

Immunoblot study of IgE binding allergens in celery roots

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Objectives: The purpose of this study was to characterize the IgE binding components of celery root by immunoblot analysis and to investigate the cross-reactive nature of the allergens.

Background: Sensitization to celery is very common in patients hypersensitive to mugwort pollen and birch pollen, and is due to cross-reactions of allergen-specific IgE antibodies. Recently, the pan-allergen profilin has been identified as an important cross-reactive allergen in celery, birch pollen and mugwort pollen. Very few data, however, on additional celery allergens are available.

Methods: Sera of 38 patients with an isolated or combined sensitization to birch pollen, mugwort pollen, and celery were investigated by immunoblot and immunoblot inhibition techniques using celery extract and purified Bet v I, the major allergen from birch pollen. The selection of sera was based on serologic sensitization, independent of clinical manifestations. Celery profilin was affinity isolated and analyzed by immunoblotting with patients' sera as well as with rabbit polyclonal antiserum raised against celery profilin.

Results: Only weak IgE binding occurred on celery immunoblots in the patient groups with a negative RAST to celery. Sera of 8% patients with a birch pollen/celery sensitization recognized a 16-kDa celery allergen that cross-reacted with Bet v I in immunoblot inhibition experiments. In the mugwort pollen/celery and birch pollen/mugwort pollen/celery patient groups, the sera of 9/16 patients contained IgE that bound to a 15 to 16-kDa double band identified as celery profilin. Furthermore, three sera of the birch pollen/mugwort pollen/celery group appeared to contain IgE antibodies against carbohydrate determinants.

Conclusions: The 16-kDa Bet v I-related celery protein appears to be an important allergen for patients sensitized to birch pollen and celery. In contrast IgE binding to celery profilin appears to be more important in patients with an additional sensitization to mugwort pollen.

INTRODUCTION

Sensitization to birch and mugwort pollen is very common in Germany and Switzerland.¹⁻⁴ In a random population sample of 8,357 Swiss adults, for example, 7.9% revealed positive skin prick tests to birch pollen.^{3,4}

Among 2,639 Swiss school children, 11.8% had positive skin prick tests to birch and 5.7% to mugwort pollen.¹

Sensitization to either type of pollen is often associated with food allergies: more than half of the patients with birch pollen allergy are also sensitized to apples, stone fruits, nuts, celery or other fruits and vegetables,⁵⁻⁹ and in about three-quarters of the mugwort-sensitive patients, sensitivities to celery, carrot, fennel, and certain spices have been found (the "celery-carrot-mugwort-spice syndrome").¹⁰⁻¹⁴ Not surprisingly, these are the most common food allergies. Sensitization to celery is associated with both birch and mugwort pollen allergy syndromes.

Approximately 40% of patients treated for food allergies at the Allergy Unit of the Department of Dermatology, University Hospital Zürich are sensitized to celery.¹⁵

Whereas the allergic reactions elicited by apples and other fruits are usually restricted to local oropharyngeal symptoms, celery allergies are often more severe, causing respiratory disorders such as bronchial asthma, gastrointestinal symptoms, urticaria, or even life-threatening systemic anaphylaxis.^{15,16}

On a molecular basis, the co-occurrence of food and pollen hypersensitivities can be explained by immunologic cross-reactions of allergen-specific antibodies of the IgE class. This is an intriguing finding, as the cross-reactions usually exceed the lines of phylogenetic development^{12,17-23} and one would not expect a priori that divergent plant families would share the same antigens. Three different allergenic structures have been proposed as being responsible for the cross-reactions between pollen and plant foods, namely Bet v I and related plant proteins, profilin, and carbohydrate determinants.¹⁷

(1) Bet v I is the major allergen of birch pollen, a glycoprotein with a molecular mass of approximately 17 kDa. Most of the birch pollen-related food hypersensitivities are caused by a family of plant proteins that share common epitopes with Bet v I. Such proteins have been identified in apples, hazelnuts and pears,^{18,24-27} but none has as yet been reported in celery.

(2) Profilins constitute a family of related plant proteins with a conserved amino acid sequence. They are found in many eukaryotic organisms, including several pollen species and a wide variety of plant foods. Together with actin, profilactin and prolyl hydroxy-

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lase, profilins are involved in a number of physiologic processes, eg, signal transduction and regulation of actin polymerization. Profilins are highly cross-reactive plant pan-allergens.²⁸⁻³² Recently, a 15-kDa allergen from celery has been identified as a profilin.³²

(3) Finally, "cross-reactive carbohydrate determinants" of food and pollen glycoproteins can cause immunologic cross-reactions involving IgE antibodies.¹⁷ This phenomenon has only been poorly investigated so far, and it is not yet known whether such structures play a role in celery/pollen cross-sensitizations.

