

Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen

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Low-temperature, acetone powder extracts were prepared from mature fruit of 16 apple strains. SDS–PAGE and immunoblot analysis revealed great variation in the relative amounts of the 18-kDa apple allergen in these extracts. EAST (RAST) scores, measured with individual and pool sera from patients allergic to birch pollen and apples, ranged from 0.2 to 4.0 and were related to the relative amount of the 18-kDa protein. These findings were confirmed by ELISA-inhibition assays, dose-related histamine release, semiquantitative evaluation of immunoblots by absorption/reflection densitometry, and skin prick tests with extracts of Golden Delicious, Boskoop, and Jamba apples (corresponding to a high, low, and very low 18-kDa allergen content). Additional open oral challenge tests were performed with two apple-allergic patients and 15 and 16 apple strains. With all methods, the deduced allergenic potency decreased in the following order: Golden Delicious > Boskoop > Jamba. Therefore, we concluded that the IgE-binding potency of apple strains depends on the occurrence of the 18-kDa allergen.

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Apples are known to be common sources of food allergens which cause mainly oropharyngeal symptoms (14). Apple allergy is closely associated with type I allergy to birch pollen, to a wide variety of other fruits and vegetables, and especially to hazelnuts (4, 6, 7, 9, 13, 16). The term “birch-pollen-related food allergy” (BPRF) can be used for this phenomenon. The observed clustering of hypersensitivities is due to immunologic cross-reactions between food and pollen proteins (2, 6, 8, 9, 11, 17). *Bet v I*, the major allergen of birch pollen, shares allergenic epitopes with a 17–18-kDa protein of Golden Delicious apples, with an 18-kDa protein from hazelnuts (6, 9), and with allergens of similar mol. mass in pears (20). Homology of *Bet v I* and the cross-reacting apple allergen could also be shown at the nucleic acid level by Northern blotting (6). Furthermore, on the basis of computer-aided sequence analysis on the DNA and protein level, the authors concluded that *Bet v I* and the cross-reacting proteins in fruit belong to a group of “pathogenesis-related proteins”, being expressed in birch pollen and fruit. Such tissues are of low metabolic activity

but may be exposed to various stress conditions; in particular, microbial attack. By immunoblotting, Ebner et al. (6) were also able to demonstrate that 81 sera of patients with IgE against *Bet v I* also showed IgE binding to the 17–18-kDa apple allergen. Minor apple allergens of low mol. mass (about 13 kDa) were recognized by only 8.4% of the 84 sera under study. In a previous investigation, we confirmed these results by IgE immunoblotting with sera from patients with BPRF (18). Additionally, we found frequently occurring allergen bands of higher mol. mass of 30–50-kDa and moderate IgE reactivities to apple extracts with a very low amount of the 18-kDa allergen. Furthermore, patients suffering from BPRF frequently report that the severity of symptoms is strongly dependent on the apple variety ingested and on the degree of maturity. Therefore, the aim of our present investigation was to study the IgE-binding potency of different apple strains with regard to the occurrence of the 18-kDa apple allergen and to confirm the results of our immunoblot analyses by increasing the number of samples.

Material and methods

Patient sera

Allergy diagnosis was performed in the medical units which participated in the study; it was based on anamnesis, skin prick test (birch pollen), scratch test (with Golden Delicious or Granny Smith apples), and RAST class determination to birch pollen (Pharmacia). All patients suffered from oropharyngeal symptoms after ingestion of "green" apples (mostly Golden Delicious or Granny Smith). EAST class determinations with Golden Delicious allergen disks were performed in our laboratory. Only sera with EAST/RAST classes of ≥ 2.5 to birch pollen and of ≥ 2.0 to Golden Delicious were used for the study. The positive pool sera ILC-1 ($n=4$) and Wn 13/32 ($n=7$) were prepared from individual sera with EAST classes ≥ 3.0 to birch pollen and Golden Delicious; they were used for ELISA inhibition experiments and for the determination of EAST classes to the apple varieties under study. The individual sera of these patients were not included in the further investigation. Negative control sera were from non-atopic subjects, including negative pool sera ILC-0 ($n=4$) and Wn 13/26 ($n=7$) and serum from an atopic patient with a RAST class of 3.0 to grass pollen and no history of food hypersensitivity. The sera were collected at the Klinik Borkum Riff der BfA, Borkum, Germany (Dr H. Aulepp), the Deutsche Klinik für Diagnostik, Wiesbaden, Germany (Dr Cl. Thiel), and the Technische Universität Berlin, Occupational Health Service, Berlin, Germany (Dr S. Berten).

Fruit extracts

Mature apples were harvested in the research field of the Institute of Fruit Culture, Technische Universität Berlin (Prof. Dr P. Lüdders). Additional samples of mature apples were either purchased at the local market or obtained from private fruit growers. Protein extracts were prepared by a low-temperature, acetone powder method, as previously described (19), and stored freeze-dried at -20°C until use. The total amount of protein was determined by a modified Bradford assay (19). Protein concentrations of the apple extracts were typically 100–400 $\mu\text{g}/\text{ml}$. Sixteen apple varieties were selected for the study: Alkmene, Altländer Pfannkuchenapfel, Apollo, Boskoop, Braeburn, Cox Orange, Ellison's Orange, Gloster, Golden Delicious, Granny Smith, Hammerstein, Jamba, Jonagold, Macoun, Prime Rouge, and Sternrenette. Commercial prick test solutions were obtained from Abelló (Bornheim, Germany), ALK (Copenhagen, Denmark), Allergo-

pharma (Reinbek, Germany), and Bencard (Neuss, Germany).

