

Hevein-like protein domains as a possible cause for allergen cross-reactivity between latex and banana

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Background: Patients allergic to natural rubber latex (NRL) frequently exhibit immediate hypersensitivity reactions to banana, but the molecular basis of these putative cross-reactions is unknown.

Objective: We wanted to examine whether proteins resembling hevein, a major NRL allergen, exist in banana and whether coexisting allergy to NRL and banana can be explained by IgE cross-reactivity to these proteins.

Methods: Allergens in banana cross-reacting with hevein were identified by IgE immunoblot inhibition. The cross-reacting proteins were purified by reversed-phase chromatography and characterized by amino acid sequencing. Allergen cross-reactivity was further assessed by IgE ELISA, IgE ELISA inhibition, and skin prick testing.

Results: In immunoblotting, 9 of 15 sera from patients allergic to NRL with IgE to hevein showed IgE binding to 32- and 33-kd banana proteins. These 2 protein bands were also targets to IgG from a hevein-immunized rabbit. IgE binding to both 32- and 33-kd protein bands was totally inhibited by hevein (10 ng/mL) in all 5 sera from patients allergic to NRL sera studied. N-terminal sequencing of the purified 32- and 33-kd proteins revealed 80% identity to the N-terminus of hevein. An internal peptide (19 amino acids) of the 33-kd protein gave over 90% identity to endochitinases of several plants. In ELISA all 15 sera from patients allergic to NRL had IgE to the purified 32-kd banana protein. In ELISA inhibition hevein (10 ng/mL) inhibited approximately 50% of IgE binding to the solid-phase 32-kd banana protein in a pool of sera from patients allergic to NRL.

Conclusion: These results suggest that the commonly occurring hypersensitivity to banana in patients allergic to NRL could be explained by cross-reacting IgE antibodies binding to epitopes in hevein and in a hevein-like domain of a previously undescribed endochitinase in banana. (*J Allergy Clin Immunol* 1998;102:1005-12.)

Key words: Latex allergy, IgE cross-reactivity, banana (*Musa acuminata*), hevein, Hev b 6.02, chitinases

Abbreviations used

ACN:	Acetonitrile
FCA:	Freund's complete adjuvant
HSA:	Human serum albumin
NRL:	Natural rubber latex
PBS-T-HSA:	PBS containing 0.02% Tween 20 and 0.2% HSA
TBS:	Tris-buffered saline
TFA:	Trifluoroacetic acid

Cross-reactive allergens are believed to be responsible for the clustering of food allergies in patients hypersensitive to various fruits and vegetables and frequently, at the same time, to various pollens.¹ The prevailing hypothesis is that allergen cross-reactivity is due to IgE antibodies that recognize structurally similar epitopes on different proteins that are phylogenetically closely related or represent evolutionary conserved structures. As a consequence of such cross-reactivity, persons may experience hypersensitivity reactions from certain allergens without previous sensitization.² Well-known examples of cross-reactive structures are profilins, highly conserved actin-binding proteins in almost all eukaryotic organisms.³ Approximately 20% of all patients allergic to birch pollen seem to be cross-sensitized to profilins in taxonomically nonrelated tree, grass, and weed pollens.^{4,5}

Allergy to natural rubber latex (NRL) proteins or peptides is a major occupational problem among glove-wearing health care workers.⁶ Also, children with spina bifida or other congenital malformations requiring repeated surgical operations and thereby frequent contacts with NRL-containing medical devices are at high risk of having NRL allergy.⁷⁻⁹ In addition to NRL products used in the medical field, persons can also become sensitized to non-medical NRL products, such as dummies, balloons, condoms, and household gloves.¹⁰⁻¹³ It has been observed that about 20% to 50% of patients allergic to NRL have experienced symptoms after eating banana.^{12,14} Evidence for cross-reacting-allergens in NRL and banana has been reported, but the molecular basis for these reactions has remained unexplained.^{12,15-19}

We²⁰ and others^{21,22} have previously shown that hevein (Hev b 6.02), the 43 amino acid chitin-binding domain of prohevein (Hev b 6.01), is a major IgE-binding allergen for patients allergic to NRL. In this study we examined whether hevein-like proteins are found in banana and whether such proteins can explain allergen cross-reactivity between NRL and banana.

