

The Russian Thistle (*Salsola kali*) Pollen Major Allergen, Sal k 1, Can Be Quantified in Allergenic Extracts and Airborne Pollen

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Key Words

Russian thistle *Salsola kali* • Sal k 1 allergen •
Amaranthaceae family • Monoclonal antibody •
Aeroallergen control • Biological standardization

Abstract

Background: Russian thistle (*Salsola kali*) pollen is an important cause of pollinosis in areas where rainfall is not abundant. Our aim was to develop an ELISA for quantification of the major allergen of *S. kali* extracts, Sal k 1, and to assess the correlation of this allergen content with the allergenic activity of extracts. **Methods:** Sal k 1 was purified by ion exchange and gel permeation chromatography and identified by mass spectrometry. Monoclonal antibody 4C11 was used for capture at 5 µg/ml and biotin-labeled specific antiserum at 0.25 µg/ml served for detection. The allergenic activity of the pollen extracts was measured by enzyme allergosorbent test inhibition. **Results:** Sal k 1 reacted to 85% of sera from 40 *S. kali*-allergic patients and was able to inhibit 92% of the IgE-binding capacity of patients' serum pool to the whole extract. The ELISA had a lineal range between 1.25 and 20 ng/ml of purified Sal k 1. The intra- and interassay coefficients of variation were lower than 5 and 10%, respectively. The assay was very sensitive since it had a detection limit of 0.08 ng/ml. No reactivity was found outside the Amaranthaceae family where only *Kochia* and *Salicornia* sp. gave sig-

nificant reactivity. A good correlation (Spearman's $\rho = 0.92$) was obtained between Sal k 1 content of different *S. kali* extracts and their IgE-binding activity. **Conclusions:** The results proved the usefulness of the two-site sandwich ELISA for aeroallergen control and for the standardization of *S. kali* pollen extracts intended for clinical use.

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Introduction

Pollens from the Amaranthaceae family (including the Chenopodioideae, Salsoloideae and Amaranthoideae subfamilies [1]) have been described as outstanding sources of pollinosis in temperate European countries, Central and Western United States, North Africa, and some Arabic countries [2–5]. Regarding the allergenic character, the most representative members of this family are Lamb's quarter (*Chenopodium album*) and Russian thistle (*Salsola kali*) [6]. Russian thistle is a tumbleweed plant typical of salty soils, where rainfall is not abundant. In Europe, it is very common in coastal areas from the Baltic Sea to the Mediterranean coast. In sev-

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eral areas of Spain (Aragón, Andalucía, Murcia and Levante) where this plant is very abundant, its pollen load may represent up to 5% of the total pollen and affects 42% of the patients with clinical sensitivity to pollens, being the third most frequent cause of pollinosis in southern Spain [7, 8]. The allergenic incidence of this weed has been increasing lately due to the advance of desertification of the south of Europe.

Three allergens have been described in *S. kali* pollen extracts: Sal k 1; Sal k 2, a protein kinase homologue, and Sal k 8, the panallergen profilin (www.allergome.org). The major allergen, Sal k 1, is a pectin methylesterase of 40 kDa with glycosylated nature and high polymorphism since up to 6 different isoforms have been described [9, 10]. It is considered an allergenic marker to discriminate among patients allergic to Amaranthaceae pollens in several areas of the south-east of Spain [10].

Current standardization methods are based on their total allergenic potency although allergen concentration should be monitored since an optimal maintenance dose of 5–20 µg of major allergens is associated with clinical improvements after immunotherapy with some specific extracts [11, 12]. Therefore, major allergen quantification is an increasing demand for products used for specific immunotherapy to clearly establish a dose-response relationship between allergens and treatment efficacy. According to this, the development of techniques to measure major allergen levels in diagnostic and therapeutic allergen products is of great interest. Monoclonal antibody-based immunoassays are the preferred tool to achieve this goal since they have the sensitivity, specificity and reproducibility required [13]. Following this aim, the European Project CREATE identified quantitative ELISAs that accurately measured Phl p 5, Bet v 1, Ole e 1, Der p 1, and Der p 2 allergens [14, 15]. In the present study, we describe for the first time a two-site sandwich ELISA for Sal k 1. This assay, based on a monoclonal antibody and a specific rabbit polyclonal antibody against purified Sal k 1, provides a specific and sensitive estimation of the content of this major allergen.

