

Allergens, IgE, mediators, inflammatory mechanisms

Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin)

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Background: In this study serum samples collected from 20 patients with birch pollen allergy were investigated. All patients had experienced allergic symptoms after contact with or ingestion of particular fresh fruits and vegetables known as birch pollen-related foods.

Methods: Serum samples were tested by means of immunoblotting for IgE reactivities with proteins in extracts of birch pollen, apple, pear, celery, carrot, and potato. Anti-Bet v 1 and anti-Bet v 2 antibodies were used to investigate cross-reactivity. Inhibition studies were performed by preincubation of sera with recombinant Bet v 1 and Bet v 2.

Results: IgE binding to proteins, corresponding to the major birch pollen allergen Bet v 1 and to Bet v 2 (birch pollen profilin) could be observed. An allergen homologous to Bet v 1 could be detected in apple, pear, and celery when a Bet v 1-specific monoclonal antibody was used. Testing a polyclonal rabbit anti-Bet v 2 antibody with extracts of the respective plants revealed the presence of profilins in every source tested. Inhibition with recombinant Bet v 1 and Bet v 2 led to complete blocking or marked reduction of IgE binding to proteins of comparable molecular weights in the respective food extracts, indicating IgE cross-reactivity.

Conclusion: Our results indicate that many plant-derived food agents contain proteins with high homology to the birch pollen allergens Bet v 1 and Bet v 2 and must therefore be considered as potentially threatening for patients with tree pollen allergy. (*J ALLERGY CLIN IMMUNOL* 1995;95:962-9.)

Key words: IgE cross-reactivity, Bet v 1, Bet v 2, fruits, vegetables, Type I allergy

The clinical observation that patients with type I allergy caused by birch pollen frequently display an intolerance to a variety of fruits and vegetables has been described in several studies.¹⁻⁶ Typically, the symptoms comprise local reactions of the mucosa

Abbreviations used

mAb: Monoclonal antibody
MW: Molecular weight

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of the upper aero-digestive tract (itching, inflammation, angioedema), but a considerable percentage of patients experience systemic symptoms (urticaria, asthma, anaphylactic shock). The food agents, which are not tolerated by patients with tree pollen allergy (birch pollen-related foodstuffs¹) include apples, pears, nuts, carrots, potatoes, celery, and many other plant-derived foods.¹⁻¹²

Recently, we demonstrated by using Western and Northern blot techniques that apples harbor

proteins with a high homology to Bet v 1, the major allergen of birch pollen.¹¹ Moreover, the existence of cross-reacting proteins could clearly be shown for hazelnuts, in which homologous proteins to Bet v 1 and Bet v 2 (birch pollen profilin) could be identified by inhibition experiments with recombinant allergens.¹² Bet v 1 is known to belong to a ubiquitous (at least in angiosperms) family of pathogenesis-related proteins.¹³ Besides Bet v 1, there are other proteins known to be ubiquitously distributed in plants and capable of eliciting an IgE response in atopic patients. In this respect, the cytoskeleton-associated profilins are potential candidates for cross-reactivity.¹⁴

In this study protein extracts from plant sources known to elicit allergic symptoms in patients with pollinosis were investigated. The aim of the work was to identify potentially dangerous foodstuffs by demonstrating the presence of Bet v 1- or Bet v 2 (profilin)-related proteins in these sources. Bet v 1 and Bet v 2 were cloned, sequenced, and expressed as recombinant proteins in our laboratory.^{15, 16} These recombinant proteins and specific antibodies^{17, 18} were used to study cross-reactivities.

Because numbers of life-threatening anaphylactic reactions caused by food allergy have been reported,^{19, 20} the identification of foods containing potential allergens seems to be very important.

METHODS

Sera

Serum samples from 20 patients with tree pollen allergy (typical case history, positive skin prick test results, and RAST class 3.5 to 5.0 in response to birch pollen) were collected. These patients were selected because they had experienced adverse reactions to at least two of the following plant-derived foodstuffs: apple, pear, celery, carrot, and potato. The symptoms after ingestion of one of the foods included mild local reactions in 19 of 20 cases (itching, burning of the lips and oral mucosa), and/or severe local symptoms that could be attributed to the consumption of the respective food; other symptoms were angioedema of the oropharyngeal tract and/or abdominal pain in eight cases. In four cases systemic anaphylactic reactions could be observed (bronchial asthma, generalized urticaria, shock). Sera from seven nonallergic individuals (negative case history, negative skin prick test results, negative RAST response to tree pollen) were pooled (normal human serum) and used for control experiments.