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METHODS

Patient and control sera

Serum specimens were obtained from 15 patients allergic to latex (12 women and 3 men; mean age, 33 years; previously shown to have IgE against hevein), including 13 adult health care workers and 2 children, of whom 1 had spina bifida and a history of multiple surgeries. Skin prick test responses to banana, performed as described previously,²³ were positive in 10 of 15 patients. Sera from 42 control subjects without NRL allergy (27 women and 17 men; mean age, 30 years) were used in ELISA studies, and 5 of these sera, all from atopic individuals, were used as controls in immunoblotting. Four individual patient sera exhibiting IgE binding to hevein and a reference serum pool from 6 patients allergic to NRL showing elevated levels of IgE to several NRL allergens²⁴ were used in ELISA-inhibition studies. All patients had positive skin prick test responses and control subjects had negative responses to an NRL extract prepared as previously described.²⁵

Extraction and purification of 32- and 33-kD banana proteins and hevein

For extracting banana proteins, pieces of fresh banana were homogenized in a Waring blender for 3 minutes at room temperature and mixed 1/5 (wt/vol) with 30% acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA). The homogenate was stirred overnight at 4°C and centrifuged at 5000g for 30 minutes, and the supernatant was filtered through a 0.45- μ m membrane (Millex HV; Millipore, Molsheim, France), concentrated 10-fold under vacuum, and filtered again through a 0.45- μ m membrane (Millex HV). To remove pectin, the preparation was chromatographed in an HPLC column (ReSource RPC, 3 mL; Pharmacia, Uppsala, Sweden) by using a linear gradient of ACN (0% to 100% in 15 minutes), and the eluting protein or peptide peaks, except the exclusion peak, were pooled and used in immunoblotting or subjected to further purification steps.

Banana proteins were purified from the banana extract by reversed phase chromatography on a ReSource RPC 3 mL column by using a linear gradient of ACN (20% to 60% in 60 minutes) in 0.1% TFA at a flow rate of 1 mL/min. Elution was monitored at 280 nm, and fractions were collected and analyzed by SDS-PAGE. The fractions containing the 32- and 33-kD proteins recognized by a hevein-immunized rabbit serum (see below) were electrophoresed and blotted to polyvinylidene difluoride membrane, and the corresponding bands were subjected to N-terminal sequencing. The 32-kD protein, purified to apparent homogeneity, was used for ELISA, ELISA-inhibition, and skin prick tests.

Hevein was purified from NRL gloves by gel filtration and reversed phase chromatography as described earlier.²⁰

Production of antihevein antibodies in rabbits

Young adult New Zealand White rabbits received 3 sequential injections of highly purified hevein.²⁰ Ten days before the immunization, rabbits were primed with subcutaneous injection of Freund's complete adjuvant (FCA) in saline (0.1 mL of a mixture containing equal parts FCA and physiologic saline) administered into the dorsum of the metatarsal area of the rear leg. The antigen injections contained 30 μ g hevein in 100 μ L PBS. The first injection was given in the popliteal lymph node as a mixture containing equal volumes of the antigen and FCA, and later injections were given at 2 week intervals without adjuvant. Serum collected 2 weeks after the third injection contained high concentrations of hevein-specific IgG antibodies, and serum from 1 rabbit was chosen to be used in immunologic recognition of hevein-like structures. The immunization protocol was approved by the Institutional Animal Care and Use Committee of the National Public Health Institute.

Immunoblotting

A method described previously in detail was used.²⁰ Optimal antigen concentrations used in immunoblotting were determined by SDS-PAGE. Antigen samples were diluted 1:3 in electrophoresis buffer containing 2% SDS and 5% 2- β -mercaptoethanol and kept in boiling water for 2 minutes. The dissolved samples, 45 μ L/cm gel, were applied to 13% acrylamide gels containing 0.1% SDS by using a 4% stacking gel. Electrophoresis was carried out for 45 minutes at a constant voltage of 200 V (Mini Protean II Cell; Bio-Rad, Richmond, California). After electrophoresis, the proteins were transferred from the gel to 0.45- μ m pore size nitrocellulose filters (Trans-Blot, Bio-Rad) in an electroblotting buffer containing 25 mmol Tris-HCl, 150 mmol glycine, and 20% methanol (pH 8.3) for 1 hour 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 3 times for 5 minutes in Tris-buffered saline (TBS) containing 0.1% Tween, and incubated for 1 hour in a blocking buffer containing 5% nonfat milk powder in TBS.

Sera from latex-sensitive individuals and control subjects, diluted 1:5 in the blocking buffer, were added, and the strips were incubated overnight at 4°C with continuous shaking. The strips were then washed 3 times with TBS-Tween and once with TBS for 5 minutes. Then biotinylated goat anti-human IgE antibody (Vector, Burlingame, Calif) diluted 1:1000 in the blocking buffer was added, and the strips were incubated for 30 minutes at room temperature. After washing 4 times as before, an additional blocking was carried out for 5 minutes with the blocking buffer, and the strips were again washed for 5 minutes. Streptavidin-conjugated alkaline phosphatase (Bio-Rad) diluted 1:24,000 in TBS was added, and the strips were incubated for 30 minutes at room temperature. The strips were again washed and then placed in a color development solution (Bio-Rad). The reaction was stopped after 8 minutes by rinsing in tap water.

Immunoblot inhibition

The antigen preparations were electrophoresed, and the proteins were transferred to nitrocellulose membrane as described above. One hundred fifty microliters of serum from latex-sensitive individuals was incubated for 2 hours at room temperature with an equal volume of inhibitor solution. Three different concentrations of inhibitor, containing (in 1 mL final volume) 1 pg, 1 ng, and 1 μ g hevein, 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% nonfat milk powder in TBS until the final dilution of the sera was 1:5. Strips were then incubated overnight at 4°C, after which the immunologic detection of IgE binding was accomplished as described above.

