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Allergic Sensitization to Native and Heated Celery Root in Pollen-Sensitive Patients Investigated by Skin Test and IgE Binding

Abstract

The rates of sensitization and allergy to four birch pollen related plant foods were investigated in a group of 167 patients who were sensitive to at least one kind of pollen and one particular food. Sensitivity was concluded from a positive skin prick test or the determination of specific IgE, whereas allergy was based on anamnestic data. The positivity rates for sensitization and allergy, respectively, were: apple, 93 and 84%; hazelnut, 90 and 78%; celery, 70 and 14%; carrot, 60 and 37%. Comparative testing by skin prick test and enzyme allergosorbent test (EAST) with extract from native and microwaved (750 W, 30 min, 100°C) celery root was performed on 46 of these patients. At least one positive test result (either prick test or EAST) was obtained for native celery in 36/46 (78%) and for heated celery in 20/46 (43%) of these patients. Although the concordance between the EAST and the skin test was very low, extended control experiments of both test procedures revealed no evidence for nonspecificity. Immunoblot analyses of extract from native celery and sera of 60 patients with a positive EAST (class ≥ 2 , ≥ 0.7 U/ml) for celery resulted in the following rates of IgE binding to known cross-reactive celery allergens: Api g 1: 33%, celery profilin: 17%; multiple bands most probably due to carbohydrate epitopes: 32%. The rate of binding to other allergens was below 10%. Since these three important structures are also present in birch pollen, no allergen could be identified as a candidate to mediate an exclusive celery/mugwort association. Investigation of extract from native and heated celery by immunoblotting pointed to a high lability of Api g 1, whereas profilin and carbohydrate epitopes appeared to be more resistant to heat. It has been concluded that sensitization to celery in German patients is without clinical significance in the majority of cases, in contrast to other birch-pollen-related plant foods such as apple and hazelnut. For the particular kind of extract used, neither the EAST nor the skin test alone represents an appropriate diagnostic method for testing sensitization to celery.

Key Words

Celery
Food allergy
Thermostable allergens
IgE
Skin prick test
Immunoblotting

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Introduction

Sensitization to celery root is highly associated with birch pollen and mugwort pollen hypersensitivity [1–4]. The cross-reactive nature of this observation has been known for many years [4–9]. When compared to allergic reactions elicited by apples and other fresh fruit and vegetables that mainly cause symptoms resembling oral allergy syndrome (OAS) [10], celery allergies are often more severe. Respiratory disorders, gastrointestinal symptoms and even life-threatening systemic anaphylaxis can be due to celery sensitization [11–14]. Celery belongs to the most frequent allergenic foods in Switzerland [14]. Clinical and immunological studies of celery hypersensitivity have been performed in Switzerland [1, 2, 13, 14] and France [11, 12, 15, 16], for example. However, there is a lack of information about sensitization and allergy to celery in Germany and its relationship to other common pollen-related food allergies, for example apple, nut and carrot hypersensitivity.

Recent studies have led to the identification and molecular characterization of cross-reactive allergens in celery, birch pollen and mugwort pollen: (1) A celery allergen that cross-reacts with Bet v 1, the major allergen of birch pollen, has been identified by immunoblot inhibition experiments [17, 18]. N-Terminal microsequencing revealed 40% sequence identity of 15 amino acid residues with Bet v 1 [19]. This degree of identity has been confirmed by the completely deduced amino acid sequence obtained by cDNA sequencing of the entire allergen [20]. The allergen has been named Api g 1. Mugwort pollen does not contain an allergen with cross-reactivity to Api g 1. (2) Cross-reactive profilins are present in celery and both birch pollen and mugwort pollen [16–18] and are candidates for explaining the clinical celery-carrot-mugwort-spice syndrome at the molecular level. (3) A third mechanism of cross-reactivity seems to be due to the existence of related sugar moieties on food and pollen glycoproteins [17, 21–23]. Celery allergens in the mass range of 35–90 kDa appear to carry these kinds of determinants [17, 23], which can be responsible for cross-reactions of specific IgE between almost all kinds of plants. As has recently been proposed for tomato and grass pollen, glycans containing α 1,3-linked fucose residues may represent important cross-reactive carbohydrate epitopes in pollen and plant food [24]. None of these structures is shared exclusively by mugwort pollen and celery.

The allergenic activity of celery has a thermostable component that appears to be associated with mugwort pollen, but not with birch pollen allergy [4]. Since dried and powdered celery root is widely used to spice processed and heated foods, the existence of stable allergens is particularly im-

portant. The molecular basis of this thermostability is unknown.

The objective of the present study was to obtain serological data about IgE binding to thermolabile and thermostable celery allergens. A further aim was to investigate the frequency of clinical relevant and latent sensitization to celery in relation to other pollen-related plant foods by comparing serological and skin test data with patients' histories.

Patients, Materials and Methods

Patients

Two hospitals participated in the clinical part of the study. All data were ascertained by the same physician. Patients were considered to be sensitized if the skin prick test and/or the determination of specific IgE was clearly positive according to the criteria described in the specific experimental sections. Sensitization to tree pollen was determined using birch pollen as the marker allergen. Weed pollen sensitization was tested with mugwort pollen extract. In the case of grass pollen, either timothy pollen or a grass pollen mix of six species was used. Patients who were sensitized to a specific food and who reported immediate type symptoms (e.g. oropharyngeal symptoms, asthma or other systemic reactions after ingestion of a specific food) were classified as being allergic to this food.

