

Hypersensitivity to mugwort (*Artemisia vulgaris*) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression

Elide A. Pastorello, MD,^a Valerio Pravettoni, MD,^a Laura Farioli, BSc,^b
Federica Rivolta, MD,^a Amedeo Conti, PhD,^c Marco Ispano, MD,^d
Donatella Fortunato, BSc,^c Anders Bengtsson, BSc,^e Matilde Bianchi, MD^a
Milan and Turin, Italy, and Uppsala, Sweden

Background: The observation of mugwort-specific IgE antibodies in patients with peach allergy suggests that mugwort sensitization might play a role in sensitization to peach.

Objective: We sought to study the clinical manifestations of mugwort hypersensitivity in patients with peach allergy, identify the common allergens, and evaluate their IgE cross-reactivity.

Methods: Patients with oral allergy syndrome for peach and specific IgE antibodies to mugwort were investigated for respiratory symptoms during the mugwort season. Peach and mugwort allergens were identified by means of SDS-PAGE and IgE immunoblotting. Immunoblotting inhibition experiments were done to study cross-reactivity between peach and mugwort and other pollens.

Results: Seventeen patients were studied, 10 with no seasonal respiratory symptoms and 7 with clear late summer respiratory symptoms. In IgE immunoblotting the 10 asymptomatic patients reacted only to a 9-kd allergen of both mugwort and peach, whereas the 7 patients with pollinosis reacted to other allergens. Ten patients with mugwort allergy, no history of allergy to peach, and negative results for peach-specific IgE antibodies were also studied. The mugwort 9-kd protein was identified as a lipid transfer protein (LTP) homologous to peach LTP. Immunoblotting inhibition showed that IgE binding to the peach 9-kd band was totally inhibited by 4 µg of peach LTP but only by 400 µg of mugwort LTP, whereas 4 µg of both mugwort and peach LTP totally inhibited the mugwort immunoblotting. The results were similar with other pollens.

Conclusions: Patients sensitized only to the 9-kd LTP of mugwort do not present hay fever symptoms, and this sensitization is a consequence of the peach sensitization. (J Allergy Clin Immunol 2002;110:310-7.)

Key words: Oral allergy syndrome, mugwort pollen, cross-reactivity, lipid transfer protein

Abbreviations used

LTP: Lipid transfer protein
SPT: Skin prick test

In southern Europe allergy to peach is not clinically associated with any kind of pollinosis,¹⁻⁴ and therefore this sensitization seems independent of any pollen hypersensitivity. This observation found its molecular basis in the demonstration that Italian patients recognize as a major allergen of *Prunus persica* a 9-kd lipid transfer protein (LTP) that has not been described in homologous form in the pollens most commonly involved in hay fever in Europe, such as grasses and birch.⁵ In the Mediterranean area the major allergens of *Parietaria judaica*, Par j 1 and Par j 2,⁶ the minor ragweed allergen Amb a 6, and Ole e 7 in olive pollen⁷ are the only allergenic LTPs thus far described in pollens and have never been reported to cross-react with food LTPs. However, Díaz-Perales et al⁸ described an LTP in *Artemisia vulgaris* pollen having an amino acid sequence with 53% identity with *P persica* LTP, although no clear IgE cross-reactivity was shown between the 2 species. These results suggest that *A vulgaris* exposure might be a factor inducing sensitization to *P persica* or, on the contrary, that sensitization to peach LTP induces cross-reactive IgE antibodies to *A vulgaris*.

A vulgaris major allergens are 2 proteins of 60 kd (Art v 1) and 28 kd (Art v 2). These allergens give rise to the so-called mugwort-spice syndrome^{9,10} but not to allergy to Prunoideae fruits. The present study was designed to evaluate the clinical and immunologic aspects of the association between *P persica* and *A vulgaris* hypersensitivity. We investigated the respiratory symptoms to *A vulgaris* of patients with peach allergy, the allergens of *P persica* and mugwort, and their cross-reactivity.

METHODS

Patients and study design

Patients referred to the Allergy Center of the 3rd Department of General Medicine of the University of Milan and to the Bizzozzero Division of Niguarda Ca' Granda Hospital of Milan for an oral

From ^aAllergy Center, 3rd Division of General Medicine, Ospedale Maggiore IRCCS, Milan; ^bUOOML, CEMOC, I.C.P., Milan; ^cNational Research Council, Turin; ^dBizzozzero Division, Niguarda Ca' Granda Hospital, Milan; and ^ePharmacia & Upjohn Diagnostics AB, Uppsala.

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Reprint requests: Elide Anna Pastorello, MD, 3rd Division of General Medicine-Padiglione Granelli, Via Francesco Sforza 35, 20122 Milan, Italy.

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allergy syndrome after eating peach were investigated for the presence of specific IgE antibodies to mugwort. In patients with positive results, skin prick tests (SPTs) with fresh peach (prick-plus-prick method¹¹) and commercial mugwort extract, peach-specific IgE antibody tests (CAP-RAST, Pharmacia), and open food challenges (a procedure we have already published^{2,12}) for peach were done.

