

Plant Lipid Transfer Protein Allergens: No Cross-Reactivity between Those from Foods and Olive and *Parietaria* Pollen

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Key Words

Panallergen • Lipid transfer protein • Cross-reactivity • Pollen-food cross-reaction • Peach • Olive • *Parietaria*

Abstract

Background: Cross-reactivity among plant food allergens belonging to the nonspecific lipid transfer protein (LTP) family is well known. In contrast, the relationship among these allergens and their putative homologs from olive (Ole e 7) and *Parietaria* (Par j 1) pollen has not been clarified. **Methods:** Sera with specific IgE to LTP allergens were obtained from peach-, mustard- and olive pollen-allergic patients. Purified LTP allergens from foods (peach, apple, mustard and wheat) and pollens (olive, mugwort and *Parietaria*) were tested by ELISA and ELISA-inhibition assays. **Results:** Plant food LTP-allergic patients showed a significantly higher number of sera (89–100 vs. 33–64%) with specific IgE and mean specific IgE levels (0.30–1.56 vs. 0.21–0.34 OD units) to the 4 food LTP allergens tested than to olive Ole e 7 and *Parietaria* Par j 1 pollen. ELISA-inhibition assays indicated cross-inhibition between food LTP allergens but no cross-reactivity between these allergens and Ole e 7 and Par j 1, or, even more, between the LTP allergens from olive and *Parietaria* pollen. **Conclusions:** LTP allergens from olive and *Parietaria* pollen

cross-react neither with allergenic LTPs from plant foods nor between themselves. Therefore, both pollens do not seem to be related with the LTP syndrome.

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Introduction

Nonspecific lipid transfer proteins (LTPs), particularly Pru p 3 from the peach fruit, are the most important allergens from plant foods in the Mediterranean adult population, as well as in some fruit- and vegetable-allergic patients from central Europe [1]. A strong cross-reactivity among different members of the LTP allergen family has been reported [2–4], leading to the so-called LTP syndrome [4, 5]. Thus, sensitization to multiple foods is common in LTP-allergic patients. Besides food allergens, some LTPs can also act as inhalant allergens from pollens, such as *Artemisia* Art v 3 and plane tree Pla a 3 [1, 2, 6]. These pollen LTPs cross-react with those from several foods, and are involved in an LTP pollen/food syndrome [1, 6], although Pastorello et al. [7] claimed that the clini-

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cal role of *Artemisia* LTP needs to be further ascertained. Allergenic members of the LTP family are also present in two other important pollens: olive tree (Ole e 7) [8] and *Parietaria judaica* (Par j 1 and Par j 2) [9]. Sensitization to Ole e 7 is particularly relevant in areas with a high exposure to olive pollen, and it is linked to severe clinical symptoms [10]. Par j 1 and Par j 2 are the major *Parietaria* pollen allergens in the European population [9]. In contrast to Art v 3 and Pla a 3, the amino acid sequence similarity of Ole e 7 and Par j 1 to LTP food allergens (that is, peach Pru p 3) is very scarce [1]. Furthermore, their role in the LTP pollen/food syndrome is unclear at present, in spite of the potential clinical implications for LTP-allergic subjects.

Methods

Patients and Sera

Sera from 3 different groups of LTP-allergic subjects were tested in this study. Peach Pru p 3+ sera (n = 9) and mustard Sin a 3+ sera (n = 11) were from patients recruited at the Servicio de Alergia, Fundación Jiménez Díaz (Madrid, Spain). Peach Pru p 3+ subjects were selected based on the following criteria: (1) a convincing clinical history of allergic reactions after peach ingestion; (2) a positive open challenge test to peach; (3) a positive SPT response to a commercial peach extract (ALK-Abelló, Madrid, Spain); (4) a positive SPT response to purified natural Pru p 3 (20 µg/ml); (5) a significant specific IgE level to recombinant Pru p 3 as determined by direct ELISA assay (see below). Clinical symptoms of the Pru p 3+ selected patients were: oral allergy syndrome (OAS; n = 6), OAS plus urticaria (U) and angioedema (AE) (n = 2), and U and AE (n = 1).

