

Crossreactivity between allergens in natural rubber latex and banana studied by immunoblot inhibition

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Summary

Background An association between allergic reactions to natural rubber latex and to banana has been reported but the immunochemical properties of the putative cross-reacting allergens remain unknown.

Objective To study extracts of banana and natural rubber latex and sera from latex-allergic patients for possible crossreacting allergens and IgE antibodies.

Methods Sera from 22 latex-allergic patients and 22 control subjects with no evidence of allergy to latex or to banana were studied. All patients had positive and controls negative reactions in skin-prick testing using an eluate of latex gloves. IgE antibodies to natural rubber latex and to banana were evaluated by immunoblotting and by radioallergosorbent test (RAST) and crossreactivity between allergens in banana and natural rubber latex by immunoblot inhibition. Skin-prick testing was used to examine *in vivo* reactivity to banana.

Results Ten of the 22 (45%) latex-allergic patients sera recognized altogether 14 allergens in banana by immunoblotting. The most frequently identified banana allergens were 23, 32, 36, 39 and 47 kDa proteins. The banana skin-prick test was positive in 14 of 18 (78%) latex-allergic patients studied and banana RAST in 12 of 14 patient sera tested. Fourteen of 21 interviewed patients reported symptoms from eating or handling bananas. In immunoblot inhibition studies a dose-dependent inhibition of IgE binding to banana extract with natural rubber latex proteins was observed in all five patient sera tested and, likewise, the binding of IgE to natural rubber latex extract was inhibited with banana proteins in four of the five patient sera.

Conclusions The present results confirm the existence of crossreacting allergens in natural rubber latex and banana and provide new information on the immunochemical nature and heterogeneity of these allergens.

Keywords: allergy, banana, crossreactivity, IgE, immunoblot inhibition, immunoblotting, natural rubber latex

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Introduction

An association between allergic reactions to natural rubber latex (NRL), banana and other fruits has been recently reported by several groups of investigators [1–

9]. Latex allergy has traditionally been recognized as an occupational problem among health care workers due to their daily exposure to surgical latex gloves or medical devices prepared of NRL [10]. People can also become sensitized to non-medical NRL products such as balloons, condoms, dummies and household gloves [10–14]. Also certain patient groups, such as children with spina bifida and other congenital malformations requiring

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multiple surgeries demonstrate a high incidence of latex allergy [15]. Coexistent allergy to NRL and banana has not been connected to any particular patient or subject group. The immunochemical basis of the observed cross-reactions is also unknown.

The aim of the present study was to examine by immunoblotting putative crossreacting allergenic proteins in banana using sera obtained from patients allergic to latex and to further characterize these allergens by an immunoblot inhibition method. Here we report that several allergens can be demonstrated by immunoblotting in banana extract and that soluble NRL proteins inhibit in a dose-dependent manner IgE binding to the majority of banana allergens. Likewise, banana proteins inhibit binding of IgE to several NRL allergens confirming immunological crossreactivity between NRL and banana.

Materials and methods

Subjects

Sera were obtained from 22 patients (aged 11–64; mean 37 years) allergic to latex and from 22 control subjects (aged 21–56; mean 26 years) with no clinical evidence of either latex or banana allergy. Of the 22 control subjects, 10 had an atopic history. All patients had positive and controls negative reactions in skin-prick test using an 1:5 w/v eluate of latex surgical gloves (Exona[®], Semperit, Vienna, Austria) prepared and performed as previously described [10]. In addition, all 22 patients had previously shown positive IgE immunoblot test results against an extract of NRL using a method described below and in [16].

