

Determination and characterization of cross-reacting allergens in latex, avocado, banana, and kiwi fruit

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Sera of 11 patients were used to characterize allergens in kiwi fruit, latex, avocado, and banana by SDS–PAGE/immunoblotting and to determine cross-reactions between these allergen extracts in EAST inhibition and immunoblot inhibition. By SDS–PAGE/immunoblotting, allergens with apparent molecular weights of 21, 38, 40, and 42 kDa were visualized in latex extract. In avocado extract, IgE-binding components of 27, 43, 52, 58, 65, 75, and 88 kDa were to be seen, whereas, in banana extract, a 40-kDa protein showed strong IgE binding. Furthermore, allergens of 52, 58, 88, and 94 kDa were detected in the extract of banana. Cross-reactions between these allergen extracts were determined by EAST inhibition. Immunoblot inhibition demonstrated that almost all IgE-reactive bands in nitrocellulose-blotted latex, avocado, and banana extracts and two components of 43 and 67 kDa in kiwi fruit shared common IgE epitopes.

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Hypersensitivity to natural rubber latex has been recognized as an important clinical problem, especially for health-care workers, but due to the wide use of natural rubber latex (NRL) products such as balloons, household gloves, and condoms, other groups of people may also suffer from latex allergy. Immediate-type hypersensitivities to latex are caused by natural, water-soluble proteins in latex sap, but investigations of proteins which cause these reactions have led to different results (1–8).

In many clinical investigations, cross-reactions between latex and tropical fruits – especially avocado, banana, and kiwi – are indicated (9–15); in contrast, there is little molecular information on the allergens causing these cross-reactions.

Recently, the allergens in avocado and banana and the cross-reacting allergens with latex were characterized by various research groups, but the investigations led to differing results (16–21). The present investigation characterized allergens in latex, avocado, banana, and kiwi fruit with regard to their molecular weights by SDS–PAGE/immunoblotting. In addition, we intended to detect

cross-reactivities between these extracts by EAST-inhibition assays. The cross-reacting allergens were finally characterized by immunoblot inhibition.

Material and methods

Patients

Patients with immediate-type hypersensitivities or sensitizations to latex, kiwi fruit, avocado, and/or banana were recruited at the Department of Dermatology and Allergy of the University Hospital Eppendorf, Hamburg, Germany, and at the Hospital Borkum Riff of the BfA, Borkum, Germany. The case reports were noted and the sera collected.

Seven of our 11 patients complained of clinical symptoms to kiwi fruit and latex. Seven patients suffered from clinical symptoms after consumption of banana, and six of them also complained of immediate-type hypersensitivities to kiwi fruit and latex. Three of the four patients who showed allergic reactions to avocado also suffered from clinical symptoms to latex, banana, and kiwi fruit.

All kiwi-allergic patients complained of oropharyngeal symptoms after consumption of kiwi fruit, whereas banana and avocado caused oropharyngeal and gastrointestinal symptoms. Latex allergy led to itching dermatitis in all of our patients with hypersensitivities to latex. The clinical symptoms of the patients are summarized in Table 1. The serum of a nonallergic subject served as a negative control.

Allergen extracts

Allergen extracts of the fruits were prepared by a low-temperature method, as described previously (22, 23), with the following minor modifications.

Pieces of avocado were homogenized with precooled (-20°C) acetone/*n*-hexane (1:1, v:v), instead of acetone, to remove fat.

The banana pulp, which was homogenized with acetone, was not filtrable. Therefore, the suspension was centrifuged (3000 g, 20 min), the supernatant discharged, and the residue washed by resuspending in acetone. Afterward, the mixture was centrifuged (3000 g, 10 min) and the residue washed once again. Finally, the residue was lyophilized.

Proteins were extracted from the acetone powder obtained, as previously described (23).

The raw material for preparation of the latex extract was ammoniated natural rubber latex (AL) containing 0.7% NH_3 ("full ammonia latex") and was kindly supplied by Kautschuk GmbH, Frankfurt (am Main), Germany. AL was centrifuged (50 000 g, 2 h), and the clear, pale yellow solution collected. Afterward, the solution was divided into aliquots of 1 ml, lyophilized, and stored at -20°C until use (2).