The first study was conducted at the Borkum Riff Rehabilitation Hospital and included 167 patients who were sensitized to at least one pollen species and who reported symptoms after ingestion of at least one plant food (group 1). All patients had severe atopic diseases (e.g. pollen asthma or atopic dermatitis), other diseases of the skin, or internal diseases, and underwent at least 4 weeks of stationary treatment and/or rehabilitation. The frequency of sensitization and allergy was evaluated for four native foods that are common elicitors of birch-pollen-related food allergies: apple, hazelnut, celery and carrot. Positivity rates among the various patient groups were compared by the one-sided and two-sided χ^2 test [25]. In 46 of these participants, skin prick tests and measurements of specific IgE by enzyme allergosorbent tests (EASTs) were carried out by simultaneously using self-prepared extracts from native and microwave-heated celery root (30 min at 100°C).

The second test series at the Department of Dermatology of the Humboldt University, Berlin, was performed during routine atopy screening of 407 patients (group 2) in whom atopic diseases were known, suspected, or had to be excluded in the differential diagnosis. Subsequent to anamnesis, all participants were skin-prick-tested with pollen extracts and extract from native celery root as above.

The control group for the skin prick test consisted of 25 patients without a history of pollen and plant food sensitization and other atopic diseases or skin diseases. The specificity of the EAST was checked with sera of 9 patients without known birch pollen, mugwort pollen and food sensitization, using self-prepared allergen disks loaded with extracts from native and heated celery. Additional controls were run with pooled sera from nonatopic donors and from patients allergic to grass pollen and by competitive EAST inhibition.

Due to the risk of severe reactions against celery and the problems of performing double-blind placebo-controlled food challenges with

fruits and vegetables in patients with mainly oropharyngeal symptoms, provocation tests were not performed.

Antisera

Rabbit polyclonal antiserum aBv directed against Bet v 1 was a gift from Allergopharma (Reinbek, Germany). Due to its cross-reactivity, this serum was suitable for immunodetection of Api g 1. Rabbit antiserum aPf against celery profilin was kindly supplied by Dr P. Deviller (Lyon, France). In addition to the sera that were taken from the participants of the study, pooled human sera with EAST scores ≥ 2 to celery were purchased from Allergopharma: serum BiP ($n = 7$, from patients allergic to tree pollen), serum BmP ($n = 12$, from patients allergic to tree pollen) and serum WeP ($n = 7$, from patients allergic to weed pollen). The EAST classes of these were: BiP native celery 2; heated celery 0; birch 4; mugwort 2; grass (timothy) 4; BmP: native celery 2; heated celery 2; birch pollen 3; mugwort 0; grass (timothy) 4; WeP: native celery 3; heated celery 3; birch 3; mugwort 3; grass (timothy) 3. In addition, pooled sera from 7 and 12 nonatopic donors and from 11 patients allergic to grass pollen (EAST class to timothy: 4; Allergopharma) were used for control experiments. Further controls were run with sera from individual patients as described in the Results section.

Allergen Extracts

Prick test solutions from pollen and hazelnut were obtained from Allergopharma. The other food extracts were prepared by a low-temperature solvent precipitation technique as described [26, 27] and stored freeze-dried at -20°C until used. Briefly, the food samples were cut into small pieces and homogenized in acetone/dry ice at -60 to -65°C . The resulting slurries were stored overnight and cooled with dry ice. Subsequently, the precipitates were washed twice with acetone at -60°C and with acetone/diethylether (1:1, v/v) at the same temperature, filtered, freeze-dried and stored at -20°C . Protein extracts were obtained by extracting approximately 2 g of these acetone powders with 30 ml of 0.01 M potassium phosphate buffer pH 7.4, containing 0.15 M NaCl (PBS), centrifugation (5°C , 20,000 g, 45 min), and filtering the supernatants through a fiberglass prefilter (No. 13400, Sartorius, Göttingen, Germany). The protein content was determined by a modified Bradford assay as described [27]. The protein extracts were stored freeze-dried until use. Celery root of the variety 'Brillant' was used native, as well as after microwave heating with 750 W for 10 min at 100°C and for 30 min at 100°C . Product temperature was monitored with a 3-channel fiber thermometer (Takaoka 1100). Golden Delicious apple extract and carrot extract were exclusively prepared from fresh foods. For prick testing, the protein extracts were reconstituted with sterile-filtered distilled water and stored in aliquots of 50 μl at -20°C until use. Optimal protein concentrations were adjusted shortly before the skin test. These diluted extracts were discarded after a maximum of 3 days.

Skin Tests

Skin prick tests were carried out on the volar side of the forearm according to the recommendations of the European Academy of Allergy and Clinical Immunology [28] as described for apple allergens [29–31]. The self-prepared prick test solutions from foods were applied at the following protein concentrations: native celery, 120 $\mu\text{g}/\text{ml}$; heated (30 min, 100°C , 750 W) celery, 145 $\mu\text{g}/\text{ml}$; carrot, 50 $\mu\text{g}/\text{ml}$; apple, 46 $\mu\text{g}/\text{ml}$. Optimal test concentrations were determined by titration of 5 different concentrations in preliminary experiments in 5 patients who were sensitized to the specific food. Protein amounts eliciting a wheal of approximately the same size as the histamine control (0.1%) were adjusted in the final test solutions.

Determination of Specific IgE

Specific IgE was routinely measured by the CAP[®] method (Pharmacia, Uppsala, Sweden). A CAP class of at least 3 (≥ 3.5 U/ml) was considered clearly positive. Additional measurements were performed with a commercial EAST according to the manufacturer's instructions (Allergopharma). In the latter assay, self-prepared allergen disks were used. Celery allergens were coupled to cyanogen-bromide-activated filter paper disks at a protein concentration of 12 $\mu\text{g}/\text{ml}$ as previously described for apple allergens [27, 30, 31]. The results were expressed either as EAST classes (corresponding to the classes of Phadezym[®] RAST) or as units/milliliter. To exclude doubtful results, EAST classes of at least 2 (≥ 0.7 U/ml) were considered positive.

