

Original article

A comparative analysis of the cross-reactivity in the polcalcin family including Syr v 3, a new member from lilac pollen

Background: Polcalcins are pollen-specific allergens with two EF-hand calcium-binding sites that exhibit strong cross-reactivity. Our objective was to isolate and express the cDNA coding of the EF-hand calcium-binding allergen from lilac pollen and to study cross-reactivity with other polcalcins from related and nonrelated pollen sources with different specific antibodies and sera from two different populations.

Methods: Specific cDNA was amplified by PCR, cloned and expressed in *Escherichia coli*. Purification was achieved by gel permeation and ion exchange chromatographies. ELISA titration and inhibition assays were performed using the recombinant forms of Syr v 3, Ole e 3, Che a 3 and Phl p 7 with sera from two Spanish regions with different sensitization profiles, as well as Phl p 7- and Ole e 3-specific polyclonal rabbit antisera, and an Ole e 3-specific monoclonal antibody.

Results: Syr v 3 displays two EF-hand consensus sites and 8863 Da of theoretical molecular mass. The allergen consists of 80 residues with identities ranging from 66 to 87% with polcalcins included in this study. Syr v 3, Ole e 3, Che a 3 and Phl p 7 showed a similar IgG- and IgE-binding capacity although differences at quantitative level were observed depending on the population of patients' sera.

Conclusion: Syr v 3 is a polcalcin with structural and antigenic similarities to the members of this family. Diagnosis of polcalcin-sensitized patients could be performed whatever polcalcin used, whereas for immunotherapy, primary sensitization to a particular allergenic source should be considered.

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Patients suffering from hay fever are frequently polysensitized to different allergenic sources. In Mediterranean countries, olive (*Olea europaea*) pollen is a major and well-determined cause of allergy, especially in regions where this tree is intensively cultivated (1). Sensitization of allergic patients to cross-reactive olive pollen allergens may be responsible for allergic symptoms upon contact with other pollens belonging to the Oleaceae family such as ash (*Fraxinus excelsior*), privet (*Ligustrum vulgare*) and lilac (*Syringa vulgaris*) (2, 3), as well as to nonrelated plant pollens of the Gramineae and Chenopodiaceae families (3, 4). Although Gramineae and Oleaceae pollens are major causes of seasonal allergic diseases worldwide (1, 5), weeds belonging to Chenopodiaceae family can be also considered as allergen sources with increasing incidence in dry areas of central and southern Europe (6).

Panallergens are proteins with highly conserved amino acid sequences and widely distributed in taxonomically nonrelated biological sources (7), thus explaining their strong implication in cross-reactivity. During the last

decade several calcium-binding proteins from pollens have been identified as cross-reactive allergens (8). These proteins present two EF-hand sequential motifs, which are composed of 12 conserved amino acid residues implicated in the binding of Ca^{2+} ions. They have been named polcalcins because of their specific expression in pollen and their calcium-binding capacity. These pollen-specific allergens have been described in rape (Bra r 1), olive (Ole e 3), birch (Bet v 4), Bermuda grass (Cyn d 7), alder (Aln g 4), Timothy grass (Phl p 7) and chenopod (Che a 3) (9–15). Interestingly, the binding of these proteins to IgE antibodies of allergic patients' sera is dependent on the presence of calcium (11, 13, 14). Recently, the three-dimensional structures of Phl p 7 and Bet v 4 have been determined, and showed a similar folding but different oligomerization state (16, 17).

Therefore, the characterization of panallergens, especially those with a high amino acid sequence similarity, from related and nonrelated plant pollens is of great interest as cross-reactions may play a role in the induction

of allergic immune responses. Moreover, the cloning and the recombinant production of allergens is a good strategy for obtaining well defined and homogeneous proteins in large quantities for diagnosis and treatment of atopic patients (18).

In this report, we describe the cloning, expression in *Escherichia coli* and purification of a new allergenic polcalcin, Syr v 3, from lilac, an Oleaceae member, whose flowers were reported to induce asthma and rhinitis symptoms (19). The aim of this work has been to carry out a comparative study including rSyr v 3 together with other EF-hand allergens belonging to related and nonrelated pollen sources, such as Ole e 3 from olive, Che a 3 from chenopod and Phl p 7 from Timothy grass, in order to evaluate their immunological relationships and elucidate their role in cross-reactivity to achieve a more effective diagnosis and treatment of allergic processes.

