

Banana allergy in patients with immediate-type hypersensitivity to natural rubber latex: Characterization of cross-reacting antibodies and allergens

Soili Mäkinen-Kiljunen, MSc Helsinki, Finland

Background: An association between allergy to latex and banana has been reported. Even though cross-reacting IgE antibodies have been demonstrated, in no study has the existence of structurally similar allergens been confirmed. In the present study banana allergy was studied in a large series of patients with latex allergy. Specific IgE antibodies were characterized for cross-reactivity and compared with pollen RAST results. Latex and banana extracts were investigated for common antigens and allergens.

Methods: Latex-, banana-, and pollen-specific (birch, timothy, mugwort) IgE were measured in 47 sera from patients with latex allergy. Thirty-one patients were skin prick tested with banana and questioned for possible reactions after eating bananas. Several RAST inhibition and immunospot inhibition studies were used to characterize cross-reacting IgE antibodies. Structurally similar antigens and allergens were evaluated with crossed-line immunoelectrophoresis and crossed-line radioimmunoelectrophoresis, respectively.

Results: Latex RAST results were positive in 31 (66%) and banana RAST results were positive in 26 (55%) of the 47 sera. Of the 31 latex RAST-positive sera, 25 (81%) were also banana RAST-positive. Results from latex RAST correlated significantly with results from banana RAST ($p < 0.001$), but not with those from pollen RAST ($p > 0.05$). Banana skin prick test results were positive in 11 (35%) of the 31 patients tested. Symptoms after eating bananas were reported by 16 (52%) of the 31 patients. In inhibition studies the binding of IgE antibodies to solid-phase banana and to several latex preparations was inhibited by latex and banana, respectively. In crossed-line immunoelectrophoresis at least one antigen from banana fused with an antigen from latex, which also bound IgE antibodies in autoradiography (crossed-line radioimmunoelectrophoresis).

Conclusions: Patients with latex allergy have symptoms caused by banana and show positive skin test and specific IgE test results. Cross-reacting IgE antibodies were confirmed by several inhibition techniques. For the first time, a structurally similar antigen/allergen was demonstrated. (*J ALLERGY CLIN IMMUNOL* 1994;93:990-6.)

Key words: Natural rubber, latex, banana, cross-reactivity, RAST inhibition, immunospot, crossed immunoelectrophoresis

The allergens in natural rubber latex (NRL) are proteins derived from the rubber tree (*Hevea brasiliensis*) and are present in NRL products used for both medical and consumer purposes. Surgical and

household gloves, enema kits, catheters, dentists' cofferdams, toy balloons, condoms, and baby pacifiers are reported to cause immediate reactions during mucosal or parenteral contact.¹⁻⁷ Reactions include contact and generalized urticaria, angioedema, rhinitis, conjunctivitis, asthma, anaphylaxis, and death.¹⁻⁶

Bananas and other fruits have been reported to elicit allergic reactions in patients with latex allergy, such as edema, collapse, urticaria, swelling of the hands, oral itching, rhinitis, cough, stridor, asthma, and anaphylaxis.⁸⁻¹⁴ In addition, subjects allergic to bananas have experienced adverse reactions after contact with latex products.¹⁴ Cross-reacting IgE antibodies have been demonstrated

From the Department of Dermatology and Allergic Diseases, Helsinki University Central Hospital, Helsinki, Finland.

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Reprint requests: Soili Mäkinen-Kiljunen, MSc, Helsinki University Central Hospital, Department of Dermatology and Allergic Diseases, Meilahdentie 2, FIN-00250 Helsinki, Finland.

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Abbreviations used

AL:	Ammoniated natural rubber latex (i.e., NRL in 0.6% ammonia)
B:	Banana extract
GE:	Latex glove extract
ISI:	Immunospot inhibition
NAL:	Nonammoniated natural rubber latex
NRL:	Natural rubber latex (includes all NRL products)
SPT:	Skin prick test

in the patients' sera,^{8-9, 12-13, 15} but the existence of common allergens in latex and bananas has not been confirmed.

