

Short communication

Tomato allergy in children and young adults: cross-reactivity with latex and potato

Background: Several studies have shown that allergy to natural rubber latex is associated with cross-reactivity to certain foods such as tomato and potato. The objective was to investigate the clinical and immunologic differences between a group of patients with clinical allergy to tomato and latex and another which had only clinical allergy to tomato. We also aimed to assess, *in vitro*, the relationship of tomato and latex allergens, which could explain the cross-reactivity.

Methods: Forty patients with histories of adverse reactions to tomato and IgE-mediated hypersensitivity were enrolled in the study. Tomato, latex, and potato components were analyzed by SDS-PAGE immunoblotting. CAP and immunoblot inhibition were used to study allergen cross-reactivity.

Results: Patients from group A had a mean age of 13.2 years, and in group B the mean age was 21.7 years. In group B, 9/10 patients belonged to the latex-fruits syndrome. All patients of both groups tolerated potato. Immunoblotting patterns obtained with patients' sera from pool A showed IgE-binding bands to tomato ranging from 44 to 46 kDa and a triple band at 67 kDa. For latex, there was a strong binding at 44 kDa, and potato showed a strong band of 44 kDa and a 67-kDa triple band. In pool B, the binding to the band of 44 kDa in latex and tomato was more intense than in pool A. In pool A, immunoblot inhibition with potato allergen showed an intense inhibition of the three allergens (potato, latex, and tomato); with latex, inhibition was partial and with tomato, a complete inhibition of tomato and latex was observed, and a partial inhibition of potato. In pool B, the inhibition pattern followed a similar tendency to pool A. The CAP inhibition confirmed the high rate of cross-reactivity between tomato, potato, and latex.

Conclusions: In our study, tomato, potato, and latex showed a common band of 44–46 kDa probably corresponding to patatin. This protein could be implicated in the high cross-reactivity between tomato, latex, and potato observed in the immunoblot and CAP inhibition.

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Fresh and cooked tomato is a basic element of the diet. Sensitization to this vegetable is particularly prevalent in the Mediterranean area, mainly in the pediatric-adolescent population. In contrast, tomato allergy seems to be of minor importance in northern Europe (1).

The simultaneous presence of IgE to allergens of tomato fruit and grass pollen is evident in many patients with pollenosis and may be caused by cross-reactivity. An Italian study reported an obvious association in children with allergy to grass pollen and tomato. (2). Cross-reactivity has also been observed between Japanese cedar allergen and tomato; this cross-reactivity may be due to a 20-kDa protein (3).

Several studies have shown that allergy to natural rubber latex is associated with cross-reactivity to certain

foods. The most commonly recognized associated food allergies were those to avocado, tomato, potato, and banana (4, 5).

The cross-reactive structures responsible for these associations have not been completely elucidated yet. Among 47 latex-allergic adults, Beezhold et al. (5) found that 17 had clinical reactivity to at least one food, 53% were positive by prick testing to avocado, and a smaller number were reactive to potato, banana, tomato, chestnut, and kiwi. Patatins are proteins found in plant species such as potato and tomato. The 46-kDa allergen in latex appears to be a *Hevea brasiliensis* homolog of patatin. This protein homology could explain the high cross-reactivity.

The aim of the study was to investigate the clinical and

immunologic differences between patients with clinical allergy to tomato and latex and another group, which had clinical allergy only to tomato but were tolerant of latex. We also aimed to assess at *in vitro* level the relationship with tomato and latex allergens of the patatin allergen from potato, which could play a role as a sensitizing allergen.

Material and methods

Subjects

Forty patients with a history of adverse reactions to tomato were enrolled in the study. The diagnosis of tomato allergy was based on food challenge or a convincing history of anaphylaxis after tomato ingestion. All patients had positive skin prick test results and/or specific serum IgE to tomato.

The patients were divided into two groups; group A comprising 30 non-latex-allergic patients, and group B comprising 10 patients with latex allergy proven by skin test, anti-IgE to latex, and the glove test, if needed.

Coexisting food and respiratory allergies were ascertained by interview and skin prick tests and *in vitro* tests with extracts of food allergens and airborne allergens present in the geographic area. Skin tests for common inhalants (mites, molds, pollen, and animal danders; Bencard Bayer, Germany, and Abello-ALK, Madrid, Spain), food and latex allergens (Leti Laboratories, Madrid, Spain), histamine (1 mg/ml), and saline solution control substances were applied to the forearms by the prick method. The mean diameters were recorded.

