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Characterization of Allergens in Kiwi Fruit and Detection of Cross-reactivities with Allergens of Birch Pollen and Related Fruit Allergens

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The sera of 29 patients who suffered from pollen-related food hypersensitivities and complained of allergic reactions to kiwi fruit and other tropical fruits were tested for specific IgE antibodies against kiwi fruit, apple, carrot, celery and birch pollen using an enzyme allergosorbent test (EAST). In 20 sera, specific IgE antibodies were detected against all five extracts. Sodium dodecyl sulphate polyacrylamide gel electrophoresis/immunoblot of kiwi fruit extract revealed two major allergens with molecular weights of approximately 43 and 67 kDa. In EAST inhibition assays, cross-reactivities between kiwi fruit, apple, birch pollen and, to a lesser degree, carrot and celery were demonstrated. The cross-reactivities seen between kiwi fruit, birch pollen and apple were not caused by the major allergen of birch pollen (Bet v 1). Allergens with molecular weights of approximately 68 kDa in birch pollen and 67 kDa in apple cross-reacted with the allergens of kiwi fruit, as demonstrated by immunoblotinhibition. Profilins, which are known plant pan-allergens, do not seem to be relevant allergens in kiwi fruit.

Keywords: Kiwi fruit, birch pollen, apple, food allergy, IgE, immunoblotting, immunoblot inhibition, enzyme allergosorbent test, enzyme allergosorbent test-inhibition

INTRODUCTION

Case reports of allergic reactions to kiwi fruit (*Actinidia chinensis*) have been described relatively frequently. The clinical symptoms range from oropharyngeal symptoms, such as

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swelling of the lips and tongue and itching in the mouth or throat (Vocks *et al.*, 1988, 1993; Gall *et al.*, 1990) to urticaria (Seifert *et al.*, 1988), contact urticaria (Fine, 1981; Veraldi & Schianchi-Veraldi, 1990), Quincke oedema (Gall *et al.*, 1994), nausea, vomiting, diarrhoea (Vocks *et al.*, 1988, 1993; Seifert *et al.*, 1988) and even anaphylaxis (Falliers, 1983; Freye, 1989; Novembre *et al.*, 1995). In contrast, little information is available about the allergens in kiwi fruit responsible for these reactions.

Gall *et al.* (1990) demonstrated cross-reactivities between allergens from kiwi fruit and birch pollen, mugwort pollen, avocado, hazelnut, apple, carrot and potato using radioallergosorbent test (RAST) inhibition assays with the sera of two patients with an allergy to kiwi fruit. In further investigations, Gall *et al.* (1994) showed that kiwi fruit is another allergenic food related to birch pollen allergy. There were detectable amounts of specific IgE antibodies to birch pollen in all 22 sera of patients who suffered from allergic reactions to kiwi fruit, but only 12 sera of patients who showed strong reactions to kiwi fruit contained specific IgE antibodies to this fruit. In RAST inhibition assays using sera from three patients, strong cross-reactivities were detected between kiwi fruit and birch pollen, apple and carrot. Very small amounts of protein in these extracts were necessary to cause 50% inhibition of IgE binding to kiwi fruit disks. However, no information was given about the allergens being responsible for these cross-reactivities; in particular, no immunoblot analysis was carried out. Vocks *et al.* (1993) used only two sera for immunoblot analysis of kiwi fruit extracts and found a broad spectrum of IgE binding components. The extract from kiwi fruit was able to inhibit IgE binding to allergens in hazelnut- and rye-flour extracts in immunoblot inhibition experiments. Cross-reactivities between kiwi fruit and birch pollen or related fruits were not investigated.

Cross-reactivities between birch pollen and fruits (e.g. apple, cherry and hazelnut) are frequently caused by allergens belonging to the 'Bet v 1 family' (Ebner *et al.*, 1991, 1995; Hirschwehr *et al.*, 1992; Vieths *et al.*, 1994; Schöning *et al.*, 1995). Bet v 1 is a 17-kDa glycoprotein and the major allergen of birch pollen. It has been hypothesized that this pollen allergen acts as a primary immunogen, and that cross-reactivities with fruits are caused by fruit allergens with epitopes having a lower affinity to the allergen-specific IgE of patients (Björkstén *et al.*, 1980; Dreborg, 1988; Ebner *et al.*, 1991). One such fruit allergen is the major allergen in apple, an 18-kDa allergen (Mal d 1), which shows structural similarities to Bet v 1 and is responsible for cross-reactivities with birch pollen (Vieths *et al.*, 1994, 1995; Schöning *et al.*, 1995).

However, profilins have been identified as the allergens being responsible for cross-reactivities between botanically unrelated pollen and fruits (Valenta *et al.*, 1992a,b, 1993; Vallier *et al.*, 1992; van Ree *et al.*, 1992). Profilins are proteins present in eucaryotic cells. They have various biological functions; for example, they can act as actin-sequestering proteins and as participants in the phosphoinositide pathway of signal transduction. Profilins are prominent allergens in the pollens of trees, grasses and weeds. They are recognized by approximately 20% of all pollen-allergic patients (Valenta *et al.*, 1992a). A profilin was also detected in birch pollen. This was called Bet v 2 (Valenta *et al.*, 1992a).

The aim of the present investigation was to characterize the allergens of kiwi fruit by their molecular weights using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblotting. In addition inhibition of an enzyme allergosorbent test (EAST) was used to establish whether there are cross-reactivities between kiwi fruit, birch pollen and related fruits. In a final step, the allergens being responsible for these cross-reactivities were detected. In this context, it had to be determined whether profilins are also involved in these reactions.