Inhibition Experiments

To control the specificity of the EAST results with self prepared celery allergen disks, competitive inhibition experiments were performed with selected sera that contained IgE against native and/or heated celery as described for allergens from apples and other plant food [23, 24, 30, 31]. Briefly, 50 μl of patient serum (diluted 1:2.5) with a positive EAST to heated and/or to native celery was incubated with 50 μl of inhibitor solution (celery extract, ovalbumin as a negative control) at a protein concentration of 250 $\mu\text{g}/\text{ml}$ overnight. Subsequently, the immunodetection of bound IgE was continued according to the manufacturer's instructions. Results were compared to uninhibited samples and expressed as percent inhibition.

Electrophoresis and Immunoblotting

SDS-PAGE, semidry blotting and immunostaining of bound antibodies were performed as described [26, 31, 32]. In short, the extracts were separated by discontinuous SDS-PAGE, using 5% stacking gels (w/v 1.0 mm, T = 5.0%, C = 2.6%) and 13% resolving gels (w/v, T = 12.8%, C = 2.7%). The protein load on the gels was 18 $\mu\text{g}/\text{cm}$ on 5.5-cm preparative slots. Thereafter, the extracts were transferred onto nitrocellulose (NC) membranes by semidry blotting. After blocking with 0.01 M PBS/0.3% (v/v) Tween 20, cut NC strips (0.4 mm) were either stained with india ink or used for immuno-detections. The latter were achieved by incubating 1 NC strip in 1.0 ml of serum, diluted in PBS containing 0.05% (v/v) Tween 20 and 0.1% bovine serum albumin. The sera were diluted as follows: human sera, 1:6.7; rabbit aBv, 1:20,000; aPf, 1:30,000. Primary incubations were carried out overnight. Bound IgE antibodies were detected by subsequently incubating the NC strips with (1) a rabbit polyclonal anti human IgE (1:4,000, 1 h), (2) a goat polyclonal affinity-purified biotinylated antirabbit IgG (1:6,000, 1 h; both from Dako, Copenhagen, Denmark), and (3) streptavidin horseradish peroxidase conjugate (1:20,000, 20 min; Medac, Hamburg, Germany). Enzymatic activity was stained with 3,3',5,5'-tetramethylbenzidine. When rabbit antisera were used for primary incubations, the anti-IgE step was omitted.

Periodate Oxidation Procedure

Periodate oxidation of carbohydrate structures was performed as previously described [23, 24]. After blotting onto NC membranes, these were soaked in 10 mM sodium acetate buffer (pH 5.0) containing 50 mM NaIO_4 . The cut NC strips were incubated in the dark at room temperature for 2 h. The reaction was stopped by adding sorbitol to a final concentration of 0.015 M. Control strips were incubated with the inactivated oxidation buffer.

Results

Examination of Patients' Data

Group 1 consisted of 167 patients with at least one pollen and one plant food sensitization who underwent at least 4 weeks of stationary treatment and/or rehabilitation at the Borkum Riff Hospital. The rates of sensitization to pollen and the four selected plant foods among these patients are shown in table 1. The total number of patients whose history indicated the presence of a food allergy are also presented. When compared to apple by the two-sided χ^2 test, the frequency of clinically relevant sensitizations indicated by patients' reports was significantly lower for carrot and celery, but not for hazelnut ($p < 0.01$). For a more detailed analysis of the birch-mugwort-celery cluster we compared sensitization frequencies to the four selected foods between birch- and mugwort-sensitized patients (table 2). Statistical analysis revealed significant differences in the rates of food sensitization between birch- pollen and the birch-pollen/mugwort-pollen group for apple, celery and carrot, but not for hazelnut ($p < 0.05$). The frequency of apple sensitization was significantly lower in the birch/mugwort group when compared to patients without mugwort pollen sensitization. In contrast, the rate of sensitization to celery and carrot was significantly higher in the patients with a birch/mugwort co-sensitization. Due to the low frequency of monosensitization to mugwort pollen (4/167 patients) we were unable to analyze food sensitization in this particular subpopulation.

The patients of group 2 were examined during routine atopy screening at the Department of Dermatology of the Humboldt University and were, in contrast to the members of group 1, not preselected for pollen and food sensitization. Among these 407 patients, 157 (39%) were sensitized to at least one kind of pollen. One hundred three of 157 (66%) were sensitive to birch pollen, 69/157 (44%) to mugwort pollen and 98/157 (62%) to grass pollen. Nineteen of 157 (12%) pollen-sensitive patients revealed a positive prick test result to native celery root. Of these 19 patients, 5 (26%) were classified as being allergic to celery. Only 3 of the 250 patients (1.2%) who were not sensitized to pollen had a positive skin prick test to celery.

The large majority of the patients whose food allergy was indicated by history reported oropharyngeal symptoms after ingestion of the specific food. Systemic or severe gastrointestinal symptoms occurred mostly in addition to local oral symptoms. In contrast, less than 10% of the patients experienced systemic reactions and/or more severe gastrointestinal symptoms exclusively. For example, in the 29 patients who were classified as being allergic to celery, the following symptoms were reported: OAS (local symp-

Table 1. Sensitization (S) and allergy (A) to pollen-related plant foods in 167 patients sensitive to at least one pollen species and at least one kind of plant food (group 1)

	Patients	
	n	%
Pollen S		
Birch	162	97
Mugwort	107	64
Grass	129	77
Apple		
S	156	93
A	141	84
Hazelnut		
S	151	90
A	131	78
Celery		
S	117	70
A	24	14
Carrot		
S	100	60
A	62	37

Table 2. Rates of combined sensitization to birch and mugwort pollen and four pollen-related plant foods among patient group 1

