ORIGINAL ARTICLE

Tomato nsLTP as an "In Vivo" Diagnostic Tool: Sensitization in a Mediterranean Population

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Abstract

Background: Tomato allergies have been extensively studied but component-resolved in vivo diagnosis with purified allergens has yet to be performed.
Objectives: To evaluate the prevalence of sensitization to Sola l 3 in a Mediterranean population, and to compare the resulting sensitization profile with that of individuals sensitized to tomato, peach, and/or purified lipid transfer protein (LTP).
Methods: Sola l 3 was purified, characterized, and used to prepare skin prick tests (SPTs). Two groups of patients were selected. Group 1 consisted of patients with at least 1 positive SPT to tomato, peach, or LTP mixture (marker extracts) who were subsequently tested with Sola l 3 (n=280). Group 2 (prevalence study) consisted of patients who underwent simultaneous SPT with the 3 marker extracts and Sola l 3 (n=658). Patients from either group who were positive to any of the 4 extracts were studied in detail (study group, n=123). ELISA and immunoblot assays were performed in individuals with a positive SPT to Sola l 3 to detect the presence of specific IgE antibodies to this allergen.
Results: Prevalence of sensitization to Sola l 3 was 3.2% overall and 54.7% in tomato-positive patients. Most tomato-sensitized patients were asymptomatic. Symptoms were more common in Sola l 3–positive individuals. Sensitization to peach and the LTP mixture did not discriminate between Sola l 3–positive and Sola l 3–negative patients.
Conclusions: This study confirms that LTP, not only from peach but also from other fruit and vegetables, including tomato, is an important allergen in the Mediterranean area. Sensitization to Sola l 3 is associated with more symptoms in tomato-sensitized patients.

Key words: Tomato. Peach. nsLTP. Sola l 3. Allergens. In vivo diagnosis. Food allergy. Molecular diagnosis.

Resumen

Antecedentes: La alergia a tomate ha sido ampliamente estudiada; sin embargo, todavía no se ha realizado diagnóstico basado en componentes *in vivo* con alérgenos purificados.
Objetivos: Evaluar la prevalencia de sensibilización a Sola l 3 en una población mediterránea y comparar su perfil de sensibilización con el de individuos sensibilizados a piel de tomate, piel de melocotón y/o nsLTP (proteínas de transferencia de lípidos) purificadas.
Métodos: Se purificó Sola l 3, se caracterizó y se utilizó para preparar pruebas cutáneas (SPT). Se seleccionaron dos grupos de pacientes. Grupo 1: a estos pacientes se les realizaron pruebas cutáneas con tomate, melocotón y una mezcla de LTPs (extractos marcadores); a los pacientes que fueron positivos al menos a uno ellos se les realizó una prueba cutánea con Sola l 3 (n=280). Grupo 2 (estudio de prevalencia): a los pacientes se les realizó una prueba cutánea simultáneamente con los tres extractos marcadores y Sola l 3 (n=658). Los pacientes de los dos grupos que fueron positivos a cualquiera de los cuatros extractos se analizaron con más detalle (grupo de estudio, n=123). Se realizaron ensayos de ELISA e inmunoblot con los individuos positivos a Sola l 3 para detectar la presencia de anticuerpos IgE específicos para este alérgeno.

Resultados: La prevalencia de sensibilización a Sola l 3 fue del 3,2%, pero incrementó hasta un 54,7% en pacientes positivos a tomate. La mayoría de los pacientes sensibilizados a tomate fueron asintomáticos. El número de pacientes sintomáticos aumentó en los individuos positivos a Sola l 3. La sensibilización a melocotón y mezcla de LTP no discriminó entre pacientes positivos y negativos a Sola l 3.
Conclusiones: Este estudio confirma que las nsLTP son alérgenos importantes en el área mediterránea, no solo relacionados con melocotón, sino también con otros vegetales, como el tomate. La sensibilización a Sola l 3 está relacionada con más síntomas en pacientes sensibilizados a tomate.

Palabras clave: Tomate. Melocotón. nsLTP. Sola l 3. Alérgenos. Diagnóstico *in vivo*. Alergia a alimentos. Diagnóstico molecular.

Introduction

Allergy to tomato (*Solanum licopersicum*), the second most important vegetable crop after potato, is one of the most prevalent vegetable allergies, with an estimated prevalence of 6.5% in a Spanish Mediterranean population [1].

