

Isolated hevein-like domains, but not 31-kd endochitinases, are responsible for IgE-mediated *in vitro* and *in vivo* reactions in latex-fruit syndrome

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Background: Individuals with natural rubber latex allergy often have immediate reactions to plant-derived foods and fresh fruits, such as avocado and banana. IgE of these patients has been shown to bind endochitinases containing an N-terminal hevein-like domain (HLD). However, evidence on 31-kd endochitinase-induced reactions *in vivo* is lacking.

Objective: We sought to assess the clinical significance of 31-kd endochitinases and isolated HLDs in latex-fruit syndrome.

Methods: The 31-kd endochitinases and corresponding HLDs were purified or produced from avocado, banana, latex, and wheat germ. Skin prick test reactivities against purified proteins were examined in 15 patients with natural rubber latex allergy. The binding efficiency of IgE to purified proteins was studied by using an inhibition ELISA. Experimentally resolved or modeled structures of the proteins were compared to clarify the molecular basis of clinical reactions.

Results: Eleven (73%) patients had skin prick test reactions to isolated HLDs of avocado and banana, but only 1 (7%) patient reacted to their corresponding 31-kd endochitinases. HLDs from avocado and banana inhibited binding of IgE to prohevein (Hev b 6.01) in 59% and 38% of patients, respectively, whereas corresponding percentages for 31-kd endochitinases were 17% and 20%, respectively. Isolated HLDs of wheat germ agglutinin and 18-kd wheat germ agglutinin did not significantly inhibit IgE binding to hevein.

Conclusion: The isolated HLD molecules alone, but not when linked to endochitinases, seem to be responsible for IgE-mediated clinical reactions in latex-fruit syndrome. Careful

selection of relevant allergens in their proper molecular form is therefore crucial in forming a reliable diagnosis of latex-fruit syndrome. (J Allergy Clin Immunol 2005;115:598-605.)

Key words: Allergy, epitopes, cross-reactivity, hevein-like domain, antibodies, structural homology, latex-fruit syndrome

Immediate hypersensitivity to natural rubber latex (NRL) is currently one of the most frequently encountered occupational diseases among health care workers and other personnel using protective gloves. A consensus exists that hevein (Hev b 6.02) is a major and, in all likelihood, the most important NRL allergen.¹⁻⁴ Hevein elicits positive skin prick test (SPT) reactions in the great majority of patients with NRL allergy, and about 70% of patients have circulating IgE antibodies to hevein.⁵⁻⁷

Endochitinases with a conserved N-terminal hevein-like domain (HLD) are found throughout the plant kingdom.⁸⁻¹⁰ These proteins are usually synthesized as preproteins, followed by the cleavage of the signal sequence in the endoplasmic reticulum and the separation of the large C domain. In the rubber tree preprohevein is processed to prohevein (Hev b 6.01), hevein (Hev b 6.02), and a C domain (Hev b 6.03), all of which are secreted into rubber latex.^{11,12} Wheat germ agglutinin (WGA), another type of hevein-like protein, is composed of 4 tandem repeats of covalently connected HLDs without an additional C domain.^{13,14}

It is now well established that 50% of patients with NRL allergy have immediate allergic symptoms to certain plant foods, especially tropical fruits.^{15,16} The main fruits implicated in the latex-fruit syndrome include avocado, banana, kiwi, and papaya. It has been suggested that HLD-containing endochitinases are presumable candidates for IgE-mediated allergic cross-reactions of patients with latex-fruit syndrome.^{17,18} Thus latex-fruit syndrome has been recognized primarily on the basis of clinical findings and by means of immunoblot analysis against 31-kd endochitinase proteins. No clinical evidence exists, how-

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Abbreviations used	
3D:	Three dimensional
HLD:	Hevein-like domain
NRL:	Natural rubber latex
RP:	Reversed phase
SPT:	Skin prick test
WGA:	Wheat germ agglutinin

ever, as to whether these endochitinase molecules are able to induce IgE-mediated reactions.¹⁸⁻²⁰

To examine the allergens responsible for cross-reactions in latex-fruit syndrome, 31-kd endochitinases and their corresponding HLDs were purified from avocado, banana, and NRL. In addition, 4 HLDs of WGA were produced separately by using recombinant DNA technology. Immunologic examination *in vitro* and *in vivo* revealed that isolated HLDs alone, but not in the context of 31-kd endochitinase molecules, are responsible for the clinical symptoms in latex-fruit syndrome.

