R. Asero[1], G. Mistrello[2], S. Amato[2]

Co-sensitisation (but co-recognition also) to novel banana and tomato allergens

[1]Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Italy
[2]Lofarma SPA, Milano, Italy.

Key words

Food allergy, banana, tomato, cross-reactivity, allergens

Summary

An unusual case of both banana and tomato allergy is reported. In vitro tests showed that both co-sensitization to and co-recognition of allergen in the two fruits were present. Interestingly, the patients showed IgE reactivity to hitherto not described, high molecular weight allergens.

Introduction

Tomato and banana allergy are not uncommon. Most cases are found in patients with seasonal airborne allergy due to the cross-reactivity between pollen profilin and the homologous food protein. Further, banana allergy has been frequently described following primary natural rubber latex allergy due to cross-reactivity between latex and banana allergens. In contrast, primary sensitisation to these two foods is rather rare. This study reports an unusual case of co-sensitisation (but of co-recognition as well) to novel tomato and banana allergens.

Patients and methods

Case report

A 17-year-old boy was recently seen at the allergy outpatient clinic of this clinic with a history of slight rhinoconjunctivitis in springtime during the last 2 years, and two distinct episodes of angioedema of the face, hypotension, and diarrhoea during the last 2 months, both occurring about 30 min after the ingestion of banana and lasting for about 1 hour. Further, the patient reported a typical oral allergy syndrome (immediate itching of oral mucosa) following the ingestion of raw tomato. Both banana- and tomato-induced symptoms were not related to the onset of the seasonal rhinoconjunctivitis, and the patient reported good tolerance of all other foods. SPT with commercial extracts (Allergopharma, Reinbeck, Germany) of the main airborne allergens present in this area including pollens (grass, mugwort, ragweed, pellitory, plantain, birch, cypress, and olive), house dust mites, molds (Alternaria, Aspergillus, Cladosporium, Candida), and danders (cat and dog) showed moderate skin reactivity to grass and ragweed pollen. SPT with a series of commercial extracts (ALK-Abello, Madrid, Spain) of food allergens including egg white and yolk, cow's milk, shrimp, pork, cod, wheat, maize, soybean, peanut, walnut, hazelnut, tomato, sunflower, carrot, orange, celery, banana, kiwi, and sesame (all 1:20 w/v), and peach LTP (30 μg/ml) were performed as well.

In-vitro assays

Tomato and banana extracts were prepared as previously described. Briefly, 100 g of fresh tomato including both pulp and peel and 100 g of banana were homogenized. Both homogenates were mixed with 300 ml of pre-cooled acetone and equilibrated at -20°C overnight. The precipitates were washed twice with acetone and once with acetone/ether (1:1, v/v) and dried. The resulting powders were extracted (1); protein concentrations of the extracts were 3 mg/ml and 0.8 mg/ml for banana and tomato, respectively (2) (Bio-Rad). In direct ELISA assays 1 μg of tomato or banana extracts both diluted in 100 μl of coating buffer (15 mM Na_2CO_3, and 35 mM $NaHCO_3$)/well, were used for coating 96-microtitre plates (Maxisorp, Nunc) (3). After washings with 0.1 M PBS, pH 7.4, and 0.05% Tween 20 (Sigma), wells were saturated with 2% BSA in PBS for 2h at RT. After further washing, 100 μl of undiluted serum were added per well and incubated for 2 h at RT. After washing bound specific IgE was detected by adding a peroxidase-conjugated anti-human IgE from goat (Biospacific, USA; 1:1500). The enzyme reaction, induced using tetramethyl-benzidine/H_2O_2 as substrate, was stopped after 20 minutes by 1 mol/L HCl. Absorbance was read at 450 nm and expressed as optical density (OD). In order to assess a possible cross-reactivity between tomato and banana allergen, ELISA cross-inhibition experiments were carried out pre-absorbing patient's serum with 10 μg of either tomato or banana protein; in case of significant inhibition, a curve was built by measuring IgE reactivity after pre-adsorption of serum with 1 μg and 0.1 μg of extract as well. Patient's IgE reactivity was further investigated by immunoblot under reducing conditions against tomato and banana extracts. Electrophoresis of extracts (15 μg/lane) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel with MES buffer according to manufacturer's instructions (Invitrogen) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane (4). The membrane was saturated with 0.1 mol/L Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with sera. After 3 washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:1000 in saturation buffer; Biospacific) using an ECL western blotting kit (Amersham) as substrate.

Results

Skin tests

SPT showed strong skin reactivity to commercial extracts of tomato (mean wheal diameter 8 mm), banana (6 mm) and hazelnut (8 mm). SPT with fresh tomato both raw and boiled at 100°C for 5 min scored intensely positive with no difference between the raw and the heat-processed food (mean wheal diameter 6 mm in both cases). In contrast, no skin reactivity to natural rubber latex extract (500 μg protein/ml) and to purified date palm profilin [(Pho d 2; 50 μg protein/ml (5)] (both by ALK-Abello) was recorded.