Material and methods

PCR-based cloning strategy and nucleotide sequence determination

Total RNA from lilac pollen (ALK-Abelló, Madrid, Spain) was obtained as described (20). cDNA was synthesized from 50 µg total RNA using modified lock-docking oligo-dT primer for the synthesis of the first strand. All reactions were performed following the protocol of the Marathon cDNA amplification kit (BD Biosciences-Clontech, Madrid, Spain) (21). For PCR amplifications, a degenerate antisense primer was designed based on the conserved C-terminal amino acid sequences of polcalcins (SV1: 5'-atgaattcRAANATYTTNGCNACRTCYTT, *EcoRI* restriction site is underlined). PCR amplification was carried out as described (20). The obtained cDNA fragments were purified, cloned into the

pCR2.1 vector included in the TA cloning kit (Invitrogen, Groningen, The Netherlands) and sequenced (20). Afterwards, a non-degenerate oligonucleotide, based on the N-terminal end of the protein deduced from the sequence obtained above, was used as a sense primer (SV2: 5'-atcatatgGCTGAGGAAGTAGCCGAG, *NdeI* restriction site is underlined). Multiple sequence alignment was performed using the CLUSTAL W program.

Expression and purification of the recombinant proteins

The cDNA included into pCR2.1 was subcloned into *NdeI/EcoRI* of pET11b expression vector rendering pET11b/Syrv3. Nonfusion recombinant protein was obtained after transforming BL21 (DE3)-competent *E. coli* cells as described (20). The soluble fraction of the disrupted cells was chromatographed onto a gel-filtration Sephadex G-50 column equilibrated in 0.2 M ammonium bicarbonate. The detection of the protein was performed with an Ole e 3-specific polyclonal antibody (pAb). Fractions containing the allergen were lyophilized and eluted from a DEAE-cellulose column with a discontinuous gradient of ionic strength from 20 mM to 0.5 M of ammonium bicarbonate at pH 8.0.

Recombinant allergens, rPhl p 7, rOle e 3, rChe a 3, rOle e 1 and rChe a 1 were purified as previously reported (14, 20, 22, 23). Natural allergens Phl p 1 and Phl p 5 were kindly donated by ALK-Abelló.

Analytical methods for protein characterization

Protein concentration of purified samples was determined by amino acid analysis after hydrolysis with 5.7 N HCl (20). Edman degradation of rSyr v 3 was performed to determine the N-terminal amino acid sequence on an Applied Biosystems model 477A sequencer (Applied Biosystems, Foster City, CA, USA). Purified protein was analysed on a Bruker Reflex II MALDI-TOF mass spectrometer equipped with an ion source with visualization optics and a nitrogen laser (337 nm) (Bruker-Franzen Analytik, Bremen, Germany). Samples were previously mixed with a matrix solution comprised of saturated α -cyano-4-hydroxycinnamic acid in 30%

Table 1. Serological data of the patients' sera

Serum number	Total IgE (kU/l)	RAST (olive)		RAST (ryegrass)		RAST (chenopod)		Ole e 1	Phl p 1/ Phl p 5	Che a 1
		Class	kU/l	Class	kU/l	Class	kU/l			
M1	1490.2	5	72.8	6	>100	4	38	—	+	—
M2	1374.6	5	88.7	6	>100	4	43.7	+	+	—
M3	909.0	5	83.7	5	93.1	0	<0.35	—	+	—
M4	365.6	5	75.1	5	94.3	0	<0.35	+	+	—
M5	192.4	5	71.4	6	>100	0	<0.35	—	+	—
M6	561.6	5	60.7	5	98.8	4	24.1	+	+	—
M7	670.4	5	90.5	6	>100	0	<0.35	+	+	—
M8	1524.0	5	61.4	6	>100	4	46.7	+	+	—
M9	1525.4	5	90.2	5	63.6	0	<0.35	+	+	—
J1	399	5	50	3	15.5	4	44.3	+	+	—
J2	475	4	21	3	7.4	3	16.5	+	+	—
J3	141	3	10.8	3	4.5	3	12.3	+	—	—
J4	213	4	33	3	14.2	4	21.4	+	—	+
J5	145	4	24	ND		ND		+	—	+
J6	323	3	8	3	4.3	ND	+	—	—	—
J7	ND	6	>100	3	8.2	4	22.4	+	—	+
J8	ND	6	>100	2	2.1	3	9.2	+	—	—
J9	115	5	54	3	17.5	ND		+	—	—

M1 to M9 patients from Madrid, J1 to J9 patients from Jaén. Specific IgE values against olive, ryegrass and chenopod are shown. Sensitization against Ole e 1, Phl p 1 and/or Phl p 5 and Che a 1 was determined by ELISA OD > 0.1 (+), OD < 0.1 (—). ND, nondetermined data.

aqueous acetonitrile and 0.1% trifluoroacetic acid. The equipment was externally calibrated employing singly, doubly and triply charged signals from either cytochrome *c* or bovine serum albumin.