METHODS

Purification and characterization of native proteins

To extract chitin-binding proteins from banana and avocado, pieces of fresh fruit were pulped in acetate buffer (50 mmol/L acetic acid, 0.2 mol/L NaCl, and 10 mmol/L tiourea, pH 4) on ice, and the mixture was stirred overnight at 4°C. The tubes were centrifuged at 5000g and 4°C for 15 minutes, and the supernatant was recentrifuged at 25,500g and 4°C for 1 hour. Filtered (0.4 μm) supernatant was affinity purified with 250 mg of chitin (Sigma Aldrich, St Louis, Mo), and proteins eluted with 1 mol/L acetic acid were immediately neutralized with 3 mol/L NH₄HCO₃.

The chitin-bound proteins were subjected to reversed-phase (RP) chromatography on a Vydac column (C8, 5 μm, 300 Å; LC Packings, Amsterdam, The Netherlands) and eluted with a linear gradient of acetonitrile (0% to 100% in 60 minutes) in 0.1% trifluoroacetic acid. Additional RP chromatography was performed, when needed, on a TSKgel TMS-250 column (C1, 10 μm, 250 Å; Tosoh Corp, Tokyo, Japan).

Prohevein was purified from the B serum of latex by using gel filtration, anion-exchange chromatography, and RP chromatography. Gel filtration was performed on a Superdex75 HR16/60 column (Amersham Biosciences, Uppsala, Sweden) in PBS. Prohevein-containing fractions were concentrated by using an Amicon Ultra-15 filter (Millipore Corp, Bedford, Mass), and the buffer was changed to 20 mmol/L Tris-Cl, pH 8.5, by using a BioGelP6 desalting column (Bio-Rad Labs, Hercules, Calif). Anion-exchange chromatography was performed on a MonoQ column (HR5/5, Amersham Biosciences) equilibrated with 20 mmol/L Tris-Cl, pH 8.5, and eluted with a linear gradient of NaCl (0-1 mol/L in 30 minutes) in the equilibration buffer. RP chromatography was performed on the TSKgel TMS-250 column.

Hevein was purified from the B serum of NRL, as previously described,⁵ and commercially available WGA was used as 18-kd WGA (Sigma Aldrich).

TABLE I. Used primers and their sequences	
Name (F/R)	Sequence
BANI_RT (F)	<u>gagcaatgcggaaggcaagccgggg</u>
BANI (F)	<u>agcagcggatccCTGGTGCCTAGAGGAAGC</u> <u>gagcaatgcggaaggcaagc</u>
BANI (R)	<u>AACACAAGCTTTTTATCCTCCGCACTG</u>
WGAI (F)	<u>ctcgccgatccCTGGTGCCTAGAGGAAGC</u> <u>cagaggtgcggcgagcaggcg</u>
WGAI (R)	<u>GCTGCAAGCTTTTTAGCTGGTCCAGCA</u> <u>GGCGCCGTT</u>
WGAII (F)	<u>ggctgcggatccCTGGTGCCTAGAGGAAGC</u> <u>aagcgtgcggcgagccagg</u>
WGAII (R)	<u>CGGACAAGCTTTTTAGTCGGCGCGGCAGG</u>
WGAIII (F)	<u>ggctgcggatccCTGGTGCCTAGAGGAAGC</u> <u>atcaagtgcggcgagccagtc</u>
WGAIII (R)	<u>CTTGCAAGCTTTTTAGTCGGTGCTGCAA</u> <u>GCACCGCT</u>
WGAIV (F)	<u>ggctgcggatccCTGGTGCCTAGAGGAAGC</u> <u>aagccgtgcggcgagcaggcc</u>
WGAIV (R)	<u>GGCGCAAGCTTTTTAAGCGTCACAGCCG</u> <u>CCGCTCTGG</u>

BamHI and HindIII sites are underlined, and thrombin sites are in bold.