Various allergens have been identified in tomato, including Sola l 1 [2], Sola l 2 [3], Sola l 3 [4], and Sola l 4 [5] (formerly known as Lyc e 1, Lyc e 2, Lyc e 3, and Lyc e 4 respectively). All of these allergens are included in the WHO/IUIS Allergen Nomenclature, but other allergens have been described in various scientific publications [6-12]. Sola l 3, or non-specific lipid transfer protein (nsLTP), is the most abundant protein in different tomato variety extracts [13] and has been identified and characterized as the most important tomato allergen in Southern Europe [14]. Sensitization to LTPs has traditionally been linked to Pru p 3 sensitization, and LTP sensitization has been related to cross-reactivity in various species. While a low degree of sequence identity (45%-65%) has been found in different allergenic members of the LTP family, their highly conserved 3-dimensional structure could explain their cross-reactivity with each other [15].

Very few population studies have examined the importance of sensitization to individual food allergens. While component-resolved diagnosis (CRD) has been established as a potent in vitro tool for determining allergen sensitization profiles and a helpful instrument for the selection of immunotherapy [16], very few data are available regarding in vivo results obtained with CRD, especially in the case of food allergens.

Consequently, the objectives of this study were to investigate the prevalence of sensitization to Sola l 3 in a Mediterranean population, and, using purified Sola l 3 for in vivo molecular diagnosis, to compare the profile of sensitization to this allergen with that of individuals sensitized to tomato peel, peach peel, and/or purified LTP (Pru p 3/ Cor a 8 mix).

Materials and Methods

Sola l 3 Purification and Characterization

Tomato peel extract was prepared as previously described [1]. Briefly, ripe tomatoes were purchased at a local market, washed in distilled water, and carefully peeled. The peel was homogenized in 0.01 M phosphate-buffered saline/polyvinylpolypyrrolidone (PBS/PVPP), extracted under continuous magnetic stirring for 4 hours at 4ºC, and centrifuged. The supernatants were collected, dialyzed, sterile filtered, and freeze-dried.

Purification of consisted of 2 chromatographic steps with a high-performance liquid chromatography system (HPLC, 1200 Series, Agilent Technologies). Briefly, 500 mg of tomato peel extract was dissolved in formic buffer (20 mM HCOOH/ $HCOO^-$ pH 4) at 1 mg/mL and purified in a HiTrap SP column (GE Healthcare). The proteins were eluted in a 0%-100% gradient formic buffer with 1 M NaCl. Fractions including Sola l 3 were mixed, concentrated, and dialyzed with phosphate buffer (40 mM $H2PO4^-$/$HPO42^-$ pH 8; 150 mM NaCl). A second purification step was performed by size-exclusion chromatography with a Superdex 75 16/60 column (GE Healthcare). The protein content was measured by the Lowry-Biuret method (Sigma).

Five micrograms of the purified protein were run on SDS-PAGE under reducing conditions in 15% acrylamide/bis-acrylamide gels and stained with Bio-Safe Coomassie gel stain (BioRad). To confirm the identity of the protein, this was digested with trypsin and sequenced by liquid chromatography-tandem mass spectrometry (LC-MS-MS).

Skin prick tests (SPTs) were prepared at a concentration of 45 µg of Sola l 3/mL.

Patient Population

A multicenter observational prospective study was performed in the following centers in South-East Spain: Complejo Hospitalario Universitario de Cartagena (Cartagena, Murcia), Hospital Marina Baixa (Villajoyosa, Alicante), Hospital Vega Baja (Orihuela, Alicante), Hospital Clínico Universitario Virgen de la Arrixaca (Murcia), and Centro de Especialidades El Españoleto (Játiva, Valencia). The study was approved by the independent ethics committee at Hospital Vega Baja under protocol number GIAT 02/09.

All patients who attended allergy clinics at the participating centers during the study periods and reported respiratory symptoms (rhinitis, rhinoconjunctivitis, and/or asthma) and/or cutaneous symptoms (urticaria and/or atopic dermatitis), with clinical indications for standard SPTs to inhalant or food allergens, were included.

SPTs with tomato peel (5 mg of freeze-dried peel/mL), peach peel (5 mg/mL) (Laboratorios LETI), and LTP mixture were performed on all patients. The LTP mixture was prepared by mixing 15 µg/mL of Pru p 3 and 15 µg/mL of Cor a 8 (total 30 µg/mL of LTP). Pru p 3 and Cor a 8 were purified using the same method as described for Sola l 3.

All patients gave informed written consent to participate in the study. Individuals without clinical indication for SPT for common allergens, on treatment with antihistamines/

corticosteroids, or who refused to provide their consent were excluded. Serum samples were obtained from all Sola l 3–positive patients who agreed to have blood taken.

