

Allergenic properties of kiwi-fruit extract: cross-reactivity between kiwi-fruit and birch-pollen allergens

Voitenko V, Poulsen LK, Nielsen L, Norgaard A, Bindslev-Jensen C, Stahl Skov P. Allergenic properties of kiwi-fruit extract: cross-reactivity between kiwi-fruit and birch-pollen allergens. Allergy 1997; 52: 136–143. © Munksgaard 1997.

**V. Voitenko^{1,2}, L. K. Poulsen¹,
L. Nielsen¹, A. Norgaard¹,
C. Bindslev-Jensen³, P. Stahl Skov¹**

¹Allergy Unit, IIR, RHIMA, National University Hospital, Copenhagen, Denmark; ²Physiology Department, Kaunas Medical Academy, Kaunas, Lithuanian Republic; ³Department of Dermatology, Odense University Hospital, Odense, Denmark

Our investigation aimed to produce and characterize a kiwi extract and to use this extract to investigate a possible cross-reactivity with birch pollen. Kiwi was extracted in two buffers: phosphate-buffered saline (PBS) and borate-buffered saline (BBS). Extraction in BBS produced a double amount of protein, and a more stabile extract. Tandem crossed-immunoelectrophoresis showed that the BBS and PBS extracts had several common, but also a few individual, proteins. The mixture of both extracts was assumed to represent the most complete allergen extract. The allergenic properties of the kiwi extract were investigated by immunoblotting (IB), RAST, and histamine-release (HR) test in 15 birch-pollen-allergic patients (eight of them with clinical kiwi allergy) and one with clinical monoallergy to kiwi. All eight birch-pollen-allergic patients with kiwi allergy and the kiwi-monoallergic patient were positive in kiwi IB binding most frequently to proteins of 10–12 and 20–25 kDa. With our extract, RAST was positive in four kiwi-allergic and one non-kiwi-allergic patient, whereas the HR test was positive in five kiwi-allergic patients and negative in all non-kiwi-allergic patients. RAST and IB inhibition demonstrated cross-reactivity between birch-pollen and kiwi allergens due to a 10–12 kDa protein. In conclusion, a kiwi extract with allergenic properties was produced, and, by the methods used, cross-reactivity was demonstrated between birch-pollen and kiwi allergens.

Key words: birch-pollen allergy; crossed-immunoelectrophoresis; cross-reactivity; food hypersensitivity; IgE immunoblotting; kiwi-fruit allergy; SDS–PAGE; specific IgE.

Valentina Voitenko, MD
Laboratory of Medical Allergology
National University Hospital
Tagensvej 20
DK-2200 Copenhagen N
Denmark

Accepted for publication 25 June 1996

Due to cross-reactivity between plant and pollen allergens, patients with allergy to pollens often report oral allergy syndrome (OAS), characterized by local itching and swelling of the mouth and throat after ingestion of certain fruits, nuts, and vegetables (1–5). Recently, several authors have suggested cross-reactivity between kiwi-fruit and plant or pollen allergens (6–8). According to the literature, increasing numbers of birch-pollen-allergic patients develop OAS after ingestion of kiwi fruit (7), and we have found 34% with OAS caused by kiwi fruit in a group of 53 birch-pollen-allergic rhinoconjunctivitis patients referred to our clinic (L. Nielsen, et al., paper in preparation). A large variation in sensitivity and specificity has been observed in comparison of different *in vitro* tests and the skin prick test (SPT) with the outcome

of buccal challenge in kiwi-allergic patients (A. Norgaard, et al., paper in preparation), indicating a varying content of relevant allergens in the different kiwi extracts. The increasing demand for establishment of the specific diagnosis of kiwi allergy implies the need for a stable, well-characterized, and validated kiwi extract. Moreover, a characterized extract is needed to investigate the possible cross-reactivity with birch and to identify one or more of the cross-reacting allergens. A well-characterized kiwi-fruit extract is still not available, and the possible components cross-reacting with birch pollen have not been identified.

Our investigation aimed to produce and characterize a kiwi-fruit extract and to use this extract to investigate possible cross-reactivity with birch pollen.

Material and methods

Patients

Sixteen patients (age range 20–54 years) were included in this study. The patients were divided into the following three groups.

Group 1 included eight (nos. 1–8) kiwi-allergic and birch-pollen-allergic patients. Kiwi allergy was defined by history of OAS to kiwi, confirmed by positive open buccal challenge with fresh kiwi fruit and positive SPT to fresh kiwi (see below). Birch-pollen allergy was documented by history of seasonal rhinoconjunctivitis, positive specific IgE ($\geq$ class 2, CAP System, Pharmacia, Sweden) to birch pollen, and positive (>3 mm in diameter) SPT to birch-pollen extract (ALK, Soluprick[®], Hørsholm, Denmark).

Group 2 consisted of seven (nos. 9–15) birch-pollen-allergic patients without allergy to kiwi (no OAS to kiwi, negative SPT, and negative open buccal challenge with fresh kiwi).