Isolation of RNA and construction of DNA sequences for banana and WGA HLDs

RNA was isolated from a 200-mg piece of banana leaf, as previously described.²¹ Total RNA was reverse transcribed by using a cDNA synthesis kit (Enhanced avian RT-PCR Kit, Sigma Aldrich) with 125 pmol of oligo-dT primer and Ban_RT_5' primer (Table I). To produce HLDs for banana and WGA (isolectin 2), their cDNAs were amplified in PCR (Table I), cloned into pBacAVs+C vector, and constructed to baculoviruses, as previously described.^{22,23} Recombinant HLD proteins were produced in insect cells according to the Bac-To-Bac Baculovirus Expression System (Gibco Life Technologies, Gaithersburg, Md), and the purifications were performed as previously described.^{22,23}

In-gel ingestion and desalting of endochitinases

Coomassie Brilliant Blue–stained protein bands were extracted from the SDS-PAGE gels, destained, and digested as previously described.²⁴ Briefly, proteins were reduced, alkylated, and digested with trypsin (Promega, Madison, Wis) overnight at 37°C. The generated peptides were extracted once with 25 mmol/L ammonium bicarbonate and twice with 5% formic acid, and finally, the peptide mixture was desalted with a Millipore μ-C18 ZipTip (Millipore Corp).

Mass spectrometry and N-terminal sequencing

Matrix-assisted laser desorption ionization time-of-flight and electrospray Q time-of-flight mass spectrometric analyses and N-terminal sequencing were performed essentially as previously described.^{25,26}

SPTs

The protein samples to be used in the SPTs were first concentrated by means of evaporation and then diluted to PBS at concentration of 500 pmol/mL. In addition to a commercial NRL preparation (Stallergenes, Fresnes, France), fresh banana and avocado fruits were

Food allergy, dermatologic diseases, and anaphylaxis

TABLE II. For prick-prick skin testing, purified proteins were adjusted to a concentration of 500 pmol/mL, and tests were performed on 15 hevein-specific patients with latex allergy

Antigen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Histamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Latex (Stallergenes)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Avocado (fruit)	–	–	–	+	–	+	–	+	–	+	+	+	–	–	–
Banana (fruit)	–	–	+	+	–	+	–	–	–	–	+	+	–	+	+
Prohevein (19 kd)	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Avocado (31 kd)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Banana (31 kd)	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
WGA (18 kd)	–	+	–	–	–	–	–	–	+	–	–	–	+	–	–
Prohevein HLD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Avocado HLD	+	+	+	+	–	+	+	+	–	+	+	+	–	+	–
Banana HLD	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–

A positive reaction (wheal diameter ≥ 3 mmol/L) is denoted by +, and a negative reaction is denoted by –.

tested with the prick-prick method.²⁷ Histamine at a concentration of 50 μ g/mL was used as a positive control.

Patients and control sera

SPTs were performed on 15 patients with latex allergy who had IgE to hevein (14 women and 1 man; mean age, 49 years), including the 5 patients (4 women and 1 man; mean age, 42 years) whose sera were used in the ELISA experiments. The diagnosis of their latex allergy was confirmed on the basis of positive NRL glove challenge.²⁸ Nine patients with banana allergy (7 women and 2 men; mean age, 35 years) with no latex sensitization were used as control subjects. They all had a positive clinical history and SPT responses to banana but negative SPT responses to NRL (Stallergenes). Four of the patients with banana allergy also reported allergic reactions (oral swelling and itching) from avocado.

In addition, sera from 17 other patients with latex allergy who had IgE to hevein (16 women and 1 man; mean age, 50 years) were used in WGA studies. In the ELISA tests 10 atopic individuals (9 women and 1 man; mean age, 49 years) with no latex or banana allergies were used as negative control subjects. Before initiation of the SPTs, approval was obtained from the Ethics Committee of the Tampere University Hospital, Tampere, Finland.

Inhibition ELISA to assess IgE cross-reactivity

Inhibition ELISA tests were performed as previously described,²⁷ but ELISA plates were coated with 31-kd endochitinases at concentrations of 100 pmol/mL, and inhibitors were used at concentrations of 500, 50, 5, and 0.05 pmol/mL.

Modeling of avocado and banana HLDs

Evolutionarily related protein sequences from avocado, banana, rubber latex, and wheat were aligned by DNAMAN using (Lynnon BioSoft, Quebec, Canada) to detect HLD-specific amino acid residues. The 3-dimensional (3D) structures of hevein (1HEV)²⁹ and HLDs in the major WGA (isolectin 2, 9WGA)³⁰ were compared by using the Bodil modeling package (Lehtonen et al, unpublished; available at www.abo.fi/fak/mnf/bkf/research/johnson/bodil.html). The homology-based models of the banana and avocado HLDs were produced with the MODELLER³¹ molecular modeling program, with hevein as a template structure. All structure representations were

prepared with the PyMOL (DeLano WL, The PyMOL Molecular Graphics System (2002), available at <http://www.pymol.org>).