The protein contents of these extracts were determined by the method of Lowry and ranged from 400 (banana) to 2000 $\mu\text{g/ml}$ (latex) (24, 25).

Enzyme allergosorbent test (EAST) and EAST inhibition

Preparation of allergen disks, EAST assay, and EAST-inhibition assay were performed as described previously (23). The results of the EAST assay were expressed as EAST classes (1–4) and in U/ml (1 U = 2.4 ng IgE), according to the manufacturer's instructions (Allergopharma, Reinbek, Germany).

Table 1. Clinical symptoms of patients and specific IgE against latex, avocado, banana, and kiwi fruit

Patient no. Sex, age (years)	Allergen	Clinical symptoms	Specific IgE (U/ml)	Patient no. Sex, age	Allergen	Clinical symptoms	Specific IgE (U/ml)
1	Avocado	—	22.4	2	Avocado	DIA	1.17
F, 28	Banana	—	19.9	F, 59	Banana	SA	0.96
	Latex	—	0.79		Latex	D	25.1
	Kiwi	—	30.2		Kiwi	IM, AV	<0.35
3	Avocado	NE	0.97	4	Avocado	NE	2.01
F, 38	Banana	—	0.37	F, 39	Banana	A	0.93
	Latex	D	0.87		Latex	A, D	32.8
	Kiwi	SL	0.78		Kiwi	SL	<0.35
5	Avocado	—	20.2	6	Avocado	DIA	4.29
F, 19	Banana	—	5.63	F, 44	Banana	DIA	2.62
	Latex	—	5.62		Latex	—	3.20
	Kiwi	IM, SL	5.01		Kiwi	IM, IT	2.84
7	Avocado	—	1.28	8	Avocado	—	6.65
F, 55	Banana	IM, DIA	1.90	M, 35	Banana	DIA	1.38
	Latex	D	1.11		Latex	D	2.20
	Kiwi	IM, SL	6.33		Kiwi	IM, IT	12.6
9	Avocado	—	6.61	10	Avocado	IM, SL	6.98
F, 29	Banana	—	28.2	F, 20	Banana	IM, SL	0.59
	Latex	D	28.3		Latex	D	7.89
	Kiwi	—	<0.35		Kiwi	IM, SL	5.01
11	Avocado	IM, DIA	2.51				
F, 32	Banana	IM, DIA	0.72				
	Latex	D	3.00				
	Kiwi	IM, IT	<0.35				

A: allergic asthma; AV: aversion; DIA: diarrhea; D: dermatitis; IM: itching in mouth; IT: itching in throat; NE: never eaten before; SA: stomachache; SL: swelling of lips; U: urticaria.

In EAST inhibition, a 10-fold dilution series of the extracts prepared in five steps was used to inhibit IgE binding to the allergen disks. Ovalbumin was used to check nonspecific inhibition.

Electrophoresis, semidry-blotting, immunostaining, immunoblot inhibition

The separation of the allergen extracts by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using a 5% stacking gel and a 13% resolving gel, semidry-blotting to nitrocellulose membranes, and immunostaining was performed as described previously (23, 26). For total protein staining and to check the efficiency of the blotting procedure, one strip of separated extract and one strip of low-molecular-weight markers (Pharmacia, Uppsala, Sweden) were stained with India ink (27).

For immunoblot inhibition, 100 μ l of the same pooled serum that was used for EAST inhibition was preincubated with 100 μ g protein of the allergen extracts. Subsequently, the solution was incubated with one strip of the blotted extract overnight. Ovalbumin was used to check nonspecific inhibition.