Group 3 included one (no. 16) kiwi-allergic patient (documented by positive open buccal challenge and positive SPT to fresh kiwi fruit) without allergy to birch pollen.

SPT and buccal challenge

SPT with fresh kiwi was performed by the prick-prick method (9). A positive SPT was defined as a wheal diameter 3 mm larger than the negative control wheal. Buccal challenge was performed by letting the patient chew 5 g of fresh kiwi for 30 s and then spitting out the fruit. A reaction was considered positive if itching or swelling occurred in the mouth or oropharynx within 15 min.

Preparation of kiwi-fruit and birch-pollen extracts

The kiwi allergens were extracted in the following two ways:

- 1) The PBS preparation was extracted in potassium phosphate buffer (10 mM potassium phosphate, 2% polyvinylpyrrolidone (PVPP), 2 mM ethylenediaminetetraacetic acid (EDTA), 10 mM diethyldithiocarbamate (DIECA), and 3 mM sodium azide (NaN_3), pH 7.5), as described by Björkstén et al. (10).
- 2) The BBS preparation was extracted in borate buffer (100 mM potassium borate, 0.5 M NaCl, 2% PVPP, 2 mM EDTA, 10 mM DIECA, 3 mM NaN_3 , pH 8.0).

In short, 100 g of peeled kiwi fruit was homogenized in 200 ml of extraction buffer. It was then centrifuged at 20000 g for 30 min at 4°C to remove

the insoluble debris, and the supernatant was dialyzed for 24 h at 4°C against a dialyzing buffer (10 mM potassium phosphate, 3 mM NaN_3 , pH 7.0), concentrated five times against PEG 2000 (Merck, Darmstadt, Germany), and finally stored at -20°C. The protein concentration was assessed by the method of Bradford (11) with a protein assay kit from BioRad (Hercules, CA, USA). Bovine serum albumin (BSA) (Sigma, St Louis, MO, USA) was used as reference protein.

Birch (*Betula verrucosa*) pollen was obtained from Allergon (Uppsala, Sweden). An amount of 500 mg crude pollen was extracted in 10 ml of 10 mM potassium phosphate buffer (pH 7.0), containing 2 mM EDTA and 3 mM NaN_3 , under continuous shaking at 4°C for 24 h. The pollen suspension was centrifuged for 30 min at 20000 g, dialyzed against PBS for 24 h, and kept at -20°C until used.

Production of antikiwi antibodies and immunoelectrophoresis

The PBS kiwi extract was used to immunize rabbits to raise polyclonal antikiwi antibodies (12) (contract immunization, Dako, Glostrup, Denmark). Two rabbits were immunized once a month with the PBS kiwi extract for 12 months. Five bleedings from the sixth month onward were pooled, and $(\text{NH}_4)_2\text{SO}_4$ -precipitated antibody was used in the experiments. Crossed-immunoelectrophoresis (CIE) was performed as described by Laurel (13); tandem crossed-immunoelectrophoresis (TCIE) and crossed-line-immunoelectrophoresis (CLIE) were performed according to Krøll (14). The quantity of allergen extracts used was 5–10 μg (in the first dimension), or 100 μg in the intermediate gel (CLIE), and 300 μl of undiluted rabbit antikiwi antiserum was used in the second dimension.

SDS-PAGE and IgE immunoblotting

SDS-PAGE was performed in a vertical slab gel apparatus (Hoefer Scientific Instruments, San Francisco, CA, USA), by the method of Laemmli (15) with a 16% separating gel and a 5% stacking gel in a discontinuous buffer system (2.5 mM Tris, 0.2 M glycine, and 0.1% SDS [w/v]). The extracts were boiled for 5 min in a sample buffer containing 2% SDS with 2% 2- β -mercaptoethanol (reducing conditions) or without 2- β -mercaptoethanol (non-reducing conditions). An amount of 100 μg of protein per 0.5 cm of gel was loaded. The molecular weights (MW) were determined with pre-stained protein standards (Amersham International, Buckinghamshire, UK). After electrophoresis, gels

were stained for protein with Coomassie brilliant blue or subjected to immunoblotting. The protein components of kiwi fruit or birch pollen were transferred onto nitrocellulose membrane, as described previously (16).

Immunoblot inhibition

Immunoblot-inhibition studies were performed with sera positive for birch or kiwi in immunoblot. Each serum was incubated overnight with birch or kiwi as inhibiting extract at protein concentrations of 2, 20, and 200 µg/ml, with a buffer and an irrelevant protein as a control. After centrifugation, the supernatants were incubated with birch or kiwi blots, as described above.

Determination of allergen-specific IgE by Maxisorp RAST

Specific IgE was measured by an immunoradiometric assay, Maxisorp RAST, according to Poulsen et al. (17). The results were expressed in cpm as the mean of duplicates. Positive results were defined as values above 400 cpm (corresponding to three times above the background). Testing by the CAP System (Pharmacia, Uppsala, Sweden) was performed with birch t-3 and kiwi f-84 allergens, according to the manufacturer's instructions. Values above 0.35 kU_A/l represented a positive result.