Antigen extracts

Natural rubber latex was prepared from deep frozen field latex of the rubber tree *Hevea brasiliensis* as previously described [16]. For extracting soluble banana proteins fresh bananas were peeled and mixed 1/10 (w/v) with phosphate buffered saline (PBS), pH 7.4 containing 0.01% of sodium azide and homogenized in a Waring blender. The homogenate was incubated overnight at +4 °C with continuous stirring, following which the eluate was centrifuged at 40 000 *g* for 60 min (Sorvall RC-5B Refrigerated Superspeed Centrifuge; Du Pont Company, Newton, Connecticut, USA). The supernatant was filtered through a 0.45 µm membrane (Millex HV; Millipore, Molsheim, France) and dialysed for two days against 20 mM ammonium bicarbonate, pH 8.0, using 3500 d dialyse tubing, filtered again through 0.45 µm membrane (Millex HV) and finally freeze-dried.

Lyophilizate was stored at +4 °C until used. The extracts were restored for use by dissolving 10 mg of lyophilizate in 1 mL of 20 mM ammonium bicarbonate buffer. Additional centrifuging at 100 000 *g* for 2 h was performed to the banana extract (BE) to remove pectin that can interfere with electrophoresis.

Protein assays

Protein concentration of the antigens (BE and NRL) were determined using the Lowry method [17] with bovine gamma globulin as the standard (Bio-Rad, Richmond, CA, USA). Total protein staining of the antigens was performed with a commercial kit (Blotting Detection Kit for Total Protein; Amersham, Amersham, UK) according to manufacturer's instructions. Protein concentrations of the antigen samples were adjusted to 450 µg/mL before immunoblotting and total protein staining.

Immunoblotting

A method described previously in detail [16] was used. Briefly, antigen samples were diluted 1:3 into electrophoresis buffer containing 2% sodium dodecyl sulphate (SDS) and 5% 2-mercaptoethanol and kept in boiling water for 2 min. The dissolved samples, 45 µL/cm of gel, were applied to 12.5% acrylamide gels containing 0.1% SDS, using a 4% stacking gel. Electrophoresis was carried out for 45 min at a constant voltage of 200 V (Mini Protean II Cell; Bio-Rad). After electrophoresis, the proteins were transferred from the gel to 0.45-µm pore size nitrocellulose filters (Trans-Blot; Bio-Rad) in an electroblotting buffer containing 25 mmol of Tris-HCl, 150 mmol of glycine and 20% methanol, pH 8.3, for 1 h at 100 V (Mini Trans-blot Cell, Bio-Rad). After blotting, the membrane was stained with Ponceau-S red to demonstrate successful transfer of proteins. The membrane was then cut into 3-mm wide strips, washed three times for 5 min in 0.1% Tween-Tris-buffered saline (TBS) and incubated for 1 h in a blocking buffer containing 5% non-fat milk powder in TBS.

Sera diluted 1:5 in the blocking buffer were added, and the strips incubated overnight at room temperature with continuous shaking. The strips were then washed four times with TBS for 5 min. Then biotinylated goat antihuman IgE antibody (Vector, Burlingame, California, USA) diluted 1:1000 in blocking buffer was added and the strips were incubated for 30 min at room temperature. After washing four times as before, an extra blocking was carried out for 5 min with the blocking buffer and the strips were again washed for 5 min. Streptavidin-conjugated alkaline phosphatase (Bio-Rad) diluted

1:24 000 in TBS was added and the strips were incubated for 30 min at room temperature. The strips were again washed and then placed in a colour development solution (Bio-Rad). The reaction was stopped after 8 min by rinsing in tap water.

Immunoblot inhibition

The antigen preparations were electrophoresed and the proteins were transferred to nitrocellulose membrane as described above. A total of 150 μ L of serum was incubated for 2 h at room temperature with an equal volume of inhibitor solution. Four different concentrations of inhibitor, containing (in 1 mL final volume) 0.1, 1, 10 and 100 μ g latex or banana proteins, were used for each serum. The inhibitor solution was replaced with an equal volume of TBS in control experiments. The mixture was then added to the strips and blocking buffer containing 5% non-fat milk powder in TBS was added until the final dilution of the sera was 1:5. Strips were

then incubated overnight at +4 °C, following which the immunological detection of IgE binding was accomplished as described above.