IgE-ELISA

ELISA was performed as described earlier.²⁰ Purified proteins (hevein or the 32-kD banana protein) diluted in 50 mmol/L carbonate buffer to a concentration of 2 μ g/mL were applied onto polystyrene microtiter plates (100 μ L/well; Nunc, Roskilde, Denmark) and incubated for 3 hours at room temperature and overnight at 4°C. The wells were then emptied, and the remaining protein binding sites were blocked for 1 hour with carbonate buffer containing 1% human serum albumin (HSA). After rinsing 3 times, 100 μ L serum from latex-sensitive individuals and control subjects, diluted 1/20 with PBS containing 0.02% Tween 20 and 0.2% HSA (PBS-T-HSA), were added to the wells and incubated for 2 hours at room temperature. The wells were then again washed, and biotinylated goat anti-human IgE (Vector; diluted 1/1000 with PBS-T-HSA) was

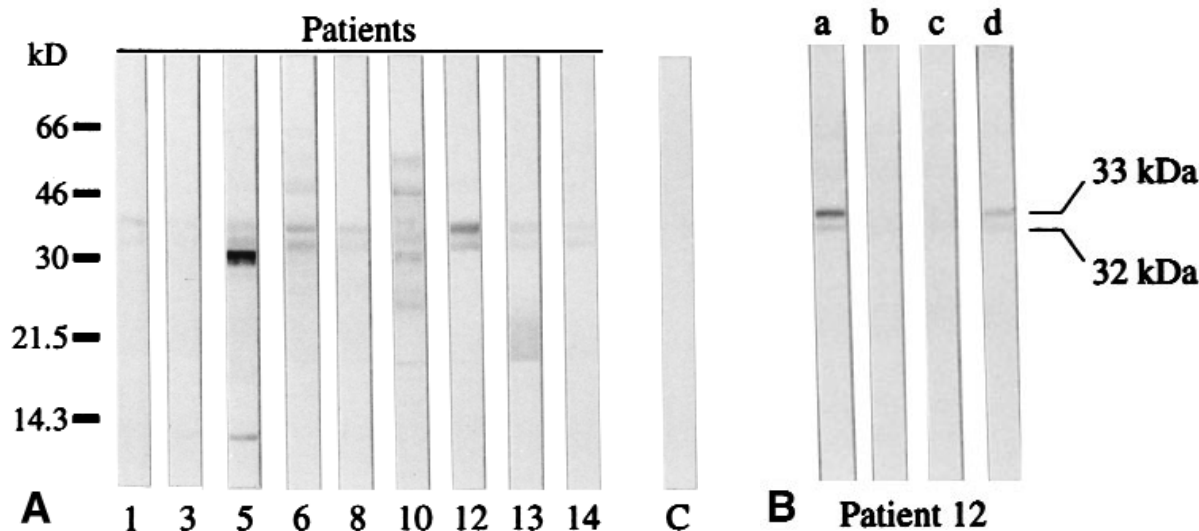


FIG 1. Immunoblot analysis of binding of IgE antibodies to banana extract. **A**, Immunoblotting shows IgE binding to 32- and 33-kD banana protein bands from sera of selected patients allergic to NRL. Sera from 1 atopic control subject remains negative (*lane C*). **B**, Inhibition of IgE binding to 32- and 33-kD banana proteins in serum from 1 patient allergic to NRL by different concentrations of hevein. Concentrations of hevein used for inhibition are as follows: *lane a*, no inhibitor; *lane b*, 1 µg/mL; *lane c*, 1 ng/mL; and *lane d*, 1 pg/mL.

added and incubated for 1 hour. Wells were again washed, and streptavidin-conjugated alkaline phosphatase (Bio-Rad; diluted 1/3000) was incubated for 1 hour. After washing, substrate (p-nitrophenyl phosphate; Sigma) was added, and the color developed was read at 405 nm by using an automated ELISA reader (Titertek Multiskan, Eflab, Finland). The mean OD value plus 3 SD of the controls was chosen as the threshold value for positivity.

ELISA inhibition

The assay was performed according to the protocol used in ELISA as described above, except that a competitive inhibition step was included. Inhibition was performed by incubating 50 µL of 4 individual sera diluted 1/10 in PBS-T-HSA for 1 hour at room temperature with an equal volume of inhibitor solution. Two or 6 different concentrations of hevein or 32-kD banana protein (protein concentrations of 10 and 1000 ng/mL or 0.01, 0.1, 1, 10, 100, and 1000 ng/mL in PBS-T-HSA) were used for inhibition. The inhibitor solution was replaced with an equal volume of PBS-T-HSA in control experiments. The mixture containing the serum and the inhibitor (or serum without inhibitor) was then added to the wells and incubated for 2 hours at room temperature. Immunologic determination of the allergen-bound IgE was performed as described above.