Materials and Methods

Pollen Extracts and Sal k 1 Purification

The pollen grains were defatted with ethyl ether for 4 h in a Soxhlet apparatus, dried, and extracted 1:5 (w/v) by magnetic stirring (4 h at 4°C) in phosphate-buffered saline (PBS) 50 mM (pH 8.0). The extract was clarified by centrifugation at 5,000 g for 30 min, filtered through AP glass fiber prefilters (Millipore, Bedford, Mass., USA), dialyzed exhaustively against water by diafil-

tration in a Pellicon System (Millipore) with a 5,000-Da cutoff, and freeze-dried.

To purify Sal k 1, Russian thistle pollen extract was dissolved in 40 mM sodium acetate (pH 5.0) and subjected to a cationic exchange chromatography on a 16/20 HF SP column attached to an AKTA prime system (GE Healthcare, Buckinghamshire, UK). Sal k 1 was eluted with a linear gradient from 0 to 300 mM NaCl in the same buffer. Fractions with IgE reactivity were pooled, concentrated in an Amicon 4 ultrafiltration device and injected into a Superdex 75 16/60 gel permeation column (GE Healthcare); fractions containing the 40-kDa protein were pooled, dialyzed, brought to 20 mM Tris (pH 8.0) and injected in a HiTrap Q column (GE Healthcare) equilibrated in the same buffer. Sal k 1 was obtained in the unbound fraction. In order to obtain enriched fractions from the 3 major isoforms, lyophilized Sal k 1 was dissolved in 20 mM sodium acetate (pH 5.0) and injected into a Resource 15S column (GE Healthcare); the isoforms were resolved with a gradient of 25 ml 0–250 mM NaCl in the same buffer.

Partially Sal k 1-depleted pollen extracts were achieved by immunoprecipitation after incubation of 0.5 mg of *S. kali* pollen extracts with anti-Sal k 1 polyclonal antibodies bound to 50 µl protein G beads (GE Healthcare). The Sal k 1/polyclonal antibody/protein G agarose complex was then removed by centrifugation. The protein content was measured by the Bradford method using Bio-Rad protein assay kit (Bio-Rad, Hercules, Calif., USA).

Patient Sera

Serum samples were obtained from 40 patients sensitized to Russian thistle pollen showing immediate hypersensitivity to Russian thistle pollen according to a clinical history of seasonal rhinitis, asthma, or both, and positive skin reaction to a biologically standardized commercial *S. kali* pollen extract (Bial-Arístegui, Bilbao, Spain).

Peptide Mass Fingerprinting

Chromatography-purified Sal k 1 was submitted to electrophoresis and stained with Coomassie blue. After electrophoresis, the 40-kDa band was manually excised from the gel and digested in situ with trypsin [16]. An aliquot (0.5 µl) of digested protein was analyzed in an Autoflex II MALDI-TOF (Bruker Daltonics, Billerica, Mass., USA) equipped with reflector using α-cyano-4-hydroxycinnamic acid as matrix in saturated conditions. Mass spectra obtained were used for protein identification in databases. The digestion was further analyzed by liquid reversed-phase chromatography coupled to an electrospray mass spectrometry with ionic trap Deca-XP (Thermo-Finnigan, Calif., USA). The fragmentation spectra obtained (MS/MS) were analyzed with Turboquest software (Thermo-Finnigan) and homologies were found using the FASTA program (University of Wisconsin, USA).

Production, Purification and Biotinylation of Antibodies

Two female BALB/c mice were immunized intraperitoneally with 10 µg of purified Sal k 1 during 2 months fortnightly. Mice spleen cells were fused with Sp2/0 myeloma line essentially as previously described [17]. Specific hybridomas were identified by ELISA employing Sal k 1-coated plates and then subcloned by limiting dilution. The monoclonal antibody isotype was determined by IsoStrip kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. Poly-

clonal antibodies were obtained by immunizing a female New Zealand rabbit with 200 µg of purified allergen emulsified in complete Freund adjuvant (Difco Laboratories, Detroit, Mich., USA). The boosts were carried out at 2-week intervals. Serum samples collected 10 days after the last injection were tested and stored at -80°C. Antibodies were purified from hybridoma culture supernatants or from rabbit serum by affinity chromatography by HiTrap Protein G columns (GE Healthcare). Anti-Sal k 1 polyclonal antibodies were labeled with biotin using the ECL protein biotinylation module (GE Healthcare).