Protein extracts

Birch pollen was obtained from Allergon AB (Engelholm, Sweden) and extracted as described previously.²¹ The following plant sources were investigated: apple

(Golden Delicious), pear, celery, carrot, and potato. Food materials were purchased from a local retail shop. Proteins from fresh, uncooked fruits and vegetables were extracted in 10% (wt/vol) by overnight shaking at 4° C in extraction buffer: 10 mmol/L potassium phosphate buffer containing 2% (wt/vol) suspended solid polyvinylpyrrolidone, 2 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L sodium diethyldithiocarbamate, and 3 mmol/L sodium azide, pH 7.0. After centrifugation at 40,000 g for 60 minutes at 4° C, the supernatant was filtered and subsequently dialyzed (Spectra/Por1, Molecularporous Dialysis Membrane, mwco:6-8,000; Spectrum, Houston, Texas) against 5 L of 10 mmol/L potassium phosphate buffer containing 3 mmol/L sodium azide, pH 7.0, for 48 hours with three changes of buffer. The dialysates were lyophilized and stored at -20° C. Protein concentrations were determined according to the method of Bradford.²²

Expression and purification of recombinant Bet v 1 and recombinant birch profilin (rBet v 2)

Escherichia coli JM 105 organisms, harboring the recombinant pKK 223-3 plasmids, were grown and harvested as described by Valenta et al.²³ Bet v 1 was purified by chromatofocusing and high-performance liquid chromatography on C8 reversed-phase columns as described in detail by Ferreira et al.¹⁵ Purification of rBet v 2 was performed by affinity chromatography to (poly) L-proline as described previously.²⁴ Protein concentration of purified recombinant proteins was determined by the Micro-Kjeldahl method with glycine as standard.²⁵

Sodium dodecylsulfate-polyacrylamide gel electrophoresis, protein transfer, and IgE immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (12% homogeneous gels, 140 × 115 × 1 mm, 5% stacking gels) was performed according to the method of Laemmli²⁶ in a vertical slab gel apparatus (Hoefer, San Francisco, Calif.) with modifications as previously described.²¹ The samples were diluted (1:4) in Laemmli buffer and subsequently loaded onto gels (14 mg extract/cm gel). Transfer of electrophoretically separated proteins to nitrocellulose sheets was done according to the method of Towbin et al.²⁷ IgE immunoblots were performed as previously described.²¹

IgE inhibition experiments with purified recombinant allergens

Serum pool for inhibition experiments. Sera from patients with reactivity to Bet v 1 and Bet v 2 in the birch pollen extract and displaying reactivity to proteins in the same molecular weight (MW) range (13 to 18 kd) in all fruit and vegetable extracts investigated were pooled (patients 2, 3, 4, 6, 8, 9, 10). This serum pool was used in 1:10 dilution for all inhibition experiments.