RAST inhibition

RAST inhibition was performed as described previously (18). As a negative control, Maxisorp RAST inhibition using unrelated proteins such as BSA, Der f, or dog-epithelium allergens (ALK, Denmark) was also performed. The percentage of inhibition in the binding of IgE antibodies was calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{cpm without inhibitor} - \text{cpm with inhibitor}}{\text{cpm without inhibitor} - \text{cpm with normal serum}} \times 100$$

Histamine release (HR)

Histamine release (HR) was analyzed in a glass fiber-based HR test according to Skov et al. (19). Histamine release >15 ng was considered positive. Patients with nonresponding basophils, i.e., basophils releasing <15 ng/ml of histamine at anti-IgE challenge, were excluded from the calculations, since their test results were inconclusive.

Results

Characterization of kiwi extract (protein content and antigenic properties)

The yield was 2 mg/ml of protein in the PBS extract and 4 mg protein/ml in the BBS extract, with the same amount of fresh kiwi fruit in both extraction procedures. The analysis of kiwi proteins on SDS-PAGE (by reducing conditions) showed several groups of proteins in both PBS and BBS kiwi extracts at regions of 30, 22–24, 15–17, and 10–12 kDa (Fig. 1). The pattern of kiwi proteins varied, depending on which running conditions – reducing or nonreducing – were applied during electrophoresis. When nonreducing conditions were used, the 30-kDa band of BBS kiwi extract split into two bands: a weak band corresponding to 30 kDa, and a stronger 27-kDa band. Kiwi extracted in the two buffers and used immediately after extraction showed identical SDS-PAGE results. The stability of the extracts varied with extraction buffer, i.e., when the extracts were incubated at 37°C for different periods. In the BBS extract, no evident changes were seen in the electrophoretic pattern after 24-h incubation at 37°C, while after 48-h incubation, the low-molecular-weight band tended to disappear (Fig. 1). After 24-h incubation of the PBS extract, all bands disappeared except the 22-kDa band, which did not change even after prolonged incubation (48 h) at 37°C (Fig. 1).

The CIE analysis showed that the kiwi extract contained both anodically and cathodically migrating proteins. More detailed analysis of tandem CIE showed that the BBS and PBS extracts had several

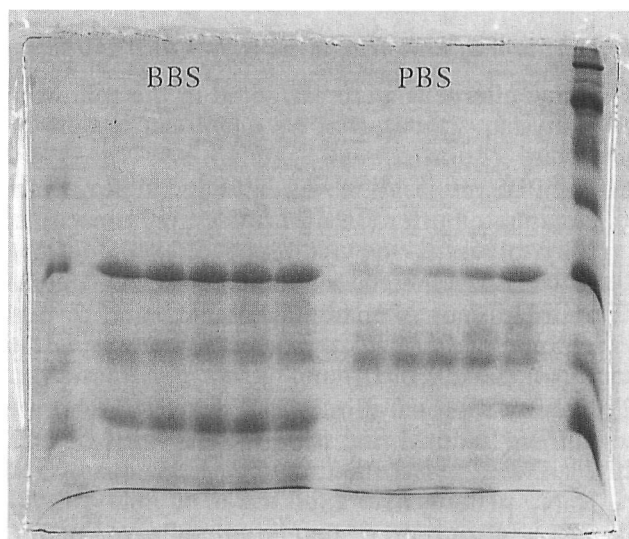


Fig. 1. SDS-PAGE of PBS and BBS kiwi extracts after incubation at 37°C for 1, 6, 24, and 48 h, run under reduced conditions. MW: molecular weight markers.

common proteins, but also a few individual ones, depending on extraction procedure (Fig. 2). Precipitate nos. 1–4 seemed to be common for both extracts, whereas precipitate no. 5 was present only in the BBS extract, and no. 6 was found only in the PBS extract. Thus, the two kiwi extracts showed a different antigen profile. Therefore, in order to use a complete extract, in the following experiments, we used a mixture of the PBS and BBS extracts with a volume ratio of 1 : 1.

Characterization of allergenic properties of the kiwi extract

The allergenic properties of the kiwi extract were investigated by Maxisorp RAST, IgE immunoblotting, and the HR test (Table 1). Of the 16 investigated sera, five were positive in Maxisorp RAST for kiwi allergens: 3/8 birch- and kiwi-allergic patients (group 1), 1/7 birch-allergic patients without kiwi allergy (group 2), and one patient with kiwi allergy and without birch-pollen allergy (group 3) (Table 1). In the CAP system, six sera were positive: 4/8 in group 1, 1/7 in group 2, and 1/1 in group 3. Four were the same as in Maxisorp RAST (Table 1).

All patient sera in group 1, two in group 2, and the same one in group 3 had various bands on kiwi IgE immunoblot, but most abundantly at the 10–12- and 20–25-kDa regions. Serum no. 16 from group 3 had a strong band in kiwi immunoblot in the 30-kDa region and a weak one at 18 kDa (Table 1).