To demonstrate the efficacy of the homologous antigen preparation in the immunoblot inhibition assay one serum (patient number 7) was tested also with inhibitor solutions containing the same proteins as were bound to the membrane. Four different concentrations of inhibitor solutions containing 0.1, 1, 10 and 100 μ g of inhibitor in one mL of final volume were used. To control possible non-specific inhibition four patient sera were also inhibited with a unrelated control protein (ovalbumin; Albumin Egg SIGMA n:o A-5503). Four concentrations of ovalbumin, containing 0.001, 0.01, 10 and 1000 μ g in 1 mL were used.

Banana skin-prick test and banana-RAST

Banana skin-prick test was performed with fresh and peeled banana by pricking first the banana and

Table 1. Main clinical manifestations and laboratory test results of 22 latex allergic patients and results of later performed banana skin-prick and RAST tests

Patient number	Sex (F/M)	Age (years)	Main clinical manifestations	Latex skin-prick test	Latex RAST (kU/L)	Serum total IgE (kU/L)	Banana skin-prick test	Banana RAST (kU/L)	Symptoms from banana
1	F	38	CU, E, FO, GU, A	4+*	20.0	396	2+*	3.9	+
2	F	65	E	3+	11.0	2442	2+	44.2	+
3	F	45	E	3+	21.0	2479	3+	3.6	+
4	F	39	CU, ES	3+	<0.4	256	0	0.4	—
5	F	49	CU, ES, FO, GU	3+	1.4	64	ND**	ND	NA***
6	F	47	CU, E	4+	5.0	797	2+	ND	—
7	M	31	CU	2+	2.2	17200	2+	18	+
8	F	37	AR	2+	3.3	641	2+	1.6	+
9	F	32	CU, E	4+	3.0	1842	3+	ND	+
10	F	43	CU, E, FO	4+	4.7	102	3+	0.7	+
11	M	32	CU	2+	<0.4	1890	0	0.9	—
12	F	37	CU, E	4+	18.0	7075	2+	ND	—
13	M	46	CU, E	3+	2.9	560	2+	1.8	+
14	F	32	CU, FO, GU, AR	3+	18.0	2867	0	0.7	+
15	F	32	CU, FO	4+	8.3	259	ND	ND	+
16	F	29	ES, FO	3+	3.4	82	0	ND	—
17	F	43	R	3+	<0.4	287	ND	<0.4	—
18	F	32	CU	3+	2.7	136	ND	ND	+
19	F	36	CU	2+	<0.4	12970	0	3.4	+
20	F	45	E, FO	3+	4.1	641	2+	1.6	+
21	F	11	E	3+	2.2	98	0	<0.4	—
22	M	12	FO	3+	2.2	110	3+	ND	+

CU. Contact urticaria. E. Eczema. ES. Eye symptoms. FO. Facial oedema. GU. Generalized urticaria. R. Rhinitis. A. Asthma. AR. Anaphylactic reaction. *skin-prick test scores 0–4+. **ND. Not done. ***NA. Information not available.

Table 2. Immunoblot cross-inhibition of IgE binding to natural rubber latex extract with banana proteins

Molecular weights of banana allergens (kDa)	Patient number 2				Patient number 3				Patient number 4				Patient number 7				Patient number 12			
	1*	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
150					++	++	-	-												
117					++	++	-	-												
78					++	++	-	-												
68					++	++	-	-												
57					++	++	-	-					+	-	-	-				
50													++	+	-	-				
47					++	+	-	-	++	++	-	-	++	+	-	-				
44					++	+	-	-	++	++	-	-								
39	++	++	++	++	++	++	-	-					+	+	-	-				
36	++	++	++	++	++	++	-	-					++	+	-	-	+	-	-	-
32					++	++	-	-					++	+	-	-	++	+	-	-
28													+	-	-	-				
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15													-	-	-	-				

1*. 100 µg banana inhibitor/mL. 2. 10 µg banana inhibitor/mL. 3. 1 µg banana inhibitor/mL. 4. 0.1 µg banana inhibitor/mL. -. No inhibition. +. Partial inhibition. ++. Total inhibition.

immediately after that the patients' skin with the same lancet (prick-prick method) [18]. A weal size equal to or greater than half of the weal size caused by histamine dihydrochloride (10 mg/mL) was considered positive. Physiological saline in 50% glycerol was used as a negative control.

