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Tomato Allergy: Detection of IgE-Binding Lipid Transfer Proteins in Tomato Derivatives and in Fresh Tomato Peel, Pulp, and Seeds

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There is an increasing consumption of tomatoes worldwide: fresh in salads, cooked in household sauces, or industrially processed. Although many tomato allergens have been identified, there is no information in the literature on the allergenic components found in commercial tomato products. The primary aim of the study was to evaluate the allergenic profile of commercial tomato products by skin prick tests (SPTs) and IgE/immunoblotting in tomato-allergic subjects. The secondary end point was the study of the IgE-binding profile of tomato peel, pulp, and seeds. Forty tomato-allergic patients, reporting oral allergy syndrome (OAS) at different grades of severity for fresh and, in some cases, also for cooked tomato, were selected on the basis of positive tomato allergy history or open food challenge (OFC). They were evaluated by SPTs with different experimental tomato extracts. SDS-PAGE/immunoblotting was performed to detect tomato allergens, which were then identified by Edman degradation. Twenty-three patients (57.5%) presented first-grade OAS at the OFC, whereas 17 (42.5%) reported severe symptoms. Ten of these 17 patients (25%) reported allergic reactions to cooked tomatoes; in immunoblotting tests, their sera reacted only to lipid transfer protein (LTP). In commercial products, LTP was the only detectable allergen. In contrast to other LTP-containing fruits, in tomato, an IgE-binding LTP was identified not only in the peel but also in the pulp and seeds. This study demonstrates that, in fresh tomato, different LTP isoforms are present and allergenic. Industrial tomato derivatives still contain LTP, thus presenting a problem for LTP-allergic patients.

KEYWORDS: ***Lycopersicon esculentum*; industrial tomato derivatives; lipid transfer protein; oral allergy syndrome; skin prick test; tomato allergy**

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is a very important dietary component, consumed worldwide. World production has doubled over the past two decades, reaching about 119 million tons in 2002−2004, and its global consumption increased by a yearly average of 4.5% between 1990 and 2004 (*1*, *2*). This may be due to the growing attention consumers have been paying to the health benefits of tomatoes. Several studies correlated a regular tomato intake with a decreased risk of prostate cancer or cardiovascular diseases (*3*, *4*). These effects have been attributed to the composition of tomatoes, which are very rich in antioxidant and free radical scavenging molecules (e.g., lycopene and β-carotene). Interestingly, industrial processing does not alter total phenolic and flavonoid content and enhances lycopene bioavailability (*5*, *6*).

Tomato is a well-known allergen source as demonstrated by several authors reporting that tomato allergy prevalence is more frequent in pollen-sensitized patients. De Martino et al. (*7*) found that 39.2% of children monosensitized to grass pollen had tomato-specific IgE. Ortolani et al. (*8*) confirmed the statistically significant association between tomato oral allergy syndrome (OAS) and grass pollen allergy; authors also reported two cases of anaphylaxis. In Germany, Foetish et al. (*9*) found a 9% prevalence of tomato OAS with detectable tomato-specific IgE in 357 mainly birch pollen allergic patients. More recently, in a study of 1734 newly investigated patients, Larramendi et al. (*10*) reported tomato sensitization accompanied by allergic symptoms in 1.85% of the overall population.

Up to now, several tomato allergens have been identified and characterized in fresh tomato fruit, in particular, Lyc e 1, a

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profilin (*11*, *12*); Lyc e 2, a β-fructofuranosidase (*13*); and Lyc e 3, a lipid transfer protein (LTP) (*9*). Lyc e 3 silenced tomato fruits have recently been produced by Lorenz et al. (*14*). No study has evaluated the clinical relevance of tomato LTP.

No data are currently available on the IgE reactivity of processed tomato which, in some countries, is the form in which tomatoes are most frequently consumed. In the United States, fresh tomato production reached about 2 million tons in 2006, whereas approximately 10 million tons were produced for processing (*1*). The European Prospective Investigation into Cancer and Nutrition Study (EPIC) found the intake of raw tomatoes ranges from an average of 5.9 g/day in The Netherlands to 112.5 g/day in Greece, whereas that of cooked and processed tomatoes goes from 9.8 g/day in The Netherlands to 51.1 g/day in Greece (*15*).