Our kiwi extract was examined by HR in seven patients from group 1, five patients from group 2,

and the same one in group 3. Three patients (nos. 2, 7, and 16) did not respond to anti-IgE (Table 1). The remaining five patients in group 1 released histamine in a dose-dependent manner after stimulation with different concentrations of kiwi extract (Fig. 3). All five patients without kiwi allergy who were available for drawing of fresh blood were negative in the HR test with kiwi extract.

Common and cross-reactivity between kiwi and birch-pollen allergens

With birch-pollen extract in Maxisorp RAST, 7/8 patients were positive in group 1 (nos. 1, 3–8), 5/7 were positive in group 2 (nos. 9–13), and patient no. 16 in group 3 was negative. All patients, except nos. 2, 15, and 16 (who were negative in Maxisorp RAST for birch), responded to birch pollen in immunoblot. The sera positive in Maxisorp RAST and immunoblot, respectively, were selected for study of the cross-reactivity between birch and kiwi by inhibition.

The cross-reactivity between birch-pollen and kiwi allergens investigated by Maxisorp RAST inhibition is shown in Fig. 4, and that by IgE immunoblot-inhibition experiments, in Figs. 5 and 6. Fig. 4 shows the level of kiwi and birch Maxisorp RAST inhibition by the birch and kiwi allergens. In all four sera of groups 1 and 2 (nos. 4–6, 12) that were positive in Maxisorp RAST for kiwi, IgE binding to adsorbed kiwi was inhibited by preincubation of sera with birch-pollen extract (Fig. 4A). The level of inhibition varied from 55 to 85%. Preincubation of the birch-negative serum (no. 16

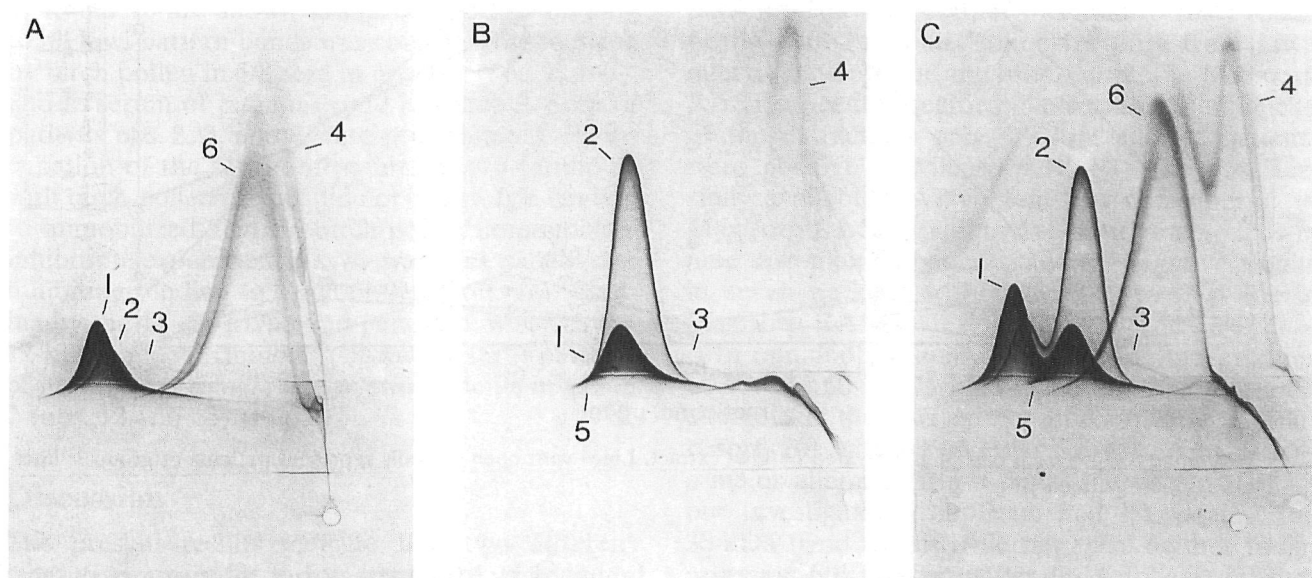


Fig. 2. Crossed-immunoelectrophoresis of 5 µl PBS kiwi extract (A) or BBS (B) against rabbit anti-kiwi antibodies. C) Tandem CIE of BBS and PBS kiwi extracts against kiwi antibodies. Numbers indicate identical protein precipitates.

Table 1. RAST, sensitivity of basophils, and IgE-immunoblot results for kiwi extract

Patient no.	Group	CAP kU _A /l	M-RAST cpm	IgE immunoblotting (kDa)						BS µg/ml
				10-12	15-18	20-25	30	38-40	> 46	
1	1	0.449	—		+	+				5
2	1	0.43	—			+		+	+	i.c.
3	1	—	—		+					0.8
4	1	0.77	1600	+		+		+	+	5
5	1	—	450	+		+				1.7
6	1	1.33	500	+	+	+		+		3.5
7	1	—	—	+						i.c.
8	1	—	—	+						n.d.
9	2	—	—							—
10	2	—	—							—
11	2	—	—							—
12	2	0.666	550	+		+			+	n.d.
13	2	—	—		+					n.d.
14	2	—	—							—
15	2	—	—							—
16	3	11.25	5000		+		+			i.c.