Results

EAST

The contents of specific IgE against latex, avocado, banana, and kiwi fruit are summarized in Table 1. In seven sera, specific IgE against all four extracts was detectable, whereas four sera contained specific IgE against latex, avocado, and banana, but not against kiwi fruit. It is interesting that some patients (e.g., nos. 1, 5, and 9) had very high contents of IgE against the allergen extracts, but did not show clinical symptoms to any of these allergens. On the other hand, patient 3 had low IgE levels, but complained of clinical symptoms to latex and kiwi fruit. Patient 11 also suffered from clinical symptoms to kiwi fruit and banana without exhibiting high IgE levels in his serum. The other patients showed a good correlation between EAST score and clinical symptoms.

SDS–PAGE/immunoblotting

Latex. In total protein staining with India ink, a strong background was observed, but faint protein bands of approximately 21, 30, and 45 kDa were slightly detected (Fig. 1). In immunoblotting of the

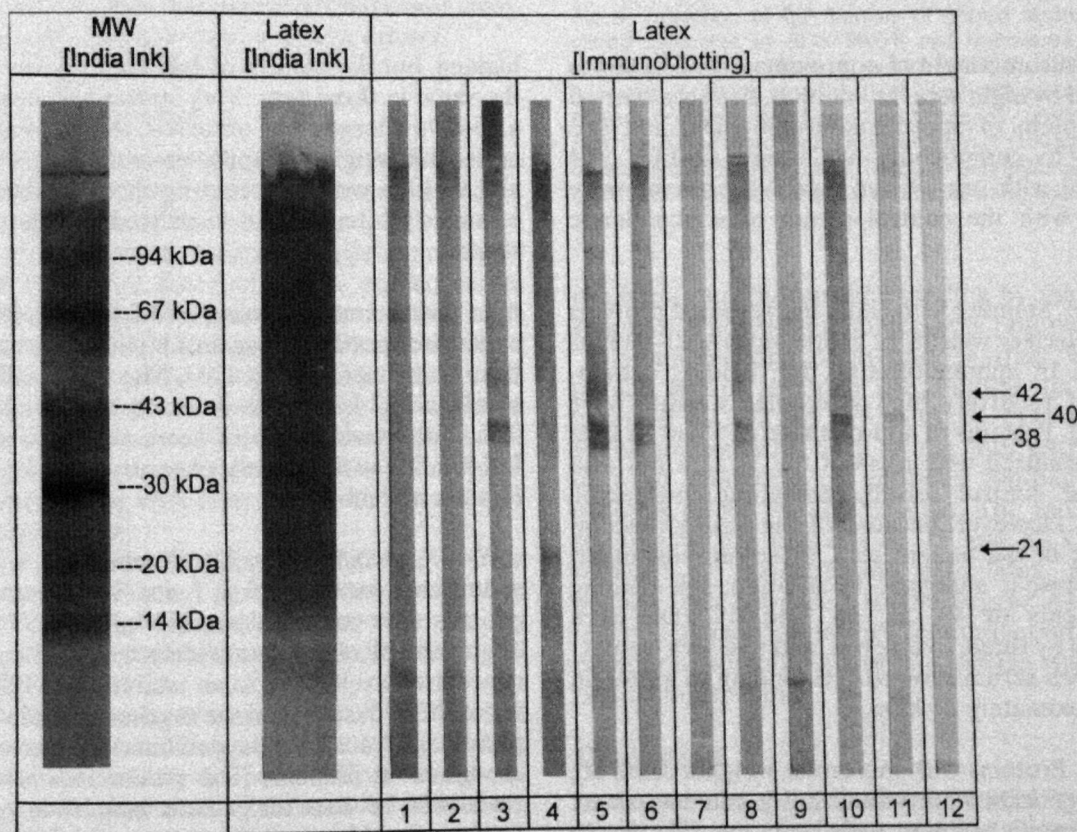


Fig. 1. India ink staining and immunoblotting of latex extract separated by SDS–PAGE and transferred to nitrocellulose membrane. Lanes 1–11) patient sera; 12) control serum of nonallergic subject.

