

Characterization of allergens in *Apiaceae* spices: anise, fennel, coriander and cumin

E. JENSEN-JAROLIM, A. LEITNER, R. HIRSCHWEHR, D. KRAFT, B. WÜTHRICH*, O. SCHEINER, J. GRAF and C. EBNER

Institute of General and Experimental Pathology, University Hospital AKH, Vienna, Austria and *Allergy Unit, Department of Dermatology, University Hospital Zürich, Switzerland

Summary

Background Symptoms elicited by IgE-mediated food allergy range from mild local to severe systemic reactions. Allergens in spices are particularly dangerous due to their hidden presence in many dishes.

Objectives and Methods According to clinical observations, mugwort and birch pollen allergy, and hypersensitivity to spices are frequently associated, but the crossreacting compounds were not defined so far. We tested sera of 15 patients who experienced adverse reactions to spiced food and characterized their IgE-binding patterns on anise, fennel, coriander and cumin extracts through immunoblot and inhibition experiments.

Results The use of anti-Bet v 1 (MoAb) and anti-profilin (rabbit) antibodies revealed the presence of crossreacting allergens in the tested spice extracts. Inhibition experiments showed that IgE-binding to allergens in *Apiaceae* spices could be blocked by preincubation of sera with rBet v 1 or rBet v 2 (birch profilin). Moreover, we detected crossreacting allergenic molecules in the molecular weight range of 60 kDa. IgE-binding to spice allergens occurred only with sera of 10/15 (66%) patients with allergy to pollen (birch, mugwort) and/or celeriac. In five out of 15 (33%) patients with a history of adverse reaction to spices, but without pollen and celeriac allergy, no IgE-binding to any spice protein could be demonstrated. It is possible that these clinical reactions could be elicited by other types of hypersensitivity (Type II, III, IV), however, as spices contain highly reactive substances, the symptoms may most likely be classified as food-intolerant.

Conclusions Bet v 1- and profilin-related allergens may, besides higher molecular weight allergenic molecules, be responsible for Type I allergy to anise, fennel, coriander or cumin, members of the *Apiaceae*.

Keywords: *Apiaceae*, spices, IgE, allergy, intolerance, immunoblot, Bet v 1, Bet v 2

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Introduction

Allergy to plant-derived food stuff may cause the oral allergy syndrome (OAS), but also affects the skin, the gastrointestinal or respiratory tract [1]. An appreciable number of patients may also experience severe anaphylactic reactions [2]. Food allergy is often associated with pollen allergy due to crossreactive allergens [3–12] and a primary

sensitization via pollen is most likely [12–14]. Mugwort and birch pollen allergic patients represent a high risk group for developing food allergy to celeriac [15–17] or related plant food, as, e.g. spices of the *Apiaceae* family [18–23]. For this clinical situation the term ‘mugwort-celery–spice syndrome’ has been proposed [17]. High total IgE and high levels of specific IgE to the inhalant allergen seem to be a prognostic factor for the development of an OAS [24]. Analysing IgE-binding in sera from patients with ‘birch-mugwort–celery syndrome’, Bauer *et al.* [25] showed that IgE-binding to Bet v 1 (or -homologues) and/or profilins

Correspondence: E. Jensen-Jarolim, Institute of General and Experimental Pathology, AKH, Erw. bau Ost, Währinger Gürtel 18–20, A–1090 Vienna, Austria.

[26–28] explains the observed clinical crossreactivities to a great part. Moreover, allergens of ≈ 60 kDa could be identified as crossreactive agents in birch and mugwort pollen, and celeriac [25]. Regarding spices, only indirect experimental evidence supported crossreactivity [15,16,20,29]. Anise, fennel, coriander and cumin, from the family *Apiaceae* are traditionally used in the European and international cuisine. The special impact of spices in food allergy may be due to the minute amounts hidden in dishes leading to allergic reactions. The characterization of the components eliciting crossreactivity between mugwort pollen, birch pollen, celeriac, and spices of the *Apiaceae* family, was the objective of the present study. However, spices also contain a number of pharmacological compounds potentially causing local irritation, obviously leading to non-IgE-mediated or food intolerance reactions in some patients.

Materials and methods

Patients

Characteristics of patients are outlined in Table 1. We selected our patients according to history of adverse reactions after the ingestion of spices or spiced food. Symptoms varied between local effects like irritation of the oral mucosa, and systemic effects as pruritus, urticaria, angioedema, exacerbation of atopic dermatitis, and gastrointestinal or respiratory symptoms. 10 patients (Nos. 6–15; Table 1) also suffered from pollen allergy to trees and/or mugwort. Two-thirds of the patients were also skin-prick tested using extracts from the spices anise, fennel, coriander and cumin.