In a previous study, sera of 41 patients with isolated or combined sensitizations against birch pollen, mugwort pollen, and celery were tested for specific IgE against 21 different spices.³³ The most intensive sensitization patterns against spices occurred in the patient group with a combined birch pollen/mugwort pollen/celery allergy. In contrast, no spice sensitization was determined in patients exclusively sensitized to birch pollen.

The aim of our present study was twofold: to characterize further the IgE binding components of celery by immunoblot analyses of the above-mentioned sera and to investigate further the cross-reactive nature of the allergens involved.

MATERIALS AND METHODS

Patient Sera

The individual sera were collected at the Allergy Unit of the Department of Dermatology, University Hospital of Zürich, Switzerland (Prof. Dr. B. Wüthrich). Additional sera were from the serum collection of the Institute of Food Chemistry, Technical University, Berlin, Germany (sera Bra, Sec, 233, MM, 216). The criteria for selection were—independent of a possible clinical relevance—significant serologic sensitizations (Pharmacia RAST-class two corresponding to 0.7 PRU/mL; PRU = Phadebas RAST Units; RAST = radioallergosorbent test) to birch pollen, mugwort pollen, birch pollen/celery, mugwort pollen/celery, or birch

pollen/mugwort pollen/celery. Negative control serum was from a non-atopic individual with no history of food allergy.

Since the amount of the serum samples was limited, only 35 sera (no. 1 to 35) of the 41 participants of the previous study³² as well as three additional sera (Bra, Sec, 233) could be subjected to immunoblot analyses. Of these 38 sera, seven had positive RASTs to birch pollen alone (1 to 17 PRU/mL) and seven to mugwort pollen alone (1 to 19 PRU/mL). Another eight sera were positive to both birch pollen (1 to 66 PRU/mL) and celery (1 to 7 PRU/mL), and five sera towards both mugwort pollen (1 to 38 PRU/mL) and celery (1 to 3 PRU/mL). The remaining 11 sera had positive RASTs to all three allergens (birch pollen 1 to 77 PRU/mL, mugwort pollen 1 to 33 PRU/mL, and celery 1 to 9 PRU/mL). In total, 24 individuals had a positive RAST towards celery (RAST 0.7 PRU/mL).

The RAST classes were determined at either the Department of Dermatology, University Hospital of Zürich or at the Institute of Food Chemistry using a commercial enzyme allergosorbent test (Allergopharma, Reinbek, Germany).

Polyclonal Antiserum

A rabbit polyclonal antiserum raised against celery profilin was kindly supplied by Dr. P. Deviller, Laboratoire de Biochimie, Hôpital Louis Pradel, Lyon, France.³²

Allergenic Extracts

Celery roots (*Apium graveolens* spp.) and Granny Smith apples (*Malus domestica* spp.) were purchased at the local market. Protein extracts were prepared by a low temperature acetone powder method as previously described,³⁴ freeze-dried and stored at -20 °C until use. The total amount of protein was determined by a modified Bradford assay using bovine serum albumin as the standard.³⁴ Protein concentrations of the celery and apple extracts were 1100 and 430 µg/mL, respectively.

Commercial birch pollen (*Betula verucosa* spp.) extract was from Allergopharma, Reinbek, Germany (concentration = 1100 µg protein/mL).

Bet v I

Bet v I was isolated from birch pollen (*Betula verucosa* spp.) by affinity chromatography using immobilized, allergen-specific monoclonal antibodies at the research laboratory of Allergopharma, Reinbek, Germany.^{35,36}

Affinity Isolation of Celery Profilin

Poly-L-proline (pLp) affinity isolation of profilin was performed by a simplified version of the procedure previously described.^{31,32} In brief, the pLp solid phase was prepared by coupling 50 mg pLp (Sigma, St. Louis, USA) to 1.5 g CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions for activated Sepharose. The pLp-Sepharose was washed twice with matrix buffer (20 mM imidazole pH 7.5, 150 mM NaCl, 0.5 mM ATP). Protein from the celery extract (15 mg) was dissolved in the matrix buffer and incubated with the pLp-Sepharose beads for three hours at room temperature to adsorb the profilin. The beads were then washed six times with matrix buffer. The celery profilin bound to the affinity matrix was desorbed in three fractions using matrix buffer containing 6 M urea. The first desorbed fraction was washed three times with 0.1 M PB (potassium phosphate buffer, pH 7.4) in a centricon 3 unit (amicon, Beverly, USA). The cleaned and concentrated sample (in the following text referred to as "profilin") was lyophilized, reconstituted in 50 µL electrophoresis sample buffer and used for preparative SDS-PAGE followed by semi-dry blotting as described below.