Patients with a positive open challenge result or a documented history of severe reactions to peach and a positive CAP-RAST result for peach and mugwort were evaluated for mugwort pollinosis by completing a diary card for respiratory symptoms during the mugwort pollen season in 2000. Briefly, in July, August, and September (the months when mugwort flowers in Italy), the patients were asked to record their symptoms daily, attributing separate values to nasal, conjunctival, and bronchial symptoms (score range, 0-3), and to enter the amount of drugs taken to reduce symptoms. The pollen count was monitored and correlated with symptoms.

Patients were screened with commercial SPTs and CAP-RASTs for other seasonal inhalant allergens (Stallergenes Italia, 1:100 wt/vol), such as grass, birch, wall pellitory, olive, and ragweed pollens. Skin reactivity was evaluated according to the Position Paper of the European Academy of Allergy and Clinical Immunology.¹³

As control subjects, we recruited 10 subjects with a documented history of mugwort allergy but no clinical symptoms and negative CAP-RAST results for peach or other fruits.

Blood was drawn from all patients, and sera were collected and stored at -80°C until used for in vitro tests to identify peach and mugwort allergens and their cross-reactivity.

In vitro methods

Peach and mugwort extracts. Peach extract was prepared according to the method described in detail in our previous study.¹⁴ The protein concentration of peach extract, determined by means of the colorimetric method of Lowry et al¹⁵ with Folin reagent, was 4.83 mg/mL. Mugwort extract (*A vulgaris*), olive (*Olea europaea*), ragweed (*Ambrosia elatior*), wall pellitory (*Parietaria officinalis*), and grass (timothy pollen) were supplied by Pharmacia & Upjohn at protein concentrations of 8 mg/mL, 3.5 mg/mL, 4.5 mg/mL, 3 mg/mL, and 3 mg/mL, respectively, determined according to the Bradford method.¹⁶

SDS-PAGE immunoblotting. Peach and pollen extracts were separated in a discontinuous buffer system in SDS-polyacrylamide 7.5% to 20% separation gradient gel with 6% stacking gel at 6 mA for 16 hours in a BIO-RAD Protein IIx vertical electrophoresis slab cell (BIO-RAD Laboratories), essentially according to the method of Neville¹⁷ and already described in detail.¹⁴ The extracts, diluted 1:2 in sample buffer, had the following protein concentrations: peach, 0.29 mg/cm gel; mugwort, 0.64 mg/cm gel; ragweed, 0.36 mg/cm gel; olive, 0.28 mg/cm gel; wall pellitory, 0.24 mg/cm gel; and grass, 0.24 mg/cm gel.

A part of the gel was then electroblotted onto nitrocellulose membrane paper (0.45 µm, Amersham) and onto a polyvinylidene fluoride hydrophobic membrane (ProBlott) by using a Trans-blot cell from BIO-RAD at 0.45 Å and 100 V for 4 hours at 4°C. The nitrocellulose paper was cut into strips and incubated overnight with each patient's serum and with a negative control serum and then incubated with iodine 125-labeled anti-human IgE antiserum and exposed on autoradiographic film (Hyperfilm, Amersham) in exposure cassettes at -70°C for 4 days.

HPLC

Purification of 9-kd mugwort and peach allergens. The peach LTP was purified by using the chromatographic conditions previously described.¹⁴ The 9-kd protein from mugwort extract was isolated and purified under the same chromatographic conditions.

Briefly, 500 µL of the pollen extract was injected into a cationic exchange column (Resource-S 1 mL, Amersham Pharmacia Biotech) connected with an HPLC system (AKTA Purifier, Amersham Pharmacia Biotech). The mobile phase was sodium acetate 3-hydrate buffer A (50 mmol/L CH₃COONa × 3 H₂O, pH 5) and buffer B (50 mmol/L CH₃COONa × 3 H₂O, pH 5, plus 1 mol/L NaCl). The gradient length was 20 column volumes, with a flow rate of 1 mL/min. Absorbance was monitored at 280 nm.

Four peaks were detected. Because the first peak analyzed by means of SDS-PAGE contained the 9-kd protein with other higher-molecular-weight impurities, further resolution was achieved by means of gel filtration with a Superdex 75 column equilibrated and eluted with 15 mmol/L NaCl in 50 mmol/L sodium acetate 3-hydrate buffer (pH 5) at a flow rate of 0.7 mL/min. A calibration curve was prepared as previously reported.¹⁴ The chromatogram showed 5 peaks that were concentrated and analyzed by means of SDS-PAGE immunoblotting to evaluate its purity and IgE-binding capacity. The protein content was measured by using the Bradford method.¹⁶

Immunoblotting cross-inhibition between peach and mugwort and peach and other pollens. An immunoblotting inhibition study was done with pooled sera of patients allergic to peach to roughly evaluate the cross-reactivity between mugwort and peach, with respiratory symptoms for mugwort (pool A). Briefly, 500 µL of pool A was incubated for 1 hour with 500 µL of peach extract at protein contents of 4, 0.4, and 0.04 mg. Four milligrams of crude peach extract totally inhibited IgE binding only to a 9-kd band of mugwort pollen. These data (not shown) indicated that a 9-kd protein was the only allergen common to mugwort and peach, and therefore all further experiments were done with purified allergen.