In ELISA the ability of purified hevein to inhibit the binding of IgE to a mixture of heterogeneous banana proteins was examined to evaluate the significance of hevein as a cross-reacting allergen. Serum pool obtained from 6 patients allergic to NRL, showing elevated levels of IgE to NRL extract in ELISA,²⁰ served as the source of IgE antibodies. Coating was performed with banana extract prepared as described above with the protein concentration adjusted to 20 µg/mL (optimized in preliminary checkerboard titrations). Six different concentrations of hevein (protein concentrations of 0.01, 0.1, 1, 10, 100, and 1000 ng/mL) were used for inhibition. For comparison, several concentrations of purified 32-kD banana allergen (protein concentrations of 0.01, 0.1, 1, 10, 100, and 1000 ng/mL) were also used for inhibition. ELISA-inhibition was performed as described above with 50 µL serum pool.

Tryptic cleavage and peptide separation, amino acid sequencing, and amino acid sequence comparison

In-gel tryptic digestion of the 33-kD protein was performed after SDS-PAGE separation as described by Hellman et al.²⁶ However, the gel piece was dried in a vacuum centrifuge instead of drying under a stream of nitrogen. The extracted peptides were separated by reversed phase chromatography on a Rexchrom C8 (5 µm, 300 Å, Regis Chemical Company) column on a Smart system (Pharmacia) by using a linear gradient of ACN (0% to 40% in 160 minutes) in 0.1% TFA, and the peptides were automatically collected and, after concentration, subjected to amino acid sequencing. N-terminal amino acid sequencing of the 32- and 33-kD proteins and 1 internal peptide from the 33-kD protein was performed on ABI 494 A Procise Sequencer (Applied Biosystems, Foster City, Calif).

Obtained amino acid sequences were compared by a computer program (FastA, Wisconsin Package Version 8.0 -OpenVMS, Genetic Computer Group, Inc) with the PIR (Protein Identification Resources) and the SwissProt databases.

Molecular modeling

Three-dimensional structure analyses of the purified 33-kD banana protein were performed by Swiss-Model (an automated knowledge-based protein modeling server).²⁷ The sequence of 30 N-terminal amino acids of the 33-kD purified banana protein was used for molecular modeling. Estimated 3-dimensional structure of the 33-kD banana protein (30 amino acids starting from the N-terminus) was visualized by RasMol (3-D molecular modeling viewer v 2.6).²⁸

Skin prick testing with purified banana proteins and purified hevein

Skin prick testing with the purified 32-kD banana protein (with concentrations of 0.01, 0.1, 1, 10, and 100 µg/mL in PBS, pH 7.2) was performed on 4 patients allergic to NRL.^{20,25} All 4 patients had

