

Hypersensitivity to black locust (*Robinia pseudoacacia*) pollen: “allergy mirages”

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Background: The allergenicity of the ornamental tree *Robinia pseudoacacia*, or black locust, is unknown.

Objective: To evaluate the prevalence of sensitization to *R pseudoacacia* pollen, its possible allergenic cross-reactivity with other common pollens, and the potential implication of pollen panallergens (profilin, polcalcin, and 1,3- β -glucanase) as a cause of sensitization to *R pseudoacacia* pollen.

Methods: Skin prick testing with *R pseudoacacia* pollen was performed in 149 patients with pollinosis. Nasal challenge with *R pseudoacacia* pollen was performed in 10 patients. The prevalence of sensitization to the recombinant forms of profilin (rChe a 2), polcalcin (rChe a 3), and the N-terminal of the 1,3- β -glucanase (rNtD of Ole e 9) was investigated. Immunoblotting, enzyme-linked immunosorbent assay, and competitive inhibition assays were performed with *R pseudoacacia* pollen and recombinant pollen allergens.

Results: Sixty-four patients (43%) had positive skin prick test reactions to *R pseudoacacia* pollen. Nasal challenge results were positive in 5 sensitized patients and negative in 4 controls and 1 sensitized patient. The allergenic profile of *R pseudoacacia* pollen comprises at least the panallergen profilin, a calcium-binding protein, and a 1,3- β -glucanase. The prevalence of sensitization to rChe a 2, rChe a 3, and rNtD of Ole e 9 was 60%, 33%, and 87%, respectively, among patients sensitized to *R pseudoacacia* pollen. Binding of IgE to *R pseudoacacia* extract was completely inhibited by *Robinia*, *Chenopodium*, *Olea*, *Cupressus*, and *Lolium* extracts.

Conclusions: The high prevalence of *R pseudoacacia* pollen sensitization in patients with pollinosis is likely to be due to cross-sensitization to panallergens (profilin, polcalcin, and 1,3- β -glucanase) from other common pollens. This phenomenon may lead to a diagnosis of “allergy mirages.”

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INTRODUCTION

Black locust, or *Robinia pseudoacacia*, is a colorful and robust tree of the Leguminosae family. It originated in North America and was introduced in Europe through Paris in 1601 by Jean Robin. Thereafter, it was brought to Spain, and now it is widely used as an ornamental tree. In Madrid, there are 22,873 black locust trees (10.6% of the tree population, according to the Parks and Forest Department of the City Hall). Because *R pseudoacacia* is mainly pollinated by insects, low concentrations of pollen grains are detected in Madrid during its flowering period (from April to July). The pollen is tricolporate, isopolar, angulaperturate, and medium sized (polar diameter = 27–34 μ m, equatorial diameter = 24–30 μ m).¹

Robinia pseudoacacia pollen was considered allergenic by Lewis and Vinay in 1979.² Two recent studies from the Iberian peninsula reported a prevalence of sensitization to this pollen, as determined by skin prick tests (SPTs), of 49% in patients with

pollinosis³ and 12% in a pediatric population.⁴ The characterization of allergens from *R pseudoacacia* has received little attention owing to the low pollen counts and its concomitance with other relevant pollens. It is unknown whether sensitization to *R pseudoacacia* pollen is a primary cause of allergy or whether it is merely due to clinically irrelevant allergenic cross-sensitization.

IgE cross-reactivity mainly arises from the presence of panallergens in different allergen sources, including the role of profilin, polcalcin, and 1,3- β -glucanase in pollen cross-sensitization.^{5–7} Each of these families of proteins possesses highly conserved amino acid sequences, thus explaining their involvement in this phenomenon.^{8–11} Ascertaining the clinical relevance of sensitization to new pollen sources that share allergens with taxonomically related or nonrelated pollens is necessary to make an accurate diagnosis and for possible specific immunotherapy.¹² We evaluated sensitization to the panallergens profilin and polcalcin from chenopod pollen and to 1,3- β -glucanase from olive pollen and its clinical significance in patients with positive SPT reactions to *R pseudoacacia* pollen.

METHODS

Patient Population

A total of 149 consecutive patients with seasonal allergic rhinoconjunctivitis, with or without asthma, were evaluated in the outpatient clinic of Fundación Jiménez Díaz in 2003.

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All these patients experienced allergic symptoms during springtime. Written informed consent was obtained from the study participants.