Inhibition with purified rBet v 1. The serum pool was

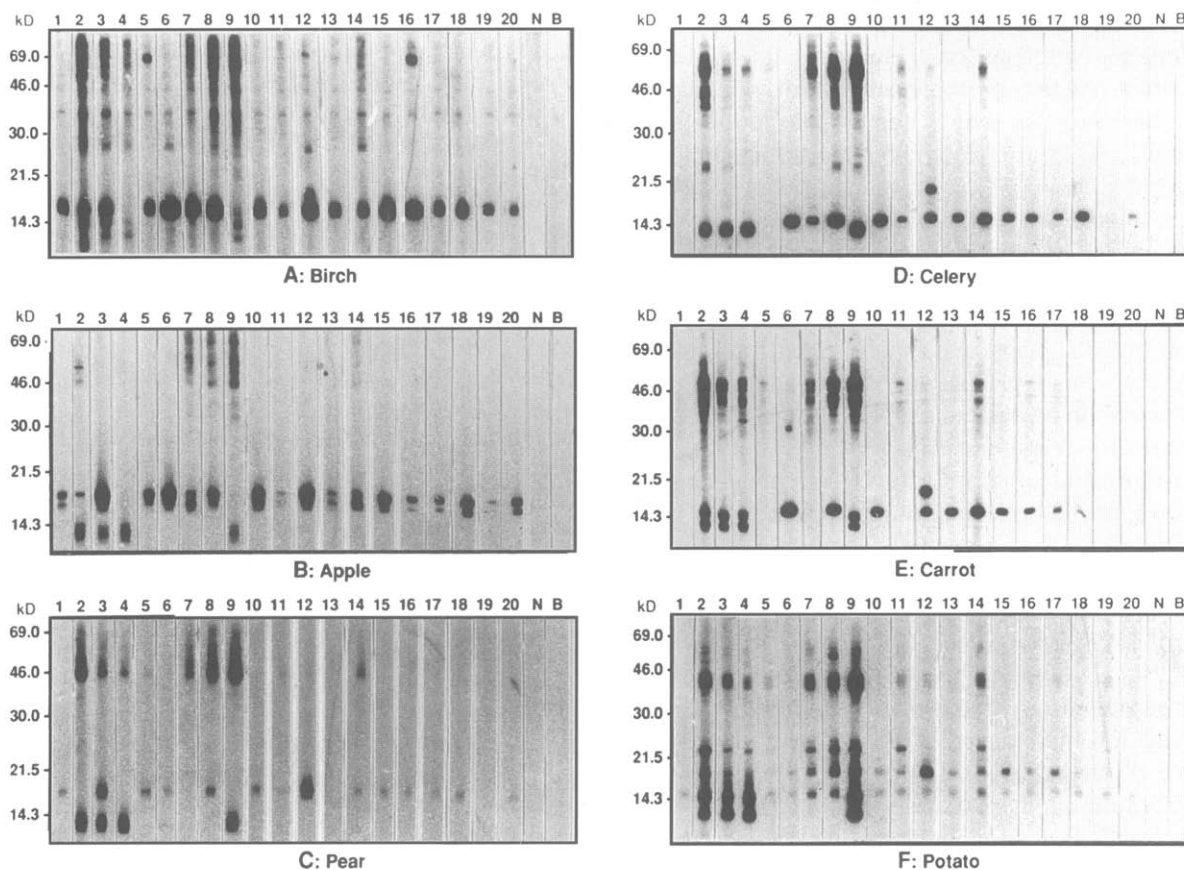


FIG. 1. A, IgE immunoblots with sera from 20 patients with birch pollen allergy on birch pollen-coated nitrocellulose strips. B, IgE immunoblots with sera from 20 patients with birch pollen allergy on apple extract-coated nitrocellulose strips. C, IgE immunoblots with sera from 20 patients with birch pollen allergy on pear extract-coated nitrocellulose strips. D, IgE immunoblots with sera from 20 patients with birch pollen allergy on celery extract-coated nitrocellulose strips. E, IgE immunoblots with sera from 20 patients with birch pollen allergy on carrot extract-coated nitrocellulose strips. F, IgE immunoblots with sera from 20 patients with birch pollen allergy on potato extract-coated nitrocellulose strips.

diluted 1:10 in buffer and preincubated overnight at 4° C with 10 µg of purified recombinant Bet v 1. Thereafter, nitrocellulose-blotted food proteins were incubated with the serum samples (1 ml/nitrocellulose strip), and bound IgE was detected as described by Jarolim et al.²¹

Inhibition with purified rBet v 2 (birch profilin). The serum pool was diluted 1:10 in buffer and preincubated overnight at 4° C with 10 µg of purified recombinant Bet v 2. Thereafter, nitrocellulose-blotted food proteins were incubated with the serum samples (1 ml/nitrocellulose strip), and bound IgE was detected as previously described.²¹

Immunoblots with monoclonal anti-Bet v 1 and rabbit anti-Bet v 2 antibodies

BIP 1, a monoclonal antibody (mAb) directed against Bet v 1,¹⁷ was tested for reactivity with all extracts investigated. Immunoblot experiments were performed as described by Jarolim et al.¹⁷ A rabbit anti-celery profilin antibody¹⁸ was used to detect homologous pro-

teins in different food sources. Nitrocellulose strips were probed with a 1:1000 dilution of the antibody. Bound rabbit antibodies were detected with an iodine 125-labeled donkey anti-rabbit antibody (Amersham International Ltd., Little Chalfont, Bucks, U.K.), diluted 1:1000.