Rye grass profilin was isolated by the same procedure at the research laboratory of Allergopharma, Reinbek, Germany.

Chemicals

Except where mentioned, all chemicals were of analytical grade.

Discontinuous Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

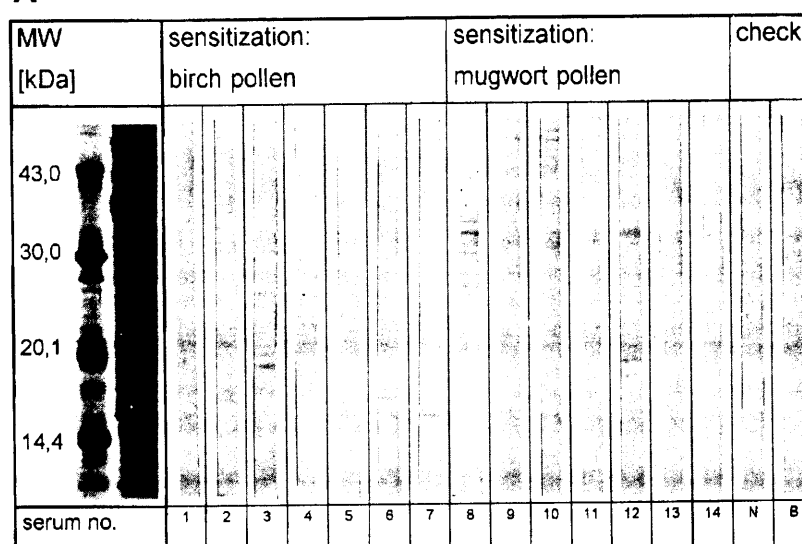
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Lämmli³⁷ in a twin vertical slab gel apparatus (Renner, Dannstadt, Germany) as previously described.³⁸ Resolving gels were 13.0% (wt/vol) homogeneous, the stacking gels contained 5% (wt/vol) acrylamide. Protein samples were reduced with β -mercaptoethanol and loaded onto the gel at a concentration of 14.0 μ g/cm.

Protein Transfer and Immunoblotting

Semi-dry electroblotting of the separated celery proteins on nitrocellulose and immunostaining of bound IgE antibodies was performed as previously described.³⁸ Briefly, nitrocellulose sheets (0.2 μ m, Schleicher & Schuell, Dassel, Germany) onto which the separated celery proteins had been transferred were cut lengthwise into strips 3 to 4 mm wide and incubated overnight at room temperature with 150 μ L human serum diluted to 1.0 mL with incubation buffer. After incubation with rabbit anti-human IgE (1:4000, 60 minutes), biotinylated goat anti-rabbit IgG (1:6000, 60 minutes, both reagents from DAKO, Copenhagen, Denmark) and streptavidin horseradish peroxidase conjugate (1:20000, 15 minutes, reagent from Medac, Hamburg, Germany), the blots were stained with 3,3',5,5'-tetramethylbenzidine/dioctylsodium-sulphosuccinate.

Non-specific binding (NSB) was checked by incubating one loaded nitrocellulose strip with buffer alone (lane B in all Figures) instead of with human serum. In addition, one strip was incubated with the serum of a non-allergic patient (lane N in all Figures). The efficiency of protein transfer was checked by staining one nitrocellulose strip containing separated celery proteins and low molecular weight marker proteins (Pharmacia, Freiburg, Germany) with India ink.