Pollen Counts

Robinia pseudoacacia pollen counts were obtained daily for 3 consecutive years (2001–2003) from the Spanish aerobiologic monitoring network, which has 3 stations in Madrid.

Skin Prick Tests

Skin prick tests were performed with a battery of aeroallergens, including common environmental pollens (*Cupressus arizonica*, *Lolium perenne*, *Platanus acerifolia*, *Olea europaea*, *Salsola kali*, *Plantago lanceolata*, *Artemisia vulgaris*, and *Parietaria judaica*) and *R pseudoacacia* pollen extract at 5% wt/vol (ALK-Abelló, Madrid, Spain). The SPTs were performed on the patient's forearm using a standardized lancet (ALK Lancet; ALK-Abelló, Hørsholm, Denmark). A 50% glycerin/saline solution and hydrochloride histamine solution at 10 mg/mL were used as negative and positive controls, respectively. The response was read 15 minutes after puncture, and the results are expressed as the mean wheal diameter in millimeters. A SPT reaction was considered positive when the wheal diameter was at least 3 mm greater than the negative control.

Nasal Challenge With R pseudoacacia Pollen

Specific nasal challenges were performed in 6 patients with positive SPT reactions to *R pseudoacacia* and in 4 patients with pollinosis and negative SPT reactions to *R pseudoacacia*. Nasal corticosteroids and antihistamines were withdrawn at least 3 weeks and 4 days, respectively, before the start of the study. Before starting the nasal challenges, patients waited for 30 minutes to allow the nasal mucosa to acclimatize to local conditions. Nasal challenges were performed first with isotonic sodium chloride solution and 15 minutes later with *R pseudoacacia* aqueous extract (10 mg/mL) by means of a nasal pump spray delivering a fixed dose of 0.125 mL of solution. The nasal response was monitored using the number of sneezes and acoustic rhinometry (SRE 2100; RhinoMetrics, Assens, Denmark) 15 minutes after each challenge. Minimal cross-sectional area measured at the head of the inferior turbinate was used as the end point. Averages of the before and after duplicate measures were used for analysis. A decrease in minimal cross-sectional area of 30% or more from baseline and 5 or more sneezes in 15 minutes was considered a positive test response.¹³

Human Serum Samples and Polyclonal and Monoclonal Antibodies

Serum samples from 15 untreated donors with rhinoconjunctivitis and with positive serum specific IgE levels and positive SPT responses to *R pseudoacacia* pollen extract were used. A serum sample from a nonatopic individual was used as a negative control. Polyclonal antisera against profilin (Ole e 2) and polcalcin (Ole e 3) from olive pollen were prepared by

immunizing New Zealand rabbits. Monoclonal antibody against 1,3- β -glucanase was donated by ALK-Abelló.

Pollen Extract and Protein Purification

Robinia pollen extract was prepared by means of saline extraction,¹⁴ and the protein concentration was determined according to the method of Lowry et al.¹⁵ rChe a 2 was recombinantly produced in *Escherichia coli* as described elsewhere.¹⁰ The protocol for rChe a 3 expression was the same as that for Ole e 3.¹¹ The N-terminal domain of the 1,3- β -glucanase from olive pollen (rNtD Ole e 9) was obtained by means of recombinant expression in *Pichia pastoris*, as reported previously.⁷

Molecular Characterization

Protein concentration and amino acid composition of purified proteins were determined by acid hydrolysis.¹⁶ Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed using 15% (wt/vol) polyacrylamide gels.¹⁷ Molecular mass determinations were performed using protein markers (MW-SDS-70L; Sigma-Aldrich Química, Madrid, Spain). The N-terminal amino acid sequence was performed by means of Edman degradation on a 494 sequencer (Applied Biosystems, Foster City, CA). Mass spectrometry analyses of proteins (1 μ g) were performed in a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (Bruker Reflex II; Bruker-Franzen Analytik, Bremen, Germany).¹⁸