Human sera and antibodies

Eighteen sera of untreated patients from two Spanish populations (Madrid and Jaén) with known reactivity to polcalcins (determined by ELISA) were used in the experiments. Among other food and pollen sensitizations all of them showed positive prick test against olive and ryegrass (Gramineae family), and display rhinitis, rhinoconjunctivitis or asthma. Patients' ages ranged between 19 and 40 years. Other characteristics of the patients' sera are shown in Table 1. A serum from a nonallergic individual has been used as control.

Two specific polyclonal antisera were obtained by immunizing rabbits with natural Ole e 3 (10) or rPhl p 7 (24). rOle e 3-specific monoclonal antibody was obtained in collaboration with Dr van Ree (Sanquin, Amsterdam, The Netherlands).

ELISA experiments

IgE-binding analysis was performed by ELISA in microtitre plates coated with 0.1 µg/well of protein and using individual sera (diluted 1 : 10), as described (15). IgG titrations were performed with dif-

ferent dilutions of pAb or mAb. For ELISA inhibition, plates were incubated alternatively with the pool of sera (*n* = 5, diluted 1 : 10), the Ole e 3-specific polyclonal antiserum (diluted 1 : 30 000), the Phl p 7-specific polyclonal antiserum (diluted 1 : 200 000) or the anti-Ole e 3 monoclonal antibody (diluted 1 : 30 000), previously preincubated with different amounts of polcalcins as inhibitors. In both experiments, the binding of human IgE was detected by mouse anti-human IgE monoclonal antibody (diluted 1 : 5000); kindly donated by ALK-Abelló, followed by horseradish peroxidase-labelled goat anti-mouse IgG (diluted 1 : 5000). The binding of IgG polyclonal antiserum was detected by peroxidase-labelled goat anti-rabbit IgG (diluted 1 : 3000; BioRad, Richmond, VA, USA); for the IgG monoclonal antibody, peroxidase-labelled goat anti-mouse IgG (diluted 1 : 5000; Pierce Biotechnology, Rockford, IL, USA) was used. Peroxidase reaction was measured as optical density (OD) at 492 nm (20). Each value was calculated as mean of two determinations. Controls without antigen or specific antibody are included. The percentage of inhibition was calculated according to the formula: Inhibition (%) = [1 - (OD with inhibitor/OD without inhibitor)] × 100.

Electrophoresis and immunoblotting

rSyr v 3 expression and purification was analysed by SDS-PAGE in 15% polyacrylamide gels according to Laemmli (25). Proteins were