A



B

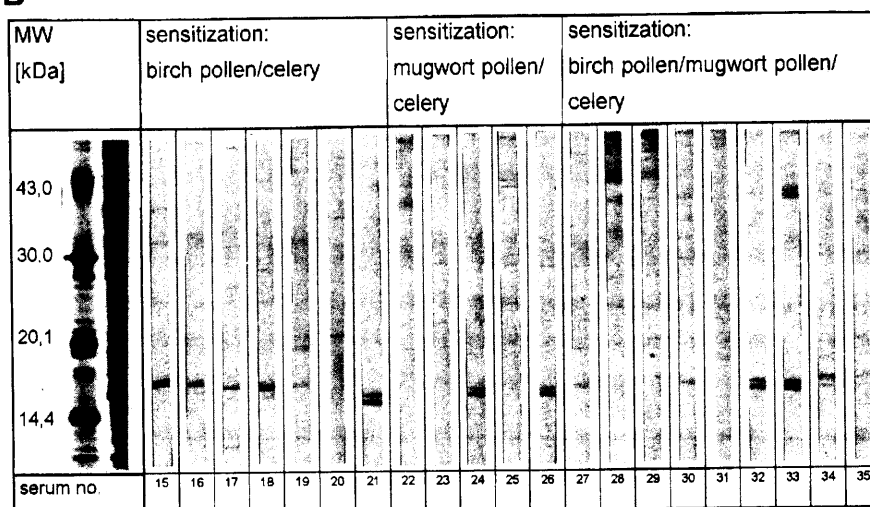


Figure 1a/1b. Binding of IgE from human sera to celery allergens separated by SDS-PAGE and transferred to NC. Sensitization: birch pollen, mugwort pollen, birch pollen/celery, mugwort pollen/celery, birch pollen/mugwort pollen/celery. Human sera as indicated, B = buffer control and N = nonallergic human control serum.

Immunoblot Inhibition

Seventy-five microliters of sera from patients 15, 16, 18, 21, 32, 33, Bra, and Sec were pre-incubated with Bet v I (15 μ g), crude celery extract (100 μ g of protein) or ovalbumin (100 μ g) (to control for non-specific inhibition) in a total volume of 1.0 mL incubation buffer for 60 minutes at room temperature in Eppendorf tubes. Afterwards, the immunodetection was performed

by the standard procedure as described above.

Periodate Oxidation Procedure

"Cross-reactive carbohydrate structures" were inactivated by treating the nitrocellulose strips-loaded with separated celery or apple proteins-with 0.1 M NaIO₄ in 0.01 M sodium acetate buffer, pH 5.0, for two hours in the dark.¹⁷ This caused a ring cleavage of

the carbohydrate sugar moieties. The reaction was stopped with sorbitol. The nitrocellulose strips were washed three times with incubation buffer and incubated with sera suspected of having IgE reactive to these carbohydrate determinants. NC strips for negative control experiments were incubated with oxidation reagent that had been inactivated by sorbitol. Sera reactive to the protein moieties of related glycoproteins in apple extracts were used as positive controls (sera MM and 216).³⁹

RESULTS

Immunodetection of IgE Binding Components in Celery Extract

Immunodetection after SDS-PAGE and semi-dry blotting was used to map the celery proteins that bind IgE antibodies from the sera of 38 patients (Fig 1). The sensitization characteristics and the binding patterns observed in the different patient groups are summarized in Table 1. In general, only weak IgE binding occurred in the patient groups with a negative RAST towards celery.

(1) Of the seven patients allergic to birch pollen alone, the sera of three showed a weak IgE reactivity to certain celery proteins [proteins of molecular weight 15 to 16 kDa (double band), 16 kDa, 18 kDa, and 26 kDa].

(2) In the group of patients sensitized to mugwort pollen alone, IgE in

three sera bound weakly to celery proteins in the range of 30 kDa. Additionally, one patient (12) had IgE specific to a protein at 18 kD, another (patient 9) had weak IgE binding to a 15 to 16-kDa double band, and a third patient (8) had antibodies that reacted to various celery proteins above 25 kDa.

(3) Among the eight patients with combined birch pollen/celery sensitization, most sera contained antibodies to the 16-kDa celery protein (six patients: 15, 16, 17, 18, and 19; results of serum Bra shown in Fig 3). In contrast, one serum (21) had IgE that bound exclusively to the 15 to 16-kDa double band.

(4) In the group of patients with a combined mugwort pollen/celery sensitization, two sera (24, 26) recognized the 15 to 16-kDa double band. Antibodies of two further patients (22, 25) bound to some proteins of approximately 40 kDa. One serum (23) showed no reaction.

(5) In the patient group with a clustered birch pollen/mugwort pollen/celery sensitization, specific binding occurred most often to either the 16-kDa protein (four sera: 27, 30, 34, 35) or the 15 to 16-kDa double band (again four sera: 32, 33, 34; results of serum Sec shown in Fig 3). The other three patients (28, 29; results of serum 233 not shown) had IgE antibodies against many bands in the range of 25 to 50

kDa. In general, no IgE binding occurred with the negative control serum.