The aim of the present study was to evaluate banana allergy by clinical history, skin prick test (SPT), and RAST in a larger group of patients with latex allergy. IgE antibodies were evaluated for their cross-reactivity and clustering with pollen IgE antibodies. Structurally similar antigens and allergens were evaluated with immunoelectrophoretic and autoradiographic methods. Some of the results have been published previously in abstract form.¹⁶

METHODS

Subjects

Sera from 47 patients with confirmed allergy to latex were used for the *in vitro* studies. The characteristics of the patients are given in Table I. All 47 patients had a positive SPT result to a latex glove eluate.¹⁷ A wheal equal to or greater than one half of the value of the histamine dihydrochloride (10 mg/ml) was considered a positive result. Saline solution in 50% glycerol was used as a negative control. Serum total IgE was determined by the CAP system (Pharmacia, Uppsala, Sweden).

Clinical history and skin prick testing with banana

Thirty-one of the 47 patients were questioned about possible symptoms after eating bananas and were skin prick tested with a freshly peeled banana by pricking first the banana and then immediately the patient's skin with the same lancet (prick-prick technique).¹⁸ Criteria for positivity were the same as those for SPT with glove eluate. Sixty-eight consecutive patients suspected of having food allergy (but not to banana) were also tested with banana and served as control subjects.

Latex proteins

Liquid-form nonammoniated natural rubber latex (NAL1 and NAL2) and ammoniated latex (AL1) from frozen field latex, as well as a freeze-dried preparation

TABLE I. Characteristics of patients with latex allergy

Total no.	47
Adults/Children	44/3
Male/Female	8/39
Atopic (%)	60
Positive skin test response to latex (%)	100
Serum total IgE (median)*	256
Symptoms caused by latex (%)	
Contact urticaria	54
Pruritus	48
Eczema	46
Erythema	39
Edema	24
Eye symptoms	11
Rhinitis	11
Generalized urticaria	11
Anaphylaxis	11
Asthma	7

*Range is 4 to 17,200 kU/L.

from ammoniated latex (AL2) were prepared as previously described.^{19, 20} The protein contents of NAL1, NAL2, and AL1 were 12.6, 3.1, and 18.1 mg/ml, respectively, and that of AL2 was 141 µg/mg.

A freeze-dried glove extract (GE1) from a brand of latex gloves (Exona; Semperit, Vienna, Austria) was prepared as described previously.² The protein content of GE1 was 140 µg/mg. In addition, an extract (1:5 wt/vol) from the same glove brand (GE2) was prepared; the protein content of this extract was 32 µg/ml.

An experimental latex skin prick test extract (1:100 wt/vol) obtained from ALK (Horsholm, Denmark) was used in immunospot studies.

Banana extracts

Banana extract was prepared from imported ripe bananas by extraction of the peeled fruit (1:5 wt/vol) with 0.1 mol/L potassium phosphate, pH 7.0. The protein content of banana extract 1 (B1) was 260 µg/ml. An aliquot (B1) was stored frozen at -20° C; the rest was dialyzed against 20 mmol/L ammonium bicarbonate for 3 days with a dialysis tube with a cutoff of 3.5 kd (Spectapor; Spectrum Medical Industries, Inc., Los Angeles, Calif.) and finally freeze-dried (banana extract 2 [B2]). The protein content of the freeze-dried banana powder was 100 µg/mg.

For the analyses, a stock solution of 10 mg of the dry weight per milliliter in phosphate buffer (0.1 mol/L, pH 7.4) was prepared from the lyophilized powders of AL2, GE1, and B2.