We assessed the cross-reactivity in an inhibition study. A pool of serum from the 10 patients without respiratory symptoms for mugwort (pool B) and single sera from 2 patients (nos. 4 and 7) were used. Briefly, 500 µL of pool B and of the 2 single sera were incubated with 500 µL of a solution containing 4 µg of purified 9-kd peach LTP and another 500 µL with 500 µL of a solution containing 4 µg of mugwort purified 9-kd allergen. After inhibition, peach and mugwort immunoblotting was done, as described above. Because the peach immunoblotting with serum pool B and the single sera was not inhibited by 4 µg of 9-kd mugwort, the experiment was repeated with 40 and 400 µg of 9-kd mugwort protein as inhibitors.

Because some patients also had a positive CAP-RAST result for other pollens, cross-inhibition experiments were done between peach and LTP-containing pollens, such as olive, ragweed, and wall pellitory, with either pool A or single positive sera by using 0.4 and 0.04 mg of the inhibiting extracts.

Amino acid sequencing. Protein and peptide sequence analysis was done on a Perkin Elmer Applied Biosystems 470A gas-phase sequencer equipped with a 120A phenylthiohydantoin-amino acid derivative analyzer. All chemicals were from Perkin-Elmer Applied Biosystems.

RESULTS

Patients

Seventeen patients (14 female and 3 male patients; mean age, 28.2 years; age range, 17-41 years) were selected. Their demographic data; peach, mugwort, and other pollen-specific IgE levels (CAP system); SPT responses for mugwort; oral allergy syndrome grading after challenge¹⁸; and pollens causing symptoms are reported in Table I.

During the mugwort pollen season (pollen counts for mugwort and ragweed are reported in Fig 1, A), 2 groups were identified: 10 patients had no symptoms, and the

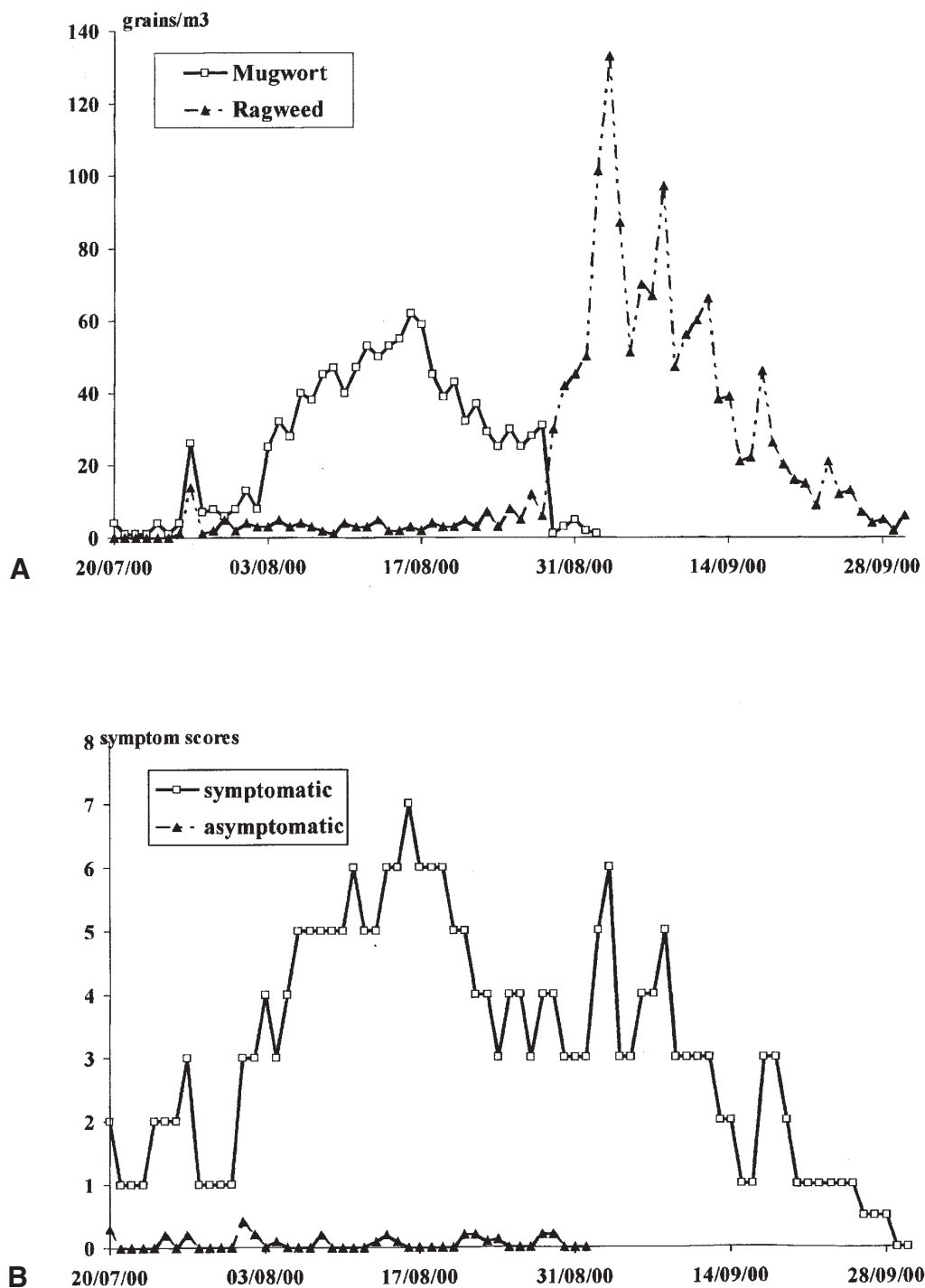


FIG 1. A, Mugwort and ragweed pollen counts in the year 2000 pollen season. B, Symptoms for mugwort and ragweed during the 2000 pollen season in symptomatic and asymptomatic patients.

other 7 had very clear late summer respiratory symptoms. A mean of the symptoms and drug consumption of the 2 populations was used to plot Fig 1, B.