RESULTS

IgE immunoblots

Birch pollen extract. All sera displayed detectable IgE binding to birch pollen proteins. Nineteen of 20 patients showed IgE binding to Bet v 1 (17 kD). Three sera (nos. 2, 3, and 9) contained IgE antibodies to birch profilin (14 kD) in addition to Bet v 1. Serum from one patient (no. 4) reacted with Bet v 2 but not with Bet v 1. Sera from 10 patients showed IgE antibodies to minor allergens with MWs higher than 30 kD (Fig. 1, A).

Apple extract. All sera displayed detectable IgE binding to apple extract. All patients reacting with Bet v 1 (Fig. 1, *A*) showed IgE binding to a double band in the 17 kd region. Sera from patients 2, 3, 4, and 9 reacted with a band in the region 13 to 14 kd. Sera from four patients recognized allergens in the higher MW range (Fig. 1, *B*).

Pear extract. Eighteen of 20 sera displayed IgE binding to pear extract. Fourteen sera reacted with a protein of 17 kd, and four sera (patients 2, 3, 4, and 9) reacted with an allergen with MW of 14 kd. Sera from seven patients recognized proteins in the higher MW range (Fig. 1, *C*).

Celery extract. Seventeen of 20 patients' sera displayed IgE binding to celery proteins. Sera from patients 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, and 20 recognized a protein of 15 kd; sera from patients 2, 3, 4, and 9 reacted with a protein of 13 to 14 kd. Eight patients reacted with allergens in the higher MW range (Fig. 1, *D*).

Carrot extract. Sixteen of 20 sera displayed IgE binding to carrot proteins. Ten patients reacted with a protein of 15 kd; sera from patients 2, 3, 4, and 9 recognized a double band in the MW range of 13 to 14 kd. Sera from nine patients reacted with allergens of higher MW (Fig. 1, *E*).

Potato extract. All patients' sera displayed IgE binding to potato proteins. The patients displayed one or several bands in the MW range of 15 to 22 kd; four patients (nos. 2, 3, 4, and 9) reacted with a molecule of 13 kd. Sera from 10 patients recognized proteins in the higher MW range (Fig. 1, *F*).

Inhibition experiments with rBet v 1 and rBet v 2 (birch profilin)

Birch pollen extract. Preincubation of a serum pool containing specific IgE to Bet v 1 and Bet v 2 with rBet v 1 led to blocking of the respective band at 17 kd, whereas IgE binding to Bet v 2 was not affected (Fig. 2). Preincubation of the serum pool with rBet v 2 led to complete inhibition of the 14 kd band representing birch profilin; IgE binding to Bet v 1 was not affected (Fig. 2).

Apple extract. Preincubation of a serum pool containing specific IgE to a 17 kd band and to a 14 kd band in apple extract with rBet v 1 led to complete blocking of the respective band at 17 kd, whereas IgE binding to the 14 kd band was not affected (Fig. 2). Preincubation of the serum pool with rBet v 2 led to complete inhibition of the 14 kd band; IgE binding to the 17 kd band was not affected (Fig. 2).

Pear extract. Preincubation of a serum pool containing specific IgE to a 17 kd band and to a 14 kd

band in pear extract with rBet v 1 led to complete blocking of the respective band at 17 kd, whereas IgE binding to the 14 kd band was not affected (Fig. 2). Preincubation of the serum pool with rBet v 2 led to complete inhibition of the 14 kd band; IgE binding to the 17 kd band and to high MW proteins was not affected (Fig. 2).

Celery extract. Preincubation of a serum pool containing specific IgE to a 15 kd band and to a 14 kd band in celery extract with rBet v 1 led to significant inhibition of the band at 15 kd, whereas IgE binding to the 14 kd band was not affected (Fig. 2). Preincubation of the serum pool with rBet v 2 led to complete inhibition of the 14 kd band; IgE binding to the 15 kd band was not affected (Fig. 2).