Sin a 3+ patients were selected based on: (1) a convincing clinical history of allergic reactions after ingestion of mustard seed-derived products; (2) a positive SPT response to a commercial (ALK-Abelló) mustard extract; (3) a significant specific IgE level to recombinant Sin a 3 as determined by direct ELISA assay. Clinical symptoms after mustard seed-derived foodstuff ingestion were: OAS (n = 3), OAS plus U and AE (n = 2), OAS plus U, AE and rhinoconjunctivitis (RC; n = 1), OAS plus U, AE, RC and gastrointestinal symptoms (GI; n = 1), OAS plus U, RC and GI (n = 1), U and AE (n = 1), AE and RC (n = 1), as well as U, AE and RC (n = 1). All Sin a 3+ patients had symptoms with fresh peach and a positive SPT response to a commercial (ALK-Abelló) peach extract.

Ole e 7+ patients (n = 4) were selected at the Servicio de Alergia, Hospital Ciudad de Jaen (Jaen, Spain), based on: (1) a clinical history of pollinosis caused by olive pollen; (2) a positive SPT response to a commercial (ALK-Abelló) olive pollen extract; (3) a significant specific IgE level to natural Ole e 7 as measured by direct ELISA assay. All four Ole e 7+ patients showed rhinitis and asthma as clinical symptoms of pollinosis. Exposure to olive pollen is very high and moderate in the Jaen and Madrid area, respectively, whereas that to *Artemisia* or *Parietaria* pollen is very low in both areas [10, 11].

A negative control serum pool was from healthy subjects (n = 4) with no specific IgE to any purified LTP allergen here assayed.

Production and Isolation of LTP Allergens

Natural LTP allergens from *Artemisia vulgaris* (nArt v 3) and *Olea europaea* (nOle e 7) pollen, and recombinant forms from the peach (rPru p 3) and the apple (rMal d 3) fruit, yellow mustard (*Sinapis alba*; rSin a 3) seed, and bread wheat (rTri a 14) were produced and isolated by standard methods previously reported [2, 8, 12–15]. nPar j 1 from *Parietaria judaica* pollen was kindly supplied by Dr. D. Barber (ALK-Abelló). Purity of the allergens was verified by SDS-PAGE analysis (fig. 1a) and by N-terminal amino acid sequencing of at least 5 residues (rPru p 3: EITCG; rMal d 3: EITXGVT; rSin a 3: ALSXG; nArt v 3: ALTCSVDV; rTri a 14: EAEIDXGHVD; nOle e 7: APSQS).

Specific IgE Determination and ELISA-Inhibition Assays

The specific IgE to purified LTP allergens was determined by a direct ELISA assay previously reported [12, 15], using 3 µg/ml of each allergen as solid phase and each individual serum (n = 24; 1:3 dilution) from LTP-allergic patients mentioned above. Blocking solution (Sigma, St. Louis, Mo., USA; catalog No. B6429; 10× casein-based blocking buffer) without solid phase (n = 12; mean $\pm$ SD = 0.112 $\pm$ 0.023 OD units), BSA (3 µg/ml) as solid phase (n = 6; mean $\pm$ SD = 0.115 $\pm$ 0.017 OD units) and a serum pool from 4 healthy subjects (n = 24; mean $\pm$ SD = 0.124 $\pm$ 0.020 OD units) were used as negative controls, and OD values >0.18 OD units (mean + 3× SD of negative control with highest value) were considered positive (fig. 2). All test were performed in quadruplicate.

ELISA-inhibition assays were carried out by using the same method, except that the appropriate serum or serum pool was preincubated (3 h at 25°C) with 3 µg/ml of the corresponding inhibitor. All tests were performed in triplicate.