For banana RAST, in-house discs were prepared as described by Ceska and Lundquist [19]. Paper discs were coupled with 50 µg of banana proteins. Standards from Pharmacia RAST kit were used for quantification. Values higher than 0.4 kU/L were considered positive.

Results

Immunoblotting and RAST to demonstrate IgE antibodies to NRL

Sera from all 22 patients who had previously shown IgE antibodies binding to NRL in immunoblotting gave positive immunoblots also in retesting. Of the 22 control sera one showed in immunoblotting IgE antibodies to latex, whereas all others were negative. The commercial latex RAST with cap method (Pharmacia, Uppsala, Sweden) was positive in 18 of the 22 patients. Seventeen of the 22 patients tested for total IgE had elevated levels. Main clinical manifestations and laboratory test results of the patients are given in Table 1.

Total protein staining and immunoblotting of banana extract

At least 25 protein bands, with apparent molecular weights ranging from 10 kDa to 150 kDa, were demonstrated in the BE by total protein staining. Of the 22 latex allergic patient sera tested, 10 (45%) demonstrated IgE antibodies binding to BE. IgE antibodies from the patient sera bound in a heterogeneous manner to 14 proteins in BE. The most frequently detected allergens, bound by at least half of the examined sera, were 23, 32, 36, 39 and 47 kDa proteins. One of the 22 control sera showed IgE binding to 32 kDa protein in BE.

Immunoblot inhibition

Inhibition of IgE binding to banana extract with natural rubber latex To examine crossreactivity between latex and banana allergens five patient sera (numbers 2, 3, 4, 7 and 12) showing strongest reactions to banana in immunoblotting were chosen for immunoblot inhibition studies. Using these five sera, 14 allergens were detected in BE and binding of IgE to 12 of these proteins (28, 32, 36, 39, 44, 47, 50, 57, 68, 78, 117 and 150 kDa) was inhibited by soluble NRL proteins. Decreased binding of IgE to BE was observed in all 5 patient sera (see Table 2). Immunoblot inhibition results for three patient sera are

Table 3. Immunoblot cross-inhibition of IgE binding to banana extract with natural rubber latex proteins

Molecular weights of latex allergens (kDa)	Patient number 2				Patient number 3				Patient number 4				Patient number 7				Patient number 12			
	1*	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
84																	++	+	-	-
75					++	++	-	-									-	-	-	-
59													++	+	-	-				
55					++	++	-	-	-	-	-	-	++	++	-	-				
54					++	++	-	-					-	-	-	-				
49					++	++	-	-	++	++	-	-								
47					++	++	-	-	++	++	-	-								
46					-	-	-	-												
44					++	++	-	-	-	-	-	-								
40													-	-	-	-				
30					+	+	-	-												
29													++	-	-	-				
20	-	-	-	-	-	-	-	-					+	-	-	-	-	-	-	-
14													++	-	-	-				

1*. 100 μ g latex inhibitor/mL. 2. 10 μ g latex inhibitor/mL. 3. 1 μ g latex inhibitor/mL. 4. 0.1 μ g latex inhibitor/mL. —. No inhibition. +. Partial inhibition. ++. Total inhibition.

shown in Fig. 1. An amount of 0.1 μ g of NRL proteins per mL in the incubation solution was able to totally inhibit IgE binding to 36 and 39 kDa proteins in one serum (number 2), whereas 10 μ g were required in another serum (number 3) and 100 μ g in a third serum (number 7). Ten microgram of latex proteins in 1 mL were sufficient to inhibit IgE binding to almost all BE proteins by two patient sera (numbers 3 and 4), but

100 μ g of latex proteins were required by two other patient sera (numbers 7 and 12). There was no detectable decrease in intensity at 15 and 23 kDa bands by any patient sera tested.