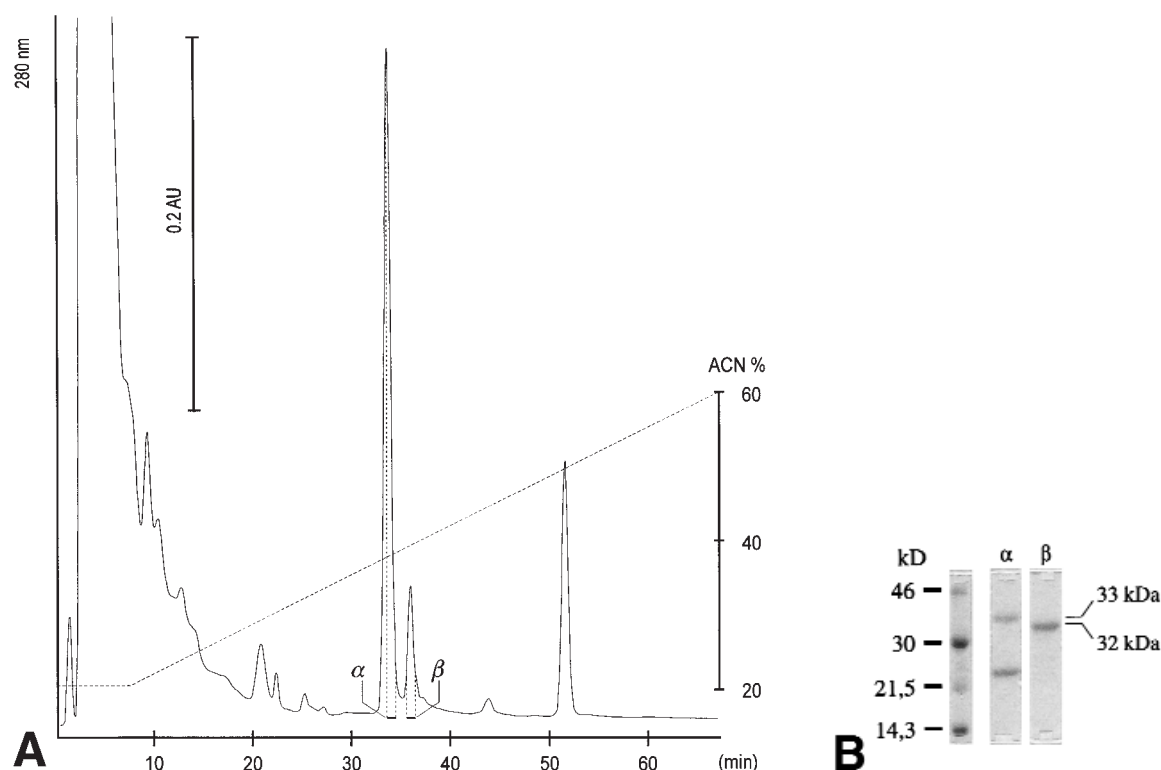


FIG 2. A, Reversed phase chromatography of the banana extract reveals several protein peaks. **B,** SDS-PAGE shows that α - and β -fractions contained protein bands of 33 and 32 kd, respectively, and the α -fraction also contained a 22-kd protein band. In immunoblotting (not shown), 33- and 32-kd protein bands bound IgE from patient sera.

TABLE I. Amino acid sequences of peptides obtained from 32- and 33-kd banana proteins

N-terminus	
Ban 33 kd*:	E Q C G R Q A G G A L C P G G L
Ban 32 kd†:	E Q C G R Q A G G A L C P G G L C C S Q Y G W C G N T D P Y
Hevein:	E Q C G R Q A G G K L C P N N L C C S Q W G W C G S T D E Y
Position:	1 16 30
Internal sequence	
Ban 33 kd‡:	R Y C D L L G V S Y G D N L D C Y N Q
Rice endochitinase§:	R Y C D M L G V S Y G D N L D C Y N Q
Position:	294 311

*Amino-terminal sequence of 32- and 33-kd banana proteins (16 residues analyzed) were identical.

†Amino-terminal sequence of 32-kd banana protein shows 80% identity to N-terminus of hevein.

‡Internal peptide obtained from the 33-kd banana protein shows 95% identity to basic endochitinase from rice (*oryza sativa*).

§Partial sequence of basic endochitinase from rice.

positive skin prick test responses to hevein and banana extract and showed antihevein-IgE as described earlier.^{20,25} As a control experiment, skin prick testing was also performed with a purified banana protein with an apparent molecular weight of 22 kd (concentrations of 0.01, 0.1, 1, 10, and 100 μ g/mL in PBS), which was not targeted by antihevein rabbit antibodies. A wheal the size of half of that of histamine dihydrochloride (10 mg/mL; ALK, Copenhagen, Denmark) or larger was regarded as a positive reaction. The study was approved by the ethical committee of Tampere University Hospital.

RESULTS

Identification of banana proteins cross-reacting with hevein

In immunoblotting, 10 (66%) of the 15 sera from patients allergic to latex showing IgE to hevein in ELISA had IgE to the banana extract (Fig 1, A). Nine of these sera showed IgE to the 32- and 33-kd banana protein

bands. The same bands bound also IgG from the hevein-immunized rabbit serum. In immunoblot inhibition, hevein, at a concentration of 1 $\mu\text{g/mL}$, totally inhibited IgE binding to the 32- and 33-kD protein bands in all 5 patient sera tested (exemplified with 1 patient serum in Fig 1, B). Binding of IgE to other banana protein bands was not affected by hevein.

Purification and molecular characterization of cross-reacting banana proteins

Reversed phase chromatography of the banana extract revealed several peaks that were collected and analyzed by SDS-PAGE and immunoblotting with rabbit antihevein serum. The fractions containing 32- and 33-kD proteins (Fig 2) recognized by the hevein-immunized rabbit serum were electrophoresed and blotted to polyvinylidene difluoride membrane, and the corresponding bands were subjected to N-terminal sequencing. Amino terminal sequencing revealed that both the 32- and 33-kD proteins (16 residues analyzed) had 80% identity to the N-terminus of hevein (Table I). Also, close structural homology between the N-terminus of hevein and the 33-kD banana protein (30 residues analyzed) was observed when the empirically determined nuclear magnetic resonance-based structure of hevein was compared with the 3-dimensional structure of the 33-kD banana protein obtained by molecular modeling (Fig 3). In addition, one 20 amino acid-long internal peptide obtained from the 33-kD banana protein gave 90% identity to endochitinase from rice and showed similar high homologies to various other endochitinases.

Binding of IgE to the purified 32-kD banana protein and ELISA inhibition studies with hevein and the 32-kD banana protein

In ELISA, IgE from all of the 15 sera from patients allergic to NRL bound to the 32-kD protein, whereas, with 1 exception, all 44 control sera were negative (Fig 4). Hevein (1 $\mu\text{g/mL}$) inhibited 77% to 100% of IgE binding to solid-phase 32-kD protein in all 4 patient sera tested (Table II). However, only weak inhibition (5% to 20%) of IgE binding to solid-phase hevein could be seen when the purified 32-kD protein (1 $\mu\text{g/mL}$) was used as an inhibitor. As an example at the individual level, purified hevein inhibited, in a dose-dependent manner, binding of IgE from the serum of 1 patient allergic to latex to solid-phase 32-kD banana protein (Fig 5, A). Hevein also inhibited the binding of IgE in a serum pool from patients allergic to NRL to solid-phase banana extract in a dose-dependent manner. Approximately 50% inhibition occurred at a hevein concentration of 10 ng/mL (Fig 5, B). However, a 100-fold higher concentration of the 32-kD protein was needed to achieve similar inhibition of IgE binding to hevein (Fig 5, B).

