

Allergens in pepper and paprika

Immunologic investigation of the celery-birch-mugwort-spice syndrome

Leitner A, Jensen-Jarolim E, Grimm R, Wüthrich B, Ebner H, Scheiner O, Kraft D, Ebner C. Allergens in pepper and paprika. Immunologic investigation of the celery-birch-mugwort-spice syndrome. Allergy 1998; 53: 36–41. © Munksgaard 1998.

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Mugwort and birch pollen allergy are frequently associated with IgE-mediated hypersensitivity to celery and spices. We analyzed 22 sera from patients with the mugwort-birch-celery-spice syndrome for IgE binding to the spices pepper and paprika by immunoblotting. Immunoblot results revealed two major allergens of 28 and 60 kDa in pepper and a 23-kDa allergen together with allergens of higher molecular weight in paprika. In immunoblot-inhibition studies, crude mugwort, birch pollen, and celery extracts significantly reduced the IgE binding to pepper and paprika allergens. However, no inhibition was achieved with rBet v 1 and rBet v 2, suggesting that no homologs of these birch proteins act as allergens in pepper or paprika extracts. N-terminal sequence analysis of the 14- and 28-kDa pepper and 23-kDa paprika allergens revealed no homology to known allergens. The 28-kDa pepper allergen showed homology to a wheat germin protein, and the 23-kDa paprika allergen was identified as a homolog of a osmotin-like or pathogenesis-related protein in tomato. Therefore, we conclude that the IgE cross-reactivity in the mugwort-birch-celery-spice syndrome to the spices pepper and paprika is not caused by homologs of Bet v 1 and profilin. N-terminal amino acid sequence analysis of the main allergens in pepper and paprika indicate a relation to frequently occurring plant proteins.

Key words: celery-birch-mugwort-spice syndrome; cross-reactivity; paprika; pepper.

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Accepted for publication 23 July 1997

The relationship of pollen allergy and IgE-mediated hypersensitivity to dietary antigens, such as those of vegetables, fruits, nuts, and spices, is well documented (1–8). Pollen- and food-allergic patients suffer from type I allergy symptoms that range from oral and pharyngeal pruritus (oral allergy syndrome [OAS]) to severe anaphylactic reactions.

Birch pollen and mugwort pollen are important causes of pollinosis in Europe, where a high incidence of associated adverse reactions to raw or cooked celery has been reported (9–14). Combined mugwort pollen and celery allergy were also found to be clinically related to type I reactions to spices (3). This was confirmed by high RAST values to spices in sera from celery-mugwort-sensitive patients (6, 12, 13). Consequently, the designation celery-mugwort-spice syndrome was proposed (3, 12). Moreover, RAST and IgE immunoblot analysis revealed an additional birch pollen

sensitization in mugwort-birch-celery-allergic patients (13, 14). Homologs of the birch pollen allergens Bet v 1 and Bet v 2 were detected in almost all plant-derived food investigated (15–22). Recently, a new class of cross-reactive allergens in celery, mugwort, and birch pollen have been demonstrated by IgE immunoblot analysis in a molecular weight range of 46–60 kDa (14).

However, regarding the molecules eliciting cross-reactivity phenomena in the celery-mugwort-birch syndrome with concomitant spice sensitization, only limited information is available. We investigated sera from patients clinically suffering from celery-mugwort-spice syndrome with additional birch pollen sensitization by immunoblotting and IgE inhibition in order to characterize potential cross-reactive allergens present in the most widely used spices: pepper (*Piper nigrum*) of the Piperaceae family and paprika (*Capsicum annum*) of the Solanaceae family.

Table 1. Summary of clinical symptoms (a: angioedema, ad: atopic dermatitis, as: asthma, c: conjunctivitis, e: eczema, n: nausea, rc: rhinoconjunctivitis, sb: shortness of breath, u: urticaria), RAST classes, and results of birch pollen, mugwort pollen, celery, pepper, and paprika IgE immunoblots, and of skin prick test results

