

## Prevalence of sensitization to *Artemisia* allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens

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### Summary

**Background** *Artemisia vulgaris* is a widespread weed in the Mediterranean area and several allergens have been detected in its pollen. One of them, Art v 3, belongs to the lipid-transfer protein (LTP) family and its prevalence in *Artemisia*-sensitized patients or its relationship with other LTP allergens is not clear.

**Objective** To assess the pattern of sensitization to an array of mugwort allergens in a Mediterranean population, and to study the cross-reactivity of Art v 3 with Pru p 3 and Par j 1, relevant LTP allergens in the area.

**Methods** Skin prick test was performed with whole extracts (*A. vulgaris*, *Parietaria judaica* and peach) and pure natural allergens Art v 1, Art v 3, Art v 60 kDa and Par j 1 in 24 mugwort-allergic patients from a Mediterranean area. *In vitro* assays included measurement of specific IgE and ELISA inhibition among LTP allergens.

**Results** The three *Artemisia* allergens elicited a positive skin response in 70–80% of the patients. Seven patients were clearly sensitized to Par j 1 and 11 to Pru p 3. There was no correlation between Par j 1 and Pru p 3 sensitization, but a highly significant correlation was found between peach extract and Art v 3 as regards the skin response. No IgE cross-reactivity was observed between Art v 3/Par j 1 or Pru p 3/Par j 1. In contrast, Art v 3 significantly inhibited the binding to Pru p 3 of IgE from three patients' sera out of six studied, but Pru p 3 was not able to inhibit the IgE binding to Art v 3. **Conclusion** Art v 3 is a major mugwort allergen and in some patients with IgE to both Art v 3 and Pru p 3, Art v 3 behaves as the primary sensitizing agent.

**Keywords** *Artemisia* pollen, cross-reactivity, lipid transfer protein, *Parietaria* pollen, skin prick test  
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### Introduction

Pollen grains of mugwort (*Artemisia vulgaris*) are an important cause of pollinosis during late summer and fall in the temperate and humid zones of the northern hemisphere and the Mediterranean basin [1]. IgE-immunoblotting experiments carried out with sera from mugwort-allergic patients reveal mainly four allergenic structures with apparent molecular weights of about 60, 25–30, 15 and 13 kDa [2].

The 60 kDa allergen was first described by de la Hoz et al. [3] as a monomeric acidic glycoprotein that is recognized by the IgE from 73% of mugwort-allergic patients. This allergen was originally named Art v I. However, no sequence data were provided and the systematic name was not kept. It will be called Art v 60 kDa throughout this paper.

The allergen currently named Art v 1 in the official allergen list of the IUIS allergen nomenclature subcommittee has recently been cloned and expressed as a recombinant protein in *Escherichia coli* [4]. It is a glycoprotein to which 90% of individuals allergic to mugwort have specific IgE. Sequence analysis showed that Art v 1 is a secreted allergen with an N-terminal defensin-like domain, and a C-terminal region rich in hydroxyproline residues, some of which are O-glycosylated. The carbohydrate moiety is highly heterogeneous and influences the electrophoretic mobility of the allergen (apparent molecular weight in SDS-PAGE 25–30 kDa).

Besides Art v 1 and Art v 60 kDa, other IgE-binding structures have been detected in *Artemisia vulgaris* pollen with described prevalence of sensitization ranging from 30 to 50%. Art v 2 [5] is a 35 kDa glycoprotein composed of two subunits linked together by a disulphide bridge(s). Art v 3 [6] belongs to the family of non-specific lipid transfer proteins (LTPs) and has an apparent molecular weight of 13 kDa in SDS-PAGE. Finally, Art v 4 corresponds to the mugwort

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profilin and has an apparent molecular weight of 15 kDa in SDS-PAGE [7].

In Southern Europe, allergy to *Rosaceae* fruits, mainly peach and cherry, is clearly associated with LTP sensitization [8, 9], which is also a marker of symptom severity. LTPs have been considered mainly as food allergens. However, allergenic LTPs have also been detected in pollens. The main *Parietaria* pollen allergens (Par j 1 and 2) belong to the LTP family [10] and have been claimed to be diagnostic markers of genuine *Parietaria* exposure [11]. In addition, other pollen LTP allergens have recently been described in *Artemisia* (Art v 3) and *Olea* (Ole e 7) [12]. All these weeds are widespread in the Mediterranean area, so it is worth considering the possibility of a link between inhalatory sensitization to pollen LTP and LTP food allergy.

In a previous work [13], it was shown that patients with adverse reactions to *Rosaceae* fruits and from the Spanish region of Murcia, which has a Mediterranean coastal climate and a high exposure to different pollen sources including *Parietaria* and *Artemisia* species, have a high frequency of mugwort sensitization. In addition, *Artemisia* LTP cross-reacts, at least partially, with LTPs from *Rosaceae* fruits [6]. On the other hand, a recent study [14] has indicated that sensitization to mugwort LTP is an epiphenomenon of peach LTP sensitization in subjects allergic to peach and with specific IgE antibodies to mugwort.