Allergen-specific IgE

Specific IgE to latex and specific IgE to pollens of birch, timothy, and mugwort were measured in the 47 sera with a commercial CAP RAST system (Pharma-

TABLE II. Clinical history and laboratory results of six patients allergic to latex

Patient No.	Serum total IgE (kU/L)	Symptoms caused by		Skin prick test*		Specific IgE (RAST, kU/L)†	
		Latex	Banana	Latex	Banana	Latex	Banana
1	73	A,R	Aw	4+	3+	11.0	4.3
2	520	R,E	I,AE	4+	3+	7.0	1.4
3	ND	U	I	3+	3+	5.0	3.8
4	15000	E,I,U	I	2+	2+	1.6	18.0
5	460	A,E,AE,U	G,I,AE	4+	2+	14.0	3.9
6	330	I,AE	AE,U	4+	ND	7.2	0.4

A, Asthma; R, rhinitis; Aw, worsening of asthma; E, eczema; I, itching (oral/eyes/ears); AE, angioedema; ND, not done; U, urticaria; G, gastrointestinal disorders.

*Results compared with histamine, 10 mg/ml; 4+, wheal size twice that of histamine; 3+, wheal size same as histamine; 2+, wheal size half of histamine.

†Positive result >0.35 kU/L.

cia). Latex ImmunoCaps (Pharmacia) were also used in inhibition studies.

For banana RAST, in-house discs were prepared according to the method of Ceska and Lundkvist.²¹ Paper discs were coupled with 50 µg of the banana protein. Standards from the Pharmacia RAST kit were used for quantitation. Values higher than 0.35 kU/L were considered positive. Banana discs were also used in inhibition studies. As a control, banana-specific IgE was measured in 42 atopic sera; most sera were from subjects allergic to pollen with no suspected allergy to latex. The mean reproducibility of the RAST test is 8.3%, expressed as a coefficient of variation from 55 determinations.

RAST inhibition

RAST inhibition studies were performed as previously described.²² Sera from six patients with latex allergy were pooled and used as a source for specific IgE. Inhibition of latex RAST was carried out with stock solution and several dilutions of the banana extract (B2). Similarly, inhibition of banana RAST was performed with NAL1 as inhibitor. Inhibition studies were also performed on the six individual sera with stock solutions of banana (B2) and latex (NAL1). In addition to NAL1, stock solutions from NAL2, AL1, AL2, and GE2 were used to inhibit banana RAST in the serum from a nonatopic patient who was allergic to latex and had symptoms caused by banana (patient 1 in Table II) and in the serum from an atopic child with allergy to pollens and banana but not to latex.

Extracts from timothy pollen and mite (*Dermatophagoides farinae*) (Soluprick, ALK) were used as control inhibitors, and latex and banana RAST-negative but mite RAST-positive serum was used as a control serum for nonspecific inhibition with latex and banana. The reproducibility of the inhibition assay is 22%, expressed as the coefficient of variation from 55 assays.

Immunospot and immunospot inhibition

To study further IgE binding and inhibition of IgE binding to several banana and latex preparations simul-

taneously, immunospot and immunospot inhibition (ISI) tests were developed.

Three microliters of the stock solution and one dilution (1:10 vol/vol) were applied to four nitrocellulose sheets (0.45 µm) (Millipore, Bedford, Mass.). The samples comprised banana extracts B1 and B2 and NRL samples AL2, latex skin test extract, GE2, NAL1, NAL2, AL1, and GE1. Skin test extract from dog dander (Soluprick) was used as a control for nonspecific inhibition. Free binding sites were blocked with 0.5% Tween 20 in 0.1 mol/L potassium phosphate buffer, pH 7.4, for 1 hour at room temperature. After washings, each sheet was incubated with preincubated pooled serum from patients with latex allergy. Four aliquots of the serum (300 µl each) were incubated for 1 hour at room temperature with 300 µl of a buffer (noninhibited immunospot), with 300 µg of banana protein (banana ISI), with 3800 µg of NAL1 protein (latex ISI), and with 300 µl of the dog dander extract (control ISI), respectively. After an overnight incubation at 4° C, sheets were washed and incubated for another night with iodine 125-labeled anti-IgE (Pharmacia) as described previously.²⁰ After exposure for 1 to 20 days at -40° C, x-ray films were developed, and IgE binding and inhibition of binding were evaluated.