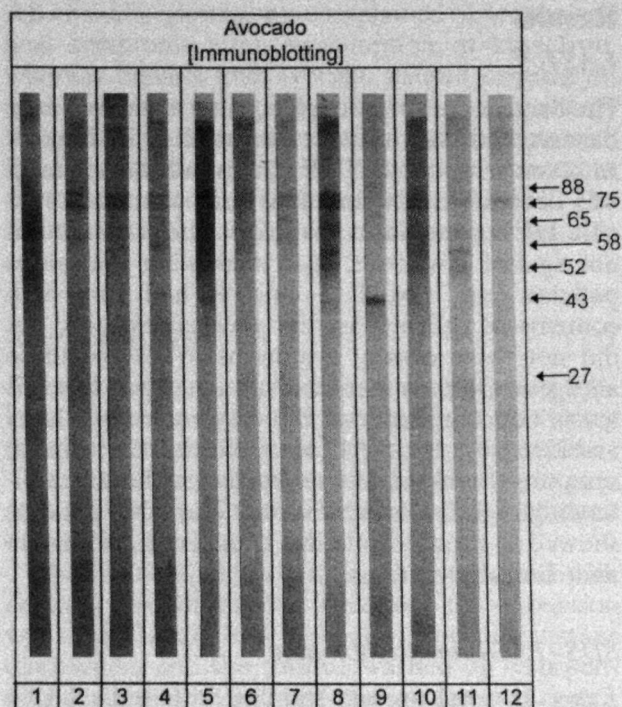


Fig. 2. India ink staining and immunoblotting of avocado extract separated by SDS-PAGE and transferred to nitrocellulose membrane. Lanes 1–11) patient sera; 12) control serum of nonallergic subject.

11 sera, a component of approximately 40 kDa was detected by eight sera. In addition, five sera reacted to a protein of approximately 38 kDa, and IgE binding to components of 21 and 42 kDa was observed with one of our sera. No proteins were stained with the control serum of a nonallergic subject.

Avocado. Staining with India ink revealed proteins with molecular weights of approximately 20–90 kDa (Fig. 2). In immunoblotting, IgE-binding components of relatively high molecular weights were detected. Proteins of approximately 75 and 88 kDa were visualized with most of the 11 sera but also with the control serum, indicating nonspecific binding. However, because of the strong reaction by some of the patient sera, these proteins might also represent allergens. In addition, IgE-binding components of 43, 52, 58, and 65 kDa were detected by three, five, seven, and five sera, respectively. One serum showed IgE binding to a protein of approximately 27 kDa.

Banana. Proteins with molecular weights of 20, 30, 61, and 65 kDa were visualized in banana extract by nonspecific staining with India ink (Fig. 3). In immunoblotting, proteins were detected by the four sera containing the highest IgE levels against

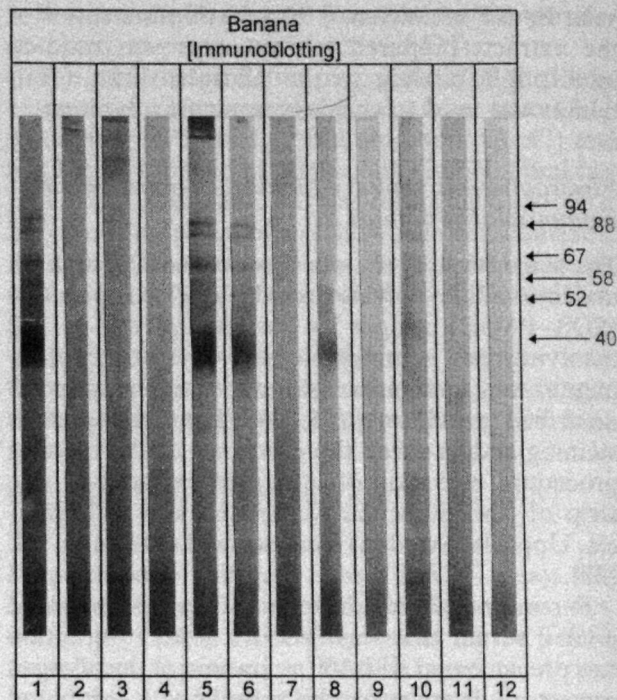


Fig. 3. India ink staining and immunoblotting of banana extract separated by SDS-PAGE and transferred to nitrocellulose membrane. Lanes 1–11) patient sera; 12) control serum of nonallergic subject.

banana, but the pattern of IgE binding was almost the same in these sera. Very strong IgE binding to a 40-kDa allergen was observed, and proteins with molecular weights of approximately 52, 58, 67, 88, and 94 kDa were detected by three of these four sera. No proteins were visualized by the control serum.