The present study aims to clarify the relevance of Art v 3 (*Artemisia* LTP) in a group of subjects from the region of Murcia, sensitized to *Artemisia* pollen. In addition, the main mugwort allergen Art v 1 and Art v 60 kDa were also assessed. The prevalence of sensitization to Par j 1 (*Parietaria* LTP) and Pru p 3 (peach LTP) were studied in the group of patients, and IgE cross-reactivity towards the different LTP allergens was evaluated by ELISA-inhibition methods.

## Materials and methods

### Patients

Patients were selected among subjects referred to the Allergy Unit of the hospital Virgen de la Arrixaca in Murcia, Spain, during the first half of the year 2002. They corresponded to consecutive patients, who presented a positive skin prick test (SPT) response (ALK-Abelló, S. A. Madrid, Spain), specific IgE (Pharmacia CAP System FEIA, Pharmacia Diagnostics, Uppsala, Sweden) and positive nasal provocation test (ALK-Abelló, S. A. Madrid, Spain) to *A. vulgaris* pollen. This test was carried out basically as indicated [15]. A result was regarded as positive when at least two of the following three conditions were met: (1) sneeze count higher than 5; (2) volume of secretion higher than 0.5 mL; (3) an increase of at least 20% in nasal resistance, measured by a technique of active anterior rhinomanometry (rhinomanometer Rhinospir-164, SibelMed, Barcelona, Spain).

A complete and careful medical history was obtained for each patient to ascertain all episodes of adverse reactions to peach and other *Rosaceae* fruits. Other pollen allergies were diagnosed by medical history and SPT.

All patients gave their consent to enter the study, which was approved by the Hospital Ethics Committee.

### Purification of allergens

Peach LTP (Pru p 3) and *Artemisia* pollen LTP (Art v 3) were purified as previously described [6].

The major *Parietaria* pollen allergen (Par j 1) was isolated by sequential size exclusion and reverse-phase chromatography of pollen extract [16].

The *Artemisia* pollen allergens Art v 1 (25–30 kDa on SDS-PAGE) and Art v 60 kDa were purified from *Artemisia* pollen extract, as indicated [3, 17].

The purity of preparations was checked by SDS-PAGE and N-terminal amino acid sequencing, using an Applied Biosystems Model 477A gas-phase sequenator (Foster City, CA, USA). Protein concentration was determined by amino acid analysis using reagents (AccQ-Tag method) and equipment from Waters (Milford, MA, USA).

### Skin prick tests

Commercially available extracts (ALK-Abelló) of *A. vulgaris* pollen (100 BU/mL, 180 µg Art v 60 kDa/mL and 150 µg Art v 1/mL), *Parietaria judaica* pollen (100 BU/mL, 6 µg Par j 1/mL) and peach (5% w/v, 75 µg Pru p 3/mL) were used. The major allergen content in the extracts was determined by mAb-based ELISAs as indicated [18–20]. Purified Par j 1 and Art v 3 were adjusted to 20 µg protein/mL, which was the optimal protein concentration for SPT found in a previous study [13]. Art v 1 and Art v 60 kDa were adjusted to 60 and 120 µg/mL, respectively, keeping the same molar concentration compared with the LTP allergens (molecular weight ~10 kDa). Either the extracts or the purified proteins were formulated with 0.9% NaCl, 50% glycerol and 0.4% phenol.

SPTs were performed in duplicate on the volar surface of the forearm using a standard 1-mm tip lancet, following the European Academy of Allergology and Clinical Immunology recommendations. Histamine hydrochloride (10 mg/mL) and saline solution (ALK-Abelló) were used as positive and negative controls, respectively. Weals were carefully outlined with a ballpoint pen and transferred to paper by means of adhesive tape. The weal areas (in mm<sup>2</sup>) were measured by planimetry. The SPT was considered positive if the weal area was greater than 7 mm<sup>2</sup> [21]. A battery of inhalants (ALK-Abelló), which included mites, moulds, epithelia, cockroach and the most relevant pollens in the area (*Lolium perenne*; *Olea europaea*; *Chenopodium album*; *Salsola kali*), was also assayed in all subjects.

