

Identification of cross-reactive and genuine *Parietaria judaica* pollen allergens

Sabine Stumvoll, PhD,^{a*} Kerstin Westritschnig, MD,^{a*} Jonas Lidholm, PhD,^b
Susanne Spitzauer, MD,^c Paolo Colombo, PhD,^d Giovanni Duro, PhD,^d
Dietrich Kraft, MD,^a Domenico Geraci, PhD,^d and Rudolf Valenta, MD^a

Vienna, Austria, Uppsala, Sweden, and Palermo, Italy

Background: The weed *Parietaria judaica* is one of the most important pollen allergen sources in the Mediterranean area. **Objective:** We sought to identify *P judaica* pollen allergen, which might be used to serologically distinguish genuine *Parietaria* sensitization and cross-reactivity to allergens from other weed species (eg, mugwort and ragweed).

Methods: The allergen profile of *P judaica* IgE-reactive sera from weed pollen-sensitized allergic individuals from the Mediterranean region (n = 36) with high *Parietaria* pollen exposure and from weed pollen-allergic patients with little or no *Parietaria* exposure (Austria, n = 42; Scandinavia, n = 8; United States, n = 19) was established by CAP FEIA measurements and by IgE immunoblot inhibition experiments with recombinant allergens.

Results: The majority (83%) of the Mediterranean weed pollen-allergic patients mounted high IgE antibody levels (mean specific IgE, 20.89 kUA/L) against recombinant (r) Par j 2, whereas only 7% of the non-Mediterranean weed-allergic patients showed low IgE reactivity to rPar j 2 (mean specific IgE, 1.03 kUA/L). The cytoskeletal protein profilin and a 2-EF-hand calcium-binding allergen were identified as cross-reactive *Parietaria* allergens, which were recognized preferentially by *Parietaria*-positive, non-Mediterranean weed pollen-allergic patients.

Conclusion: rPar j 2 might be used as a diagnostic marker allergen to identify weed pollen-allergic patients who are genuinely sensitized against *Parietaria* pollen and thus would be particularly suited for specific immunotherapy with *Parietaria* pollen extract. (J Allergy Clin Immunol 2003;111:974-9.)

Key words: *Parietaria*, recombinant allergens, rPar j 2, cross-reactivity, diagnosis

Pollens of weeds represent important elicitors of type I allergy during late summer and fall.¹ Reported prevalences of sensitization to certain weeds differ according to the highly divergent distribution area of those species: Ragweed (*Ambrosia* spp) is rare in central Europe but constitutes the most prominent allergenic weed in North America.² Whereas mugwort (*Artemisia vulgaris*) is the predominating weed in northern, western, and eastern parts of Europe,³ the closely related *Parietaria judaica* and *Parietaria officinalis* are the most important causes of pollinosis in Mediterranean countries.⁴⁻⁶

Parietaria flowers from spring to autumn, with peaks from May to June; it thus has an unusually long pollen season.³ For this reason, up to 80% of pollinosis patients in the Mediterranean region are sensitized against *Parietaria* allergens.^{6,7} *Parietaria* pollen allergens have been characterized by immunochemical and molecular-biological techniques. The major *P judaica* pollen allergens, Par j 1⁸ and Par j 2,⁹ exhibit significant sequence homology with each other and belong to a family of plant proteins that are known to transfer lipid molecules across membranes. Allergens related in sequence to Par j 1 and Par j 2 occur in pollens of various plants and fruits and have been termed *nonspecific lipid-transfer proteins* (nsLTPs).^{10,11}

Whereas the LTP-like allergens from *Parietaria* are well described,^{8,9} little information is available regarding other *Parietaria* allergens. We used serum samples of *Parietaria*-sensitized allergic subjects from *Parietaria*-exposed Mediterranean regions and from non-Mediterranean areas without significant *Parietaria* exposure to identify *Parietaria* pollen-specific and cross-reactive allergens.⁶ Both populations were tested for IgE reactivity to rPar j 2, the major *Parietaria* allergen. Highly cross-reactive pollen allergens from birch (ie, birch profilin, rBet v 2) and from timothy grass (ie, calcium-binding allergen, rPhl p 7) were used for IgE inhibition studies and specific antisera allowed us to identify cross-reactive and genuine allergens in *Parietaria* pollen. Our finding that *Parietaria*-exposed patients reacted predominantly to rPar j 2, whereas *Parietaria*-reactive patients without exposure were sensitized mainly to cross-reactive *Parietaria* allergens, suggests that rPar j 2

From ^athe Department of Pathophysiology, AKH, University of Vienna;

^bPharmacia Diagnostics AB, Uppsala; ^cthe Department of Medical and Clinical Chemistry, AKH, University of Vienna; and ^dIstituto di Biomedicina e Immunologia Molecolare "Alberto Monroy," Palermo.