Immunologic Assays

Proteins separated by SDS-PAGE were transferred to nitrocellulose membranes and were immunodetected by using individual serum samples ($n = 15$) from patients with positive SPT reactions to *R pseudoacacia* pollen (diluted 1:10) or olive profilin specific polyclonal antiserum (diluted 1:10,000), olive polcalcin specific polyclonal antiserum (diluted 1:5,000), and 1,3- β -glucanase monoclonal antibody (diluted 1:1,000). A mouse monoclonal anti-human IgE antibody (ALK-Abelló) (diluted 1:5,000) and a horseradish peroxidase-labeled goat anti-mouse IgG (Pierce Chemical Co, Rockford, IL) (diluted 1:5,000) were used to detect bound IgE antibodies. Alternatively, for the detection of *R pseudoacacia* protein specific IgG binding, horseradish peroxidase-labeled goat anti-rabbit IgG (BioRad, Richmond, CA) (diluted 1:3,000) and anti-mouse IgG (diluted 1:5,000) were used as second antibodies. The signal was developed by means of enhanced chemiluminescence Western blotting reagent (Amersham Biosciences, Barcelona, Spain).¹⁹

Immunoblotting inhibition of IgE binding to *R pseudoacacia* pollen extract (40 μ g of total protein) using a pool of 4 allergic serum samples (diluted 1:10), which were previously incubated with 2 μ g of profilin from chenopod pollen or with 500 μ g of pollen extracts from *O europaea*, *Chenopodium album*, *C arizonica*, and *L perenne*, or bovine serum albumin as a negative control was performed as described previously.¹⁰ Quantification of IgE levels in the serum samples from allergic patients was performed by means of enzyme-linked immunosor-

bent assay (ELISA). Plates were coated with *R pseudoacacia* extract (20 µg) or purified proteins (0.1 µg) and incubated with individual allergic serum samples (diluted 1:10) for 2 hours. Binding of IgE was detected by means of the antibodies described previously herein. Peroxidase reaction was developed with *o*-phenylenediamine reagent.²⁰ All the determinations were performed in duplicate. Optical density at 492 nm values less than 0.1 were considered negative responses.

RESULTS

Pollen Counts

Robinia pseudoacacia pollen is present in the atmosphere of Madrid from April to June. The median daily count during this season in 2003 was 0 grains/m³ (range, 0–33 grains/m³), and total annual counts ranged from 2 to 142 grains/m³ in the different stations in Madrid. Similarly low pollen counts were measured in 2001 and 2002. During these months, the most frequent pollen types collected were grass and olive pollens.

SPT Results

Sixty-four (43%; 95% confidence interval, 35%–51%) of the 149 patients had a positive SPT reaction to *R pseudoacacia*. No patients were monosensitized to this pollen. Patients with positive SPT responses to *R pseudoacacia* showed a higher prevalence of sensitization to other pollens than those with negative SPT responses to *R pseudoacacia*: *L perenne*, 95% vs 83% ($P < .01$); *O europaea*, 92% vs 73% ($P < .001$); *P acerifolia*, 88% vs 42% ($P < .001$); *C arizonica*, 78% vs 63% ($P < .01$); *S kali*, 83% vs 36% ($P < .001$); *P lanceolata*, 75% vs 38% ($P < .001$); *A vulgaris*, 57% vs 19% ($P < .001$); and *P judaica*, 42% vs 7% ($P < .001$).

Nasal Challenge With *R pseudoacacia* Pollen

Five (83%) of 6 patients with positive SPT reactions to *R pseudoacacia* had a positive nasal challenge result with *R pseudoacacia* pollen extract at 10 mg/mL (Table 1). The 4 patients with negative SPT reactions to *R pseudoacacia* pollen had a negative response to nasal challenge testing.

Recognition Patterns of *R pseudoacacia*–Allergic Serum Samples in Robinia Pollen Extract

Robinia specific IgE reactivity was measured by means of ELISA using serum samples from 15 patients with positive SPT reactions to *R pseudoacacia* pollen extract; IgE reactivity was positive in 9 of the samples (60%) (Table 1). These serum samples were assayed for their IgE-binding capability to *R pseudoacacia* extract in immunoblotting after SDS-PAGE (Fig 1). Most of the serum samples recognized bands of 10 to 15 kDa, whose electrophoretic mobility suggests that they may correspond to profilin or polcalcin. Furthermore, IgE-binding bands of high molecular mass suggest the presence of allergens such as 1,3-β-glucanase (32–46 kDa), which is present in different pollens and vegetables.