In initial immunoblot inhibition experiments with the sera of patients Bra, 32 and 33, the pre-incubation with 100 µg of protein from crude celery extract totally quenched the IgE binding to both the 16-kDa band as well as the 15 to 16 kDa proteins on celery immunoblots. This indicated that the IgE binding was specific (results not shown).

Identification of Allergens Related to Bet v I

In order to examine whether any of the protein bands recognized by the patients' sera were related to the birch pollen allergen *Bet v I*, immunoblot inhibition experiments were performed using purified *Bet v I*. The purity of the *Bet v I* sample, isolated by affinity chromatography, was checked by SDS-PAGE analysis as shown in Figure 2. Preincubation with the pure birch pollen allergen completely inhibited IgE binding to the 16-kDa band, but did not influence IgE binding to the 15 to 16-kDa double band (Fig 3; sera 15, 16, 18, and Bra, versus sera 21, 32, 33, and Sec). It was therefore concluded that the 16-kDa celery allergen shared common epitopes with the major birch pollen allergen *Bet v I*, whereas the 15 to 16-kDa double band represented allergens of unrelated structure.

Table 1. Number of Patients with IgE Reactivity to Known Cross-Reactive Allergens or Other Proteins from Celery

	Sensitization					Total Patient Panel (n = 38)
	Birch Pollen (n = 7)	Mugwort Pollen (n = 7)	Birch/Celery (n = 8)	Mugwort/Celery (n = 5)	Birch/Mugwort/Celery (n = 11)	
Known cross-reactive allergens from celery						
Bet v I related allergen (MW = 16 kDa)	1*	0	6	0	4	11
Profilin (MW = 15-16 kDa)	1*	1*	1	2	4	9
HMW-bands† (MW 25 kDa)	0	1	0	1	3	5
Other proteins						
Very weak reaction and/or other individual allergens‡	2	3	6	2	6	19
No IgE binding	4	3	0	1	1	9

* Weak IgE binding.

† Suggested IgE binding to cross-reactive carbohydrate determinants (CCD) of high molecular weight (HMW) allergens was confirmed by periodate oxidation for three sera (28, 29, and 233).

‡ Some patients were also counted in the other categories.

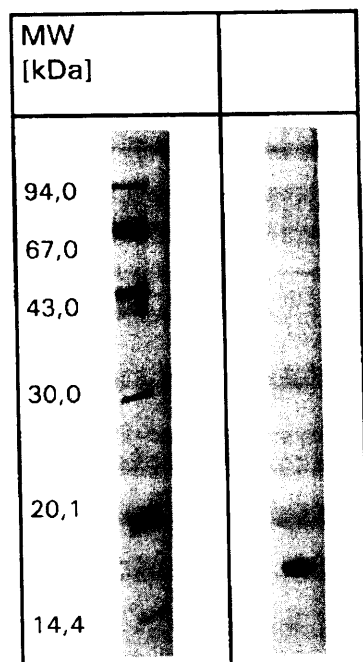


Figure 2. SDS-PAGE analysis of Bet v I from birch pollen isolated by affinity chromatography using an immobilized monoclonal antibody. The gel was silver stained.

Identification of Celery Profilin

On the basis of previous work by Valier et al,³² the 15 to 16-kDa double band was suspected to be celery profilin. Two further experiments were performed to confirm this hypothesis. First, celery blots were incubated with a rabbit antiserum raised against celery profilin. The rabbit antibodies showed intensive binding to both (iso)proteins at 15 to 16 kDa (Fig 4).

Second, we had hoped to perform immunoblot inhibition experiments with celery profilins purified by pLp affinity chromatography. Our yield (15 μ g), however, was too low to guarantee efficient inhibition. Instead, direct IgE binding was investigated by separating the profilin fraction with SDS-PAGE, and incubating with selected sera from the patient collective (Fig 5). The four sera (21, 32, 33, Sec) that reacted to the 15 to 16-kDa double band in the crude celery extract reacted specifically with an identical double band at the same molecular weight in the purified profilin fraction. In con-

trast, neither the nonallergic control serum nor the sera 15 and 16, which contained IgE against the Bet v I related celery allergen, showed any IgE binding to the isolated profilins.