Electrophoresis, Agarose Isoelectric Focusing and Immunoblotting

Pollen extracts or Sal k 1 were separated by 12.5% SDS-PAGE under reducing conditions [18]. Isoelectric focusing of Sal k 1 (3 µg) was performed on Isogel Agarose plates with a pH range from 3 to 10 (Lonza, Rockland, Me., USA) following the manufacturer's recommendations. The separated protein bands were visualized by Coomassie brilliant blue staining or blotted onto PVDF transfer membranes (Hybond-P, GE Healthcare). After blocking, blots were incubated with a pool of sera (diluted 1/5) from *S. kali*-allergic patients, with anti-Sal k 1 polyclonal antibodies (0.05 µg/ml) or with the monoclonal antibody 4C11 (0.5 µg/ml). Then, membranes were incubated with anti-human IgE (Southern Biotech, Birmingham, Ala., USA), anti-rabbit immunoglobulins (Dako, Copenhagen, Denmark) or anti-mouse IgG antibodies (Sigma Chemical Co., St. Louis, Miss., USA) linked to horseradish peroxidase. Finally, blots were developed by the ECL Plus Western Blotting Detection System (GE Healthcare).

Quantification of Sal k 1 by a Two-Site Sandwich ELISA

The two-site sandwich ELISA was performed by coating microtiter 96-well plates (Greiner, Frickenhausen, Germany) overnight at room temperature with 100 µl/well of monoclonal antibody 4C11 at 5 µg/ml in PBS. The coated wells were blocked with 200 µl of PBS containing 1% BSA and 0.05% Tween 20. Afterwards, they were sequentially incubated with 100 µl/well of Sal k 1 or *S. kali* pollen extract dilutions, biotin-labeled anti-Sal k 1 polyclonal antibodies (0.25 µg/ml), and streptavidin-peroxidase (0.25 µg/ml; Sigma). All incubations were performed at 37°C for 1 h, with 3 washes of 200 µl/well of PBS-Tween 20 between successive steps. Finally, wells were incubated at room temperature in the dark with 200 µl/well of a commercial solution of *o*-phenylenediamine (Sigma). The reaction was stopped after 30 min by adding 50 µl of 3 M H₂SO₄ and the optical density was then measured at 492 nm.

A standard curve was obtained ranging from 80 to 0.08 ng/ml of Sal k 1, and it was run per day for 10 days to evaluate interassay variations. In order to determine intra-assay variations, 5 Sal k 1 concentrations corresponding to the lineal portion of the standard curve (1.25, 2.5, 5, 10 and 20 ng/ml) were incubated 20 times during the same day. Crude extracts were assayed as dilution series in triplicate, and the concentrations were calculated by interpolating from the lineal portion of the standard curve generated with the purified Sal k 1. The detection limit was calculated as the amount of Sal k 1 which corresponded to the median plus 3.33 times the standard deviation obtained after 30 measurements of the zero standard. Specificity of the antibodies against a broad battery of different allergenic plant and nonplant extracts was evaluated by adding to the assay 8 µg/ml of the mentioned ex-

tracts which corresponded to a concentration 10-fold higher than the highest assayed with *S. kali* extract.

Extraction and Quantification of Sal k 1 from Pollen

The quantification of the number of pollen grains per milligram of pollen was performed using a Neubauer counting chamber (Brand, Wertheim, Germany). Decreasing known numbers of pollen grains (from 170,000 to 10 grains per milliliter) were extracted for 2 h at room temperature with 50 mM phosphate buffer (pH 7.4) containing 150 mM NaCl, 125 mM NH₄HCO₃, 3 mM EDTA, and 0.005% Tween 20 [19]. The extract was separated from the particulate matter by centrifugation at 5,000 g for 10 min and then 100 µl of the supernatant was analyzed by two-site sandwich ELISA.

Enzyme Allergosorbent Test and Enzyme Allergosorbent Test Inhibition

Specific IgE levels were quantified by the enzyme allergosorbent test (EAST; Hytec-specific IgE EIA; Hycor Biomedical Inc., Kassel, Germany) as described by the manufacturer. *S. kali* extract and Sal k 1 were coupled to CNBr-activated paper disks [20] at 3 mg/ml and 100 µg/ml, respectively.

The relative IgE-binding activity of *S. kali* pollen extracts and Sal k 1 was evaluated by comparison with an in-house reference (IHR) *S. kali* pollen extract, previously calibrated by the skin prick test according to the Nordic Guidelines and EAACI recommendations and using 10 mg/ml histamine as control [21, 22]. Briefly, aliquots of a serum pool from 26 high-reactive *S. kali* pollen-sensitized patients were preincubated with serial dilutions of *S. kali* extracts or purified Sal k 1. Next, these aliquots were incubated with IHR *S. kali* extract-coupled disks and after washing, the disks were incubated with alkaline phosphatase-labeled mouse anti-human IgE (Hycor Biomedical) and developed following the instructions of Hytec-specific IgE EIA (Hycor Biomedical). The relative IgE-binding activity of extracts was estimated on the basis of the 50% inhibition values by comparison with the IHR extract.