Therefore, tomato allergy studies ought to include a clinical evaluation not only of fresh tomatoes but also of the processed derivatives, identifying the modification of the IgE-binding property of the different allergens when subjected to different technological treatments. In the present study the primary aim was to evaluate the IgE-binding capacity of commercially available tomato products as compared to fresh tomatoes in vivo and in vitro. The secondary end point was to investigate whether LTP was detectable in fresh tomato peel, pulp, and seed extracts.

MATERIALS AND METHODS

Patients. Forty patients (27 females and 13 males, mean age = 28.9), whose clinical data are summarized in **Table 1**, were admitted to the study on the basis of a clear history of allergic reactions to tomato, positive fresh tomato prick + prick test (*16*), and specific IgE titration.

Tomato allergy history was evaluated as reliable when at least typical first-grade OAS occurred (*17*). Subjects with allergic symptoms strictly localized in the oral mucosa were enrolled if presenting a positive open food challenge (OFC). Patients reporting more severe reactions were not challenged because of the potential risk of reactions. According to symptom severity, we identified four OAS grades, as already published (*17*).

The Ethics Committee approved the study, and all selected patients were enrolled after providing their informed consent.

Study Design. The subsequent steps of the present study were (1) to determine the IgE-binding profile of 'Galeon' fresh tomato; (2) to characterize the IgE-binding pattern of six different commercial tomato products using a pool serum; (3) to study skin reactivity to two fresh tomato cultivars ('Galeon' and 'Joy') and three tomato derivatives (canned peeled tomatoes, tomato puree, and tomato paste) in tomato-allergic subjects; (4) to determine the IgE-binding profile of the main fresh tomato tissues, that is, peel, pulp, and seeds using a pool serum; and, finally, (5) to detect and identify the 9 kDa protein band in different experimental extracts (tomato tissues and industrial derivatives).

Skin Tests. To evaluate the IgE reactivity of industrial tomato products and their basic materials, skin tests were performed with experimental in-house extracts from fresh tomatoes ('Galeon' and 'Joy' cultivars) and commercial tomato products (canned peeled tomatoes, tomato puree, and tomato paste); the extracts were prepared according to the method of Primavesi et al. (*18*). Histamine dihydrochloride (10 mg/mL) and saline solution were also tested as positive and negative controls, respectively. Briefly, 300 g of homogenized material was extracted 1:1 with phosphate buffer saline (PBS) 0.1 M, pH 7.4, and centrifuged at 6000 rpm for 20 min; the supernatant was then dialyzed versus PBS and concentrated using an Amicon ultrafiltration cell (membrane cutoff 3000 Da) up to $^1/_{10}$ of the initial volume. The extract was finally diluted (1:1 v/v) with glycerol to make it suitable for skin testing. To be considered positive, a skin test had to induce a wheal and flare reaction of at least 3 mm diameter (*19*).

Results were transferred to a thin tape; after scanning, the wheal areas were analyzed by Wilcoxon signed-rank test (exact two-tailed p value).

Tomato Open Food Challenge (OFC). OFC (*20*) was performed with 'Galeon' fresh tomato, administering doses at 15 min intervals. A minimum starting dose of 250 mg was given; the following doses were then doubled until 128 g. We based the maximum dose delivered on previous observations that positive reactions occur at higher tomato doses than other fruits (unpublished data). The test was considered to be positive when objective symptoms appeared. In the case of subjective symptoms, the challenge was considered to be positive when the same symptoms occurred twice.

Tomato IgE Determination. All of the selected patients were submitted to serum-specific IgE determination for tomato by the ImmunoCAP System (Phadia, Milan, Italy), according to the manufacturer's instructions. Results were expressed as kUA/L.

SDS-PAGE and IgE Immunoblotting. SDS-PAGE was carried out as described by Pastorello et al. (*20*), using a stacking gel of 6% and a gradient separation gel of 7.5−20%. The samples (0.16 mg protein × cm of gel) were run at 6 mA/gel for about 16 h in a Protean II xi Cell (Bio-Rad Laboratories, Hercules, CA). After separation, a part of the gel was stained with Coomassie Blue R-250.