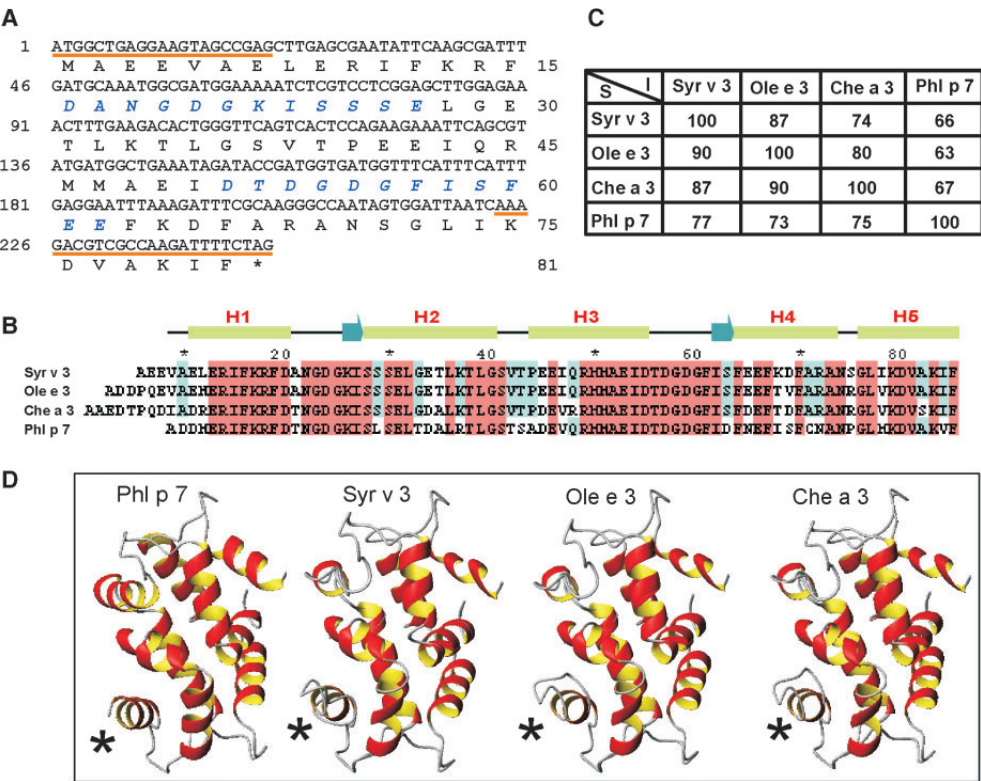


Figure 1. (A) Nucleotide sequence of the cDNA encoding Syr v 3 and its deduced amino acid sequence. Primers used are underlined and the stop codon is indicated by an asterisk. The two calcium-binding domains are in blue. (B) Alignment of the amino acid sequences of the four polcalcins used in the study. The secondary structure plot is according to the three-dimensional structure of Phl p 7 (16). α-Helix is represented by green tubes and β-strand by blue arrows. (C) Percentage of identity (I) and similarity (S) between pairs of proteins are indicated. (D) Backbone ribbon-type representation of the three-dimensional modelled structure of Che a 3, Ole e 3 and Syr v 3 determined by using the services of the Swiss-Model Protein Modelling Server, using as template the structure of Phl p 7 (PDB entry 1K9U) (16). Asterisks show the N-terminal end of the molecules.

either visualized by Coomassie Blue staining or electrophoretically transferred onto nitrocellulose membranes for immunodetection as described (15). Human sera and IgG antibodies were the same used in ELISA experiments. Controls without monoclonal antibody or polyclonal antiserum, but including the corresponding peroxidase-labelled second antibody are included, as well as the serum of a nonallergic individual. The signal was developed by the ECL-Western-blotting reagent (Amersham Biosciences, Barcelona, Spain).

Results

Cloning and sequencing of Syr v 3, a calcium-binding allergen belonging to the polcalcin family

cDNA synthesized from total RNA of *S. vulgaris* pollen was used as template to amplify a specific DNA encoding Syr v 3, which resulted in 243 bp (Fig. 1A). The polypeptide displays an open reading frame of 81 residues and a deduced molecular mass of 8994 Da. No polymorphism was detected in the sequence of Syr v 3, which contains two segments with a consensus sequence of 12-amino acid residues of calcium-binding sites of EF-hand type. The comparison of the amino acid sequence with those of proteins included in database banks confirmed that it belonged to the polcalcin family (Fig. 1B). Identities ranging between 66 and 87% and similarities between 77 and 90% were found among polcalcins used in this study (Fig. 1C). Three-dimensional structures of Che a 3, Ole e 3 and Syr v 3 were modelled on the basis of the experimental determined structure of Phl p 7 (16) (Fig. 1D). Although similar structures were obtained for the four polcalcins, differences can be mainly detected at the N-terminal end of the proteins.

Recombinant expression in *E. coli* of Syr v 3, purification and molecular characterization

Intracellular protein expression in *E. coli* of Syr v 3-encoding cDNA was evaluated by means of SDS-PAGE and Coomassie Blue staining of the soluble fraction from the harvested and disrupted cells (Fig. 2A).

Two chromatographic steps in Sephadex G-50 and DEAE-cellulose columns were performed to purify rSyr v 3 (Fig. 2B). The protein eluted at 0.3 M ammonium bicarbonate of ionic strength in the ion exchange chromatography rendering 20 mg/l of cell culture of soluble polcalcin. The purified recombinant allergen was analysed in immunoblotting for its IgG- and IgE-binding capacities against Ole e 3-specific monoclonal antibody and polyclonal antiserum, and for a pool of olive-allergic sera. rSyr v 3 was strongly recognized by all of these antisera (Fig. 2C). No signal was observed for the controls.

To evaluate the purity of rSyr v 3, mass spectrometry analysis and Edman degradation of the protein were performed. The N-terminal amino acid sequence of rSyr v 3 rendered AEEVA, which is in agreement with the sequence deduced from the cDNA encoding Syr v 3, but

