

Identification of hevein (Hev b 6.02) in *Hevea* latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy

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Background: Recent studies demonstrated that allergy to natural rubber latex is frequently associated with hypersensitivity to avocado fruit. The responsible cross-sensitizing allergen has not been identified.

Objective: The purpose of this study was to investigate the cross-reactivity of a latex major allergen, hevein, with avocado proteins.

Methods: Serum samples from 118 health care workers (HCWs) allergic to latex (HCW group) and 78 patients with spina bifida (SB) allergic to latex (SB group) were included in this study. Anti-hevein and anti-avocado IgE antibodies were measured by enzyme-linked allergosorbent assay. Cross-reactivity of hevein to avocado proteins was assessed by inhibition of the IgE binding in individual patients' sera containing IgE antibodies to both hevein and avocado.

Results: The prevalence of seropositive IgE antibodies to avocado was found to be strongly associated with the presence of hevein-specific IgE antibodies in subjects of both groups ($P < .001$). Sixty-seven of 91 (73%) subjects from the HCW group and all 19 subjects in the SB group with positive IgE antibodies to hevein also had elevated IgE values to avocado. Competitive RAST inhibition with 42 sera showed that IgE binding to avocado could be completely inhibited in 27 (64%) sera by preincubation with hevein. By contrast, the degrees of inhibition of IgE to hevein by avocado extract ranged from 0% to 36% ($n = 16$). These results indicate that sensitization to avocado in most patients allergic to latex is caused exclusively by IgE-binding epitopes present in hevein. Results of immunoblots and immunoblot inhibition with 11 serum samples confirmed that a 30-kd protein in avocado was the major IgE-binding component; the IgE-binding reactivity to this protein could be inhibited by hevein in all sera tested.

Conclusion: Hevein is the major cross-reacting allergen with avocado in subjects with latex allergy. (*J Allergy Clin Immunol* 1998;102:476-81.)

Key words: Latex allergy, avocado allergy, hevein, cross-reactivity, IgE, immunoblotting

Abbreviations used

EAST:	Enzyme allergosorbent test
HCW:	Health care worker
NRL:	Natural rubber latex
SB:	Spina bifida
TBS:	Tris-buffered saline

IgE-mediated immediate-type allergic reactions to proteins of natural rubber latex (NRL) represent an important occupational problem, especially among health care workers (HCWs) because of the frequent use of latex gloves for protection from bacterial and viral infections.¹⁻⁴ Up to 17% of clinic employees were estimated to be sensitized to latex.^{4,5} Recent studies have shown that allergy to NRL is significantly associated with hypersensitivity to foods or fruits, including avocado, chestnut, papaya, kiwi, and banana.⁶⁻¹¹ The high prevalence of seropositive IgE antibodies to avocado proteins in patients allergic to latex is particularly evident. Previous reports showed that 46.3% to 78% of patients with verified latex allergy also had IgE antibodies to avocado.^{10,12-15} Cross-reacting IgE antibodies to both latex and avocado allergens were demonstrated by immunoblot inhibition and RAST inhibition, in which the IgE binding to avocado could be inhibited with latex extracts to a high degree. However, less information is available regarding the nature of the major allergens that are responsible for the IgE cross-reactivity.

Hevein (Hev b 6.02), a small protein with only 43 amino acids, is the mature form of hevein family proteins in NRL, which have been identified as the most common allergens for HCWs allergic to latex.¹⁶⁻¹⁹ In our previous work²⁰ we demonstrated that about 75% of HCWs allergic to latex had hevein-specific IgE antibodies. High-sequence homology of hevein to a broad family of plant defense proteins has been also reported^{21,22} and is believed to contribute to the increased prevalence of food allergies in individuals allergic to latex.²³

The purpose of this study was to investigate the cross-reactivity of hevein with avocado proteins. One hundred ninety-six serum samples from HCWs allergic to latex ($n = 118$) and patients with spina bifida (SB) ($n = 78$) were tested for IgE antibodies to hevein and avocado extracts.

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Samples showing strongly positive IgE antibodies to both hevein and avocado proteins were then tested by competitive inhibition assays to investigate the role of hevein in the development of sensitization to avocado in patients allergic to latex.