Patients			Symptoms		RAST/IgE-immunoblot					Skin prick test	
No.	Age (years)	Sex	Local	Systemic	Birch pollen	Mugwort pollen	Celery	Pepper	Paprika	Pepper	Paprika
1	49	F	—	n, sb, u	4/+	2/+	nd/+	nd/—	nd/+	—	—
2	48	F	+	sb	6/+	2/+	2/+	0/+	1/+	—	+
3	46	M	+	a, sb	3/+	3/+	2/+	2/+	2/+	—	—
4	30	M	+	as, u	5/+	3/+	0/+	0/—	1/+	—	—
5	39	F	+	a, u	3/+	1/+	3/+	0/—	0/+	nd	nd
6	38	F	—	a, sb, u	3/+	4/+	2/+	2/+	3/+	—	—
7	15	F	+	e, rc, sb	nd/+	nd/+	nd/+	nd/+	nd/+	nd	nd
8	29	F	+	sb, rc	6/+	3/—	nd/+	nd/+	nd/+	—	—
9	32	M	+	sb, u	5/+	3/+	2/+	0/—	0/+	—	+/-
10	48	F	+	e	4/+	4/+	2/+	0/+	2/+	—	+
11	52	M	+	a, rc	0/+	3/+	0/+	0/—	0/+	—	—
12	36	M	+	sb, u, rc	2/+	3/+	1/+	2/+	2/+	—	—
13	40	F	—	c, sb	3/+	2/+	0/+	0/—	0/—	—	—
14	36	M	+	—	2/+	2/+	2/+	nd/+	nd/+	+/-	+/-
15	35	F	+	as, u, rc	4/+	3/+	2/+	1/+	2/+	—	—
16	27	M	+	as	2/+	3/+	2/+	nd/+	nd/+	+/-	—
17	32	F	+	a, sb, u	3/+	3/+	2/+	2/+	2/+	—	—
18	49	F	+	rc, u	4/+	1/+	3/+	2/+	2/+	+/-	+/-
19	42	F	—	ad	0/+	4/+	0/+	2/+	2/+	—	—
20	27	M	—	e, c, u	4/+	3/+	2/+	1/+	2/+	—	+
21	27	F	+	sb, rc	6/+	3/+	2/+	2/+	2/+	—	—
22	41	F	—	rc	6/+	2/+	4/+	2/+	3/+	nd	nd

nd: not done, +: positive, -: negative.

Material and methods

Patients

Serum samples from 22 patients with mugwort-birch-celery-spice syndrome from Austria and Switzerland were investigated. All patients suffered from inhalant allergy during the flowering season of mugwort and birch pollen and had experienced adverse reactions to food, especially celery tuber and spiced food. Some 21/22 patients' sera displayed IgE binding to mugwort pollen, celery tuber, and birch pollen proteins in immunoblotting. In 30/34 (88%) experiments, RAST results with pepper and paprika correlated with immunoblotting. The patients' characteristics are summarized in Table 1.

A serum pool from persons without indications of type I allergy and 10 sera collected from patients with high total IgE levels and suffering from other allergies were used for control experiments, showing no IgE binding to blotted pepper and paprika extract (data not shown).

Protein extracts

Pickled green peppercorns, ground green and ground black peppercorns, and paprika powder were purchased from a local retail shop. Proteins

were extracted by overnight shaking at 4°C in 10 mmol/l potassium phosphate buffer (pH 7.0) containing 2% (w/v) suspended solid polyvinylpyrrolidone (PVPP), 2 mmol/l ethylenediaminetetraacetic acid (EDTA), 10 mmol/l sodium diethyldithiocarbamate (DIECA), and 3 mmol/l sodium azide. After centrifugation at 40 000 g for 60 min at 4°C, supernatants were extensively dialyzed (molecular porous dialysis membrane, Spectra/Por 1, cutoff: 6–8 kDa; Spectrum, Houston, TX, USA) against 10 mmol/l potassium phosphate buffer (pH 7.0) containing 3 mmol/l sodium azide. Dialysates were lyophilized and stored at -20°C. Protein concentrations were determined by the method of Bradford (23). The protein content of 1 g paprika powder was about 5 mg and the protein yield of 1 g of peppercorns was about 0.5 mg. For further analysis, extracts of pickled and ground peppercorns were used in pooled form.