The study comprised 2 groups:

Group 1. Patients were recruited in 3 centers: Complejo Hospitalario Universitario de Cartagena, Hospital Marina Baixa, and Hospital Clínico Universitario Virgen de la Arrixaca. Each center collected data on the first 95 patients fulfilling the inclusion criteria. All the patients initially underwent SPT with tomato peel, peach peel, and the LTP mixture, and those positive to at least 1 of the 3 extracts underwent SPT with Sola l 3.

Group 2 (prevalence study). For the comparative analysis of the prevalence of Sola13 in this Mediterranean population, tomato peel, peach peel, LTP mixture, and Sola l 3 were tested simultaneously on all patients fulfilling the inclusion criteria over a 3-month period.

Study Group

All patients from the 2 groups who tested positive to at least 1 of the SPTs (tomato peel, peach peel, LTP mixture, and Sola13) were further studied. The study group was used to compare the profile of sensitization to Sola13 with that of individuals sensitized to tomato peel, peach peel, and/or LTP.

Specific IgE

Testing for IgE antibodies specific to Sola13 was performed by direct ELISA. Briefly, Immulon IV microplates (Thermo Fisher Scientific) were coated with purified Sola13 (10 µg of protein per well), and each serum sample (diluted 1:1 with 0.01 M PBS) was incubated for 2 hours at room temperature. After 3 washes with PBS-0.1% Tween, peroxidase-conjugated monoclonal antihuman IgE (Ingenasa) (dilution 1:800) was added. After 2 hours, the reaction was developed with TMB (3,3',5,5'-tetramethylbenzidine), stopped with 0.16 M sulfuric acid, and read at 450 nm with a plate reader (Thermo Fisher Scientific). Serum with an optical density value equal to or below 0.15 was considered negative and not used for further assays.

Immunoblot

Immunoblot assays were performed to observe the recognition of Sola l 3 by ELISA-positive sera. Briefly, 2 µg of Sola l 3 run on SDS-PAGE were electrotransferred to an Immobilon-P membrane (Millipore) and dried at room temperature. The membranes were then incubated overnight with individual patient sera diluted 2/3 in PBS. After incubation with monoclonal antihuman IgE-peroxidase (Ingenasa), the reaction was developed with luminol (BioRad) and visualized by chemiluminescence.

Statistical Analysis

Descriptive statistical analyses (mean and SD) were used for the analysis of numerical variables (age and wheal size). $\chi 2$ or Mann-Whitney tests were used to compare groups of individuals (those sensitized to Sola13 vs those not sensitized). SigmaStat 3.5 (Point Richmond) software and Open Epi version 2.3.1 (http://www.openepi.com/Menu/OE_Menu.htm) were used for the statistical analysis.

Results

Sola l 3 Purification and Characterization

Sola13 was purified in 2 steps with a final yield of 4.4 µg per mg of tomato peel extract. The SDS-PAGE of the protein obtained in the final purification step is shown in Figure 1A. The peptide sequences obtained by LC/MS-MS aligned perfectly with different zones of 2 isoforms of Sola l 3 (UniProtKB Q4A1N1 and P93224) (Figure 1B). Alignment

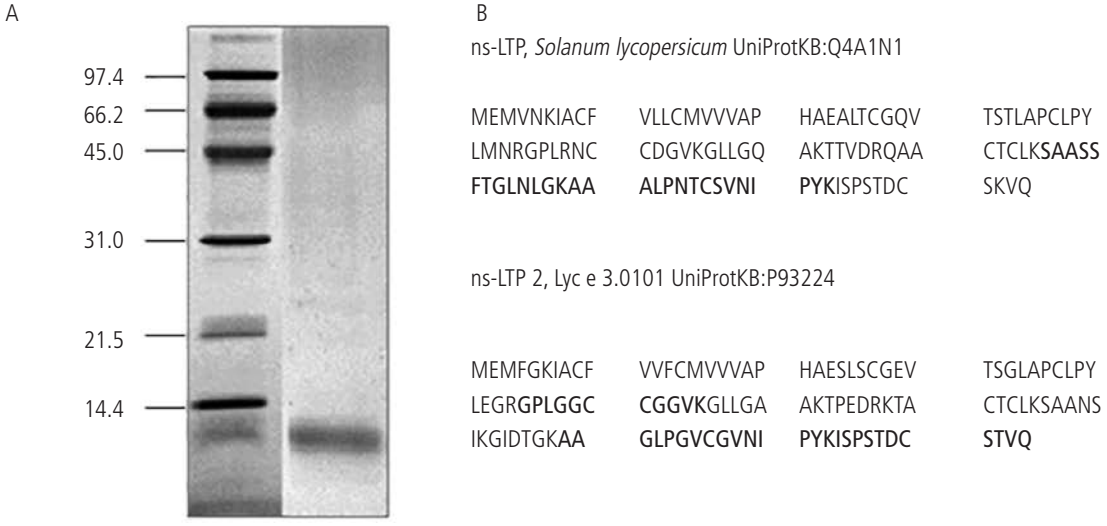

Figure 1. A, SDS-PAGE of purified Sola l 3 (5 µg from the last purification step). B, Sequences of tomato non-specific lipid transfer proteins (UniProtKB), peptides obtained by liquid chromatography-tandem mass spectrometry in this study are marked in bold.