Oral challenge tests

Open oral challenge tests were performed with samples of all 16 apple strains. Small pieces (10 g) of the apple samples from which extractions were subsequently made were ingested by three members of our laboratory staff who also participated in the histamine-release assay. Patients Schö and So were allergic to birch pollen and experienced itching and swelling of the lips, tongue, and throat after eating fresh Golden Delicious and Granny Smith apples. Patient Spe was allergic to grass pollen and had no history of food allergy (for serologic data and skin test results, see Table 2). The severity of symptoms after oral challenge was scaled in four classes ranging from no symptoms (–) to very strong symptoms (+++). On one day, not more than two positive challenge tests were performed. If no symptoms occurred in the apple-allergic patients, rechallenge with selected apple strains was performed with a greater amount of sample (150 g or one small apple). For practical (e.g., a relatively high number of challenge test dates which were dependent on the times of ripening, since only mature apples were used for extraction) and ethical reasons, the number of participants of the challenge tests could not be increased.

Skin tests

Skin tests were performed by the prick test method on the forearms with low-temperature extracts of mature apples (Golden Delicious, Boskoop, and Jamba), diluted 1:10 with distilled water and the four commercial prick test solutions. Histamine (0.1%) and NaCl (0.15 M) served as positive and negative controls. The average diameter of each wheal was established by measuring the longest diameter and the diameter perpendicular to it. Positivity was rated 3+ in the presence of a wheal with a diameter greater than the average diameter of the wheal induced by histamine, 2+ if the average diameter was the same as the average of the histamine wheal, and 1+ if the average diameter exceeded half of the average diameter of the histamine wheal. The positive patient group consisted of 11 subjects (four men and seven women) with hypersensitivity to birch pollen and apples. Most of them suffered from allergies to other fruit, nuts, and vegetables. The negative control group consisted of 12 subjects (seven men and five women), including seven nonallergic subjects and five atopic persons without food hypersensitivity (four patients allergic to grass pollen, and one patient with atopic dermatitis).

RAST class determinations

Commercial filter paper disks ($d = 6$ mm, no. 589/3, Schleicher & Schüll, Dassel, Germany) were activated with cyanogen bromide according to Ceska & Lundkvist (3). Apple proteins were coupled to the activated disks as previously described (19), with 2 μ g of protein per disk and a protein concentration of 12 μ g/ml in the coupling solution. RAST classes were determined by a commercially available ELISA (EAST) containing a monoclonal anti-IgE alkaline phosphatase conjugate and *p*-nitrophenylphosphate (PNPP) as the substrate, according to the manufacturer's instructions (Allergopharma, Reinbek, Germany). Absorbances were measured at 405 nm, and the reference system of the manufacturer was used for quantitation. The results were expressed in EAST classes (RAST classes) with one decimal place.

ELISA-inhibition assays

Patients' sera were diluted 1:5 or 1:10 in 0.05 M Tris buffer, pH 7.4, containing 0.15 M NaCl, 0.3% (w/v) bovine serum albumin (BSA), and 0.1% (v/v) Tween 20 (TBS/BSA/Tween). A 10-fold dilution series of the inhibitor extracts was prepared (five steps) in the same incubation buffer. Volumes of 50 μ l diluted serum and 50 μ l inhibitor solution were added to an allergen disk and incubated overnight at room temperature. After three washes with 0.15 M NaCl/0.1% (v/v) Tween 20, 100 μ l goat affinity-purified anti-IgE alkaline phosphatase conjugate (Tago, Burlingame, CA, USA; code no. 2494, lot no. 1601), diluted 1:10 000 in TBS/BSA/Tween, was added and incubated overnight. The disks were washed again, and the bound enzymatic activity was stained with PNPP for 3 h at 37°C with an alkaline phosphatase staining kit (Bio-Rad, Munich, Germany). Absorbance was measured at 405 nm.

Since the amount of protein in the fruit extracts was not standardized, the allergenic activity of the inhibitor extracts was characterized by calculating the absolute amount of protein (μ g) causing 50% inhibition of IgE binding (C_{50}) (17).

SDS-PAGE

SDS-PAGE was performed according to the method of Lämmli (12) in a twin vertical slab gel apparatus (Renner, Dannstadt, Germany) with 5% stacking gels and homogeneous 13% resolving gels, as previously described (18). Protein samples were reduced with β -mercaptoethanol and loaded on the gel at a concentration of 14 μ g/cm (5.5-cm preparative slots for subsequent immunoblotting and 0.5-cm analytic slots for total protein staining). For silver staining, 4–6 μ g/cm of protein was applied.

Protein transfer and immunoblotting

Semidry electroblotting of the separated allergens and immunostaining of the bound IgE-antibodies were done by a procedure slightly modified from that previously described (18). After protein transfer onto nitrocellulose (NC) (0.2 μ m, Schleicher and Schüll, Dassel, Germany), the dried NC membranes were blocked with 0.3% (v/v) Tween 20 in 0.01 M potassium phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS). Human sera and all immunochemicals were diluted with 0.01 M PBS/0.05% (v/v) Tween 20/0.1% (w/v) BSA (incubation buffer). Each incubation step was followed by three washes with 0.05% (v/v) Tween 20 in 0.01 M PBS. Cut NC strips (4 mm) were incubated overnight at room temperature with 150 μ l human serum, made up to 1 ml with incubation buffer, in disposable mini-incubation trays (Bio-Rad, Munich, Germany). After incubation with rabbit anti-IgE (1:4000, 1 h), goat biotinylated anti-rabbit IgG (1:6000, 1 h, both from DAKO, Copenhagen, Denmark), and streptavidin horseradish peroxidase conjugate (1:20 000, 20 min; Medac, Hamburg, Germany), the blots were stained with 3,3',5,5'-tetramethylbenzidine/dioctyl sodium sulfoxsuccinate (TMB/DONS). To check the efficiency of the protein transfer, a total protein stain of one NC strip with separated apple extract and low mol. mass marker proteins (Pharmacia, Freiburg, Germany) was done with amido black.

Semiquantitative evaluation of immunoblots by reflection densitometry

After simultaneous development, stained immunoblots were scanned with a CS 930 dual-wavelength, thin-layer chromatoscanner (Shimadzu, Duisburg, Germany) in the dual wavelength absorption/reflection mode at 370 nm (measuring wavelength) and 500 nm (reference wavelength).