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS–PAGE), protein transfer, and immunoblotting

SDS–PAGE (12% homogeneous gel, 140×115×1 mm, 5% stacking gel) was performed by the method of Laemmli (24) in a vertical slab gel

apparatus (Hoefer, San Francisco, CA, USA) with modifications, as previously described (25), loading 1 mg of protein extract. Protein samples were mixed with Laemmli's sample buffer in 4 : 1 ratio before heating at 95°C for 5 min, and subsequently loaded onto gels. The electrophoretic transfer of proteins from gels to nitrocellulose sheets was performed by the method of Towbin et al. (26). IgE was detected by immunoblotting, as previously described (25). Briefly, nitrocellulose strips were saturated with 50 mmol/l sodium phosphate buffer (pH 7.5) containing 0.5% (w/v) bovine serum albumin, 0.05% (w/v) sodium azide, and 0.5% (v/v) Tween 20. Patients' sera were diluted 1 : 4 with the same buffer, and nitrocellulose strips were incubated with 1 ml diluted serum under continuous shaking overnight at 4°C. Bound IgE was detected by ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden), 300 000 cpm/ml.

IgE-inhibition experiments

Inhibition with crude extracts of birch pollen, mugwort pollen, celery tuber, pepper, and paprika. Patients' sera reacting with the major allergens of pepper and paprika in IgE immunoblot analysis were diluted as described above. A volume of 1 ml diluted serum was preincubated with 50 µg of crude protein extract of birch pollen, mugwort pollen, celery tuber, pepper, and paprika (overnight, 4°C) before application to nitrocellulose strips. Incubations with sera were carried out under continuous shaking overnight at 4°C. After washing, bound IgE was detected as described above.

Inhibition with recombinant Bet v 1 (rBet v 1) and recombinant Bet v 2 (rBet v 2). Alternately, diluted patients' sera were preincubated (overnight, 4°C) with 10 µg rBet v 1 (27) or 10 µg rBet v 2 (28), subsequently added to nitrocellulose strips with blotted proteins, and incubated overnight at 4°C. Detection of bound IgE was again performed as described above.

Amino (N)-terminal amino acid sequencing of proteins

Amounts of 1 mg of pepper protein extract and 1 mg of paprika protein extract were separated by 12% SDS-PAGE and electroblotted onto polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). IgE-binding protein bands (50–100 µg protein/band) were excised and subjected to protein sequence analysis by the Hewlett-Packard HP G1005A protein-sequencing system, as described previously (29).

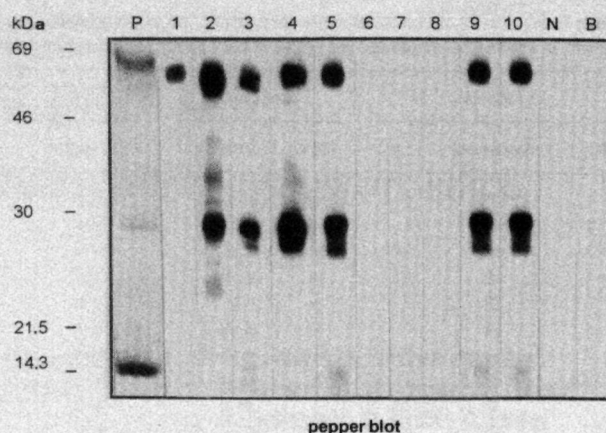


Fig. 1. SDS-PAGE analysis and IgE binding to pepper extract. Coomassie brilliant blue-stained 12% SDS-polyacrylamide gel showing separated pepper protein extract (lane P). Representative IgE immunoblots of five sera from patients with celery-birch-mugwort-spice syndrome on pepper extract (lanes 1–5). Inhibition of IgE immunoblots: one representative serum (no. 5) preincubated with 50 µg birch pollen, mugwort pollen, or celery tuber protein extracts (lanes 6–8), 10 µg rBet v 1 (lane 9), and 10 µg rBet v 2 (lane 10); lanes N and B) normal human serum and buffer controls.

Results

SDS-PAGE, IgE-immunoblot, and IgE-inhibition studies

Pepper. Pepper extract was first analyzed in Coomassie brilliant blue-stained SDS-polyacrylamide gels, where proteins ranged from 14 to 70 kDa (Fig. 1). Some 16/22 (73%) sera of patients with celery-birch-mugwort-spice syndrome demonstrated IgE binding on blotted pepper extract (Fig. 1, lanes 1–5). The most prominent IgE-binding protein bands that were recognized by 15/16 (93%) patients' sera were detected at 28 and 60 kDa. Two sera showed additional IgE binding to minor allergens of 14, 25, 30, 35, and 40 kDa; one serum reacted only to the 60-kDa allergen.