Further evidence for the identity of the bands was obtained by incubating the same six sera with purified profilin from rye grass pollen and with crude protein extracts from apples and birch pollen (results not shown). Sera 21, 32, 33, and Sec had IgE that bound to the grass pollen profilin at approximately 15 kDa. No IgE binding was observed with sera 15, 16, and the non-allergic control. The first four sera also recognized allergens of similar molecular weights on the birch pollen and apple immunoblots. Taken together, these results strongly suggest that the 15 to 16-kDa double band was celery profilin.

IgE Binding to Cross-Reactive Carbohydrate Determinants

Immunoblot analyses of the sera from patients 28, 29, 233 and (to a lesser degree) 22 revealed strong binding to many allergen bands with molecular weights above 25 kDa. Similar IgE binding occurred on blot strips loaded with birch pollen, mugwort pollen, apple extract, and aniseed extract (results not shown). We surmised that these unusual patterns could be due to IgE binding to cross-reactive carbohydrate determinants on a series of plant glycoproteins. To test this hypothesis, the ring structures of the carbohydrate sugar moieties were destroyed by periodate oxidation, and IgE binding experiments with the relevant sera were repeated. IgE binding by sera 28, 29, and 233 (result of last serum not shown) to bands of high molecular weight was strongly reduced by the oxidation procedure (Fig 6). In contrast, the IgE binding activity of the protein epitopes of related apple allergens was not affected by the oxidation procedure.

DISCUSSION

The immunoblot procedure used here consisted of SDS-PAGE under dissociating and reducing conditions, semi-dry electrophoretic transfer of the sep-

arated proteins onto nitrocellulose membranes and peroxidase immunostaining. Although SDS and reductive treatment can reduce the antigenic and probably the allergenic activity of the proteins, in most cases the residual activity is sufficient to be detected by this sensitive method (detection limit <1 ng of target protein^{38,40,41}). In addition, renaturation processes on the nitrocellulose membrane surface and the existence of sequential epitopes may help to compensate for the denaturing effects of sample preparation and electrophoretic separation. In this study, "negative" results (very weak or no IgE binding to celery proteins) were obtained from $\frac{2}{24}$ celery-sensitive patients (25%). Other authors have reported negative immunoblot results for $\frac{3}{36}$ celery-sensitive patients.²³ To rule out losses due to denaturation of conformational epitopes, further studies should include blot analyses under native conditions, for example using the IEF-immunoprint technique.

Binding of IgE antibodies to celery profilin (15 to 16-kDa double band) was most prominent in the sera of patients who were also allergic to mugwort pollen: $\frac{9}{16}$ sera (38%) from the mugwort pollen/celery and birch pollen/mugwort pollen/celery patient groups were positive. In a comparable study of sera from patients with a positive RAST to celery and mugwort pollen, celery and birch pollen, or all three, $\frac{21}{36}$ samples (58%) recognized a 15-kDa double band, recently identified as profilin, from celery extract.^{23,33} A cross-reactive 15-kDa allergen has also recently been found in extracts of stalk celery, cucumber, carrot and watermelon.²² It is tempting to speculate that these cross-sensitivities may also be due to profilins.

In contrast to the mugwort/celery group, the sera of patients with combined birch pollen/celery sensitization reacted predominantly with a 16-kDa celery allergen with cross-reactivity to Bet v I from birch pollen (IgE binding detected in $\frac{8}{8}$ sera). A further $\frac{4}{11}$ patients from the combined birch pollen/mugwort pollen/celery group were sensitized to this allergen. These re-

sults indicate that the Bet v I related celery protein is an important allergen for birch pollen-sensitive patients. Other authors have reported finding similar allergens in celery: initial studies of Valenta et al⁴² pointed to a Bet v I related celery allergen with a molecular weight of 16 to 17 kDa. In another report, a cross-reactive 17-kDa allergy shared by celery, carrot, and birch pollen was identified using the serum of one patient sensitized to birch, grass, and ragweed pollen as well as to celery, carrots and other plant foods.²¹ Cross-reactivity was confirmed by immunoblot inhibition with crude birch pollen extract, but no experiments were carried out with isolated Bet v I. These allergens are probably identical to the 16-kDa allergen identified in our study. This view is further supported by recent sequencing data: using two-dimensional immunoblotting and N-terminal amino sequencing, Bet v I related allergens of 16-kDa molecular weight were identified in celery and carrot. Based on the 15 N-terminal amino acid residues, sequence homologies of 47% and 33% with Bet v I were calculated for the two food allergens, respectively.⁴³

Interestingly, one serum (21) of the birch pollen/celery group of the present study had IgE directed against celery profilin and grass pollen profilin, rather than the Bet v I related protein. Repeated experiments failed to reveal a positive RAST to mugwort pollen. This result may be due to sequence variations among the different plant profilins.