Presence of Homologous Allergens From Different Sources in Robinia Pollen

Robinia pseudoacacia pollen extract (40 µg per lane) was applied in 15% polyacrylamide gel, and SDS-PAGE was performed. The gel was stained with Coomassie brilliant blue to visualize proteins (Fig 2A). Alternatively, electrophoresed samples were blotted to membranes and immunostained with Ole e 2 and Ole e 3 specific polyclonal antisera and 1,3-β-glucanase specific monoclonal antibody. The presence of

Table 1. Clinical Characteristics and Results of Specific IgE and Challenge Tests

Participant No.	SPT response to common aeroallergens	Seasonal symptoms	Nasal challenge with <i>Robinia pseudoacacia</i> pollen	Specific IgE, OD			
				To <i>Robinia</i> pollen	To rChe a 2	To rChe a 3	To rNtD of Ole e 9
1	C, P, O, G, D	RC/A	ND	0.03	0.14	0	0.01
2	C, P, O, G, D, HDM	RC/A	ND	1.13	0.74	0	0.27
3	C, P, O, G, D	RC/A	–	0.28	0	3.23	0.39
4	P, O, G, D, M	RC	ND	0.13	0.29	0	0.19
5	C, P, O, G, W	RC/A	+	0.63	1.79	1.08	0.08
6	C, O, G, M, Co, D	RC	ND	0.05	0.16	0	0.37
7	C, P, O, G, D, M	RC/A	ND	0.15	0.23	0	0.19
8	P, O, G, W	RC	ND	0.02	0	1.69	0.34
9	P, O, G, D	RC/A	ND	0.21	0	0	0.21
10	C, G, W, D	RC	+	0.06	0.22	0	0.83
11	G	RC	ND	0.06	0.21	1.11	0.27
12	P, O, G, HDM, D	RC/A	ND	0.23	0.23	0	0.13
13	P, O, G	RC	+	0	0	0	0.25
14	C, P, O, G, W, D	RC/A	+	0.29	0	1.89	0.29
15	P, O, G, D	RC	+	0.30	0	0	0.30
16	Control subject	–	–	0	0	0	0

Abbreviations: A, asthma; C, cypress pollen; Co, cockroach; D, cat or dog dander; G, grass pollen; HDM, house dust mites; M, moulds; ND, not determined; O, olive pollen; OD, optical density; P, plane tree pollen; RC, rhinoconjunctivitis; SPT, skin prick test; W, weed pollen; +, positive; –, negative.

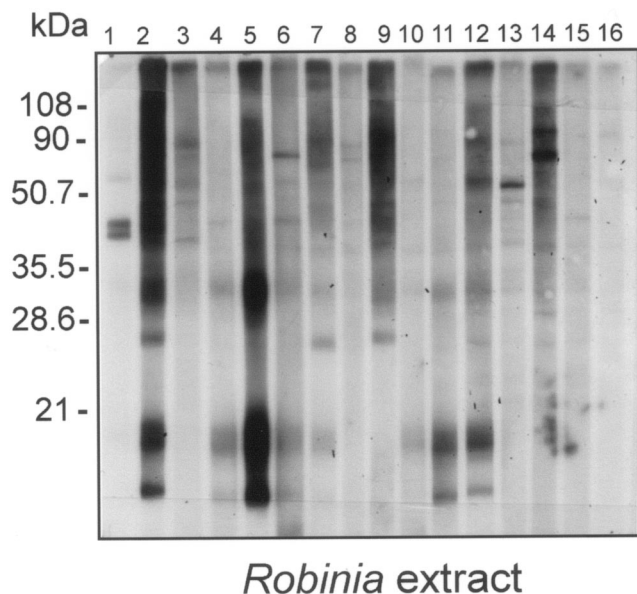


Figure 1. Analysis of IgE reactivity for 15 serum samples from patients with *Robinia* sensitization (lanes 1–15) and a nonallergic serum sample (lane 16).

profilin, 1,3- β -glucanase, and polcalcin was demonstrated using specific antibodies (Fig 2B).

Significance of Profilin, Polcalcin, and 1,3- β -Glucanase in the IgE Response to *R pseudoacacia* Pollen

Quantification of the IgE response of the 15 serum samples from *R pseudoacacia*-allergic patients was performed by means of ELISA against purified rChe a 2, rChe a 3, and rNtD of Ole e 9. A serum sample from a nonatopic individual was used as a negative control, and *R pseudoacacia* pollen extract-coated wells were used as positive controls. Nine serum samples (60%) had a positive response to rChe a 2, 5 (33%) to rChe a 3, and 13 (87%) to the rNtD of Ole e 9 (Table 1).

