

Identification, Cloning, and Sequence of a Major Allergen (Hev b 5) from Natural Rubber Latex (*Hevea brasiliensis*)*

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Proteins in commercial latex products, derived from the rubber tree *Hevea brasiliensis*, cause anaphylaxis in susceptible individuals, especially health care workers and children with spina bifida. To identify latex allergens, we utilized IgE from the serum of a latex-allergic health care worker to screen a cDNA library from *Hevea* latex. The identified cDNA clone, cDNA Hev b 5, encodes an open reading frame of 163 peptide residues. Hybridization analysis of cDNA Hev b 5 with RNA extracted from *Hevea* tissue indicates that the full-length transcript is about 1000 bases. The nucleotide and deduced protein sequences have significant homology to sequences from kiwi and potato, which are known to cause allergic reactions in some latex-allergic patients. Fifty-six percent of spina bifida patients and 92% of health care workers with latex allergy have IgE specific to the protein encoded by cDNA Hev b 5. A monoclonal antibody raised from a mouse immunized with Hev b 5 binds to a protein in *Hevea* latex with an M_r identical to that of the expressed and cleaved recombinant protein. Taken together, these results establish that the antigen Hev b 5 contains a major epitope for IgE-mediated reactions to *H. brasiliensis* latex products.

Natural rubber latex in medical devices and consumer products has been implicated in over 1100 anaphylactic reactions that resulted in at least 15 deaths between 1988 and 1992 (1, 2). These reactions are caused by proteins that elute from the surfaces of certain latex products, especially gloves, barium enema catheters, balloons, and condoms. The proteins bind to antigen-specific IgE on tissue mast cells and trigger an anaphylactic response. While the production of IgE to latex proteins can occur in any individual, some individuals with uniquely high exposure to latex products appear to be at significantly greater risk for the production of these antibodies than the general population. These include health care workers and children with spina bifida (3–5). Interestingly, there appears to be significant clinical and immunochemical cross-reactivity between some latex proteins and allergens in certain fruits and vegetables, such as banana, kiwi, avocado, and potato, and patients with fruit and vegetable allergy may be at

increased risk for reacting to latex proteins (6–10).

Latex allergy can be a devastating disease, with serious and occasionally fatal outcomes. The only treatment is avoidance (11, 12), and health care workers with severe latex allergy may be required to change careers or leave health care entirely to avoid potentially dangerous exposure. Since anaphylactic episodes have occurred following skin testing with crude latex extracts (13–16), immunotherapy is not possible with currently available crude preparations. The identification and isolation of pure antigens, and the analysis of the B- and T-cell epitopes of these antigens, will facilitate the design and testing of safer regimens of immunotherapy.

The proteins in natural rubber latex are derived from the commercial rubber tree, *Hevea brasiliensis*. Latex is produced in laticifers, which are specialized structures that consist of anastomosed latex-producing cells. Harvested *Hevea* latex is a complete cytosol with a high protein content (17, 18). *Hevea* latex is typically ammoniated when tapped to prevent premature coagulation and bacterial growth. Ammoniated latex is the source material for most of the products that elicit allergic reactions; thus, it clearly contains immunogenic material. However, immunochemical studies with ammoniated latex have failed to reveal the wealth of IgE-binding proteins found in non-ammoniated latex (NAL)¹ (19), which is collected into a liquid nitrogen-cooled container.

From the first observations that products made from *Hevea* latex can elicit catastrophic allergic reactions in susceptible individuals, the component allergens have eluded conclusive identification. In part, this has been due to the complexity and instability of the proteins of *Hevea* latex. In addition, many investigators have been unable to obtain quantities of fresh material for examination and have been limited to the use of finished products or ammoniated latex (19) for their studies. In spite of these difficulties, several potential allergens have been identified in *Hevea* latex, including rubber elongation factor (Hev b 1) (20–24), hevein preprotein (25, 26), heveamine (26, 27), β -1,3-glucanase (Hev b 2) (28), a 24-kDa protein (Hev b 3) (23, 29, 30), and a component of the microhelix protein complex (Hev b 4) (28). In each of these investigations, protein has been isolated from natural *Hevea* latex or from finished products (usually gloves) and identified as an allergen by Western blot, radioallergosorbent testing (RAST), or RAST inhibition, using

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) U42640.

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¹ The abbreviations used are: NAL, non-ammoniated latex; cDNA Hev b 5, screened DNA sequence from the *H. brasiliensis* cDNA library; rHev b 5, recombinant *Hevea* peptide encoded by cDNA Hev b 5; MBP, maltose-binding protein; rHev b 5/MBP, fusion protein expressed by pMAL c-2/Hev b 5, with rHev b 5 on the COOH terminus, and MBP on the NH₂ terminus; PVDF, polyvinylidene difluoride; PCR, polymerase chain reaction; RAST, radioallergosorbent test; PAGE, polyacrylamide gel electrophoresis; CAPS, 3-(cyclohexylamino)propanesulfonic acid; HCW, health care worker; SB, spina bifida; bp, base pair(s); RACE, rapid amplification of cDNA ends.

sera from latex-allergic individuals.