Results

Purification and Analysis of Sal k 1

Purification of Sal k 1 was performed by means of 3 chromatographic steps consisting of 2 ion exchange and 1 size exclusion chromatographies. The purified Sal k 1 was analyzed by SDS-PAGE and consisted of a protein with an apparent molecular mass of 40 kDa and an estimated purity >95% (fig. 1a). The identity of purified Sal k 1 was confirmed by mass spectrometry, as the sequences obtained for MS/MS-derived peptides matched registered sequences of databases. The highest homology was found to Sal k 1.0301 covering 66% of the reported sequence (fig. 1e). A purification yield of about 2.1 mg of pure protein per gram of dry pollen was obtained. Isoelectric focusing analysis of the purified Sal k 1 indicated that the protein possesses several isoforms in a range of

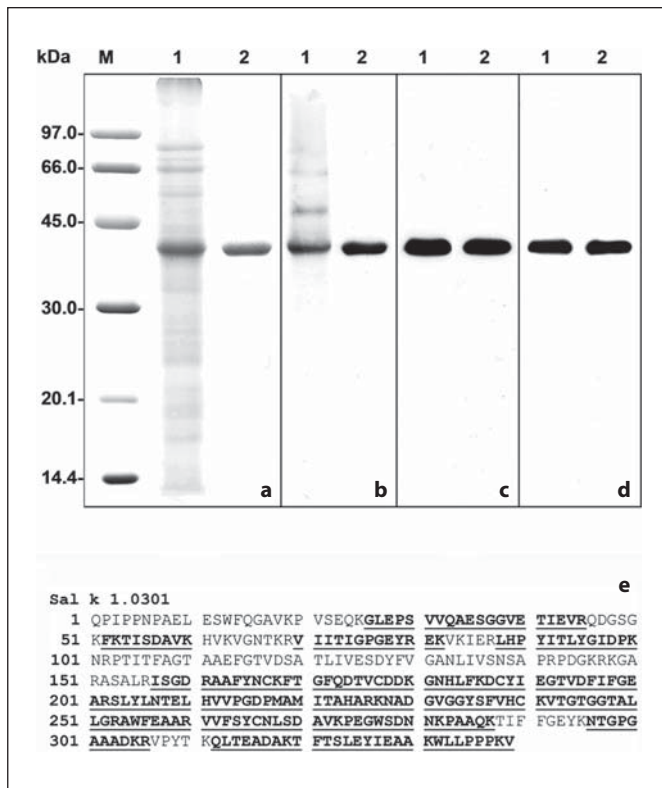


Fig. 1. Coomassie brilliant blue-stained SDS-PAGE (a) and immunoblots under reducing conditions of *S. kali* pollen extract (lane 1) and Sal k 1 (lane 2), incubated with a pool of sera (b), anti-Sal k 1 polyclonal antibodies (c) or 4C11 monoclonal antibody (d). e Peptides from the purified 40-kDa protein identified by peptide mass fingerprinting are marked (bold and underlined) on the amino acid sequence of Sal k 1.0301.

pI from 6.3 to 8.8. The purified protein was recognized by a pool of sera from *S. kali*-allergic patients in immunoblotting (fig. 1b).

Clinical Relevance of Sal k 1

In order to confirm the allergenic significance of the purified Sal k 1, sera from *S. kali*-allergic patients were investigated by the EAST showing that 34 out of 40 tested patients (85%) had Sal k 1-specific IgE (data not shown). The IgE-binding activity of Sal k 1 in the *S. kali* extract was estimated by EAST inhibition. Purified Sal k 1 was able to inhibit the binding of specific IgE to the whole extract, up to 92% (fig. 2). This result demonstrated that Sal k 1 is responsible for most of the IgE-binding activity in *S. kali* extracts and the development of an assay for quantification of this important allergen is of great interest.