METHODS

Study subjects

Serum samples from 118 HCWs allergic to latex (HCW group: 26 men/92 women) and 78 patients with SB (SB group: 43 men/35 women) were included in this study. The serum samples were consecutively collected between July 1993 and December 1996. All of the subjects were previously given a diagnosis of latex allergy on the basis of clinical history and showed the presence of specific IgE antibodies to latex in their sera by latex-RAST (CAP system, Pharmacia). In the HCW group, the mean age of the subjects was 29.9 years (range, 19 to 52 years). The level of latex-specific IgE antibodies ranged from 0.73 to greater than 100 kU/L (median, 7.87 kU/L), and all of the subjects in the HCW group also had positive skin responses to latex extract as determined by skin prick testing. According to the results from skin prick tests with 5 environmental allergens, 89% of the HCWs allergic to latex were found to be atopic (positive response to at least 1 allergen). In the SB group the mean age of the patients was 10.4 years (range, 2.4 to 21.7 years), and the IgE antibodies to latex ranged from 0.46 to greater than 100 kU/L (median, 10.20 kU/L). On the basis of questionnaire data and positive IgE levels to environmental allergens tested by CAP system (positive SX1-test), 32% of them were atopic. A serum pool from 10 individuals (HCWs) with latex allergy was also prepared and studied. Twelve additional sera, 10 from nonallergic adults and 2 from patients with SB but without latex allergies, were used as negative controls.

Preparation of allergen and allergen extracts

Latex protein extract. Soluble latex protein extract was prepared by centrifugation of fresh, ammoniated (0.7%) latex sap from Malaysia as described previously.²⁰ Results of 2-dimensional electrophoresis of the latex proteins and immunostaining with mAbs directed against Hev b 1²⁴ indicated that the latex protein extract contains a corresponding protein spectrum as previously reported.²⁵

Hevein. Highly purified hevein (Hev b 6.02, 4.7 kd) was isolated from the latex protein extract mentioned above and characterized by HPLC and mass spectrometry as previously described.²⁰

Avocado extract. For preparation of avocado protein extract, 750 g of avocado pear pulp were mixed with 1 L of PBS containing 500 mg EDTA and 1760 mg ascorbic acid (pH 7.4) at 4° C for 12 hours. The resultant mixture was first centrifuged at 3600 g and 4° C for 15 minutes to remove the most precipitate and further centrifuged at 12,000 g and 4° C for 45 minutes to remove the residual particles. The supernatant was then collected and dialyzed against distilled water for 24 hours at 4° C and finally freeze-dried. The protein content in extracts was determined by Lowry's method,²⁶ with ovalbumin as the reference protein.

Determination of specific IgE antibodies

IgE antibodies to the whole latex allergens were determined by the CAP system by using commercial solid-phase latex allergen (latex ImmunoCAP) from Pharmacia Upjohn (Uppsala, Sweden), according to the manufacturer instructions.

The detection of specific IgE antibodies against avocado in patients with latex allergy was carried out with the enzyme allergosorbent test (EAST) by using the method described previously.²⁰ Briefly, the avocado protein extract was coupled onto cyanogen bromide-activated paper discs in coupling buffer (0.1 mol/L sodium

bicarbonate solution, pH 8.4) at a protein concentration of 2 mg/mL. The allergen-coated discs were then used as target antigen and incubated with 50 μ L of each patient's serum for 180 minutes at room temperature. A Phadezym RAST test kit (Pharmacia) was used to estimate the specific IgE values in the EAST. Serum samples with IgE concentrations of 0.35 kU/L or greater were regarded as positive, as recommended by Pharmacia. All positive samples were assayed a second time to confirm the results by using the CAP system with avocado ImmunoCAP as the solid-phase allergen. In addition, the samples with anti-avocado IgE concentrations between 0.35 and 0.7 kU/L were further examined with the CAP system by means of an autoinhibition assay to verify the binding specificity. The results were then regarded as positive if the IgE binding was able to be completely inhibited by avocado protein solution.

Hevein-specific IgE antibodies in serum samples were determined by using EAST with discs coated with purified hevein as the target allergen as previously described.²⁰

Immune inhibition assay

The cross-reactivity of latex-allergen hevein with IgE-binding proteins in avocado was assessed by competitive immune inhibition assays by means of the CAP system. Ten microliters of hevein solution at a concentration of 0.7 mg/mL in dilution buffer (PBS containing 1% BSA) was mixed with 50 μ L patient serum. The individual antigen-antibody mixes were allowed to equilibrate overnight at 4° C and were then transferred into wells containing solid-phase allergen avocado-ImmunoCAP. The remaining IgE antibodies binding to avocado allergens were then determined by means of the CAP system as described above. As control, the avocado-specific IgE concentration in sera was predetermined with the same process by mixing 50 μ L patient serum with 10 μ L dilution buffer. In addition, latex allergen Hev b 1 and an enzyme allergen Asp o 2 (*Aspergillus oryzae*) were also used as inhibitors for control purposes. To assure analogous conditions, the sera with specific IgE concentrations greater than 15 kU/L were prediluted to give a final IgE concentration of about 5 to 10 kU/L. Results were expressed as the percent inhibition of the reaction of IgE in the absence of inhibitor.