Electrophoretic methods

To demonstrate the presence of cross-reacting antigens and allergens in latex and banana, crossed-line immunoelectrophoresis and crossed-line radioimmuno-electrophoresis were performed.²⁰ Forty-seven micrograms of NAL1 protein was applied to the sample well, and 100 µg of banana protein was mixed with agarose on the lower intermediate gel. The upper intermediate gel was plain agarose as were both intermediate gels on the control plate in NRL crossed immunoelectrophoresis. Rabbit antiserum against a latex glove extract (GE1) was used as a precipitating antibody in the upper gel (3.3 µl/cm²).²⁰ Banana antigens and allergens precipitable with the rabbit anti-latex serum were studied with 15 µg of banana protein as a sample in banana

crossed immunoelectrophoresis and crossed radioimmuno-electrophoresis, respectively.

To demonstrate allergens by IgE binding, autoradiography was performed for crossed radioimmuno-electrophoresis and crossed-line radioimmuno-electrophoresis in a manner identical to that described for immunospot.

Statistics

Statistical analysis was performed with the Medstat program (The Astra Group A/S, Denmark). To calculate the rank correlation coefficient, the nonparametric Spearman test was used. The significance of the correlation coefficient was calculated by the chi square test. The significance of the correlation of positive and negative results in SPT and RAST was calculated in the contingency table by Fisher's exact test. Values of p less than 0.05 were considered significant.

RESULTS

Banana SPT and symptoms from banana

Results of SPT with fresh banana were positive in 11 of 31 (35%) patients with latex allergy tested. Of the 68 control subjects, four (6%) had positive results. The difference between the groups was highly significant ($p < 0.001$).

Symptoms after eating bananas were reported by 16 of 31 (52%) patients interviewed. The correlation between symptoms and banana SPT results was significant ($p < 0.001$). The clinical histories and laboratory findings of six patients whose sera were used in RAST inhibition studies are collected in Table II as examples. In addition to these, one patient experienced anaphylaxis caused by banana, and another experienced worsening of eczema. Information on possible symptoms caused by banana among the 68 control subjects was not available.

Allergen-specific IgE

Of the 47 sera from patients allergic to NRL, 31 (66%) were latex RAST-positive and 26 (55%) were banana RAST-positive. Further, of the 31 latex-positive sera, 25 (81%) were banana RAST-positive, whereas of the 16 latex RAST-negative sera, only one (6%) was banana RAST-positive. The correlation between the latex and banana RAST results was highly significant ($p < 0.001$). The correlation between the quantities of latex-specific and banana-specific IgE in the 47 sera is illustrated in Fig. 1.

The results from banana RAST correlated with those of banana SPT ($p < 0.01$) and with symptoms caused by banana ($p < 0.001$) but did not correlate with RAST results to birch, timothy, or mugwort ($p > 0.05$).

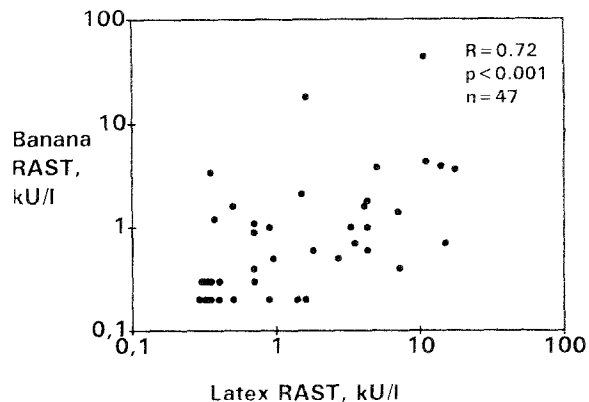


FIG. 1. Correlation of the quantity of IgE in latex and banana RAST.

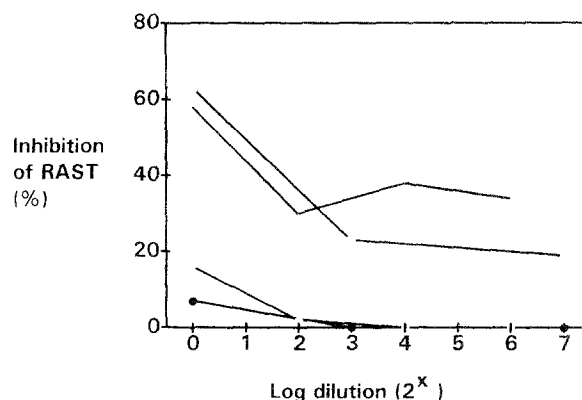


FIG. 2. Inhibition of banana RAST by latex (—) and latex RAST by banana (—□—) in pooled serum from patients with latex allergy. Inhibition of latex RAST by timothy (—●—) and banana RAST by mite (—◇—) were used as controls.

