

Molecular and immunologic characterization of new isoforms of the *Hevea brasiliensis* latex allergen Hev b 7: Evidence of no cross-reactivity between Hev b 7 isoforms and potato patatin and proteins from avocado and banana

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Background: Hev b 7 is a *Hevea brasiliensis* latex allergen with sequence identities of 39% to 42% to patatins recently identified as potato allergens. The complementary DNAs encoding 2 different Hev b 7 isoforms were previously reported.

Objective: The aim of this study was to determine the sequence variation of Hev b 7 and to compare the IgE reactivity of individual isoforms in vitro and in vivo. A further objective was to evaluate possible cross-reactivities between Hev b 7 and patatins and proteins from banana and avocado.

Methods: An *H brasiliensis* λ ZAP complementary DNA (cDNA) library was screened with use of a Hev b 7 cDNA probe. Four Hev b 7 isoforms were produced in recombinant form and their IgE-binding capacities were compared. IgE immunoblot inhibitions and ELISA inhibition assays were used to investigate the possible cross-reactivity between Hev b 7 and recombinant potato patatin and proteins from avocado and banana.

Results: Two new isoforms, S2 and D2, were identified by sequencing 32 cDNA clones with full-length coding regions. All 4 recombinant isoforms displayed esterase activity and identical IgE-binding capacities. The new isoforms S2 and D2 were evaluated in skin prick tests and provoked responses equivalent

to natural Hev b 7. No cross-reactivity was observed between Hev b 7 isoforms and potato patatin and proteins from avocado and banana.

Conclusions: All 4 recombinant Hev b 7 isoforms have equivalent IgE-binding capacity and therefore represent suitable reagents for the development of in vitro and in vivo diagnostic tests. Hev b 7, patatins, and their homologs appear not to contribute to cross-reactivity in the latex-fruit syndrome. (J Allergy Clin Immunol 1999;104:1302-10.)

Key words: Type I allergy, natural rubber latex, Hev b 7, isoforms, polymorphism, patatins, cross-reactivity, recombinant allergens

Since the first clinical description of latex allergy,¹ IgE-mediated hypersensitivity to natural rubber latex (NRL) proteins has been acknowledged to impose serious medical problems, especially among occupationally exposed persons (eg, health care workers) and patients who have to undergo repeated surgeries such as patients with spina bifida.^{2,3} An increasing number of NRL allergens are known.^{4,5} In our previous work we reported the complementary DNA (cDNA) cloning and immunologic and biochemical characterization of the NRL allergen Hev b 7.^{6,7}

Rational design of strategies for diagnosis and immunotherapy of allergic diseases with recombinant allergens is complicated by the fact that many plant allergens exist as multiple closely related isoforms with potentially differing immunologic properties. On the other hand, the very existence of naturally occurring isoforms with different immunomodulatory capacities offer exciting possibilities for the design of immunotherapy strategies.^{8,9} Sequence polymorphism has been identified for a number of major allergens, including the birch pollen allergen Bet v 1,¹⁰ the hazel pollen allergen Cor a 1,¹¹ the house dust mite allergen Der p 2,¹² and the major NRL allergen Hev b 2 (Sowka, unpublished results).

Nishiyama et al¹³ demonstrated that all three Der f 2 isoforms were equally recognized by IgE antibodies from a panel of 14 sera from dust mite-allergic patients.

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The nucleotide sequences reported in this article have been submitted to the GenBank/EBI Data Bank and assigned the accession numbers AJ223039 (Hev b 7/S2) and AF113546 (Hev b 7/D2).

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Abbreviations used

cDNA:	Complementary DNA
GST:	Glutathione-S-transferase
nHev b 7:	Natural Hev b 7
NRL:	Natural rubber latex
RAST:	Radioallergosorbent test
rHev b 7:	Recombinant Hev b 7
SPT:	Skin prick test

In contrast, Breiteneder et al¹¹ showed that 4 different isoforms of Cor a 1, the major allergen of hazel pollen, differed in IgE binding, although these isoforms had amino acid identities of 96% to 99% with each other. In addition, the four Cor a 1 isoforms differed in their ability to induce activation of allergen-specific T-cell clones.¹⁴ It was shown in a number of studies that even single conservative exchanges inside and outside the T-cell epitopes can have profound effects on the immune response.¹⁵⁻¹⁷ In the case of Bet v 1, the existence of many isoforms with different allergenic characteristics could be shown. Naturally occurring or engineered Bet v 1 isoforms that have almost no capacity to bind IgE but maintain their T-cell stimulatory capacities form the base for new concepts in immunotherapy.^{8,9} Identification of immunodominant isoforms are of special interest because their use could improve reproducibility and comparability of routine tests.^{18,19}