Histamine-release assays

Peripheral blood (20 ml), anticoagulated with ethylenediaminetetraacetic acid (EDTA), was separated by dextran sedimentation and the leukocyte fraction, including the basophils, was washed once with 35 ml 0.025 M piperazine-*N,N'*-bis-(2-ethanesulfonic acid) buffer, pH 7.4, containing 0.11 M NaCl, 0.05 M KCl, 0.003% human serum albumin, and 4 mM EDTA (PIPES A/EDTA), and once with pure PIPES A. The cells were resuspended in PIPES A/1 mM CaCl_2 /0.25 mM MgCl_2 (PIPES ACM) (10). A 250- μ l volume of a 10-fold serial dilution (1:10–1:100 000) of the allergenic extracts in PIPES ACM was added to 250 μ l of the cell suspension and incubated at 37°C for 30 min. Released histamine was extracted

with 1-butanol, reacted with *o*-phthalaldehyde, and determined automatically by fluorescence photometry according to the method of Siraganian & Hook (15). The spontaneous histamine release (HR) was determined by incubating cells in buffer alone. Total histamine content was assayed in a cell suspension treated with 3% perchloric acid. IgE-mediated HR was checked by addition of anti-IgE (400 U/ml). The potency of the fruit extracts was quantified by estimating the protein concentration corresponding to a 30% HR (C_{30}) from the resulting dose-release plots in μg protein/ml fruit extract dilution (cell sensitivity).

Results

SDS-PAGE

Extracts of the selected 16 apple strains, all from mature fruit, were submitted to SDS-PAGE, and the resulting gels were silver stained. All extracts produced intensive protein patterns with up to 30 individual bands. Somewhat weaker protein patterns were obtained with the apple extracts of lower "allergenicity" (i.e., Jamba or Gloster; see below). The presence of the 18-kDa apple protein band and a subjective estimation of its intensity are summarized in Table 1. For example, Fig. 1 shows SDS-PAGE analyses of apples with high (lanes 1–4, 6), moderate (lane 7), and very low contents (lanes 8, 9, 11) of the 18-kDa protein.

To check the reproducibility of our results, four samples of Granny Smith apples and three samples of Boskoop apples from different sources (Granny Smith: samples A1 (Chile) and A27 (France), purchased at the local market; A15 (Italy), "organically grown", purchased in a local health food shop; A17 (Germany), grown in the research field of the Institute of Fruit Culture, Berlin; Boskoop: sample A12, obtained from a private grower, Germany; A13 (Germany), purchased at the local market; A16 (Germany), "organically grown", purchased in a local health food shop) were extracted and analyzed.

Table 1. Occurrence of 18-kDa apple protein band in low-temperature extracts of 16 apple strains. Bands were classified by relative intensity after silver staining of SDS-PAGE gels

Relative amount of the 18-kDa protein band			
High	Medium	Low	Very low
Granny Smith	Sternrenette	Boskoop	Jamba
Golden Delicious	Alkmene		Macoun
Jonagold			Ellison's Orange
Braeburn			Gloster
Prime Rouge			Altlander
Cox Orange			Hammerstein
Apollo			

All Granny Smith apples contained the 18-kDa protein, whereas in the Boskoop samples the allergen band could not be detected by SDS-PAGE and silver staining (results not shown).

EAST class determinations

IgE binding to Golden Delicious, Granny Smith, and Boskoop allergen disks was examined for seven individual sera of patients allergic to birch pollen and apples, the pool serum Wn 13/32 and the negative pool serum Wn 13/26 (Fig. 2). The "positive" sera revealed high EAST classes between 2.0 and 4.0 to Golden Delicious and Granny Smith, whereas the corresponding classes with the Boskoop disks were generally lower with all sera (0.7–2.8). No IgE binding was observed for the negative control serum.

Specific IgE to all of the 16 apple strains was determined in three sera: the positive pool sera ILC-1 and Wn 13/32 and serum from patient Bra. IgE antibodies of patient Bra bound strongly to the 18-kDa apple allergen and to a protein band of about 15–16 kDa. The resulting EAST classes are presented in Fig. 3. The results agreed closely for the three sera, but strong differences in the IgE binding to the various apple strains occurred (EAST classes of 0.2–4.0).

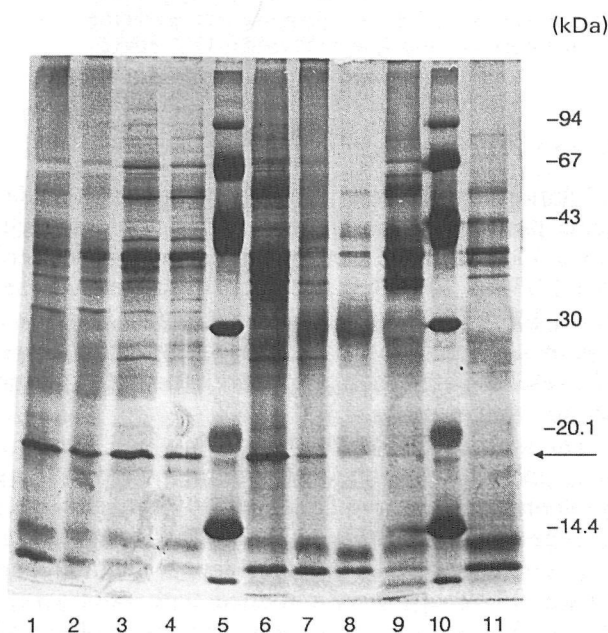


Fig. 1. SDS-PAGE analyses of apple extracts. Protein bands were made visible by silver staining. Lane 1: Apollo, lane 2: Braeburn, lane 3: Granny Smith, lane 4: Golden Delicious, lane 5: LMW marker, lane 6: Prime Rouge, lane 7: Sternrenette, lane 8: Jamba, lane 9: Boskoop, lane 10: LMW marker, lane 11: Alkmene.