In our earlier work using NAL, monoclonal antibodies detected peptides of several sizes by Western blot analysis and affinity purification techniques (31), an observation confirmed by other investigators (32). One possible explanation of this result is that many of the peptide bands observed in NAL were cleavage products of larger, parent proteins; other explanations are that several proteins contain the epitopes recognized by the monoclonals or that latex proteins aggregate under conditions of standard electrophoretic analysis. This limits the value of Western blot analysis (19, 33–35) in the definitive identification of latex allergens. We therefore screened a *Hevea* cDNA library with IgE from a latex-allergic patient, with the intent of isolating and expressing the gene product of the cDNA clone for further study.

EXPERIMENTAL PROCEDURES

Patients—Sera were obtained from health care workers ($n = 38$) and children with spina bifida ($n = 82$), as part of a study approved by the Children's Hospital Institutional Review Board. Except for 14 of the health care workers (nHCW), all of the health care workers (HCW) in the study had reported symptomatic type I allergic reactions to latex and were tested for evidence of latex-specific IgE by RAST (19). Because of their high risk of developing symptomatic latex allergy (5), sera were obtained from consecutive spina bifida (SB) patients without regard to clinical history. The serum of one HCW was selected for use in screening the cDNA library. This patient had experienced recurrent episodes of urticaria, rhinoconjunctivitis, and wheezing following exposure to latex gloves, and had been asymptomatic since following strict latex avoidance measures. The patient had no history of allergy or intolerance to fruits or other foods. The serum was positive for latex-specific IgE by RAST (19).

Screening the cDNA Library—The cDNA library, cloned in bacteriophage λ gt11, had been derived from mRNA isolated from the latex of *H. brasiliensis* clone RRIM 600 (22). *Escherichia coli* Y1090 cells were infected with recombinant λ phage containing the *Hevea* cDNA library and plated. After 4 h at 42 °C, the cultures were overlaid with nitrocellulose filters that had been impregnated with 10 mM isopropyl-1-thio- β -D-galactoside, and incubated overnight at 37 °C. The filters were lifted, blocked, and incubated in *E. coli*-adsorbed patient serum (1:5, v/v, in blocking solution) overnight at 4 °C. Filters were then washed and incubated in goat anti-human IgE conjugated to alkaline phosphatase (1:500; Kirkegaard & Perry, Gaithersburg, MD). After extensive washing, the filters were developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Life Technologies, Inc.) (36). Positive clones were verified, and the plaques were isolated and expanded. The target sequences of the clones were amplified by polymerase chain reaction (PCR) using λ forward and reverse primers (Promega), and analyzed using agarose gel electrophoresis and partial thermocycle sequencing (CircumVent, New England Biolabs) with the λ primers. One of these PCR-amplified products, designated cDNA Hev b 5 (37), was chosen for further analysis.

Generation of Subclones—The sequence cDNA Hev b 5 was inserted into the pMAL-c2 vector according to manufacturer's directions (New England Biolabs). The ligated pMAL-c2/Hev b 5 was inserted into competent XL1 blue *E. coli* (Stratagene), which were then grown and replica-plated onto an isopropyl-1-thio- β -D-galactoside/5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside plate containing ampicillin. In order to identify those clones containing sense inserts, white colonies were screened by the binding of IgE from the serum used to screen the library (38). As expected, about half of the white colonies that were screened expressed protein that bound to the IgE of the test patient.

Northern Analysis—RNA was extracted from *H. brasiliensis* leaf and stem tissue (39), (gift of K. Cornish and D. Siler, Albany, CA) obtained from 4-year-old trees grown from clone PB260. The RNA was separated by electrophoresis in a 1.2% agarose/formaldehyde gel and transferred to a GeneScreen Plus membrane (DuPont NEN). The membrane was then probed overnight at 42 °C with cDNA Hev b 5 that had been ^{32} P-labeled by the random primer labeling technique, using $\text{exo}(-)$ Klenow enzyme and [α - ^{32} P]dCTP (Prime-It II, Stratagene).

Extension of the cDNA Ends of cDNA Hev b 5—The ends of cDNA Hev b 5 were extended by the 5' and 3'-RACE techniques (Life Technologies, Inc.). For the 5' end extension, 1 μ g of *Hevea* RNA was incubated with reverse transcriptase (SuperScript, Life Technologies, Inc.) using the primer sequence 5'-AGAGTCAGTCTTTGAGCC-3'. The

cDNA was isolated from the primer and RNA, and a poly(C) tail was formed with terminal deoxynucleotidyltransferase according to manufacturer's directions. The cDNA was amplified by PCR using an anchor primer (5'-CUACUACUACUAGGCCACGCTCGACTAGTACGGGII-GGGIIGGGIIG-3') and 5'-AGAGTCAGTCTTTGAGCC-3', and then again with the nested primers 5'-CUACUACUACUAGGCCACGCTCGACTAGTAC-3' and 5'-CAUCAUCAUTCTCTGGAGTCGTTT-GATCTGC-3'. The PCR product was separated on a 2% agarose gel (NuSieve GTG, FMC Bioproducts); the largest band (about 400 bp) was excised and the DNA isolated using a centrifugal filtration device (Ultrafree-MC, Millipore).