To date, 2 isoforms of Hev b 7 have been published that differ in their sequences by 7 amino acids.^{6,7} In addition, one of the peptide sequences determined for the purified natural Hev b 7 did not completely match the published amino acid sequences, indicating the existence of still other Hev b 7 isoforms.⁷

Hev b 7 isoforms and potato patatins share 39% to 42% amino acid sequence identities. Cross-reactivity between potato proteins and a 46-kd patatin-like protein from latex has been observed.²⁰ IgE-mediated allergic reactions to potato have been reported,²¹⁻²³ and potato patatin was identified as a first allergen in 20 of 27 (74%) children with positive skin prick test (SPT) responses to potato.²⁴ Thus the question arises whether the cross-reactivity between Hev b 7 isoforms and patatins contributes to the clinical association between allergy to NRL and potato proteins.^{20,25,26}

In this study we present polymorphism data for the NRL allergen Hev b 7 obtained by sequencing of 32 cDNA clones with full-length coding regions. Two new isoforms, S2 and D2, were identified and characterized. All 4 known isoforms of Hev b 7 were produced in recombinant form and compared for their IgE binding capacity in vitro and in vivo. The lack of cross-reactivity between Hev b 7 and potato patatin and proteins from avocado and banana was assessed by IgE immunoblot inhibitions and ELISA inhibition assays.

METHODS

Preparation of protein extracts

C-serum proteins were prepared from nonammoniated NRL freshly collected from *Hevea brasiliensis*, clone RRIM 600, as

described previously.⁷ To prepare avocado extracts, 10 g of avocado pear mesocarp tissue (*Persea americana* Mill cv Haas) were homogenized in a Waring blender and mixed with 20 mL of extraction buffer containing 50 mmol/L TRIS-hydrochloric acid, pH 8.0, 10 mmol/L EDTA, 10 mmol/L diethyldithiocarbamate, and 10 mmol/L sodium sulfate. The mixture was then centrifuged at 40,000g for 1 hour. The supernatant was collected and filtered through a Millex-HV filter (Millipore, Bedford, Mass) and stored at -20°C. Banana extracts were prepared according to Alenius et al.²⁷

Preparation of the hybridization probe and library screening

The sequence corresponding to the full-length open reading frame of Hev b 7 (1164 bp) reported by Sowka et al⁷ was amplified by PCR with use of the primers 5'-ATGGCTACTGGTAGTACTACTTTGAC-3' (sense) and 5'-TTTGAGTTGACGGAGCTTTCGTTTC-3' (antisense) and labeled with phosphorus 32 deoxycytidine triphosphate with the Random Primed DNA labeling kit (Boehringer Mannheim, Mannheim, Germany). A previously constructed *H brasiliensis* λ ZAP cDNA library⁷ was used to isolate new Hev b 7 isoforms according to standard procedures.^{28,29} The cDNA for the library construction was synthesized from RNA isolated from the RRIM clone No. 600 of the Rubber Research Institute of Malaysia to represent the range of diversity of Hev b 7 sequences of this clone because it is the most important commercial *H brasiliensis* cultivar in use today.

DNA sequencing

Sequence analysis was performed by the dideoxy chain termination method³⁰ with use of the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing Kit (Amersham Life Science). After a denaturing step at 94°C for 2 minutes, the sample was subjected to 25 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 70°C for 30 seconds in the GeneAmp PCR System 2400 (Perkin Elmer) with use of fluorescent-labeled standard primers and the Li-Cor DNA sequenator model 4000L (Li-Cor, Lincoln, Neb). Both strands of every clone were analyzed to yield the sequences.

Computer-aided sequence analyses

The FASTA algorithm implemented in the Wisconsin Package (Genetics Computer Group, Madison, Mich) was used to search for protein sequence similarities within the isoforms of Hev b 7 and to other proteins in the GenBank database. The deduced amino acid sequences of the new Hev b 7 isoforms S2 and D2 were analyzed for possible cleavage sites with the program SIGSEQ1.³¹ This program applies a set of empirically derived rules to generate predictions for signal peptidase cleavage sites.