Carrot extract. Preincubation of a serum pool containing specific IgE to a 15 kd band and to a 14 kd band in carrot extract with rBet v 1 led to significant blocking of the respective band at 15 kd, whereas IgE binding to the 14 kd band was not affected (Fig. 2). Preincubation of the serum pool with rBet v 2 led to complete inhibition of the 14 kd band; IgE binding to the 15 kd band was not affected (Fig. 2).

Potato extract. After preincubation of a serum pool containing specific IgE to proteins with 18 to 22 kd and to proteins of 13 and 15 kd band in potato extract with rBet v 1, no inhibition could be observed (Fig. 2). Preincubation of the serum pool with rBet v 2 led to complete inhibition of the 12 to 14 kd band; IgE binding to the 18 to 22 kd band and to a high MW band was not affected (Fig. 2).

Immunoblots with Bet v 1- and Bet v II-specific antibodies

BIP 1. The anti-Bet v 1 mAb BIP 1 displayed strong reactivity to a 17 kd protein present in birch pollen and apple. Weak reactivity was observed with a 17 kd protein in the pear extract. The mAb recognized a 15 kd protein in the celery extract. No reactivity was observed when BIP 1 was tested with carrot and potato extracts (Fig. 3).

Rabbit-anti-profilin antibody. The polyclonal rabbit-anti-profilin antibody reacted with all extracts investigated in the MW range of 14 kd (Fig. 4).

DISCUSSION

Tree pollen allergy is a common disease in countries of the northern hemisphere. In this respect, the pollens of trees of the order *Fagales* (e.g., birch, hazel, alder, hornbeam) represent an important source of allergens. The major allergens of these pollens are highly homologous at the

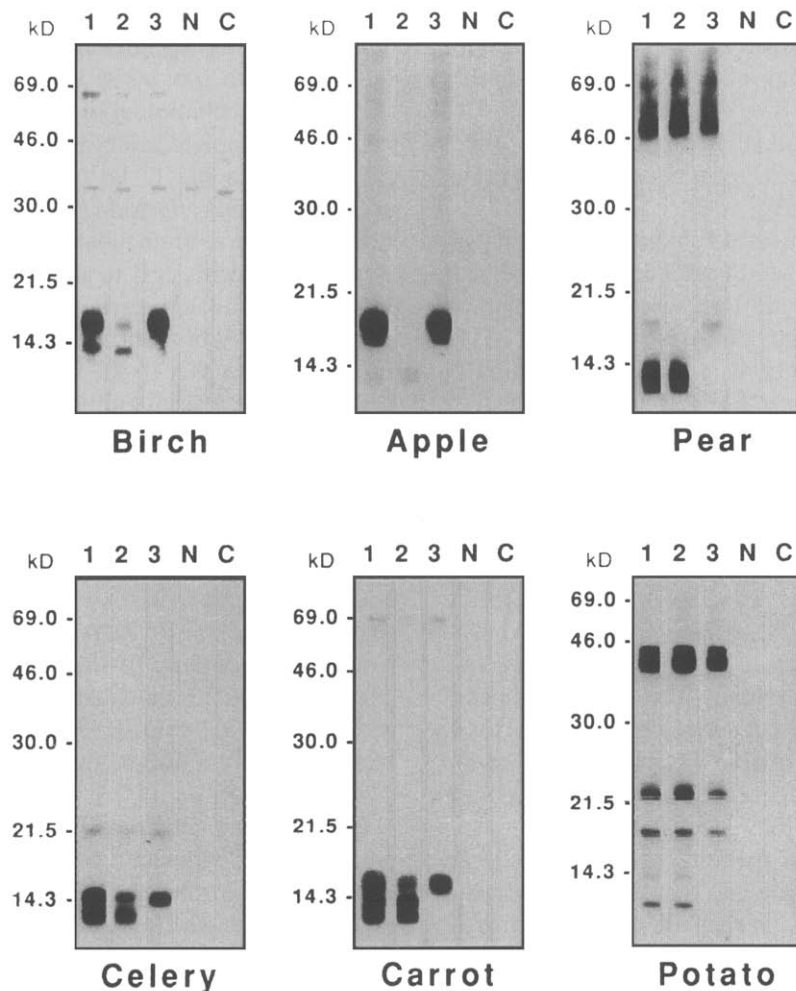


FIG. 2. Inhibition experiment. *Lane 1*, Sera from patients reactive with Bet v 1, Bet v 2, and the putative cross-reacting allergens in fruit and vegetable extracts were pooled and tested for IgE binding to birch, apple, pear, celery, carrot, and potato extracts. *Lane 2*, Immunoblot with the Bet v 1/Bet v 2-positive serum pool after preincubation with 10 µg of recombinant Bet v 1. *Lane 3*, Immunoblot with the Bet v 1/Bet v 2-positive serum pool after preincubation with 10 µg of recombinant Bet v 2. *Lanes N and C*, Normal human serum and buffer controls.