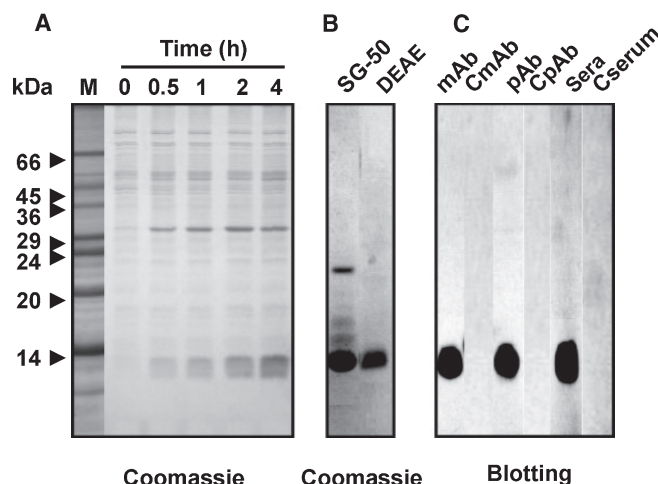


Figure 2. Analysis of the expression, purification and immunological recognition of rSyr v 3 by SDS-PAGE. (A) Protein-staining of the soluble fractions collected at different times after induction of the *E. coli* cells; M, molecular mass markers. (B) Protein-staining of the fractions from Sephadex G-50 and DEAE-cellulose columns. (C) Western blotting of purified rSyr v 3 with Ole e 3-specific monoclonal antibody (mAb), Ole e 3-specific polyclonal antiserum (pAb) or with a pool of olive-polcalcin sensitized patients' sera. Controls without mAb (CmAb) or pAb (CpAb), but including the corresponding peroxidase-labelled second antibody are shown, as well as a control with the serum of a non-allergic individual (Cserum).

lacking the initial Met due to the biological processing by the bacteria. Mass spectrometry analysis rendered a single peak with a molecular mass of 8865.8 Da, which is closely near to the theoretical value deduced from the amino acid sequence without the initial Met (8863 Da), and in agreement with the Edman degradation result.

Polcalcin-specific antibodies recognize to rSyr v 3

ELISA titration analyses with Ole e 3- and Phl p 7-specific polyclonal antisera, and Ole e 3-specific monoclonal antibody were performed in order to compare the immunological properties of Syr v 3 with those of polcalcins from different plant families: olive from Oleaceae, Timothy grass from Gramineae and chenopod from Chenopodiaceae. A very similar behaviour was observed for Syr v 3, Ole e 3 and Che a 3 polcalcins when the polyclonal antisera were used (Fig. 3A,C), and was different with Phl p 7. Otherwise, when the monoclonal antibody was utilized, a similar recognition was observed for all polcalcins indicating that the epitope recognized by this antibody is conserved in all of them (Fig. 3B).

ELISA inhibition experiments among polcalcins were also performed by coating the plates with rOle e 3 for the assay with the antibodies raised against Ole e 3 or by coating the plates with rPhl p 7 when the antibody