### Specific IgE determination and ELISA inhibition

Specific IgE to *Artemisia* pollen extract was determined by the Pharmacia CAP System. Specific IgE to the purified allergens was determined by ELISA. Polystyrene 96-well plates (Costar 3590, Corning, NY, USA) were coated overnight at 4 °C with 50 µL of purified allergen at 2 µg/mL in phosphate-buffered saline (PBS) buffer. The coated wells were blocked with 1% bovine serum albumin (BSA) in PBS, and then incubated overnight at 4 °C with 50 µL of patient's serum. After washing with 0.1% Tween-20 in PBS, wells were incubated with a peroxidase-labelled anti-human IgE (code P0295, Dako A/S, Denmark; 1/1000 dilution) for 1 h at room temperature. Plates were washed again, and then developed by incubating with 50 µL of peroxidase substrate buffer (0.012% H<sub>2</sub>O<sub>2</sub>,

0.66 mg/mL *o*-phenylenediamine (OPD); Dako, code S 2045). The reaction was stopped after 30 min with 50 µL of 2 M H<sub>2</sub>SO<sub>4</sub>, and the optical density (OD) was measured at 492 nm. Assays were performed in duplicate. As negative control, it was used blocking buffer and a serum from a non-atopic individual, showing an OD less than 0.05 U in all cases.

Results were expressed in kilounits per litre using a control curve obtained with a *L. perenne* pollen extract on solid phase and a pool of sera from grass-allergic patients previously calibrated against the Pharmacia CAP System.

For the ELISA-inhibition assays, 96-well plates were coated with 50 µL of purified allergen at 2 µg/mL as indicated above. After blocking, wells were incubated with 25 µL of the patient's serum and 25 µL of inhibitor at 4 µg/mL. After washing, wells were incubated with peroxidase-labelled anti-human IgE, and developed with OPD buffer as indicated above.

The inhibition (%) of IgE-binding was determined as:

$$\text{Inhibition (\%)} = 100 \times \left( 1 - \frac{\text{OD} - \text{OD}_{100\%}}{\text{OD}_{0\%} - \text{OD}_{100\%}} \right)$$

OD is the result obtained with a specific serum and allergen inhibitor; OD<sub>0%</sub> is the result with BSA as inhibitor and OD<sub>100%</sub> is the blank of the assay.

Inhibitor concentration to reach plateau level was determined from preliminary dose-response experiments.

### Statistical analysis

It was performed using SPSS software (version 11.5). Correlations were calculated by the Spearman rank test (*r<sub>s</sub>*). Results were considered significant at *P* < 0.05.

### Results

The clinical data, SPT results with pollen extracts and peach extract, and nasal provocation test results to *A. vulgaris* pollen are indicated in Table 1. All of the 24 patients had respiratory symptoms during the mugwort pollen season, which has a characteristic two-peak pattern in the region of Murcia (Fig. 1).

Nine patients (9/24 = 37.5%) had no sensitivities to other pollens apart from *A. vulgaris* (#1, 3, 4, 8, 10, 16, 18, 20, 24; mean age, 27.3 years; SD = 11.1) (pollen monosensitized group). The rest of the patients (15/24 = 62.5%) were sensitized to other pollens, mainly *Parietaria*, *Lolium*, *Chenopodium* and *Olea*, apart from *Artemisia* (mean age, 30.7; SD = 7.8) (polysensitized group). Twenty-one (21/24 = 87.5%) of the patients had an *Artemisia* IgE level of class 3 or higher. No significant differences in the IgE levels to *A. vulgaris* were found between these two groups (monosensitized, geometric mean = 8.3 kU/L, geometric SD = 2.7;

**Table 1.** Clinical data of the patients included in the study

| Patient no. | Age (years)/sex | Symptoms* |          | SPT†               |                   |                   |        |           |       | Nasal provocation test <i>A. vulgaris</i> ¶ |
|-------------|-----------------|-----------|----------|--------------------|-------------------|-------------------|--------|-----------|-------|---------------------------------------------|
|             |                 | Pollens   | Peach    | <i>A. vulgaris</i> | <i>P. judaica</i> | <i>L. perenne</i> | Weeds‡ | Trees§    | Peach |                                             |
| 1           | 49/F            | R, C, As  | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |
| 2           | 33/F            | R, C      | U, A, GE | +                  | —                 | +                 | —      | —         | +     | +                                           |
| 3           | 31/M            | R, C, As  | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |
| 4           | 17/M            | R         | FA       | +                  | —                 | —                 | —      | —         | +     | +                                           |
| 5           | 16/M            | As        | —        | +                  | —                 | +                 | Ch     | O         | +     | +                                           |
| 6           | 21/M            | R, C      | U, A     | +                  | —                 | —                 | Ch     | O         | +     | +                                           |
| 7           | 25/M            | R, As     | OAS      | +                  | —                 | +                 | Ch     | O         | +     | +                                           |
| 8           | 18/M            | R, C      | U        | +                  | —                 | —                 | —      | —         | +     | +                                           |
| 9           | 28/M            | R, C      | —        | +                  | +                 | +                 | Ch     | O         | —     | +                                           |
| 10          | 28/M            | R, C      | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |
| 11          | 41/F            | R, C, As  | —        | +                  | +                 | +                 | Ch     | O         | +     | +                                           |
| 12          | 36/F            | R, C, As  | U, A     | +                  | +                 | —                 | Ch     | O, Pa     | +     | +                                           |
| 13          | 41/F            | R, C      | —        | +                  | +                 | —                 | Ch     | —         | +     | +                                           |
| 14          | 29/M            | R, C      | —        | +                  | —                 | +                 | Ch     | O         | —     | +                                           |
| 15          | 41/M            | R, C, As  | —        | +                  | +                 | +                 | Ch, Pl | O, Pa, Cu | +     | +                                           |
| 16          | 26/F            | R, C      | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |
| 17          | 21/F            | R, C      | —        | +                  | +                 | +                 | Pl, M  | O, Pa, Cu | +     | +                                           |
| 18          | 27/F            | R, C      | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |
| 19          | 29/F            | R, C      | An       | +                  | —                 | +                 | Ch     | O         | +     | +                                           |
| 20          | 37/F            | R, C, As  | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |
| 21          | 33/M            | As        | —        | +                  | +                 | +                 | Ch     | —         | —     | +                                           |
| 22          | 30/F            | R, C      | OAS      | +                  | +                 | —                 | Ch     | —         | +     | +                                           |
| 23          | 37/M            | R, C, As  | OAS      | +                  | —                 | +                 | Pl     | O         | +     | +                                           |
| 24          | 13/F            | As        | —        | +                  | —                 | —                 | —      | —         | —     | +                                           |