*S. S. and K. W. contributed equally and are listed in alphabetical order.

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Reprint requests: Rudolf Valenta, MD, Department of Pathophysiology, General Hospital of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria.

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TABLE I. Frequency of IgE reactivity to pollen extracts from different weeds in 4 populations

Allergen source	Percent reactive sera (mean IgE levels, kU _A /L)			
	Mediterranean (n = 36)	Scandinavia (n = 30)	Austria (n = 8)	USA (n = 19)
<i>Parietaria</i>	100 (30.40)	100 (4.42)	100 (4.42)	100 (4.81)
Ragweed	38.9 (9.72)	92.8 (10.72)	100 (7.35)	94.7 (20.58)
Mugwort	30.6 (5.67)	97.6 (7.79)	100 (9.05)	94.7 (5.36)

might be used as a diagnostic marker for a genuine sensitization to *Parietaria*. The implications of this finding to the selection of patients for specific immunotherapy of *Parietaria* pollen allergy are discussed.

METHODS

Patients

Sera were obtained from allergic subjects sensitized to weed pollen. Their origin was either the Mediterranean area (Spain, Greece, France, and Italy), with its heavy exposure to *Parietaria* pollen, or non-Mediterranean areas (Austria, Scandinavia, and the United States), where little or no exposure to *Parietaria* pollen occurs.⁶ Sensitization to weed pollen was confirmed by IgE serology (CAP FEIA, Pharmacia Diagnostics, Uppsala, Sweden) through use of tests for mugwort, ragweed, and *Parietaria* pollen (Table I). Sera containing IgE antibodies reactive to *Parietaria* pollen extract (Spain, n = 19; Greece, n = 15; Italy, n = 1; France, n = 1; Austria, n = 30; Scandinavia, n = 8; the United States, n = 19) were tested for IgE reactivity to rPar j 2 by CAP FEIA measurements. Skin prick tests with *Parietaria* pollen extracts were performed in a subset of the Austrian subjects to confirm biological reactivity.

Natural weed pollen extracts

Pollens from *P. judaica* and mugwort were purchased from Allergon (Välinge, Sweden). Protein extracts were prepared by homogenization of 0.5 g pollen in 50 mL distilled water through use of an ultraturrax (Ika, Heidelberg, Germany). Proteins were extracted by shaking at 4°C for 3 hours. Extracts were then centrifuged at 16,000g for 15 minutes to remove insoluble particles. The supernatants were frozen as 1-mL aliquots in liquid nitrogen, lyophilized, and stored at -20°C until use.¹² Protein extracts were analyzed by SDS-PAGE¹³ and Coomassie Brilliant Blue staining (Bio-Rad, Richmond, Calif) to estimate quantity and quality of proteins.¹⁴

Recombinant allergens

Recombinant *P. judaica* allergen, rPar j 2, which had been expressed in *Escherichia coli*, was purified as described.⁹ Experimental ImmunoCAP tests were prepared by covalent immobilization of the purified allergen onto CnBr-activated cellulose at a concentration chosen to achieve an adequate linear measuring range and a background for negative sera well below the conventional cut-off value of 0.35 kU_A/L.¹⁵ Recombinant timothy grass pollen allergen, rPhl p 7, was expressed in *E. coli* and purified as described.¹⁶ Recombinant birch pollen profilin, rBet v 2,¹⁷ was purchased from Biomay (Vienna, Austria).