To determine which molecules were responsible for IgE cross-reactivities between mugwort pollen, celery tuber, birch pollen, and pepper, we performed immunoblot inhibition experiments. Preincubation of patients' sera with crude extract of mugwort pollen, celery tuber, or birch pollen significantly inhibited IgE binding to blotted pepper extract (Fig. 1, lanes 6–8). Preincubation of patients' sera with rBet v 1 and rBet v 2 did not reduce IgE binding to any pepper allergen (Fig. 1, lanes 9–10). IgE binding to blotted mugwort pollen proteins could be blocked by preincubation of patients' sera with pepper extract (not shown).

Paprika. In Coomassie brilliant blue-stained SDS-polyacrylamide gels, proteins of paprika extract ranged from 14 to 48 kDa (Fig. 2). Some 21/22

Table 2. N-terminal amino acid sequences of major allergens in pepper and paprika extracts

14-kDa pepper allergen: no homology found
 28-kDa pepper allergen: 58% identity with/70% similarity to wheat germin
 23-kDa paprika allergen: 100% identity with pathogenesis-related protein PR P23

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    1 A I T D X S A V T K S T A Q X M N Y L V G K E P T P S27
    2 D P S P L Q D F X V A D L K S - P I R V N G F V23
      | | | | | | | | | | | | | | | | | | | |
    25 D P D P L Q D F C V A D L D G K A V S V N G H T48
      | | | | | | | | | | | | | | | | | | | |
    27 A T F E V R N N X P10
      | | | | | | | | | | | | | | | | | | | |
    29 A T F E V R N N C P23
    
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X: not determined.

(95%) sera of patients with celery-birch-mugwort-spice syndrome recognized paprika allergens in the range of 23 to 50 kDa in IgE immunoblots (Fig. 2, lanes 1–5).

On blotted paprika extract, IgE binding was significantly inhibited by preincubating patients' sera with crude mugwort pollen, celery tuber, and birch pollen extract (Fig. 2, lanes 6–8). IgE binding to blotted paprika extract was not affected by preincubation of patients' sera with rBet v 1 and rBet v 2 (Fig. 2, lanes 9–10).

IgE binding on mugwort pollen blot was inhibited by preincubation of patients' sera with paprika extract (not shown).

Characterization of pepper and paprika allergens by N-terminal amino acid sequence analysis

Pepper and paprika allergens found in these IgE immunoblot experiments were further characterized by N-terminal sequencing. Single amino acids were obtained for the 14- and 28-kDa pepper proteins as well as for the 23-kDa paprika allergen.

The analyzed N-terminal amino acids of the 14-kDa pepper allergen did not indicate significant homology to described proteins (Table 2).

The analyzed 23 N-terminal amino acids of the 28-kDa pepper allergen showed 70% similarity to and 58% identity with the N-terminus of the wheat germin protein (SWISS-PROT: P15290) (30).

The N-terminal amino acid sequence (9 aa) of the 23-kDa paprika allergen showed 100% identity with the N-terminal sequence of the tomato protein called osmotin-like protein or pathogenesis-related protein PR P23 (SWISS-PROT: Q01591) (31).

Discussion

The clinical observation of an association of celery-mugwort-birch pollen allergy with hypersensitivity to spices has been verified by RAST analysis (8, 13). In a previous study analyzing 402 patients with food allergy, 23 (5.7%) had food allergy to paprika/pepperoni and four (1%) to pepper (11). However, in an investigation of patients allergic to mugwort