*A. vulgaris*, *Artemisia vulgaris*; *P. judaica*, *Parietaria judaica*; *L. perenne*, *Lolium perenne*.

\*R, rhinitis; C, conjunctivitis; As, asthma; U, urticaria; A, angioedema; GE, glottis oedema; OAS, oral allergy syndrome; FA, facial angioedema; An, anaphylaxis.

†Positive, +, or negative, —, SPT response as indicated in Materials and methods.

‡Species with positive SPT response: Ch, *Chenopodium album*, Pl, *Plantago lanceolata*, M, *Mercurialis alba*.

§Species with positive SPT response: O, *Olea europaea*, Pa, *Platanus acerifolia*, Cu, *Cupressus arizonica*.

¶+, as indicated in Materials and methods.

polysensitized, geometric mean = 8.9 kU/L, geometric SD = 2.0).

Tables 2 and 3 display the results of SPT carried out with *Artemisia* pollen whole extract and the purified *Artemisia* allergens Art v 3, Art v 1 and Art v 60 kDa. All the patients had a positive SPT to *Artemisia* extract, in agreement with the study inclusion criteria (see Methods). The 3 *Artemisia* allergens had a prevalence of sensitization above 70% in the total group of patients, indicating that they are clearly major allergens.

No significant differences were observed in the mean weal areas obtained with *Artemisia* extract and Art v3/Art v 1/Art

v 60 kDa, in the monosensitized and polysensitized groups. There was a significant correlation between the weal areas obtained with *Artemisia* extract and purified Art v3 ( $r_s = 0.499$ ,  $P = 0.013$ ), and a highly significant correlation between *Artemisia* extract/Art v 1 and *Artemisia* extract/Art v 60 kDa ( $r_s = 0.635$ ,  $P = 0.001$  and  $r_s = 0.749$ ;  $P < 0.001$ , respectively).

In addition to *Artemisia*, another important allergenic pollen in the geographical area, *Parietaria judaica*, was assayed, along with the major allergen Par j 1, by SPT in the group of patients studied (Table 2). The skin responses were positive in nine patients and there was a highly significant correlation ( $r_s = 0.869$ ,  $P < 0.001$ ) between the weal areas corresponding to the *Parietaria* extract and those of Par j 1.

Peach extract was also tested by SPT (Table 2). Fourteen patients (14/24 = 58.3%) had a positive SPT response, two (2/9 = 22.2% confidence interval 95%: 3–60%) in the monosensitized group and 12 (12/15 = 80% CI95: 52–96%) in the polysensitized group. Interestingly, a highly significant correlation ( $r_s = 0.727$ ,  $P < 0.001$ ) was found between the skin responses to the peach extract and those to Art v 3. No other significant correlations were found.

