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 susceptible individual, health care workers.

Fifty-six percent of spina bifida patients and 92% of HCW, health care workers with latex allergy have IgE specific for latex proteins. 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 immunological 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 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, immunological 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), hevamine (26, 27), 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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1 The abbreviations used are: NAL, non-ammoniated latex; cDNA, 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; PVD, polyvinylene 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 from 10 consecutively sampled (SB) dogs were not screened. These sera are expressed in the open reading frame of the λ EcoRI site, as a fusion protein (rHev b5/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 b5/MBP was adsorbed to an amyllose resin, electrophoresed in a 12% SDS-bis gel, and transferred to a polyvinylidene difluoride membrane (PVDF, Millipore) in a CAPS/methanol buffer (42). The bands were cut out and ready to Edman degradation on an automated gas-phase sequencer (42).

**Amino Acid Sequencing of Cleaved rHev b5/MBP**—Cleaved rHev b5/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 b5/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 32P-labeled 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 b5/MBP at control buffer and incubated overnight at 4°C. The sera were then added to polyvinyl chloride wells coated with rHev b5/MBP, and developed as above.

**Production of Mouse Hybridomas**—Hybridoma lines were produced from a BALB/c mouse immunized with rHev b5/MBP in Freund’s adjuvant, as described previously (31). Cell lines were tested for production of antibody to rHev b5/MBP, MBP, and NAL. In preliminary studies, hybridomas 7C10 and 17A10 produced detectable antibodies to all three antigens by enzyme-linked immunosorbent assay, but only the antibody from 17A10 detected specific bands on Western analysis of crude 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

The cDNA was isolated from the primer and RNA, and a poly(C) tail was added. cDNA was synthesized using reverse transcriptase (SuperScript, Life Technologies, Inc.) using the primer sequence 5'-AGAGTCAGTCTTTGAGCC-3' and 5'-GAAGCTCTGGTTAGGCC-3', and then again with the nested primers 5'-CACUCAUCUAAGGGCCACGGT-GCATGAC-3' and 5'-CAGUCAUCAUACUTCCGTGAGCT-3'. The PCR product was separated on a 2% agarose gel and purified.

Both the 3'-RACE and 5'-RACE products were inserted into the pAM1 plasmid using uracil DNA glycosylase (Life Technologies, Inc.).

**Sequence Analysis**—Both the pAM1 RACE products and the pMAL-c2 vector were sequenced by the University of California San Diego DNA Sequencing Facility using T7 DNA polymerase (Sequenase 2.0, U. S. Biochemical Corp.) using overlapping forward and reverse primers.
Fig. 2 Northern analysis of cDNA Hev b 5. RNA was extracted from H. brasilensis 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 32P-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 polycadenylation signal AATAAT 19 bases upstream of the (polyA) 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 (pKWI501) 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 pKWI501 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 pKWI501 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 Mr of affinity-purified rHev b 5/MBP was 81,000. Cleavage of rHev b 5/MBP with Factor Xa yielded two prominent bands of Mr 44,000 and 36,000 (Fig. 4). Sequence analysis indicated that the Mr 44,000 peptide is MBP, which is the leader sequence in pMAL fusion products. Edman analysis of the Mr 36,000 fragment yielded a sequence derived from the polylinker site in pMAL (49). 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).

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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...
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 Mr of Hev 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 4M urea, the Mr 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 Mr 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 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 μg/ml rHev b 5/MBP, 98% inhibition of solid phase binding was achieved with both the spina bifida and...
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).

**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
Hcv b 5, a Major Allergen from H. brasiliensis Latex

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