Protein extracts

Anise, fennel, coriander and cumin were purchased in a local shop and were homogenized in a food processor. Proteins were extracted 10% (w/v) in 10 mmol/L potassium phosphate buffer (pH 7.0) containing 2% (w/v) suspended solid polyvinylpyrrolidone (PVPP), 2 mmol/L ethylene diamine tetra-acetic acid (EDTA), 10 mmol/L sodiumdiethyldithiocarbamate (DIECA), and 3 mmol/L sodium azide, by overnight shaking at 4 °C. After centrifugation at 40 000 *g* for 60 min at 4 °C, supernatants were extensively dialysed (molecularporous dialysis membrane, Spectra/Por 1, cut-off: 6–8000 kDa; Spectrum, Houston, Texas, 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 according to the method of Bradford [30].

SDS-PAGE and immunoblotting

SDS-PAGE (12% separation gel, 5% stacking gel) was

performed according to the method of Laemmli [31] in a vertical slab gel apparatus (Hoefer, San Francisco, California, USA), briefly: samples were mixed with Laemmli sample buffer in 4:1 ratio prior to heating at 95 °C for 5 min and subsequently loaded onto gels (100 μ g per cm gel). The electrophoretical transfer of proteins from gels to nitrocellulose sheets was performed according to the method of Towbin *et al.* [32].

IgE-immunoblotting

IgE-detection by immunoblotting was done as previously described [33], briefly: nitrocellulose strips were saturated with a 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 sodium phosphate buffer (pH 7.5) and incubated with nitrocellulose blotted proteins (1 mL diluted sera/strip) under continuous shaking overnight at 4 °C. Bound IgE was detected by ^{125} I-rabbit antihuman IgE (Pharmacia, Uppsala, Sweden), 300 000 cpm/mL. Blots were then washed, dried and exposed to Kodak X-omat film at –70 °C.

IgE-inhibition with crude extracts of birch pollen, mugwort pollen, and celeriac

Patients' sera were selected according to their IgE-binding in immunoblots and diluted as described above. One millilitre diluted serum was preincubated with 50 μ g of crude protein extract of birch pollen, mugwort pollen or celery tuber (overnight, 4 °C) prior to application to anise, fennel, coriander and cumin blots. Incubation with native or inhibited sera was carried out under continuous shaking overnight at 4 °C. After washing, bound IgE was detected as described above.

IgE-inhibition with rBet v 1 and rBet v 2

Inhibition experiments were essentially carried out as described above, but this time sera were pre-incubated with 15 μ g rBet v 1 [23] or 15 μ g rBet v 2 [24] per mL diluted serum per strip with blotted spice extract.

Immunoblotting with monoclonal and polyclonal monospecific antibodies

The mouse monoclonal antibody BIP 1 detecting an epitope of Bet v 1 (17 kDa) [34] or alternately a polyclonal monospecific rabbit anti-celery profilin antibody (kindly provided by Dr Philippe Deviller, Lyon, France) were used. Briefly: after blocking, as described above, 1 mL BIP hybridoma supernatant or 1 mL antiprofilin antibody (1:1000) were