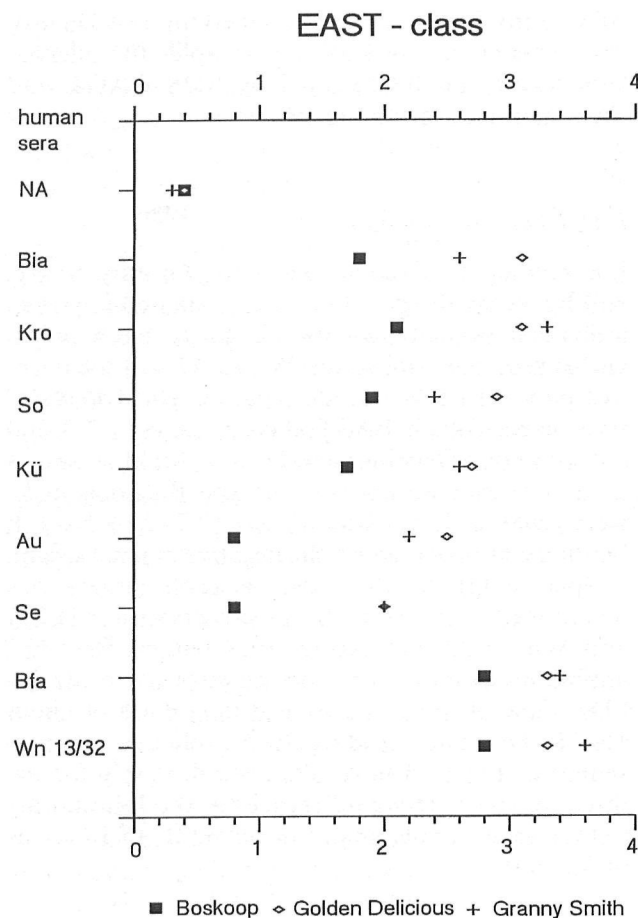


Fig. 2. Results of EAST to Golden Delicious, Granny Smith, and Boskoop apples for seven individual sera of patients allergic to birch pollen and apples, birch/apple-positive pool serum Wn 13/32, and negative control pool serum Wn 13/26 (NA).

ELISA inhibition experiments

Inhibition assays were done to determine the allergenic potency of the apple varieties. Golden Delicious allergen disks were incubated with pool sera Wn 13/32 (1:5) and ILC-1 (1:10). Boskoop, Braeburn, Ellison's Orange, Jamba, Golden Delicious, and ovalbumin (control) were used as the inhibitors. The resulting inhibition graphs with the ILC-1 serum are presented in Fig. 4.

The absolute amounts of protein which caused a 50% inhibition of the IgE binding were 0.12 μ g (Golden Delicious and Braeburn), 0.71 μ g (Ellison's Orange), and > 7 μ g (Jamba) with the ILC-1 serum. C_{50} amounts of 0.36 μ g (Golden Delicious) and 2.0 μ g (Boskoop) were obtained with pool serum Wn 13/32. No significant inhibition was observed with ovalbumin. Birch pollen extract typically revealed C_{50} amounts of 0.01 μ g with the sera/allergen disks used, indicating its high allergenic activity and cross-reactivity in patients with BPRF (17, 19). The results of the inhibition assays agreed closely with

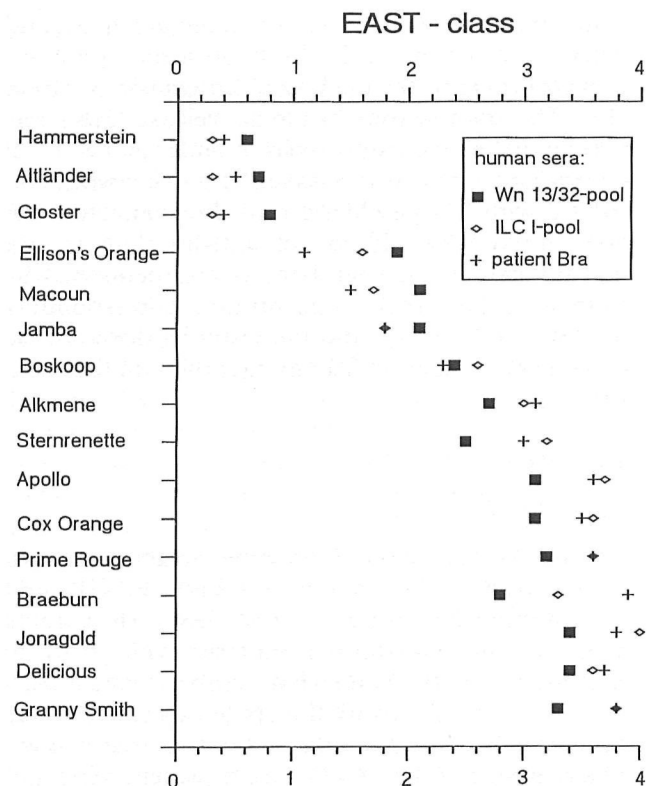


Fig. 3. Results of EAST to 16 apple varieties for pool sera Wn 13/32, ILC-1, and serum from patient Bra.

the direct IgE binding to the apple varieties observed by EAST class determination.

In vitro histamine release

The immunologic properties of our apple extracts were further evaluated by measuring the *in vitro* HR capacity from washed blood basophils. Blood

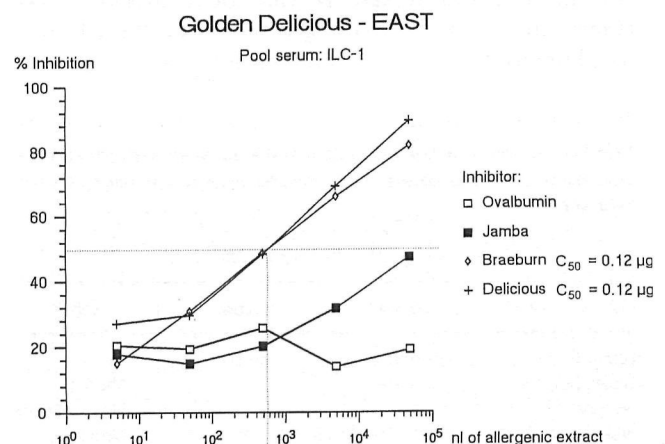


Fig. 4. ELISA-inhibition experiments with Golden Delicious allergen disks and pool serum ILC-1 (1:10), performed to quantify allergenic potency of apple strains.

samples were taken from three atopic donors: patients Schö and So, both allergic to birch pollen, apples, nuts, and several other fruit, and patient Spe, who was allergic only to grass pollen. Additional patient data are summarized in Table 2. Protein concentrations causing 30% HR (C_{30}) as well as the total HR resulting from incubation with anti-IgE (400 U/ml) were calculated (Table 3).