In-vitro assays

Direct ELISA showed significant IgE reactivity to both banana (854 OD) and tomato (3285 OD). Cross-inhibition experiments showed that pre adsorption of serum with banana extract caused a dose-dependent reduction of IgE reactivity to tomato, whereas pre adsorption with 10 μg of tomato extract caused very little inhibition of IgE reactivity to banana (Tab. 1), thus showing partial cross-reactivity between banana and tomato allergens and sug-

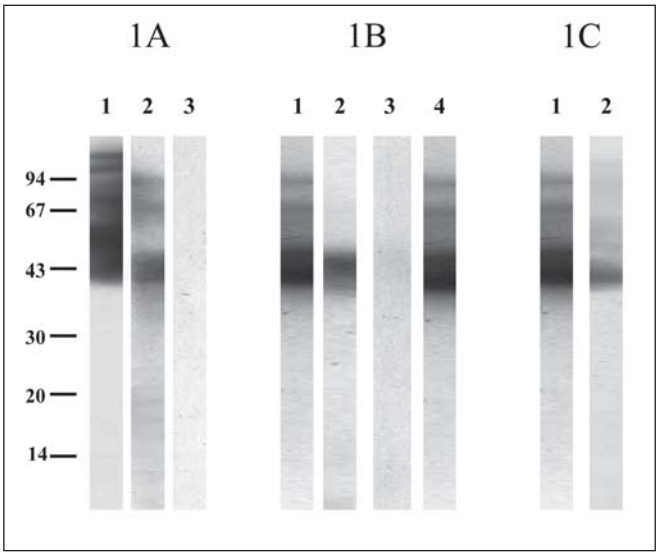
Table 1 - ELISA and cross-inhibition studies results.

	OD	% inhibition	
IgE reactivity to tomato extract	Uninhibited serum	3284	
	Serum pre adsorbed with banana extract (10 μg)	1203	63
	Serum pre adsorbed with banana extract (1 μg)	1999	39
	Serum pre adsorbed with banana extract (0.1 μg)	3079	6
IgE reactivity to banana extract	Uninhibited serum	854	
	Serum pre adsorbed with tomato extract (10 μg)	563	34
	Serum inhibited with house dust mite extract	889	0

IgE reactivity is expressed as optical density (OD); based on the mean values found in normal sera levels < 150 OD were considered negative

gesting banana as the possible primary sensitizer. On immunoblot analysis IgE reactivity against proteins from 43 to 90 kDa in banana extract and against 43, 67, and 94 kDa proteins in tomato extract was found (Fig. 1A). Pre-absorption of patient's serum with banana abolished IgE reactivity to 67 and 94 kDa tomato allergens, whereas IgE reactivity against the 43-kDa-zone remained unchanged (Fig. 1B). In view of the reported presence of cross-reactive carbohydrate determinants (CCD) in tomato extracts (6) we investigated whether patient's IgE-reactivity to tomato and banana extract was at least in part directed to CCD. To this end we treated tomato extract-blotted nitrocellulose strip with sodium periodate in order to oxidise possible glycoprotein oligosaccharides (7). The IgE-binding pattern was then compared with that from the untreated strip. Periodate treatment induced the loss of IgE-binding to 67 and 94 kDa tomato components while IgE-reactivity against 43 kDa zone allergen was only partially reduced (Fig. 1C), suggesting that the 2 higher m.w. components were expressed as glycoproteins and that the IgE reactivity to 67 and 94 kDa was possibly due to CCD in both tomato and banana.

Figure 1 - A) Immunoblot analysis of patient's serum IgE reactivity to banana and tomato. Lane 1: IgE reactivity to banana extract; lane 2: IgE reactivity to tomato extract; lane 3 : IgE reactivity to banana or tomato extract of a normal control serum. B) Lane 1: IgE reactivity to tomato extract; lanes 2-4: IgE reactivity to tomato extract after pre-absorption of serum with banana extract, tomato extract, and house dust mite extract, respectively. C) IgE reactivity to tomato extracts before (lane 1) and after (lane 2) treatment of extract with sodium periodate.



Unfortunately the lack of patient's serum did not allow us to perform same experiments with banana extract to reinforce our hypothesis. The persistence of IgE reactivity to tomato 43 kDa allergen following pre-absorption of serum with banana extract suggests a co-sensitization to both foods.

Discussion

Several tomato allergens have been described to date, including Lyc e 1 [profilin, m.w. 14 kDa (8)], Lyc e 2 (fructofuranosidase; 50 kDa), Lyc e 3 (lipid transfer protein, 6 kDa), Lyc e chitinase (31 kDa), Lyc e glucanase (55 kDa), and Lyc e peroxidase (44 kDa). The clinical relevance of each of these allergens is ill defined, with the exception of profilin which may cause oral allergy syndrome (9). The IgE profile of our patients does not correspond to any of these allergens with the possible exception of peroxidase. If so, this would be the proof that tomato peroxidase sensitisation can be clinically relevant. Patient's hypersensitivity to banana is very interesting as well. Banana allergy has been mostly described following primary natural rubber latex allergy due to hypersensitivity to 1,3-beta-glucanase (Hev b 2) and class-1 chitinase (Hev b 6/Mus a 2), or in patients with pollen allergy due to cross-reactivity with profilin (Mus a 1), but in this case reactivity to both natural rubber latex and profilin was ruled out. This patient seemingly reacted to hitherto not yet described tomato and banana allergens.

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