Table 1. Characteristics of patients with adverse reactions to spices

Patient	Symptoms	After ingestion of	IgE in immunoblot to:										Age			
			M	B	C	A	F	Co	Cu	1	2	3	PRIST	Sex	(years)	
1	Pruritus, dyshidrosis	Spices, beer	0	0	0	0	0	0	0	0	0	0	0	ND	F	65
2	Otolaryngeal irritation, angioedema, chronic urticaria	Spices, vegetable food	0	0	0	0	0	0	0	0	0	0	0	85	M	34
3	Otolaryngeal irritation, angioedema, pruritus, cephalaea	Spiced salad	0	0	0	0	0	0	0	0	0	0	0	242	M	33
4	Atopic dermatitis	Spices, nuts, poppy seed, egg, fish	0	0	0	0	0	0	0	0	0	0	0	95	F	45
5	Otolaryngeal irritation, angioedema, urticaria, wheezing	Gulyas, spiced meat and sausages, spiced bread, pizza, chocolate	0	0	0	0	0	0	0	0	0	0	0	528	M	29
6	Rhinitis, asthma, wheezing, diarrhoea	Spiced fish and cheese, apples, nuts, chocolate	+	+	+	+	+	+	+	0	+	+	0	87	M	30
7	OAS, angioedema, wheezing	Spiced beef, goat cheese, maizeflour	+	+	+	+	0	0	0	0	0	+	+	926	M	52
8	OAS, angioedema, urticaria, rhinitis, wheezing	Pizza bread, celeriac, honey	+	+	+	+	+	+	+	0	+	+	+	280	F	38
9	OAS, angioedema, rhinitis	Spiced bread, vegetable food and fruits	+	+	+	+	+	0	+	+	+	0	0	1411	F	48
10	OAS, angioedema, rhinitis, abdominal pain	Paprika, tomatoes, kiwi, apples	+	+	+	+	+	+	+	0	+	+	+	1372	F	44
11	Abdominal pain	Spiced bread, celeriac, raw carrots, honey	0	+	+	+	+	+	+	0	0	+	+	79	F	53
12	Sickness, diarrhoea, rhinitis	Spices (paprika), tomatoes, instant soup, apples, nuts	0	+	+	+	+	0	0	+	0	0	0	114	F	29
13	Sickness, vomiting, diarrhoea	Spices, celeriac, carrots, apples, nuts, plums	0	+	+	+	+	0	+	+	+	0	0	90	F	51
	Anaphylactic shock after:	Fish														
14	Abdominal pain, eczema, wheezing, rhinitis	Spiced drinks, tea, apples, nuts	0	0	+	+	+	+	0	0	+	0	0	262	F	54
15	Sickness, rhinitis, asthma	Spiced vegetable food, apples, kiwis, apricots	0	+	0	+	+	+	+	+	+	0	0	81	F	25

M, mugwort pollen; B, birch pollen; C, celeriac; A, anise seed; F, fennel; Co, coriander; Cu, cummin; 1, Bet v 1 homologue in spices; 2, Bet v 2 homologue in spices; 3, higher molecular weight allergens (50–70 kDa) in spices.

applied, overnight at 4 °C. Bound BIP mouse antibody was detected by ^{125} I-labelled sheep anti-mouse immunoglobulin (Amersham International Ltd, UK); bound rabbit antibody was detected by ^{125} I-labelled donkey anti-rabbit antibody (Amersham International Ltd, UK), 100 000 cpm/mL.

Results

Patients' IgE-binding to blotted spice extracts

Patients' sera were tested in IgE immunoblot using anise,

fennel, coriander or cumin extracts as antigens (Fig. 1). Ten patients (Nos. 6–15), who also suffered from pollen allergy with positive IgE in mugwort pollen, birch pollen and celeriac immunoblots and showed respective symptoms with spiced food, revealed intense IgE-binding to 14, 17 and 50–70 kDa allergens in anise and fennel extracts. 6/10 patients (No. 6, 9 and 12, 13 and 14, 15; Fig. 1) displayed IgE-binding to a 17 kDa allergen, 2/10 (Nos. 8, 10; Fig. 1) reacted with a 14 kDa molecule, three patients (Nos. 8, 10 and 11; Fig. 1) recognized allergens with molecular weights ranging from 50 to 70 kDa. Patients who reacted strongly