The CAP-RAST values for mugwort for the 10 control patients ranged from 8.5 to 14.5 kU/L (mean, 10.8 kU/L).

SDS immunoblotting

Fig 2, A, shows the IgE immunoblotting of mugwort extract. Even though all the patients reacted to a 9-kd band of mugwort, the 10 who had no seasonal respiratory

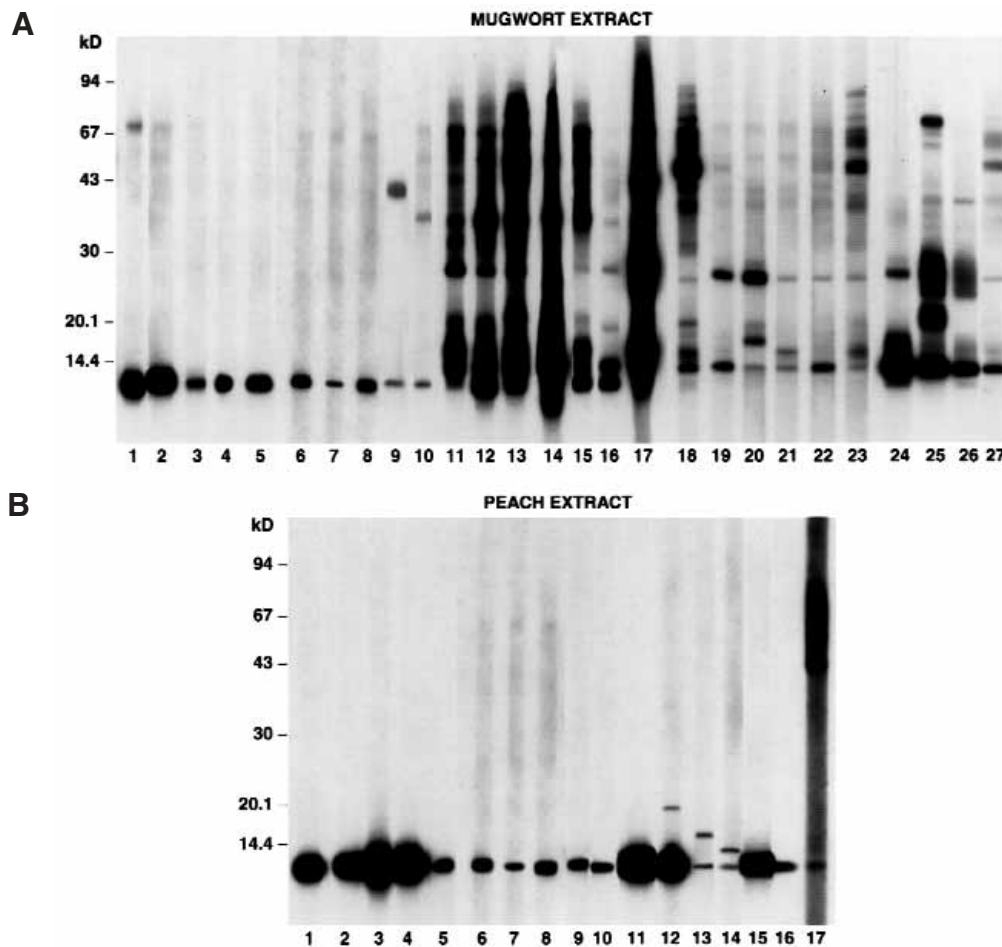


FIG 2. A, IgE immunoblot of mugwort extract with the sera of 27 mugwort-sensitive patients. Patients 1 to 17 were allergic to peach: 1 to 10 were asymptomatic for mugwort pollinosis, and 11 to 17 had seasonal respiratory symptoms. Patients 18 to 27 were asymptomatic for peach. B, IgE immunoblot of peach extract with the sera from all peach-sensitive patients (nos. 1-17).

symptoms (nos. 1-10) reacted only to this protein, whereas the 7 with seasonal respiratory symptoms also reacted to higher-molecular-weight proteins (nos. 11-17), particularly to allergenic components with molecular weights of 77, 60, 40, 20, 28, 16.5, and 14 kD. No reactivity to the 9-kD allergen was detected with the sera from patients allergic to mugwort but not to peach (nos. 18-27).

Immunoblotting analysis of peach extract showed IgE reactivity against a 9-kD protein (Fig 2, B), recently identified as an LTP, from all 17 sera. The 10 patients with no seasonal respiratory symptoms who only reacted to the 9-kD band of the mugwort extract also reacted only to the 9-kD allergen of peach, whereas the 7 patients with seasonal mugwort symptoms reacted to the higher-molecular-weight allergens of peach, such as the 15-, 17-, and 20-kD proteins, and only one at 40 to 70 kD.