A

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Sola l 3 (Q4A1N1)       ---MEMVNKIACFVLLCMVVVAPHAEA-LTCGQVTSTLAPCLPYLMNRGPLR-NCCDGVK   55
Sola l 3 (P93224)       ---MEMFGKIACFVVFCMVVVAPHAES-LSCGEVTSGLAPCLPYLEGRGPLG-GCCGGVK   55
Pru p 3 (B6CQU3)        MAYSAMT-KLALVVALCMVVSVPIAQA-ITCGQVSSSLAPCIPYVRGGGAVPPACCNGIR   58
Pru p 3 (P81402)        ----------------------------ITCGQVSSALAPCIPYVRGGGAVPPACCNGIR   32
Cor a 8 (Q9ATH2)        ---MGSL-KLVCAVLLCMMVAAPVARASLTCPQIKGNLTPCVLYLKNGGVLPPSCCKGVR   56
                                                    ::* ::.. *:**: *: . * :   ** *::

Sola l 3 (Q4A1N1)         GLLGQAKTTVDRQAACTCLKSAASSFTGLNLGKAAALPNTCSVNIPYKISPSTDCSKVQ   114
Sola l 3 (P93224)         GLLGAAKTPEDRKTACTCLKSAANSIKGIDTGKAAGLPGVCGVNIPYKISPSTDCSTVQ   114
Pru p 3 (B6CQU3)          NVNNLARTTPDRQAACNCLKQLSASVPGVNPNNAAALPGKCGVSIPYKISASTNCATVK   117
Pru p 3 (P81402)          NVNNLARTTPDRQAACNCLKQLSASVPGVNPNNAAALPGKCGVHIPYKISASTNCATVK   91
Cor a 8 (Q9ATH2)          AVNDASRTTSDRQSACNCLKDTAKGIAGLNPNLAAGLPGKCGVNIPYKISPSTNCNNVK   115
                            : . ::*. **::**.***. : .. *:: . **.**. *.* ******.**:* .*
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B

% Homology	Sola l 3 (Q4A1N1)	Sola l 3 (P93224)	Pru p 3 (B6CQU3)	Pru p 3 (P81402)	Cor a 8 (Q9ATH2)
Sola l 3 (Q4A1N1)					
Sola l 3 (P93224)	74%				
Pru p 3 (B6CQU3)	51%	47%			
Pru p 3 (P81402)	52%	49%	97%		
Cor a 8 (Q9ATH2)	52%	49%	55%	59%	

Sequences have been obtained from UniprotKB (http://www.uniprot.org/)

Figure 2. A, Alignment of lipid transfer protein sequences from the proteins used in the study. B, Percentage of homology between each pair of proteins.

of tomato Sola l 3 with the LTPs used as markers (Pru p 3 and Cor a 8) is shown in Figure 2A. The percentage of homology is shown in Figure 2B.

Patient Population and Clinical Characteristics

The study comprised 938 individuals in total; there were 280 individuals in group 1 and 658 in group 2.

Study Group

A total of 123 patients (13.1%), 34 from group 1 (12.1%) and 89 from group 2 (13.5%), were positive to at least 1 of the 4 SPTs performed (Sola l 3, tomato peel, peach peel, and LTP mixture) and formed the study group (mean [SD] age, 28.8 [12.3] years; 59 males/64 females). In this group the characteristics of Sola l 3–sensitized patients were compared with those of patients sensitized to tomato peel, peach peel, or LTP mix. Their clinical characteristics are presented in Table 1.

Thirty-five individuals from the study group tested positive to purified Sola l 3 (28.5%). Sensitization of these 35 patients to the 3 different extracts used as selection criteria is shown in Figure 3A.