PATIENTS, MATERIALS AND METHODS

Patients

Twenty-nine patients with birch pollen hypersensitivity and clinical symptoms of allergy to kiwi fruit or other tropical fruits were recruited at the Borkum Riff Hospital of the BfA

(Borkum, Germany) and at the Department of Dermatology and Allergy and Experimental Dermatology and Allergology, University Hospital Hamburg-Eppendorf (Hamburg, Germany). The case history of each patient was noted and sera were collected. One additional serum (sample Mast 103) was obtained commercially from Mast Diagnostica GmbH (Reinfeld, Germany). Serum from one non-atopic individual with no history of food hypersensitivity was used as a negative control.

Allergenic Extracts

Allergenic extracts from kiwi fruit, apple, celery and carrot were prepared by a low-temperature method, as described by Vieths *et al.* (1992a) with some minor modifications. Briefly, pieces of fresh fruit were homogenized in acetone at -40°C (cooling in dry ice), and the mixture was stored overnight at -20°C . The precipitates were washed twice with acetone (-20°C) and once with acetone:diethylether (1:1, v:v, -20°C), filtered and lyophilized. Protein extracts were obtained by extracting approximately 1.5 g of this acetone powder with 30 ml of 0.01 M-potassium phosphate buffer (PBS) containing 0.15 M-NaCl, pH 7.4 for 60 min by ice cooling. The suspension was centrifuged (4°C , $10\,500 \times g$, 60 min) and the supernatant was filtered. The solution was divided into aliquots of 1 ml, lyophilized and stored at -20°C until use.

The protein content of these extracts was determined by the method of Lowry *et al.* (1951), modified by Bensadoun and Weinstein (1976). The protein content ranged from 250 (apple) to $1500\,\mu\text{g ml}^{-1}$ (kiwi fruit).

Birch pollen extract was obtained commercially from Allergopharma (Reinbek, Germany). The protein content of this extract was $1000\,\mu\text{g ml}^{-1}$. The purified major birch pollen allergen Bet v 1 was kindly supplied by Allergopharma (Dr B. Weber). The allergen was isolated by affinity chromatography using an immobilized, allergen-specific monoclonal antibody.

EAST

Preparation of allergen disks. Paper disks were activated with cyanogen bromide according to the method described by Ceska and Lundquist (1972). Proteins of the allergenic extracts from kiwi fruit, apple, birch pollen, carrot and celery were coupled to these disks by a procedure slightly modified to that described by Ceska and Lundquist (1972). Approximately 300 allergen disks were stirred in 100 ml of coupling solution ($20\,\mu\text{g ml}^{-1}$ of protein in double-distilled water) overnight at 4°C . The solution was aspirated and the disks were washed with 100 ml 0.5 M-sodium hydrogen-carbonate (pH 8.4). Afterwards, paper disks were deactivated by stirring with 100 ml of ethanolamine buffer (1% by volume, pH 9.2) for 120 min. The disks were then washed three times with 100 ml of 0.1 M-sodium bicarbonate (pH 9.5), twice with 100 ml of 0.1 M-sodium acetate (pH 4.0) and twice with 0.5 M-sodium bicarbonate (pH 8.4). Finally, the disks were lyophilized and stored at -20°C .

Prior to use, the allergen disks were reconstituted with an incubation buffer (4.6% ethylene diamine tetraacetic acid, 0.3% bovine serum albumin (BSA), 0.25% sodium azide and 0.25% Tween 20 in 0.1 M-sodium phosphate buffer, pH 7.4).

EAST. Specific IgE antibodies against kiwi fruit, birch pollen, apple, celery and carrot in patients sera were measured using a commercial test kit (Allergopharma) according to the manufacturer's instructions. The absorbance was measured at 405 nm. The results were expressed in EAST classes (1–4) and specific IgE levels (in U ml^{-1} (1 U = $2.4\,\text{ng ml}^{-1}$ of IgE)) using the manufacturer's reference system.

EAST inhibition assay. Pooled serum was diluted 1:2 (kiwi fruit disks) or 1:5 (birch pollen and apple disks) in incubation buffer (0.3% BSA plus 0.1% Tween 20 in PBS). A 10-fold dilution series of the inhibitor extracts from kiwi fruit, birch pollen, celery, carrot and apple was prepared in five steps using the same incubation buffer (undiluted, 1:10, 1:100, 1:1000 and 1:10 000). Ovalbumin was used to check non-specific inhibition. A total of 50 μ l of diluted serum was mixed with 50 μ l of the inhibitor solution. After 15 min, an allergen disk was added and incubated overnight at room temperature in the dark. After three washes with 1% Tween 20 in PBS (v/v), 50 μ l of anti-human IgE alkaline phosphatase conjugate (Sigma, St Louis, MO, USA), diluted 1:200 in incubation buffer, were added and incubated overnight. The disks were washed again and the bound enzyme activity was stained with *p*-nitrophenylphosphate (PNPP) for 60 min at 37°C using an alkaline phosphatase staining kit (Bio-Rad, Munich, Germany). Absorbance was measured at 405 nm. Inhibition of <5%, <50% and >50% were considered non-significant, weak inhibition and strong inhibition respectively.