HPLC: Purification of 9-kD mugwort and peach allergens

The purity of the peach LTP was analyzed by means of SDS-PAGE, with results similar to those we have already reported.¹⁴ After cationic exchange HPLC, we identified

a mugwort LTP in the first peak, but it was not pure. Therefore we collected 10 mL of this impure fraction (protein content, 0.03 mg/mL) by repeated runs and concentrated it to a final volume of 1 mL (protein content, 0.3 mg/mL). This was injected onto a gel filtration column, obtaining the 5 peaks. After comparison with the chromatographic profile of the markers, we collected the fraction corresponding to the second peak, which had an appropriate molecular weight. The peak corresponding to the purified 9-kD protein was collected by repeated runs, concentrated again to a final volume of 1 mL with a protein content of 0.18 mg/mL, and analyzed by means of SDS-PAGE and IgE immunoblotting. We demonstrated its purity and its IgE-binding activity (data not shown).

Immunoblotting cross-inhibition between peach and mugwort and peach and other LTP-containing pollens

IgE binding to the 9-kD component of peach was totally inhibited by means of preincubation of serum pool B with 4 μ g of peach LTP but not with 4 μ g of mug-

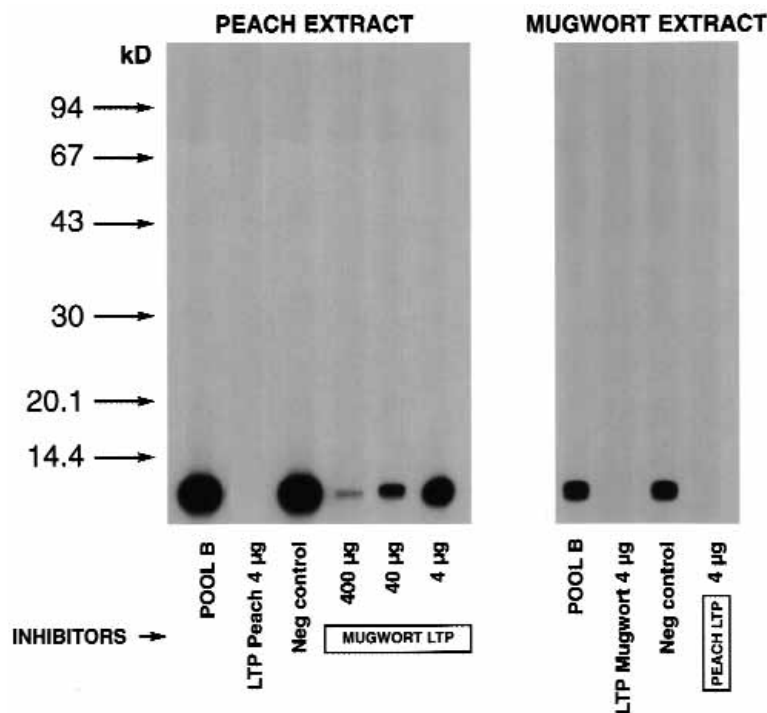


FIG 3. *Left*, Inhibition of IgE immunoblotting of the raw peach extract by mugwort-purified LTP at different protein contents. *Right*, Inhibition of IgE immunoblotting of mugwort extract by peach-purified LTPs. Serum pool from patients asymptomatic for mugwort was used (pool B).

TABLE I. Characteristics of patients

Patient no.	Sex	Age (y)	Peach CAP system (kU/L)	Mugwort CAP system (kU/L)	Mugwort SPT	Peach OAS grading after challenge	Grass CAP system (kU/L)	Wall pellitory CAP system (kU/L)	Birch CAP system (kU/L)	Ragweed CAP system (kU/L)	Olive CAP system (kU/L)	Other pollens causing symptoms
1	F	32	2.42	3.5	3+	III	<0.35	<0.35	<0.35	<0.35	1.27	—
2	F	38	3.75	3.3	2+	III	<0.35	<0.35	<0.35	<0.35	0.80	—
3	M	18	56.4	7.32	2+	III	1.33	5.49	<0.35	7.32	<0.35	—
4	F	29	27.8	10.6	1+	IV*	<0.35	1.72	<0.35	<0.35	<0.35	—
5	F	29	7.49	4.48	1+	IV*	<0.35	<0.35	<0.35	<0.35	<0.35	—
6	F	17	8.05	1.7	1+	III	0.51	0.36	<0.35	1.56	1.70	—
7	F	35	21	1.06	3+	IV*	<0.35	<0.35	<0.35	<0.35	<0.35	—
8	F	30	16.4	2.02	1+	III	<0.35	0.90	<0.35	<0.35	<0.35	—
9	F	37	2.77	1.19	2+	III	0.49	<0.35	0.53	<0.35	<0.35	—
10	F	23	14	1.22	1+	III	<0.35	<0.35	1.24	<0.35	<0.35	—
11	F	29	2.35	44.3	3+	III	59.3	12.3	28.2	4.03	2.51	Grass, birch
12	M	21	11.2	87.7	4+	III	>100	35.6	>100	5.88	<0.35	Grass, wall pellitory
13	M	17	1.39	36	3+	III	>100	5.67	18.6	10.6	<0.35	Grass, ragweed
14	F	41	14	100	4+	IV*	>100	82.4	>100	79.8	82.2	Birch, ragweed
15	F	23	7.74	44.9	2+	III	50.1	1.53	24.9	8.25	<0.35	Grass, birch, ragweed
16	F	24	6.85	20.5	2+	III	13.9	2.68	6.63	4.30	2.95	Grass, birch, ragweed
17	F	37	2.76	99.8	4+	III	>100	3.2	8.82	24.6	17.5	Grass

Patients 11 to 17 were symptomatic for mugwort.