Table 4 shows the levels of specific IgE to *A. vulgaris*, to *Artemisia* allergens and to Par j 1 and Pru p 3. The rate of sensitization of Art v 3, Art v 1 and Art v 60 kDa were 83%, 50% and 92%, respectively. There was a significant correlation between the *Artemisia* whole extract IgE titre and the IgE levels for each of the purified mugwort allergens ( $r_s = 0.709$ ,  $P < 0.001$  for Art v 3;  $r_s = 0.465$ ,  $P = 0.022$  for Art v 1;

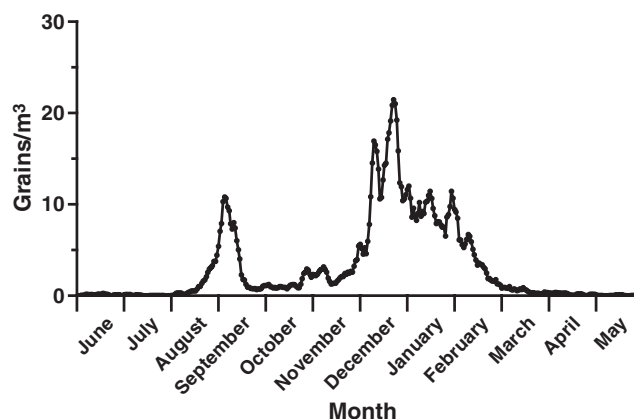


Fig. 1. *Artemisia* pollen count in the Murcia region. Each point corresponds to a 5-day mobile average for years 1993–2002.

Table 2. Skin prick test results

| Patient no. | Weal area (mm <sup>2</sup> )* |         |         |              |                   |         |       | Histamine |
|-------------|-------------------------------|---------|---------|--------------|-------------------|---------|-------|-----------|
|             | <i>Artemisia</i>              | Art v 3 | Art v 1 | Art v 60 kDa | <i>Parietaria</i> | Par j 1 | Peach |           |
| 1           | 101                           | 12      | 38      | 52           | –                 | 7       | –     | 44        |
| 2           | 263                           | 125     | 166     | 36           | –                 | –       | 142   | 36        |
| 3           | 569                           | 37      | 522     | 70           | –                 | –       | –     | 36        |
| 4           | 58                            | 130     | –       | –            | –                 | –       | 62    | 30        |
| 5           | 44                            | 36      | –       | –            | –                 | –       | 54    | 19        |
| 6           | 26                            | 16      | –       | 7            | –                 | –       | 49    | 16        |
| 7           | 48                            | 59      | –       | 21           | –                 | –       | 50    | 38        |
| 8           | 82                            | 53      | –       | 12           | –                 | –       | 75    | 33        |
| 9           | 20                            | –       | 17      | 9            | 25                | 36      | –     | 17        |
| 10          | 120                           | 11      | 104     | 38           | –                 | –       | –     | 20        |
| 11          | 27                            | 25      | 20      | –            | 30                | 46      | 22    | 25        |
| 12          | 50                            | 42      | –       | –            | 10                | –       | 21    | 20        |
| 13          | 66                            | 15      | 74      | 43           | 58                | 52      | 17    | 33        |
| 14          | 37                            | –       | 13      | 35           | –                 | –       | –     | 28        |
| 15          | 101                           | 96      | 27      | 33           | 44                | 80      | 17    | 24        |
| 16          | 145                           | 21      | 76      | 47           | –                 | 9       | –     | 34        |
| 17          | 147                           | 13      | 75      | 133          | 106               | 148     | 22    | 35        |
| 18          | 30                            | –       | 26      | 10           | –                 | –       | –     | 41        |
| 19          | 56                            | 29      | 23      | 14           | –                 | –       | 29    | 22        |
| 20          | 30                            | –       | 19      | 13           | –                 | –       | –     | 27        |
| 21          | 19                            | –       | 10      | –            | 30                | 55      | –     | 25        |
| 22          | 33                            | –       | 25      | 16           | 24                | 23      | 36    | 20        |
| 23          | 73                            | 140     | 42      | 41           | –                 | –       | 83    | 36        |
| 24          | 50                            | –       | 8       | 18           | –                 | –       | –     | 24        |

\*–, Weal area lower than 7 mm<sup>2</sup>.

**Table 3.** Descriptive statistics of SPT data for *Artemisia* allergens

|                                 | <i>n</i> | %    | 95 CI     | Mean  | SEM  |
|---------------------------------|----------|------|-----------|-------|------|
| Total ( <i>n</i> = 24)          |          |      |           |       |      |
| <i>Artemisia</i> whole extract  | 24       | 100  | 85.8–100  | 91.5  | 23.6 |
| Art v 3                         | 17       | 70.8 | 48.9–87.4 | 50.6  | 10.7 |
| Art v 1                         | 18       | 75.0 | 53.3–90.2 | 71.4  | 28.2 |
| Art v 60 kDa                    | 19       | 79.2 | 57.8–92.9 | 34.1  | 6.8  |
| Histamine                       | 24       | 100  | 85.8–100  | 28.5  | 1.6  |
| Monosensitized ( <i>n</i> = 9)  |          |      |           |       |      |
| <i>Artemisia</i> whole extract  | 9        | 100  | 66.4–100  | 131.7 | 56.2 |
| Art v 3                         | 6        | 66.7 | 29.9–92.5 | 44.0  | 18.4 |
| Art v 1                         | 7        | 77.8 | 40.0–97.2 | 113.3 | 69.3 |
| Art v 60 kDa                    | 8        | 88.9 | 51.7–99.7 | 32.5  | 8.0  |
| Histamine                       | 9        | 100  | 66.4–100  | 32.1  | 2.6  |
| Polysensitized ( <i>n</i> = 15) |          |      |           |       |      |
| <i>Artemisia</i> whole extract  | 15       | 100  | 78.2–100  | 76.7  | 16.7 |
| Art v 3                         | 11       | 73.3 | 44.9–92.2 | 59.9  | 12.9 |
| Art v 1                         | 11       | 73.3 | 44.9–92.2 | 56.5  | 17.5 |
| Art v 60 kDa                    | 11       | 73.3 | 44.9–92.2 | 35.6  | 11.6 |
| Histamine                       | 15       | 100  | 78.2–100  | 27.6  | 2.0  |