For extension of the 3' end, 1 μ g of *Hevea* RNA was incubated with reverse transcriptase and an adapter primer (5'-GGCCACGCTCGACTAGTAC(T)₁₇-3'). The sequence was then amplified by PCR using 5'-CAUCAUCAUTGCGCAGTGTGAGGTTGAA-3' and 5'-CUACUACUACUAGGCCACGCTCGACTAGTAC-3'. The PCR product was separated on an agarose gel and purified.

Both the 3'-RACE and 5'-RACE products were inserted into the pAMP1 plasmid using uracil DNA glycosylase (Life Technologies, Inc.).

Sequence Analysis—Both the pAMP1 RACE products and the pMAL c-2/Hev b 5 plasmids were inserted into competent XL1 blue *E. coli*, and the plasmids were isolated from broth cultures using Qiagen tips according to manufacturer's directions. DNA sequences were determined using T7 DNA polymerase (Sequenase 2.0, U. S. Biochemical Corp.) using overlapping forward and reverse primers.

Expression and Purification—Broth cultures of the XL1 blue *E. coli* containing pMAL-c2/Hev b 5 were grown in the presence of isopropyl-1-thio- β -D-galactoside. Inserts cloned into the pMAL plasmid are expressed in the open reading frame of the λ EcoRI site, as a fusion protein (rHev b 5/MBP) with the *E. coli* maltose-binding protein (MBP) on the amino terminus and the expressed insert gene on the carboxyl terminus, with a Factor Xa cleavage site between the two sequences. rHev b 5/MBP was adsorbed to an amylose resin, eluted with 10 mM maltose, and concentrated with a Centrprep 3 device. Cleavage of rHev b 5/MBP was performed by incubation with Factor Xa (1%, w/w) for 4 h at 20 °C in the presence of 0.03% SDS. Cleavage products were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) after denaturation in electrophoresis sample buffer containing 0.0625 M Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, and 5% β -mercaptoethanol. Alternatively, samples were electrophoresed in SDS-PAGE gels containing 4 M urea (40) after denaturation in sample buffer containing 6.7 M urea instead of glycerol (41).

Amino Acid Sequencing of Cleaved rHev b 5/MBP—Cleaved rHev b 5/MBP was separated on a 12% SDS-PAGE and transferred to a polyvinylene difluoride membrane (PVDF, Millipore) in a CAPS/methanol buffer. After staining with Coomassie Brilliant Blue, the bands indicated were cut out and subjected to Edman degradation on an automated gas-phase sequencer (42).

Binding of Human IgE to rHev b 5/MBP—rHev b 5/MBP was bound to polyvinylchloride microtiter plates in coating buffer (0.1 M sodium carbonate/bicarbonate pH 9.6). Optimal binding of IgE in several sera was found to occur at 10 ng/well for rHev b 5/MBP, as compared with 1,000 ng/well for NAL. After overnight coating, the wells were blocked, incubated with patient sera diluted 1:3 in phosphate-buffered saline containing 3% bovine serum albumin, and finally with ^{125} I-rabbit anti-human IgE (10 ng or 6,000 cpm total; Pharmacia Biotech Inc.). Positive binding was >1% of the total and at least 300% of negative control. Base-line counts were <20 cpm. For RAST inhibition, pooled sera were premixed with rHev b 5/MBP or control buffer and incubated overnight at 4 °C. The sera were then added to polyvinyl chloride wells coated with rHev b 5/MBP, and developed as above.

Production of Mouse Hybridomas—Hybridoma lines were produced from a BALB/c mouse immunized with rHev b 5/MBP in Freund's adjuvant, as described previously (31). Cell lines were tested for production of antibody to rHev b 5/MBP, MBP, and NAL. In preliminary studies, hybridomas 7C10 and 17A10 produced detectable antibody to all three antigens by enzyme-linked immunosorbent assay, but only the antibody from 17A10 detected specific bands on Western analysis of latex proteins. Both cell lines generate antibody of the IgM class.

Animal care and protocols were reviewed and approved by the Animal Research Committee of the Children's Research Institute.