Separated proteins were electroblotted onto a nitrocellulose membrane, which was cut into strips and incubated overnight with individual or pooled serum. IgE binding was detected by incubation with ^{125}I-labeled anti-human IgE antibodies and exposure on X-ray film at −70 °C for 4 days.

For the in vitro tests, tomato extracts, starting from 300 g of raw material according to Björksten et al. (*21*), were obtained from 'Galeon' and 'Joy' selected tomato cultivars intended for industrial processing; six commercial tomato derivatives, in particular from two different brands of canned peeled tomatoes, two tomato purees, and two tomato pastes; and different tissues (peel, pulp, and seeds) of the 'Galeon' cultivar, starting from 300 g of fresh tomato that yielded about 30 g of peel, 260 g of pulp and 10 g of seeds, respectively. In particular, seeds were separated from the surrounding moisture, then gently soaked in physiological solution, allowed to drain in a sieve, and ground in a mortar. Finally, the ground material was used for extraction (*21*).

Protein concentration of all tomato extracts was determined according to the Lowry method (*22*).

Identification of Tomato Allergens. Some bands corresponding to the IgE-binding proteins of the various tomato extracts ('Joy' peel tomato, purified 9 kDa proteins from 'Galeon' tissues, commercial tomato derivatives) were excised from the SDS-PAGE gel, passively eluted by a slightly modified Zieske technique, and microsequenced on a Procise 492 protein sequencer (Applied Biosystems, Fullerton, CA), as described elsewhere (*23*). To identify sequence homologies MPsrch software was used (http://www.ebi.ac.uk/MPsrch/).

Purification of Tomato 9 kDa Allergen. The 9 kDa protein was purified from separated extracts of 'Galeon' tomato peel, pulp, and seeds by following chromatographic steps (AKTA Purifier, Pharmacia Biotech, Uppsala, Sweden). First, proteins were separated by a cation-exchange Resource-S column (Pharmacia Biotech, volume = 6 mL), equilibrated with 50 mM sodium acetate and eluted with a linear gradient of 0−1 M NaCl, pH 5. The concentrated fractions were then separated on a gel filtration Superdex 75 HR 10/30 column (Amersham Biosciences). The eluted peaks were concentrated and analyzed by SDS-PAGE and immunoblotting. A solution at 10 μg/mL of purified protein was also tested by SPT to confirm its allergenic potency.

RESULTS

Patients. Twenty-three of 40 selected patients presented first-grade OAS. Among them, 16 patients reacted to OFC with fresh tomato at the maximum test dose (128 g), whereas 7 patients reacted at 64 g. Seventeen patients with a history of more serious clinical symptoms (i.e., urticaria, angioedema, gastrointestinal symptoms, and glottis edema) did not undergo the challenge. Ten of the 17 patients had a history of symptoms after eating either fresh or cooked tomatoes and commercial tomato products (patients 16, 18, 20, 21, 27, 30−32, 34, and 40). Patients reporting symptoms with cooked tomato were not challenged either with fresh or cooked tomatoes because all of them had severe reactions.

Patients with first-grade OAS were recommended not to consume fresh tomatoes, but they were allowed to eat cooked

Table 1. Demographic Data, Allergic Symptoms to Fresh Tomatoes, OAS Grade, Symptoms for Cooked Tomatoes, Open Food Challenge (OFC)-Eliciting Dose, Prick + Prick (P + P) Tests, and Specific Tomato IgE Levels