*n*, number of patients with positive SPT; % frequency of positive SPT in the group; 95 CI, 95% confidence interval; mean, mean weal (mm<sup>2</sup>) of positive results; SEM, standard error of mean.

**Table 4.** Specific IgE levels (kU/L) to *A. vulgaris* whole extract and purified allergens

| Patient no. | <i>A. vulgaris</i> | Art v 3 | Art v 1 | Art v 60 kDa | Par j 1 | Pru p 3 |
|-------------|--------------------|---------|---------|--------------|---------|---------|
| 1           | 7.42               | 2.16    | 2.68    | 2.22         | –       | –       |
| 2           | 5.43               | 0.86    | 1.06    | 1.55         | –       | 1.86    |
| 3           | 3.48               | 1.02    | 2.43    | 0.96         | –       | –       |
| 4           | 2.60               | 0.62    | –       | –            | –       | 0.42    |
| 5           | 6.38               | 0.52    | –       | 0.49         | –       | 1.51    |
| 6           | 21.4               | 1.56    | –       | 1.44         | –       | 1.14    |
| 7           | 5.59               | 1.20    | –       | 1.05         | –       | 1.94    |
| 8           | 6.60               | 1.66    | –       | –            | –       | 0.78    |
| 9           | 10.4               | 3.11    | 3.51    | 3.91         | > 17.5  | –       |
| 10          | 90.4               | > 17.5  | > 17.5  | > 17.5       | –       | –       |
| 11          | 9.28               | –       | –       | 1.39         | > 17.5  | –       |
| 12          | 8.66               | 0.86    | –       | 0.45         | –       | 0.36    |
| 13          | 12.5               | 3.30    | 3.80    | 5.17         | > 17.5  | –       |
| 14          | 3.73               | 0.64    | 1.33    | 1.00         | –       | –       |
| 15          | 43.5               | 3.28    | 6.86    | 0.85         | > 17.5  | 0.36    |
| 16          | 9.37               | 2.38    | 3.91    | 3.52         | –       | –       |
| 17          | 24.5               | 3.21    | 8.70    | 6.13         | > 17.5  | 0.64    |
| 18          | 6.25               | –       | –       | 0.50         | –       | –       |
| 19          | 6.18               | –       | –       | 0.43         | –       | 0.42    |
| 20          | 11.5               | 3.17    | 4.79    | 2.96         | –       | –       |
| 21          | 3.43               | –       | –       | 0.40         | 6.15    | –       |
| 22          | 5.49               | 0.58    | –       | 0.94         | 7.92    | 2.78    |
| 23          | 8.54               | 1.10    | –       | 1.33         | –       | –       |
| 24          | 7.01               | 0.38    | 0.67    | 2.54         | –       | –       |

–, < 0.35 kU/L.

$r_s = 0.643$ ,  $P = 0.001$  for Art v 60 kDa). No significant correlation was found with Par j 1 and Pru p 3. No correlation was found between the variables weal area and specific IgE concentration, neither for *Artemisia* whole extract nor for the purified allergen Art v 3. However, the correlation

was significant for Art v 1 ( $r_s = 0.546$ ,  $P = 0.006$ ), Art v 60 kDa ( $r_s = 0.570$ ,  $P = 0.004$ ), Par j 1 ( $r_s = 0.852$ ,  $P < 0.001$ ), and for peach weal/ Pru p 3 IgE ( $r_s = 0.764$ ,  $P < 0.001$ ).

ELISA inhibition experiments were carried out with the purified allergens Art v 3, Par j 1 and Pru p 3, all belonging to the LTP family, and individual serum from patients. For technical reasons, only sera with a suitable IgE concentration were analysed (higher than 1.0 kU/L to at least one purified allergen, except sera 20 and 23, which were not included because of the low volume of sample available). The allergen was adsorbed to the solid phase and IgE-binding was inhibited by itself (positive control of inhibition) and by the other(s) IgE-positive LTP allergen(s). The results are summarized in Table 5. In the case of Art v 3 or Par j 1 on the solid phase, no significant inhibition (higher than 15%) by the other LTPs was detected. However, in three out of six patients studied from the polysensitized group, a significant inhibition (two patients higher than 60% and one 19%) of the binding of serum IgE to Pru p 3 was achieved by Art v 3, with no parallel inhibition in the reverse experiments.