In much the same way, the ability of the whole latex allergens (inhibitor concentration: 1 mg/mL) to inhibit the IgE binding to avocado proteins or that of avocado proteins to block the IgE binding to hevein (performed by EAST inhibition) or to latex proteins (performed by CAP-system) were also investigated.

Electrophoresis, immunoblotting, and immunoblot inhibition

One-dimensional SDS-PAGE and immunoblot analysis of the protein extract from avocado were performed as described before,²⁰ with minor modification. Briefly, 120 μ g of avocado proteins were separated on homogenous 10% NuPAGE gel (Novex, San Diego, Calif) under reducing conditions. For semidry blotting, proteins on the gel were transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, Mass) by using a semidry system (16 \times 16 cm) of Roth (Karlsruhe, Germany), with a discontinuous buffer system at 0.8 mA/cm² membrane for 1 hour at room temperature. After blocking with a solution consisting of 1% BSA and 1% polyvinylpyrrolidone (both from Sigma) in Tris-buffered saline (TBS; 50 mmol/L Tris/HCl and 150 mmol/L NaCl [pH 7.4], containing 5 mmol/L Na₂SO₄ and 1 mmol/L EDTA) and washing with TBS, membranes were cut into individual 4-mm wide strips. By immunoassay, the strips were incubated individually with 800- μ L serum samples diluted 1:10 in TBST-BSA (2% BSA dissolved in TBS containing 0.05% [vol/vol] Tween 20) for 18 hours at 4° C. The binding of specific IgE antibodies to the proteins was detected with alkaline phosphatase-conjugated anti-human IgE antibodies

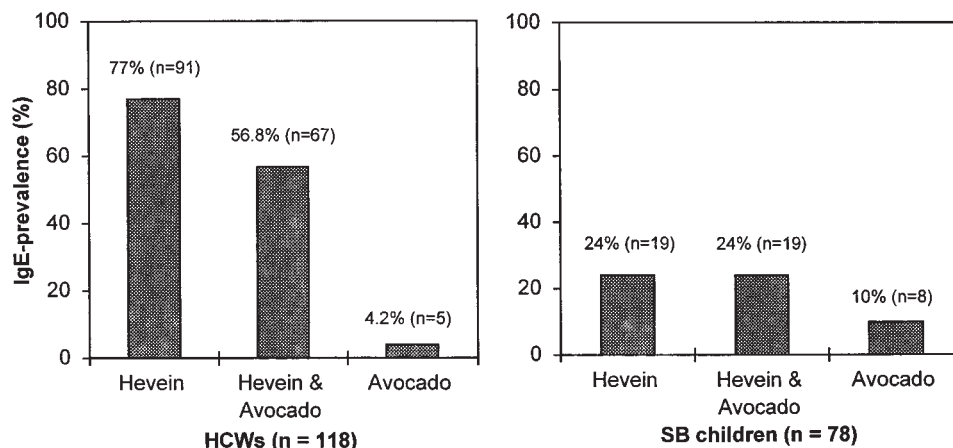


FIG 1. Prevalence of IgE antibodies to hevein (left column), to hevein and avocado (middle column), and to avocado proteins alone (right column) among HCWs and children with SB who have latex allergy.

TABLE I. Results of reciprocal inhibition of IgE binding in sera from patients with IgE antibodies to latex, hevein, and avocado

Number of sera tested	Solid-phase antigen	Inhibitor	Degree of inhibition in sera					Total
			100%	75%-95%	50%-75%	25%-50%	0%-25%	
42	Avocado*	Latex	35	2	4	1	0	42
42	Avocado*	Hevein	27	5	3	4	3	42
16	Latex*	Avocado	0	0	3	3	10	16
16	Hevein†	Avocado	0	0	0	2	14	16

*Immune inhibition assays were performed with the CAP system.

†EAST-inhibition with disks coated with hevein as solid-phase antigen.