patient	age/sex	tomato symptoms[a]	OAS grade	cooked tomato symptoms (OAS grade)	OFC-eliciting dose (g)	P + P	tomato CAP (kUA/L)
1	41/F	A, U	3	N	nt[b]	+++	38.4
2	27/M	OAS	1	N	128	++	0.37
3	31/F	OAS, lips AE	1	N	nt	++	10.2
4	21/F	OAS	1	N	128	++++	12.5
5	27/F	OAS	1	N	64	+++	3.01
6	21/F	OAS	1	N	128	+++	9.13
7	58/F	OAS	1	N	128	+++	2.12
8	32/F	OAS	1	N	128	++++	2.83
9	32/F	OAS	1	N	128	+++	1.62
10	25/F	OAS	1	N	128	++++	4.41
11	49/F	OAS, GI	2	N	nt	+++	14.7
12	42/F	OAS	1	N	64	+++	1.81
13	23/M	OAS	1	N	128	++++	2.09
14	40/F	OAS	1	N	128	+++	1.18
15	20/M	OAS, e	1	N	128	+++	19.7
16	31/M	GI, U	3	Y (3)	nt	++	27.9
17	15/F	U-AE	3	N	nt	++++	1.52
18	29/F	OAS, AE	3	Y (2)	nt	++++	60.6
19	19/F	OAS, D	3	N	nt	++	1.98
20	22/F	OAS, AE, D	3	Y (3)	nt	+++	0.43
21	30/F	OAS, GE, GI	4	Y (3)	nt	+++	4.88
22	16/M	U, AE, GI	3	N	nt	++	4.23
23	26/M	OAS, GI, D	3	N	nt	+++	1.77
24	12/M	OAS	1	N	128	+++	4.04
25	35/M	OAS	1	N	128	+++	3.08
26	37/F	OAS	1	N	128	+++	2.82
27	34/F	OAS, GI	2	Y (2)	nt	++	5.67
28	40/F	OAS	1	N	64	+++	12.5
29	19/F	OAS, E	1	N	128	++	7.01
30	11/M	OAS, AE	3	Y (3)	nt	+++	1.88
31	26/M	OAS, AE	3	Y (2)	nt	+++	4.52
32	17/M	OAS, GE	4	Y (3)	nt	+++	7.09
33	14/F	OAS	1	N	128	+++	2.99
34	41/F	AE, U	3	Y (2)	nt	++++	15.5
35	46/F	OAS	1	N	128	+++	1.23
36	30/F	OAS, AE, D	3	N	nt	++	1.26
37	23/F	OAS	1	N	64	++	2.9
38	41/M	OAS	1	N	128	++	7.3
39	28/M	OAS, E	1	N	64	++++	14.8
40	25/F	OAS, GI	2	Y (2)	nt	++++	30.8

[a] A, asthma; AE, angioedema; D, dyspnea; E, eczema; GE, glottis edema; GI, gastrointestinal symptoms; OAS, first-grade of oral allergy syndrome; U, urticaria. [b] nt, not tested.

tomato or industrial tomato products without any restriction. Patients reporting severe reactions with tomato were strongly recommended to avoid fresh and cooked tomato, in the forms of both household sauce and industrial derivatives. Moreover, they received autoinjectable epinephrine and were carefully trained with regard to behavior in the event of an emergency.

Fresh 'Galeon' Tomato IgE Immunoblotting. Figure 1 shows IgE immunoblotting of fresh 'Galeon' tomato using each patient's serum. Preliminary analyses did not show any difference between 'Galeon' and 'Joy' cultivars either in SDS-PAGE bands or in IgE-binding pattern (data not shown). Furthermore, as even the N-terminal sequences of 9 kDa proteins of the two tomato cultivars peel were similar, we decided to perform further analyses only with 'Galeon' tomato.

Many IgE-binding proteins were highlighted; in particular, 26 patients responded to a 14 kDa protein (65%) and 24 patients to a 35 kDa protein (60%), whereas 20 patients reacted to bands at 24 and 26 kDa (50%), 18 patients recognized a 9 kDa protein (45%), and 13 patients responded to a 30 kDa allergen (32.5%).

Allergen Characterization. N-terminal sequencing was performed on all allergenic proteins of fresh tomato extracted from gel as indicated in **Figure 1** (bands A−E). The 9 kDa band corresponds to an LTP (A), the 24 kDa band to the β-fructofuranosidase precursor (B), the 26 kDa band to the osmotin-like protein (C), the 30 kDa band to the basic endochitinase precursor (D), and the 35 kDa band to the pectinesterase 1 precursor (E). Unfortunately, we were not able to characterize the band at 14 kDa, due to several N-terminal analysis failures, but its molecular mass and the fact that it was the most widely recognized allergenic band in our study population led us to hypothesize a profilin.