OAS, Oral allergy syndrome.

*According to severity on the basis of history and not to the challenge test.

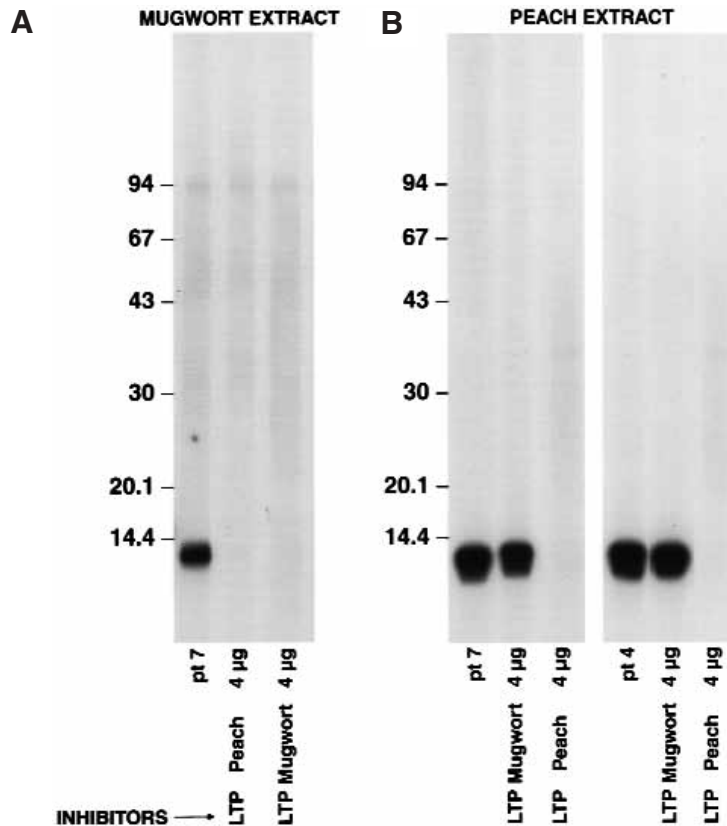


FIG 4. A, Inhibition of IgE immunoblotting of mugwort extract by 4 µg of peach and mugwort-purified LTPs with sera from patient 7. B, Inhibition of IgE immunoblotting of the raw peach extract by 4 µg of mugwort and peach LTPs with sera from patients 7 and 4.

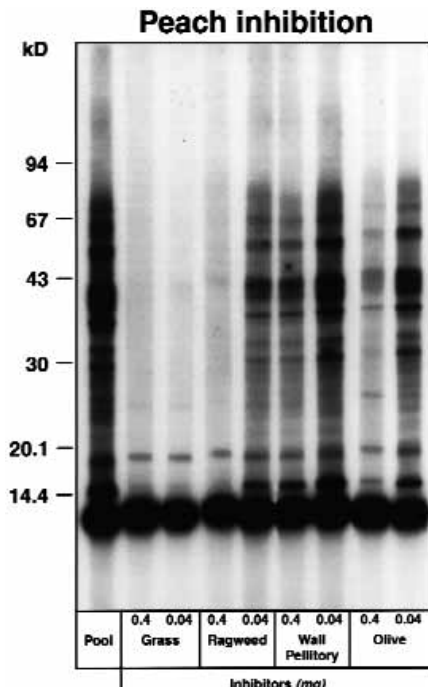


FIG 5. Inhibition of IgE immunoblotting of the raw peach extract by 0.4 and 0.04 mg of grass, ragweed, wall pellitory, and olive extract using serum pool A.

wort LTP (Fig 3, *left*). Only 400 µg of mugwort LTP totally inhibited the IgE binding to the 9-kD component of peach. Fig 3, *right*, shows that 4 µg of peach LTP totally inhibited the IgE binding of pool B to the 9-kD allergen of mugwort pollen, exactly like 4 µg of mugwort LTP. In each experiment a negative serum, tested as a control, did not inhibit IgE binding to the different extracts. Fig 4, A shows the results of cross-inhibition with a single patient's sera, confirming that 4 µg of both peach and mugwort LTP completely inhibited IgE binding to the mugwort 9-kD protein. However, 4 µg of mugwort LTP did not inhibit the IgE binding to peach LTP, whereas 4 µg of peach LTP did (Fig 4, B). Only 400 µg of mugwort LTP inhibited the IgE binding to peach LTP (data not shown).

No inhibition of IgE binding to peach LTP was observed after preincubation of pool A with the extracts of grass, olive, ragweed, and wall pellitory pollens (Fig 5). However, grass and ragweed inhibited binding to the higher-molecular-weight allergens.

Using single sera for the cross-inhibition experiments with olive pollen (Fig 6), we found that IgE binding to the 9-kD allergen of the olive extract was completely inhibited by the lowest concentration of peach extract, whereas the highest olive extract concentration only weakly inhibited IgE binding to the 9-kD allergen of peach.