RESULTS

Purification and characterization of chitin-binding hevein-related allergens from avocado, banana, and latex

HLD-containing allergens were purified from fresh avocado and banana fruits. Proteins recovered by means of chitin affinity chromatography were further separated with RP chromatography. HLD-containing proteins were recognized by rabbit polyclonal hevein antibodies in ELISA tests. The major immunoreactive proteins were identified as avocado and banana endochitinases according to their determined N-terminal sequence (EQCGR QAGGALCPGGLCCSQ...) and peptide mass fingerprinting.

By using mass spectrometry, 2 forms (4.3 and 31.1 kd) of HLD-containing proteins with an identical N-terminal sequence were detected from avocado, but only the larger form (31.2 kd) was detected from banana. Similarly, we also purified prohevein (18.5 kd) and hevein (4.7 kd) from rubber latex B serum.

Our goal was to assess the immunologic differences between the 2 forms of HLD-containing proteins from the 3 species. Because we were unable to purify the smaller form (4.3 kd) from banana, we extracted total RNA from banana leaf, constructed its cDNA, and produced the recombinant protein with an insect cell system that has previously been shown to be a reliable method for the production of HLDs.^{22,23,32}

N-terminal HLDs alone, but not in the context of 31-kd endochitinases, elicited positive SPT reactions

To test the ability of different proteins to induce symptoms *in vivo*, we performed SPTs with purified