IgE Binding Properties of Different Tomato Extracts (Peel, Pulp, Seeds and Commercial Tomato Derivatives). To evaluate LTP localization in tomato, as has already been done for several fruits belonging to the Rosaceae family, such as apple, plum, peach, and apricot (*24*), protein extracts of peel, pulp, and seeds were analyzed by IgE immunoblotting. **Figure 2a** shows the IgE-binding profile of extracts from 'Galeon' tomato peel, pulp, and seeds, using a pooled serum from patients 1, 3, 20, 21, and 34, selected on the basis of their tomato IgE-binding profiles. An IgE

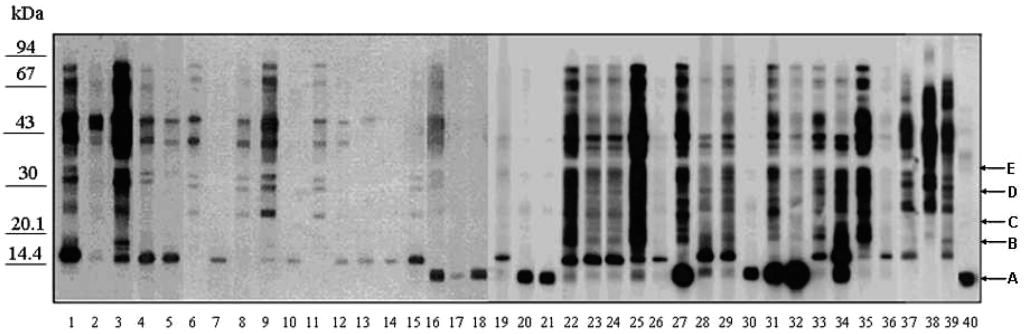

Figure 1. IgE immunoblotting results, by patient, for fresh raw 'Galeon' tomato extract. The arrows indicate the protein bands analyzed by Edman degradation: A, lipid transfer protein; B, β-fructofuranosidase precursor; C, osmotin-like precursor; D, basic endochitinase precursor; E, pectinesterase 1 precursor.

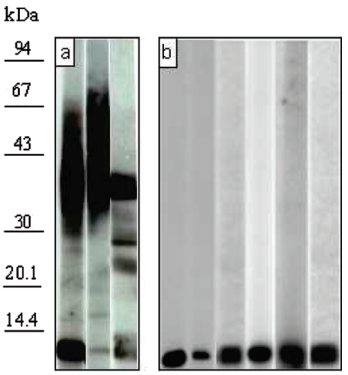

Figure 2. IgE immunoblotting results for different tomato extracts: (**a**) 'Galeon' tomato peel (lane 1), pulp (lane 2), and seeds (lane 3); (**b**) industrial tomato derivatives [canned peeled tomatoes (lanes 4 and 5), tomato puree (lanes 6 and 7), and tomato paste (lanes 8 and 9)].

reactivity with the 9 kDa band was detectable in all three extracts. **Figure 2b** reports the allergenic profile of six different industrial tomato products (two brands of canned peeled tomatoes, two tomato pastes, and two purees), which typically underwent mild to high thermal treatment. The IgE Immunoblotting of these extracts revealed a binding only to the 9 kDa band.

Purified 9 kDa protein from tomato tissues was also assessed by IgE immunoblotting using the pooled serum described above, showing a single allergenic band. **Figure 3C** shows IgE immunoblotting results for the purified tomato peel LTP.

Comparison of 9 kDa Allergen Sequences from Different Tomato Extracts (Peel, Pulp, Seeds, and Commercial Tomato Derivatives). The purified 9 kDa proteins from different sources were N-terminally sequenced, and the results are shown in **Table 2**.

The N-terminal sequence of the 9 kDa protein in peel and pulp of 'Galeon' tomato was L-S-C-G-E-V-T-S-G-L; this allergen was identified as LTP 2 (Swiss-Prot accession no. P93224). The same N-terminal sequence was found in 'Joy' tomato peel extract.

In 'Galeon' tomato seed extract the 9 kDa protein sequence was V-I-T-C-D-T-V-F-N-D-L-K-P-C-L. This sequence presents a high homology with LTPs from other species, such as sesame (64.3%), wheat (57.1%), rice (57.1%), sunflower (57.1%), and lentil (57.1%), whereas the homology percentage with the tomato peel and pulp LTPs is lower.