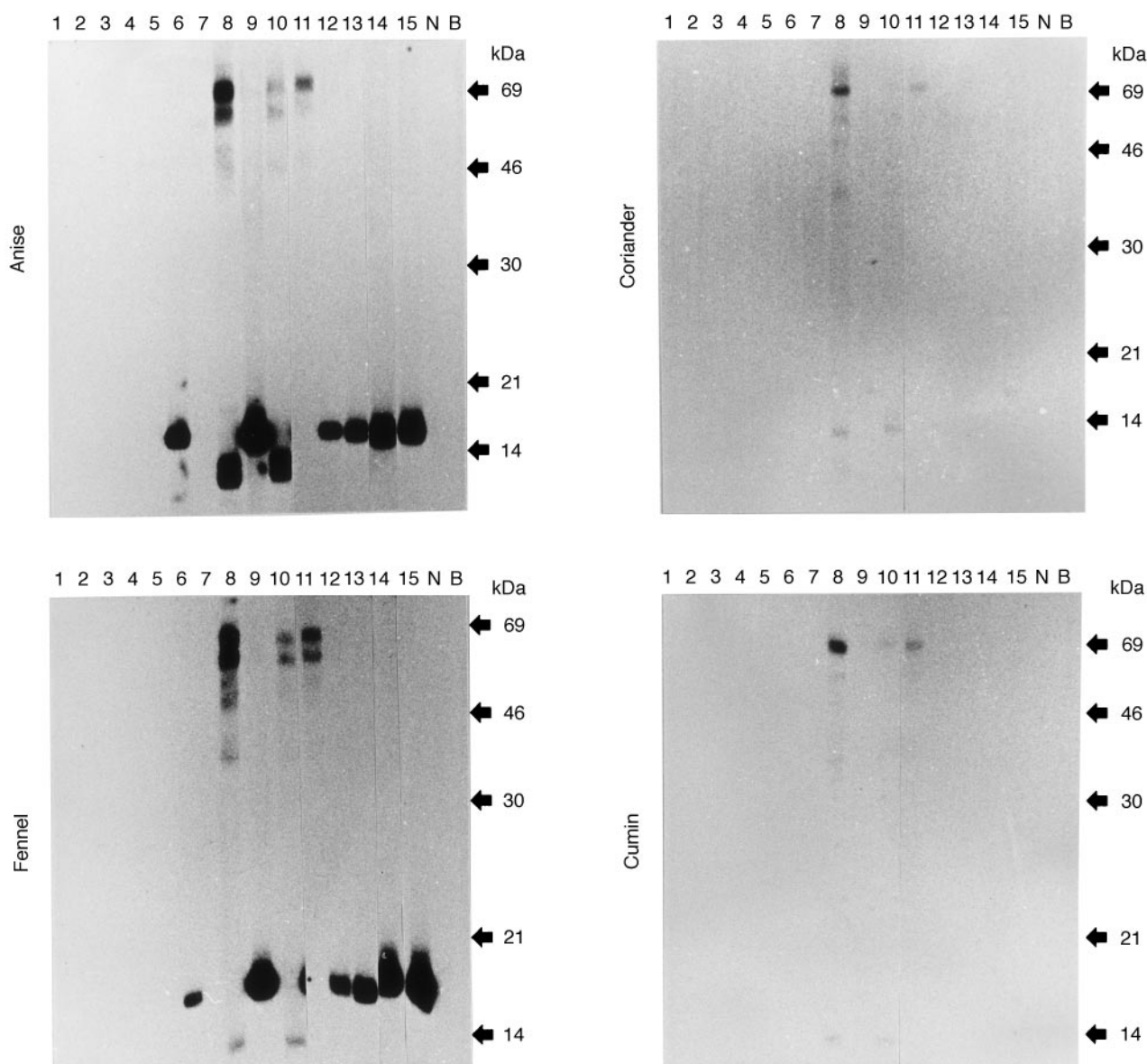


Fig. 1. IgE immunoblots on extracts of anise, fennel, coriander and cumin. Numbers on top correspond to patients' numbers (see Table 1). N, Serum from a healthy non-allergic volunteer. B, Buffer control.

with a 17 kDa allergen in anise and fennel did not detect a 17 kDa allergen in coriander or cumin, except for patients 9 and 15 who showed very weak IgE-binding to a 17 kDa allergen. Patients 1–5 had no IgE-binding to mugwort, birch pollen and celeriac extracts, and exhibited also no IgE-binding to the tested spice extracts.

Detection of Bet v 1 and Bet v 2 homologues in spices

The monoclonal anti-Bet v 1 antibody BIP1 showed binding to 16–17 kDa compounds in all spices tested, thus pointing at the presence of molecules immunologically related with Bet v 1 in extracts from anise, fennel, coriander and cumin (Fig. 2). The polyclonal rabbit anticelery profilin antibody bound to 13 kDa (in coriander) to 14 kDa (in anise and cumin) proteins in immunoblot experiments, indicating the presence of profilins in the spices tested.

IgE-inhibition experiments using native allergen extracts and recombinant allergens (rBet v 1 and rBet v 2)

Two patients' sera displaying typical IgE-binding patterns on blotted spice extracts were used for inhibition experiments. Patient 15 exhibited IgE-binding to a 17 kDa allergen

exclusively (Fig. 3, lanes 1). This IgE-binding was inhibited by preincubation with rBet v 1 (Fig. 3, lanes 2). Patient 8 was selected for IgE binding to 13 and/or 14 kDa molecules, and to allergens with higher molecular weights ranging from 25 to 70 kDa on blotted anise, fennel, coriander and cumin extracts. Binding of IgE to the 14 kDa allergens in anise, was completely blocked by pre-absorption of IgE with rBet v 2 (Fig. 3, lanes 4). Using extracts from mugwort pollen, birch pollen and celeriac, IgE-binding to all allergens in fennel, coriander and cumin was completely extinguished, to anise allergens markedly reduced (Fig. 3, lanes 5, 6, 7). Complete inhibition of the 60 kDa structure in anise could be achieved by the use of higher doses of inhibitor (not shown).