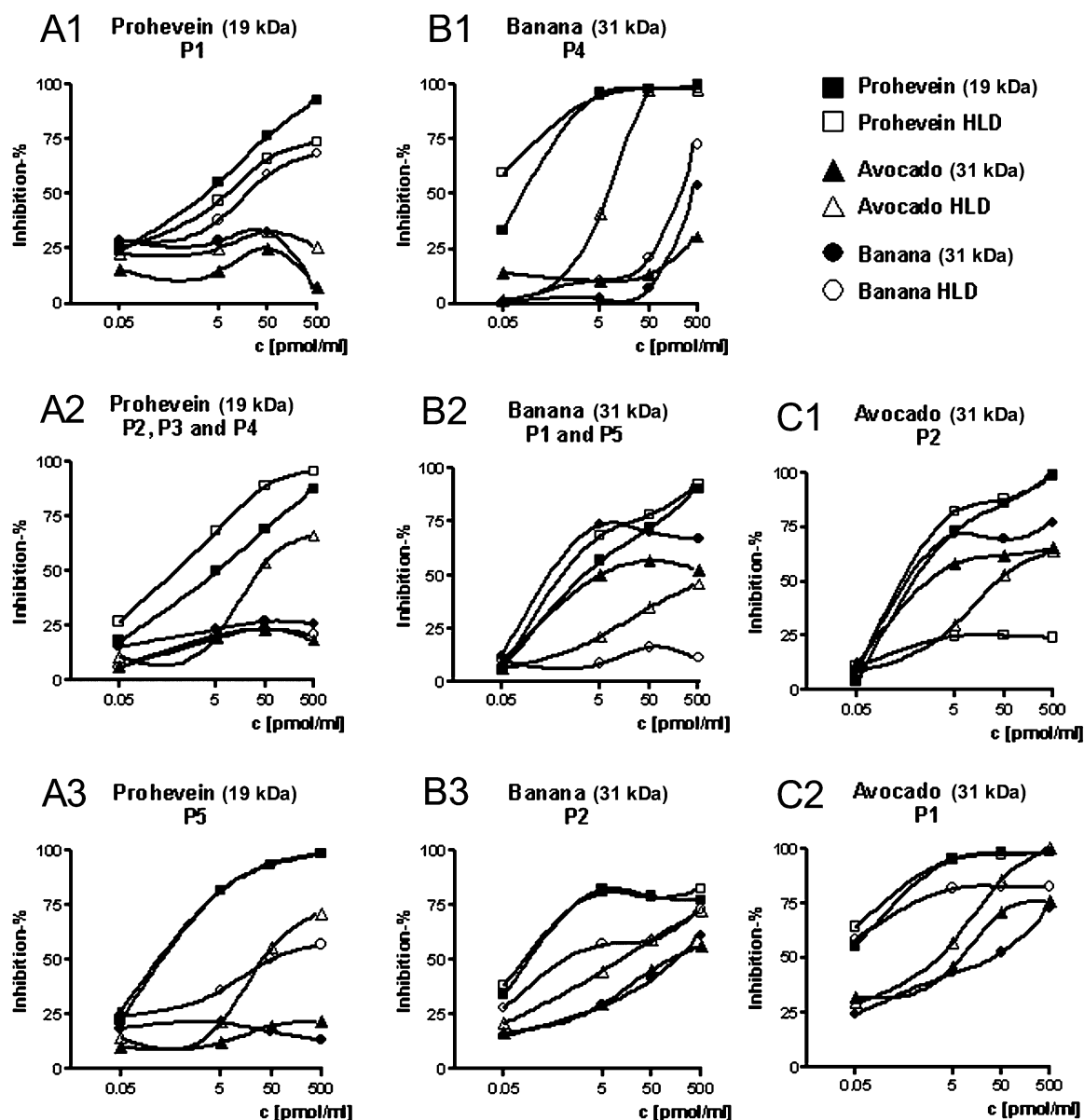


FIG 1. Three or two different trend groups were identified with the sera from 5 patients with NRL allergy (P1-P5) in ELISA inhibitions against immobilized prohevein (A1-A3) and immobilized 31-kD endochitinases from banana (B1-B3) and avocado (C1-C3).

HLD-containing proteins on 15 patients with NRL allergy (Table II) and 9 patients with banana allergy but without NRL allergy. Eleven (73%) of the 15 patients with NRL allergy had a positive reaction to isolated HLDs from both avocado and banana, whereas only 1 (7%) patient reacted to the corresponding 31-kD endochitinases. All patients with NRL allergy had positive reactions to hevein, and 13 (87%) of them also had reactions against prohevein. Control subjects with banana allergy with no latex sensitization had negative reactions to 31-k endochitinases, as well as to isolated HLDs. Seven (47%) and 6 (40%) of the 15 patients with NRL allergy had reactions to fresh banana and avocado fruit, respectively (Table II).

Three of the patients with NRL allergy had positive reactions to the 18-kD WGA.

IgE of the patients bound more efficiently to isolated HLDs from avocado and banana than to their corresponding 31-kD endochitinases

To examine the binding efficiency of IgE, 31-kD endochitinases from avocado, banana, and rubber latex and their N-terminal HLDs were used in ELISA inhibitions against solid-phase prohevein or the 31-kD proteins of avocado and banana. Strong inhibition of IgE binding to solid-phase prohevein was detected with prohevein and

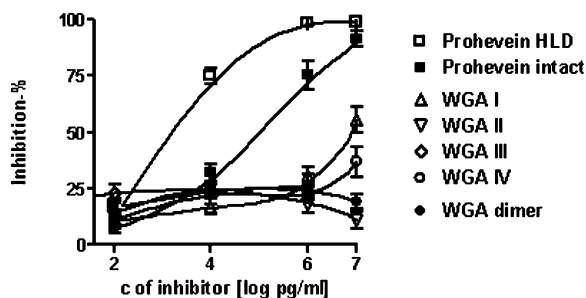


FIG 2. Average inhibitions of HLDs from WGA (WGA I-WGA IV) and 18-kd WGA against solid-phase hevein are presented, with 17 patients with NRL allergy showing immune responses to hevein.

hevein, as well as with HLDs from avocado (59%) and banana (38%). However, the 31-kd endochitinases from avocado and banana revealed only slight inhibition, 17% and 20%, respectively, in equimolar concentrations. Avocado HLDs also exhibited strong inhibition, 77% and 73%, respectively, of IgE binding to solid-phase 31-kd endochitinases from avocado and banana, respectively.

Hevein and prohevein were always the most efficiently recognized by IgE, although considerable variation from patient to patient was observed. The patients were divided into groups of 3 or 2 according to their inhibition patterns (Fig 1). In the first group (P1) only the banana HLD showed inhibition (68%) of IgE binding to solid-phase prohevein, whereas in the second group (P2, P3, and P4) only HLD from avocado inhibited IgE binding (71%; Fig 1, A). In the third group (P5) HLDs from both avocado and banana showed 71% and 57% inhibition, respectively. The 31-kd endochitinases from fresh banana and avocado fruits were virtually nonreactive with respect to their ability to inhibit IgE binding to prohevein.