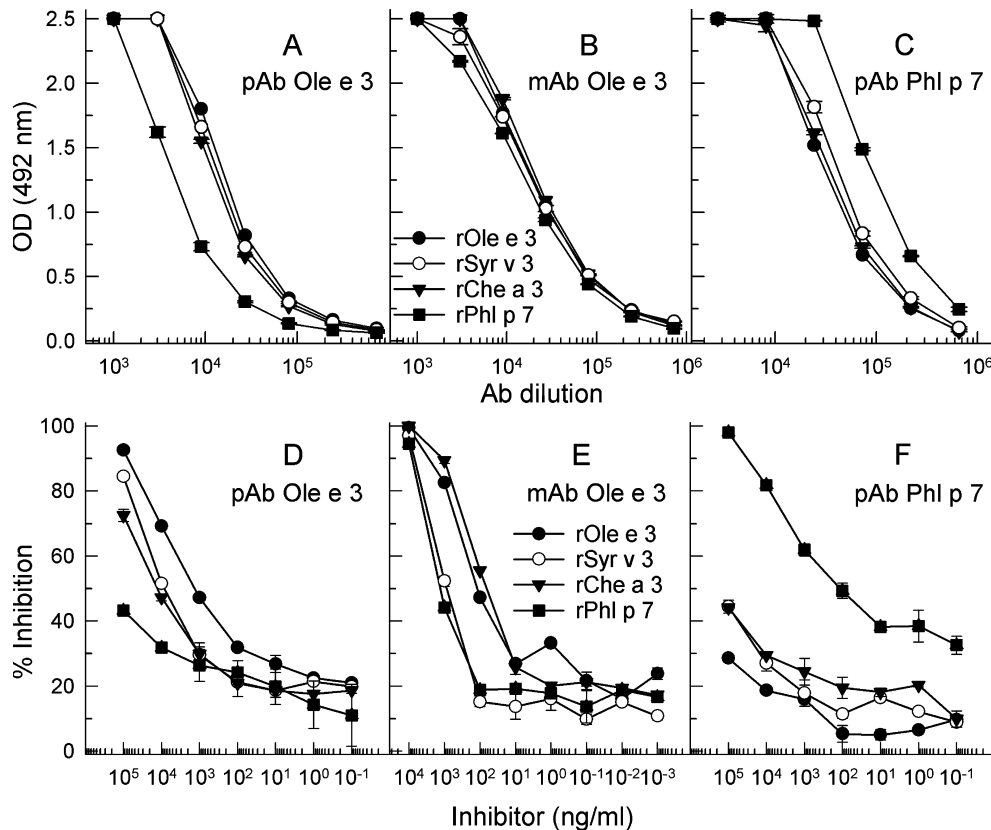


Figure 3. ELISA titration of rOle e 3, rChe a 3, rSyr v 3 and rPhl p 7 using: (A) Ole e 3-specific polyclonal antiserum, (B) Ole e 3-specific monoclonal antibody or (C) Phl p 7-specific polyclonal antiserum. ELISA inhibition of: (D) Ole e 3-specific polyclonal antiserum to rOle e 3-coated wells, (E) Ole e 3-specific monoclonal antibody to rOle e 3-coated wells and (F) Phl p 7-specific polyclonal antiserum to rPhl p 7-coated wells, by using rOle e 3, rChe a 3, rSyr v 3 and rPhl p 7 as inhibitors.

specific for this polcalcin was used. In all cases, rOle e 3, rChe a 3, rSyr v 3 and rPhl p 7 were used as inhibitors. In the inhibition performed with Ole e 3-specific polyclonal antiserum, rPhl p 7 produced less inhibition (43%) than rChe a 3, rSyr v 3 and rOle e 3, which reached up 73, 85 and 93% of inhibition, respectively (Fig. 3D). When the experiment was performed with the monoclonal antibody raised against Ole e 3, 100% of inhibition was obtained with all polcalcins, but less binding to rPhl p 7 and rSyr v 3 was detected at 50% of inhibition (Fig. 3E). At last, when the inhibition was performed with the polyclonal antiserum raised against Phl p 7, the maximum inhibition (98%) was obtained only with rPhl p 7; the inhibition obtained for the other polcalcins was lower, reaching values of around 40% (Fig. 3F).

Cross-reactivity studies indicate differences in IgE-reactivity to polcalcins in different populations

Eighteen allergic patients' sera from Madrid (nine patients) and Jaén (nine patients) – two geographical regions of Spain with different pollen sensitizations (26)

– and with IgE reactivity to polcalcins, determined by ELISA, were used. To define the primary sensitization sources of these patients, all sera were tested by ELISA against Ole e 1, used as a marker for olive sensitization, Che a 1 as a marker for chenopod, and Phl p 1 and Phl p 5 used as markers for grass pollen sensitization (Table 1). Jaén population resulted originally sensitized to olive as all patients had Ole e 1-specific IgE and only two sera (22%) had Phl p 1- and/or Phl p 5-specific IgE. By contrast, most of the patients from Madrid resulted with both sensitizations, as all of them (100%) showed Phl p 1- and/or Phl p 5-specific IgE, and six of nine sera (67%) had Ole e 1-specific IgE. The percentage of primary sensitization in these patients against chenopod was not significant, as only three patients from Jaén but none from Madrid exhibited specific IgE against Che a 1.

The same 18 sera were tested by means of ELISA against each polcalcin (Fig. 4). In both populations, a similar recognition for all proteins was obtained.

IgE binding to polcalcins was also studied by ELISA inhibition experiments. For this purpose, ELISA plates were coated with polcalcins from the pollen sources which