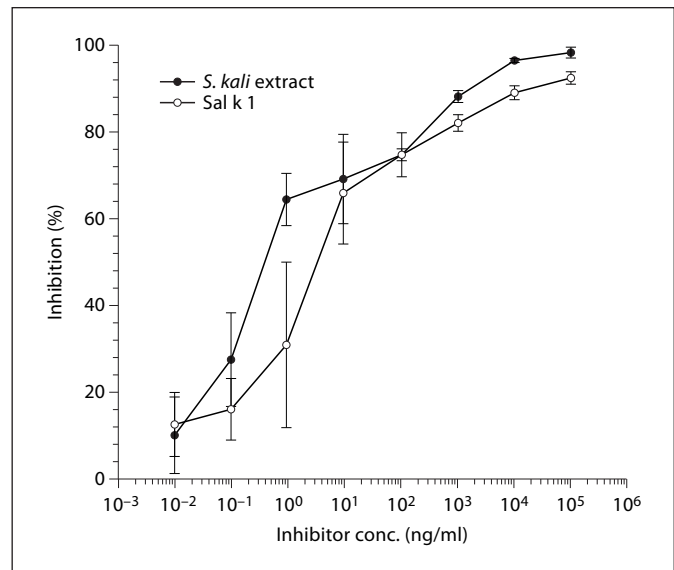


Fig. 2. IgE inhibition curves. The binding of specific human IgE from the serum pool to *S. kali* pollen extract was inhibited using the same extract and the purified Sal k 1. Values represent mean $\pm$ SD (n = 3).

Production and Characterization of Antibodies

After fusion of spleen cells from BALB/c mice immunized with purified Sal k 1 and myeloma cells, 18 hybridomas secreting IgG anti-Sal k 1 monoclonal antibodies were obtained which showed different recognition patterns by immunoblotting against Sal k 1 (data not shown). Monoclonal antibodies were assayed to determine their capacity to capture Sal k 1 in solution when biotin-labeled polyclonal antibodies were used as tracer. The 4C11 monoclonal antibody, IgG1 subtype with *k* light chains, was selected because it rendered the highest optical density at minor Sal k 1 concentrations. Antibody specificities were demonstrated by immunoblotting of *S. kali* crude extract (fig. 1c, d) where both antibodies recognized a unique 40-kDa band in both purified protein and extract samples.

ELISA for Sal k 1 Quantification

A two-site sandwich ELISA to quantify Sal k 1 was developed by using 4C11 monoclonal antibody as capture and biotin-labeled anti-Sal k 1 polyclonal antibodies as tracer. A standard curve was created by using natural purified Sal k 1 in the interval from 80 to 0.08 ng/ml. The assay was dose dependent and the curve revealed a sigmoid shape when plotted on a semi-logarithmic scale (fig. 3a). The correlation was linear between 20 and 1.25