A similar trend was seen also in the inhibition of IgE binding to the 31-kd endochitinase from banana (Fig 1, B). In the first group (P4) isolated HLD from avocado showed total inhibition, whereas all other proteins showed 100-fold reduced inhibition capacity. In the third group (P2) N-terminal HLDs of avocado and banana showed strong inhibition against banana endochitinase. Also in the second group (P1) the same HLDs showed stronger inhibitions against avocado endochitinase than against 31-kd endochitinases (Fig 1, C).

IgE from patients with NRL allergy bound weakly to the 4 hevein isoforms in WGA

When compared with fruit endochitinases, WGA, the 3D structure of which is known, presents an entirely different domain constellation. We therefore separately produced and purified 4 HLDs from WGA isolectin 2 and tested their ability to inhibit the binding of IgE to hevein (Fig 2). The first and the fourth domains showed a 10,000- to 1000-fold reduced average inhibition capacity at a fairly high concentration of the inhibitors, whereas the other WGA proteins were virtually nonreactive in this setup.

HLDs have conserved sequences and structures

To examine the molecular basis for the different immunologic responses, all HLDs from NRL, avocado, banana, and wheat were compared in multiple sequence alignment (Fig 3). Significant amounts of conserved small (Gly and Ser) and aromatic amino acid residues (Trp and Tyr), in addition to 8-disulfide bridge-forming cysteines, were detected. In reverse, the typical extra loop (His³⁵-Asn³⁶) of hevein was absent in all the other HLD proteins.

In addition to the primary sequence, the 3D structures also are usually homologous between proteins that serve as targets for allergic cross-reactions. In the absence of coordinates for 31-kd endochitinases, we modeled HLDs from avocado and banana. They provided a good foundation for comparative modeling because they showed 76% and 63% sequence identity with hevein, respectively. As expected, their 3D structures were highly similar (Fig 4, A), showing exceptions only at sites that also differed in primary sequence (Arg⁵ and Ser³²-Asn³⁶). Further reliability for the models was acquired by comparing the location of their chitin-binding residues with those of the hevein (PDB: 1HEV) and the WGA (PDB: 9WGA) structures (not shown). When the key amino acid residues on the surface of our previously identified IgE epitope of hevein³² (Arg⁵, Lys¹⁰, Glu²⁹, Tyr³⁰, His³⁵, and Gln³⁸) were located on the modeled HLDs, they seemed to remain in the conserved positions (Fig 4, B-D).

Structures of hevein and the 4 HLDs of WGA (isolectin 2) were superimposed almost identically throughout the C- α traces (Fig 5, A). Major differences were observed at the N-terminus and near the C-terminal end between hevein residues.³⁴⁻³⁷ Notably, charged amino acid residue Arg⁵ on hevein pointed in a slightly different direction from the corresponding residues on the WGA subunits, and only the fourth HLD in WGA had a positively charged amino acid residue (Lys) in the corresponding position. The side chain of Lys¹³⁴ of WGA was missing in the PDB file and was modeled for the purpose of visualization (Fig 5, A). Again, when compared with the IgE-binding residues of hevein, corresponding residues on the 4 HLDs in WGA structure were found at a highly solvent-exposed location (Fig 5, B).

DISCUSSION

Several studies have reported that about one half of patients with NRL allergy have hypersensitivity to various plant foods, in particular to tropical fruits, such as avocado, banana, papaya, and kiwi.^{16,17,33} Hevein, the major latex allergen (Hev b 6.02) representing the N-terminal region of prohevein (Hev b 6.01), and its homologues in avocado^{6,34,35} and banana^{27,36} are thought to be the major proteins participating in these reactions.