Skin prick test reactivity of the purified 32-kD banana protein

In skin prick testing, 1 of the 4 patients allergic to NRL chosen for tests had a positive reaction to the 32-kD banana protein, whereas a control protein (22-kD banana protein) elicited negative reactions in all patients.

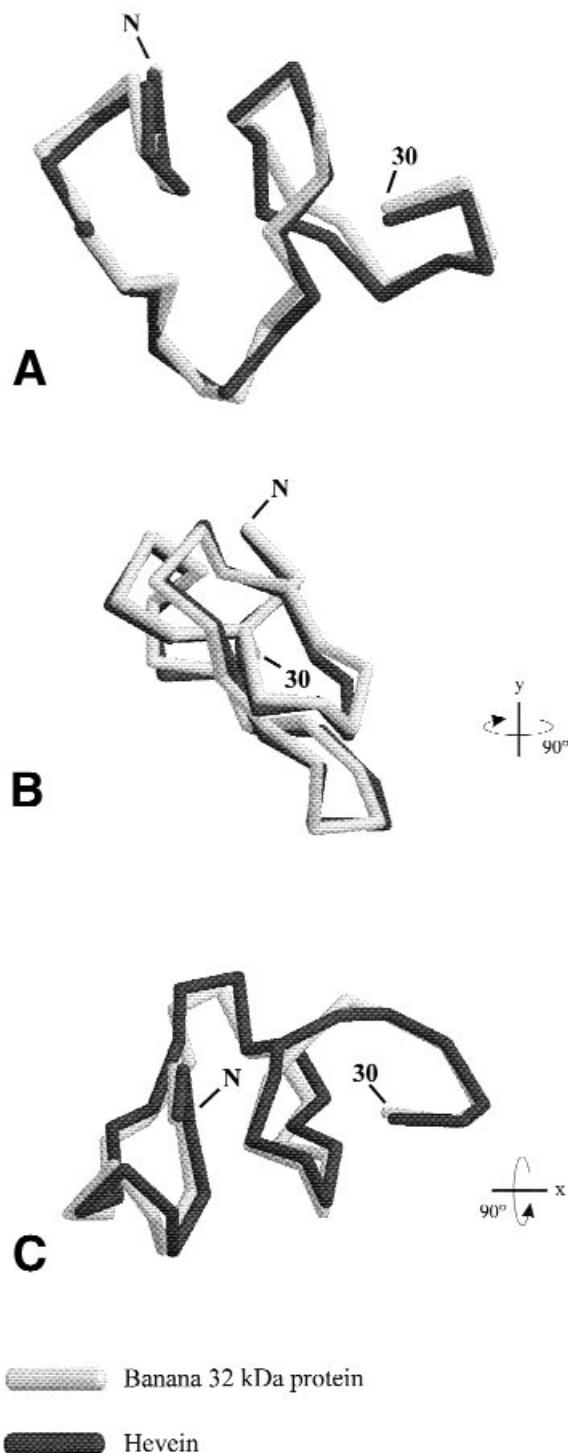


FIG 3. Comparison of 3-dimensional structures of N-terminal (30 residues) parts of hevein and 32-kD banana protein. Molecular modeling of banana protein was performed by Swiss-Model server (an automated knowledge-based protein modeling server). **A**, **B**, and **C** present so-called backbone structures of molecules viewed from different angles. *N* denotes amino terminus of both proteins.

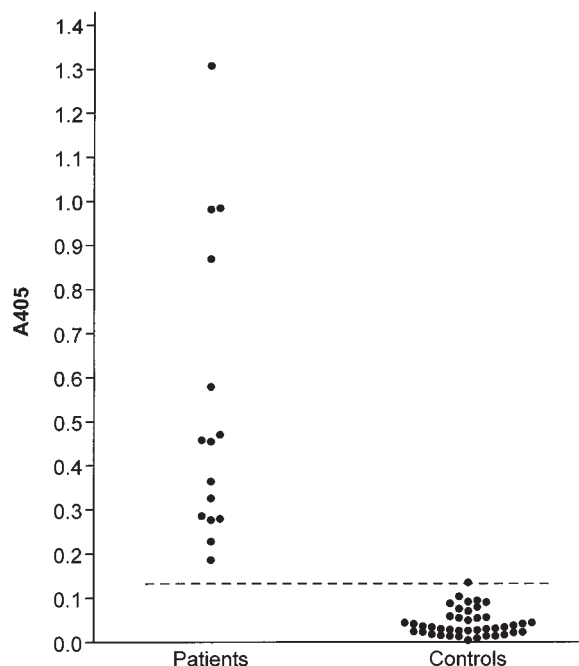


FIG 4. IgE binding to the purified 32-kd banana protein in ELISA. All 15 sera from patients allergic to NRL (previously shown to have IgE against hevein) show IgE binding, whereas all but 1 of the 44 control sera remain negative. Threshold value for positivity (mean OD value + 3 SD of controls) is indicated by the dotted line.