Serum samples obtained from the patients were stored in 1-ml aliquots until use. A volume of 1 ml of each serum was pooled in two pools, A and B, and then divided into 500- μ l aliquots and stored at -70°C with the individual sera until used.

Specific serum IgE to allergens was determined by an enzymatic immunoassay CAP-FEIA (Pharmacia Diagnostic, Uppsala, Sweden), according to the manufacturer's instructions.

Total serum IgE was determined by MEIA IMX System (Abbot Diagnostics, Chicago, IL, USA) following the manufacturer's instructions.

Preparation of extracts

Tomato and potato extract. An amount of 200 g of ripe tomato or peeled raw potato was washed carefully, ground, and soaked for 1 h in 500 ml of PBS with 10 mM ascorbic acid as an antioxidant (6). The mixture was stirred for 2 h at 4°C and centrifuged at 7000 g for 40 min at 4°C . The supernatant was dialyzed (molecular weight cutoff: >3500 Da; Spectrapor; Spectrum Medical Industries, Inc, Los Angeles, CA, USA) at 4°C for 24 h against glycine, centrifuged at 15000 g for 30 min at 4°C , and filtered through a 0.45- μm filter (Millipore Corp, Bedford, MA, USA). Finally, the material was lyophilized. The protein content was determined by the Bradford (Bio-Rad) method (7). The lyophilized extract was stored at -20°C in a desiccator.

Latex extract. The liquid ammoniated latex from *Hevea brasiliensis* (kindly donated by Aristegui Laboratory, Bilbao, Spain) was centrifuged at 44000 g in a Sorvall sc high-speed centrifuge (Du Pont, Wilmington, DE, USA) for 90 min according to Yeang et al.'s protocol (8). The proteins from serum and rubber cream were sampled together, dialyzed, lyophilized, and stored, as were the other allergens.

CAP inhibition

The assay was carried out in duplicate, and each sample was tested twice. The pool serum was used at 1:2 dilution and then diluted 1:1 with the three extracts (tomato, latex, and potato), purified. Ovalbumin was used as negative inhibition control. The concentration of competing fluid-phase allergens, diluted with PBS, was centrifuged and incubated with tomato, potato, and latex ImmunoCAP, according to the test procedure. Percent inhibition was calculated by plotting the mean inhibition values, and this was validated if the linear correlation coefficients were higher than 0.95.

SDS-PAGE and immunoblot

SDS-PAGE was performed with a 12% polyacrylamide separating gel and a stacking gel of 4%, as reported elsewhere (6). Silver staining (Bio-Rad Laboratories, Hercules, CA, USA) was done on gels to ensure proper protein separation and visualizations. Proteins were electrophoretically transferred from the separating gel to a PDVF (Millipore, USA) membrane in a transfer buffer (Tris-glycine) with 20% methanol. This procedure was done in a minitransfer chamber (Bio-Rad Laboratories, Hercules, CA, USA). The three blotted allergens were incubated with pool sera diluted 1:1 and with a negative pool.

Alkaline-phosphatase-conjugated goat anti-human IgE (1:2000 v/v of TBS; Caltag Laboratory, San Francisco, CA, USA) was added and incubated at room temperature for 3 h. The blot was developed with an alkaline-phosphatase-conjugate substrate kit (Bio-Rad Laboratories) of nitroblue tetrazolium and 5-bromo-4-chloro-3 indolyl-phosphatase, as reported elsewhere (6).

Immunoblot inhibition

Aliquots (1.5 ml) of the serum pool (1:1 v/v dilution in TTBS) were incubated overnight with equal volumes of tomato, latex, and potato extracts at final concentrations of 0.01, 0.1 and 1 mg/ml.

The mixtures were centrifuged, and each supernatant was incubated with tomato, potato, and latex followed by immunodetection as described above. The immunoblot was also inhibited with ovalbumin (1 mg/ml final concentration) (Sigma Chemical Co., St Louis, MO, USA) as a negative control.

Statistical methods

Because of the skewed distribution of specific IgE levels, data were compared by nonparametric means with the Mann-Whitney *U*-test. Probability (*P*) values of less than 0.05 were considered statistically significant.