Electrophoresis, Transfer, and Immunostaining of Non-ammoniated Latex Proteins—NAL (E8 extract) was obtained from Dr. Yuan DeVries (FDA/CBER, Bethesda, MD). NAL was prepared for analysis in sample buffer containing 6.7 M urea and electrophoresed by SDS-PAGE in a SDS-PAGE gel containing 4 M urea as described above. The separated peptides were transferred to a PVDF membrane in Towbin's buffer (43), stained with Coomassie Brilliant Blue, cut into strips, and blocked for

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1 ACTTCACCTCC CTAGCATCTCT TCCTCTCCTT AAAACCATCT CTATATATTC TTTCAAGCTT
1 [S S L
61 ACACACATCC TTCATTTTGG CTTTCCAA ATG GCC AGT GTT GAG GTT GAA TCA GCT
4 H T S F I F A F Q J M A S V E V E S A>

116 GCA ACA GCA TTG CCA AAG AAT GAG ACA CCT GAG GTG ACC AAG GCT GAG GAG
22 A T A L P K N E T P E V T K A E E>

167 ACG AAG ACC GAA GAA CCT GCA GCA CCA CCT GCC TCT GAG CAA GAA ACC GCC
39 T K T E E P A A P P A S E Q E T A>

218 GAT GCT ACA CCT GAA AAA GAG GAA CCT ACA GCT GCT CCC GCA GAA CCG GAA
56 D A T P E K E E P T A A P A E P E>

269 GCT CCA GCT CCT GAA ACC GAG AAG GCT GAA GAA GTA GAA AAG ATT GAG AAG
73 A P A F E T E K A E E V E K I E K>

320 ACC GAG GAG CCT GCA CCA GAA GCA GAT CAA ACG ACT CCA GAG GAA AAG CCA
90 T E E P A P E A D Q T T P E E K P>

371 GCT GAG CCT GAG CCT GTT GCA GAG GAG GAG CCC AAA CAT GAG ACC AAA GAG
107 A E P E P V A E E E P K H E T K E>

422 ACT GAA ACA GAG GCC CCT GCA GCT CCT GCA GAG GGA GAG AAG CCA GCT GAA
124 T E T E A P A A P A E G E K P A E>

473 GAA GAG AAG CCA ATT ACT GAA GCA GCA GAG ACA GCC ACC ACA GAA GTT CCG
141 E E K P I T E A A E T T T E V P>

524 GTG GAA AAA ACA GAG GAA TAA ATAGGT CTATTATGCA TATCAAGAA
158 V E K T E End>

571 AGGGGGGGAT CGAGTTTCCTG TTATTTTAATTA TAFTTTTCTT ATTAATTTAT
621 GATTATGATT ATTAAGGATC GATTTCGATG GTTTTGAGAG ATAGAACCTA
671 GAGTCAGTCT TTGAGCAAAA ACTGGGATCC ATCTATGTCG ATCAGGGCAC
721 TTGCCAGAGA TGAGTTACTG TGAGGTCTAC ATGGATTATG GTATGTAGAT
771 GGTAGTAGAT ATTTATGGTG TGATGTGATG ATGTGATTG TTGTTTGTAT
821 AATAATGATT TCCTCTGCCA

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FIG. 1. Nucleotide and deduced peptide sequence clone cDNA Hev b 5. The DNA sequence was determined using T7 DNA polymerase (Sequenase 2.0, U. S. Biochemical Corp.). Additional extended 5' and 3' sequences were determined using the 5'- and 3'-RACE techniques (Life Technologies, Inc.) and *Hevea* RNA as template. Extended sequences are underlined. The poly(A) signal sequence (nucleotides 821–826) is in boldface type. The deduced peptide sequence is indicated below the encoding nucleotides. The bracketed amino acid residues are expressed when Hev b 5 is part of the pMAL c-2/Hev b 5 expression product, but are upstream of the putative authentic start codon (nucleotide 89). These sequence data are available from GenBank under accession number U42640.

30 min in 50 mM Tris, 150 mM NaCl, pH 7.5, containing nonfat dry milk (5%, w/v). After overnight incubation with tissue culture supernatant from either the 7C10 or 17A10 cell lines (10^{-2} , v/v, in Tris/NaCl containing 1% bovine serum albumin), strips were washed and incubated for 2 h with peroxidase-labeled goat anti-mouse IgM (Kirkegaard & Perry) (10^{-5} , in 1% bovine serum albumin). Strips were developed with a chemiluminescent peroxidase substrate (Supersignal CL-HRP, Pierce) according to manufacturer's directions.

RESULTS

Isolation and Characterization of Clone cDNA Hev b 5—The *Hevea* latex cDNA library was screened with IgE from a latex-allergic health care worker. Five immunoreactive clones were identified and purified; DNA inserts were amplified by PCR using λ forward and reverse primers. All five clones contained inserts of about 900 bp. Partial sequence analysis confirmed clone homology. The complete DNA sequence of one of these clones, cDNA Hev b 5, was determined after insertion of the sequence into the pMAL-c2 vector. The sequence of clone cDNA Hev b 5 consists of 757 nucleotides, with an open reading frame 492 nucleotides in length that is in-frame with λ gt11 fusion protein expression (Fig. 1).