DISCUSSION

This study shows that proteins structurally and immunologically resembling hevein, a major NRL allergen, exist in banana and bind IgE from sera of patients allergic to NRL. Banana proteins with apparent molecular weights of 32 and 33 kd were purified and shown to exhibit high sequence homology to the N-terminus of hevein. Because both the 32- and 33-kd banana proteins gave identical N-terminal sequences, it is reasonable to assume that they both represent isoforms or modifications of 1 protein. Internal peptide sequence analysis of the 33-kd banana protein revealed high homologies to endochitinases from various plants, thereby suggesting that these proteins are banana endochitinases containing a hevein-like domain. IgE antibodies to the 32- and 33-kd protein bands were demonstrated by immunoblotting in sera from 9 of 15 patients allergic to NRL showing IgE to hevein. With 1 exception (patient 3, Fig 1, A), all 9 of these patients had positive skin prick test responses to banana extract. In ELISA all of the 15 patients showed IgE binding to the purified 32-kd banana protein. Hevein inhibited, in a dose-dependent manner, the binding of IgE to a solid-phase 32-kd banana protein in sera from all 5 patients tested. Although this study recognized at least 8 IgE binding proteins in banana (Fig 1, A), the role of hevein is emphasized by the fact that it inhibited in ELISA more than 70% of IgE binding of pooled sera from patients with NRL allergy to solid-phase banana extract. It is thus tempting to

assume that the previously documented hypersensitivity reactions of patients with NRL allergy to banana can be explained by cross-reactivity between the major latex allergen, the 4.7-kd hevein, and a previously undescribed 32-kd endochitinase in banana.

In cross-wise inhibition experiments it was noted that solutions of purified hevein inhibited the binding of IgE from patients with NRL allergy to solid-phase 32-kd banana protein in the nanogram range (Table II and Fig 5, A). In contrast, a 100-fold higher concentration of the 32-kd banana protein was required to bring even a weak inhibition in the binding of IgE to solid-phase hevein. A similar phenomenon was also observed when IgE binding from pooled sera of patients with NRL allergy to solid-phase banana extract was inhibited by purified hevein; 50% of IgE binding to banana extract was inhibited by 10 ng of purified hevein, whereas more than 1 μ g of the 32-kd banana protein was needed to obtain similar inhibition. Although general physical chemical properties of hevein and the 32-kd banana protein could partly explain their different inhibitory capacities, our results suggest that the primary sensitizer in the present patients allergic to NRL has been hevein or hevein-like structures in natural rubber products. Hence binding of IgE to the 32- and 33-kd banana proteins could be, in immunologic terms, a secondary phenomenon.

The reason why only 1 of the 4 patients allergic to hevein reacted in skin prick testing to the *in vitro* cross-reacting 32-kd banana protein is not known. Conformational changes induced by protein purification methods used in the present study (eg, reversed phase chromatography) may reduce the protein's ability to cause *in vivo* reactivity. It could also be that before the banana protein is able to achieve its full allergenic *in vivo* potency, it requires enzymatic digestion in the oral mucosa or intestine. It may also be pointed out that the 32-kd banana protein obviously is an endochitinase that contains a chitin-binding domain, whereas the hevein molecule (4.7 kd), as a whole, is a chitin-binding domain that is cleaved from its precursor protein, prohevein (20 kd).

Hevein-like chitin-binding domains (about 43 amino acids) represent highly conserved protein structures existing in many defense proteins of the plant kingdom.²⁹ The amount of such proteins increases greatly after the plant has been attacked by, for example, fungi or insects. There is increasing evidence on the possible role of chitin-binding proteins as allergens. In addition to proteins in NRL,^{20-22,30} wheat germ agglutinin, containing 4 hevein-like domains, has been reported to bind IgE from sera from patients allergic to NRL,^{21,31} to inhibit the binding of IgE to purified hevein,³¹ and to induce positive skin prick test reactions in patients allergic to NRL.³¹ Recently, IgE-binding chitinases with hevein-like domain have also been recognized in avocado and chestnut.³² Because similar defense proteins presumably occur in all kinds of plants, it is reasonable to assume that chitin-binding proteins could be important sensitizing or cross-reactive allergens.