The basophils of the apple-allergic patients showed a strong dose-related HR with Golden Delicious apples (results not shown). The C_{30} concentrations of Boskoop and Jamba apples were significantly higher because of a lower allergenic potency. HR was negative with basophils from the atopic patient Spe (control). The results of anti-IgE incubation indicated that all three participants were responders. The allergenic potency of the extracts, expressed in terms of C_{30} concentrations, agrees with the results of the ELISA-inhibition experiments.

In addition, we examined the HR capacity of a commercially available prick test solution ("apple", Bencard, Neuss, Germany) with cells from patient Schö. No significant HR was obtained, indicating that active apple allergens were not present (results not shown).

Immunoblotting

To confirm the observation that, in comparison with literature data (6), our apple extracts contained more IgE-reactive minor allergens, NC strips with separated Golden Delicious and Granny Smith apple allergens were incubated with sera of >20 patients with apple hypersensitivity. The binding frequencies of IgE antibodies from these sera to apple allergens of different mol. mass are shown in Fig. 5. Note that the intensity of IgE binding cannot be recognized from the figure. On Granny Smith immunoblots, seven individual apple allergens were recognized by more than 10% of the sera.

Table 2. Patient data of participants of histamine-release assay

Patient:	Schö	So	Spe
<i>EAST classes</i>			
Birch pollen	3.9	3.0	0
Golden Delicious	2.4	2.9	0
Grass pollen	0	0	2.9
<i>Oral symptoms after ingestion of</i>			
Golden Delicious	+++	+++	--
Boskoop	+	+	--
Jamba	--	--	--
<i>Skin test results</i>			
Golden Delicious	++	++	--
Boskoop	+	--	--
Jamba	--	--	--

Table 3. 30% histamine-release concentrations (μ g of protein/ml allergenic extract dilution) and total histamine release caused by anti-IgE incubation (400 U/ml)

Patient:	Schö	So	Spe
<i>Allergenic extract</i>			
Golden Delicious	0.003	0.07	--
Boskoop	0.29	0.32	--
Jamba	2.4	7.0	--
Anti-IgE	36%	48%	26%

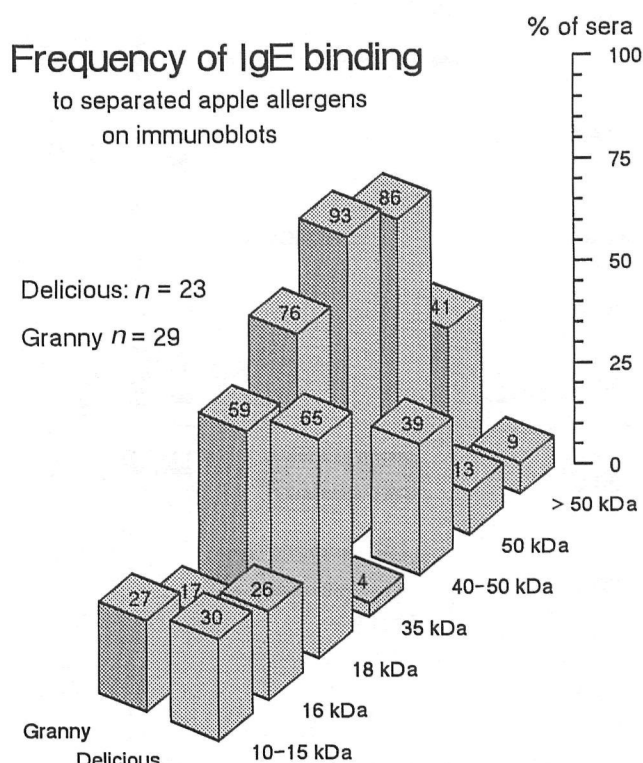


Fig. 5. Frequency of IgE binding to separated Golden Delicious and Granny Smith allergens for sera of 23/29 patients allergic to birch pollen and apples.

Although both apple strains (Golden Delicious and Granny Smith) are strongly allergenic and produced similar EAST classes with most of the positive sera (Figs. 2 and 3), the resulting IgE-binding patterns were significantly different (Fig. 5). More than 50% of the sera revealed strong IgE reactivities to the 18-kDa allergens of both apple extracts.

The allergen patterns of apples with different allergenic potency were studied by immunoblotting with Golden Delicious and Boskoop extract and the positive sera which were used for EAST class determinations (Fig. 2). The resulting IgE-binding patterns are presented in Figs. 6A and B. Two individual sera from nonatopic donors served as negative controls (lanes 9 and 10).