Kiwi fruit. Immunoblotting of the seven sera which contained specific IgE against kiwi fruit has already been shown previously (23). The major allergens of 43 and 67 kDa were detected by five and four sera, respectively. Two of these sera also showed IgE binding to the protein of approximately 22 kDa (results not shown).

EAST inhibition. For EAST inhibition, a pooled serum from patients 1, 5, 6, 7, and 8 was used. These patients' sera contained specific IgE (EAST classes ≥ 2) against all of the four extracts tested. The protein concentrations leading to an inhibition of IgE binding at the 50% level and the maximum inhibition (in percentage) caused by the undiluted allergen extracts are given in Table 2. The results indicate cross-reactivities between the extracts from latex, avocado, banana, and kiwi fruit, because an inhibition of at least 50% was observed with all allergen disks by preincubation with all allergen extracts.

Table 2. EAST inhibition with extracts of latex, avocado, banana, and kiwi fruit for pooled serum. Protein concentration (in µg/ml) for 50% inhibition and – in parentheses – maximum inhibition (in percentage) caused by undiluted extract are given

Allergen disk	Latex	Avocado	Banana	Kiwi fruit
Latex	11.0 (88%)	64.1 (67%)	40.0 (55%)	1768.2 (50%)
Avocado	731.5 (61%)	5.7 (90%)	400.0 (51%)	176.6 (71%)
Banana	160.0 (84%)	2.6 (94%)	31.1 (83%)	34.3 (94%)
Kiwi fruit	1505.5 (56%)	55.7 (70%)	214.8 (59%)	81.8 (90%)

IgE binding to latex disks was strongly inhibited by latex, but also by avocado and banana, whereas a very high protein concentration of kiwi extract was necessary for 50% inhibition. This result indicates that kiwi fruit extract shows IgE epitopes rather different from avocado, banana, and latex.

EAST inhibition with kiwi fruit disks demonstrated that cross-reactivity between kiwi fruit and latex was relatively weak, because a very high concentration of latex protein was necessary to cause 50% inhibition. In comparison of inhibition to avocado and banana disks, it is worth mentioning that lower protein concentrations of the four extracts were necessary for an inhibition at the 50% level for banana than for avocado disks. These results indicate that banana extract has fewer cross-reacting IgE epitopes than avocado extract.

Immunoblot inhibition

Immunoblot inhibition with the same pooled serum confirmed the cross-reactivities demonstrated in EAST inhibition. In the latex extract, IgE-reactive bands with molecular weights of approximately 40 and 36 kDa were detected by the pooled serum (Fig. 4). The 36-kDa protein may be the same protein as the 38-kDa allergen which was detected in immunoblot analysis with the individual sera. The slight difference in molecular weight might be explained by the reproducibility of the method. IgE binding to these components was totally inhibited by preincubation with latex, avocado, banana, and kiwi fruit extract.

In kiwi fruit extract, allergens of 43 and 67 kDa were strongly detected by the pooled serum (Fig. 5). Preincubation with any of these four extracts revealed a strong inhibition of antibody binding to these allergens.

In immunoblotting of banana extract, IgE-binding proteins of approximately 40, 52, 58, 67, 88, and 94 kDa were detected by the pooled serum. Antibody binding was again totally inhibited by kiwi fruit, avocado, banana, and latex extract (Fig. 6).