(Sigma) in TBST-BSA (diluted 1:2000 for 2 hours at room temperature), and bands were visualized with a substrate solution containing 0.4 mmol/L 5-bromo-4-chloro-3-indolyl phosphate disodium salt and 0.37 mmol/L p-nitro blue tetrazolium chloride in 0.1 mol/L NaHCO₃ buffer, containing 10 mmol/L MgCl₂ (pH 9.6). Sera from patients allergic to latex but showing no IgE antibodies to avocado were used as controls.

To access the cross-reacting allergens in avocado with hevein, immunoblot inhibition assays were performed with hevein as the inhibitor. The procedure of immunoblot inhibition assay was the same as immunoblotting described above except that each diluted serum sample was incubated with 20 μ L of hevein solution (0.7 mg/mL) for 16 hours at 4° C before exposure with the blotted avocado proteins.

RESULTS

Prevalence of sensitization to hevein and avocado

To analyze the prevalence of sensitization to hevein and avocado in patients with latex allergy, 196 serum samples from HCWs allergic to latex ($n = 118$) and patients with SB ($n = 78$) were tested by EAST for specific IgE antibodies against hevein and avocado proteins. Twelve sera from individuals without a history of latex allergy were also used as controls. As shown in Fig 1, in the HCW group 91 (77%) samples exhibited elevated IgE antibodies to hevein, and 72 (61%) were found to have positive IgE antibodies to avocado proteins. Of them, 67 exhibited IgE antibodies to both hevein and

avocado proteins. The IgE concentrations to hevein and avocado ranged from 0.47 to 26.7 kU/L (median, 2.90 kU/L) and 0.36 to 19.6 kU/L (median, 1.12 kU/L) by EAST, respectively. In the SB group 19 of 78 serum samples (24%) tested showed positive IgE antibodies against hevein, and the IgE concentration ranged from 0.55 to 20.9 kU/L (median, 4.07 kU/L and geometric mean, 8.03 kU/L). Twenty-seven (34.6%) serum samples, including the 19 hevein-positive sera, showed positive IgE antibodies to avocado. The IgE concentration to avocado ranged from 0.37 to 21.91 kU/L (median, 1.46 kU/L and geometric mean, 3.86 kU/L). The positive IgE results to avocado as determined by EAST were confirmed by RAST in the CAP system. All 12 control sera showed negative IgE results to hevein, as well as to avocado.

Competitive RAST inhibition

Competitive inhibition assays were performed to investigate the cross-reactivity of latex or hevein with avocado proteins by using 42 serum samples from patients with IgE antibodies to both hevein and avocado (30 from HCWs and 12 from children with SB). The inhibition results shown in Table I indicate that in 35 serum samples (83%) the IgE binding to avocado proteins could be completely inhibited by preincubation of 50 μ L serum with 10 μ g latex protein extract, and in the other 6 sera the inhibition ranged from 55% to 95%. By

using hevein as an inhibitor, a complete inhibition of IgE binding to avocado was observed in 27 serum samples (64%), suggesting that the sensitization to avocado in these 27 patients was caused exclusively by IgE-binding epitopes present in hevein. In 5 serum samples the inhibition of IgE binding to avocado by hevein ranged from 75% to 95%, and in the other 10 sera the maximum inhibition by hevein was found to be smaller than 70% (range, 10% to 65%).

The ability of avocado proteins to inhibit the IgE binding to latex or hevein was also investigated. Sixteen of the above 42 sera, 8 from HCWs and 8 from patients with SB, showing complete inhibition of IgE binding to avocado by latex, as well as by hevein, were selected for the inhibition assays. Interestingly, in none of these 16 sera could the IgE antibodies to latex or to hevein be completely inhibited by preincubation of serum with 20 μ g of avocado proteins. In the 8 sera from HCWs, the inhibition of IgE binding to latex by avocado with the CAP system ranged from 3% to 52%, and in the other 8 sera from the SB group, the maximum inhibition of IgE binding to latex by avocado was 22%, with no inhibition detected in 2 subjects. In EAST inhibition the maximum inhibition of IgE binding to hevein by avocado was 36%. Moreover, 8 ($n = 16$) sera showed no detectable inhibition of IgE binding to hevein by preincubation of the sera with avocado proteins. These results indicate that besides the shared IgE-binding epitopes with avocado, hevein also possesses IgE-binding determinants that cannot be blocked by avocado proteins.

