pru p3 or recognized by specific polyclonal antibodies against plant LTP. The allergen detected by the patient's serum was identified as a cysteine protease inhibitor belonging to the family of soybean trypsin inhibitors Kunitz type (Sol t 4).

Potatoes represent an important part of the worldwide diet. Allergic reactions to this foodstuff are uncommon, and usually result from ingestion, mainly in children. Castell et al. (3) reported anaphylaxis to white potato in a girl, and they demonstrated specific IgE antibodies directed against several potato proteins ranging from 14,000 to 40,000 kDa. Allergic reactions to contact with raw potato has been reported more frequently in adults, usually in the form of an oral contact dermatitis or contact urticaria (4,5), but asthma, rhinoconjunctivitis, wheezing or even anaphylaxis had also been described (6,7).

Potato contains a number of allergens, ranging from 16 to 65 kDa in size, of which a few have been characterized. Smith et al. (8) demonstrated that 75% of potato-sensitized subjects reacted to Sol t 1 (patatin) a 43 kDa allergen. Sol t 2, Sol t 3 and Sol t 4 have molecular masses ranging from 16 to 20 kDa and have been identified as cathepsin D-, cysteine-, and aspartic protease-inhibitors belonging to the family of Soybean trypsin inhibitors (Kunitz type); Seppala et al. (9) showed IgE binding to Sol t 4 in 67%, Sol t 2 in 51%, and to Sol t 3 in 43% of the sera of atopic children.

A study in children up to 4 year old with suspected food allergy showed that 70% of children had positive SPT to potato and

IgE antibodies to a Kunitz-type soy trypsin inhibitor (KSTI) and the 75% of children with suspected soya allergy, had IgE antibodies to Sol t 2-4. A marked allergenic cross-reactivity was demonstrated between Sol t 2-4 and these KSTI allergens. The study concluded that in children with positive SPT and serum IgE to soy, there may be cross-reactive IgE antibodies to potato allergens and vice versa (10). Our patient developed oral allergy transient symptoms to legumes and we demonstrated low levels of specific IgE to them.

On the other hand, although previously the patient had tolerated kiwi, the study showed an intense sensitization particularly to nAct d 1, a cysteine protease. After the reported reaction, we could not verify that the child tolerates this fruit because his mother didn't approve a controlled challenge.

We conclude that in food allergic reactions the causative allergens should be thoroughly investigated, even in patients with a previous diagnosis of allergy to common foodstuffs.

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