We were unable to obtain RNA from *Hevea* leaf and stem tissue by any of the standard, guanidinium thiocyanate-based techniques (44). However, using the method described by Schultz *et al.* (39), in which contaminating polysaccharides and other secondary products are selectively precipitated, we isolated 0.25 mg/g of high quality *Hevea* leaf RNA for Northern analysis and extension of the cDNA ends. Hybridization analysis indicated that the full-length transcript of the cDNA Hev

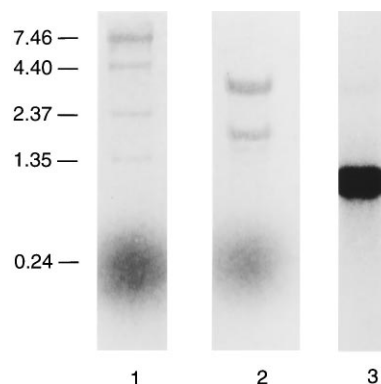


FIG. 2. Northern analysis of cDNA Hev b 5. RNA was extracted from *H. brasiliensis* tissue. On agarose gel electrophoresis, two sharp bands were observed at 3.55 and 1.97 kb, corresponding to plant ribosomal RNA. The RNA was transferred to a GeneScreen Plus membrane (DuPont NEN) and probed at 42 °C with ^{32} P-labeled pMAL c-2/Hev b 5. Lane 1, RNA standards (stained with methylene blue); lane 2, total *Hevea* RNA (stained with methylene blue); lane 3, autoradiogram after hybridization with labeled pMAL c-2/Hev b 5.

b 5 sequence is about 1000 nucleotides (Fig. 2). Using the RACE technique, we were able to identify an additional 50 bp at the 5' end, and 32 bp on the 3' end from first strand DNA, for a total of 839 bp. The 3'-RACE extension contains a putative polyadenylation signal AATAAT 19 bases upstream of the poly(A) sequence (45). The 5'- and 3'-untranslated regions are 89 and 295 bp long, respectively (Fig. 1).

In order to ascertain whether cDNA Hev b 5 is unique, a nucleotide sequence search was performed (46). Although cDNA Hev b 5 appears to be unique, three strongly homologous regions with a recently reported cDNA sequence (pKIWI501) from *Actinidia deliciosa*, the kiwi fruit, were identified (Fig. 3A). The degree of identity is 80% over a region 75 bp long, 64% over a region 54 bp long, and 67% over a region 117 bp long. All three homologous regions lie within the putative open reading frames of both sequences (47).

The deduced amino acid sequences of Hev b 5 and pKIWI501 were analyzed for maximal alignment by a weighted dynamic programming method (48). Overall, 46% of the deduced Hev b 5 sequence is identical to the aligned pKIWI501 sequence, with greatest homology at the amino and carboxyl termini (Fig. 3B). Other sequences with which Hev b 5 has significant homology are potato stolon tip protein (38%), pig neurofilament triplet L protein (36%), and rat myristoylated alanine-rich kinase C substrate (35%).

Expression and Analysis of the Recombinant Hevea Protein—In order to examine the immunoreactivity of the encoded protein, cDNA Hev b 5 was expressed as part of the pMAL-c2 fusion protein, rHev b 5/MBP. The yield was between 7 and 10 mg of rHev b 5/MBP/liter of broth, and purity after affinity chromatography on an amylose column was greater than 95% on SDS-PAGE. Under standard SDS-PAGE conditions, the M_r of affinity-purified rHev b 5/MBP was 81,000. Cleavage of rHev b 5/MBP with Factor Xa yielded two prominent bands of M_r 44,000 and 36,000 (Fig. 4). Sequence analysis indicated that the M_r 44,000 peptide is MBP, which is the leader sequence in pMAL fusion products. Edman analysis of the M_r 36,000 fragment yielded a sequence derived from the polylinker site in pMAL, the *Eco*RI adapters used in the initial library construction (22), and serine, which is the first amino acid residue of the encoded recombinant peptide. Thus, the M_r 36,000 peptide is the recombinant *Hevea* polypeptide (rHev b 5) encoded by cDNA Hev b 5 (Fig. 5).

The deduced amino acid sequence of rHev b 5 has a calculated molecular mass of 17,455 Da and a pI of 3.894, and

A

Hev b 5: 77 TTTGCTTTCCAAATGGCCAGTGTGAGGTTGAATCAGCTGCAACAGCATTGCCAAAG
 pKIWI501: 53 TTTGCTTCCCAATGGCCACTGTGAGGTTACACCTGCTGTGACAGCATTTGCCAGAA
 AATGAGACACCTGAGGTG 151
 AATGAAACAGCTGATGAG 127

Hev b 5: 216 CCGATGCTACACCTGAAAAAGAGGAACCTACAGCTGCTCCCGCAGAACCGGAAG 269
 pKIWI501: 435 CAGAGGCTCCGGCAGCAGCTGAGGAACCTACTGATGCACAGAGGCTCCGGCAG 488

Hev b 5: 428 ACAGAGGCCCTGCGAGCTCCTGCAGAGGAGAGAGCCAGCTGAAGAAGAGAGCCCA
 pKIWI501: 503 ACCAATGCACCGAGGCTCCGGCAGTGGGTGAGGAACAGAGGCCAAGAAGGGA
 ATTACTGAAGCAGCAGACAGACCCACCAAGTTCGGTGGAAAAACAGAGGAATAA 544
 CCAGATGAAGCGGTGGAGGAAGCAAGCAGACAGAGTTCAGTTGATAAACTGAGGAATAA 619