Results

Peach Pru p 3 as LTP allergen model [1], together with apple Mal d 3, mustard Sin a 3 and wheat Tri a 14 as cross-reactive food allergens [2, 13–16] and *Artemisia* Art v 3 as cross-reactive pollen allergen [2, 7, 17, 18] were purified (fig. 1a) to compare their IgE-binding capacity with that of isolated olive Ole e 7 and *Parietaria* Par j 1 (fig. 1a) pollen allergens.

The alignment and valuation of closeness of amino acid sequences of the seven isolated LTP allergens (fig. 1b, c) strongly indicated a very close relationship between Pru p 3 and Mal d 3 (81% of identity), a true similarity between these fruit allergens and those from mustard seed (Sin a 3), wheat flour (Tri a 14) and *Artemisia* pollen (Art v 3) (54–43% of identity), but a low and doubtful closeness (32–19% of identity) to olive (Ole e 7) and *Parietaria* (Par j 1) allergens.

Joint consideration of the specific IgE levels to the seven purified LTP allergens in two groups of plant food LTP-allergic patients (peach Pru p 3+ and mustard Sin a 3+; fig. 2) showed a substantially higher number of sera

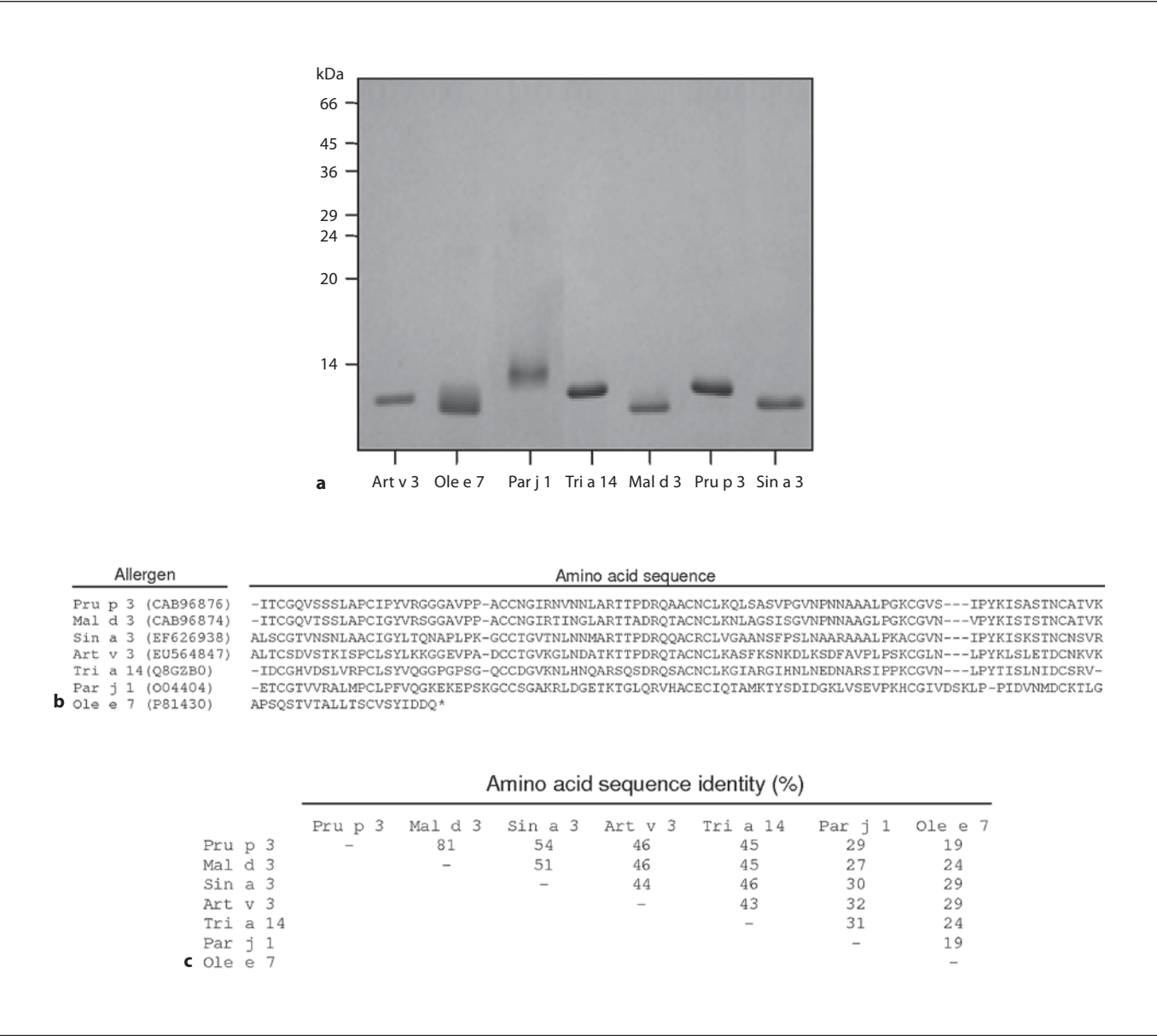