Production of recombinant proteins

The cDNA corresponding to the full-length open reading frame of the Hev b 7 isoform S2 was amplified by PCR with use of the following primers: sense 5'-ATTATCTCGAGAAAAGAGGGGCTACTGTAGTACTACTTTGAC-3' (*Xho* I) and antisense 5'-GCGGCCGCTTATCATTTGAGTTGACGGAGC-3' (*Not* I), digested with *Xho* I and *Not* I and inserted into the *Xho* I/*Not* I-digested *Pichia pastoris* expression vector pPIC9. Hev b 7/S2-producing clones were obtained according to the manufacturer's instructions (*Pichia* Expression Kit, Invitrogen). The cDNA encoding the coding region of the isoform D2 was amplified by PCR with the primers sense 5'-TTAATGAATTCCGATGGCTACTGGTAGTACTCC-3' (*Eco*RI) and antisense 5'-TTAATGAATTCTCATTTGAGTTGACGGAGCTTTTCG-3' (*Eco*RI) and cloned in the plasmid pGEX2T. The protein was produced as a fusion to glutathione-S-transferase (GST) as described for another Hev b 7 isoform.⁶

Recombinant S1 and D1 isoforms were produced as described previously.^{6,7} Purified recombinant potato patatin produced in transgenic tobacco was a gift from Monsanto Company (St Louis, Mo).

Purification of the recombinant Hev b 7 isoforms and natural Hev b 7

The Hev b 7 isoform S2 was purified from culture supernatant of *P. pastoris* cells secreting the recombinant protein. After 6 days of induction the culture was centrifuged to pellet the cells, and ammonium sulfate was added to the supernatant to give 90% saturation. After it was stirred for 1 hour at 4°C, the precipitate was collected by centrifugation at 10,000*g* for 10 minutes, redissolved in 5 mL of 20 mmol/L TRIS–hydrochloric acid, pH 7.5, and 0.2 units PNGase F per 10 µg of recombinant Hev b 7 (rHev b 7) were added to remove *N*-linked sugars. The sample was dialyzed twice against 20 mmol/L TRIS–hydrochloric acid, pH 7.5 (buffer A). This crude preparation was loaded onto a Resource Q anion-exchange column (Pharmacia Upjohn) equilibrated with 20 mmol/L TRIS–hydrochloric acid, pH 7.5. The column was washed thoroughly with the same buffer and the rHev b 7/S2 was eluted with a linear gradient between 0% and 50% buffer B (20 mmol/L TRIS–hydrochloric acid, 1 mol/L sodium chloride, pH 7.5) at a flow rate of 1 mL/min. Fractions of 0.5 mL were collected. The Hev b 7 isoform D2 was purified after cleaving of the fusion part by thrombin according to Kostyal et al.⁶ Recombinant S1 and D1 isoforms were purified as described previously.^{6,7} Natural Hev b 7 (nHev b 7) was purified according to Yusof et al.³²

Esterase activity

Esterase activity was determined with the chromogen substrate *p*-nitrophenyl palmitate (ICN Biomedicals) as described previously.⁷ The protein concentration was assessed with the protein assay kit from BioRad with BSA as a standard.

SDS-PAGE, immunoblotting, and inhibition experiments

Proteins were separated on 12% SDS-PAGE gels under reducing conditions and transferred to Protran nitrocellulose membranes (Schleicher and Schuell). IgE immunoblots were performed according to procedures described previously.³³ IgE antibodies were detected with iodine 125-labeled rabbit antihuman IgE (RAST RIA, Pharmacia Upjohn) diluted 1:10. For immunoblot inhibition experiments, individual sera and serum pools were incubated overnight at 4°C with 20 µg purified rHev b 7. The preincubated sera were then used to probe the nitrocellulose strips containing latex C-serum proteins, potato extracts, recombinant potato patatin, or banana or avocado extracts.