The characteristics of individuals in the study group were quite similar, regardless of Sola l 3 positivity or negativity; they differed in terms of sensitization to *Artemisia* pollen ($P<.05$) and, in particular, symptoms provoked by tomato. Nine (25.7%) of the Sola l 3–positive patients reported symptoms after the ingestion of tomato compared with just 6 (6.8%) of the Sola l 3–negative patients ($P<.001$). Seven (77.8%) of the 9 Sola l 3–positive patients with symptoms reported systemic symptoms including urticaria and anaphylaxis. Of the Sola l 3–negative patients with symptoms, 3 (50%) had oral allergy syndrome, 2 had digestive symptoms (33%) (1 of these was sensitized to the LTP mix), and 1 had urticaria (17%). More than half of the individuals sensitized to tomato peel (54.7%) were sensitized to Sola l 3, and most of the Sola l 3–positive individuals (82.9%) were sensitized to tomato peel. There was a significant difference between individuals with a positive and a negative test to Sola l 3 in terms of sensitization to tomato ($P<.001$). Neither sensitization to peach peel nor to the LTP mix (both nonsignificant) helped to distinguish between Sola l 3–positive and Sola l 3–negative individuals. Of the 3 markers studied, the only association found for sensitization to Sola l 3 was with tomato peel ($P<.001$) (Table 1).

The mean (SD) wheal size obtained with Sola l 3 was 38.2 (23.6) mm^2. The wheal sizes for the other 3 markers used as selection criteria are shown in Figure 3B and Table 1.

Table 1. Description of Study Group[a]

	Total	Sola l 3–Positive	Sola l 3–Negative	χ^2 (*P* Value)
Patients, No. (%)	123	35 (28.5)	88 (71.5)	
Mean age (SD), y	28.9 (12.1)	30.2 (12.2)	28.3 (12.1)	NS
Male/female, No. (%)	59/64 (48/52)	20/15 (57.1/42.9)	39/49 (44.3/55.7)	NS
Sensitization to aeroallergens				
Pollen	104 (84.6)	32 (91.4)	72 (81.8)	NS
Mites	55 (44.7)	19 (54.3)	36 (40.9)	NS
Epithelia	38 (30.9)	13 (37.1)	25 (28.4)	NS
Molds	14 (11.4)	5 (14.3)	9 (10.2)	NS
Inclusion criteria				
LTP mix	95 (77.2)	26 (74.3)	69 (78.4)	NS
Tomato peel	53 (43.1)	29 (82.9)	24 (27.3)	*P*<.001
Peach peel	95 (77.2)	27 (77.1)	68 (77.3)	NS
Tomato symptoms	15 (12.2)	9 (25.7)	6 (6.8)	*P*<.05
Peach symptoms	51 (41.5)	12 (34.3)	39 (44.3)	NS
Pollen				
Olea europaea	75 (61)	21 (60)	54 (61.4)	NS
Cupressus arizonica	17 (13.8)	4 (11.4)	13 (14.8)	NS
Grass mix	37 (30.1)	12 (34.3)	25 (28.4)	NS
Cynodon dactylon	28 (22.8)	10 (28.6)	18 (20.5)	NS
Platanus spp.	18 (14.6)	6 (17.1)	12 (13.6)	NS
Artemisia vulgaris	52 (42.3)	22 (62.9)	30 (34.1)	*P*<.05
Parietaria judaica	30 (24.4)	12 (34.3)	18 (20.5)	NS
Chenopodium album	50 (40.7)	17 (48.6)	33 (37.5)	NS
Salsola kali	51 (41.5)	16 (45.7)	35 (39.8)	NS
Plantago lanceolata	19 (15.4)	6 (17.1)	13 (14.8)	NS
Mean (SD) SPT wheal size, mm^2				
LTP mix	56.1 (37.8)	61.3 (52.5)	54.1 (30.7)	NS
Tomato peel	38.1 (23.9)	45.5 (26.6)	29.1 (16.9)	*P*<.05
Peach peel	53.5 (38)	56 (30.5)	52.5 (40.7)	NS
Sola13	38.2 (23.6)	38.2 (23.6)	-	

Abbreviations: LTP, lipid transfer protein; NS, nonsignificant; SPT, skin prick test.
[a]Data presented as number (%) of patients in each group unless stated otherwise.