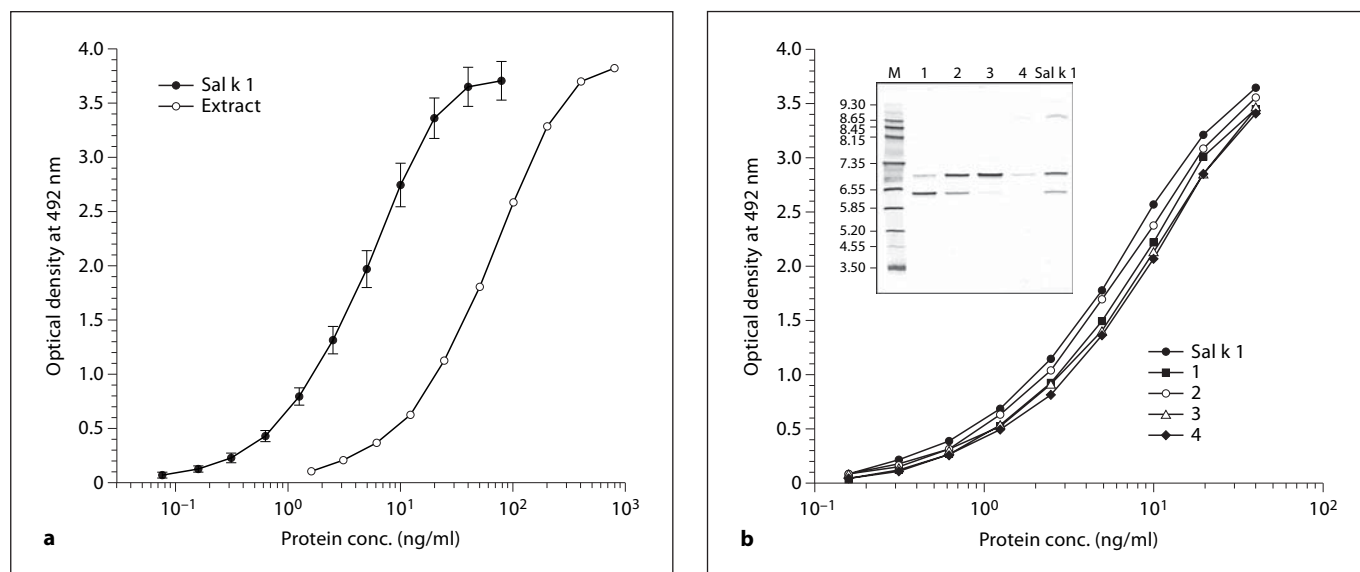


Fig. 3. **a** Dose-response curves in two-site sandwich ELISA of natural purified Sal k 1 and *S. kali* pollen extract. Values represent mean $\pm$ SD ($n = 10$). **b** Dose-response curves of natural purified Sal k 1 and partial purified isoforms shown in the isoelectric focusing gel (inset).

ng/ml with a coefficient of regression of 0.998 and a detection limit of 0.08 ng/ml. The assay was highly reproducible, the interassay coefficient of variation was less than 20% between 80 and 0.16 ng/ml, being 5.4, 7.5, 8.3, 8.9 and 9.9% at 20, 10, 5, 2.5, and 1.25 ng/ml, respectively. The intra-assays coefficients of variation were 1.5, 2.9, 4.9, 3.6 and 4.7% at the same concentrations of the linear portion of the standard curve. A recovery study was made to determine the assay accuracy. For this purpose, known incremental amounts of Sal k 1 were added to a *S. kali* pollen extract and the total Sal k 1 concentration was calculated. The percentage of recovery was $95 \pm 2.9\%$. Several isoforms of Sal k 1 partially purified by ionic exchange chromatography were detected by the described ELISA (fig. 3b).

Interference of the skin prick diagnostic solution components glycerol and phenol on ELISA was tested. No effect of these components was detected at 1% of glycerol and 0.005% of phenol, which corresponded to a dilution of 50 and 100 times, respectively, when according to the sensitivity of the ELISA, dilutions of up to 1:600,000 could be allowed.

The specificity was evaluated against a broad battery of different allergenic extracts from: tree pollen (*Platanus acerifolia*, *Cupressus arizonica*, *Phoenix dactylifera*), grass pollen (*Lolium perenne*, *Phleum pratense*, *Dactylis glomerata*), fruits (*Actinidia chinensis*), weed pollen includ-

ed in different taxonomic families (*C. album*, *Atriplex lentiformis*, *Kochia scoparia*, *Beta vulgaris*, *Amaranthus* sp., *Salicornia* sp., *Parietaria judaica*, *Helianthus annuus*, *Artemisia vulgaris*, *Plantago lanceolata*), and nonplant extracts (*Alternaria alternata*, *Dermatophagoides pteronyssinus*, *Anisakis simplex*). In all cases, except in the members of the Amaranthaceae family, the optical density was lower than the detection limit at 8 μ g/ml of extract protein, a concentration 10-fold higher than the highest assayed with *S. kali* extracts. Among the Amaranthaceae family, *C. album*, *A. lentiformis*, *B. vulgaris*, and *Amaranthus* sp. pollen extracts gave an optical density lower than the detection limit at 0.8 μ g/ml. On the other hand, pollen extracts from other Amaranthaceae family members such as *Salicornia* sp. and *K. scoparia* contained 0.6 and 4.1 μ g of Sal k 1-like proteins per milligram of extract, respectively. In direct ELISA, specific polyclonal antibodies reacted with proteins in all Amaranthaceae extracts, but 4C11 monoclonal antibody recognized proteins only in *S. kali*, *Salicornia* sp. and *K. scoparia* extracts. Polyclonal antibodies and the pool of sera from *S. kali*-allergic patients detected proteins around to 40-kDa in the Amaranthaceae extracts, but the recognition was weak in the *Chenopodium* pollen, so this protein is different or it is in minor proportion in the extract (fig. 4).