Inhibition of IgE binding to nondenatured purified nHev b 7 was performed in an ELISA assay with use of the Allercoat 6 Conjugate Kit (Sanofi Diagnostic Pasteur, Redmond, Wash). Microtiter plates (Nunc, Wiesbaden, Germany) were coated overnight at 4°C with 4 µg/mL nHev b 7 in 50 mmol TRIS, pH 8.0, washed, and blocked. A serum pool of 10 latex-allergic patients sensitized to Hev b 7 was preincubated with increasing amounts (1 µg/mL up to 60 µg/mL) of rHev b 7/S2 or rHev b 7/D2 for 5 hours at room temperature. Subsequently, alkaline phosphate-conjugated horse antihuman IgE (Sanofi Diagnostic Pasteur) was added and left overnight at room temperature. ELISA plates were developed with 1.5 mg/mL *p*-nitrophenylphosphate. The enzymatic reaction was stopped by adding 50 µL of 2 mol/L sodium hydroxide and absorbance was measured at 405 nm.

To test cross-reactivity between Hev b 7 and potato patatin in ELISA, 4 µg/mL potato patatin in PBS, pH 8.0, was coated and the

inhibitions were performed as described above. A serum pool of 5 latex- and potato-allergic individuals with IgE to potato patatin was used in this experiment.

Patients' sera

Two groups of patients were used in this study. The first group consisted of 15 sera from NRL-allergic patients with positive case histories, positive SPTs, positive radioallergosorbent test (RAST) results (Stallergènes, Antony, France), characteristic type I allergy to latex, and reacting to Hev b 7 in IgE immunoblot. Nine of these patients had allergic reactions to potato. The patients' data of this group are summarized in Table I. The second group comprised sera of 29 patients allergic to latex and sensitized to potato (with positive SPTs and positive RASTs, RAST class at least 2) but with no IgE antibodies specific for Hev b 7. Fourteen of these 29 potato-sensitized patients also demonstrated allergic cutaneous and mucosal reactions such as contact urticaria or itching in the mouth in response to handling or ingestion of potato. In addition, sera from 10 patients allergic to latex and banana or latex and avocado were used individually or pooled in IgE immunoblot inhibitions. Sera from 20 nonallergic individuals were pooled and used for negative control experiments.

SPTs

Fifteen latex-allergic patients with IgE directed against Hev b 7 and 5 control patients were included in this study. Nine of these patients had allergic reactions to potato. SPTs were performed on the volar aspect of the forearm. A 25-gauge sterile needle was used to prick the epidermis beneath the drop of allergen solution. The test sites were wiped clean after 15 minutes, and the wheal-and-flare reaction in millimeters was recorded. The test solutions used for the SPT included a standard latex skin test solution (Bencard, low ammoniated raw extract, 7–10 mg/mL), a commercial potato extract (Bayer, 1:10 wt/vol), and solutions of the rHev b 7 isoforms S2 and D2 and nHev b 7, all at 50 µg/mL. SPT with fresh potato was also conducted by lancing a piece of the raw potato with a sterile 25-gauge needle and then pricking the epidermis. Histamine and 0.9% saline solution were used as positive and negative controls, respectively. SPTs with wheal-and-flare reactions at least half the size of the histamine control were considered positive. Five individuals with no history of any type I allergy were tested as controls.

RESULTS

Sequence polymorphisms of Hev b 7 cDNA clones

Thirty-two cDNA clones containing the full-length coding region of Hev b 7 were sequenced. Two new isoforms, S2 and D2, were identified for the first time. Seventeen sequences out of 32 corresponded to the isoform S1,⁷ 8 of 32 to the isoform S2, 2 of 32 to the isoform D2, and 5 of 32 had single nucleotide exchanges each. These 5 sequences could be the result of PCR artifacts and were therefore not considered as new isoforms. The coding regions of S2 and D2 were of identical length and encoded proteins of 388 amino acids (Fig 1). No leader peptide could be identified with use of the program SIGSEQ1, which is consistent with the proposed cytoplasmic localization of Hev b 7.⁷ Comparison of the deduced amino acid sequences of the Hev b 7 isoforms revealed between 4 and 8 amino acid residue differences corresponding to identities between 97.9% and 99.0% (Fig 1). The 3' non-coding regions of all 4 isoforms differed in both