SDS-PAGE

Discontinuous SDS-PAGE was performed according to the method of Laemmli (1970) with a 5% (w/v) acrylamide stacking gel and a 13% (w/v) acrylamide resolving gel. Protein samples were reduced with β -mercaptoethanol (sample buffer according to Vieths *et al.* (1992b)) and loaded on to the gel at a concentration of 10 μ g cm^{-1} for immunoblot analysis. The gels (250 $\times$ 120 $\times$ 0.5 mm) were developed in a horizontal slab gel apparatus (Desaphor HF; Desaga, Heidelberg, Germany), first at 200 V until the samples had entered the resolving gel and then at 600 V until the dyes were at the anode.

Protein Transfer

Proteins were transferred to nitrocellulose (NC) membranes (0.2 μ m; Schleicher and Schüll, Dassel, Germany) according to the method of Kyse-Andersen (1984), using a Novablot semi-dry blotting apparatus (Pharmacia, Freiburg, Germany). The blotting time was 40 min at 0.8 mA cm^{-2} .

Immunostaining

Immunostaining was performed according to Vieths *et al.* (1992b) with minor modifications. Briefly, cut strips (4 mm) of the dried NC membranes were blocked twice with 5% skimmed milk powder and 0.1% Tween 20 in PBS (Ahlroth *et al.*, 1995) in mini-incubation trays (Bio-Rad). The strips were then incubated with 100 μ l of serum diluted in a total volume of 1.5 ml of incubation buffer (0.3% BSA, 0.1% Tween 20 in PBS) overnight at room temperature. After incubation with rabbit anti-human IgE (1:4000, 60 min), biotinylated goat anti-rabbit IgG (1:6000, 60 min; both reagents from Dako, Copenhagen, Denmark) and streptavidin-horseradish peroxidase conjugate (1:20 000, 20 min; reagent from Medac, Hamburg, Germany), the blots were stained with 3,3',5,5'-tetramethylbenzidine-dioctylsodiumsulphosuccinate.

One strip was incubated with serum from a non-allergic patient to check unspecific binding. To check the efficiency of protein transfer, one strip with separated extract and one strip with low molecular weight marker proteins (Pharmacia) were stained with Indian ink according to the method described by Hancock and Tsang (1983).

Immunoblot Inhibition

A total of 100 μ l of the same pooled serum used for EAST inhibition was incubated with 100 μ g of protein of the allergenic extracts and 10 μ g of Bet v 1 respectively in a total volume of 1.5 ml of incubation buffer for 60 min at room temperature. Afterwards, immunostaining was performed by the procedure described earlier.

Detection of Profilins

Strips of the NC membranes with separated kiwi fruit proteins were incubated with polyclonal rabbit antisera against profilins from ragweed and celery diluted 1:500 in incubation buffer. The bound antibodies were visualized as described earlier, omitting the incubation step with anti-human IgE. The polyclonal antisera against ragweed and celery profilins were kindly supplied by Dr P. Deviller, Laboratoire de Biochimie, Hôpital Louis Pradel, Lyon, France.

RESULTS

Patients

Of the patients, 23 and 25 complained of oral symptoms of allergy to kiwi fruit and apple respectively. Twenty-one patients showed oral symptoms to both fruits. Two patients (C. H. and Bo 207 K) suffered from urticaria to kiwi fruit. No patients showed gastrointestinal symptoms or anaphylaxis.

Seven patients and one patient suffered from clinical symptoms after eating carrot and celery respectively. These patients also showed clinical symptoms to kiwi fruit and apple. The results indicate that allergens from kiwi fruit may be particularly strongly related to allergens from birch pollen and apple. Clinical symptoms to kiwi fruit and apple are presented in Table 1.

EAST

The sera of 30 patients and the commercially obtained serum (Mast 103) were examined for specific IgE antibodies against birch pollen, kiwi fruit, apple, carrot and celery. The IgE content of sera against birch pollen, kiwi fruit and apple are summarized in Table 1. Apple was included as a food that regularly provokes symptoms in a high percentage of birch pollen-allergic patients. A total of 29 sera contained specific IgE antibodies against birch pollen, 26 against apple, 24 against celery and 23 each against kiwi fruit and carrot. In 20 sera, specific IgE against all five extracts was detectable. It is remarkable that most of the sera had higher contents of specific IgE against birch pollen and apple than against kiwi fruit. The IgE levels against carrot and celery were lower than those against birch pollen and apple in most cases, but higher than the IgE levels against kiwi fruit.

Immunoblotting

Sera with EAST classes ≥ 2 (corresponding to an IgE level of ≥ 0.7 U ml^{-1}) were used for immunoblot investigations to characterize the allergens in the extracts from kiwi fruit, birch pollen and apple with regard to their molecular weights.

Kiwi fruit. Up to 10 proteins with apparent molecular weights ranging from 20 to 70 kDa were detectable in the kiwi fruit extract by staining the blot for total protein with Indian ink. The most intense bands appeared at approximately 20, 23 and 26 kDa (Figure 1). For immunoblotting, 22 sera were tested. Two allergens with molecular weights of approximately 43 and 67 kDa were detected by 10 and 8 of the 16 sera shown in Figure 1 respectively. Moreover, IgE-binding proteins were seen at approximately 22 kDa (detected by five sera), 30 kDa (three sera) and 13 kDa (two sera). No proteins were detected by the control serum of the non-allergic patient. Of the immunoblots not shown here, the allergens with molecular weights of 43 and 67 kDa were also detected by five and four of the six sera respectively. One serum also showed IgE binding to the allergens with 30 and 22 kDa.