nucleic acid and protein levels and are therefore cross-reacting, so that virtually all patients with tree pollen allergy have type I reactions during the flowering season of all trees of this particular order in spring.²⁸⁻³⁰ The clinical observation that patients with tree pollen allergy frequently experience symptoms after ingestion of particular fruits and vegetables is well established.¹⁻⁶ The symptoms have mostly local character, but in several cases systemic symptoms such as urticaria, angioedema, or anaphylaxis have been reported. For particular food sources (i.e., apples, hazelnuts) the presence of allergens, cross-reacting with allergen Bet v 1, the major birch pollen allergen, and with Bet v 2 (birch profilin) have been demonstrated.⁵⁻¹² This study addresses the question of whether cross-

reacting proteins to these two molecules could be detected in all of the plant-derived foods that are frequently not well tolerated by individuals with tree pollen allergy. Twenty patients with typical case history and positive skin prick test and RAST responses to birch pollen were investigated. All of the patients displayed adverse reactions to at least two of the foods investigated in this study (apple, pear, carrot, celery, and potato). IgE immunoblot analysis revealed IgE binding to extracts produced from the plant sources in almost every patient; proteins of similar MW were recognized by patients' IgE in all extracts. Nineteen of 20 patients displayed IgE specific for Bet v 1, the major birch pollen allergen; 10 of 20 patients (50%) reacted with proteins of 15 to 18 kD in all the other extracts

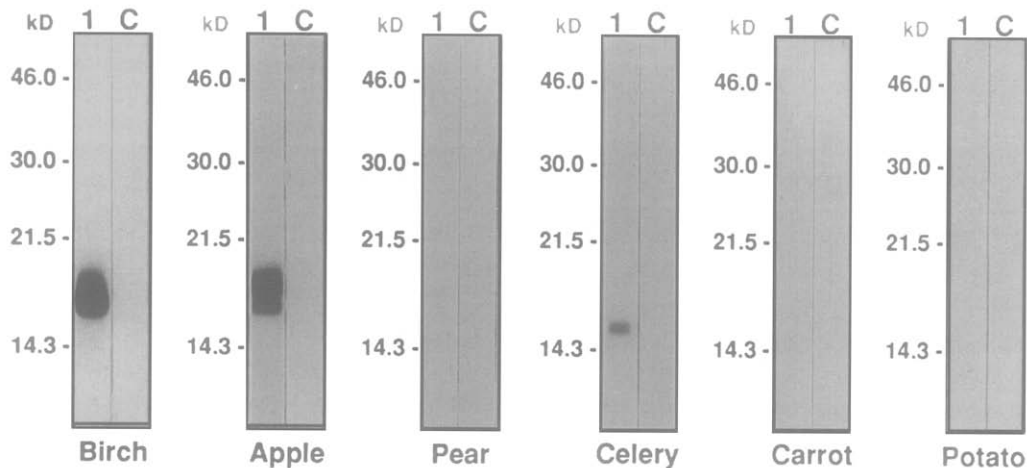


FIG. 3. Immunoblot with an anti-Bet v 1 mAb. Reactivity with fruit and vegetable extracts. C, Control.