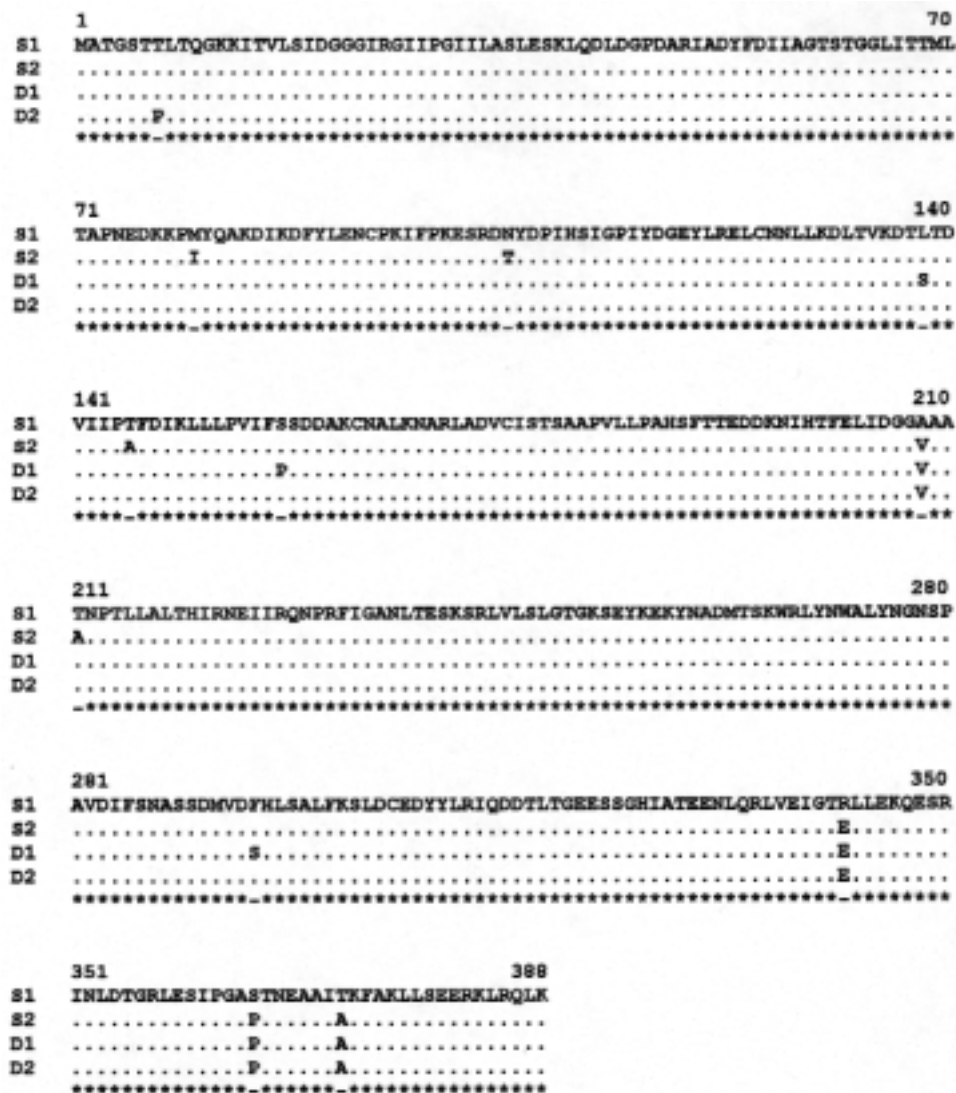


FIG 1. Protein sequence alignment. Deduced amino acid sequences of Hev b7 isoforms were aligned. Dots, identical residues; asterisks, residues identical in all sequences compared. Accession numbers are AJ223038 for S1, AJ223039 for S2, U80598 for D1, and AF113546 for D2.

sequence and length for each isoform pair compared (data not shown). A consensus polyadenylation signal AATAAA was present in the 3' noncoding regions of all 4 isoforms.