Industrial tomato products showed 9 kDa proteins with the following sequences: L-S/T-C-G-Q/E-V-E-S/L-G, identified as LTP 1 (Swiss-Prot accession no. P27056), and V-I-T-C-D-T-V-F-N-D-L-K−P-C-L, corresponding to tomato seed LTP.

Skin Prick Tests with Different Tomato Extracts. The protein contents of tomato extracts used for skin tests were 2.0 and 2.2 mg/mL for whole 'Galeon' and 'Joy' extracts and 2.4, 3.9, and 6.3 mg/mL for canned peeled tomato, tomato puree, and tomato paste, respectively. SPTs were performed in two groups of patients selected on the basis of the immunoblotting results: the first group of patients (patients 1, 4, 9, 11, and 37) were LTP negative (black columns, **Figure 4**) and the second (patients 18, 20, 21, 30, and 40) exclusively LTP positive (white columns, **Figure 4**). No statistically significant difference in skin reactivity to 'Galeon' and 'Joy' whole tomato extracts was detected between the two groups of patients, that is, LTP positive and LTP negative. On the contrary, a strong difference in skin reactivity for canned peeled tomato, puree, and paste (**Figure 4**, right side) was observed: skin tests were completely negative in LTP-negative patients and highly positive in LTP-positive patients.

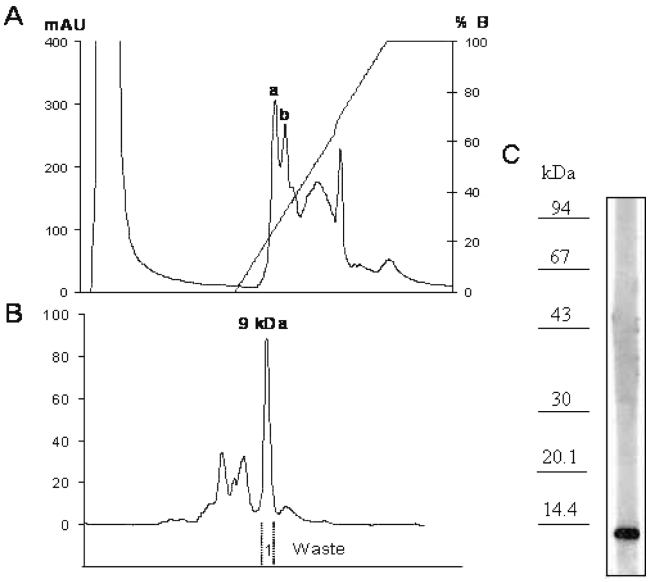

Figure 3. Purification of the 9 kDa protein from tomato tissues: (**A**) cationic exchange chromatography profile; (**B**) gel filtration profile of the peaks obtained with the cationic exchange column; (**C**) IgE immunoblotting results for purified 9 kDa protein from tomato peel.

Table 2. N-Terminal Sequences of IgE-Binding Proteins at 9 kDa Found in Different Tomato Extracts

tomato extract	LTP N-terminal sequence	Swiss-Prot accession no.
peel	L-S-C-G-E-V-T-S-G-L	LTP2 (P93224)
pulp	L-S-C-G-E-V-T-S-G-L	LTP2 (P93224)
industrial products	(a) L-S/T-C-G-Q/E-V-E-S/L-G	(a) LTP1 (P27056)
	(b) V-I-T-C-D-T-V-F-N-D-L-K-P-C-L	
seeds	V-I-T-C-D-T-V-F-N-D-L-K-P-C-L	

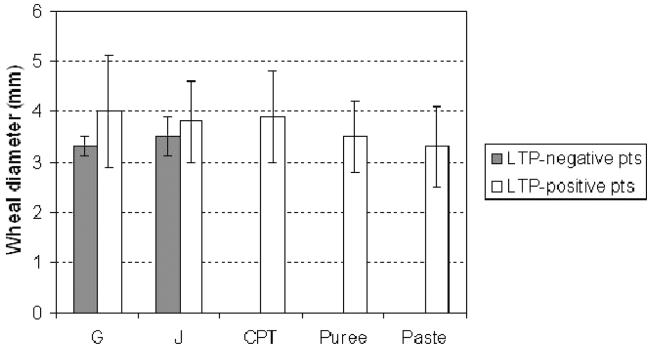

Figure 4. SPT results, expressed as mean wheal diameter ± standard deviation, for the in-house tomato extracts (G, 'Galeon' tomato; J, 'Joy' tomato; CPT, canned peeled tomatoes). SPTs were performed in two groups of tomato-allergic patients, the first including patients without LTP reactivity (black columns) and the second, LTP-reactive patients (white columns).