In immunoblot inhibition of latex, kiwi fruit, and banana extract, neither nonspecific inhibition by

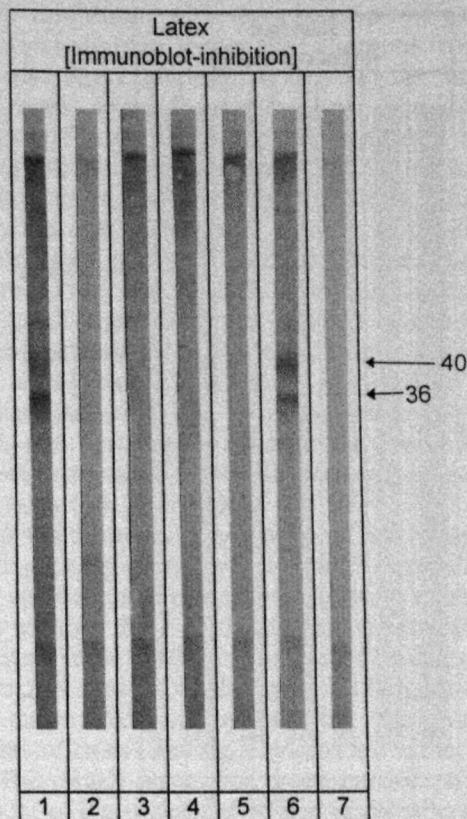


Fig. 4. Inhibition of IgE binding of pooled serum to latex extract separated by SDS-PAGE and transferred to nitrocellulose membrane. Lane 1) noninhibited; 2) inhibited with latex extract (100 µg protein); 3) inhibited with avocado extract (100 µg protein); 4) inhibited with banana extract (100 µg protein); 5) inhibited with kiwi fruit extract (100 µg protein); 6) inhibited with ovalbumin (100 µg) to determine nonspecific inhibition; 7) control serum to determine nonspecific binding.

ovalbumin nor nonspecific binding by the control serum of a nonallergic subject was observed.

Immunoblotting of avocado extract with the pooled serum revealed IgE binding to components of 52, 58, 65, 75, and 88 kDa (Fig. 7). Preincubation with each of the four extracts led to total inhibition of antibody binding to the proteins of 52, 58, and 65 kDa. The intensity of staining of the proteins of 75 and 88 kDa was reduced by preincubation with latex, avocado, banana, and kiwi fruit to the degree which was reached by incubation with the control serum. The binding to components of 20–25 kDa was identified as nonspecific.

Discussion

In comparison of IgE levels with clinical symptoms in our patients, it is interesting that many patients showed clinical symptoms to several of these extracts without containing high IgE levels. This

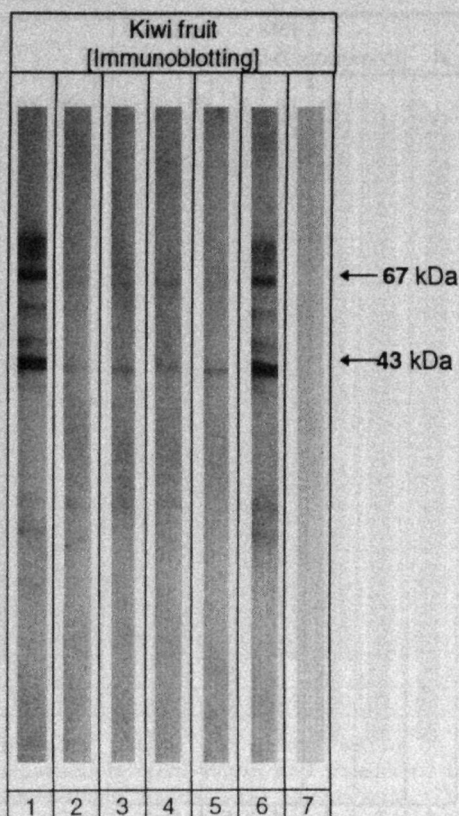


Fig. 5. Inhibition of IgE binding of pooled serum to kiwi fruit extract separated by SDS-PAGE and transferred to nitrocellulose membrane. Lane 1) noninhibited; 2) inhibited with kiwi fruit extract (100 μ g protein); 3) inhibited with latex extract (100 μ g protein); 4) inhibited with avocado extract (100 μ g protein); 5) inhibited with banana extract (100 μ g protein); 6) inhibited with ovalbumin (100 μ g) to determine nonspecific inhibition; 7) control serum to determine nonspecific binding.