	Apple	Hazelnut	Celery	Carrot
Birch pollen n = 59/167 (35)	59/59 (100)	52/59 (88)	35/59 (60)	29/59 (49)
Mugwort pollen n = 4/167 (2)	3/4 (75)	1/4 (25)	3/4 (75)	2/4 (50)
Birch/mugwort cosensitization n = 104/167 (62)	93/104 (89)	97/104 (93)	79/104 (76)	69/104 (66)

Numbers in parentheses indicate percentages.

toms at the mucosa of the lips, mouth, tongue and/or throat) were elicited in 27 patients (93%). Severe gastrointestinal symptoms such as diarrhea or vomiting were reported by 4 patients (14%), asthma by 4 patients (14%), Quincke edema by 2 two patients (6.9%) and urticaria by 1 patient (3.5%). Anaphylactic shock due to celery ingestion had not been experienced by any individual within the study pop-

ulation. Nineteen of the 29 patients (66%) exclusively reported OAS-like symptoms.

Comparison of Skin Prick Test and EAST with Native and Heated Celery Root

Forty-six patients of group 1 were comparatively investigated by skin prick test and EAST with extracts from native celery and celery root heated at 100°C for 30 min. Thirty-six of 46 patients (78%) had at least one positive test result (either prick test or EAST) to native celery and 20/46 (43%) to heated celery.

Thirty-eight of 46 participants (83%) had a positive prick test result with at least 1 celery extract. Among these 38 positive patients, 27/38 (71%) exclusively reacted with native celery, 4/38 (11%) were only positive with heated celery and 7/38 (18%) revealed a positive prick test with both extracts.

Twenty of the 46 participants (43%) had an EAST class of ≥ 2 (≥ 0.7 U/ml) that was taken as cutoff for positivity to at least 1 of the celery extracts. Nineteen of the 20 positive sera (95%) contained IgE directed against native celery and 11/20 (55%) were EAST-positive with heated celery. Generally, the sera exhibited a stronger reactivity to native celery than to heated celery, except 1 serum that reacted with heated celery but had a doubtful result with native celery (class 1). The results of EAST to native and heated celery in relation to the patients' sensitization against birch pollen and mugwort pollen are compared in figure 1. The graph shows that only 2 of the 15 patients who were sensitized to birch pollen but not to mugwort pollen had a clearly positive EAST to native celery, and all of these patients exhibited no IgE or very low levels of specific IgE against heated celery. Since the 3 patients who were exclusively sensitized to mugwort were also negative to celery (EAST ≤ 0.7 U/ml), IgE reactivity against heated celery was only present in patients with polyvalent pollen sensitization.

The disagreement between skin test and EAST results was striking. For example, only 17/34 (50%) of all patients who were skin-test-positive with native celery also had a positive EAST with the same extract. Only sera from 2 of 11 patients (18%) with a positive prick test to heated celery contained IgE specific for proteins from heated celery root. Nine of 35 sera (26%) from patients with a negative skin test result to heated celery contained IgE that reacted with the corresponding allergen disk. Six of the 46 patients (13%) reported OAS-like symptoms after ingestion of celery. Five of these patients had at least one positive test result (either skin test or EAST) to 1 of the celery extracts. One patient allergic to celery (Bo 172) was negative according to the criteria described under Materials and Methods, but had a slightly positive EAST of class 1 (0.6 U/ml) to native and class 1

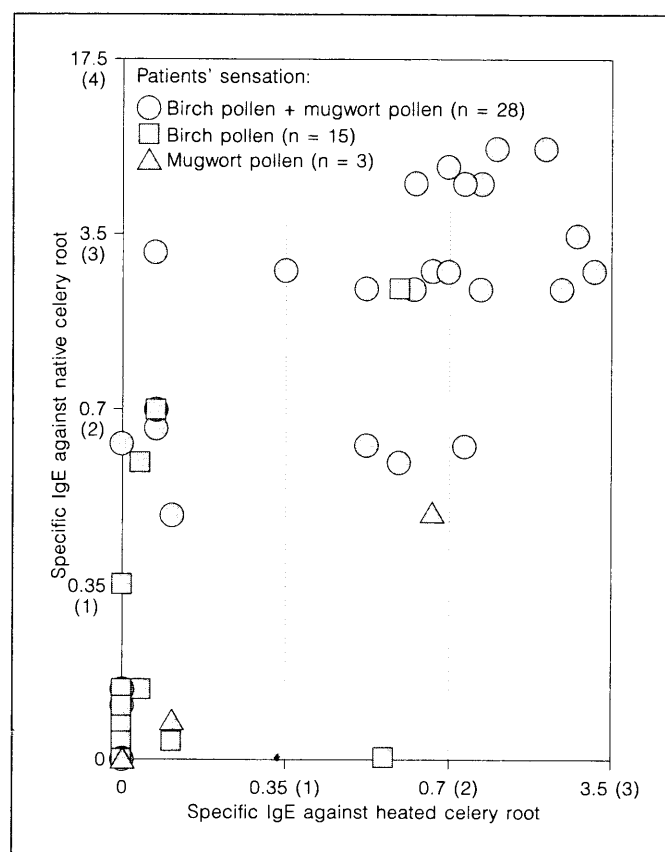


Fig. 1. Comparison of specific IgE binding to allergens from native and heated celery root in relation to the pollen sensitization of 46 patients of group 1. Results are expressed in units/milliliter. In addition, whole EAST classes are given in parentheses.

(0.5 U/ml) to heated celery. Both the EAST and the skin test revealed positive as well as negative results among these patients, but the number of tests did not allow a statistical analysis. Six of the 46 patients (13%) were negative in both the EAST and the skin test.