An inhibition assay with a serum pool from 10 HCWs with latex allergy was also performed. As shown in Fig 2, the inhibition of IgE to avocado by hevein was dose dependent, and the IgE binding to avocado was inhibited up to 80%. In fact, the maximum inhibition was achieved by less than 10 ng of hevein; increasing the amount of inhibitor up to 10 μ g of hevein resulted in no additional inhibition of IgE binding to avocado. However, by using the same serum pool as the IgE source, less than 20% of the IgE binding to latex allergens (measured by CAP system) and to hevein (measured by EAST inhibition) could be inhibited by preincubating the serum with up to 20 μ g of avocado proteins (Fig 2). As controls, 6 serum samples were randomly selected and tested by EAST inhibition with avocado discs as solid-phase antigen and latex Hev b 1 (10 μ g, $n = 6$) and Asp o 2 (10 μ g, $n = 3$) as inhibitors. Only 1 serum sample showed a slight inhibition (21%) of IgE binding to avocado by Hev b 1.

Immunoblotting and immunoblot inhibition

An immunoblot inhibition assay was performed to characterize the possible cross-reacting proteins to hevein in avocado. The avocado protein extract was first separated by SDS-PAGE, and the proteins were then transferred onto a polyvinylidene difluoride membrane. Ten sera and 1 serum pool with increased IgE antibody levels to avocado proteins, as well as to hevein, were used in the study. In addition, in 6 of these 10 sera (lanes 1 to 6 in Fig 3), the IgE-binding reactivities to avocado

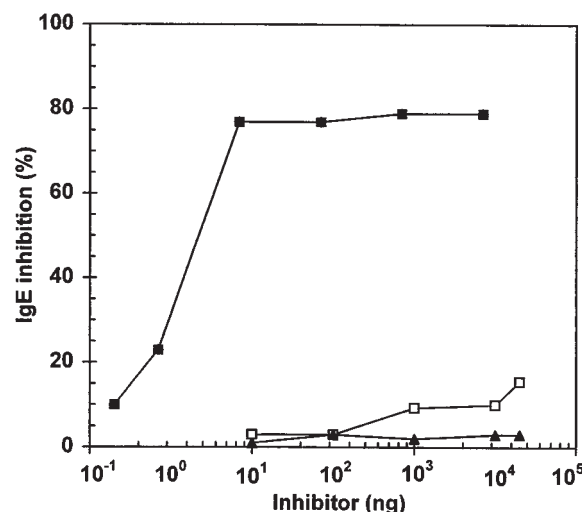


FIG 2. Dose-related inhibition of IgE binding to avocado proteins by hevein (filled squares) and inhibition results of IgE binding to hevein (open squares) and latex (filled triangles) by avocado extract by using a serum pool.

could be completely inhibited by hevein in EAST. The immunoblotting results shown in Fig 3 indicate that IgE antibodies in 9 of the 10 sera and the serum pool recognized a 30-kd protein of avocado. Some sera also showed binding reactivity to components with molecular weights of 26 kd (lanes 7 to 10), 36 kd (lanes 6 to 9), and about 55 kd (lanes 7 and 9). After preincubation with hevein solution, all serum samples lost their IgE-binding reactivity to the 30- and 36-kd proteins on the membrane blots, whereas the IgE-binding reactivity to other protein bands of 26 and 55 kd were not affected by hevein. These results indicate that most subjects allergic to latex have elevated IgE antibody levels to a 30-kd protein in avocado, but sensitization to hevein is the main reason for the observed cross-reactivity and the development of latex-associated avocado allergies. Four control sera from non-allergic subjects showed no binding reactivity to avocado proteins on the membrane blots (data not shown).

DISCUSSION

The aim of this study was to investigate the cross-reactivity potential of hevein from *Hevea* latex in patients with allergies to both latex and avocado. Recently,²⁰ we demonstrated that hevein (4.7 kd, Hev b 6.02) is one of the most common allergens in latex. However, among the 2 risk groups (HCWs and patients with SB) exhibiting the highest risk of having latex allergies, the prevalence of sensitization to hevein is rather different. Although about 75% of HCWs with latex allergy are sensitized to hevein, less than 30% of the patients with SB who have latex allergy showed IgE antibodies against hevein in their sera. Results of serologic measurement presented in this study confirmed our prior findings: 91 of 118 sera from HCWs and 19 of 78 from patients with SB showed positive anti-hevein IgE antibodies for a prevalence of 77% and 24%, respectively. It is obvious that a similar