M-RAST: Maxisorp RAST; BS: sensitivity of basophils, expressed as lowest concentration of kiwi extract in µg/ml which releases >15 ng of histamine; n.d.: histamine release from basophils was not performed with patients whose fresh blood was not available; i.c.: inconclusive results. Negative results are marked as —.

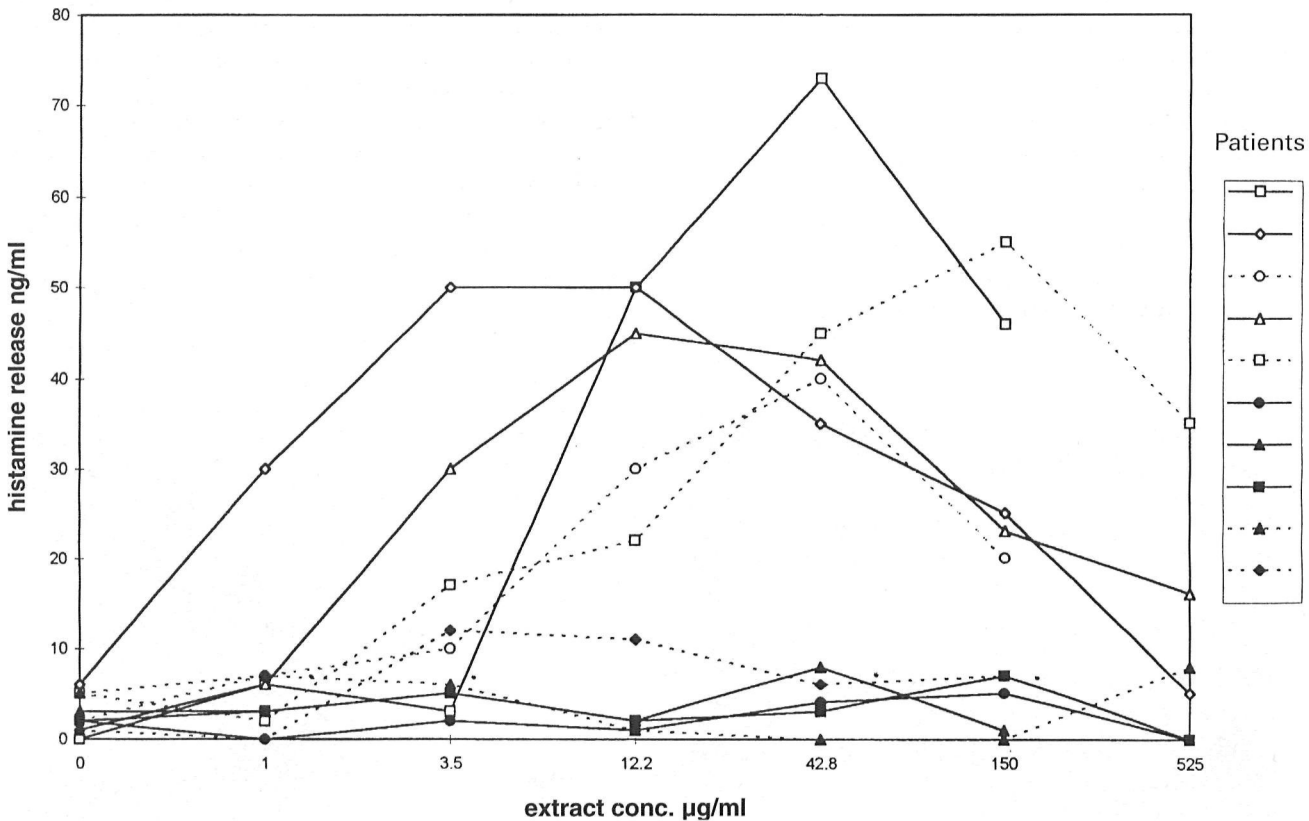


Fig. 3. Histamine release from basophils activated by kiwi extract. Lines with open symbols represent patients of group 1; lines with filled symbols, patients of group 2.

of group 3) with birch-pollen extract did not produce inhibition (Fig. 4A). All seven patient sera of group 1 positive in Maxisorp RAST for birch were

inhibited by kiwi extract, but a lower percentage of inhibition was obtained (Fig. 4B). In 2/5 sera in group 2, preincubation of sera with kiwi extract