B

Hev b 5: MASVEVESAA TALPKNETPE VTKAETKTE EPAAPPASEQ ETADATPEKE
 pKIWI501: MATVEVTPAV TALPENET-E VTKPQEPQPE AAVAAPPAPA EPVTEEPEKA

Hev b 5: EPTAAPAEPE APAPETEKAE EVEKIEKTEE PAPEADQTPP EEKPAEPEPV
 pKIWI501: APEAVEAPEE PAA-AEAAAK EVEATEGKAE FTGEMKDKTP EATDAPEAPA

Hev b 5: AEEEPKHETK ETETEAPAAP AEKEKPAEEE KPITEAAETA TTEVPVEKTE E
 pKIWI501: AAEEPTDAP- EEPNAPAP AVGEEPEAKE GKPDEAVEEA STEVPVDKTE E

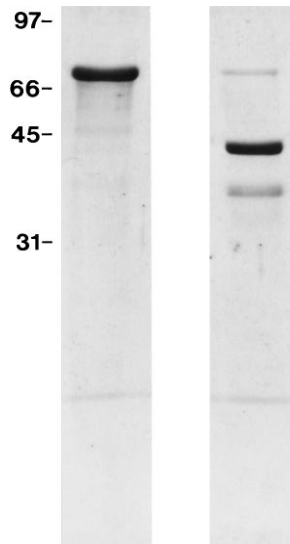


FIG. 4. **Electrophoretic analysis of Factor Xa cleavage products of rHev b 5/MBP.** rHev b 5/MBP (20 μ g) was incubated with Factor Xa (0.2 μ g) for 4 h at 20 $^{\circ}$ C in the presence of 0.03% SDS, electrophoresed, and stained with Coomassie Blue. *Left lane*, undigested rHev b 5/MBP; *right lane*, rHev b 5/MBP digested with Factor Xa. The migration of molecular mass markers is indicated on the left.

contains 163 amino acid residues, of which 46 (28%) are glutamic acid, 29 (18%) are alanine, 23 (14%) are proline, and 21 (13%) are threonine. The deduced molecular mass is in contrast to the M_r of rHev b 5 of 36,000 on SDS-PAGE (Fig. 4); this disparity has been noted for other proteins with high proline content (49) and low pI (40). When digested rHev b 5/MBP was analyzed by SDS-PAGE in the presence of 4 M urea, the M_r of the rHev b 5 band decreased by 33% to 24,000; the MBP band remained at 44,000, but the undigested fusion protein migrated at about 66,000 (Fig. 6). This indicates that most of the discrepancy between the deduced molecular mass and the M_r of rHev b 5 (and rHev b 5/MBP) is due to incomplete denaturation resulting in aberrant migration (41).

Immune Reactivity of rHev b 5/MBP—The antigenicity of

MBP M-K-T-E-E-G-K-L-V-I- W -I-N-G-D-K-
 81 kDa M-K-T-E-E-G-K-L-V-I- (N,A) -I-N-G-D-K-
 45 kDa M-K-T-E-E-

pMAL c-2 polylinker -I-E-G-R-I-S-E-
 ↓
 Eco RI adapter F-E-D-P-G-T-M-V
 ↓
 start of insert sequence S-S-L-H-T-S-
 ↓
 36 kDa I-S-E-F-E-D-P-G-T-M-V-S-

FIG. 5. **Amino-terminal sequence analysis of rHev b 5/MBP and rHev b 5.** The separated peptides shown in Fig. 4 were subjected to Edman degradation using an automated gas-phase sequencer. The published sequence for MBP is shown at the top. The experimentally determined amino-terminal sequences of the 81- and 45-kDa bands are shown below the MBP sequence in *boldface type*. The experimentally determined sequence of the 36 kDa band is shown at the bottom in *boldface type* as well, and is aligned with the predicted amino acid sequences of the expressed pMAL-c2 polylinker, EcoRI adapter (22) and rHev b 5 (Fig. 1), which are *underlined* and shown above.

the rHev b 5 portion of rHev b 5/MBP was assessed by direct binding to rHev b 5/MBP by IgE of latex-allergic patients. When the sera of NAL RAST-positive patients were examined directly, significant binding to rHev b 5/MBP was noted in 12/13 (92%) of HCW and 32/57 (56%) of SB patients. In contrast, among NAL RAST-negative patients, only 2/10 (20%) of HCW and 3/25 (12%) of SB patients had rHev b 5-specific IgE by this assay (Fig. 7). None of the sera bound to MBP alone (data not shown).