Among the 42 atopic control subjects, IgE reactivity to banana was detectable in six (28%) sera. The difference between the patient group and the control group was significant ($p < 0.05$). In the pollen-allergic control group, banana RAST results did not correlate with RAST results to birch, timothy, or mugwort ($p > 0.05$).

RAST inhibition

Inhibition curves of latex RAST by banana and that of banana RAST by latex (NAL1) with a pooled serum are shown in Fig. 2. Single-point inhibitions of latex RAST by banana and banana RAST by latex in six individual sera are presented in Table III.

There was no significant inhibition by the control extracts (mite, 16%; timothy, 8%), nor did latex (8%) or banana (10%) inhibit mite RAST in the control serum.

The results of single-point inhibition of banana RAST in the serum from a nonatopic patient with

TABLE III. Latex RAST inhibition by banana and banana RAST inhibition by latex in sera of six patients with latex allergy

Serum no.	Inhibition (%)	
	Latex RAST by banana	Banana RAST by latex
1	18	78
2	41	50
3	53	82
4	0	19
5	43	45
6	14	0

Inhibitions greater than 20% are considered significant.

latex allergy and in the serum from a child with banana allergy, when various latex preparations were used as inhibitors, are presented in Table IV.

Immunospot and ISI

Results of immunospot and ISI are shown in Fig. 3. In immunospot, IgE antibodies were bound to banana and to all latex preparations; the strongest binding was to NAL1, and the weakest was to glove extracts.

In ISI the inhibition of the IgE binding was more efficient with NAL1 than with banana. IgE binding to latex or banana was not inhibited by the control extract.

Immunoelectrophoretic studies

Latex (NAL1) and banana antigens were precipitated with rabbit antibodies to latex in CIE and in CLIE. In addition, the presence of allergens was demonstrated with a pooled serum after autoradiography in crossed radioimmuno-electrophoresis and in crossed-line radioimmuno-electrophoresis (Fig. 4). At least one antigen from NAL1, which was also an allergen, fused with an antigen/allergen from banana.

DISCUSSION

Several methods were used to scrutinize the reported cross-reactivity between latex and banana. Banana allergy in patients with latex allergy was evaluated by clinical history, skin testing, and measurement of specific IgE antibodies. Several inhibition studies were used to characterize cross-reacting IgE antibodies, together with immunoelectrophoretic methods to demonstrate common antigen(s), followed by autoradiography to confirm the allergenicity by IgE binding.

TABLE IV. Banana RAST inhibition by latex preparations in serum from a patient with latex allergy and in serum from a child with banana allergy

Latex inhibitor	Inhibition (%) of banana RAST in serum	
	Adult with latex allergy	Child with banana allergy
NAL1	78	49
NAL2	79	30
AL1	80	19
AL2	80	4
GE2	77	0

Commercial latex RAST was positive in 66% of the 47 sera, which agrees with a previous finding.¹⁷ The number of positive banana RAST results was surprisingly high, 55% of all sera and 81% of latex RAST-positive sera. Ross et al.¹⁵ did not find banana IgE in sera from 12 patients with latex sensitivity. They used an ELISA method in which polystyrene was used as the solid phase for banana allergens. Moreover, they analyzed sera from patients with histories of anaphylaxis to latex, whereas only five of our patients had experienced an anaphylactic reaction to latex; the others had had rather mild symptoms (Table I).

About half (52%) of the 31 patients interviewed reported symptoms caused by bananas, ranging from mild reactions such as gastrointestinal disorders, itching, and swelling to one episode of severe anaphylaxis. In addition, one patient described the disappearance of rhinitis after he had stopped eating bananas. It is obvious that patients with latex allergy may have symptoms caused by bananas and other fruits. However, the clinical significance of fruit avoidance in relation to latex allergy remains open.