Fig. 1. a Protein staining of purified LTP allergens separated by SDS-PAGE. **b** Alignment of the amino acid sequences of the LTP allergens assayed in this work. Accession number of each sequence is indicated in parenthesis. An asterisk indicates the end of the only N-terminal amino acid sequence of Ole e 7 available at present. The C-terminal domain of Par j 1 is not included. **c** Identities (%) among the amino acid sequences shown in **b**.

with specific IgE (89–100 vs. 33–64%) and mean specific IgE levels (0.30–1.56 versus 0.21–0.34 OD units) to food LTP allergens from peach, apple, mustard and wheat than to olive nOle e 7 and *Parietaria* rPar j 1 pollen. The partially cross-reactive pollen LTP allergen Art v 3 rendered intermediate values of specific IgE prevalence (56–82%; fig. 2). The analysis of sera from olive tree pollen-allergic

patients (Ole e 7+ in fig. 2) further confirmed the low specific IgE reactivity of nPar j 1 compared with those of food LTP allergens.

Three different subpools (Pru p 3+, Sin a 3+ and Ole e 7+) formed by 3–4 individual sera with a specific IgE to all seven LTP allergens (fig. 3a–c) were tested by ELISA-inhibition assays against rPru p 3 and rSin a 3, selected as

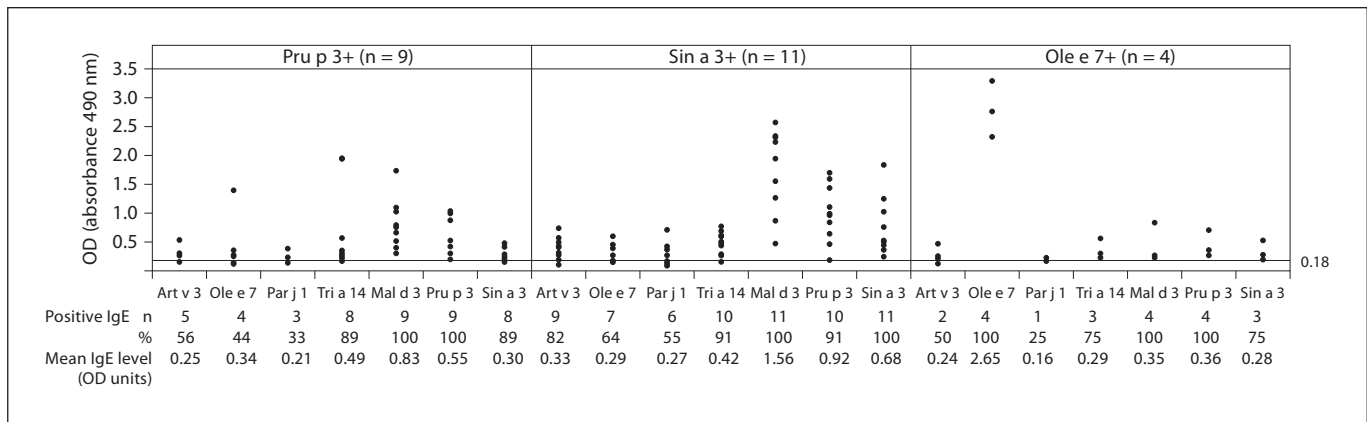


Fig. 2. Specific IgE levels to purified LTP allergens in sera from patients with allergy to peach (Pru p 3+), yellow mustard (Sin a 3+) or olive pollen (Ole e 7+).