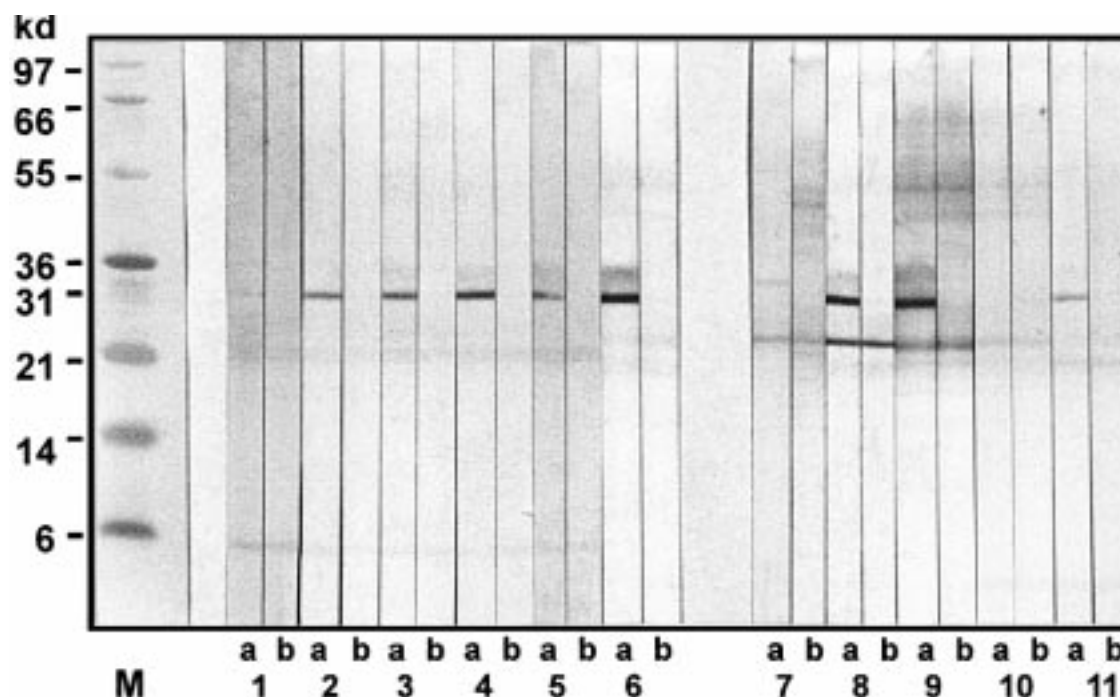


FIG 3. Immunoblotting and immunoblot inhibition results showing IgE-binding reactivity to avocado proteins in 10 sera (lanes 1 to 10) and serum pool (lane 11) from patients allergic to latex. Lane M, molecular weight markers. Strips indicated with *a* show the serum IgE-immunoblot reactivity to avocado proteins. Strips marked with *b* show results of immunoblot inhibition assay in which sera were individually preincubated with hevein solution before reacting with avocado proteins on membrane blots.

prevalence of sensitization to avocado was found among both patient groups; the rate of sensitization to avocado in HCWs and patients with SB with latex allergy was 61% (72 of 118) and 34.6% (27 of 78), respectively. In addition, in both patient groups the subjects with positive anti-hevein IgE antibodies were at significantly higher risk to be seropositive for anti-avocado IgE. The odds ratio for anti-avocado IgE, given positive IgE responses to hevein, was 6.63 (95% CI, 2.57 to 17.1; $P = .0001$) in HCWs, and in patients with SB all 19 subjects with positive anti-hevein IgE antibodies also had IgE antibodies to avocado ($P < 1 \times 10^{-8}$).

Avocado pear is an exotic fruit in Germany. According to the results of a questionnaire answered by parents about the exposure to avocado in the children with SB studied, only 1 of the 30 avocado-positive subjects with SB had a history of eating avocado. The sensitization to avocado is therefore unlikely to be induced by avocado itself. The strong association of IgE antibodies to hevein and avocado suggests that sensitization to hevein might play a key role in the development of seropositivity to avocado in patients with latex allergy.