Immunoblot experiments and quantitative IgE measurements

P. judaica pollen extract, equivalent to 0.2 mg protein per centimeter of gel, was separated by preparative SDS-PAGE and blotted on nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).¹⁸

For IgE inhibition experiments, sera from Austrian patients resembling typical IgE reactivity profiles were diluted 1:10 in buffer A (50 mmol/L sodium phosphate [pH 7.5], 0.5% w/v BSA, 0.5% v/v Tween 20, and 0.05% w/v NaN₃) and preincubated with either natural weed pollen (mugwort or *Parietaria*) extract (100 μ g weed pollen extract/1 mL serum dilution) or recombinant allergens (5 μ g of each recombinant allergen/1 mL serum dilution) or, for control purposes, with BSA (5 μ g/1 mL serum dilution) overnight at 4°C. Preadsorbed sera were then incubated overnight at 4°C with nitrocellulose-blotted *Parietaria* pollen extracts, and bound IgE antibodies were detected with ¹²⁵I-labeled anti-human IgE antibodies (Pharmacia Diagnostics) and visualized by autoradiography.¹⁹

Rabbit antisera raised against Par j 2, rPhl p 7, and rBet v 2 were used to detect cross-reactive allergens in nitrocellulose-blotted *Parietaria* and mugwort (*Artemisia vulgaris*) pollen extracts.¹⁹

Specific IgE antibody levels (kU_A/L) to weed pollen extracts and to rPar j 2 were determined through use of the Pharmacia CAP system (Pharmacia Diagnostics). rPar j 2 and *Parietaria* pollen extract-specific IgE levels were plotted against each other in a diagram, and the differences in IgE levels in the populations were analyzed by means of the Mann-Whitney test.

RESULTS

Weed pollen-allergic patients from non-Mediterranean populations exhibited IgE reactivity to *P. judaica* pollen extracts

Table I summarizes results from IgE ImmunoCAP measurements performed with *Parietaria* pollen-reactive sera from the Mediterranean region, Austria, Scandinavia, and the United States. *Parietaria* pollen extract-specific serum IgE levels were significantly higher in the Mediterranean population (mean IgE level, 30.4 kU_A/L; Table I), whereas significantly lower levels were found in the other populations (Austria, 4.42 kU_A/L; Scandinavia, 4.42 kU_A/L; United States, 4.75 kU_A/L; *P* < .001). However, when we tested the *Parietaria* extract-positive sera for IgE reactivity to ragweed and mugwort pollen extract, striking differences regarding the number of ragweed- and mugwort-reactive sera were found among the tested populations. More than 90% of the sera from Austria, Scandinavia, and the United States showed IgE reactivity to ragweed or mugwort pollen extract, whereas ragweed- and mugwort-specific IgE antibodies were detected in only 38.9% and 30.6%, respectively, of the Mediterranean subjects. The levels of mugwort pollen-specific IgE were comparable in sensitized individuals from all 4 populations, but the levels of ragweed pollen extract-specific IgE were highest in patients from the United States (Table I). The biological reactivity associated with *Parietaria* pollen-specific IgE