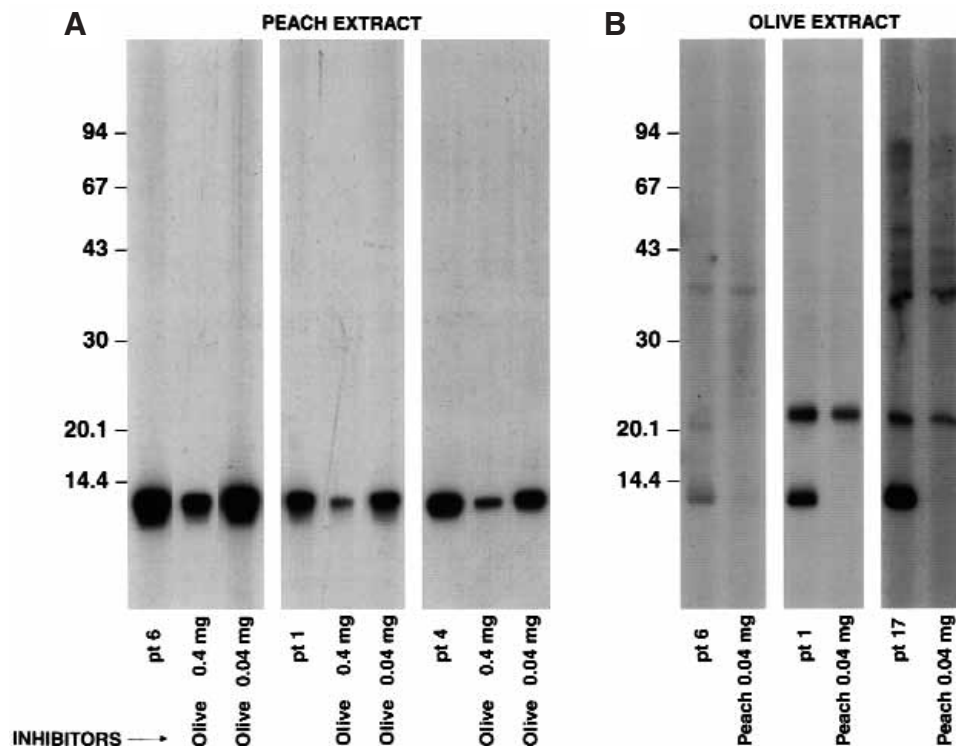


FIG 6. A, Inhibition of IgE immunoblotting of the raw peach extract with 0.4 and 0.04 mg of olive extract using sera from patients 6, 1, and 4. B, Inhibition of IgE immunoblotting of olive extract by 0.4 and 0.04 mg of raw peach extract with sera from patients 6, 1, and 17.

There was no IgE cross-reactivity between peach and ragweed and peach and wall pellitory, either with serum pool A or with single sera (data not shown).

Amino acid sequencing

The N-terminal sequence of the 9-kd purified mugwort protein (14 amino acids) was Leu-Thr-Cys-Ser-Asp-Val-Ser-Asn-Lys-Ile-Thr-Pro-Cys-Leu. A database search indicated that this protein belongs to the LTP family with 64% homology and 43% identity with peach LTP (accession number, SWISS PROT P81402). Díaz-Perales et al⁸ reported a similar sequence with only 2 different amino acids: Ala extra in position 1 and Ser in position 12, corresponding to Thr in position 11 in our sequence.

DISCUSSION

In this study we set out to elucidate the relationship between sensitization to mugwort pollen and allergic reactions to peach. We recruited 17 subjects with clear allergic symptoms after eating peach and mugwort-specific IgE antibodies to assess their symptoms during the mugwort season and to study the pattern of IgE reactivity to mugwort and peach and their cross-reactivity.

The seasonal study showed that 7 patients had pollinosis, and 10 had no seasonal rhinitis or asthma, which was confirmed by comparing the seasonal diary records and the pollen count. IgE immunoblotting showed that these 10 patients only reacted to a 9-kd band in peach and

mugwort extracts, whereas the 7 symptomatic patients responded to several proteins in both extracts. The 9-kd peach allergen is already recognized as an LTP and is its major allergen.¹⁴ It has also been recognized in homologous form in mugwort because N-terminal sequence analysis of the mugwort 9-kd protein confirmed its high degree of sequence identity with the peach LTP (43% identity and 64% homology), and it was cross-reactive with the 9-kd peach allergen (Fig 4).

Because all the patients reacting only to this mugwort protein were completely free of seasonal symptoms, sensitization to this allergen presumably has no clinical significance. The low positivity of mugwort-specific IgE antibodies in patients with allergic reactions to Prunoideae fruits should therefore be considered a form of cross-reactivity because of the sensitization to peach LTP and not vice versa. This emerged clearly from the lack of IgE sensitization to the mugwort 9-kd LTP in patients allergic to mugwort but not to peach (Fig 2, patients 18-27). The fact that no one in this group reacted to this allergen strengthens our conclusions.

This cross-sensitization can be ascribed to the presence of common allergenic determinants on mugwort and peach LTPs that are partially homologous, even though the strongest allergen is probably peach LTP, as can be inferred from the immunoblotting cross-inhibition experiments (Figs 3 and 4) with the serum pool and with single sera. The LTP in peach immunoblotting was totally inhibited only by 400 µg of mugwort LTP, hardly at all

by 40 µg, and not at all by 4 µg and was complete with 4 µg of peach LTP. This indicates that only a few allergenic epitopes of peach LTP are present on the mugwort LTP.