These findings were further confirmed by the results of RAST inhibition assays. Our cross-inhibition experiments demonstrated that hevein shares common IgE-binding epitopes with avocado proteins because addition of hevein to serum samples significantly inhibited the binding of IgE antibodies to avocado. Of 42 sera tested in the inhibition experiments, anti-avocado IgE antibodies could be completely inhibited by hevein in 27 (64%) serum samples. As

compared with the inhibition obtained with latex proteins as an inhibitor in which a complete inhibition of anti-avocado IgE by latex was observed, a complete inhibition by hevein was found in 77% of serum samples (27 of 35). These results clearly indicated that latex hevein is the dominant cross-reacting allergen to avocado.

By contrast, the binding of IgE antibodies to hevein, as well as to latex, could only be weakly inhibited in the case of avocado proteins used as inhibitors. In all 16 sera studied that showed complete inhibition of IgE binding to avocado by latex and hevein, the rate of inhibition of IgE to latex or to hevein by avocado did not exceed 60%. Furthermore, in 8 of these 16 sera, no evident inhibition of the IgE binding to hevein by avocado was observed. From these results we conclude that hevein and avocado proteins share some, but not all, of their IgE-binding epitopes. The hevein molecule contains individual IgE-binding epitopes that are not present on the avocado proteins, and these allow hevein to strongly bind with IgE antibodies, even if some binding sites have been blocked by the shared epitopes on avocado proteins.

Results from immunoblot and immunoblot inhibition assays suggest that the major IgE-binding component in avocado is a 30-kD protein. In 9 of 10 sera and in a pooled serum sample, this protein was strongly bound with IgE antibodies. After preincubation of the sera with hevein in fluid phase, the IgE-binding reactivity to this protein was completely inhibited, indicating that this 30-kD protein is the major cross-reacting allergen to hevein in avocado. In 4 serum samples (lanes 7 to 10 in Fig 3)

FIG. 4. Comparison of amino acid sequence of avocado 30-kd allergen (1 to 15) obtained by N-terminal sequencing, with avocado class I endochitinase (1 to 35) and latex hevein. ; indicates identical amino acid residues.