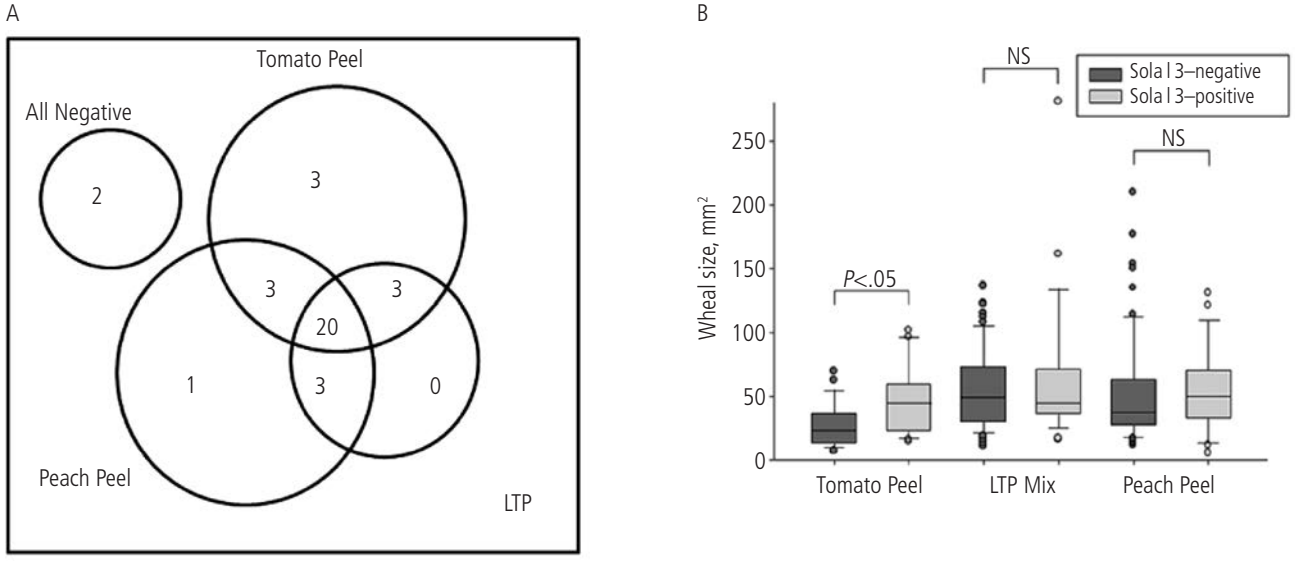

Figure 3. Characterization of the population. A, Number of patients sensitized by skin prick tests to the 3 extracts used as selection criteria. B, Skin prick test wheal sizes used as inclusion criteria. LTP, indicates lipid transfer protein; NS, nonsignificant.

Prevalence

The prevalence of sensitization to Sola l 3 was 3.2% (21 of the 658 individuals in group 2). The prevalence to the different test extracts analyzed was as follows: tomato peel 5.3% (n=35), peach peel 10.2% (n=67), and LTP 10.2% (n=67). Two of the 21 Sola l 3–positive patients in group 2 (9.5%) were negative to the 3 marker extracts analyzed.

Sola l 3–Specific IgE

Serum samples were obtained from 29 (82.9%) of the Sola l 3–positive individuals; 17 (58.6%) had positive specific IgE against the purified Sola l 3, with a mean (SD) value of 0.9 (0.8). There were no significant differences between IgE-positive/negative individuals regarding symptoms. More patients were sensitized to the LTP mixture and tomato peel in the IgE-positive group ($P<.05$). Wheals induced by Sola l 3 and LTP mix were larger in patients who tested positive for IgE to Sola l 3 ($P<.05$) (Table 2).

Immunoblot

Fifteen positive sera to Sola l 3 by ELISA were used in immunoblot assays (Figure 4). Not enough sera were collected from patients 198 and 203 to perform these analyses. Sola l 3 was detected in 12 sera (80%). Sera from 3 patients with negative sIgE to Sola l 3 (407, 763, and 803) were used as negative controls. Patients who did not recognize Sola l 3 in the immunoblot (94, 165, and 186) reported no symptoms with either tomato or peach.

Discussion

Tomato allergies have been extensively studied by different groups in the last 5 years. Numerous publications have characterized the allergenic composition of tomato extracts, properties of tomato allergens, and allergenic profiles of sensitized patients [7,11,13,16-19]. However, in vivo allergenic sensitization with purified Sola l 3 has never been investigated.

Table 2. Comparison of Sola l 3 IgE–Positive and Sola l 3 IgE–Negative Individuals[a]