Allergic cross-reactivity has been in most instances demonstrated by IgE immunoblotting with 31-kd endochitinase proteins as targets. However, it is not known whether these HLD-containing endochitinase molecules

HEV	ECCGRQAGGKLCNNLCCSQWCGSTDEYCSPDHNCQSN.CKD	43
AVO	ECCGRQAGGALCPGGLCCSQFGWCGSTSDYCGPT..CQSQ.CGG	41
BAN	ECCGRQAGGALCPGGLCCSQYCWGNTDPYCGKG..CQSK.CGG	41
WGADI	QRCGEQGSNMECPNNLCCSQYCYCGMGDDYCGKG..CONGACWT	42
WGADII	KRCGSQAGGATCPNNHCCSQYGHCGFGAEYCGAG..CQGGPCRA	42
WGADIII	IKCGSQSGGKLCNNLCCSQWCFGLGSEFCGGG..CQSGACST	42
WGADIV	KPCGKDAGGRVCINNYCCSKWCSGIGPGYCGAG..CQSGGQDA	42

FIG 3. Sequence alignment of HLDs from latex (HEV), avocado (AVO), banana (BAN), and WGA (WGA-I/WGA-IV) shows identical residues in black and homologous residues in gray.

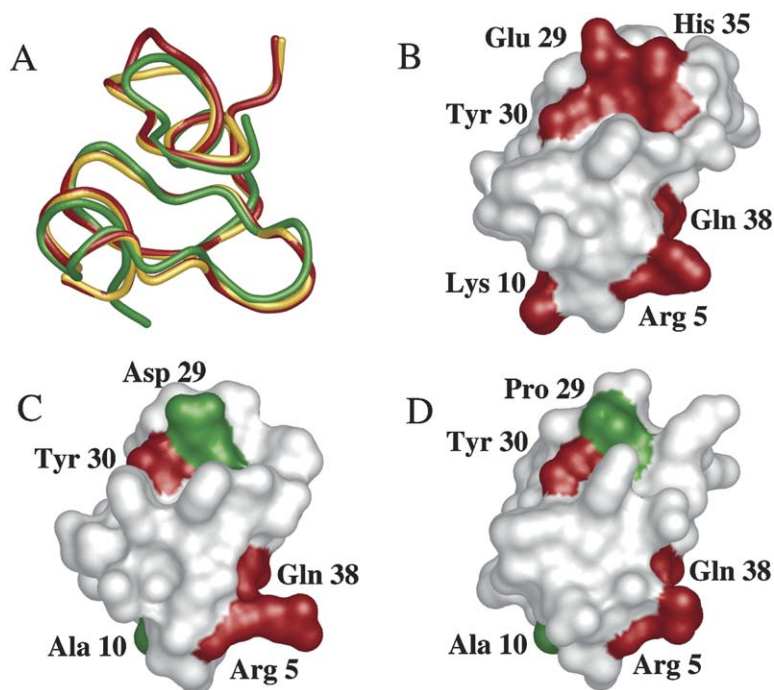


FIG 4. Ribbons present native hevein (red) and modeled HLDs from avocado (green) and banana (yellow; A). On the surface picture of hevein (B) and HLDs from avocado (C) and banana (D), red amino acid residues are identical to the critical residues on the hevein epitope, whereas green residues are substituted in HLDs.

are able to induce clinical symptoms.¹⁸⁻²⁰ To clarify the molecular basis for the cross-reactivity and to assess its clinical significance, we purified or produced recombinant forms of endochitinases (18-31 kD) and their corresponding HLDs (about 4.5 kD) from avocado, banana, and rubber latex and tested their immunologic properties *in vivo* and *in vitro*.

SPTs with patients with NRL allergy revealed that, unexpectedly, only 1 (7%) patient had a reaction against the 31-kD endochitinases from avocado and banana, whereas 11 (73%) patients had positive SPT reactions against their corresponding isolated HLDs. None of the patients with banana allergy had reactions against either 31-kD endochitinases or the isolated HLDs. As anticipated, all the patients with NRL allergy had SPT reactions against hevein, and 13 of the 15 patients also had reactions against prohevein. To clarify the immunologic basis for these differences, we assessed the binding efficiency of IgE to the HLD-containing proteins by means of inhibition ELISA. Isolated HLDs from avocado and banana effectively inhibited the binding of IgE to

solid-phase prohevein, whereas only weak inhibition was seen with 31-kD endochitinases at equimolar concentrations. Taken together, our results show that isolated HLD molecules alone, but not in the context of 31-kD endochitinases, are able to induce type I cutaneous allergic reaction in patients with NRL allergy. Given that HLDs produced clear-cut SPT reactions, it is also likely that they could provoke more generalized symptoms. Controlled exposure studies are, however, needed to further substantiate the clinical manifestations in latex-fruit syndrome. These findings suggest that considerable variation exists in the binding affinity of IgE antibodies to isolated HLDs when compared with 31-kD endochitinases.