DISCUSSION

The primary end point of the study was the evaluation of the allergenicity of industrial tomato products in a study population of 40 tomato-allergic patients, some of them reporting adverse reactions to cooked tomato, too. Using IgE immunoblotting, we found several tomato allergens already described in the literature, allowing us to evaluate their epidemiological relevance in the Italian population. In particular, this is the first study evaluating the allergenic role of tomato LTP, which, although it is only a minor allergen, proved to be clinically relevant in our study population, being the only recognized allergen in 15% of the patients (patients 17, 18, 20, 21, 30, and 40). Generally, these patients experienced more severe reactions to tomato, such as angioedema, urticaria, and dyspnea, and showed symptoms also with tomato-derived products.

In Rosaceae fruits, LTP is mainly concentrated in the peel, with lower amounts detectable in the pulp, too(*24*); on the basis of this observation, we decided to investigate IgE reactivity of the three main tomato tissues in the 'Galeon' cultivar. IgE immunoblotting illustrates that peel, pulp, and seeds contained a 9 kDa IgE-binding band (**Figure 2a**); the N-terminal sequences confirmed that these proteins are all LTPs. We could not exclude the hypothesis that, even if we carefully separated each tomato part from the others, a minimal part of tomato peel LTP had contaminated pulp during lancet peeling. Nevertheless, other previous authors isolated a tomato LTP from fruit pericarp, showing its role in activating the polygalacturonase multiprotein complex, responsible for pectin degradation and pulp softening during tomato ripening (*25*). In particular, the sequences of tomato peel and pulp LTPs corresponded to the already described Lyc e 3, whereas the tomato seed LTP sequence proved to be a different isoform, with a high percentage of homology with other species' LTPs: sesame, wheat, rice, sunflower, lentil, and barley, for example. Interestingly, Sheoran et al. (*26*) first described tomato seed LTP, as well as other storage proteins, as having the function of transporting lipids from endosperm to embryo and defending the seed against infections during germination.

Our major finding was that all of the commercial tomato derivatives considered, such as canned peeled tomatoes, tomato puree, and tomato paste, contain a single detectable IgE-binding protein, which proved to be an LTP (accession number P27056, tomato seed LTP). According to these observations, we found that skin reactivity to tomato derivatives was positive only in patients with in vitro IgE reactivity to LTP and negative in patients without LTP sensitization. Moreover, all of the patients reacting to tomato sauce were positive to LTP, thus implying that these products are not safe for LTP-sensitized patients.

This was not surprising because the thermal treatment denatures many allergens, but not LTP, as demonstrated for peach (*27*) and maize (*28*). Industrial tomato processing can involve various steps, depending on which of the wide range of final products present on the market is being made. Generally, in the production of peeled whole tomatoes, after washing and sorting, fresh tomatoes are scalded in boiling water, mechanically peeled, packed, and thermally treated; for pulp or paste production fresh tomatoes are crushed, thermally treated, strained to remove seeds and peel, and finally concentrated by evaporation, until the desired content of soluble solids (°Brix) is reached. Industrial tomato products are thus typically produced using mild to intense thermal treatment. Considering these findings, a simple prick + prick test performed with commercial tomato derivatives, such as tomato sauce, might be useful to identify tomato LTP-positive patients.

In conclusion, this study has demonstrated that (a) in industrial derivatives LTP is the only allergenic protein still present; (b) industrial tomato derivatives, which are commonly consumed worldwide, could be a significant allergen source for LTP allergic patients; and (c) different allergenic LTPs are present not only in tomato peel but also in tomato pulp and seeds.

ABBREVIATIONS USED

LTP, lipid transfer protein; OAS, oral allergy syndrome; OFC, open food challenge; PBS, phosphate buffer saline; SPT, skin prick test.

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