In the present study the correlation of latex IgE antibodies with banana IgE was significant ($p < 0.001$), whereas latex RAST results did not correlate with RAST results to birch, mugwort, or timothy pollens ($p > 0.05$), which is in accord with the reported clinical finding.²³

In several inhibition studies (RAST inhibition, ISI) both latex and banana showed distinct inhibitions (Figs. 2 and 3, Tables III and IV). To my knowledge, this is the first time that immunospot and ISI have been used to characterize specific and cross-reacting IgE antibodies. The more efficient inhibition with NAL compared with GE may

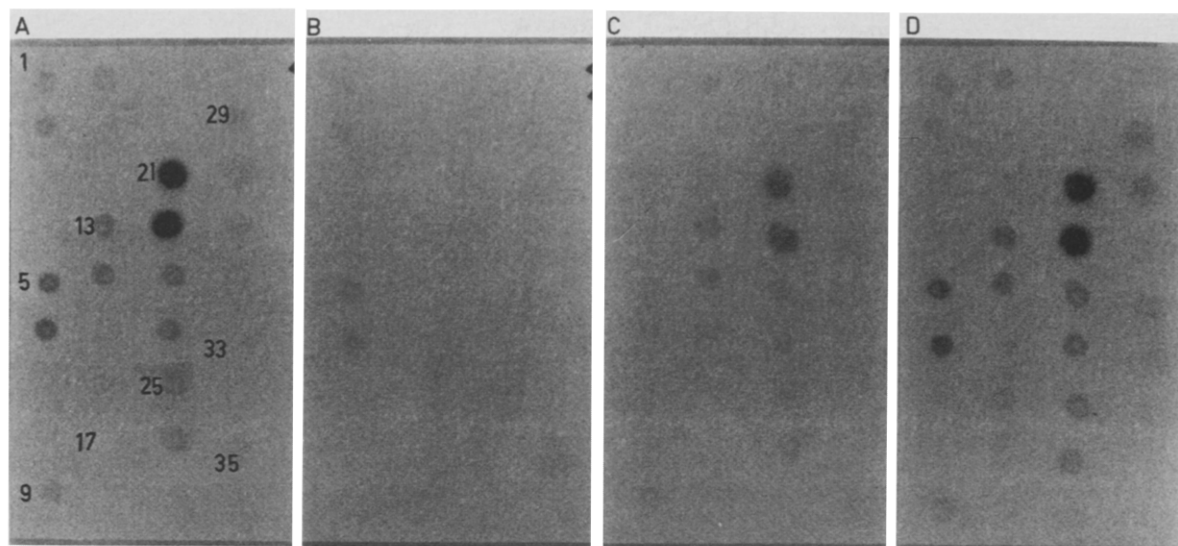


FIG. 3. Immunospot and immunospot inhibition after autoradiography. Replicates of undiluted samples and a 10-fold dilution were applied on each sheet. Samples comprised 1-4, B1; 5-8, B2; 9-12, AL2; 13-16, latex skin test extract; 17-20, GE₂; 21-24, NAL1; 25-28, NAL2; 29-32, AL1; 33-34, GE1 (1:1 only); and 35-36, dog dander extract as control (1:1 only). A pool of sera from patients with latex allergy was preincubated with buffer (A), NAL1 (B), banana (C), and dog dander extract (D).