IgE Binding to Robinia Pollen Mainly Due to Cross-reactive Allergens

Immunoblotting inhibition assays of IgE binding to *R pseudoacacia* pollen extract was performed with an equivolumetric pool of 5 serum samples and using rChe a 2 (2 μ g) and *Lolium*, *Olea*, *Robinia*, *Cupressus*, and *Chenopodium* pollen extracts (500 μ g) as inhibitors (Fig 3A). Phosphate-buffered saline was used as a negative control of inhibition. Preincubation of the pool of serum samples with rChe a 2 totally inhibited IgE binding to *R pseudoacacia* profilin. With the different pollen extracts used as inhibitors, IgE binding to *R pseudoacacia* extract was completely removed. On the other hand, preincubation of the same pool of serum samples with *R pseudoacacia* extract (500 μ g) only eliminated the positive bands corresponding to profilin and a band of 45 kDa (that likely would correspond to 1,3- β -glucanase) in the olive pollen, as well as most of the bands in the *Chenopodium*

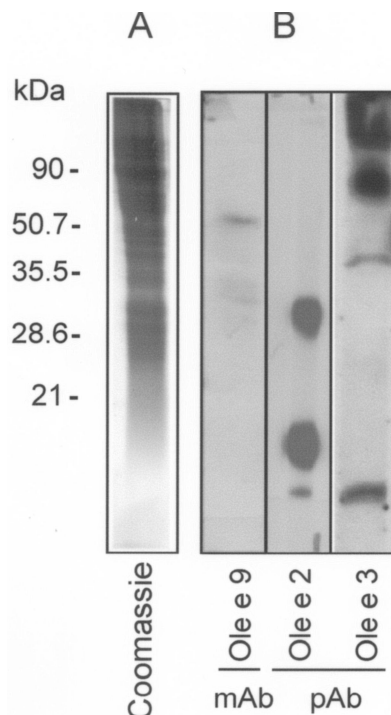


Figure 2. A, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie blue staining of *Robinia pseudoacacia* extract. B, Immunoblotting analysis of IgG reactivity of *Robinia* extract (40 μ g of total protein) against the N-terminal of Ole e 9 (1,3- β -glucanase) specific monoclonal antibody (mAb) and Ole e 2 (olive profilin) and Ole e 3 (olive polcalcin) specific polyclonal antisera (pAb).

pollen extract (Fig 3B). No IgE-binding inhibition was detected in either *Lolium* or *Cupressus* extracts.

DISCUSSION

Although *R pseudoacacia* is a common tree in Madrid, the levels of pollen collected in different stations in the city varied from null to very low. The *R pseudoacacia* flower, similar to other members of the Leguminosae family, is papilionaceous, with the anthers tightly enclosed by petals, releasing few grains directly into the atmosphere. We found a high prevalence of SPT positivity to *R pseudoacacia* pollen in patients with pollinosis despite the low counts of this pollen found in Madrid. To explain this finding, it could be argued that *R pseudoacacia* pollen is very allergenic or, more likely, that it might be due to cross-sensitization to panallergens from other common pollens. We demonstrated the second hypothesis by means of ELISA and immunoblotting inhibition experiments.

Lewis and Vinay² investigated North American pollinosis due to insect-pollinated plants, and they suggested that *R pseudoacacia* has moderate allergenicity. We show that nasal challenge with *R pseudoacacia* pollen extract may give rise to positive responses, but there is no proof that this pollen elicits symptoms during natural pollen exposure. It has been shown