	Total	Positive	Negative	χ^2 (*P* Value)
Patients, No. (%)	29	17 (58.6)	12 (41.4)	
Mean age (SD), y	30.1 (10.2)	26.9 (8.2)	34.6 (11.4)	NS
Male/female, No. (%)	18/11 (62.1/37.9)	12/5 (70.6/29.4)	6/6 (50/50)	NS
Sensitization to aeroallergens				
Pollen	27 (93.1)	16 (94.1)	10 (83.3)	NS
Mites	16 (55.2)	10 (58.8)	6 (50)	NS
Epithelia	13 (44.8)	7 (41.2)	6 (50)	NS
Molds	4 (13.8)	1 (5.9)	3 (25)	NS
Inclusion criteria				
LTP mix	23 (79.3)	16 (94.1)	7 (58.3)	*P*<.05
Tomato peel	23 (79.3)	16 (94.1)	7 (58.3)	*P*<.05
Peach peel	23 (79.3)	15 (88.2)	8 (66.7)	NS
Symptoms				
Tomato	7 (24.1)	6 (35.3)	1 (8.3)	NS
Peach	11 (37.9)	6 (35.3)	5 (41.7)	NS
Pollen				
Olea europaea	19 (65.5)	13 (76.5)	6 (50)	NS
Cupressus arizonica	4 (13.8)	3 (17.6)	1 (8.3)	NS
Grass mix	10 (34.5)	7 (41.2)	3 (25)	NS
Cynodon dactylon	9 (31)	6 (35.3)	3 (25)	NS
Platanus spp.	4 (13.8)	3 (17.6)	1 (8.3)	NS
Artemisia vulgaris	18 (62.1)	12 (70.6)	6 (50)	NS
Parietaria judaica	11 (37.9)	9 (52.9)	2 (16.7)	NS
Chenopodium album	16 (55.2)	10 (58.8)	6 (50)	NS
Salsola kali	16 (55.2)	11 (64.7)	5 (41.7)	NS
Plantago lanceolata	6 (20.7)	3 (17.6)	3 (25)	NS
Mean (SD) SPT wheal size, mm^2				
LTP mix	65 (54.9)	78.8 (60.7)	60.6 (77.9)	*P*<.05
Tomato peel	43 (24.6)	48.5 (25.8)	31.4 (17.5)	NS
Peach peel	58 (32.5)	65.8 (31.1)	43.4 (31.8)	NS
Sola l 3	38.6 (24.7)	45.8 (27.1)	28.6 (17.1)	P<.05

Abbreviations: LTP, lipid transfer protein; NS, not significant; SPT, skin prick test.
[a]Data presented as number (%) of patients in each group unless stated otherwise.

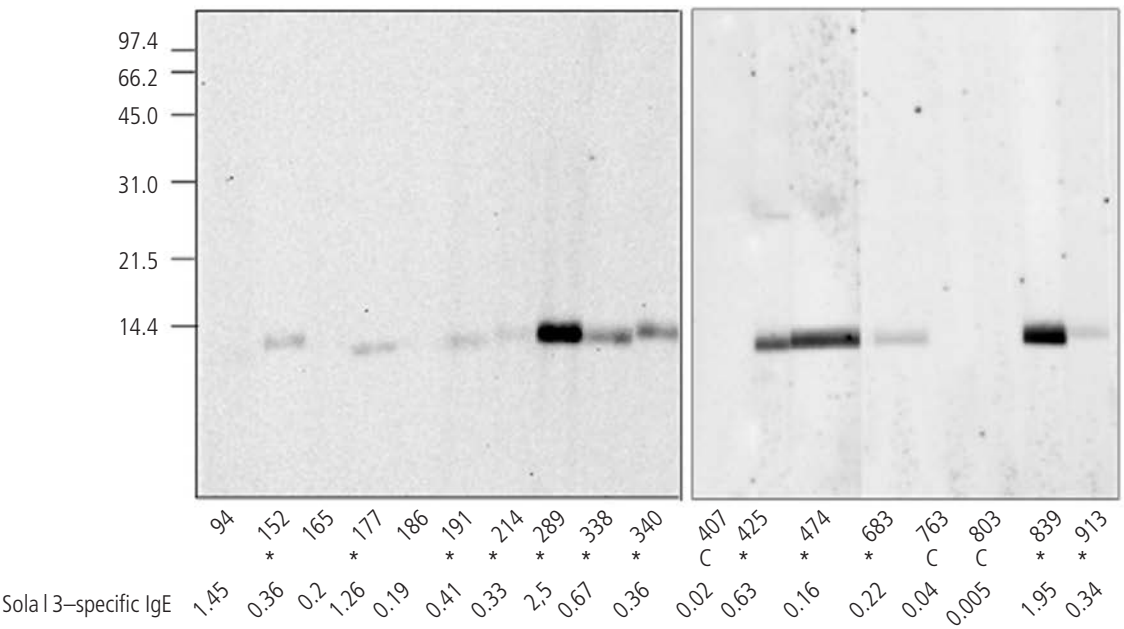

Figure 4. Immunoblot with the selected sera. Purified Sola l 3 was run in the solid phase. Different samples are shown with patient numbers. Negative controls are marked with a "C". Positive samples are marked with an asterisk (*). Sola l 3 specific IgE values (optical density) are shown under each sample.