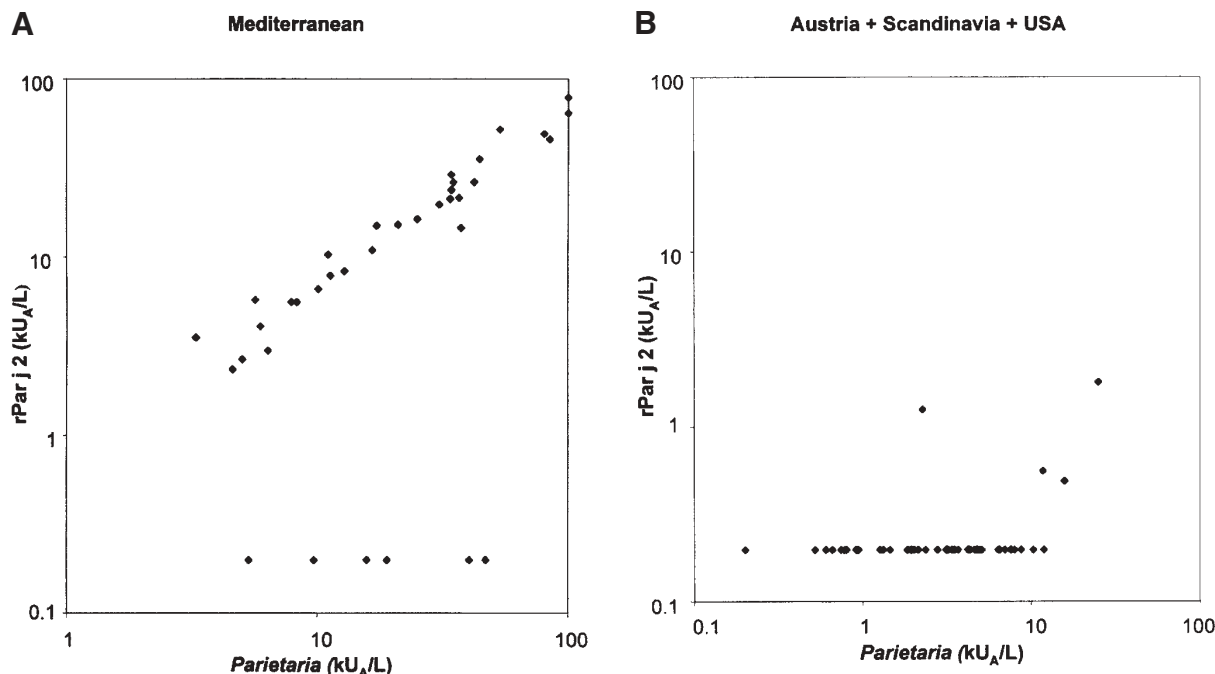


FIG 1. Serum IgE levels in weed pollen-sensitized allergic individuals from the Mediterranean region (A) and from Austria, Scandinavia, and the United States (B). Serum IgE levels (kU_A/L) specific for rPar j 2 (y axis) and for *Parietaria judaica* pollen extract (x axis) are plotted for each serum sample.

was confirmed by skin prick testing in the Austrian patients (data not shown).

Mediterranean *P judaica* pollen-sensitized patients reacted predominantly to rPar j 2, whereas non-Mediterranean patients are mainly sensitized to other cross-reactive pollen allergens

Sera from the Mediterranean subjects ($n = 36$), Austria ($n = 30$), Scandinavia ($n = 8$), and the United States ($n = 19$) that were positive for *Parietaria* pollen extract according to CAP FEIA testing were analyzed for the presence of IgE antibodies to rPar j 2, an 11.4-kd major allergen from *Parietaria* pollen (Fig 1, A and B). In the Mediterranean population, 30 (83%) of 36 sera showed IgE reactivity to rPar j 2; this compared with only 4 (7%) of 57 of the non-Mediterranean sera. Furthermore, the mean IgE levels to rPar j 2 were significantly higher in the Mediterranean (20.89 kU_A/L) than in the non-Mediterranean (1.03 kU_A/L) rPar j 2-reactive patients ($P < .001$).

Using specific rabbit antisera against Par j 2 and against the 2 cross-reactive allergens (profilin, rBet v 2; 2-EF-hand allergen, rPhl p 7), we demonstrated that Par j 2 can be detected in *Parietaria* pollen but not in pollen of other weeds (mugwort, Fig 2, A; ragweed, data not shown). Profilin (Bet v 2, 14 kd) and 2-EF-hand pollen allergens (Phl p 7, 9 kd) were detected as cross-reactive antigens in *Parietaria*, mugwort (Fig 2, A), and ragweed (data not shown).

To characterize the *Parietaria* pollen allergens recog-

nized by the non-Mediterranean patients, IgE immunoblot inhibition experiments were performed with purified recombinant allergens and allergen extracts.

Preincubation of sera from Austrian weed pollen-allergic patients (Fig 2, B; patients A-D) with mugwort pollen extract strongly inhibited IgE binding to nitrocellulose-blotted *Parietaria* pollen extract. Two of the cross-reactive *Parietaria* allergens could be identified by IgE inhibition experiments with purified recombinant allergens: IgE binding to components of 11 to 14 kd (Fig 2, B; patients A, B, and C) could be completely abolished (Fig 2, B; patients A and B, lanes rBet v 2) or strongly reduced (Fig 2, B; patient C, lanes rBet v 2) after preincubation of sera with birch pollen profilin (rBet v 2). Preincubation of sera with rPhl p 7, a highly cross-reactive calcium-binding protein from timothy grass, led to a complete inhibition of IgE binding to the 9-kd component in *Parietaria* pollen extract (Fig 2, B; patient B, lane rPhl p 7). Preincubation of sera with BSA (Fig 2, B) or without addition of protein (data not shown) did not affect IgE reactivity to *Parietaria* allergens.