Cross-allergies have recently received increasing attention. At the primary structure level, detailed infor-

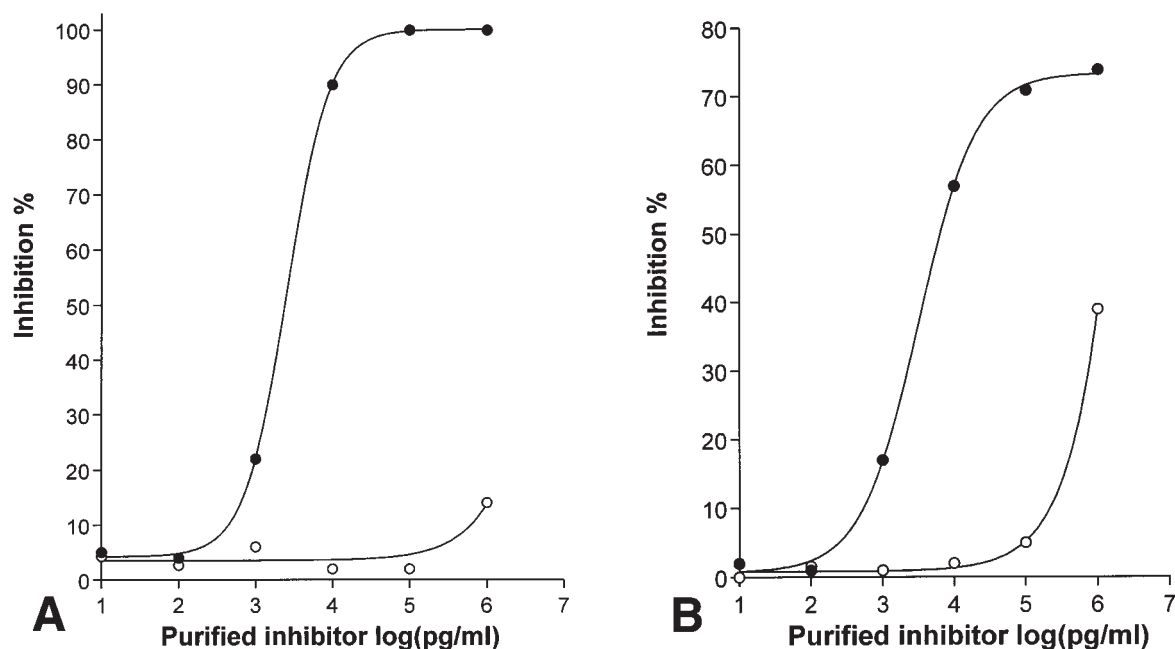


FIG 5. ELISA inhibition experiments with hevein and banana proteins. **A**, Inhibition of IgE binding to solid-phase 32-kd banana protein by different concentrations of hevein and vice versa in serum from 1 patient (number 1 in Fig 1). Closed circles, 32-kd banana protein in solid phase and hevein as an inhibitor; open circles, hevein on solid phase and 32-kd banana protein as an inhibitor. **B**, Inhibition of IgE binding to banana extract with hevein (closed circles) and 32-kd banana protein (open circles) in reference serum pool from 6 patients allergic to NRL.

TABLE II. Inhibition of IgE binding to solid-phase 32-kd banana protein by different concentrations of hevein and vice versa

Solid-phase antigen	Inhibitor		Inhibition percent*			
			Patient 1	Patient 6	Patient 8	Patient 10
Ban 32 kd† (2 µg/mL‡)	Hevein	1.00 µg/ml	100	77	100	100
		0.01 µg/mL	90	54	100	89
	Ban 32 kd†§	1.00 µg/mL	59	36	100	25
		0.01 µg/mL	6	3	11	14
Hevein (2 µg/mL‡)	Hevein§	1.00 µg/mL	100	99	99	98
		0.01 µg/mL	92	88	83	84
	Ban 32 kd†	1.00 µg/mL	20	19	5	16
		0.01 µg/mL	10	7	5	4

*Four sera from patients allergic to NRL (patients 1, 6, 8, and 10 in Fig 1, A) were studied in ELISA.

†Purified 32-kd banana protein (fraction b in Fig 2, B).

‡Concentration of antigen used in coating.

§Results from autologous inhibition experiments.

mation on cross-reacting pollen allergens, especially on profilins, have been published.^{4,5,33-37} The term “panallergen” was introduced to denote the commonness of the allergen cross-reactivity between profilins derived from different plant species and also from other organisms, even humans.⁵ Another example of molecules suggested to represent so-called panallergens is manganese superoxide dismutase from *Aspergillus fumigatus*,³⁸ an enzyme shown to be a potent allergen with highly homologous counterparts in a variety of species in the plant kingdom.³⁹ In this study we describe cross-reacting allergens that are derived from taxonomically distant plant species (ie, *Hevea brasiliensis* and *Musa acuminata*). In

contrast to allergenic cross-reactivity between different plant profilins and within manganese superoxide dismutases, which are structurally related molecules of reasonably similar size, we here demonstrated cross-reactivity residing in homologous protein domains that are functionally related. Further studies are needed to assess whether chitin-binding proteins, or more precisely their hevein-like chitin-binding domains, represent a novel panallergen family explaining a considerable proportion of allergen cross-reactions in plant kingdom.

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