may be explained by the rapid degradation of IgE in the serum. In addition, with EAST, only free IgE molecules, but not IgE bound to mast cells or basophils, are determined. On the other hand, some patients showed only sensitization to the extracts, as seen in high IgE levels, but not clinical symptoms. This discrepancy is also reported for other food allergens and might be explained by the following facts. For clinical symptoms to occur, two epitopes must be located on the allergen, allowing the "bridging" which causes the degranulation of basophils and mast cells. In EAST and immunoblotting, only a *single* antigen-antibody reaction is determined. The location and the number of epitopes on the allergen cannot be predicted.

Investigations of allergens in latex by various groups revealed different results. Rubber elongation factor (REF), a 14.6-kDa protein, was identified as the major allergen in latex (5), but prohevein, a 20-kDa protein, was also described as a major allergen (4).

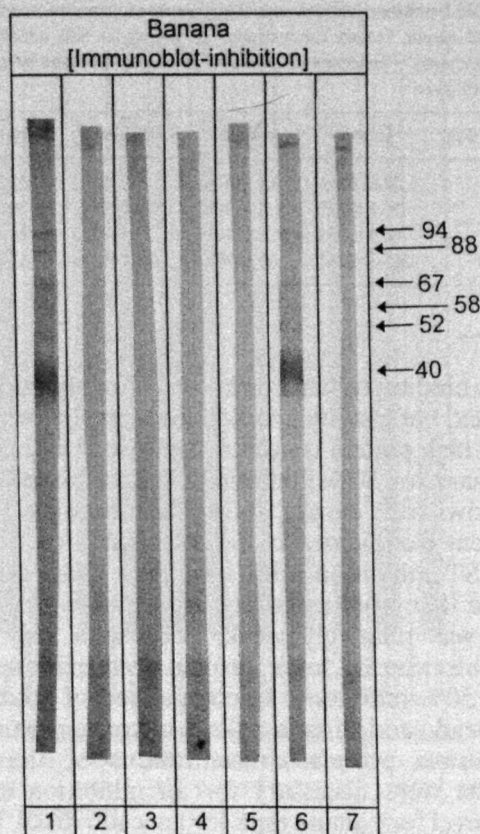


Fig. 6. Inhibition of IgE binding of pooled serum to banana extract separated by SDS-PAGE and transferred to nitrocellulose membrane. Lane 1) noninhibited; 2) inhibited with banana extract (100 μ g protein); 3) inhibited with avocado extract (100 μ g protein); 4) inhibited with latex extract (100 μ g protein); 5) inhibited with kiwi fruit extract (100 μ g protein); 6) inhibited with ovalbumin (100 μ g) to determine nonspecific inhibition; 7) control serum to determine nonspecific binding.

In our investigations, latex allergens with apparent molecular weights of 38, 40, and 42 kDa were detected by SDS-PAGE/immunoblotting. This agrees with the results of other groups describing allergens of similar molecular weights (2, 4, 6–8, 28).

It is worth mentioning that REF was not detected in latex extract, although we used the same raw material as Czuppon et al. (1992) (4). Recently, it was reported that the yellow, aqueous phase obtained by ultracentrifugation of raw latex, the C-serum, which was also used in our investigations, does not contain REF (1). Because of the high relevance of this allergen, we prepared a new latex extract from air-dried AL (29), but also in this preparation REF was not detected in immunoblotting. This discrepancy might be explained by the sensitization of the latex-allergic patients.