Control of the Test Procedures

Due to the obvious differences between the skin test results and specific IgE determination, even when the same celery extracts were used in both procedures, extended controls had to be performed.

The skin prick tests with both extracts were negative in the control group ($n = 25$). The celery EAST was controlled with sera of 9 patients without known birch pollen, mugwort pollen and food sensitization and was negative in all cases. Among these, 1 patient was monosensitized to grass pollen. In addition, no specific IgE to native and heated celery was detected in the pooled sera from 7 and 12 nonatopic

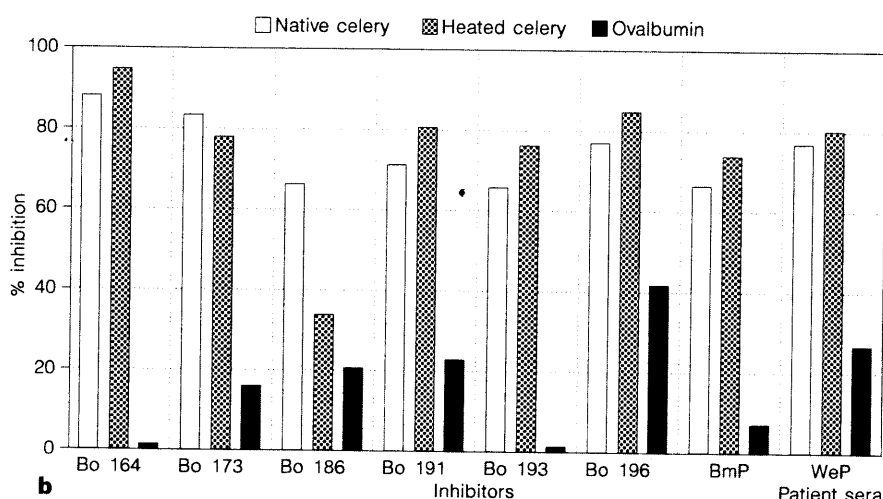
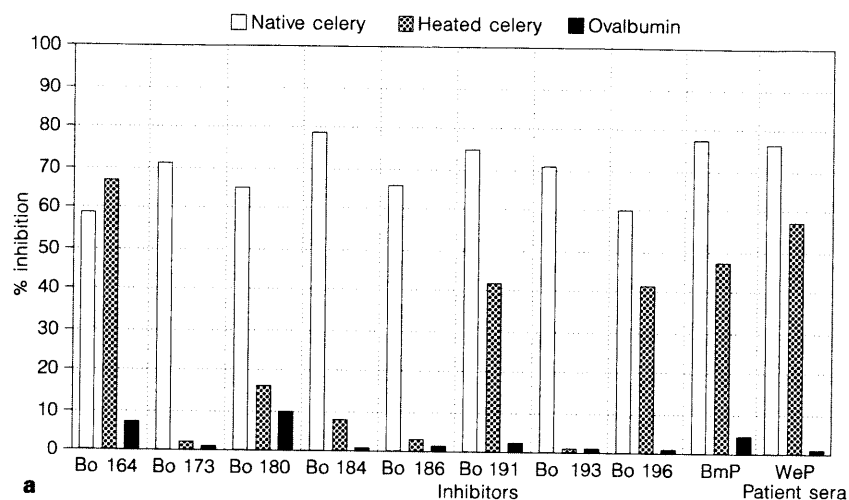


Fig. 2. a Results of specific inhibition of IgE binding to allergen disks with native celery allergens. Patient sera (diluted 1:2.5) as indicated. The amount of protein applied for inhibition was 250 µg/ml. Results are expressed as percent inhibition. **b** Results of specific inhibition of IgE binding to allergen disks with heat-denatured celery allergens. Patient sera (diluted 1:2.5) as indicated. The amount of protein applied for inhibition was 250 µg/ml. Results are expressed as percent inhibition.

donors, respectively, and in a serum pool from 11 patients allergic to grass pollen. Besides direct IgE measurements, the possibility of nonspecific interactions of IgE with celery allergen disks was also tested by cross-inhibition experiments (fig. 2). The celery-positive pooled sera WeP (n = 7) and BmP (n = 12) as well as sera from 8 patients with a positive EAST to native and/or heated celery were diluted 1:2.5, incubated with 250 µg/ml of protein from native or heated celery and allergen disks with both celery extracts overnight. Due to the relatively weak IgE binding, sera Bo 180 and Bo 184 were not tested with allergen disks of heated celery. Subsequent determination of bound IgE revealed significant inhibition of $\geq 50\%$ in all cases when native celery allergens were used for inhibition. Heated celery caused only weak or no inhibition of IgE binding to native celery pro-

teins at the solid phase with the 5 sera Bo 173, Bo 180, Bo 184, Bo 186, Bo 193. In 1 case (Bo 186) heated celery also inhibited weakly (34%) with disks carrying the same extract. Control inhibitions with an equal amount of ovalbumin were negative ($\leq 20\%$), except IgE binding of 3 sera (Bo 196, WeP, Bo 191) to heat-denatured celery.

Immunoblotting

Sixty sera with an EAST class of at least 2 to native celery were investigated by immunoblotting (results not shown). Twenty of the 60 sera (33%) contained IgE against Api g 1, 10/60 (17%) against celery profilin, and 19/60 (32%) sera reacted with multiple bands in the mass range of 35–95 kDa that are suspected of carrying carbohydrate determinants [17, 23, 24]. Besides these structures which are

known to mediate cross-reactivity between pollen and plant food, no additional important celery allergens could be identified. Only 5 of 60 sera (8.3%) reacted weakly with other individual celery allergens. Despite a positive EAST, 19/60 (32%) of the sera recognized no allergen bands in extract of native celery which was subjected to SDS-PAGE immunoblotting.