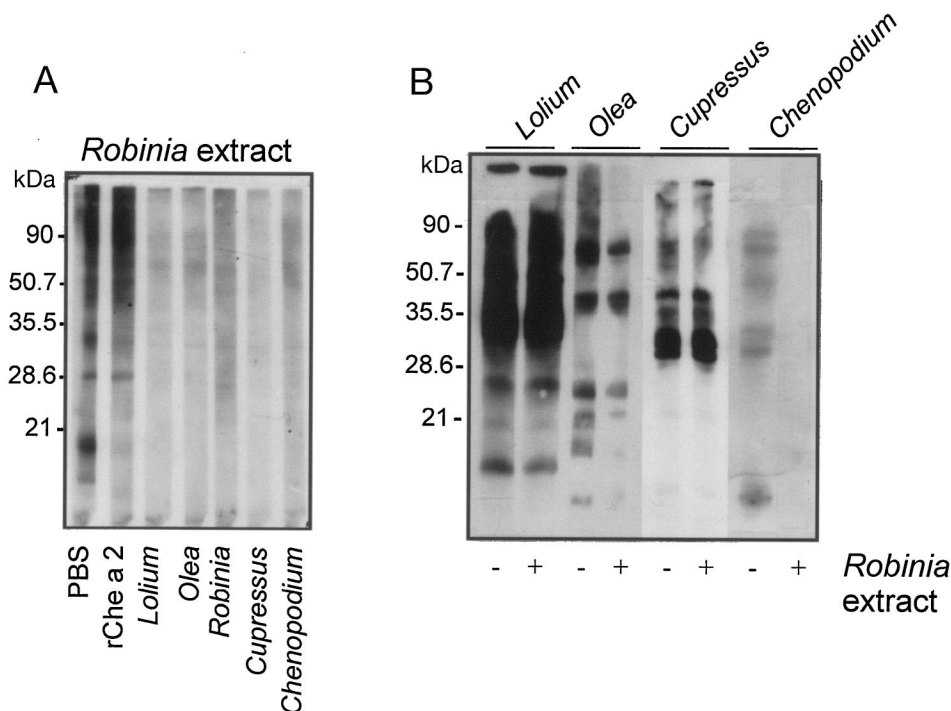


Figure 3. A, Immunoblotting inhibition assays with *Robinia pseudoacacia* extract using a pool of serum samples sensitized to *Robinia* preincubated with 2 μ g of rChe a 2 and 500 μ g of rye grass (*Lolium*), olive (*Olea*), black locust (*Robinia*), cypress (*Cupressus*), and chenopod (*Chenopodium*) pollen extracts as inhibitors and phosphate-buffered saline (PBS) as a negative control. B, Immunoblotting inhibition assays with *Lolium*, *Olea*, *Cupressus*, and *Chenopodium* using a pool of serum samples sensitized to *Robinia* preincubated with *R pseudoacacia* extract as inhibitor (lanes +) and PBS as a negative control (lanes -).

that provocation challenge with water-soluble allergen extracts can elicit positive asthmatic,^{21,22} nasal,²³ and conjunctival²⁴ responses in sensitized individuals who have never experienced spontaneous symptoms of asthma, rhinitis, or conjunctivitis.

It has been hypothesized that profilin, polcalcin, and, more recently, 1,3- β -glucanase may play a significant role as panallergens causing sensitization to taxonomically different pollens.^{7,11,25} Che a 2 shows high allergenic cross-reactivity with profilins from other sources, and it is a good marker for profilin sensitization.¹⁰ Studies on the allergenicity of pollen profilins showed prevalence rates of sensitization to these proteins of approximately 20% to 30% in patients with pollinosis^{26–30} and revealed the importance of profilin in pollen-food cross-sensitization.^{5,31–33} We had almost complete abolition of IgE binding of allergic serum to *R pseudoacacia* profilin with rChe a 2 as inhibitor. These data indicate that rChe a 2 shares most of the IgE epitopes with the *R pseudoacacia* profilin and suggest an important role for this allergen in cross-reactivity between these pollens. We also found a high prevalence of sensitization (33%) to polcalcin rChe a 3 in patients with positive SPT reactions to *R pseudoacacia*. Members of the polcalcin family have been identified as relevant allergens (Aln g 4 and Ole e 3), and the prevalence of sensitization to them is approximately 17% to 35% in patients with pollinosis.^{18,34} The fact that the signal of

Ole e 3 specific polyclonal antibody recognizes so slightly the *R pseudoacacia* polcalcin and 1,3- β -glucanase could be attributed to the low levels of these allergens in the *R pseudoacacia* pollen.

The enzyme 1,3- β -glucanase is also a relevant plant panallergen.³⁵ Despite the low level of this protein in the extracts observed in the immunoblotting with NtD specific monoclonal antibody in Figure 2B, a high prevalence is obtained when the purified protein is tested with serum samples from patients with positive SPT responses to *R pseudoacacia*, although with low levels of specific IgE in most cases.

The fact that several grass, tree, and weed common pollens completely abolished IgE binding to *R pseudoacacia* pollen extract indicates that panallergens from these pollens play a significant role in the positivity of SPTs to *R pseudoacacia*. Contrarily, most of the IgE reactivity remains after inhibition of these common pollen extracts with *R pseudoacacia* extract, with only a few IgE-binding bands in the olive pollen extract, whose molecular masses could correspond to profilin and 1,3- β -glucanase, being inhibited. Not surprisingly, IgE binding to *Chenopodium* extract, which in Madrid is considered an allergenic pollen mainly owing to cross-sensitization, was completely inhibited by *R pseudoacacia* pollen.