Inhibition of IgE binding to natural rubber latex extract with banana IgE antibodies from the same five patient sera used in banana inhibition studies bound to a total of

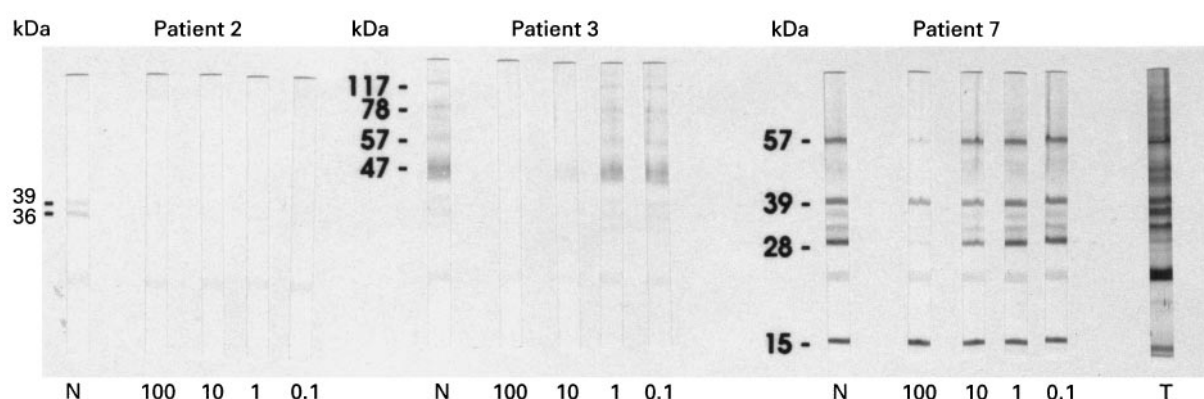


Fig. 1. Immunoblot inhibition analysis of IgE binding to natural rubber latex extract with soluble banana proteins in sera from three latex-allergic patients. Patient numbers are equal to those given in Table 1. Inhibitor concentrations (100, 10, 1 and 0.1 μ g/mL) are given below the patient column. The non-inhibited strip is denoted as N and the strip used for total protein staining as T. Molecular weights (kDa) of the allergens are indicated on the left side column of each patient.

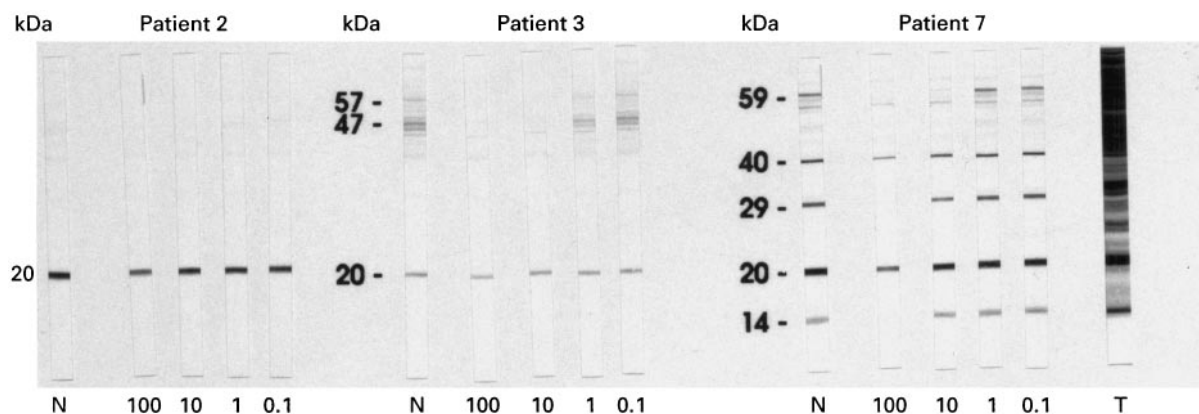


Fig. 2. Immunoblot inhibition analysis of IgE binding to banana extract with soluble latex proteins in sera from three latex-allergic patients. Inhibitor concentrations (100, 10, 1 and 0.1 $\mu\text{g/mL}$) are given below the patient column. The non-inhibited strip is denoted as N and the strip used for total protein staining as T.