In order to analyze IgE binding to cross-reactive carbohydrate determinants, a method combining ELISA using various oligosaccharides and polysaccharides coupled to paper disks with an immunoblot/periodate oxidation procedure was developed (only the latter was applied in this study). With these methods, IgE binding to carbohydrate determinants could be clearly identified in 19% of 54 sera from patients sensitized to two or more pollen species and five or more plant foods.³⁹ Since the amount of sera was limited in this study, it was not possi-

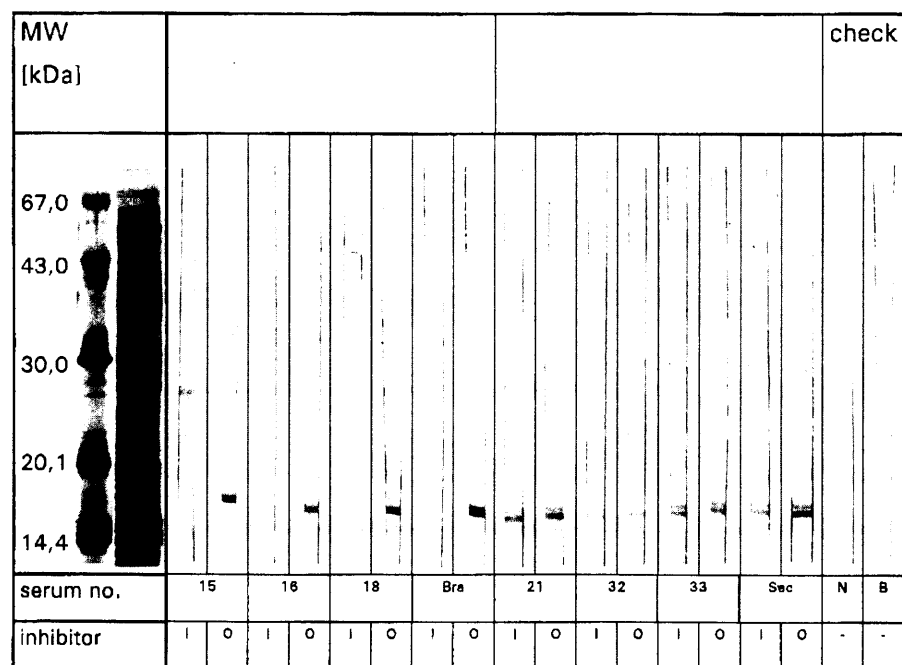


Figure 3. Immunoblot inhibition: Binding of IgE from human sera to celery allergens was inhibited by preincubation of serum with Bet v I or ovalbumin: human sera as indicated, B = buffer control, and N = nonallergic human control serum. I = inhibited with 15 µg Bet v I, and O = inhibited with 100 µg ovalbumin.

ble to confirm the immunoblot results of patients 28, 29, and 233 by ELISA. Since immunoblot and ELISA results were in agreement for most of the patients previously investigated, however, it is tentatively concluded that the IgE antibodies of the three patients were directed against carbohydrate determinants of glycoproteins from celery.

The allergenic activity of celery has a thermostable component (suggesting a carbohydrate rather than a protein moiety) that is associated with mugwort pollinosis, but not with birch pollen allergy.¹⁴ Furthermore, sensitizations to spices are prominent in connection with mugwort pollen and celery allergies, but rare in patients exclusively sensitized to birch pollen and celery.^{9,10,33} In the future, it will be interesting to evaluate whether these phenomena are related, and whether they are due to profilins and/or cross-reactive carbohydrate determinants.

In summary, our results suggest that three distinct structures are probably involved in cross-reactions between celery

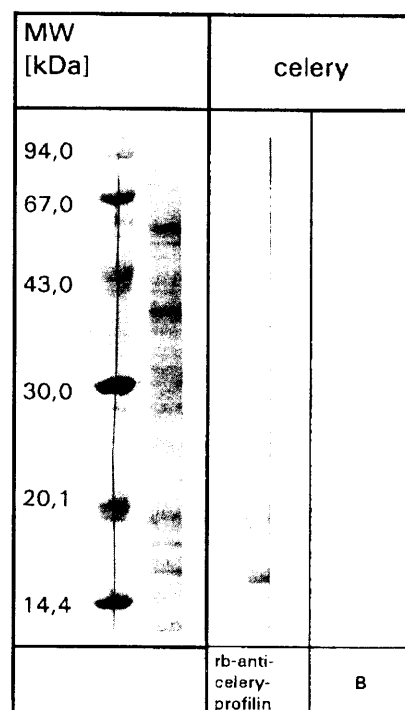


Figure 4. Binding of profilin specific rabbit antiserum (1:16000) to celery extract separated by SDS-PAGE and transferred to NC. B = buffer control.