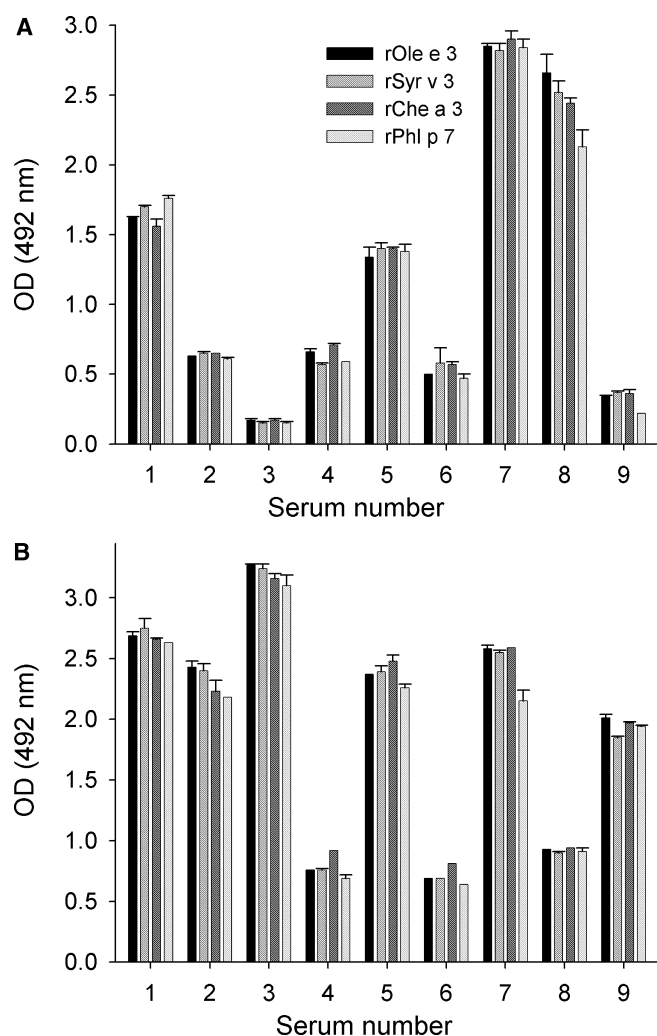


Figure 4. IgE-binding quantitation to the four polcalcins used in the study in the sera of patients from two different regions of Spain: (A) Jaén ($n = 9$) and (B) Madrid ($n = 9$).

dominate in a particular region. Thus, olive and lilac polcalcins were alternatively used in the experiment performed with a serum pool from patients from Jaén and Timothy grass polcalcin with a serum pool from patients from Madrid. All the polcalcins were used as inhibitors in each experiment (Fig. 5). When rOle e 3 is coating the wells, the inhibition reached by itself was the highest value (97%), and rSyr v 3, rChe a 3 and rPhl p 7 rendered 78, 69 and 48%, respectively (Fig. 5A). When rSyr v 3 was the polcalcin coating the wells, rOle e 3 and rSyr v 3 produced around 95% of inhibition, and rChe a 3 and rPhl p 7 were able to inhibit 76 and 59%, respectively (Fig. 5B). In the population from Madrid, the higher inhibition was observed with rPhl p 7 (95%) (Fig. 5C).

Discussion

Oleaceae is a broad family of plants for which allergenic cross-reactivity among many of their members has been reported (2). Olive and ash pollens are known as important causes of allergy (1, 27), whereas privet and lilac have been described as elicitors of allergic symptoms in conditions of local exposure (e.g. when smelling lilac flowers or when cutting privet hedges) (19, 27). Solid candidates to exert the cross-reactivity are the Ole e 1-like family of allergens, profilins and polcalcins. We have performed a cross-reactivity study among polcalcins from olive, Timothy grass, lilac and chenopod pollens that are present in the Mediterranean area using sera from two different populations of allergic patients.

The existence of a polcalcin in lilac pollen had been detected by our group testing the presence of Ole e 3-homologous mRNAs and antigens in different pollens by using Northern blot and Western blot experiments, respectively (20). In this work, the cDNA encoding this

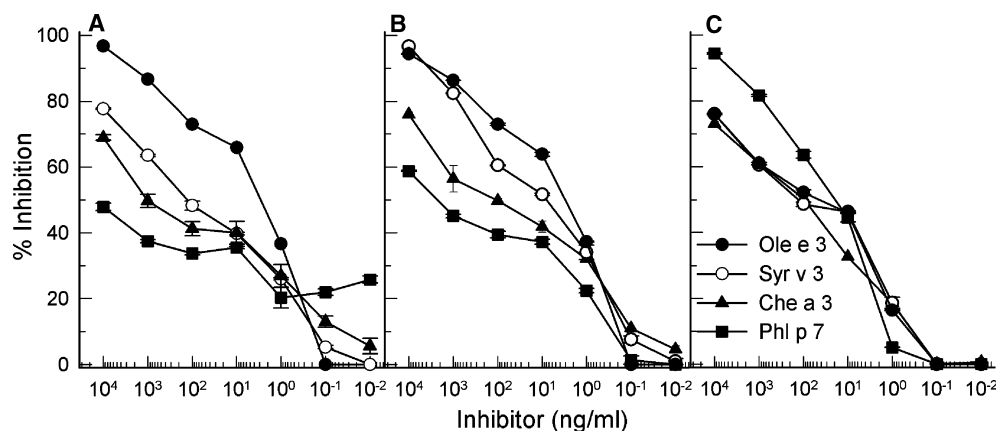


Figure 5. ELISA inhibition of a pool of polcalcin-sensitized patients' sera: from Jaén to (A) rOle e 3-coated wells, (B) rSyr v 3-coated wells and (C) from Madrid to rPhl p 7-coated wells using rOle e 3, rChe a 3, rSyr v 3 and rPhl p 7 as inhibitors.