The thermostability of the celery allergens was also investigated. Equal amounts of protein (18 µg/cm) from native celery and of the 2 samples from microwave-heated celery root were separated by SDS-PAGE, transferred onto NC membranes and subsequently tested with patient sera and specific rabbit antibodies. The influence of heating on the immunoreactivity of Api g 1 is shown in figure 3a. The IgE antibody binding of the 3 human sera (patients Bo 155, S69 and serum pool BiP from 7 patients) to Api g 1 at 16 kDa completely disappeared after heating the celery roots for 10 min. The reactivity of the rabbit antiserum aBv specific for Bet v 1 was reduced to a faint band and disappeared after prolonging the heating time to 30 min.

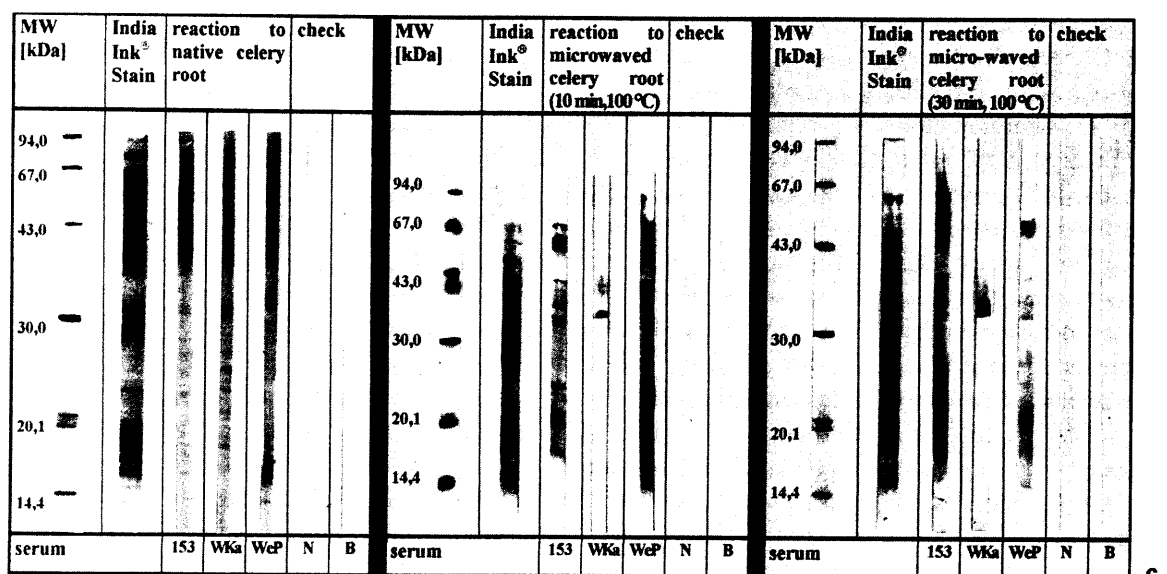
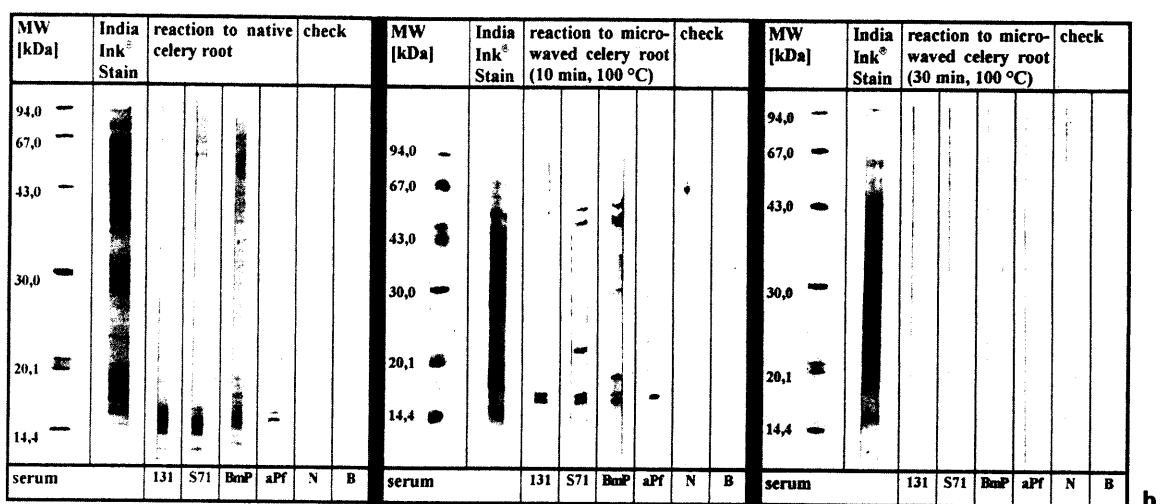
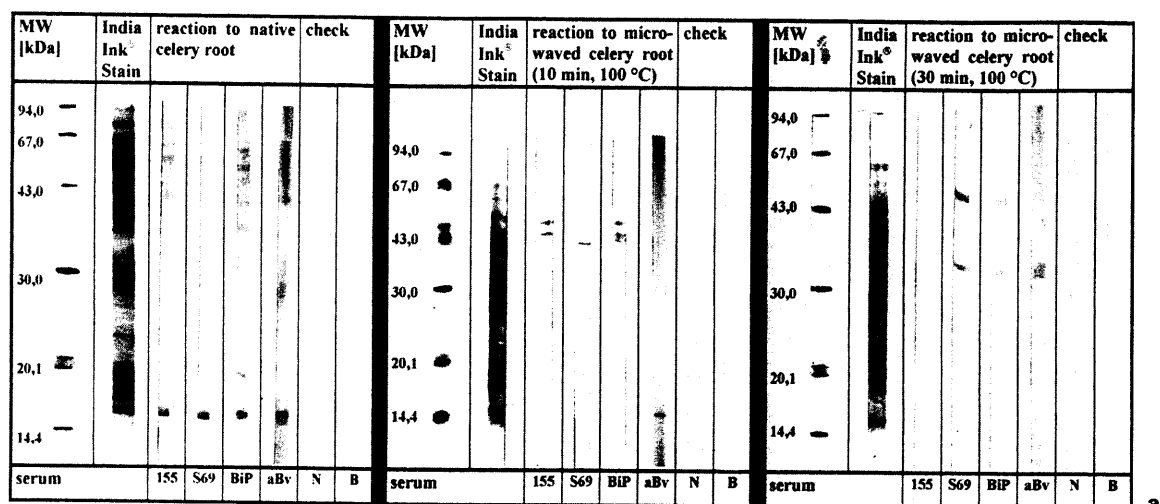
The same analyses were carried out with sera from pollen-allergic patients containing profilin-specific IgE (patients Bo 131, S71 and serum pool BmP from 12 patients) as well as with the rabbit polyclonal antiserum aPf against celery profilin (fig. 3b). All 4 sera retained some reactivity after 10 min at 100°C treatment, but the profilin double band at 15–16 kDa disappeared for celery root heated for 30 min. IgE binding to multiple bands probably carrying carbohydrate epitopes was investigated with 3 sera (patients Bo 153, WKa and the serum pool WeP from 7 patients) (fig. 3c). Sera Bo 153 and WeP clearly bound to multiple bands even after microwave treatment (30 min, 100°C, 750 W). For the serum pool WeP, the intensity of the multiple band pattern was reduced. In the case of patient WKa, application of heat reduced the complex pattern recognized in native celery to a single band at approximately 35 kDa. Whether the reactivity of these sera could be directed against sugar moieties was further tested by oxidative cleavage of the glycans on NC blots. As illustrated in figure 4 for native celery, periodate oxidation drastically reduced the reactivity of IgE to the multiple high-molecular-weight bands for all 3 sera. Similar effects were also obtained when blot strips containing proteins from heated celery root were subjected to the oxidation procedure (results not shown). The additional reactivity of serum WeP to Api g 1 was not influenced by the oxidation procedure. This observation is most likely due to the recognition of protein epitopes on this celery allergen related to Bet v 1 (Api g 1).