## Discussion

The main objective of this study was to clarify the relevance of an array of *A. vulgaris* pollen allergens including Art v 3, which is a member of the LTP family, in mugwort-allergic patients. A second objective was to determine if Art v 3 could act as a primary sensitizing allergen in mugwort-allergic patients with specific IgE to Pru p 3, peach LTP, and/or specific IgE to Par j 1, *Parietaria* LTP.

The patients enrolled in the study were from Murcia (SE Spain), an area with a Mediterranean climate and rich in weeds, including *Artemisia*, *Parietaria* and *Chenopodium* species. The 24 patients selected were allergic to *A. vulgaris*, as demonstrated by skin prick and nasal provocation tests, serum-specific IgE and symptoms of rhinitis and/or asthma during the mugwort pollen season. Nine patients had no sensitivities to other pollens (pollen monosensitized group) and 15 patients were sensitized to other pollens, apart from mugwort (polysensitized group).

The prevalence of sensitization to three representative *Artemisia* allergens was evaluated by *in vivo* and *in vitro* techniques. Art v 1 and Art v 60 kDa had a rate of sensitization above 70%, by SPT. Interestingly, the prevalence of sensitization to the mugwort LTP, Art v 3, was in the same range (about 70%), indicating that it is clearly a major allergen too. There was, in general, a high variability in the weal area obtained with either the *Artemisia* extract or the purified allergens. This variability was because of the different skin reactivity of the patients to the extract/allergens and not to a lack of reproducibility of the SPT technique, taking into account the low variability achieved with the histamine control solution (Table 3).

No significant differences in the rate of sensitization to the three allergens were observed in the pollen monosensitized and polysensitized groups (Table 3). A significant correlation was found between *Artemisia* pollen whole extract SPT response (or IgE titre) and purified allergen SPT response (or IgE titre), thus supporting the relevance of these three

**Table 5.** ELISA inhibition results (% inhibition IgE-binding) with purified allergens

| Art v 3 on solid phase |           |         |         | Pru p 3 on solid phase |           |         |         | Par j 1 on solid phase |           |         |         |
|------------------------|-----------|---------|---------|------------------------|-----------|---------|---------|------------------------|-----------|---------|---------|
| Serum no.              | Inhibitor |         |         | Serum no.              | Inhibitor |         |         | Serum no.              | Inhibitor |         |         |
|                        | Art v 3   | Pru p 3 | Par j 1 |                        | Art v 3   | Pru p 3 | Par j 1 |                        | Art v 3   | Pru p 3 | Par j 1 |
| 2                      | 100       | –       | NR      | 2                      | –         | 98      | NR      | 9                      | NR        | –       | 94      |
| 6                      | 99        | –       | NR      | 5                      | –         | 99      | NR      | 13                     | –         | NR      | 95      |
| 7                      | 84        | –       | NR      | 6                      | 63        | 98      | NR      | 15                     | –         | –       | 93      |
| 8                      | 99        | –       | NR      | 7                      | 66        | 84      | NR      | 17                     | –         | –       | 86      |
| 9                      | 97        | NR      | –       | 17                     | 19        | 96      | –       | 22                     | –         | –       | 99      |
| 13                     | 100       | –       | NR      | 22                     | –         | 94      | –       |                        |           |         |         |
| 15                     | 98        | –       | –       |                        |           |         |         |                        |           |         |         |
| 17                     | 96        | –       | –       |                        |           |         |         |                        |           |         |         |

–, less than 15% inhibition.

NR, not relevant because no IgE reactivity to the inhibitor allergen in the serum sample.

allergens. Compared with the SPT data, the prevalence of serum-specific IgE to the *Artemisia* allergens was slightly higher in the case of Art v 3 and Art v 60 kDa, but clearly lower (50% vs. 75%) for Art v 1. There are no clear reasons for this discrepancy, but it has been reported that Art v 1 skin tests reflect the clinically relevant sensitization to a higher degree than *in vitro* tests [22].

In a previous work, we have shown that in peach-allergic patients from the region of Murcia, in contrast with other regions with a low exposure to weed pollens, a pattern of skin sensitization to both Pru p 3 and Art v 3 was found in a significant number of patients [13]. With this background, we have also studied in *Artemisia*-allergic patients the possible sensitization to other important LTP allergens in the geographical area: Par j 1, the main *Parietaria judaica* pollen allergen which is also a member of the LTP family [10], and Pru p 3.