14 proteins in NRL. BE proteins inhibited IgE binding to NRL in four of the five sera examined (Table 2). A decreased IgE binding was observed to 12 NRL proteins (14, 20, 29, 30, 44, 47, 49, 54, 55, 59, 75 and 84 kDa). The immunoblot inhibition results of three patient sera are demonstrated in Fig. 2. The amount of BE required for this inhibition varied from 10 μg to 100 μg in 1 mL final volume. There was no detectable decrease in intensity at 40 kDa band.

Inhibition experiments with homologous extracts One patient serum (number 7), showing strong IgE binding in immunoblotting to both BE and NRL extracts, was inhibited also with soluble proteins homologous to the

membrane bound proteins. Soluble banana proteins were able to inhibit binding of IgE to seven to nine BE proteins detected by this patient serum (Fig. 3). Ten microgram of BE per mL were sufficient to inhibit binding of IgE to 32, 36, 39, 47, 50 and 57 kDa BE proteins and 100 μg to 28 kDa banana protein. No inhibition was found at any inhibitor concentrations used in IgE binding to 15 and 23 kDa BE proteins.

When the patient serum was incubated with soluble latex proteins IgE binding was inhibited to all seven NRL proteins detected in this patient serum (Fig. 3). One microgram of NRL proteins per mL was sufficient to inhibit the binding of IgE to 14 and 20 kDa NRL proteins, 10 μg to 29 and 40 kDa proteins and 100 μg to 54, 55 and 59 kDa proteins.

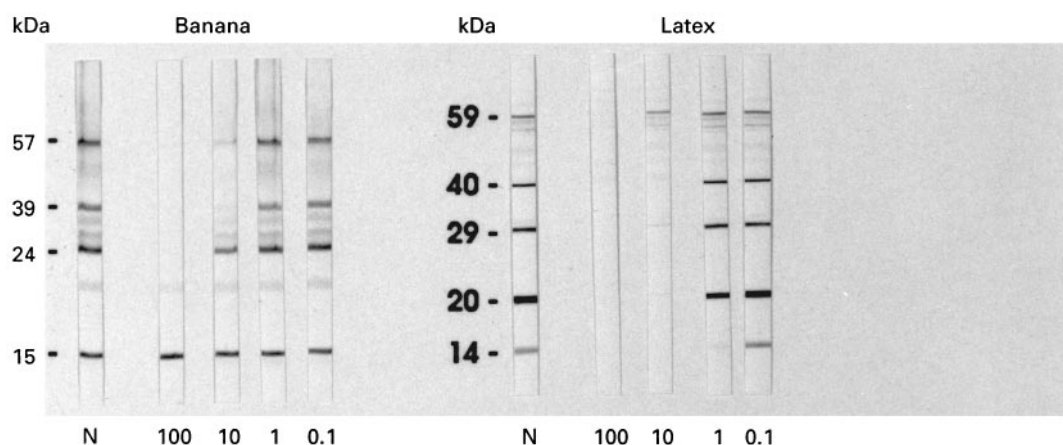


Fig. 3. Homologous inhibition of binding of IgE to solid phase latex with soluble latex proteins (latex) and to solid phase banana with soluble banana proteins (banana) in serum from a latex allergic patient (number 7). Inhibitor concentrations (100, 10, 1 and 0.1 $\mu\text{g/mL}$) are given below the patient column. The strip without inhibition is denoted as N.

To exclude possible non-specific inhibition the sera of four patients were examined also with an unrelated protein (ovalbumin). No inhibition of IgE binding to any of the NRL proteins was observed at any ovalbumin concentration (0.001, 0.1, 10 and 1000 μg in 1 mL final volume) used.

Banana skin-prick test and banana-RAST

Skin-prick test with banana extract, performed to 18 of the 22 latex allergic patients, was positive in 12 of the 18 (82%) latex allergic patients tested and banana-RAST in 12 of 14 patients tested (Table 1).