Discussion

Our study confirms celery as being an important allergenic plant food that can be recognized by specific IgE from pollen allergic patients even in a heat-denatured state. However, on the basis of subjective patient reports, our results indicate a very high rate of clinically insignificant sensitization to celery in contrast to other pollen-related plant foods such as apple or hazelnut. In patient group 1, 90% and 87% of the patients sensitized to apple and hazelnut, respectively, reported inability to tolerate these foods. In contrast, this was the case in only 21% of all patients who were sensitized to celery (table 1). This high degree of latency corresponds to the results of patient group 2 with 5 of 19 (26%) celery-sensitized patients being allergic to celery. However, these rates are possibly somewhat underestimated since the evaluation was based on anamnestic data. Many of the patients rarely consumed celery root and were not aware of the ubiquitous presence of celery as a spicing ingredient in processed food. Nevertheless, it is unlikely that these factors would lead to an estimation of the rate of celery allergy comparable to apple and hazelnut.

Since all members of group 1 were selected on the basis of a known food and pollen sensitization, the total positivity rates are not representative for the general pollen-sensitive population. The importance of the patient group selection on epidemiological data is underlined by the results in the patients of group 2, who were routinely tested during atopy screening. Only 12% of the 157 pollen-sensitive patients belonging to this group were sensitized to celery and 3% were classified as allergic to celery. These data are more likely representative and are closer to the anamnestic data of Pi-

Fig. 3. a Immunoblot analyses of extracts from native and heated celery root. Extracts were separated by SDS-PAGE and transferred to NC. Antibodies bound to Api g 1 at approximately 16 kDa. Human sera are from patients Bo 155, S69 and serum pool BiP from 7 patients, diluted 1:6.7. aBv = Rabbit antiserum against Bet v 1, diluted 1:2,500; N: nonallergic human control serum; B = buffer control. **b** Immunoblot analyses of extracts from native and heated celery root. Extracts were separated by SDS-PAGE and transferred to NC. Antibodies bound to the celery profilin double band at approximately 15–16 kDa. Human sera are from patients Bo 131, S71 and serum pool BmP from 12 patients, diluted 1:6.7. aPf = Rabbit antiserum against celery profilin, diluted 1:30,000; N = Nonallergic human control serum; B = Buffer control. **c** Immunoblot analyses of extracts from native and heated celery root. Extracts were separated by SDS-PAGE and transferred to NC. IgE antibody binding is shown for sera that were suspected to recognize carbohydrate epitopes. Human sera are from patients Bo 153, WKa and serum pool WeP from 12 patients, diluted 1:6.7. N = Nonallergic human control serum; B = buffer control.