Birch pollen. Twenty-eight sera were used for immunoblot investigations of birch pollen extract. Allergens with 17 kDa (Bet v 1) and 34 kDa were detected by 15 of the 16 sera

TABLE 1. Clinical symptoms of patients in reaction to kiwi fruit and apple and levels of specific IgE in their sera

Patient (Sex, age)	Clinical symptoms ^(a)	Specific IgE (U ml ⁻¹)	Patient (Sex, age)	Clinical symptoms ^a	Specific IgE (U ml ⁻¹)
Bo 57 (F, 38)	Kiwi: IM Apple: IM, IT	Birch pollen: 52.1 Kiwi: 36.3 Apple: 37.7	Bo 157 (F, 30)	Kiwi: IM, IT Apple: IM, IT	Birch pollen: 8.10 Kiwi: <0.35 Apple: 0.73
Bo 171 (F, 29)	Kiwi: IM Apple: IM, IT	Birch pollen: 11.5 Kiwi: 0.50 Apple: 7.74	Bo 173 (F, 10)	Kiwi: IM, IT Apple: IM, IT	Birch pollen: 43.1 Kiwi: 24.0 Apple: 35.4
Bo 185 (M, 36)	Kiwi: IM, SL Apple: IM, IT, SL	Birch pollen: 28.5 Kiwi: 0.81 Apple: 33.3	Bo 186 (F, 35)	Kiwi: — Apple: —	Birch pollen: 26.3 Kiwi: 1.91 Apple: 25.3
Bo 187 (M, 36)	Kiwi: IM, IT Apple: IM, IT, ST	Birch pollen: 42.3 Kiwi: 0.79 Apple: 33.2	Bo 191 (M, 20)	Kiwi: IM, IT Apple: IM, IT	Birch pollen: 51.1 Kiwi: 5.02 Apple: 42.3
Bo 50K (F, 28)	Kiwi: — Apple: SL, ST	Birch pollen: 38.9 Kiwi: 30.2 Apple: 38.4	Bo 195K (F, 59)	Kiwi: AV Apple: IM, IT	Birch pollen: <0.35 Kiwi: <0.35 Apple: <0.35
Bo 201K (F, 38)	Kiwi: IT Apple: IT	Birch pollen: 5.90 Kiwi: <0.35 Apple: 0.84	Bo 202K (F, 24)	Kiwi: — Apple: IM, IT	Birch pollen: 51.2 Kiwi: 45.8 Apple: >60
Bo 203K (F, 38)	Kiwi: SL Apple: SL	Birch pollen: 23.2 Kiwi: 0.78 Apple: 10.7	Bo 204K (F, 33)	Kiwi: — Apple: SL	Birch pollen: 47.6 Kiwi: 6.41 Apple: >60
Bo 205K (F, 24)	Kiwi: SL, IT Apple: SL, IT	Birch pollen: 5.1 Kiwi: <0.35 Apple: 3.66	Bo 207K (F, 39)	Kiwi: SL, U Apple: SL, U	Birch pollen: <0.35 Kiwi: <0.35 Apple: <0.35
Bo 208K (M, 23)	Kiwi: SL Apple: —	Birch pollen: 1.11 Kiwi: 1.22 Apple: 1.18	J. A. (F, 19)	Kiwi: IM, SL Apple: IM, SL	Birch pollen: 1.67 Kiwi: 5.01 Apple: 7.37

TABLE 1. (continued)

Patient (Sex, age)	Clinical symptoms ^(a)	Specific IgE (U ml ⁻¹)	Patient (Sex, age)	Clinical symptoms ^a	Specific IgE (U ml ⁻¹)
K. B. (F, 25)	Kiwi: IM Apple: IM	Birch pollen: 17.8 Kiwi: 11.7 Apple: 1.31	H. B. (F, 24)	Kiwi: IM, SL Apple: IM, SL	Birch pollen: 6.89 Kiwi: 1.91 Apple: 0.64
C. H. (F, 28)	Kiwi: U Apple: —	Birch pollen: <0.35 Kiwi: 1.41 Apple: <0.35	C. I. (F, 44)	Kiwi: IM, IT, SL Apple: IM, IT	Birch pollen: 11.2 Kiwi: 2.84 Apple: 5.90
E. M. K. (F, 55)	Kiwi: IM, IT, SL Apple: —	Birch pollen: 35.5 Kiwi: 6.33 Apple: 37.6	K. N. (M, 35)	Kiwi: IM, IT Apple: IM, IT	Birch pollen: >60 Kiwi: 12.6 Apple: 28.5
C. N. (F, 23)	Kiwi: IM, IT, SL Apple: IM	Birch pollen: 48.9 Kiwi: <0.35 Apple: 30.8	Cl. N. (F, 20)	Kiwi: IM, SL Apple: IM, SL	Birch pollen: 27.5 Kiwi: 5.01 Apple: 52.9
G. S. (F, 31)	Kiwi: IM, IT, RH Apple: IM, RH	Birch pollen: 55.1 Kiwi: 1.24 Apple: 8.09	D. S. (F, 32)	Kiwi: IM, IT Apple: IM, IT	Birch pollen: 7.38 Kiwi: <0.35 Apple: 3.63
M. T. (F, 29)	Kiwi: IM Apple: IM	Birch pollen: 49.2 Kiwi: 3.91 Apple: 10.5	Mast 103 ?	Kiwi: ? Apple: ?	Birch pollen: 40.7 Kiwi: >60 Apple: 51.2

^aAV, aversion; IM, itching in mouth; IT, itching in throat; RH, rhinitis; SL, swelling of lips; ST, swelling of throat; U, urticaria.