To our knowledge, data about allergens in avocado, banana, and kiwi fruit are rather rare. In this

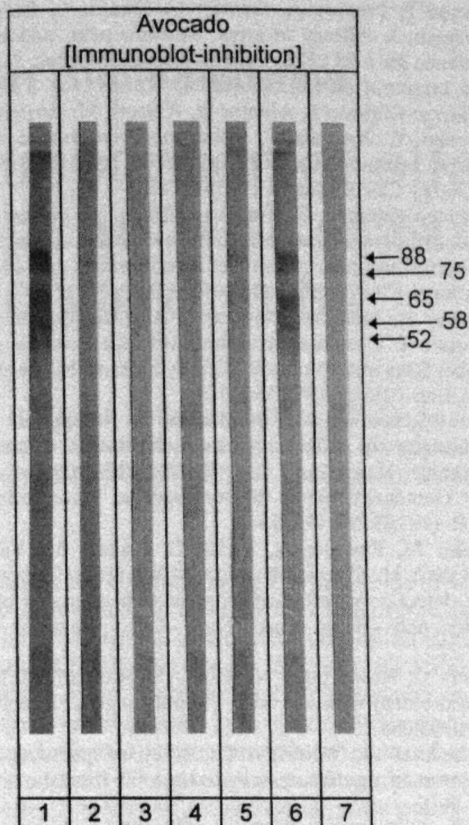


Fig. 7. Inhibition of IgE binding of pooled serum to avocado extract separated by SDS-PAGE and transferred to nitrocellulose membrane. Lane 1) noninhibited; 2) inhibited with avocado extract (100 µg protein); 3) inhibited with latex extract (100 µg protein); 4) inhibited with banana extract (100 µg protein); 5) inhibited with kiwi fruit extract (100 µg protein); 6) inhibited with ovalbumin (100 µg) to determine nonspecific inhibition; 7) control serum to determine nonspecific binding.

investigation, IgE-binding components with molecular weights of 27, 43, 52, 58, 65, 75, and 88 kDa were detected in avocado extract. Similar results were obtained by Ahlroth et al. (16). In banana extract, IgE-binding proteins with molecular weights of 40, 52, 58, 67, 88, and 94 kDa were detected by four of our 11 patient sera. These results agree with the results of other groups (17, 20, 21).

For EAST inhibition and immunoblot inhibition, a pooled serum was used, because the individual sera all contained specific IgE against these four extracts and revealed the detection of almost the same allergens in immunoblotting.

In EAST inhibition, we have demonstrated cross-reactivities between extracts from latex, avocado, and banana. This agrees with the results of other groups (9, 10, 13, 14, 18), but, in contrast, no cross-reactions between kiwi fruit and these extracts have been described with EAST inhibition and

RAST inhibition, with the exception of avocado (11). Here we have shown that allergens from kiwi fruit also share common epitopes with allergens from latex, avocado, and banana. The different degrees of inhibition may be explained by the primary sensitization of the individual patients. A botanic reason for these observations cannot be given, because kiwi fruit, banana, avocado, and rubber latex belong to different botanic families. The results of immunoblot inhibition clearly show that all allergens detected in latex, avocado, and banana extracts and the major allergens in kiwi fruit of 43 and 67 kDa are involved in cross-reactions between these extracts. Other groups also point out that many allergens in avocado and banana extracts are responsible for cross-reactivities with latex (16, 17, 19–21).

It is worth mentioning that there are many cross-reacting allergens in these extracts, especially avocado and banana. Further investigations must determine whether there are relationships between these allergens. Some bands may be caused by degradation products or precursors of only a few allergens or even one major allergen. In addition, the molecular weights of cross-reacting allergens are rather different. This clearly shows that cross-reactions are independent of the molecular weight of the allergens.

To our knowledge, no information that allergens in kiwi fruit are responsible for cross-reactions with latex, avocado, and banana is available. In a recent investigation, we demonstrated that the major allergens in kiwi fruit cross-reacted with birch pollen. This investigation has shown that the same allergens are also responsible for cross-reactions with latex, avocado, and banana. Recently, the 43-kDa allergen in kiwi fruit has been isolated and characterized by its isoelectric point and its N-terminal amino acid sequence (31).

Further investigations must determine the clinical relevance of these results by prick tests with the same allergen extracts and some individual, isolated allergens.

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