Seven of the eight positive sera demonstrated IgE

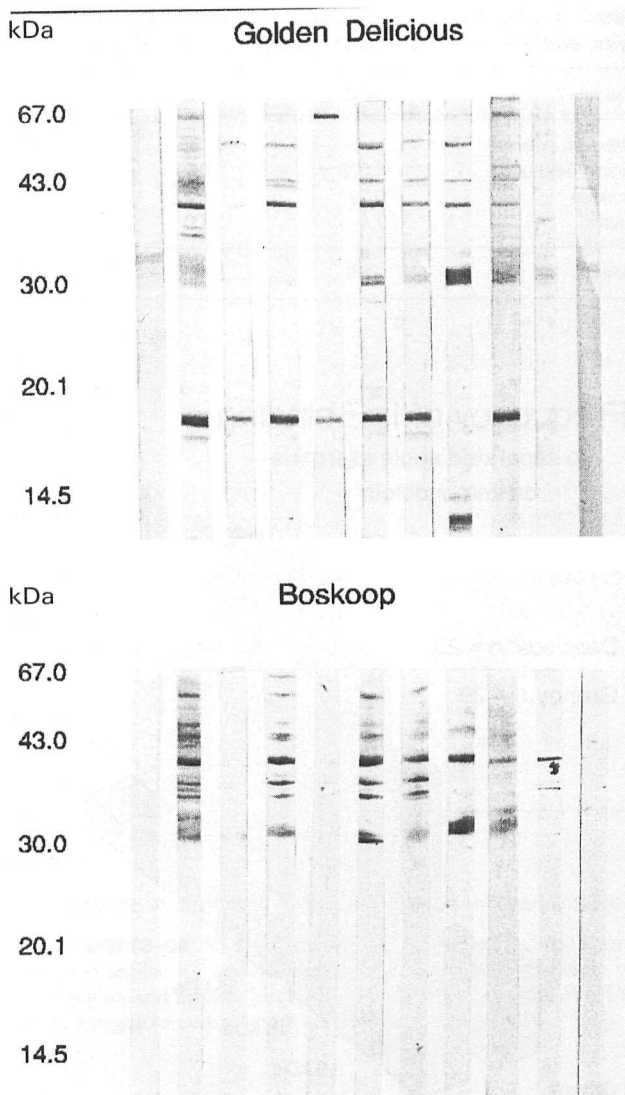


Fig. 6. IgE-binding patterns to extracts of Golden Delicious (A) and Boskoop (B) apples, separated by SDS-PAGE and transferred to NC. Human sera: 1 birch pollen pool Wn 13/32, 2 Bia, 3 Kro, 4 So, 5 Kü, 6 Au, 7 Se, 8 Bfa, 9–10 nonallergic control sera. C control (incubation buffer instead of human serum).

binding to the 18-kDa Golden Delicious apple allergen. In addition, all positive sera recognized more than one allergen. No IgE reactivity appeared in the 18-kDa region of Boskoop extract, but six of the eight positive sera bound to allergens of higher and lower mol. mass (Fig. 6B). Since staining of Golden Delicious and Boskoop immunoblots was not stopped simultaneously, the band intensities of Figs. 6A and B cannot serve as a criterion for quantitative IgE binding to the different apple strains.

Evaluation of immunoblots by reflection densitometry

For more objective comparison of the allergen band intensities, immunoblots of Golden Delicious, Boskoop, and Jamba apples were incubated with the pool

serum Wn 13/32, and developed and stopped simultaneously, and the band intensity was scanned. Similar amounts of protein were loaded on the gels, separated, and transferred to NC membranes. Amido black staining of cut NC strips for total protein yielded patterns of similar intensities for the three apple strains. The obtained absorption/reflection graphs are presented in Fig. 7.

Again, the Golden Delicious strain produced the strongest reaction with patients' IgE antibodies. The 18-kDa band and more than five "minor" allergen bands of high intensity were detected. The allergen bands were clearly weaker on the Boskoop and Jamba immunoblots. A very weak allergen band in the 18-kDa region appeared on the Boskoop immunoblot.

Skin prick tests

Skin prick tests (SPT) with Golden Delicious extract were positive in all patients with apple hypersensitivity (minimum 1+). Of the apple-positive patients, 4/11 (36%) had a positive prick test to Boskoop extract, whereas the SPTs with the Jamba strain were negative in all patients with apple allergy. In addition, no patient reacted to any of the four commercial apple prick test solutions. All SPTs were negative in the members of the control group. In general, the patients presented a positive skin reaction to histamine and a negative one to the NaCl control.

Oral challenge tests

The evaluation of symptoms by the two apple-allergic patients after oral challenge with 10 g fresh apple tissue was nearly identical: (+ + +) symptoms were provoked by Golden Delicious, Granny Smith, Jonagold, Braeburn, Prime Rouge, and Apollo (exception: patient So was not challenged with the Prime Rouge variety). Cox Orange elicited (+ +) symptoms in patient So and (+ + +) symptoms in patient Schö. Sternrenette, Alkmene, Boskoop, and Ellison's Orange produced moderate (+) symptoms in both patients. Jamba, Macoun, Gloster, Altländer Pfannkuchenapfel, and Hammerstein were tolerated by the patients. Rechallenge experiments were conducted with one small apple (150 g) for three apple strains (Jamba, Macoun, and Altländer Pfannkuchenapfel), which were again tolerated by the patients. All challenge tests were negative with the "control" patient Spe.

Discussion

In a recent study, Ebner et al. (6) clearly demonstrated that a 17–18-kDa protein is an important

Detection of separated apple allergens by immunoblotting

Human serum: birch pollen pool Wn 13/32 ($n=7$)