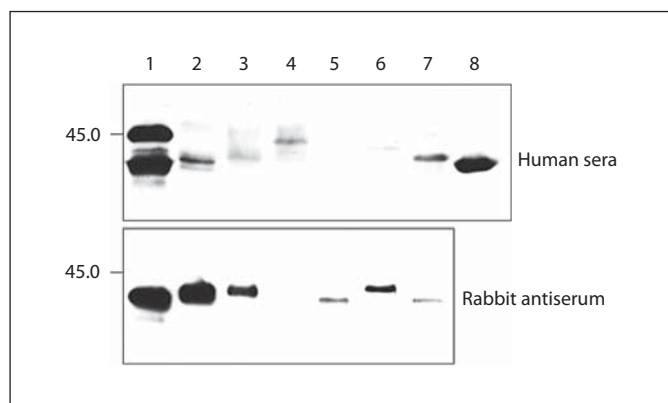


Fig. 4. Immunoblot of pollen extracts from different Amaranthaceae incubated with a pool of sera from *S. kali*-allergic patients and anti-Sal k 1 rabbit polyclonal antibodies. Pollen extracts: *S. kali* (lane 1), *K. scoparia* (lane 2), *Salicornia* sp. (lane 3), *C. album* (lane 4), *B. vulgaris* (lane 5), *A. lentiformis* (lane 6), and *Amaranthus* sp. (lane 7). As reference, purified Sal k 1 was loaded in lane 8.

Quantification of Sal k 1 in Pollen Extracts and Correlation with IgE-Binding Activity

The described ELISA was applied to assess Sal k 1 content in 5 *S. kali* pollen extracts. The curve obtained showed good parallelism with the standard curve (fig. 3), an indication that the assay was measuring the same molecule in the different preparations and that the allergen had not been altered during the purification process. The concentration of Sal k 1 ranged from 22.5 to 28.7 $\mu\text{g}/\text{mg}$ with an average of 25.3 $\mu\text{g}/\text{mg}$. Three Russian thistle pollen extracts were subjected to 1–3 immunoprecipitation rounds and partially Sal k 1-depleted extracts with different Sal k 1 content were obtained. In some cases, the amount of allergen after depletion was reduced more than 99%. Immunoblotting assays showed a proportional decrease in the IgE reactivity to the Sal k 1 band in the depleted extracts (data not shown). The Sal k 1 concentration calculated in the depleted and nondepleted extracts had a significant correlation (Spearman's $\rho = 0.92$) to the IgE-binding activity of the extracts, determined by EAST inhibition (fig. 5), demonstrating that the Sal k 1 content is highly representative of the overall allergenic activity of the whole *S. kali* extract.

Quantification of Sal k 1 in Pollen Grains

To determine whether the Sal k 1 ELISA could be used for detection of airborne *S. kali* pollen, decreasing amounts of this pollen were extracted and the superna-

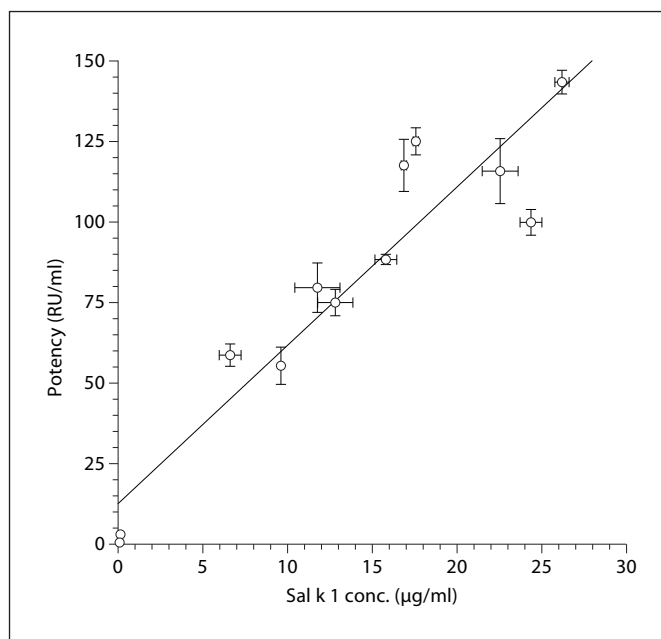


Fig. 5. Correlation between IgE-binding activity values determined by EAST inhibition and Sal k 1 content measured by the two-site ELISA in 12 *S. kali* pollen extracts. Values represent mean $\pm$ SD ($n = 3$).