In order to demonstrate that the bound IgE in this assay was binding specifically to rHev b 5/MBP, we examined the effect of preincubating the pooled sera with soluble rHev b 5/MBP before adding the sera to the solid phase protein. At inhibiting concentrations of 1 μ g/ml rHev b 5/MBP, 98% inhibition of solid phase binding was achieved with both the spina bifida and

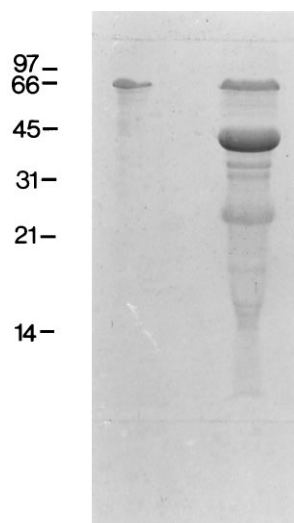


FIG. 6. Electrophoretic analysis of rHev b 5/MBP, rHev b 5, and MBP under strongly dissociating conditions. rHev b 5/MBP and its Factor Xa digest were prepared as in Fig. 4. Samples were prepared for electrophoresis in a sample buffer containing 6.7 M urea, electrophoresed by SDS-PAGE in a gel containing 4 M urea, and stained with Coomassie Blue. Left lane, undigested rHev b 5/MBP; right lane, rHev b 5/MBP digested with Factor Xa. The migration of molecular mass markers is indicated on the left.

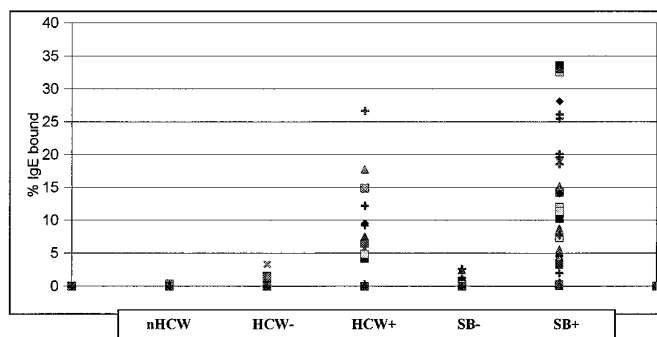


FIG. 7. Binding of human IgE to rHev b 5/MBP. Sera from HCW and SB patients were tested for binding to rHev b 5/MBP by RAST. Positive binding was $>1\%$ of the total and at least 300% of negative control. A high IgE control serum (Pharmacia, 330–350 kU/L) exhibited no binding to rHev b 5/MBP. No binding was noted by any of the sera to MBP alone (data not shown). nHCW ($n = 14$), HCW with no history or symptoms of latex allergy; HCW- ($n = 10$), HCW with a history of latex allergy but a negative NAL RAST; HCW+ ($n = 13$), HCW with a history of latex allergy and a positive NAL RAST; SB- ($n = 25$), spina bifida patients with a negative NAL RAST; SB+ ($n = 57$), spina bifida patients with a positive NAL RAST. Each patient serum is represented by a single point.

health care worker serum pools (Fig. 8).

In order to confirm that rHev b 5 is a true latex antigen, and not just an antigen to which latex-allergic patients react coincidentally with native latex allergens, we immunized mice with rHev b 5/MBP and measured the anti-latex response. When BALB/c mice were immunized with rHev b 5/MBP, they made a vigorous IgG response to rHev b 5/MBP as well as to non-ammoniated latex (50). NAL was separated by SDS-PAGE under dissociating conditions and a Western blot was performed using a monoclonal antibody (17A10) from one such mouse. The E8 NAL extract used in this experiment is the reference material prepared by the FDA, and it contains both soluble and particle-bound *Hevea* proteins (24, 51). The antibody detected a distinct peptide band at about 23 kDa, as well as several fainter bands from 30–60 kDa; the control monoclonal antibody (7C10) failed to bind to any of the NAL proteins (Fig. 9).

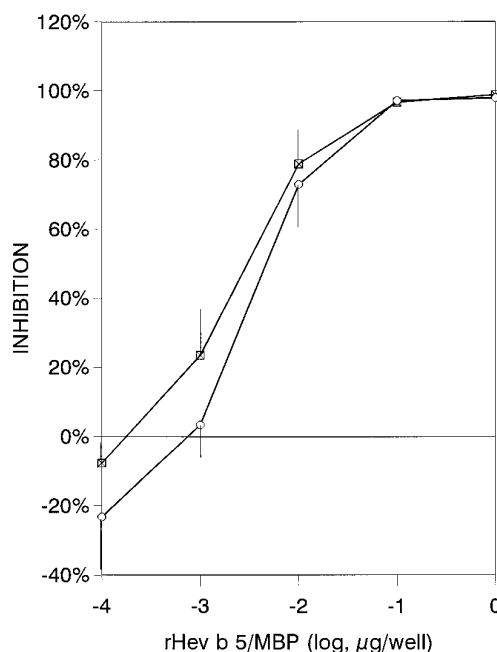


FIG. 8. Specificity of IgE binding to rHev b 5/MBP. Pooled sera were premixed with rHev b 5/MBP (in micrograms of protein as estimated by absorbance at 280 nm) or control buffer and incubated overnight at 4 °C. The sera were then added to polyvinyl chloride wells coated with rHev b 5/MBP (10 ng/well), and IgE bound to the well was determined by RAST as in Fig. 7. Percent inhibition is calculated by the formula: $(B_{\text{control}} - B_{\text{rHev b 5/MBP}})/B_{\text{control}} \times 100$, where B_{control} is the percent IgE bound of sera preincubated with control buffer, and $B_{\text{rHev b 5/MBP}}$ is the percent IgE bound of sera preincubated with rHev b 5/MBP. Circles, health care workers; squares, spina bifida patients.