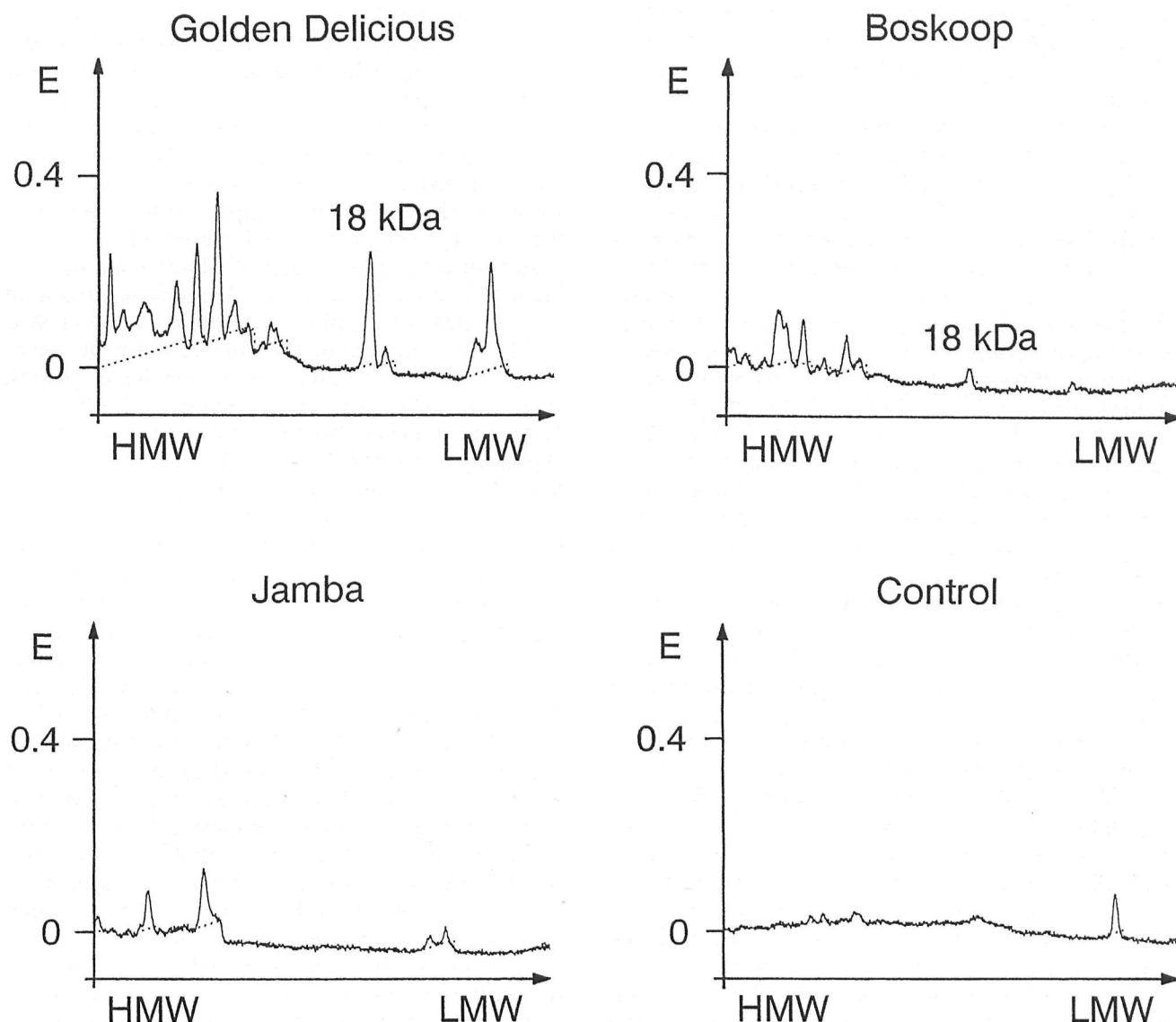


Fig. 7. Evaluation of simultaneously developed Golden Delicious, Boskoop, and Jamba apple immunoblots by reflection densitometry. Human serum: pool serum Wn 13/32. Negative control (use of incubation buffer instead of human serum) for Golden Delicious immunoblots is shown.

allergen in Golden Delicious apples and that this protein shares common epitopes with *Bet v I*, the major allergen of birch pollen. The cross-reacting fruit and pollen proteins probably belong to a group of "pathogenesis-related proteins" the function of which is unknown. To our knowledge, the IgE-

binding properties of various apple strains previously have been studied with five (1) and two (5) apple strains only with RAST. Golden Delicious, Granny Smith, and Ingrid Marie apples were found to be highly allergenic. However, in our present investigation, we studied the IgE-binding potency of

16 apple strains with regard to the occurrence of the 18-kDa allergen, assuming that the genes coding for the 18-kDa proteins of apples are constitutively transcribed and translated in the fruit. Our finding that Granny Smith and Boskoop apples from different sources contained, in general, high (Granny Smith) or low (Boskoop) relative amounts of the 18-kDa protein supports this view. Furthermore, in an extended study, we were able to show that increased synthesis of the allergen occurred during ripening of Golden Delicious apples, but not in the Boskoop strain (21). From these data, it may be concluded that the capability of allergen synthesis depends on the apple strain.

Seven of the 16 apple strains studied (44%) contained considerable amounts of the 18-kDa protein (Table 1, Fig. 1) and had a high IgE-binding capacity in the EAST (EAST classes of ≥ 3 with the sera used). The six apple varieties with a very low relative amount of the 18-kDa protein also produced low EAST classes (≤ 2). Confirmation experiments were conducted with three apple strains with different relative amounts of the 18-kDa allergen: Golden Delicious, Boskoop, and Jamba, corresponding to a high, low, and very low 18-kDa allergen concentration. The results of the ELISA-inhibition and histamine-release assays, as well as the skin test results, agreed closely and were further confirmed by the absorption/reflection densitometry measurements of simultaneously developed immunoblots. All methods revealed that the allergenic activity decreased in the following order: Golden Delicious > Boskoop > Jamba. Therefore, we concluded that the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen.

Additional open oral challenge tests were performed with the three participants of the histamine-release assay. The degree of symptoms produced by the different apple strains agreed closely with the results of the *in vitro* studies and the skin prick tests, with the exception of Ellison's Orange which produced EAST classes of ≤ 2.0 but elicited moderate symptoms in the two apple-allergic patients. Since the ELISA inhibition potency of Ellison's Orange was approximately 10 times that of Jamba, which produced higher EAST classes, difficulties in the allergen disk preparation might have led to an underestimation of the allergenic potency of Ellison's Orange extract from direct IgE binding in the EAST. However, the symptoms were much less severe than for highly allergenic varieties such as Golden Delicious and Granny Smith. Since only two allergic patients were studied, general conclusions on the degree of symptoms produced by the different apple strains cannot be drawn from these experiments. For this, challenge tests with an increased number of

patients and selected apple varieties would be required.

Interestingly, five of the seven "highly allergenic" apple strains are very common on the German market (Braeburn, Cox Orange, Golden Delicious, Granny Smith, Jonagold). In contrast, most of the "low allergenic" apple strains are of minor economic importance in Germany (i.e., Altländer, Ellison's Orange, Hammerstein, Macoun). It is tempting to speculate that properties which make an apple strain suitable for intensive agricultural and commercial use (resistance to microbial attack?) depend on the presence of the 18-kDa allergen. Possibly, new "low allergenic" apple strains may be developed by fruit cultivation. In addition, our results may help patients with moderate apple hypersensitivity and persons sensitized only to birch pollen to select apple varieties which cause no symptoms or to minimize the risk of developing apple hypersensitivity.