The present study had 2 objectives and involved 2 different groups of patients. One group (comprising patients sensitized, according to SPT, to tomato peel, peach peel, and/or LTPs [a mixture of peach and hazelnut LTPs]) was used to study the relationship between sensitization to Sola l 3 and sensitization to whole tomato extract and other LTPs. The second group was used to study the prevalence of sensitization to Sola l 3 in a Mediterranean population and to detect patients specifically sensitized to Sola l 3.

Our data confirm previously published results regarding tomato sensitization in the Mediterranean area. We observed a prevalence of 5.9%, which is very similar to a previous finding of 6.5% by our group [1]. Sensitization to peach was also very high in our population (10.2%), but still lower than a rate of 26% reported elsewhere in pollen-allergic patients [20]. Even though the populations studied were different ("all allergic" in our study vs "pollen-allergic" in the second study), when we considered only pollen-sensitized individuals, the prevalence was still lower (14.2%, 57 of 401 pollen- sensitized individuals). These differences may reflect the heterogeneity of peach sensitization in Spain, which is probably less dependent on pollen sensitization along the Mediterranean coast (as in our study) than in the inland plateau region analyzed in the other study.

Pru p 3 sensitization in our study was 10.2%, which is similar to previously published rates for Spain (12%-13%) [21,22].

In our population, sensitization to Sola l 3 was 3.2%. However, this rate increased to 54.7% when only tomato-positive individuals were considered, indicating that Sola l 3 is a major tomato allergen in our area.

In group 2 (the prevalence study group), there were 2 patients who were positive to Sola l 3 and negative to the 3 selection extracts used in group 1. One of these patients had a small wheal (12 mm^2) and the other was sIgE-negative to Sola l 3. The positive SPT result could therefore be due to cross-reactivity to a fruit other than peach. Even if these 2 patients had been lost due to the restrictive criteria for group 1, neither showed significant sensitization to Sola l 3, suggesting the soundness of the selective criteria used to study purified proteins with limited availability.

As in previous studies [1], most of the tomato-sensitized individuals in our group were asymptomatic (only 20.8% of tomato-sensitized patients in the study group reported symptoms). In the present study, this percentage was slightly higher (25.7%) in Sola l 3–positive patients, 1 of whom tested negative to the complete tomato extract by SPT. However, there were significant differences on comparing Sola l 3–positive and –negative patients reporting symptoms to tomato ($P<.05$; OR, 4.731; 95% CI, 1.539-14.55). One-third (34.3%) of Sola l 3–positive patients also had symptoms with peach. All of them were sensitized to peach and LTP (except 1 who was sensitized only to tomato), suggesting probable cross-reactivity between Sola l 3 and Pru p 3. In the group of Sola l 3–negative individuals, 44.3% reported symptoms to peach.

Patients with a negative immunoblot reported no symptoms, and 58% of patients who recognized Sola l 3 by immunoblot reported symptoms (5 to tomato and 6 to peach). There were no differences in symptoms reported by patients who tested positive for sIgE and negative for peach and tomato.

The purified tomato LTP used in this study has a homology of around 50% with Pru p 3 and Cor a 8, probably explaining the high frequency of sensitization to the 2 LTPs used: Sola l 3 and the LTP mixture. The preparation of purified Sola l 3 used in SPT contained 2 of the 4 Sola l 3 isoforms described in the databases (http://www.allergome.org/); both share around 50% homology with Pru p 3 and Cor a 8 (Figure 2B).

In summary, we have confirmed that LTP from peach and other fruit and vegetables is a dominant allergen in the Mediterranean area. Sensitization to Sola l 3 is common in tomato-sensitized individuals in the Mediterranean area and is associated with more symptoms. In vivo molecular diagnosis with high-quality products such as those used in our study is a good tool for analyzing patient sensitization. This study supports the theory that not all nsLTPs are allergenically similar, and that the use of panallergens from different sources could help to differentiate between genuine sensitization and cross-reactivity. Consequently, more studies are necessary with natural or recombinant purified panallergens.

Acknowledgments

We thank Beatriz Martínez for her technical work

Funding

The authors declare that no funding was received for this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

Part of the results reported in this article were presented as oral communications at the 30th Congress of the European Academy of Allergy and Clinical Immunology, Istanbul, Turkey (June 2011) and at the Simposio Internacional de Alergia Alimentaria 100 años de Inmunoterapia in Barcelona, Spain (November 2011).

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Manuscript received March 4, 2014; accepted for publication, July 29, 2014.

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