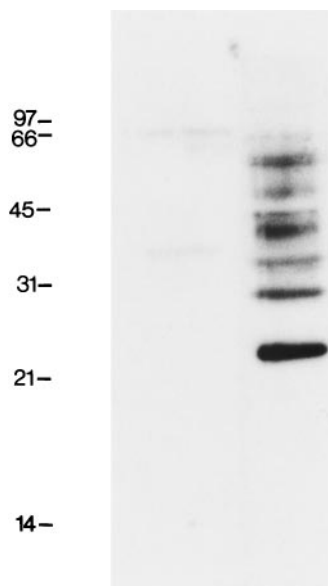


FIG. 9. Western blot of NAL with a monoclonal antibody raised to rHev b 5. NAL was separated on a 15% SDS-PAGE containing 4 M urea and transferred to PVDF. The membrane was then incubated overnight with tissue culture supernatant of hybridoma 17A10 or control cell line 7C10, diluted 10^{-2} in blocking buffer. Bound antibody was detected with peroxidase-labeled goat anti-mouse IgM, diluted 10^{-5} and a chemiluminescent peroxidase substrate (Supersignal CL-HRP, Pierce). Left lane, 7C10; right lane, 17A10. Migration of molecular weight markers is indicated on the left.

DISCUSSION

The use of the *Hevea* latex cDNA library has allowed us to examine a single, highly purified latex antigen in great detail. Northern analysis indicated that the full-length transcript of

cDNA Hev b 5 is about 1000 nucleotides long; our attempts to extend the ends of the isolated cDNA clone yielded a sequence of 839 bp. It is likely that the remaining difference represents the poly(A) tail, although it is formally possible that cDNA Hev b 5 does not encode the full length of the antigen sequence. Nonetheless, two independent experiments indicate that the expressed protein will be a valuable tool for the analysis of the immune response to latex. First, most patients with latex allergy, and a large majority of HCWs with latex allergy, have IgE specific for Hev b 5 (Fig. 7). Second, a monoclonal antibody raised from a mouse injected with the Hev b 5 fusion protein recognizes protein bands in a natural latex extract, especially a band with an apparent molecular mass of 24,000, identical to that of the cleavage product of the fusion protein itself (Figs. 6 and 9).

A surprising result is the striking homology between cDNA Hev b 5 and a recently described cDNA clone from the kiwi (Fig. 3). Whether this homology is the reason for the observed clinical cross-reactivity of latex and certain fruit allergies is uncertain. Others have suggested that cross-reactivity among diverse plant species may be due to the widespread appearance of the actin inhibitor profilin (52), but we were unable to find any profilin-like sequences in either cDNA Hev b 5 or pKIWI501. The homology with the stolon tip protein of potato is also notable and may account for some reports of potato allergy among latex-allergic individuals (10). The mRNA for both the kiwi and potato proteins peak early in plant development, but persist in the mature plant, and the expressed proteins may be present in the ingested products and elicit clinical reactions.

The role of Hev b 5 in *H. brasiliensis* is uncertain. The appearance of this message in at least 5 out of only 1000 plaques screened from a latex cDNA library indicates that the message is abundant in latex cytosol. In addition to its homology to kiwi and potato sequences of unknown function, Hev b 5 is also homologous with neurofilament triplet L protein and the myristoylated alanine-rich kinase C substrate. These observations, along with its resistance to standard denaturation techniques, suggest that Hev b 5 is a cytoplasmic structural protein. Subcellular localization studies, using monoclonal antibodies, will help better define the biological function of Hev b 5.

Latex allergy is an increasingly important problem, affecting the lives and careers of thousands of health care providers and their patients. It is notable that, in spite of the large number of *Hevea* proteins that have been described, Hev b 5 appears to be a newly described protein. While it is unlikely that Hev b 5 represents the only allergen in *Hevea* latex, a large majority of patients with latex allergy, regardless of risk category, have IgE that recognizes the Hev b 5 sequence. Thus, Hev b 5 is a major antigen in *Hevea* latex (37). Among the unresolved issues in the care of people with latex allergy are the extreme difficulty of complete latex protein avoidance and the absence of effective and safe immunotherapy regimens. The molecular identification of latex protein antigens, and the precise identification of the T- and B-cell epitopes of these antigens, will help address these issues and may serve as a model for the treatment of other life-threatening allergic disorders.

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