allergen was cloned and sequenced, and it was named Syr v 3 according to the IUIS Nomenclature Committee rules. The protein is 80 amino acids in length, three residues shorter than Ole e 3, and displays two EF-hand sites. No polymorphism was detected in the amino acid sequence. The recombinant Syr v 3 was obtained in *E. coli* cells and purified at homogeneity with a high yield (20 mg/l of cell culture). The molecular mass obtained by MS was 8865.8 Da, and the theoretical pI 4.41, which are features that agree with those expected for polcalcins (13, 15, 20). The N-terminal amino acid sequence of purified Syr v 3 showed the absence of the initial Met residue as reported for other polcalcins (14, 20). Ole e 3-specific monoclonal antibody and polyclonal antiserum, as well as IgE from patients allergic to olive-polcalcin were able to recognize Syr v 3 indicating that it shares IgG and IgE epitopes with Ole e 3.

ELISA titration and inhibition experiments showed a binding behaviour against polyclonal antibodies quite similar for Che a 3, Syr v 3 and Ole e 3, whereas Phl p 7 exhibited the most different reactivity in these assays. This different IgG-inhibition capacity of Phl p 7 could be explained because this polcalcin has the lowest degree of identity and is the shortest molecule (Fig. 1B,C). Regarding the modelled three-dimensional structures of polcalcins, common epitopes could be expected as a result of their similar global folding. The small differences detected in the recognition by the antibodies can be attributed to differences observed at the N-terminal end of the polypeptides, which has been reported as a putative immunodominant region (14, 28), and to local changes in the surface of the protein.

The monoclonal antibody specific to Ole e 3 was able to discriminate by inhibition assay between the pair Ole e 3/Che a 3 and the pair Syr v 3/Phl p 7. Only positions 4, 6, 7, 68, 75 and 78 (Fig. 1B) showed identical residues in the former pair and different in the latest; thus, it can be suggested that some of these residues could be involved in the binding to this antibody.

The reported allergenic prevalence of this family of proteins varies between 5 and 46% for Bet v 4 and Che a 3, respectively (11, 15) and is around 20–30% for Ole e 3 (10, 29) and 10% for Phl p 7 (14). The prevalence for each allergen seems to depend on the geographical area of the patients. When Moverare et al. (30) compared different European populations regarding the reactivity of Bet v 4, prevalence values between 5 and 11% for patients from North and Central Europe, and 27% for Italian patients were obtained. All these data point to the existence of a certain correlation between the poly-sensitization degree and the prevalence to these minor allergens.

The IgE response of the four polcalcins was analysed using two populations of allergic individuals: (i) sera from Jaén, which are mainly sensitized against olive pollen, and (ii) sera from Madrid sensitized against both

grass and olive pollen. Because of the unusual incidence of lilac pollinosis, skin prick tests against lilac pollen extract are not commonly performed. However, nasal provocation tests and skin prick test with lilac pollen were performed in four patients from the population of Jaén and all of them gave a positive response (data not shown). The different sensitivity of the two allergic populations to olive and grass pollen could be due to the pollen levels in each region. Thus, in Jaén olive pollen counts reached 36 000 grains/m³ in 2004, and 110 000 grains/m³ in 2003, whereas grass pollen reached only 3000 grains/m³. In Madrid, pollen levels of these species are not so different, being in 2004 of 500 grains/m³ for olive and 2000 grains/m³ for grass pollen (<http://www.polenes.com>).

Regarding to the ELISA experiment performed with polcalcins, similar responses were observed in the binding of each individual serum to the four allergens as a result of their panallergenic character. However, the inhibition curves showed significant differences in the populations of Madrid with respect to that of Jaén for which Ole e 3 – and to a lesser extent Syr v 3 – was the best inhibitor. This result could be explained by (i) the clear dominance of the olive pollen in Jaén and therefore the subsequent dominance of olive sensitization, and (ii) the narrow relationships between Syr v 3 and Ole e 3, both members of the same taxonomical family. For patients from Madrid, the inhibition capability of different polcalcins is more similar, but Phl p 7 seems to be the best inhibitor. This is probably because patients from Madrid show sensitization to olive and grass pollens, and the latter is the main sensitization source, as the nine sera (100%) have Phl p 1- and/or Phl p 5-specific IgE and only six of nine (67%) have Ole e 1-specific IgE.

In conclusion, and although the cross-reactivity among polcalcins is high, they do not present a complete immunological equivalence. Therefore, the diagnosis of patients sensitized to polcalcins can be performed with any member of the family. Regarding specific immunotherapy, our findings indicate that polcalcins from different sources may be substituted for each other due to their high degree of sequence identity and immunological cross-reactivity. However, it may be of advantage to include in therapeutic vaccines the polcalcin from that allergen source against which the patient was genuinely sensitized.

Acknowledgments

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