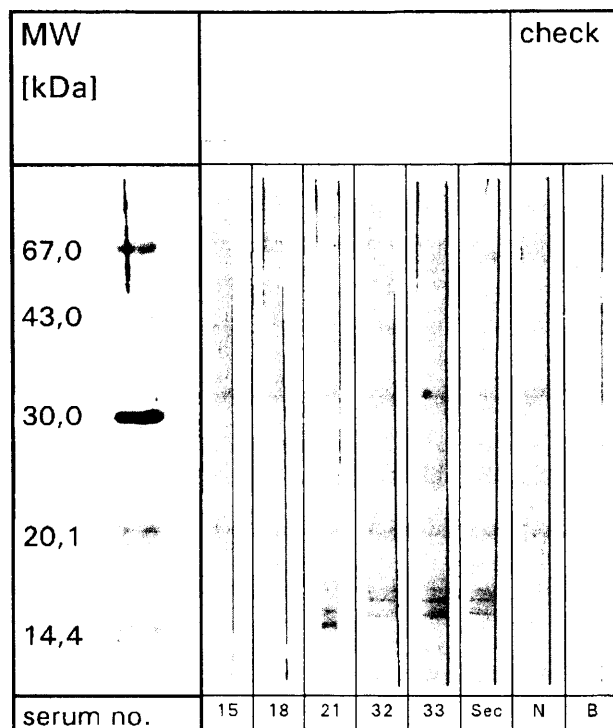


Figure 5. Binding of IgE from human sera to profilin from celery isolated by affinity chromatography. Human sera as indicated, B = buffer control, and N = nonallergic human control serum.

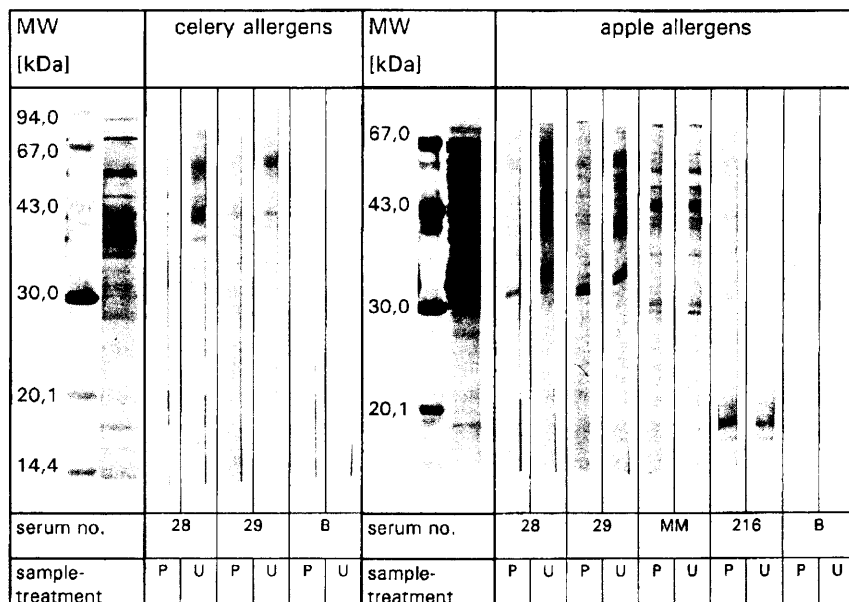


Figure 6. Binding of IgE from human sera to celery and apple allergens separated by SDS-PAGE and transferred to NC. Sample treatment: P O, 1 mol/l periodate-treated strips, and U untreated strips.

and pollen allergens: (1) a 16-kDa protein with structural analogy to the known allergen Bet v I, (2) a 15 to 16-kDa

celery profilin and (3) cross-reactive carbohydrate structures. The first allergen seems to be important for birch pollen/

celery sensitive patients without mugwort pollen sensitization, whereas sensitization to profilin is probably more common in patients with an additional sensitization to mugwort pollen. Further sensitizations associated with the carbohydrate moieties remain to be elucidated.

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