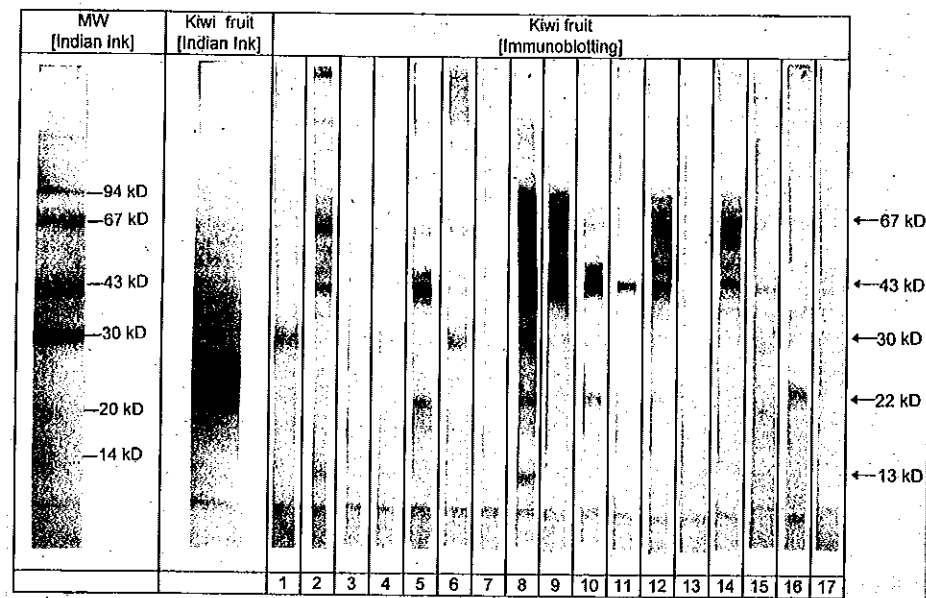


FIG. 1. Indian ink staining and immunoblotting of kiwi fruit extract separated by SDS-PAGE and transferred to a NC membrane. Patient sera: 1, Bo 57; 2, Bo 173; 3, Bo 185; 4, Bo 187; 5, Bo 50 K; 6, Bo 203 K; 7, Bo 204 K; 8, Mast 103; 9, J. A.; 10, K. B.; 11, H. B.; 12, C. I.; 13, E. M. K.; 14, K. N.; 15, Cl. N.; 16, M. T.; 17, control serum from a non-allergic individual.

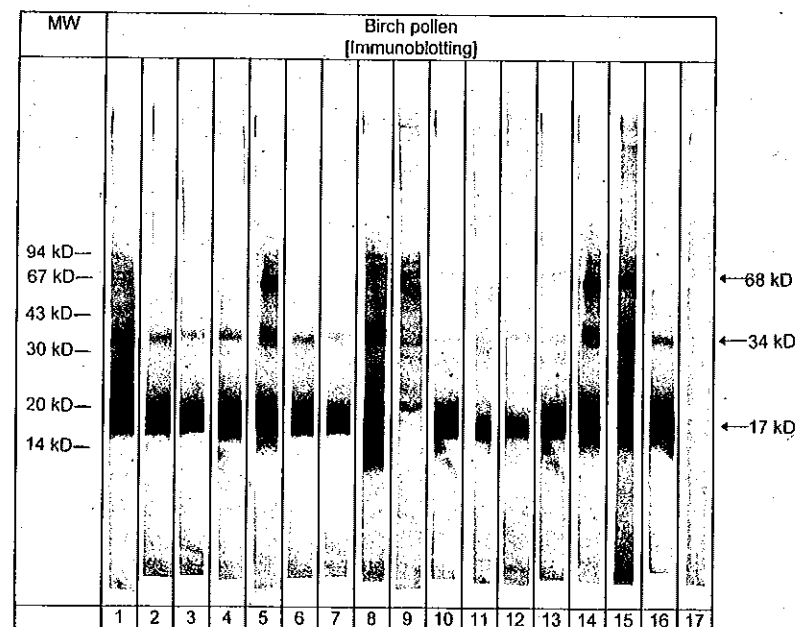


FIG. 2. Immunoblotting of birch pollen extract separated by SDS-PAGE and transferred to a NC membrane. Patient sera: 1, Bo 57; 2, Bo 173; 3, Bo 185; 4, Bo 187; 5, Bo 50 K; 6, Bo 203 K; 7, Bo 204 K; 8, Mast 103; 9, J. A.; 10, K. B.; 11, H. B.; 12, C. I.; 13, E. M. K.; 14, K. N.; 15, Cl. N.; 16, M. T.; 17, control serum from a non-allergic individual.