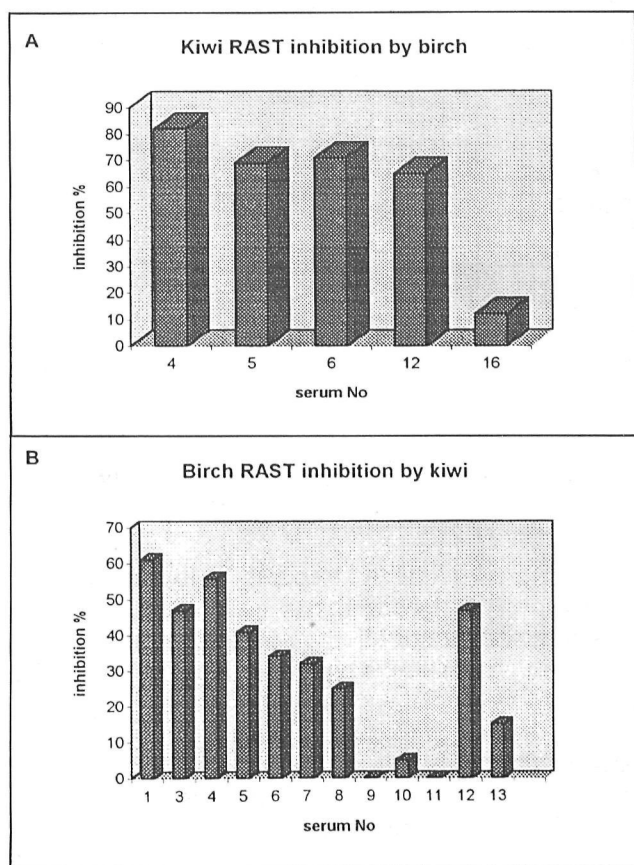


Fig. 4. Kiwi (A) and birch (B) RAST inhibition.

inhibited IgE binding to adsorbed birch pollen, whereas inhibition of sera no. 13 was very low (17%).

Immunoblot-inhibition experiments showed inhibition of both kiwi and birch immunoblots. Kiwi immunoblots with the representative patients nos. 1, 4, and 16 are shown in Fig. 5. The IgE binding to all kiwi pattern bands was considerably reduced by birch pollen in 5/8 sera in group 1 (nos. 1, 4–7), and in serum of patient no. 12 in group 2. Sera of patients nos. 2, 3, and 8 were not inhibited. Preincubation of the serum of patient no. 16 (group 3) with birch-pollen extract did not inhibit IgE binding to immobilized kiwi. In birch-pollen immunoblot-inhibition experiments, kiwi was less capable of inhibiting binding to birch-pollen blots: IgE binding by the 17–18-kDa band persisted, whereas the 12-kDa band was inhibited by kiwi in three patients of group 1 (nos. 5–7) and in two patients in group 2 (nos. 12 and 13) (Fig. 6).

Discussion

The present results indicate that two different extraction procedures for kiwi fruit produce a similar protein profile in SDS-PAGE, but a different antigen profile in tandem CIE. Extraction in

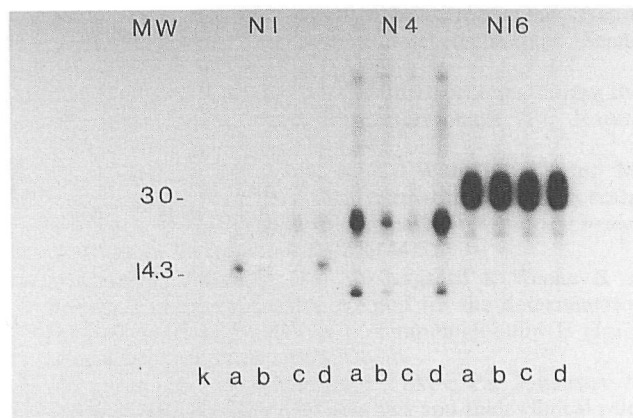


Fig. 5. Kiwi IgE-immunoblot inhibition. Lane a) IgE binding by immobilized kiwi after preincubation of sera with BSA (60 µg/ml); b) IgE-binding after prior serum incubation with birch pollen 20 µg/ml; c) inhibition by birch pollen (200 µg/ml); d) immunodetection of kiwi extract with 1:10 dilution of serum; k) negative control serum; 1 represents blots incubated with serum no. 1 diluted 1:20 in TBS buffer; 4, blots incubated with serum no. 4 diluted 1:20; and 16, blots incubated with serum no. 16 diluted 1:40.

BBS, instead of PBS, produced a double amount of protein, and this extract was more stable when treated at 37°C. Since both extracts were able to bind specific IgE from patient sera (data not shown), we suppose that the mixture of both extracts constitutes the most complete allergen extract. The reason for the difference may be that BBS inhibits proteases, but it is also possible that the difference in ionic strength accounts for the improved yield.

IgE immunoblotting and Maxisorp RAST demonstrated that our kiwi extract was able to recognize kiwi-specific IgE antibodies in kiwi-allergic patients. The kiwi extract was able to react specifically with basophil leukocytes only from kiwi-allergic patients. On the other hand, the Maxisorp RAST procedure seemed to affect the allergenicity of the extract, as only 4/9 kiwi-allergic patients were positive in Maxisorp RAST. The commercially available CAP System was not superior to Maxisorp RAST, yielding five positive reactions in nine kiwi-allergic patients, and six negative results in seven patients without allergy to kiwi (as in Maxisorp RAST).