- and in the serum pool, additional reactive bands with molecular weights of about 26 and 55 kd are present, and their IgE-binding reactivity could not be blocked by hevein. These findings explain the partial inhibition of anti-avocado IgE by hevein in these sera.
- Anaphylactic reactions to avocado have been reported in patients presensitized to latex and vice versa.²⁷⁻²⁸ Several studies have investigated cross-reactivity between latex and avocado or other food allergens.¹³⁻¹⁵ By using immunoblot and immunoblot inhibition methods, Ahlroth et al.¹⁵ demonstrated that a number of avocado proteins were able to bind with IgE antibodies from patients with allergy to latex and avocado, and the major allergen in avocado seemed to be a protein with a molecular weight of about 30 kd. Moreover, they found that the IgE-binding reactivity to this 30-kd protein could be inhibited by latex extract.^{14,15} But in these studies the molecular nature of the components responsible for the cross-reactivity was not identified. Our IgE-binding data for hevein and the allergens in avocado represent a first example of cross-reactivity between a fully characterized latex allergen and its counterpart from avocado. Investigations on the molecular immunologic properties of the cross-reacting 30-kd allergen in avocado are in progress. Our preliminary results by protein sequencing suggest that the 30-kd avocado allergen is a class I endochitinase containing a hevein domain at the N-terminus (Fig 4). The detailed results about cross-reacting allergens in avocado will be published separately.
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- ## REFERENCES
1. Turjanmaa K, Alenius H, Mäkinen-Kijunen S, Reunala T, Palosuo T. Natural rubber latex allergy. *Allergy* 1996;51:593-602.
 2. Yassin MS, Lierl MB, Fisher TJ, O'Brien K, Cross J, Steinmetz C. Latex allergy in hospital employees. *Ann Allergy* 1994;72:245-9.
 3. Grzybowski M, Ownby DR, Peyser PA, Johnson CC, Schork MA. The prevalence of anti-latex IgE antibodies among registered nurses. 1996;98:535-44.
 4. Turjanmaa K. Incidence of immediate allergy to latex gloves in hospital personnel. *Contact dermatitis* 1987;17:270-5.
 5. Lagier F, Vervloet D, Lhermet I, Poyen D, Charpin D. Prevalence of latex allergy in operating room nurses. *J Allergy Clin Immunol* 1992;90:319-22.
 6. Lavaud F, Cossart C, Reiter V, Bernard J, Deltour G, Holmquist I. Latex allergy in patients with allergy to fruit. *Lancet* 1992;339:492-3.
 7. Blanco C, Carrillo T, Castillo R, Quirarte J, Cuevas M. Avocado hypersensitivity. *Allergy* 1994;49:454-9.
 8. Vandenplas O, Vandezande LM, Hallqvist JL, Delwiche JP, Jamart J, Looze Y. Association between sensitization to natural rubber latex and papain. *J Allergy Clin Immunol* 1996;97:1421-4.
 9. Baur X, Chen Z, Rozynek P, Duser M, Raulf-Heimsoth M. Cross-reacting IgE antibodies recognizing latex allergens, including Hev b 1 as well as papain. *Allergy* 1995;50:604-9.
 10. Beezhold DH, Sussman GL, Liss GM, Chang NS. Latex allergy can induce clinical reactions to specific foods. *Clin Exp Allergy* 1996;26:416-22.
 11. Ross BD, McCullough J, Ownby DR. Partial cross-reactivity between latex and banana allergens. *J Allergy Clin Immunol* 1992;90:409-10.
 12. Blanco C, Carrillo T, Castillo R, Quirarte J, Cuevas M. Latex allergy: clinical features and cross-reactivity with fruits. *Ann Allergy* 1994;73:309-14.
 13. Brehler R, Theissen U, Luger T. "Latex-fruit syndrome": frequency of cross-reacting IgE antibodies. *Allergy* 1997;42:404-10.
 14. Lavaud F, Prevost A, Cossart C, Guerin L, Bernard J, Kochman S. Allergy to latex, avocado pear, and banana: evidence for a 30 kd antigen in immunoblotting. *J Allergy Clin Immunol* 1995;95:557-64.
 15. Ahlroth M, Alenius H, Turjanmaa K, Mäkinen-Kijunen S, Reunala T, Palosuo T. Cross-reacting allergens in natural rubber latex and avocado. *J Allergy Clin Immunol* 1995;96:167-73.
 16. Beezhold DH, Sussman GL, Kostyal DA, Chang NS. Identification of a 46-kD latex protein allergen in health care workers. *Clin Exp Immunol* 1994;98:408-13.
 17. Alenius H, Kalkkinen N, Lukka M, Reunala T, Turjanmaa K, Mäkinen-Kijunen S, et al. Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. *Clin Exp Allergy* 1995;25:659-65.
 18. Chen Z, Posch A, Raulf-Heimsoth M, Baur X. Isolation and identification of hevein as a major IgE-binding polypeptide in *Hevea* latex [abstract]. *J Allergy Clin Immunol* 1996;97:982.
 19. Alenius H, Kalkkinen N, Reunala T, Turjanmaa K, Palosuo T. The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43 amino acid fragment, hevein. *J Immunol* 1996;156:1618-25.
 20. Chen Z, Posch A, Lohaus CH, Raulf-Heimsoth M, Mayer HE, Baur X. Isolation and identification of hevein as a major IgE-binding polypeptide in *Hevea* latex. *J Allergy Clin Immunol* 1997;99:402-9.
 21. Soedjanaatmadja UMS, Subroto T, Beintema JJ. Processed products of the hevein precursor in the latex of the rubber tree (*Hevea brasiliensis*). *FEBS Lett* 1995;363:211-3.
 22. Beintema JJ, Peumans WJ. The primary structure of stinging nettle (*Urtica dioica*) agglutinin—a two-domain member of the hevein family. *FEBS Lett* 1992;299:211-3.
 23. Beezhold DH, Kostyal DA, Sussman GL. IgE epitope analysis of the hevein preprotein: a major latex allergen. *Clin Exp Immunol* 1997;108:114-21.
 24. Chen Z, van Kampen V, Raulf-Heimsoth M, Baur X. Allergenic and antigenic determinants of latex allergen Hev b 1: peptide mapping of epitopes recognized by human, murine and rabbit antibodies. *Clin Exp Allergy* 1996;26:406-15.
 25. Posch A, Chen Z, Wheeler C, Dunn MJ, Raulf-Heimsoth M, Baur X. Characterization and identification of latex allergens by two-dimensional electrophoresis and protein microsequencing. *J Allergy Clin Immunol* 1997;99:385-95.
 26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. *J Biol Chem* 1951;193:265-75.
 27. Lavaud F, Cossart C, Reiter V, Bernard J, Deltour G, Holmquist I. Latex allergy in patient with allergy to fruit [letter]. *Lancet* 1992;339:492-3.
 28. Ceuppens JL, Van Durme P, Doms-Gooms A. Latex allergy in patient with allergy to fruit [letter]. *Lancet* 1992;339:493.