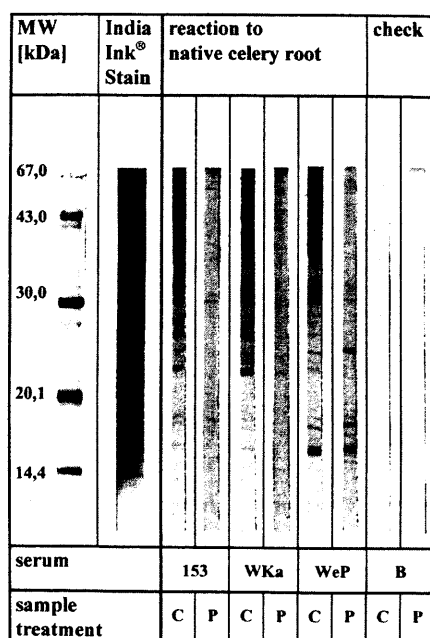


Fig. 4. Identification of patients' IgE reactivity to glycans of extract from native celery root. After Western blotting NC strips were treated under the following conditions: C = Control treatment with sorbitol-inactivated NaIO₄ solution; P = treated with 50 mM NaIO₄ solution. Patient sera and additional controls as in figure 3c.

chler and Stich [13], who found 22% of their mugwort-sensitized patients to be sensitized to 'vegetables', 11% of them being allergic to celery. However, the data of our German patients also indicate a higher degree of latency when compared to results of other groups from Switzerland: Bircher et al. [33] analyzed plant food sensitization in 238 pollen-allergic patients in a serological study using the CAP method. IgE specific for celery allergens was found in 27% of these patients. In response to a questionnaire, 22/130 (17%) of pollen-allergic patients reported symptoms after ingestion of celery. Although the rate of celery allergy is comparable to the 14% determined in our patient group 1, the rate of celery sensitization is drastically lower, indicating a lower degree of latency. These differences might be partly due to higher sensitization rates that would be determined by skin test and to differences between nutritional habits. For example, in contrast to most parts of Germany, celery is a common ingredient of fresh salads in Switzerland.

The frequencies of sensitization to celery found in the patients sensitive to birch pollen and in the group-1 patients with a concomitant birch/mugwort sensitization may indicate that there is no mugwort-specific influence on celery sensitization which is independent from birch pollen sensi-

tization. However, there seems to be an additive effect of a concomitant mugwort pollen sensitization on the rate of sensitization to celery in patients allergic to birch pollen. Due to our immunoblot studies [17], this effect is possibly due to a higher number of patients sensitized to profilin [34] or carbohydrate determinants [23, 24] in patients sensitive to more than one phylogenetically nonrelated pollen species.

After comparison of the EAST and skin prick test results, those in 46 patients of group 1 were suspected to be nonspecific. This is particularly the case for heated celery since only 2 of 12 patients (17%) with a positive prick test to this extract revealed IgE specific for the same material and vice versa, 9 of 34 sera (26%) of patients with negative prick test results to heated celery were found to be IgE-reactive with the corresponding allergen disk. However, our control experiments revealed no evidence for nonspecific effects. All skin prick tests with both celery extracts were negative in the control group of 25 patients. Moreover, during atopy screening of group 2, only 3 positive prick tests to celery occurred in the pollen-negative group of 250 patients. In the case of specific IgE determination with celery allergen disks and sera of 9 individuals without celery sensitivity as well as pooled sera from 7 and 12 nonatopic donors and from 11 patients allergic to grass pollen, all measurements were negative. Additional IgE inhibition pointed to the specificity of IgE binding even with allergen disks of heated celery. Weak inhibition of IgE binding to native celery by heated celery was measured with 5 sera. This effect is most probably due to epitope denaturation by heating and not to nonspecificity. However, inhibition with the control protein ovalbumin exceeded 20% in 3 cases when allergen disks of heated celery were used. Since ovalbumin is glycosylated, this may be due to IgE reactivity with thermostable glycans, which was indeed confirmed for the serum pool WeP. Finally, it must be kept in mind that a highly sensitized patient group was studied, with most patients being allergic to more than one kind of pollen and at least one plant food, and that only 6 of these 46 patients (13%) were negative for celery in all test procedures. It is therefore suggested that positive results in the different tests are due to a sensitization to celery. The results of the different procedures may be additive for unknown reasons. However, in accordance with many other food allergies [35], skin test and determination of specific IgE only indicates sensitization and not allergy, but may be a helpful tool in studying properties of celery allergens during heating and food processing.

The immunoblot experiments reported here were performed on extracts from native celery root and from celery root microwave-heated for 10 or 30 min at 100°C, by mon-

itoring the product temperature. During blot screening of 60 sera with NC strips loaded with proteins from native celery, we were again unable to identify celery allergens without cross-reactive homologues being present in birch pollen. Only the known cross-reactive structures of proteins homologous to Bet v 1 (Api g 1), profilins and carbohydrate determinants were detected as important IgE binding components. Therefore, at the serological level we confirmed the existence of a 'birch-mugwort-celery syndrome'.

Approximately one third of those sera with a positive EAST to celery were not reactive on immunoblots that were produced under reducing and denaturing conditions. To a lesser extent this observation has also been made in our previous study [17]. Possible explanations are the disruption of conformational epitopes or a lower detection limit of blotting when compared to EAST.

Finally, heating of celery under carefully controlled conditions and subsequent probing of immunoblots with sera of known specificity revealed that the immunoreactivity of the major celery allergen Api g 1 is highly sensitive to heat, while celery profilin is somewhat more stable under these conditions. Furthermore, with 2 of 3 sera, IgE reactivity of

carbohydrate determinants survived even 30 min at 100°C. The thermostable allergenic activity of celery that was suspected to be associated with mugwort pollen but not with birch pollen sensitization [4] may therefore be due to a co-sensitization to mugwort of patients allergic to birch pollen. Profilins and carbohydrate structures of birch and mugwort pollen may represent the more stable IgE binding material. Profilins are known to trigger histamine release in allergic patients [34]. Therefore, when investigating symptoms after ingestion of heated celery, profilin again represents an interesting candidate to explain thermostable allergenic activity. In contrast, sensitizations to carbohydrates are thought to be without clinical significance [21, 22]. Further work is in progress to obtain more detailed information about the labile and stable allergenic structures of celery.

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