In contrast to the findings of Ebner et al. (6), who identified only two important allergens in extracts of Golden Delicious apples, both with mol. mass below 20 kDa, our immunoblot studies revealed the existence of up to seven important allergen bands of mol. mass of 12–67 kDa in various apple varieties (Golden Delicious, Granny Smith, Boskoop). Considering especially our results with Boskoop apples, we believe that these bands do represent allergens, for the following reasons. Apples of this strain contain only low amounts of the 18-kDa allergen; immunoblot analyses revealed nearly negligible IgE reactivities in the 18-kDa region, but up to six allergen bands in the region of 30–67 kDa (i.e., Fig. 6B, lane 1). Furthermore, EAST classes of up to 2.8, positive histamine-release assays, and skin tests were obtained with Boskoop extract in this study. In addition, the two apple-allergic patients who participated in the HR reported moderate symptoms after the ingestion of Boskoop apples. These findings support our view that the allergenic potency of these apples which we quantified as "medium" is mediated by the 30–67-kDa allergen bands. Possibly, the association constant of patients' IgE to these proteins is relatively low, as compared with the 18-kDa allergen of other apple strains. The observed differences from the results of other authors may be due to the composition of the allergenic extracts: Ebner et al. applied the Björkstén method (1), whereas we used a low-temperature, acetone powder method (19). The influence of extract quality can clearly be recognized from the skin test results. Although it has been known since 1980 that the preparation of active apple extracts requires special methods, the four commercially available prick test solutions were unreliable for the diagnosis of apple allergy.

In addition, the results of HR may also reflect the high activity of our acetone powder extracts. In a

recent study, Kleine-Tebbe et al. investigated the dose-related HR in birch-pollen-allergic patients with and without fruit hypersensitivity, using Granny Smith extract prepared by the Björkstén method (10). In basophils from the fruit-sensitive patients, a 30% HR was elicited by about 1 µg/ml of protein from apple peel and apple pulp. In contrast, 0.003 and 0.07 µg/ml protein of our Golden Delicious extract triggered a 30% HR in the two participants of the HR assay reported in this paper. It might be interesting to determine whether stable prick test solutions of apples and other fruit can be established on the basis of our acetone powder extracts.

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References

1. BJÖRKSTÉN F, HALMEPURO L, HANNUKSELA M, LAHTI A. Extraction and properties of apple allergens. *Allergy* 1980; 35: 671–7.
2. CALKHOVEN PG, AALBERS M, KOSHTÉ VL, POS O, OEI HD, AALBERSE RC. Cross-reactivity among birch pollen, vegetables and fruits is due to at least three distinct cross-reactive structures. *Allergy* 1987; 42: 382–90.
3. CESKA M, LUNDKVIST U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972; 9: 1021–30.
4. DREBORG S. Food allergy in pollen-sensitive patients. *Ann Allergy* 1988; 61: 41–6.
5. EBNER H, HEMSTRÖM C, KRAFT D, SCHROEDER H. Apfel-unverträglichkeit und Birkenpollenallergie. Beitrag zur Frage eines Zusammenhanges dieser Phänomene. 4. Kölner RAST-Symposium, Berichtsband, 1983: 128–33.
6. EBNER C, BIRKNER T, VALENTA R, et al. Common epitopes of birch pollen and apples – studies by Western and Northern blot. *J Allergy Clin Immunol* 1991; 88: 588–94.
7. ERIKSSON NE, FORMGREN H, SVENONIUS S. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982; 37: 437–43.
8. 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.
9. HIRSCHWEHR R, VALENTA R, EBNER C, et al. Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992; 90: 927–36.
10. KLEINE-TEBBE J, GALLEANI M, JEEP S, PILZ B, BAISCH A, KUNKEL G. Basophil histamine release in patients with birch pollen hypersensitivity with and without allergic symptoms to fruits. *Allergy* 1992; 47: 618–23.
11. LAHTI 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.
12. LÄMMLI UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680–5.
13. MÖLLER C. Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. *Ann Allergy* 1989; 62: 343–5.
14. ORTOLANI C, ISPANO M, PASTORELLO E, BIGI A, ANSALONI R. The oral allergy syndrome. *Ann Allergy* 1988; 61: 47–52.
15. SIRANGANIAN RP, HOOK WA. Histamine release assay methods for the study of human allergy. In: ROSE NR, FRIEDMAN H, eds. *Manual of clinical immunology*. American Society of Microbiology, Washington, DC: 1980: 808–21.
16. THIEL NC. Nahrungsmittelallergien bei Pollenallergikern (sogenannte pollenassoziierte Nahrungsmittelallergien). *Allergologie* 1988; 11: 397–410.
17. VIETHS S, BROCKMANN S, SCHÖNING B. Nahrungsmittelallergie gegen Obst und Gemüse: Eine aktuelle serologische Untersuchung zur Sensibilisierung von Pollenallergikern. *Allergologie* 1992; 15: 367–79.
18. VIETHS S, SCHÖNING B, BALTES W. Allergy to fruits and vegetables in pollen-sensitive patients: allergen characterization by IgE immunoblotting and peroxidase staining. *Food Agric Immunol* 1992; 4: 181–97.
19. VIETHS S, SCHÖNING B, BROCKMANN S, AULEPP H. Untersuchungen zur Allergie gegen Lebensmittel pflanzlicher Herkunft: Herstellung und Charakterisierung von Obst- und Gemüseextrakten für serologische Untersuchungen. *Dtsch Lebensm Rundsch* 1992; 88: 239–43, 273–9.
20. VIETHS S, SCHÖNING B, AULEPP H, BALTES W. Identifizierung kreuzreagierender Allergene in Pollen und pflanzlichen Lebensmitteln. *Lebensmittelchemie* 1993; 47: 49–53.
21. VIETHS S, SCHÖNING B, JANKIEWICZ A. Occurrence of IgE binding allergens during ripening of apple fruits. *Food Agric Immunol* (in press).

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