DISCUSSION

The weed *Parietaria* is one of the most common allergen sources in the Mediterranean countries, but it also can be found sparsely in the southern United Kingdom, Austria, the temperate regions of central and eastern Europe, Australia, and California, where other weeds predominate.⁶ The choice of an appropriate

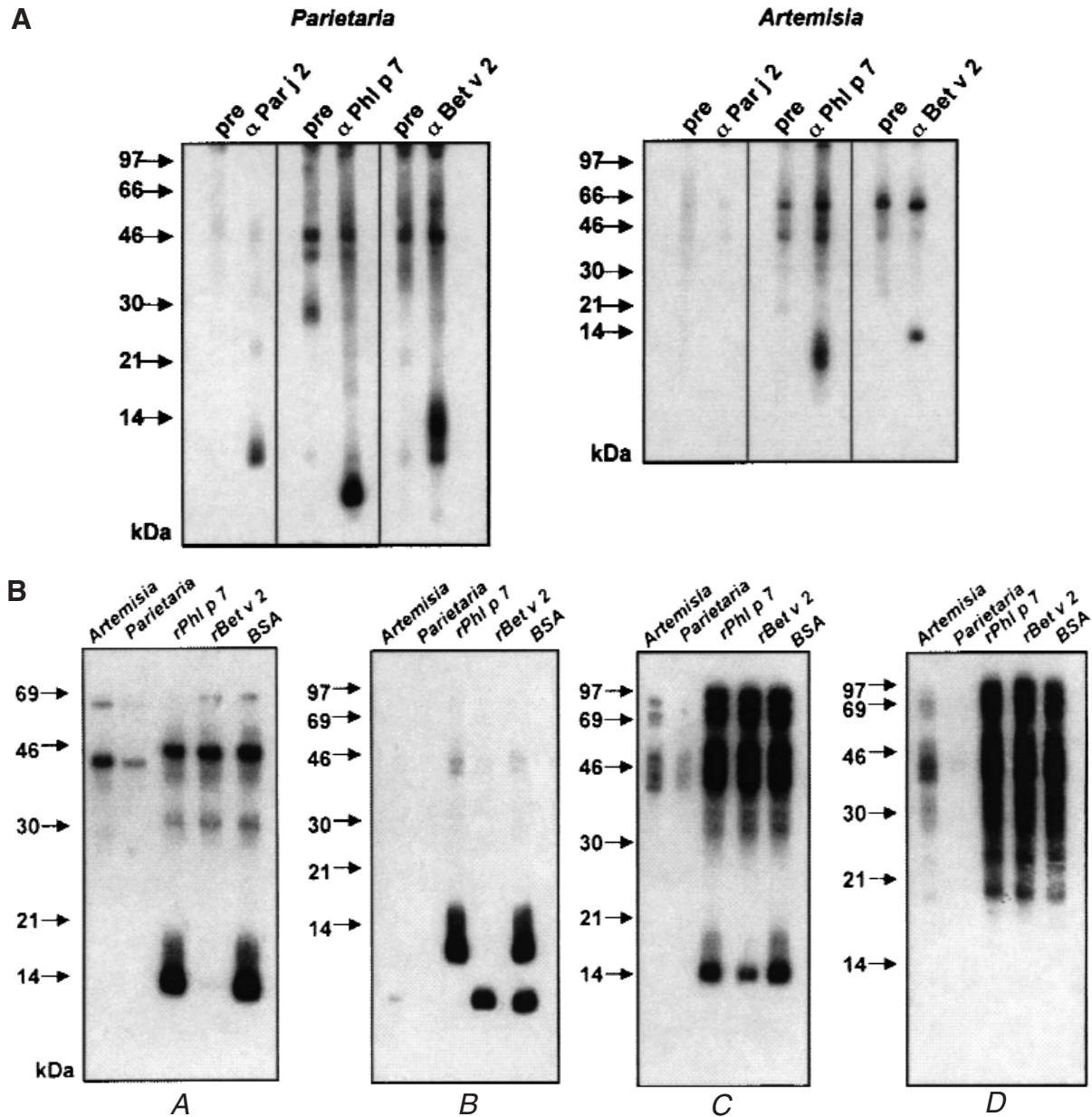


FIG 2. Identification of allergens in nitrocellulose-blotted *Parietaria* and *Artemisia* extract (**A**). Rabbit anti-sera (lanes α Par j 2, α Phl p 7, and α Bet v 2) or the corresponding preimmune sera (pre lanes) (**A**) were used to detect allergens. Identification of cross-reactive weed pollen allergens by IgE immunoblot inhibition (**B**). Serum IgE reactivity of 4 Austrian weed pollen-allergic patients (panels **A-D**) to nitrocellulose-blotted *Parietaria judaica* pollen extract. Sera were preadsorbed with pollen extracts from mugwort (*Artemisia*) or *Parietaria*, with recombinant allergens (lanes rPhl p 7 and rBet v 2) or an unrelated control protein (BSA lanes). Molecular weights are indicated on the left margin.