Results

Subjects

The clinical features of the studied subjects are shown in Table 1. In group A, the most frequent clinical symptom with tomato was oral allergy syndrome (OAS), although urticaria was the most frequent symptom in group B. In this group, anaphylaxis occurred in 20% of the patients.

Sensitization to pollens was more frequent in group A than in group B. In both groups, grass and olive-tree pollen were the most often implicated.

In group A, 17/30 patients had sensitization to latex with skin test and/or specific IgE positive to latex, but none of them had clinical symptoms with latex.

Clinical symptoms to fruits were very common among patients in group A (28/30). In group B, 9/10 patients

Table 1. Clinical symptoms and sensitizations to inhalant and food allergens and total and specific IgE

	Group A Tomato and non-latex-allergic patients	Group B Tomato and latex-allergic patients
Number of patients	30	10
Age (range)	13.2 (1.5–48)	21.7 (5–34)
Sex (M/F)	14/16	1/9
Symptoms with tomato	OAS 73% U 30% RWC 10% AP/V 10% A 0%	OAS 10% U 60% RWC 10% AP/V 0% A 20%
Sensitization to pollens		
Grass	97%	70%
Olive-tree	27/30	7/10
Compositae	25/30	7/10
Others	21/30	5/10
Sensitization to other inhalants	20/30	5/10
Sensitization to other foods		
Milk	3/30	1/10
Egg	5/30	1/10
Fish	5/30	0
Legumes	13/30	3/10
Fruits	28/30	10/10
Nuts	22/30	2/10
Crustacea	2/30	0
Total IgE	493.3	584.7
CAP tomato	11.5 kU/l	8.60 kU/l
CAP potato	4.7 kU/l	2.61 kU/l
CAP latex	2.4 kU/l	20.60 kU/l*

OAS: oral allergy syndrome; U: generalized urticaria; R: rhinitis; W: wheezing; C: cough; AP: abdominal pain; V: vomiting; A: anaphylaxis.

* Statistical significance ($P \leq 0.0001$).

exhibited the latex-fruits syndrome. In both groups, sensitization to fruits was the most frequent, followed by nuts and legumes. All patients tolerated potato.

In vitro studies

For pool A and pool B, specific IgE mean values are shown in Table 1. In the blotting of pool A, several bands could be recognized with different molecular mass for tomato, latex, and potato (Fig. 1, upper panel). Tomato showed a double protein band at 44 and 46 kDa and a triple band at 67 kDa. There were several protein bands at 20 and 30 kDa. A protein between 14 and 20 kDa was also detected. Latex had a strong binding at 44 kDa, and it also showed a weaker binding at 36 and 30 kDa. In addition, another protein band of 14 kDa was recognized. Potato showed a strong band at 44 kDa and a triple band at 67 kDa; likewise, there were several bands between 20 and 30 kDa and a double band of approximately 17 kDa.

In pool B (Fig. 2, upper panel), the profile obtained from potato and tomato was similar to pool A, except that it had two more bands at 14 and 17 kDa. The binding to the band of 44 kDa in latex, and tomato was more intense in pool B than in pool A. An unspecific binding to potato in atopic children without any pollen sensitization was excluded (data not shown).