Discussion

Results of the present study using immunoblotting, RAST and immunoblot inhibition techniques for sera from 22 patients allergic to latex confirm the existence of crossreacting allergens in natural rubber latex and banana. A dose-dependent inhibition of the binding of IgE to BE with NRL was observed in all five patient sera chosen for immunoblot inhibition studies. In agreement with this the binding of IgE to NRL was inhibited by BE in four of the five patient sera. IgE antibodies from 10 of the 22 (45%) latex allergic patients sera bound in a heterogeneous manner to 14 proteins in BE. The most frequently detected allergens were 23, 32, 36, 39 and 47 kDa banana proteins.

In earlier studies [1–4,9] RAST-inhibition, enzyme linked immunosorbent assay (ELISA)-inhibition, immunospot-inhibition and immunoelectrophoretic methods have been used to examine the crossreactivity between latex and banana. In the study of M'Raihi *et al.* [1] using serum from a nurse allergic to latex and banana, banana extract inhibited in a dose-dependent manner the binding of IgE to latex RAST discs. According to the study of Ross *et al.* [4], all of the 12 banana RAST positive patient sera studied demonstrated in ELISA IgE antibodies to latex. However, none of the 12 latex RAST positive sera had IgE to banana in the same assay which result is in contrast to the present study. We found IgE antibodies to banana in 10 of the 22 latex allergic patient sera to by immunoblotting and 12 of 14 patient sera tested were positive in banana RAST. In the RAST inhibition study by Lavaud *et al.* [2] using serum from a patient with coexisting allergy to latex, banana and avocado, binding of IgE to banana and avocado discs was inhibited by latex extract, but no inhibition of IgE binding to latex was observed by avocado and banana extracts. In contrast to the negative results of Lavaud *et al.* [2], we demonstrated that soluble BE proteins inhibited IgE binding to 12 of 14 NRL proteins in four of the five

sera studied by immunoblot inhibition. The presence of structurally similar antigens and allergens in NRL and banana, demonstrated with electrophoretic methods, were recently reported [9].

Of 22 control sera tested one serum showed IgE binding to a 32 kDa protein in BE in immunoblotting. The same serum showed IgE antibodies also to 20 kDa protein in NRL but the patient was negative in both banana and latex skin-prick tests. This finding could be due to crossreacting IgE antibodies showing affinity both to 20 kDa protein in NRL and to 32 kDa protein in BE. In homologous inhibition experiments no inhibition of IgE binding was observed to 15 kDa and 23 kDa BE proteins. This could be due to differences in epitope conformation of proteins existing in soluble form in comparison to immobilization onto solid surface.

Results of the present study indicate that banana and NRL contain structurally similar allergens. In general, smaller amounts of NRL proteins (w/v) were sufficient to inhibit IgE binding to banana than *vice versa*. This phenomenon could be due to IgE antibodies having different affinities to banana and NRL proteins. However, since the protein compositions of the antigen extracts are heterogenic, purified proteins are needed for more detailed analysis of the sensitizing processes. It has been reported that latex-allergic patients may experience adverse reactions from banana [1,2,5,8,9]. Furthermore, the present study as well as our recent studies [9] showed that more than half of latex-allergic patients may have symptoms while eating or handling bananas. It is thus possible that these crossreacting structures in banana are responsible for causing the type I hypersensitivity reactions in latex-allergic patients. This contention is also supported by the observation that most latex-allergic patients in the present study had positive results in skin-prick tests to banana.

In conclusion, the present findings show that cross-reacting allergens and antigens can be found in banana and NRL by means of immunoblotting, RAST and immunoblot inhibition techniques. We detected in BE altogether 14 allergens of different molecular sizes which reacted with IgE antibodies in latex-allergic patient sera. NRL caused a dose dependent inhibition of IgE binding to BE and *vice versa*. Further studies are needed to settle whether an association exists between type I latex allergy and other, especially fruit and vegetable allergies.

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