Discussion

IgE-crossreactivity to homologues of Bet v 1 and/or profilin (Bet v 2-related proteins) in plant-derived food is critical in patients with allergy to birch pollen, mugwort pollen and celeriac [12,25,35,36]. Furthermore, the major allergen of celeriac, Api g 1, was recently shown to be 40% identical with Bet v 1, thus explaining clinical crossreactivity of birch pollen and the food allergen celeriac [12]. Homologues of Bet v 1 were, however, not found in mugwort pollen so

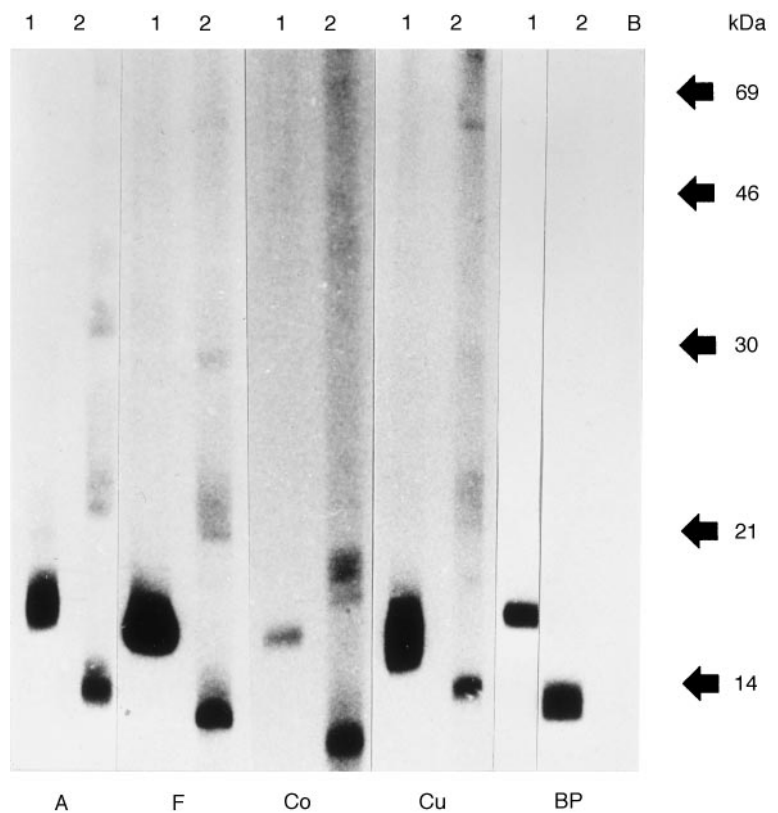


Fig. 2. Monoclonal anti-Bet v 1 antibody BIP 1 (lanes 1) and polyclonal rabbit anticelery profilin antibody (lanes 2) bind to proteins of anise (A), fennel (F), coriander (Co), cumin (Cu) and birch pollen (BP) extracts. B, Buffer control.