model food LTP allergens, and nOle e 7 and nPar j 1, in order to find out whether or not actual cross-reactivity exists among all these LTP molecules. Pru p 3+, as well as Sin a 3+, serum pools (fig. 3a, b), and Pru p 3 or Sin a 3 as solid phase or inhibitor, showed self-inhibition and inhibition by the other food allergens, but not by those from pollens. This result was further confirmed using Ole e 7 and Par j 1 as solid phase. A pool of sera and an individual serum (no. 4) from olive pollen-allergic patients, tested against Ole e 7 or Par j 1 as solid phase (fig. 3c, d), fully supported the lack of inhibition between food and olive or *Parietaria* pollen LTPs, as well as between Ole e 7 and Par j 1 (only self-inhibition was obtained in fig. 3c, d).

Discussion

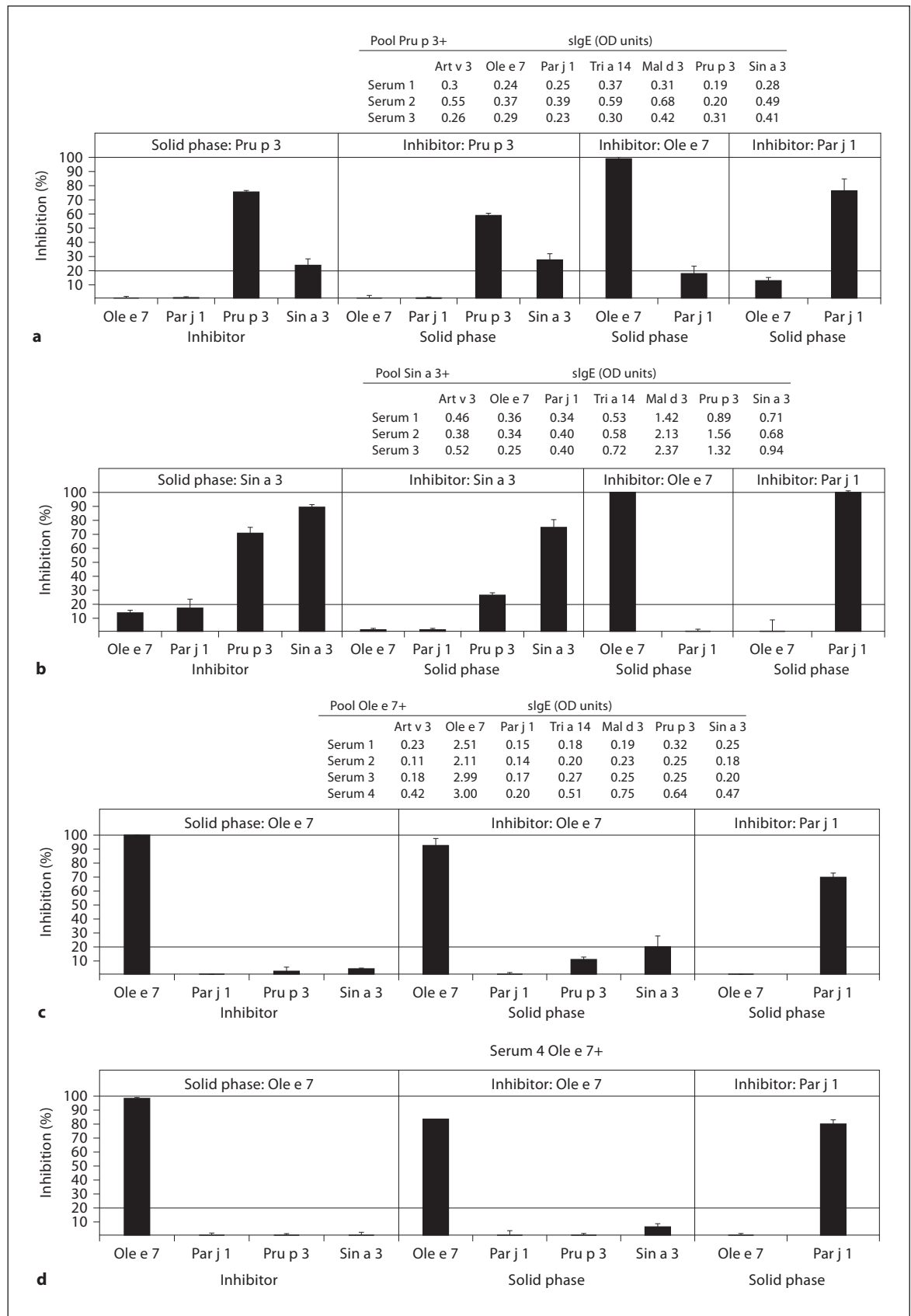
The status of LTPs as pollen allergens and their structural and immunological (cross-reactivity) relationship with their putative homologs from plant foods are crucial matters regarding their involvement in the LTP syndrome as well as the potential routes of sensitization of this allergen family [1, 5, 6]. Taking into account previous data, two different types of allergenic LTP from pollens can be considered. Mugwort Art v 3 and plane tree Pla a 3 seem to be clearly implicated in plant food-pollen co-sensitization, share around 50% of amino acid sequence with peach Pru p 3 and display partial cross-reactivity with this and other food LTP allergens [1, 2, 17–19]. In contrast, olive Ole e 7 presents less than 20% of sequence identity with Pru p 3, and *Parietaria* Par j 1 and Par j 2 show larger molecular sizes (14.7 and 11.3 kDa, respec-