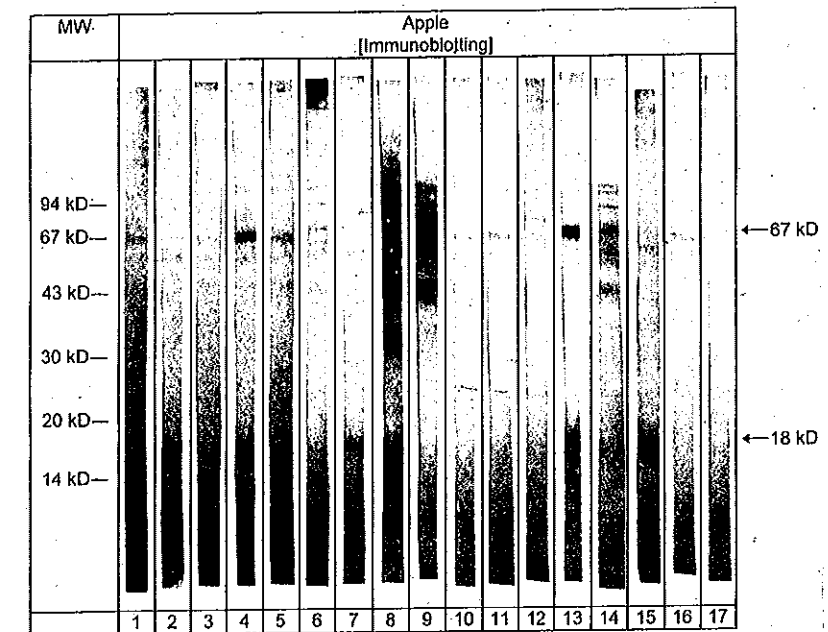


FIG. 3. Immunoblotting of apple extract separated by SDS-PAGE and transferred to a NC membrane. Patient sera: 1, Bo 57; 2, Bo 173; 3, Bo 185; 4, Bo 187; 5, Bo 50 K; 6, Bo 203 K; 7, Bo 204 K; 8, Mast 103; 9, J. A.; 10, K. B.; 11, H. B.; 12, C. I.; 13, E. M. K.; 14, K. N.; 15, Cl. N.; 16, M. T.; 17, control serum from a non-allergic individual.

shown in Figure 2. Seven sera also reacted with an allergen of approximately 68 kDa. IgE binding to this protein was also observed by four of the 12 additional sera whose immunoblots are not shown. All patients sera that detected the 68-kDa protein also contained specific IgE antibodies against kiwi fruit. No proteins were detected with the control serum.

Apple. Twenty-two sera were used for immunoblot analysis of the apple extract. The known major allergen at approximately 18 kDa (Mal d 1) was detected by seven of the 16 sera shown in Figure 3. Furthermore, IgE binding to a protein with a molecular weight of approximately 67 kDa was observed for most of the patient sera, but was also seen, though less intensively, with the control serum. The detection of this component by the control serum indicated non-specific binding; however, because of the strong reaction of some patient sera (e.g. Bo 187, E. M. K.) with the 67-kDa protein, this component will also be an allergen. Further allergens with molecular weights ranging from 30 to approximately 100 kDa were detected by four sera. Two of the six sera whose immunoblots are not shown revealed IgE binding to the 67 kDa allergen and one to Mal d 1.

Profilins. Using polyclonal antisera, a protein of approximately 13 kDa was detected in an extract from kiwi fruit (not shown). As already seen, only two of 22 samples of patients sera contained specific IgE antibodies against a component of the same molecular weight as the component detected by the polyclonal antisera. No further investigations were therefore performed to find out if this component really was a profilin.

TABLE 2. EAST inhibition with extracts of birch pollen, kiwi fruit, apple, celery and isolated Bet v 1 for the pooled serum and kiwi fruit, birch pollen and apple. The protein concentration (in $\mu\text{g ml}^{-1}$) for 50% inhibition or—if 50% inhibition was not reached—the maximum inhibition caused by the non-diluted extracts (in %) is given

Allergen disk	Kiwi fruit	Birch pollen	Bet v 1	Apple	Carrot	Celery
Kiwi fruit	18.0	75.4	(15%)	14.9	106.9	213.8
Birch pollen	(13%)	56.1	6.4	(26%)	(26%)	(17%)
Apple	(27%)	4.8	0.5	32.3	(23%)	(20%)

EAST Inhibition

For EAST inhibition, a pooled serum from patients J. A., K. B., H. B., C. I., K. N., E. M. K. and Cl. N. was used. The protein concentrations leading to an inhibition of IgE binding to the allergen disks at the 50% level are given in Table 2. If 50% inhibition was not reached, the maximum percentage inhibition caused by the undiluted extracts is given.

IgE binding to kiwi fruit allergens is strongly inhibited by preincubation with kiwi fruit extract itself, birch pollen and apple extract. Bet v 1 causes a strong inhibition of IgE binding to birch pollen and apple disks, but not to kiwi fruit disks. Preincubation with kiwi fruit extract causes a weak inhibition of IgE binding to birch pollen disks (approximately 13%) and to apple disks (approximately 27%). Extracts from celery and carrot only led to a weak inhibition of IgE binding to apple and birch pollen disks (17–26%), and the inhibition potency of celery and carrot protein was lower than the protein of kiwi fruit, birch pollen and apple to kiwi fruit disks. No significant inhibition was observed with ovalbumin in all cases.

Immunoblot Inhibition

The pooled serum used in EAST inhibition was also used for immunoblot inhibition to characterize the allergens being responsible for cross-reactivities. IgE binding to the allergens with molecular weights of about 43 and 67 kDa in kiwi fruit was almost totally inhibited by preincubation with kiwi fruit, birch pollen and apple. Extracts from carrot and celery only revealed a weaker reduction of antibody binding to the allergens in kiwi fruit. Bet v 1 had no observable inhibition effect on IgE binding to these allergens (Figure 4).

On immunoblots of birch pollen extract, IgE binding to Bet v 1 and the 34-kDa allergen was only inhibited by birch pollen extract and Bet v 1, but not by kiwi fruit extract (Figure 5). Antibody binding to the 68-kDa allergen was inhibited by kiwi fruit, apple and birch pollen extract, but not by isolated Bet v 1. Furthermore, IgE binding to Bet v 1 and the 34-kDa allergen was reduced, but not totally inhibited, by apple extract.