The fact that panallergens share structural and immunologic properties leads us to suggest the use of representative members of such families, which carry most of the significant

cross-reactive epitopes, as “marker allergens” of polysensitization, which may or may not have clinical relevance. In the latter scenario, panallergens may simply act as confounding factors in the diagnosis of allergy. Profilin and polcalcin from chenopod and 1,3- β -glucanase from olive pollen can be considered representative members of these panallergen families and, therefore, are suitable candidates to evaluate broad cross-sensitization to pollen allergens.

Allergy tests that detect specific IgE using “whole” allergen extracts at best allow the identification of allergen-containing sources. Sensitization to panallergens further complicates the clinical diagnosis of allergy because these allergens can be present at different sources. The classic approach to allergy diagnosis using SPT (or radioallergosorbent test) with allergen extracts may lead to the diagnosis of “allergy mirages” that do not represent true sensitizations, as likely happens with *R pseudoacacia* pollen, at least in our geographic area. However, *R pseudoacacia* can still have allergenic potential because it contains cross-reactive allergens with well-established allergen sources, and exposure may be the key determinant factor to trespass the threshold of clinical relevance.

Provocation challenge tests in the mucous membranes have been considered definitive proof of relevant allergy sensitization.³⁶ However, it is now clear that these tests may also have false-positive results when high concentrations of the allergen extracts are applied to sensitized individuals who do not show allergic symptoms during natural allergen exposure.^{21,22} These facts raise the issue of the sensitization to some allergens (eg, with low specificity to bind IgE, panallergens, or cross-reactive carbohydrate determinants) that may mimic true allergy in the diagnostic tests.^{37,38} Component-resolved diagnosis using purified or recombinant allergens³⁹ or reciprocal inhibition assays¹³ may help identify the patient’s allergen sensitization profile and aid in distinguishing true or primary sensitizations with clinical significance from those situations in which “allergy is not always allergy” owing to clinically irrelevant IgE cross-sensitization.

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REFERENCES

- Subiza J, Jerez J, Jiménez JA, et al. Allergenic pollen and pollinosis in Madrid. *J Allergy Clin Immunol*. 2003;112:229–239.
- Lewis WH, Vinay P. North American pollinosis due to insect-pollinated plants. *Ann Allergy*. 1979;42:309–318.
- Cosmes Martin PM, Moreno Ancillo A, Dominguez Noche C, et al. Sensitization to *Castanea sativa* pollen and pollinosis in northern Extremadura (Spain). *Allergol Immunopathol*. 2005;33:145–150.
- Loureiro G, Rabaca M, Blanco B, et al. Aeroallergens sensitization in an allergic paediatric population of Cova da Beira, Portugal. *Allergol Immunopathol*. 2005;33:192–198.
- van Ree R, Voitenko V, Van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol*. 1992;98:97–104.
- Valenta R, Duchene M, Vrtala S, et al. Calcium-binding allergens: from plants to man. *Int Arch Allergy Immunol*. 1998;117:160–166.
- Palomares O, Villalba M, Quiralte J, et al. 1,3- β -glucanases as candidates in latex-pollen-vegetable food cross-reactivity. *Clin Exp Allergy*. 2005;35:345–351.
- Ebner C, Hirschwehr R, Bauer L, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol*. 1995;95:962–969.
- Huecas S, Villalba M, Rodríguez R. Ole e 9, a major olive pollen allergen is a 1,3- β -glucanase: isolation, characterization, amino acid sequence, and tissue specificity. *J Biol Chem*. 2001;276:27959–27966.
- Barderas R, Villalba M, Rodríguez R. Recombinant expression, purification and cross-reactivity of chenopod profilin: rChe a 2 as a good marker for profilin sensitization. *Biol Chem*. 2004;385:731–737.
- Ledesma A, Villalba M, Batanero E, Rodríguez R. Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca²⁺-binding proteins (polcalcins) involved in allergy. *Eur J Biochem*. 1998;258:454–459.
- Kazemi-Shirazi L, Niederberger V, Linhart B, et al. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. *Int Arch Allergy Immunol*. 2002;127:259–268.
- Sastre J, Lluich-Bernal M, Bustillo AM, et al. Allergenicity and cross-reactivity of Russian olive pollen (*Eleagnus angustifolia*). *Allergy*. 2004;59:1181–1186.
- González E, Villalba M, Rodríguez R. Allergenic cross-reactivity of olive pollen. *Allergy*. 2000;55:658–663.
- Lowry O, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193:265–275.
- Barderas R, Villalba M, Pascual CY, et al. Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of *Chenopodium album* pollen: isolation, amino acid sequences, and immunologic properties. *J Allergy Clin Immunol*. 2004;113:1192–1198.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227:680–685.
- Ledesma A, Rodríguez R, Villalba M. Olive pollen profilin: molecular and immunologic properties. *Allergy*. 1998;53:520–526.
- Huecas S, Villalba M, González E, et al. Production and detailed characterization of biologically active olive pollen allergen Ole e 1 secreted by the yeast *Pichia pastoris*. *Eur J Biochem*. 1999;261:539–546.
- Batanero E, Villalba M, Ledesma A, et al. Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. *Eur J Biochem*. 1996;241:772–778.
- Ahmed T, Fernández RJ, Wanner A. Airway responses to antigen challenge in allergic rhinitis and allergic asthma. *J Allergy Clin Immunol*. 1981;67:135–145.
- Chang JH, Chan H, Quirce S, et al. In vitro T-lymphocyte response and house dust mite-induced bronchoconstriction. *J Allergy Clin Immunol*. 1996;98:922–931.
- Bodtger U, Poulsen LK, Malling HJ. Asymptomatic skin sensitization to birch predicts later development of birch pollen