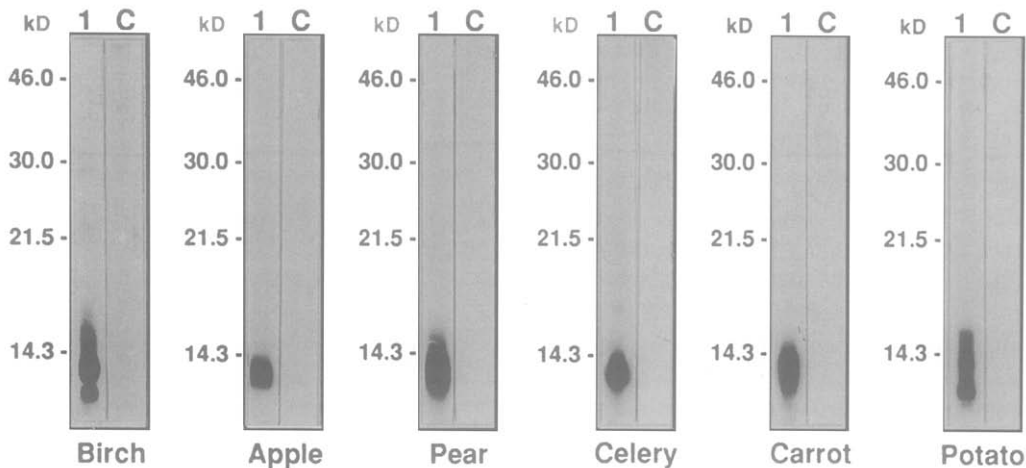


FIG. 4. Immunoblot with an anti-celery-profilin antibody from rabbit. Reactivity with fruit and vegetable extracts. C, Control.

tested. However, for some patients individual binding patterns could be observed. For example, sera from patients 1, 2, 3, 4, 5, and 19 displayed IgE binding to Bet v 1 in the birch pollen extract, but did not recognize corresponding proteins in celery or carrot extracts in spite of strong reactivity with 17 kD proteins in apple and pear. This phenomenon correlated with the clinical situation of the respective patients. On the other hand, serum from patient 7, reacting with Bet v 1 in the birch pollen extract also recognized the 15 kD Bet v 1 homologue in celery¹⁰ and the 17 kD homologue in apple¹¹ but showed no IgE reactivity in this MW range in pear and carrot extracts. Serum from patient 11 obviously reacts with Bet v 1 and corresponding molecules, but not with the homologue in carrot. This phenomenon was independent of the IgE titer and indicates individual

recognition patterns for particular patients. Also, the serum pool used for all inhibition experiments confirms this observation: the same serum pool in some extracts reacted more strongly with the Bet v 1 homologue than with the Bet v 2 homologue (e.g., in the apple extract, Fig. 2); in others the Bet v 2 homologue was strongly recognized, but reactivity with the Bet v 1 homologue was hardly detectable (e.g., in pear extract, Fig. 2). Molecular biology methods could provide information about homologies between the allergens and help to characterize regions on these molecules that are crucial for antibody binding and thus for the allergic reaction. Cross-reactivity of homologous proteins with Bet v 1 was proved by inhibition experiments. By preincubation with recombinant Bet v 1 it was possible to block or markedly reduce IgE binding to homologous proteins in apple, pear,

celery, and carrot; no significant inhibition could be demonstrated in potato (Fig. 2). BIP 1, an mAb raised to Bet v 1,¹⁷ displayed a positive signal in birch, apple, celery, and pear (Fig. 3) but did not bind to proteins present in carrot or potato extracts, again indicating shared epitopes but also providing evidence for differences in the epitope composition of the different allergens. Four of 20 sera from our patients-collective displayed IgE binding to Bet v 2 (birch profilin) in the birch pollen extract. In all four cases, reactivity of patients' sera proved the presence of a profilin homologue in all plant extracts tested. Also in this case, cross-reactivity was proven by cross-inhibition (positive for all extracts including potato, Fig. 2) and by cross-reactivity of a profilin-specific antibody (positive signal in every extract tested, Fig. 3). In all cases the food hypersensitivity named in the case history could be confirmed by an IgE-binding band in the respective food extract. It is well known that some of these foods can be safely eaten by sensitive patients if cooked, indicating thermolability of the allergens. However, 10 minutes of cooking in sodium dodecylsulfate-containing sample buffer²⁶ did not abolish IgE-binding capacities of our extracts. For many patients, additional potential allergens could be identified (i.e., IgE-binding proteins in food extracts other than those named by the patient). Thus it can be concluded that many fruits and vegetables contain birch pollen-related allergens, which can lead to symptoms in predisposed (birch pollen-allergic) patients. We consider these foods potentially harmful for birch pollen-allergic individuals with specific IgE to either Bet v 1 or Bet v 2.^{19, 20, 23}

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