Preincubation with extracts from birch pollen and apple as well as isolated Bet v 1 led to inhibition of IgE binding to the 18-kDa allergen (Mal d 1) in apple extract. Kiwi extract only inhibited antibody binding to the 67-kDa allergen, but had no effect on Mal d 1 (Figure 6). No significant inhibition with ovalbumin or non-specific binding with the control serum was observed.

DISCUSSION

In the present paper, allergens in kiwi fruit were investigated by SDS-PAGE/immunoblot. Two allergens with molecular weights of approximately 43 and 67 kDa were detected by 15 and 12 of 22 sera tested respectively. To the author's knowledge, this is the first report on these new birch pollen-related food allergens. These allergens are only slightly detected by total protein staining of the blot with Indian ink or by silver staining of the gel, showing that the content of these allergens in kiwi fruit extract is low. On the other hand, proteins

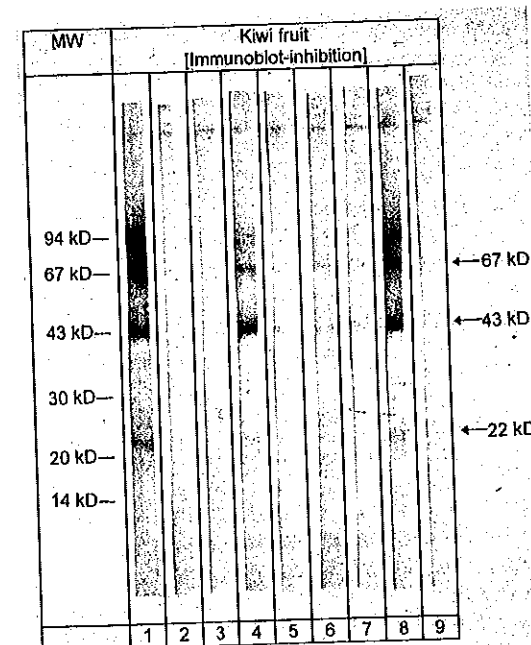


FIG. 4. Inhibition of IgE binding of pooled serum to kiwi fruit extract separated by SDS-PAGE and transferred to a NC membrane. 1, Uninhibited; 2, inhibited with kiwi fruit extract (100 $\mu\text{g/inhibition}$); 3, inhibited with birch pollen extract (100 $\mu\text{g/inhibition}$); 4, inhibited with Bet v 1 (10 $\mu\text{g/inhibition}$); 5, inhibited with apple extract (100 $\mu\text{g/inhibition}$); 6, inhibited with carrot extract (100 $\mu\text{g/inhibition}$); 7, inhibited with celery extract (100 $\mu\text{g/inhibition}$); 8, inhibited with ovalbumin (100 $\mu\text{g/inhibition}$) to check non-specific inhibition; 9, control serum to check non-specific binding.

of higher content in the extract (proteins with molecular weights of 20, 23 and 26 kDa) do not represent potent allergens.

For EAST inhibition, a pooled serum from patients J. A., K. B., H. B., C. I., K. N., E. M. K. and Cl. N. was used. All these patients showed clinical symptoms of allergy to kiwi fruit, birch pollen and apple, and had EAST classes ≥ 2 against all five extracts tested here. Furthermore, the same allergens were detected in kiwi fruit, birch pollen and apple using these sera. The intensity of staining was different, but this was caused by differences in the content of specific IgE in these sera. EAST inhibition was also carried out with individual sera from J. A. and K. N., and led to similar results as those obtained using the pooled serum (data not shown).

In EAST inhibition, cross-reactivities were found between kiwi fruit, birch pollen and apple, carrot and celery as members of common pollen-related allergenic plant foods. This is in agreement with previous studies by other groups (Gall *et al.*, 1990, 1994). In contrast to other birch-pollen-related food allergens, there is no immunological relationship between the allergens in kiwi fruit and Bet v 1 as demonstrated in EAST and immunoblot inhibition. In addition, profilins do not seem to be important allergens in kiwi fruit because only two of the 22 patient sera contained IgE antibodies against a protein with a molecular weight similar to one detected by polyclonal antisera directed against profilins from ragweed and celery. Instead, a protein of approximately 68 kDa in birch pollen extract seems to be responsible for cross-reactivities between kiwi fruit and birch pollen. These results should be confirmed by EAST and immunoblot inhibition with the isolated allergen. A 68-kDa allergen has already been described by other groups as a minor allergen in birch pollen (Jarolim *et al.*, 1989a,b).

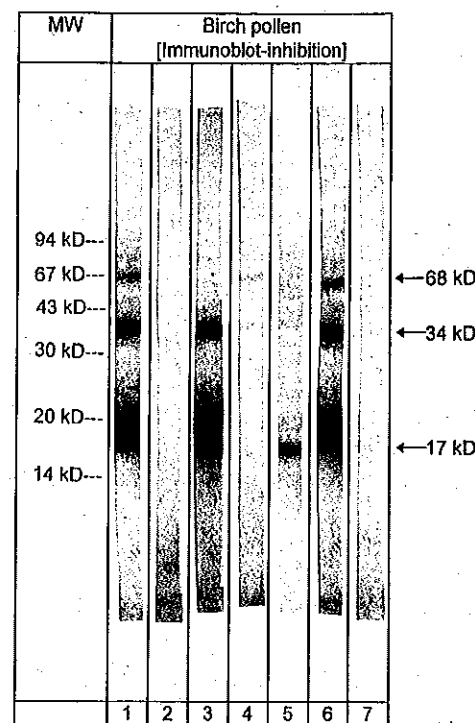


FIG. 5. Inhibition of IgE binding of pooled serum to birch pollen extract separated by SDS-PAGE and transferred to a NC membrane. 1, Uninhibited; 2, inhibited with birch pollen extract (100 μ g/inhibition); 3, inhibited with kiwi fruit extract (100 μ g/inhibition); 4, inhibited with Bet v 1 (10 μ g/inhibition); 5, inhibited with apple extract (100 μ g/inhibition); 6, inhibited with ovalbumin (100 μ g/inhibition) to check non-specific inhibition; 7, control serum check non-specific binding.