Table 1. Average values (mean $\pm$ SD) of Sal k 1 released from *S. kali* pollen grains using the developed Sal k 1 ELISA

Pollen grains	Sal k 1, ng
2	0.099 $\pm$ 0.015
9	0.172 $\pm$ 0.001
34	0.661 $\pm$ 0.065
136	2.267 $\pm$ 0.026
680	2.811 $\pm$ 0.058
3,400	3.027 $\pm$ 0.039
17,000	2.872 $\pm$ 0.020

Values in italics are those that fit within the lineal range of the assay.

tants analyzed. We estimated, using a Neubauer counting chamber, that 1 mg of *S. kali* pollen contains an average of 170,000 pollen grains. Taking into account these data, the Sal k 1 ELISA was able to detect the allergen released from 2 pollen grains, showing that this assay is sensitive enough for detection of airborne Sal k 1 (table 1).

Discussion

Crude allergen extracts are currently used for allergy diagnosis and immunotherapy although their allergen composition and allergenic potency may vary. This is likely due to the variability of the natural source materials, which in the case of pollens may depend on variety differences or environmental conditions. The prevalence of Russian thistle allergy has a special relevance in countries with a desert climate, high temperatures and a dry soil [5] and has been increased lately due to the advance of desertification of the south of Europe. Sal k 1 shows a reaction in most of *S. kali*-allergic patients [this work; 9, 10] and highly contributes to the total allergenic activity of *S. kali* extracts since it is responsible for 92% IgE-binding activity of the whole extract. In addition, the good correlation obtained between the allergen content and the total IgE-binding activity supports that Sal k 1 is a very relevant allergen of Russian thistle pollen extracts. These results demonstrated that the present immunoassay could be useful for standardization of *S. kali* pollen allergenic extracts intended for clinical use.

In the present study, a solid-phase ELISA method was set up to measure the major allergen, Sal k 1, from *S. kali* pollen. The assay is highly reproducible, as demonstrated by the low intra- and interassay coefficients of variation obtained (<5% and <10%, respectively), and valid to detect several isoforms of Sal k 1. Additionally, the described assay is highly specific within the Amaranthaceae family since it enables the detection of Sal k 1-like proteins in *Salicornia* and *Kochia* pollen extracts. Therefore, it would be a useful tool for the allergen quantification of these 2 pollen extracts. Nevertheless, the corresponding purified allergen for each extract should be used for an accurate quantification of the allergen content expressed in mass units.

The specific ELISA described here could also be applicable for the routine measurement of Sal k 1 content in production batches of skin prick test diagnostic solutions since prick test components had no effect on the assay. Quantification of major allergens is needed for optimal specific immunotherapy as low-dose immunotherapy is usually ineffective, and high doses may induce an unacceptable rate of systemic reactions [11]. There is preliminary evidence for immunotherapy with ragweed, grass, cat, mite, and venom allergens, since a maintenance dose of 5–20 µg of major allergens per injection is associated with a significant improvement in patient symptom records [11, 12]. The average Sal k 1 content in our manufactured immunotherapy maintenance doses is 15 µg,

which is in accordance with these data. A successful double-blind, placebo-controlled study with a modified vaccine of *S. kali* has recently been reported, where a maintenance dose containing 43 µg Sal k 1 (evaluated with densitometry) was used [23]. Nevertheless, more clinical trials using specific immunotherapy with allergen-quantified extracts should be performed in order to correlate immunotherapy efficacy and major allergen content.

In patients with pollinosis, allergic symptoms are often correlated with the number of airborne pollen grains, although this correlation is not always so close. A lag period between total Amaranthaceae pollen counts and clinical symptoms in *S. kali* monosensitized patients has been described [24]. Several factors may have influenced the results, especially the fact that in all aerobiological surveys, pollens of this family seem to be indistinguishable from a morphological point of view and therefore are grouped together. Although variable degrees of cross-reactivity have been suggested among members of this family [6, 25], the allergenicity of many of the species has not been evaluated. Sal k 1-like protein is not present in *Chenopodium* pollen extract which restates the allergenic marker characteristic of Sal k 1 as previously suggested [10] and shows that the high cross-reactivity observed between *Chenopodium* and *Salsola* is due to other allergens. Therefore, Amaranthaceae pollen counts may not reflect the exact pollen season when *S. kali* produces clinical symptoms. The direct measurement of the concentration of aeroallergens has only recently been introduced, and is an important advance in public health information systems [19, 26]. The high sensitivity of the developed ELISA would allow the quantification of Sal k 1 levels in collected samples of airborne pollen and correlate them with clinical symptoms.

In conclusion, for the first time, an ELISA procedure has been described for the quantification of the major and important allergen from Russian thistle pollen. The assay is highly sensitive and specific for this allergen and could be very useful for aeroallergen control and for quality control of Russian thistle pollen extract preparations intended for clinical use.

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