In immunoblot analyses, we found the proteins of 10–12 and 20–25 kDa to be the most common allergens in the kiwi extract, in contrast to another report (20), which found that the 30-kDa band was a major allergen in kiwi fruit. The only patient in our investigated group who had IgE against the 30-kDa band on kiwi blotting, was neither birch- nor grass-pollen allergic (no. 16). We suppose that anti-kiwi antibodies differ in birch-pollen-associated and birch-pollen-non-associated allergy to kiwi fruit.

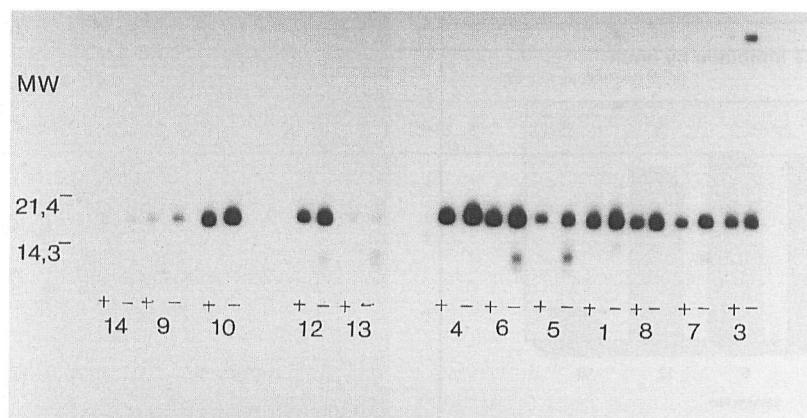


Fig. 6. Birch-pollen IgE-immunoblot inhibition. All samples carried identical samples of blotted birch pollen. Lanes marked - show IgE-binding profile with different sera; lanes marked + show IgE binding after preincubation of sera with 200 µg/ml kiwi extract.

The different numbers of positive reactions in RAST and immunoblotting might be due to the presence in the kiwi-fruit extract of heat-labile allergenic proteins which do not bind to the matrix in Maxisorp RAST, due to degradation (as the Maxisorp RAST procedure involves incubation at 37°C overnight, in contrast to immunoblotting). The thermolability of the kiwi allergens has been previously demonstrated (21): some kiwi allergens begin to lose their reactivity in SPT after the extract is heated to 60°C. However, similar experiments were not done with RAST.

By Maxisorp RAST inhibition and immunoblot inhibition, we demonstrated that there is indeed cross-reactivity between birch-pollen and kiwi-fruit allergens. Our results imply that kiwi extract contains the cross-reactive determinants, although the degree of IgE binding to the cross-reactive determinants varied from serum to serum. An interesting finding was that patient no. 12 of group 2 did not develop clinical symptoms upon ingestion of kiwi fruit, but showed specific IgE to kiwi which was inhibited by birch (in Maxisorp RAST and immunoblot experiments).

Other studies have shown similar cross-reactions between pollens and other fruits and vegetables (1, 3–5, 22). Similar allergens or allergenic determinants or lectins have been assumed to play a role in cross-reactions between birch pollen and fruits (2, 23–26). The identification of allergens is essential for understanding fruit and pollen-associated hypersensitivity. A few studies have been done on the characterization of kiwi-fruit allergens as compared with other fruit and pollen allergens: a protein of 20 kDa has been reported to be a cross-reactive allergen in hazelnut and kiwi fruit (27). Gall et al. (28) have demonstrated a very weak cross-reactivity between thiolproteases in kiwi fruit and papain or bromelain, isolated from papaya and

pineapple, respectively. Recently, a low-molecular-weight protein, profilin, has been identified as a cross-reactive minor allergen in birch pollen, celery, and other fruits and vegetables (26, 29, 30). The kiwi fruit was not investigated.

Our findings indicate that the cross-reactivity between birch-pollen and kiwi allergens is partly explained by a protein of 10–12 kDa: a protein of this size was inhibited in most birch-pollen immunoblots after preincubation of sera with kiwi. However, the inhibition of birch immunoblots was poorly expressed and observed in only 4/8 patients of group 1 and in two patients of group 2. The suggestion that only a minor allergen was involved in the cross-reactivity might explain the poor inhibition. It could also be that kiwi extract loses some allergenic determinants during the extraction procedure, while birch-pollen extract contains many more allergenic components, and these are able to inhibit more cross-reactive components in kiwi extract.

With our rabbit antikiwi antibodies, we could not demonstrate cross-reactivity between birch-pollen and kiwi allergens in CLIE by using kiwi extract in the first dimension and birch-pollen extract in the second dimension. Birch extract did not change the pattern of immunoprecipitates (data not shown). The binding of rabbit antibodies to the kiwi RAST solid phase or to kiwi immunoblots could not be inhibited by preincubation with birch-pollen extract (data not shown). Cross-reactivity in human sera and absence of cross-reactivity with rabbit antibodies could be due to the route of immunization, a different class of antibodies, or other species-inherent differences.