Recombinant expression and purification of Hev b 7 isoforms

The isoform S2 was expressed in secreted form in the *P. pastoris* expression system. During the processing through the *P. pastoris* secretory pathway, the protein becomes *N*-glycosylated (data not shown). Because the natural protein is not glycosylated,⁷ the recombinant S2 was deglycosylated by peptide *N*-glycosidase F treatment before purification. The protein was then purified over a Resource Q anion-exchange column and eluted at

125 to 150 mmol/L sodium chloride. The yield of the purified protein was approximately 10 mg/L culture supernatant. The Hev b 7 isoform D2 was produced as a GST fusion protein in the cytoplasm of *Escherichia coli*, purified over glutathione agarose beads and the GST moiety cleaved off with thrombin as described for D1.⁶ The yield of affinity-purified rHev b 7/D2 was 1.5 mg/L of culture broth. The isoforms D1 and S1 were produced as described previously.^{6,7}

Esterase activity

Both isoforms identified in this study, S2 and D2, displayed esterase activity. The specific activity of the purified rHev b 7 isoforms S2 and D2 with *p*-nitrophenyl palmitate as substrate were 443 ± 5 and 43 ± 5 mU/mg

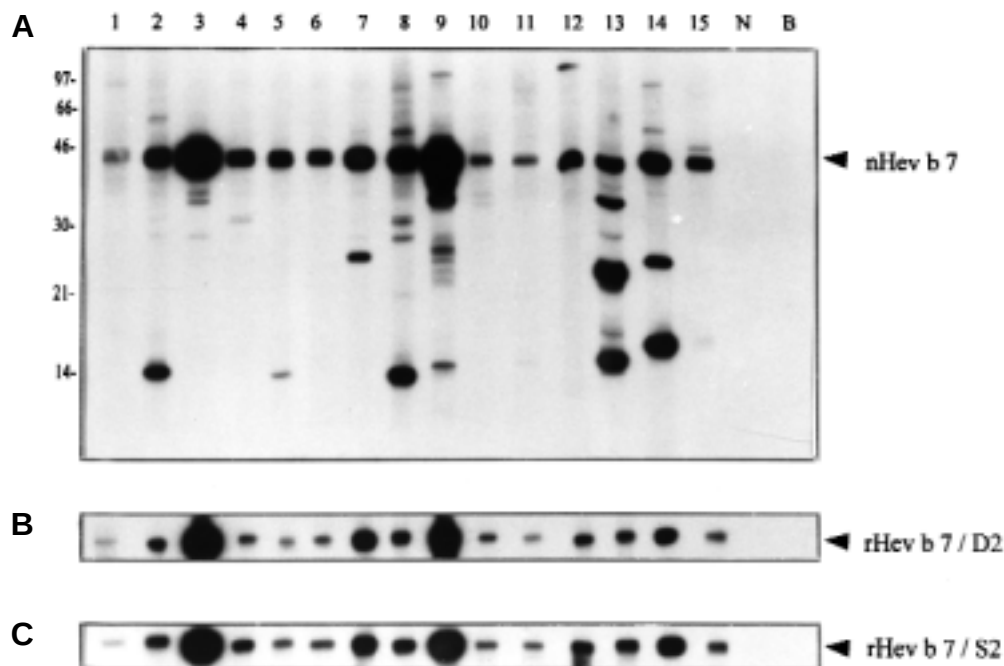


FIG 2. IgE binding of sera from latex-allergic patients sensitized to Hev b 7 tested on latex C-serum (**A**), rHev b 7 isoform D2 (**B**), and rHev b 7 isoform S2 (**C**). Controls are nonallergic human serum pool (N) and buffer instead of serum (B).

protein or, expressed in ΔA_{400} , 8.195 ± 0.100 and $0.797 \pm 0.100/10 \text{ min} \times (\text{mg protein})^{-1}$, respectively. Thus the specific activity measured for S2 was slightly lower than the previously reported specific activity for S17 ($508 \pm 6 \text{ mU/mg protein}$) and approximately 10-fold higher than for the *E coli* product D2.

In vitro and in vivo IgE binding capacity of Hev b 7 isoforms

The previously described Hev b 7 isoforms S1 and D1^{6,7} and the S2 and D2 isoforms presented in this study were all produced in recombinant form and compared for their IgE binding capacities to nHev b 7 and to each other. All isoforms displayed equal IgE binding capacity. IgE immunoblots with a panel of 15 Hev b 7 positive sera with the isoforms S2 and D2 are shown in Fig 2, B and C. IgE binding of the individual recombinant isoforms correlated with the intensity of the 43-kD band of the latex C-serum IgE immunoblot (Fig 2). By use of rHev b 7/S2 or rHev b 7/D2, we could achieve a complete inhibition of IgE binding to nHev b 7 present in latex C-serum (Fig 3, A). In contrast, the IgE binding to a 14-kD allergen did not change (Fig 3, A). The same result was obtained with the isoforms S1 and D1 (data not shown). In an ELISA assay total inhibition of IgE directed to nHev b 7 was achieved by preincubating a serum pool from 10 Hev b 7-sensitized individuals with $1 \mu\text{g/mL}$ rHev b 7/S2 or rHev b 7/D2. In addition, purified nHev b 7 and the recombinant isoforms S2 and D2 were tested in SPT and led to equivalent reactions (data not shown).