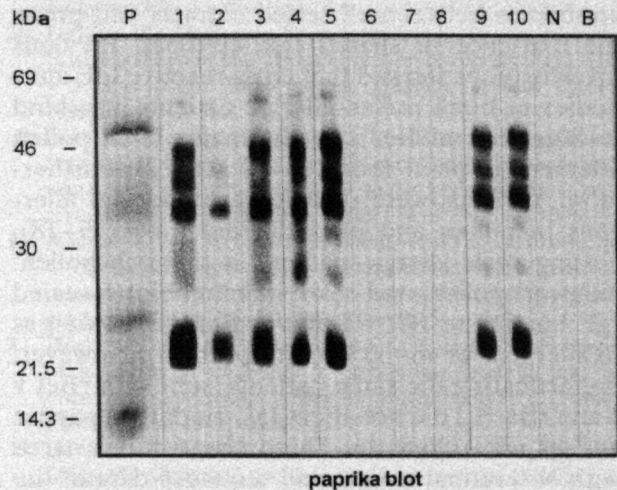


Fig. 2. SDS-PAGE analysis and IgE binding to paprika extract. Coomassie brilliant blue-stained 12% SDS-polyacrylamide gel shows separation of paprika protein extract (lane P). Representative IgE immunoblots of five sera from patients with celery-birch-mugwort-spice syndrome on paprika extract (lanes 1–5). Inhibition of IgE immunoblots: one representative serum (no. 5) preincubated with 50 µg birch pollen, mugwort pollen, or celery tuber protein extracts (lanes 6–8), 10 µg rBet v 1 (lane 9), and 10 µg rBet v 2 (lane 10); lanes N and B) normal human serum and buffer.

pollen, Wüthrich et al. showed that 52% of them displayed RAST classes between 1 and 3 for black pepper and paprika (13). Moreover, the spices pepper and paprika were chosen for this investigation because of their frequent use in the European cuisine. We report here that 16/22 (73%) of patients who were clinically suffering from the mugwort-birch-celery-spice syndrome displayed IgE binding to pepper and 21/22 (95%) to paprika proteins in immunoblotting. However, in most cases, it was not possible to elicit positive SPT with commercially available test substances, a fact which limits the diagnostic power of those extracts in the diagnosis of spice allergy. In our study, in 3/16 (19%) pepper- and 6/21 (29%) paprika-sensitive subjects, SPT with commercially available protein extracts were positive (Table 1).

As expected, IgE binding to pepper and paprika allergens were markedly inhibited by preincubating patients' sera with crude celery tuber, mugwort, and birch pollen extracts (Figs. 1 and 2). In contrast to data described for apple–birch cross-reactivity – where preabsorption of apple-specific IgE could not completely inhibit IgE reactivity to birch pollen (15) – inhibition with pepper and paprika extract displayed the capacity to block IgE binding to birch pollen, mugwort pollen, and celery allergens completely (data not shown). These findings indicate cross-reactivity of IgE antibodies between all tested extracts and prove the existence of shared IgE epitopes. Previous investigations showed that cross-reactive IgE antibodies of birch pollen-allergic patients also bind to homologs of Bet v 1 (the major birch pollen allergen) in plant food (15–17, 21, 22). Furthermore, profilins were revealed as important allergens in pollen and plant-derived food (16–18). Testing spice-allergic patients with birch pollen, mugwort pollen, and celery immunoblot revealed IgE binding to Bet v 1 and/or Bet v 2, as well as to allergens of 46–60 kDa (not shown). However, preincubating the same patients' sera with rBet v 1 and rBet v 2 did not affect IgE binding to pepper and paprika allergens. These observations agree with N-terminal amino acid sequence data of the 14- and 28-kDa pepper allergens and the 23-kDa paprika allergen, indicating no sequence similarity to either Bet v 1 or Bet v 2. As inhibition with complete extracts led to blocking of IgE binding to spice allergens, other proteins, e.g., the recently described cross-reactive allergen group of 46–60 kDa in the mugwort–birch–celery spice syndrome (which is recognized by all our patients' IgE), might play a crucial role in cross-reactivity to pepper and paprika (14).

Selected patients' sera displaying IgE binding to the 23-kDa paprika allergen also recognized a 23-kDa protein in tomato and potato extracts (not shown). The N-terminal sequence analysis of the 23-kDa paprika allergen indicated 100% identity with the pathogenesis-related tomato protein P23, belonging to a class of intracellular pathogenesis-related proteins of the botanic family Solanaceae (31). The pathogenesis-related protein P23 in Solanaceae has properties important for the survival of the plant (31).

Furthermore, on the basis of N-terminal sequence analysis, we conclude that the 28-kDa pepper allergen represents a member of the germin gene family (30). Germin-like proteins were detected in plants of botanically distinct families, e.g., in wheat and barley or white mustard, where they were found to be associated with the cell wall metabolism in young leaves or roots (32, 33).

In conclusion, we describe new cross-reacting pollen-associated food allergens. Interestingly, in the case of pepper and paprika, the cross-reactivity did not occur on the basis of panallergens such as profilins or Bet v 1.

Acknowledgment

This work was supported by grants S06704-MED and S06707-MED from the Fonds zur Förderung Wissenschaftlicher Forschung (FWF), Vienna, Austria.

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