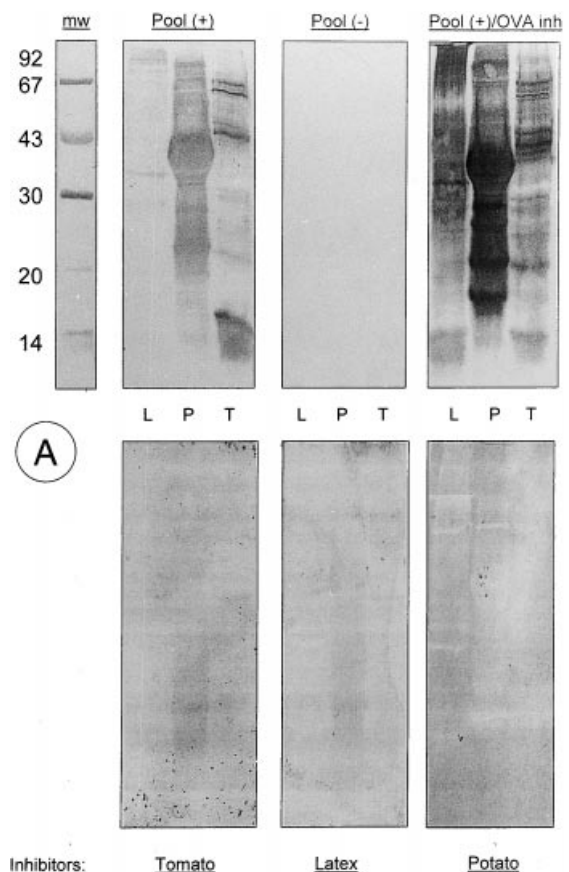


Figure 1. Upper panel. In pool A, SDS-PAGE immunoblot analysis. On left, molecular weight standards, followed by immunodetection with specific IgE to latex (L), potato (P), and tomato (T) in serum pool; negative control sera and inhibition with OVA in serum pool. Lower panel. In pool A, immunoblot from SDS-PAGE extracts (latex, potato, and tomato) inhibited with tomato, latex, and potato.

To detect cross-reactivity between allergens, immunoblot inhibition was done with potato, latex, and tomato with a maximum concentration of 1 mg/ml (Fig. 1, lower panel). In group A, all three allergens showed quasi-total inhibition by potato. With latex inhibition, it was only partial for the three allergens. With tomato, a complete inhibition of tomato and latex was observed as well as a partial inhibition of potato.

In group B, the inhibition pattern followed a similar tendency to group A (Fig. 2, lower panel), except the lower latex inhibition with tomato and the lower inhibition of tomato at 44 kDa and higher MW bands by latex.

Blot-immunoprint patterns obtained with the individual sera of 30 patients from pool A demonstrated IgE-binding to tomato at 17, 22, 44, and 75 kDa (data not shown).

The most recognized bands in individual sera from pool B were 46 kDa (70%) and 17 and 44 kDa (60%) (data not shown).

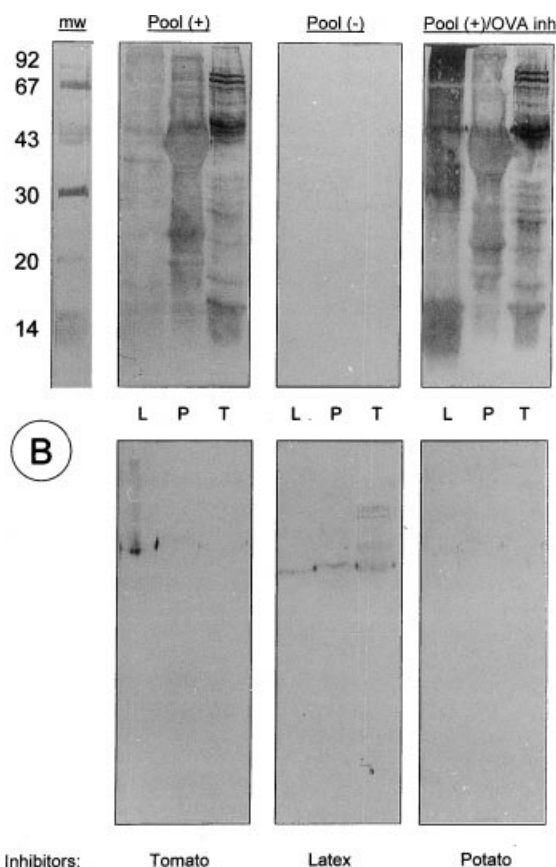


Figure 2. Upper panel. In pool B, SDS-PAGE immunoblot analysis. On left, molecular weight standards, followed by immuno-detection with specific IgE to latex (L), potato (P), and tomato (T) in serum pool; negative control sera and inhibition with OVA in serum pool. **Lower panel.** In pool B, immunoblot from SDS-PAGE extracts (latex, potato, and tomato) inhibited with tomato, latex, and potato.

CAP inhibition

CAP inhibition was performed after blot inhibition in order to confirm the high rate of cross-reactivity among tomato, potato, and latex.

In pool A, this cross-reactivity was very high, following the same lineal tendency shown by the inhibition of tomato with tomato (data not shown). The inhibition values were generally higher than 20%, even with dilutions as low as 1 ng/ml.