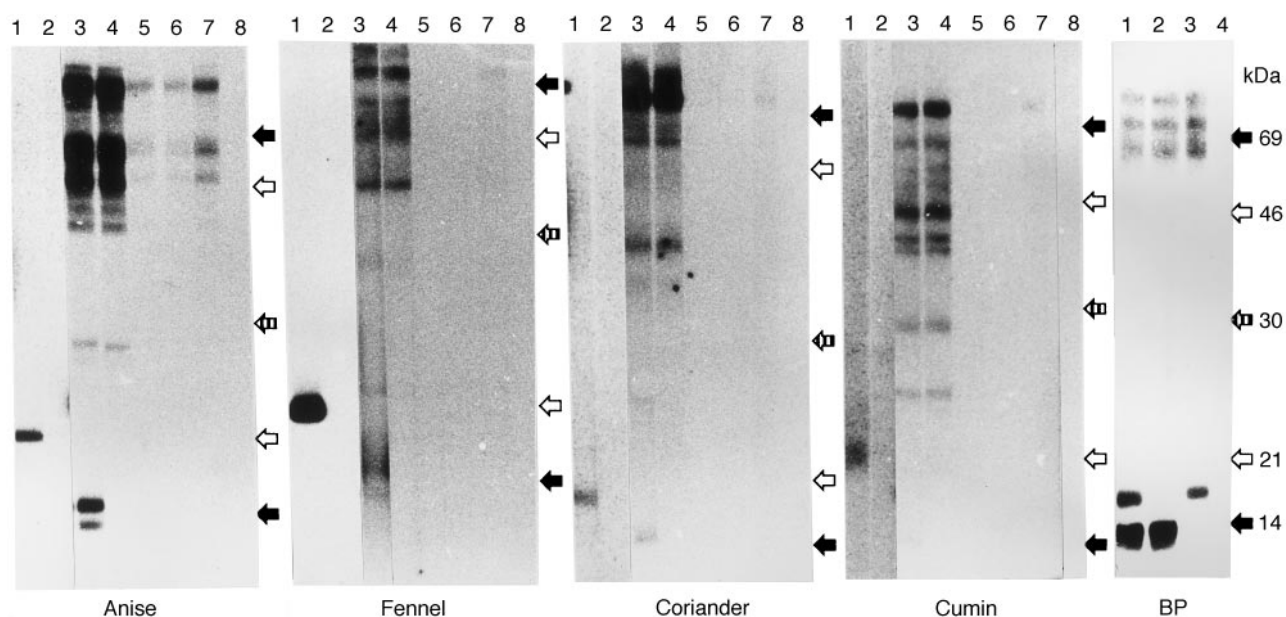


Fig. 3. IgE-immunoblot inhibition on anise, fennel, coriander, cumin and birch pollen (BP) extracts. IgE-binding from patient no. 15 is shown before (lanes 1) and after preincubation with rBetv1 (lanes 2). Serum from patient no. 8 is shown before (lanes 3) and after preincubation with rBetv2 (lanes 4), crude mugwort pollen (lanes 5), birch pollen (lanes 6) or celeriac extracts (lanes 7). Lanes 8: Serum from a healthy non-allergic volunteer.

far. In this case, IgE crossreactivity to profilins can be assumed to play a significant role [28,35]. Besides Bet v 1 and Bet v 2, crossreactive allergens with higher molecular weight (≈ 60 kDa) represent a third group of crossreactive allergens in the birch-celery-mugwort syndrome [25,32]. We revealed that homologues of Bet v 1, of Bet v 2, and of this third group of allergens are also existing in spices of the *Apiaceae*. This explains the frequent coincidence of the birch-mugwort-celery syndrome with food allergy to spices (mugwort-celery-spice syndrome [17]).

Results of our skin-prick tests using *Apiaceae* spices as antigen (data not shown) did not regularly correspond with the clinical situation of the patients, however, the specificity of skin-prick tests for diagnosing food allergy is still controversial [1].

In immunoblot, rather high IgE-binding capacity was observed in extracts from seeds of anise and fennel, as compared to coriander and cumin at corresponding protein concentrations. The IgE-binding patterns in immunoblot (especially regarding extracts from anise and fennel), were similar to those observed on blotted birch pollen, mugwort and celeriac extract (data not shown). There was, however, a marked difference regarding the IgE-binding to a 17 kDa allergen among *Apiaceae* spices: of the six patients showing IgE-binding to this Bet v 1-homologous in anise and fennel extracts, only two detected weakly this protein in extracts of

coriander and cumin. In contrast, the monoclonal anti-Bet v 1 antibody, BIP 1 [34] detected Bet v 1 homologues in all tested spice extracts. Thus, the 17 kDa anise and fennel (Bet v 1-related) allergen may harbor epitopes, which are present on Bet v 1, but which are not similarly present on the coriander or cumin homologue.

The patient group classified as spice-allergic was dominated by females (80%), an as yet unexplained phenomenon, which is in agreement with earlier reports [17]. Only patients with birch and/or mugwort pollinosis and/or celeriac allergy showed IgE-binding to the tested spices. Therefore, spicy food has to be considered dangerous for birch pollen- and mugwort pollen-allergic individuals. In this respect, the use of rBet v 1, rBet v 2 or 'r60 kDa allergen(s)' might be helpful to identify patients at high risk for developing spice allergy. Moreover it is tempting to speculate that only few recombinant allergen molecules might be sufficient for a 100% diagnosis of allergy to vegetable food and *Apiaceae* spices.

Most interestingly, we failed to detect spice-specific IgE in patients with adverse reactions to spices, but without pollinosis. Since we found IgE antibodies to spice proteins in approximately two-thirds of the patients, the pathomechanisms in one third of patients remain obscure. This group of patients might also be classified as food-intolerant.

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