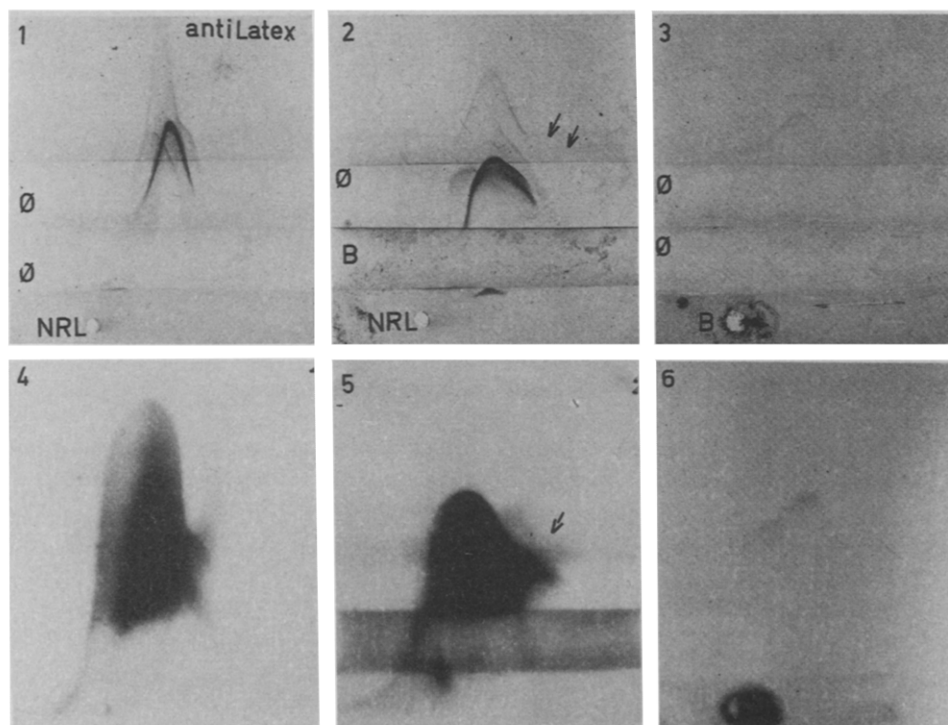


FIG. 4. Crossed immunoelectrophoresis (1 and 3), crossed radioimmuno-electrophoresis (4 and 6), crossed-line immunoelectrophoresis (2), and crossed-line radioimmuno-electrophoresis (5) of latex and banana. Latex proteins (47 μ g) in the sample well were precipitated with rabbit antibodies to latex in the upper gel (3.3 μ l/cm²) without addition to the intermediate gels (1,4) and with an addition of 100 μ g banana (B) proteins (2,5) in the lower intermediate gel. Cross-reacting antigens and allergens are indicated with an arrow. In plates 3 and 6, 15 μ g of banana protein was run into rabbit anti-latex serum-containing gel with no addition to the intermediate gels.

result not only from the different allergen concentration, but also from the different form of allergens; native allergens in NAL may inhibit banana allergens more efficiently than modified allergens in GE because of rubber processing.^{20, 24} Similar latex RAST inhibition results with banana have been reported,^{8, 15} but negative results have also been obtained.⁹ However, in no study has the inhibition been complete. Nonspecific inhibition, (e.g., by lectins in latex and banana), was excluded in control studies.

Our previous immunoelectrophoretic studies have demonstrated that latex contains at least 10 antigens, six of which bind human IgE antibodies in CRIE.²⁰ In the present study at least one of the NAL allergens, albeit faintly, fused with an allergen from banana, which confirms the presence of structurally similar allergens in both extracts (Fig. 4). However, the pattern of cross-reacting allergens may be more complicated, because rabbit IgG and human IgE do not necessarily recognize the same epitopes.

The methods used in this study did not allow the determination of the molecular weight of the cross-reacting allergens. Banana allergens have not been characterized, but our preliminary results suggest several cross-reacting allergens in latex and banana (manuscript submitted).²⁵

In conclusion, about half of patients with latex allergy have symptoms from bananas and show positive results in banana skin testing and/or in banana RAST. Cross-reactivity of the IgE antibodies is demonstrated in several inhibition assays, and the existence of cross-reacting antigen and allergen is confirmed by immunoelectrophoretic and autoradiographic analyses. However, the stimulus for the cross-reacting antibodies remains unknown.

Because severe allergic reactions are known to be caused by latex and bananas, patients allergic to either of these allergens should be interviewed, and when necessary, also tested with the cross-reacting allergen to avoid unexpected reactions when in contact with the allergen.

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