weed pollen extract for specific immunotherapy in the latter areas might therefore represent a difficult task, because it will not be possible to unambiguously identify the sensitizing weed species.²⁰⁻²² In this context it has been demonstrated that an unexpectedly high percentage of mugwort-allergic patients from Austria exhibited IgE reactivity to ragweed pollen because of the presence of cross-reactive allergens, although ragweed rarely occurs in Austria.²³

The current study identified cross-reactive allergens (eg, profilin^{19,24} and calcium-binding proteins^{16,25,26}) in *P. judaica* and a genuine *Parietaria*-specific marker allergen, rPar j 2,⁹ as potential diagnostic marker molecules by which to distinguish patients with primary sensitization to *Parietaria* from those who exhibit IgE reactivity and clinical sensitivity (ie, skin sensitivity) to *Parietaria* because of cross-reactivity with other weed species (eg, mugwort and ragweed).

Sera from non-Mediterranean allergic individuals with little or no exposure to *Parietaria* reacted predominantly with cross-reactive weed pollen allergens, which also occur in mugwort and ragweed pollen extracts. Using purified recombinant allergens with high cross-reactivity (ie, rBet v 2, profilin from birch pollen²⁴; rPhl p 7, a calcium-binding timothy grass pollen allergen¹⁶) for IgE immunoblot inhibition experiments, we could identify profilin and a 2-EF-hand calcium-binding allergen as cross-reactive *Parietaria* allergens sharing IgE-binding epitopes with allergens from other weed species. In addition, several not-yet-characterized high-molecular-weight components (40–100 kD) present in *Parietaria* pollen were found to cross-react with mugwort pollen allergens.²⁷

A markedly different IgE reactivity profile was found for the Mediterranean population. The analysis of these sera revealed a predominant IgE response to Par j 2, a member of the family of nonspecific LTPs. Using rPar j 2, we could demonstrate that 83% of the Mediterranean sera, but only 7% of the non-Mediterranean sera, showed IgE reactivity to this allergen.

The reason for the different recognition frequencies of Par j 2 in the 2 populations is most likely the occurrence of detectable Par j 2 only in *Parietaria* pollen, as demonstrated by the binding of a specific antiserum raised against Par j 2, whereas no cross-reactive protein could be found in the other weed pollens (Fig 2, A). The non-Mediterranean patients' sera contained significantly lower levels of *Parietaria* pollen extract-specific IgE than did the Mediterranean but still exhibited skin sensitivity to *Parietaria* pollen. It was thus not possible to distinguish the 2 populations on the basis of allergen extract testing alone.

We therefore suggest that rPar j 2 be used for diagnostic testing to unambiguously identify the disease-eliciting allergen sources for all of those areas where *Parietaria* coexist with other allergenic weeds. Furthermore, we believe that the Par j 2-based test will be useful to discriminate those Mediterranean patients, though rare, who are not sensitized against the major allergen of *Parietaria* and hence might benefit less from *Parietaria*-specific immunotherapy than those who are sensitized to the major allergen, Par j 2.²⁸ In this scenario, immunotherapy with *Parietaria* pollen extract would be indicated if the clinical criteria for immunotherapy, as specified in a World Health Organization position paper,²⁹ are fulfilled and sensitization to rPar j 2 can be demonstrated. *Parietaria* pollen extracts used for immunotherapy are mainly standardized regarding their major allergen contents (eg, Par j 2), not regarding the presence of profilin or calcium-binding allergens.^{30–32} We therefore suggest that immunotherapy with *Parietaria* pollen extract be avoided if a patient is not sensitized against the major *Parietaria* pollen allergen, Par j 2, but reacts only with cross-reactive allergens. The molecules identified in our study (ie, rPar j 2, profilin, and 2-EF-hand allergens) might therefore be useful to assist the clinician in selecting patients for immunotherapy with *Parietaria* pollen extract²⁸ and perhaps to detect the possible development of new sensitizations during and after treatment, as was recently reported for birch pollen-specific immunotherapy.³³

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