Thus considering the lack of seasonal symptoms to mugwort pollen in patients IgE sensitized exclusively to mugwort LTP and the considerable amount of mugwort LTP necessary to inhibit the LTP in the peach immunoblotting, we can conclude that sensitization to mugwort LTP is only an epiphenomenon of the sensitization to peach LTP and not a factor favoring peach allergic reactivity. The same observations hold for the sensitization toward olive LTP that seems to depend exclusively on sensitization to peach LTP. Cross-inhibition experiments showed that peach at a very low concentration totally inhibited IgE binding to the olive LTP but not vice versa. Even though few patients had positive IgE antibodies toward olive pollen (this tree is not frequent in the Milan area), they allow us to extend our considerations to olive, positivity to which could be clinically totally silent when exclusively caused by LTP sensitization.

The absence of cross-reactivity with wall pellitory and ragweed LTPs once again demonstrates that the sensitization to peach is not dependent on sensitization to any LTP-containing pollens but is a real food allergy. We can thus conclude that, unlike the model of birch pollen and apple sensitization, allergy to peach is not pollen dependent or at least does not depend on the pollens we usually consider in the Mediterranean area. In the future, we will try to clarify why so many people in Italy and Spain are sensitized to LTPs in all possible forms.

We cannot, however, explain why patients with positive results only to mugwort LTP have no respiratory symptoms to mugwort pollen, especially because this protein seems amply present in mugwort extract, as shown in SDS-PAGE analysis. We believe it is only the low level of mugwort-specific IgE (Table I), explained by the sensitization to only some epitopes of the mugwort LTP, that elicits a mugwort-positive RAST result with no clinical counterpart. These findings agree with those of our previous report that an RAST positivity of less than 10 kU/L specific IgE antibodies for pollens such as grass and birch is often not associated with seasonal respiratory symptoms, whereas higher values are so associated.¹⁹

In conclusion, this study showed that sensitization to mugwort LTP is a consequence of sensitization to peach LTP and that CAP-RAST positivity to mugwort at a low titer in subjects allergic to peach without any other pollinosis has no clinical expression and can be considered an epiphenomenon of peach LTP sensitization.

REFERENCES

1. Kivity S, Dunner K, Marian Y. The pattern of food hypersensitivity in patients with onset after 10 years of age. *Clin Exp Allergy* 1994;24:19-22.
2. Pastorello EA, Ortolani C, Farioli L, Pravettoni V, Ispano M, Borgia A, et al. Allergenic cross-reactivity among peach, apricot, plum, and cherry in patients with oral allergy syndrome: an in vivo and in vitro study. *J Allergy Clin Immunol* 1994;94:699-707.
3. Ortolani C, Ispano M, Pastorello EA, Bigi A, Ansaloni R. The oral allergy syndrome. *Ann Allergy* 1988;61:47-52.
4. Cuesta-Herranz J, Lazaro M, de las Heras M, Lluch M, Figueredo E, Umpierrez A, et al. Peach allergy pattern: experience in 70 patients. *Allergy* 1998;53:78-82.
5. Sanchez-Monge R, Lombardero M, Garcia-Sellés FJ, Barber D, Salcedo G. Lipid transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;103:514-9.
6. Colombo P, Duro G, Costa MA, Izzo V, Mirisola M, Locorotondo G, et al. *Parietaria* pollen allergen. *Allergy* 1998;53:917-21.
7. Rodriguez R, Villalba M, Monsalve RI, Batanero E. The spectrum of olive allergens. *Int Arch Allergy Immunol* 2001;125:185-95.
8. Díaz-Perales A, Lombardero M, Sánchez-Monge R, García-Selles FJ, Pernas M, Fernández-Rivas M, et al. Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, Castanea nut and Rosaceae fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000;30:1403-10.
9. Bauer L, Ebner C, Hirschwehr R, Wüthrich B, Pichler C, Fritsch R, et al. IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens. Immunoblot investigations of the birch-mugwort-birch-celery syndrome. *Clin Exp Allergy* 1996;26:1161-70.
10. Leitner A, Jensen-Jarolim E, Grimm R, Wüthrich B, Ebner H, Scheiner O, et al. Allergens in pepper and paprika. Immunologic investigation of the celery-birch-mugwort-spice syndrome. *Allergy* 1998;53:36-41.
11. Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1982;38:167-72.
12. Pastorello EA, Pravettoni V, Farioli L, Ispano M, Fortunato D, Monza M, et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999;104:1099-106.
13. Dreborg S. Skin tests used in type I allergy testing. Position paper of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989;44(suppl 10):27-8.
14. Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Ispano M, Monza M, et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999;103:520-6.
15. Lowry OH, Rosenbrough NS, Fair AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;93:265-75.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
17. Neville DM. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J Biol Chem* 1971;246:6328-63.
18. Pastorello EA, Pravettoni V, Farioli L, Ispano M, Fortunato D, Monza M, et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999;104:1099-106.
19. Pastorello EA, Incorvaia C, Pravettoni V, Marelli A, Farioli L, Ghezzi M. Clinical evaluation of CAP System and Rast in the measurement of specific IgE. *Allergy* 1992;47:463-6.