In conclusion, we have been able to produce a kiwi extract with allergenic properties. We demonstrated cross-reactivity between birch-pollen and kiwi allergens in some kiwi-allergic and birch-

pollen-allergic patients, a result which could be due to common allergenic structures recognized by human IgE.

Acknowledgments

We thank Dr T. Bøg-Hansen for his kind advice on immunoelectrophoretic methods. Financial support for this work was received from the Scholarship Program Between Norway and the Baltic Republics of 1993 and the Allergy Foundation of 1981.

References

1. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. *Clin Allergy* 1987;17:33-42.
2. Calkhoven PG, Aalbers M, Kosthe VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987;42:382-90.
3. Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch allergy. *Allergy* 1983;38:167-72.
4. Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982;37:437-43.
5. Halmepuro L, Vuontela K, Kalimo K, Björkstén F. Cross-reactivity of IgE antibodies with allergens in birch pollen, fruits and vegetables. *Int Arch Allergy Appl Immunol* 1984;74:235-40.
6. Boccafogli A, Vicentini L, Camerani A, Cogliati P, D'Ambrosi A, Scolozzi R. Adverse food reactions in patients with grass pollen allergic respiratory disease. *Ann Allergy* 1994;73:301-8.
7. Eriksson E. Clustering of foodstuffs in food hypersensitivity. An inquiry study in pollen allergic patients. *Allergol Immunopathol (Madr)* 1984;12:28-32.
8. Ortolani C, Pastorello E, Farioli L, et al. IgE-mediated allergy from vegetable allergens. *Ann Allergy* 1993;71:470-6.
9. Dreborg S. Skin tests for diagnosis of IgE-mediated allergy. *Allergy* 1989;44 Suppl 10:31-7.
10. Björkstén F, Halmepuro L, Hannuksela M, Lathi A. Extraction and properties of apple allergens. *Allergy* 1980;35:671-7.
11. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal Biochem* 1976;72:248-54.
12. Harboe N, Ingild A. Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand J Immunol* 1973;Suppl. 2:161.
13. Laurel C-B. Electrophoretic microheterogeneity of serum α -antitrypsin. *Scand J Clin Lab Invest* 1965;17:271.
14. Krøll J. Tandem crossed-immunoelectrophoresis. Handbook of immunoprecipitation-in-gel techniques. *Scand J Immunol* 1983;Suppl 10:113.
15. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
16. Jensen-Jarolim E, Poulsen LK, With H, Kieffer M, Ottevanger V, Skov PS. Atopic dermatitis of the face, scalp, and neck: type I reaction to the yeast *Pityrosporum ovale*? *J Allergy Clin Immunol* 1992;89:44-51.
17. Poulsen LK, Malling H-J, Søndergaard I, Weeke B. A sensitive and reproducible method for the determination of subnanogram quantities of immunoglobulin E (IgE). *J Immunol Methods* 1986;92:131-6.
18. Johansen CR, Abrahamsen L, Skov PS, Johansen N, Poulsen LK. Aeroallergen analyses and their clinical relevance. *Allergy* 1991;46:492-501.
19. Skov PS, Norn S, Weeke B. Histamine release and pathological states: a new method for detection of histamine release. *Agents Actions* 1984;14:414-16.
20. Pravettoni V, Farioli L, Ispano M, et al. Allergenic pattern of kiwi and cross-reactivity with grass-pollen. Abstracts EAACI '94, Stockholm, 1994:74.
21. Gall H, Kalveram K-J, Forck G, Sterry W. Kiwi fruit allergy: a new birch pollen-associated food allergy. *J Allergy Clin Immunol* 1994;94:70-6.
22. Thiel C. Food allergy in pollen allergic individuals (pollen-associated food allergies). *Allergologie* 1988;11:397.
23. Andersen KE, Løwenstein H. An investigation of the possible immunological relationship between allergen extracts from birch pollen, potato and apple. *Contact Dermatitis* 1978;4:73-9.
24. Halmepuro L, Løwenstein H. Immunological investigation of possible structural similarities between pollen antigens and antigens in apple, carrot and celery tuber. *Allergy* 1985;40:264-72.
25. Lathi A, Björkstén F, Hannuksela M. Allergy to birch pollen and apple, and cross-reactivity of the allergens studied with the RAST. *Allergy* 1980;35:297-300.
26. Vallier P, Dechamp C, Vial O, Deviller P. A study of allergens in celery with cross-sensitivity to mugwort and birch pollens. *Clin Allergy* 1988;18:491-500.
27. Vocks E, Borge A, Szliska C, et al. Common allergenic structures in hazelnut, rye grain, sesame seeds, kiwi, and poppy seeds. *Allergy* 1993;48:168-72.
28. Gall H, Kalveram K-J, Forck G, Tümmlers U. Cross-allergenicity between kiwi fruit and thiol proteinases, pollen and foodstuffs. *Allergologie* 1990;12:447-51.
29. van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a crossreactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992;98:97-104.
30. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-82.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.