In pool B, the tomato inhibition of tomato, latex, and potato followed a similar tendency to pool A, although in low dilutions the grade of inhibition was lower (data not shown). In both pool A and pool B, the maximum CAP inhibition (with tomato as inhibitor) at 1 mg/ml was over 90%.

Discussion

Our patients were mostly children and adolescents. Patients from group A had a mean age lower than group B. In both groups, females were dominant, although more so in group B.

Previous studies of tomato allergy in a young population with allergic pollenosis have shown oral allergy syndrome to be the most common clinical symptom in these patients (9–12). These results are in concordance with our study, in which oral allergy syndrome was predominant in group A, and generalized urticaria in group B. None of the patients in group A had anaphylaxis, but this symptom was present in 20% of patients from group B.

It is known that food allergy is more frequent in patients with pollen allergy. In our study, the sensitization to pollens occurred in 97% in group A and 70% in group B. The main allergens were grass and olive-tree pollens.

It is interesting that the sensitization to pollens appeared before the sensitization to tomato in 20 patients in group A, but in only one patient in group B.

In group B, 9/10 patients exhibited the latex-fruit syndrome, and in these patients sensitization to tomato occurred simultaneously with sensitization to latex. Allergies to fruits, legumes, and vegetables usually appear after the first 2 years of life, when the majority of these foods have already been introduced into the diet. These data show that food allergy constitutes a dynamic disorder, with some foods changing etiology as a function of the child's age (13).

Patatins are storage proteins found in plant families such as tomato and potato (14). Patatin from tomato (Sol t 1) and patatin from latex (Hev b 7) represent defense-related proteins, which have been connected to cross-reactions between latex and fruits and vegetables. In their study on latex-allergic patients, Seppälä et al. (15) showed that 49% of the latex-allergic adults had IgE antibodies to Hev b 7 and 43% had antibodies to Sol t 1 from potatoes. Moreover, 73% of these patients had a positive skin prick test to raw potato, but all of them were tolerant of potato.

These results agree with our study; tomato from pool A showed bands with molecular mass of 44, 46, and 67 kDa. Potato and latex also had a band with a molecular mass of 44 kDa. It has been reported that the serum of some latex-allergic patients recognized a 46-kDa protein found in nonammoniated latex (15). The N-terminal acid sequence of the 46-kDa protein had sequence homology (60%) with patatin, a protein of potato. In the immunoblotting, we had two bands of 44 and 46 kDa that could be patatin. In the immunoblot inhibition, potato inhibited tomato and latex, but our patients were tolerant of potato. A possible explanation of this tolerance is the loss of allergenicity due to the cooking of the potato. Patients usually eat cooked potato, not raw potato.

Because of the intensive cross-reactivity shown in the immunoblot inhibition, CAP inhibition was done to determine the degree of cross-reactivity more accurately.

Both groups of patients (A and B) were tolerant to

cooked potato, but in the immunoblot inhibition and CAP inhibition we observed a full inhibition between potato and tomato and latex.

In our study, all three, tomato, latex, and potato, had several bands with a molecular mass between 14 and 30 kDa. There are several latex allergens with these molecular masses, such as Hev b 1 (rubber elongation factor), Hev b 5 (acidic latex protein), Hev b 6 (prohevein), and Hev b 8 (latex profilin), and some of them have been described as the cause of cross-reactivity (16, 17). Another relevant latex allergen, Hev b 2, is a 35-kDa basic β -1,3-glucanase, and was recognized by specific IgE from food-allergic patients with hypersensitivity to tomato (18).

PR-5 proteins are designated thaumatin-like proteins (TLPs). One of these proteins was found to be identical to a corresponding portion of the osmotin-like protein P23 from tomatoes (19). There has been evidence in the past few years of 30–35-kDa allergens with common

epitopes present in latex and in several fruits. These common allergens are class I chitinases and are the major panallergens involved in the latex-fruit syndrome (19). In both pool A and pool B, we recognized bands in these areas.

In summary, patatin, a protein of 44–46 kDa, may be implicated in the high cross-reactivity between tomato, latex, and potato. Clinical symptoms with tomato and not with potato could be explained by the loss of allergenicity that results from cooking the potato. Further studies are needed to establish clearly the major allergens of tomato, because of the significance of this food as a staple in the European diet.

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