From the molecular weight, it could be suspected that the 68-kDa allergen is a tetramer of Bet v 1 (17 kDa), but, in contrast, isolated Bet v 1 is not able to inhibit IgE binding to the 68-kDa allergen. Furthermore, other groups have demonstrated using monoclonal antibodies that the allergen with an apparent molecular weight of 68 kDa is not a tetramer of Bet v 1 (Jarolim *et al.*, 1989a,b).

Recently, a 35-kDa allergen has been detected as a new cross-reacting structure in birch pollen, exotic fruits and other plant foods (Wellhausen *et al.*, 1996). It was demonstrated that this allergen had epitopes which are different from those of Bet v 1. Furthermore, a dimer of Bet v 1 appeared as a diffuse band on immunoblots of birch pollen extract. In the present investigations, a protein of about 34 kDa was detected as a sharp band on the birch pollen immunoblot. As this protein was detected by all patient sera which contained specific IgE antibodies against Bet v 1 and IgE binding to this allergen was inhibited by preincubation with Bet v 1, the 34-kDa allergen seems to be a dimer of Bet v 1.

In the EAST inhibition studies carried out by Wellhausen *et al.* (1996), the inhibitable portions of IgE binding to kiwi fruit disks caused by Bet v 1 and the 35-kDa allergen taken together were less than the inhibition caused by whole birch pollen extract. The difference may be explained by the influence of the 68-kDa allergen in birch pollen.

In the present investigation, cross-reactivities between kiwi fruit and apple were demonstrated by EAST and immunoblot inhibition. In immunoblot inhibition, the IgE binding to the major allergen from apple (Mal d 1) was not influenced by preincubation with kiwi fruit. This was expected because all epitopes from Mal d 1 are also localized on Bet v 1 (Vieths *et al.*, 1995), which itself does not cross-react with allergens from kiwi fruit. However, antibody binding to an 67-kDa allergen in apple was inhibited by kiwi fruit and apple

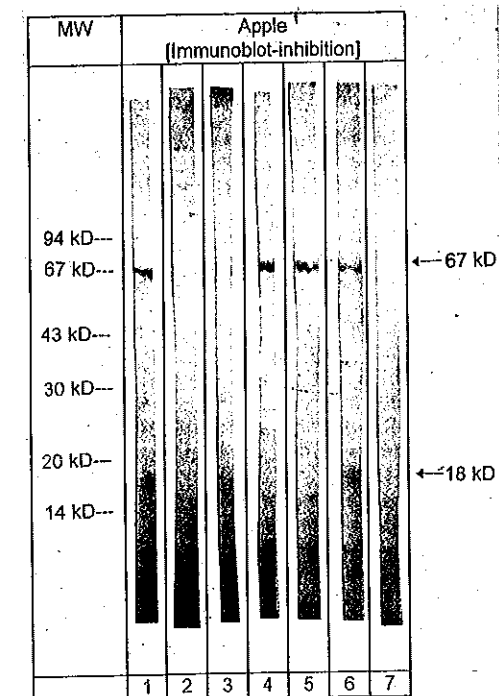


FIG. 6. Inhibition of IgE binding of pooled serum to apple extract separated by SDS-PAGE and transferred to a NC membrane. 1, Uninhibited; 2, inhibited with apple extract (100 μ g/inhibition); 3, inhibited with kiwi fruit extract (100 μ g/inhibition); 4, inhibited with birch pollen extract (100 μ g/inhibition); 5, inhibited with Bet v 1 (10 μ g/inhibition); 6, inhibited with ovalbumin (100 μ g/inhibition) to check non-specific inhibition; 7, control serum to check non-specific binding.

extract, but not by birch pollen and Bet v 1. It was remarkable that in EAST inhibition, IgE binding to kiwi fruit disks was inhibited by pre-incubation with apple and birch pollen extract to a higher degree than vice versa. The former shows that epitopes of the kiwi fruit allergens are also localized on the allergens from birch pollen and apple. The latter may be explained by IgE in patients sera which is directed against Mal d 1 and Bet v 1, since the binding of these molecules to the allergen disks is not inhibited by kiwi fruit extract.

The results of cross-reactivities between apple and birch pollen are in agreement with previous investigations (Björkstén *et al.*, 1980; Ebner *et al.*, 1991; Vieths *et al.*, 1992c). Although these results have been obtained using sera of patients who showed clinical symptoms to kiwi fruit, apple and birch pollen, the clinical relevance of these results has to be confirmed by skin-prick tests or oral challenge.

Further studies are now being undertaken to obtain amino acid sequence data of these new food allergens. In investigations of other groups, it has been pointed out that in a considerable number of cases hypersensitivity to Latex can also be associated with hypersensitivity to kiwi fruit (Blanco *et al.*, 1994; Dousson *et al.*, 1994; Laxenaire, 1995). The cross-reacting allergens of kiwi fruit will be identified.

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