Identification of the primary sensitizers has been a difficult task in latex-fruit syndrome.^{15,37,38} The present results demonstrate that IgE from patients with latex allergy bound with much higher efficiency to hevein and prohevein than to proteins from avocado and banana, suggesting that at least in these patients HLD-containing NRL proteins are the primary sensitizers. None of the control subjects with banana allergy but without NRL

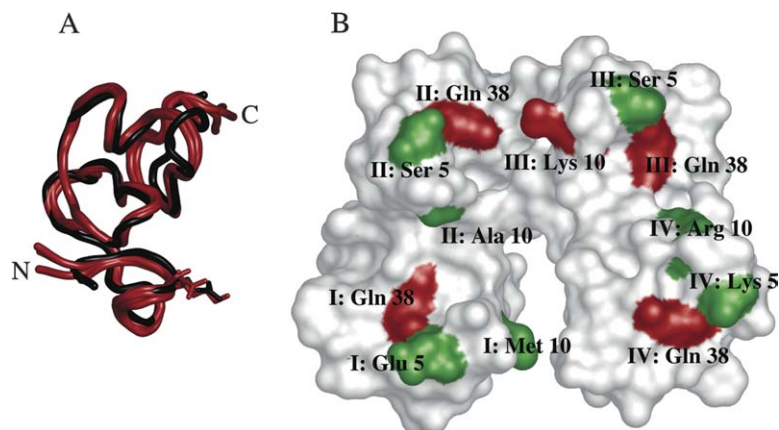


FIG 5. C- α traces of native hevein (green) and 4 WGA domains (red) are superimposed (A). The Arg⁵ of hevein and corresponding amino acid residues in WGA are shown in the stick model. On the surface of WGA (B), red amino acid residues are identical to the critical residues on the hevein epitope, whereas green residues are substituted in WGA.

allergy had measurable IgE antibodies against any HLD-containing proteins from banana, avocado, or NRL. It thus appears that patients with NRL allergy are sensitized primarily to hevein in NRL and that these patients have a high probability of development of cross-allergies against isolated HLDs in avocado and banana. Banana allergy without simultaneous NRL allergy is likely to be caused by banana proteins without N-terminal HLDs.

Among the interactions of HLDs with their corresponding IgE antibodies in latex-fruit syndrome, WGA is of particular interest because its 3D structure has been solved and shown to consist of 8 separate HLDs. To assess the efficiency of IgE binding to WGA in comparison with that of hevein and prohevein, we separately produced covalently connected HLDs in WGA (WGA I-WGA IV) and tested their inhibition properties *in vitro*. Only the first and the fourth domains showed weak inhibition of IgE binding to hevein, whereas the second and third domains were completely inactive. The 18-kD WGA was unable to exhibit any inhibition of IgE binding to hevein. Our recent location of the IgE-interacting amino acid residues on hevein enables us to suggest a possible explanation for our results.³² Maximally, 2 of the 6 IgE-interacting key residues (Arg⁵, Lys¹⁰, Glu²⁹, Tyr³⁰, His³⁵, and Gln³⁸) on hevein were not substituted or missing in HLDs of WGA (Fig 5, B). Interestingly, Arg⁵ of hevein was the only amino acid residue present on the surface of the modeled structures of HLD from avocado and banana (Fig 4, B), whereas it was absent from all the WGA HLDs. Slight IgE binding with the fourth HLD of WGA might be related to 2 conserved mutations in which Arg⁵ of hevein is changed to Lys¹³⁴ and Lys¹⁰ is changed to Arg¹³⁹. These observations could indicate the existence of epitopes on WGA to which IgE from patients with latex allergy binds with low affinity.

In conclusion, our results demonstrate that isolated HLD molecules alone, but not in the context of endochitinase molecules, are able to induce immediate allergic

reactions in patients with NRL allergy. These differences are probably due to variation in the efficiency with which the IgE antibodies bind to isolated HLDs and to their corresponding 31-kD endochitinases. The present findings also suggest that patients with latex-fruit syndrome are sensitized primarily to NRL hevein and that allergic cross-reactivity *in vivo* occurs if anti-hevein IgE antibodies have a strong enough affinity to cross-link other HLDs in certain fruits. To avoid false-negative responses in the diagnosis of latex-fruit syndrome, careful selection of relevant allergens in their proper molecular form is crucial.

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