-
- allergy in adults: a 3-year follow-up study. *J Allergy Clin Immunol.* 2003;111:149–154.
24. Sastre J, Lluich-Bernal M, Quirce S, et al. A double-blind, placebo-controlled oral challenge study with lyophilized larvae and antigen of the fish parasite, *Anisakis simplex*. *Allergy.* 2000;55:560–564.
25. Valenta R, Duchêne M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med.* 1992;175:377–385.
26. Hirschwehr R, Heppner C, Spitzauer S, et al. Identification of common allergenic structures in mugwort and ragweed pollen. *J Allergy Clin Immunol.* 1998;101:196–206.
27. Laffer S, Vrtala S, Duchêne M, et al. IgE-binding capacity of recombinant timothy grass (*Phleum pratense*) pollen allergens. *J Allergy Clin Immunol.* 1994;94:88–94.
28. Pauli G, Osler JP, Deviller P, et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, r Bet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol.* 1996;97:1100–1109.
29. Asturias JA, Arilla MC, Gómez-Bayón N, et al. Cloning and immunological characterization of the allergen Hel a 2 (profilin) from sunflower pollen. *Mol Immunol.* 1998;35:469–478.
30. Wopner N, Willeroidee M, Hebenstreit D, et al. Molecular and immunological characterization of profilin from mugwort pollen. *Biol Chem.* 2002;383:1779–1789.
31. Enrique E, Alonso R, Bartolome B, et al. IgE reactivity to profilin in *Platanus acerifolia* pollen-sensitized subjects with plant-derived food allergy. *J Invest Allergol Clin Immunol.* 2004;14:335–342.
32. Fernández-Rivas M, van Ree R, Cuevas M. Allergy to *Rosaceae* fruits without related pollinosis. *J Allergy Clin Immunol.* 1997;100:728–733.
33. Elfman L, Svensson M, Lidholm J, et al. Different profiles in specific IgE to rBet v1 and rBet v2 in patients allergic to birch pollen from six countries. *Int Arch Allergy Immunol.* 1997;113:249–251.
34. Hayek B, Vangelista L, Pastore A, et al. Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, AIn g 4: structural basis for calcium-modulated IgE recognition. *J Immunol.* 1998;161:7031–7039.
35. Wagner S, Breiteneder H. The latex-fruit syndrome. *Biochem Soc Trans.* 2002;30:935–940.
36. Melillo G. EAACI provocation tests with allergens. *Allergy.* 1997;52(suppl 35):5–35.
37. Ebo DG, Hagendorens MM, Bridts CH, et al. Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clin Exp Allergy.* 2004;34:137–144.
38. Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol.* 2002;129:286–295.
39. Heiss S, Mahler V, Steiner R, et al. Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol.* 1999;113:830–837.
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