Seven patients were clearly sensitized to Par j 1 (positive SPT and specific IgE) and 11 patients (nine of them in the polysensitized group) were sensitized to peach, as determined by SPT, and had specific IgE to Pru p 3. There was no correlation between Par j 1 and Pru p 3 sensitization, but a highly significant correlation was found between peach extract and Art v 3 as regards the SPT response.

ELISA inhibition experiments with the three purified LTP allergens and patient's individual sera were performed to study the possible cross-reactivity at the IgE level. Two main conclusions were drawn from these experiments. The first one is the absence of cross-reactivity between Art v 3 / Par j 1 or Pru p 3/Par j 1, indicating that there are no common IgE epitopes on these allergens, and that sensitization to Par j 1 is independent of Art v 3 or Pru p 3 sensitization. The second conclusion is that in some *Artemisia*-allergic patients, with IgE to both Art v 3 and Pru p 3, Art v 3 behaves as the primary sensitizing agent. This conclusion is based on the fact that we have found Art v 3 sensitization in mugwort-allergic patients with no Pru p 3 sensitization, and that Art v 3 is able to partially inhibit the IgE binding to Pru p 3 in three out of six patients studied (in two patients clearly above 50% inhibition), but Pru p 3 does not inhibit the IgE-binding to Art v 3. The level of cross-reactivity detected among Par j 1, Art v 3 and Pru p 3 is in line with the percent sequence identity found in these molecules [6].

The fact that in some *Artemisia*-allergic patients with Art v 3 and Pru p 3 sensitizations, Art v 3 behaves as the strongest allergen contrasts with the results of Pastorello et al. [14]. These authors studied the relevance of Art v 3 in peach-allergic patients and concluded that sensitization to mugwort LTP is a consequence of the sensitization to peach LTP. Immunoblotting inhibition showed that IgE-binding to the Pru p 3 band was totally inhibited by 4 µg of peach LTP, but only by 400 µg of mugwort LTP, whereas 4 µg of both mugwort and peach LTP totally inhibited the IgE-binding to the Art v 3 band. In addition, no Art v 3 sensitization was found in mugwort-sensitized patients with no Pru p 3 IgE. These discrepancies could be explained taking into account the different patient profiles in the studies. We recruited the patients based on *Artemisia* sensitization, whereas in Pastorello's work, the patients were selected by peach allergy. Another difference could be the *Artemisia* pollen pressure in Milan and Murcia. Finally, different techniques were used to assess IgE cross-reactivity. We think that ELISA inhibition is more adequate than immunoblotting inhibition in cases of partial cross-reactivity, as is the case with Art v 3 and Pru p 3. In addition, it provides more quantitative information.

LTPs have been considered mainly as food allergens. The resistance of LTPs to proteolytic attack [18, 23] indicates that these proteins can sensitize and induce systemic symptoms by the digestive route and, consequently, LTPs can be considered as true food allergens, in contrast with Bet v 1-like allergens and profilins. Moreover, LTPs can also lead to sensitization by the inhalation route. The pollen LTP Par j 2 is a marker by which to distinguish patients with primary sensitization to *Parietaria* from those who exhibited IgE reactivity to *Parietaria* extract without previous pollen exposure [11]. Furthermore, we have found a high prevalence of sensitization to the mugwort LTP Art v 3 in patients with primary allergy to *Artemisia* pollen. It is reasonable to consider a possible link between respiratory and food allergies mediated by LTPs. In this sense, we have shown in this work that in a group of mugwort-allergic patients with IgE to both Art v 3 (inhalant LTP) and Pru p 3 (food LTP), the inhalant allergen behaves as the primary allergen. Besides, it has been reported that Cor a 8 (hazelnut LTP) is a major allergen for Spanish patients allergic to hazelnuts, in contrast with Northern Europe patients [24]. All the patients studied in that work

were also allergic to *Platanus acerifolia* pollen, and it is possible that the existence of an LTP in the pollen could account for a primary sensitization through the inhalation route and a subsequent cross-reaction with Cor a 8. Finally, it has been reported very recently [25] that in one patient, Pru p 3 inhalation from peach tree leaves caused respiratory symptoms that were previous to the clinical expression induced by Pru p 3 by the digestive route.

An important issue to consider for future work is the role of T cell cross-reactivity between inhaled and food LTPs. It is likely that the cross-reactivity at the T cell epitope level is higher than at the B-cell epitope level, and continuous stimulation with different LTPs could have an enhancing effect because of the generation and/or survival of memory T cells.

As a final conclusion, the importance of the *Artemisia* allergens previously described, Art v 1 and Art v 60 kDa, has been confirmed. In addition, the mugwort LTP, Art v 3, is also an important allergen for *Artemisia*-sensitized patients and may act as a primer for peach LTP sensitization in some patients.

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