tively) than typical 9-kDa LTP allergens, and low levels of sequence identity (27–29%) with the peach LTP allergen [1]. No cross-reaction between olive or *Parietaria* pollen LTP and any food or even pollen (Art v 3 or Pla a 3) allergen of the LTP family has been reported.

The data on the specific IgE levels to different purified LTP allergens summarized in figure 2 indicate a close immunological relationship between peach Pru p 3, apple Mal d 3, wheat Tri a 14 and mustard Sin a 3, a moderate one with *Artemisia* pollen Art v 3, and a doubtful cross-reactivity with olive pollen Ole e 7 and *Parietaria* pollen Par j 1, which is in line with the previous reports discussed above. Furthermore, the results from ELISA-inhibition assays (fig. 3) confirmed the cross-reactivity between LTP allergens from peach and mustard, and further indicate a lack of immunological relationship at the IgE level between both allergens and those from olive and *Parietaria* pollen. On the other hand, no cross-reactivity between Ole e 7 and Par j 1 has been found in this study.

Two main conclusions arise from the results presented herein. Firstly, the status of Ole e 7 and Par j 1 in the LTP panallergen family should be revised. These pollen allergens present neither a strong structural nor a close immunological relationship with representative LTP food

Fig. 3. ELISA-inhibition assays using purified LTP allergens as solid phase and inhibitor, and serum pool from peach- (a), yellow mustard- (b) and olive pollen-allergic patients (c) and an individual serum (serum 4) from an olive pollen-allergic patient (d). Means (n = 3) and SD (bars) are represented.



3

allergens, such as peach Pru p 3. This fact could also be extensive to other putative LTP allergens, such as ragweed Amb b 6 [1]. Secondly, both olive tree and *Parietaria* pollen do not seem to be included in the LTP syndrome, and, therefore, probably do not lead to co-sensitization to plant foods (such as *Rosaceae* fruits and nuts) in LTP-sensitized patients. However, the limited number of patients analyzed here clearly stressed these conclusions. Future studies on patients with the LTP syndrome and

different sensitization profiles will further clarify whether or not Ole e 7 and Par j 1 are true members of the LTP allergen family.

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