Evidence of no cross-reactivity between Hev b 7 and potato patatin and proteins from avocado and banana

To test the cross-reactivity between Hev b 7 and food proteins, we performed IgE immunoblot inhibition and ELISA inhibition assays with the purified rHev b 7 isoform S2. Sera from 15 latex-allergic patients reacting to Hev b 7 (group 1, clinical data summarized in Table I) were pooled into 2 subsets and used in IgE immunoblot inhibition experiments. The first subset included 9 patients with positive case histories of latex and potato allergy, positive RAST, and positive SPT to latex and potato extracts (patients 2, 3, 5-7, and 12-15 in Table I). The pattern of IgE binding to potato proteins did not change after preincubation of the serum pool with $20 \mu\text{g}$ of rHev b 7/S2 (Fig 3, B, lanes 1 and 2). The second subset included 6 patients with positive case histories of latex allergy, positive RAST, and positive SPT to latex (patients 1, 4, and 8-11 in Table I) and IgE binding to potato proteins but no adverse reactions to potato and negative SPTs to potato extracts. Again, the IgE binding pattern did not change after preincubation of the serum pool with $20 \mu\text{g}$ of rHev b 7/S2 (Fig 3, B, lanes 3 and 4). We individually tested 15 sera from Hev b 7-sensitized latex allergic patients (Table I) for their IgE binding to recombinant potato patatin in immunoblots, and none of the sera showed any antipatatin reactivity. There was no clear correlation between specific IgE to potato proteins and positive SPT to potato (Table I). The 5 controls, who had no history of latex allergy and did not show IgE bind-

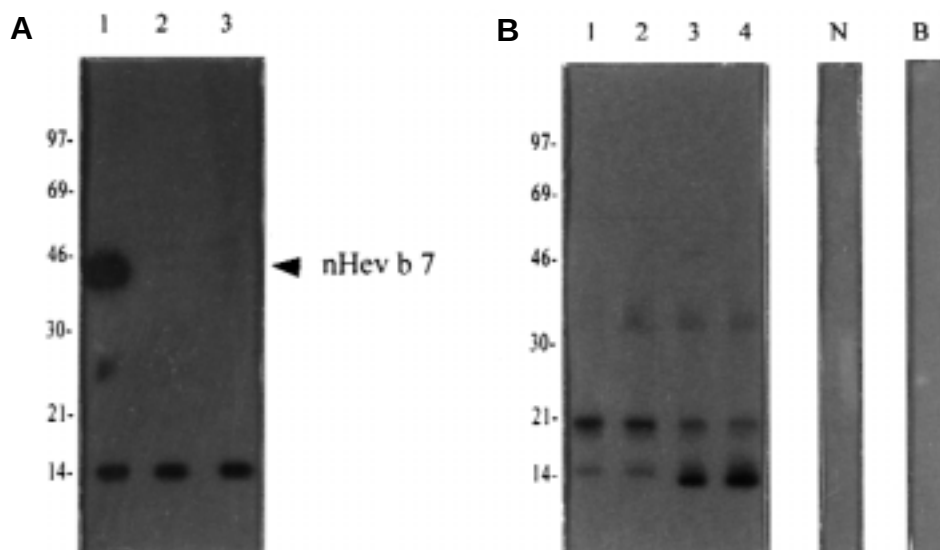


FIG 3. Inhibition experiments. **A**, Inhibition of IgE binding to natural Hev b 7 by rHev b 7 isoforms. *Lane 1*, Serum pool of 10 latex-allergic patients reacting to Hev b 7 tested on latex C-serum proteins; *lane 2*, inhibition of IgE binding to latex C-serum proteins by preincubation with 20 μ g of purified rHev b 7 isoform S2; *lane 3*, inhibition of IgE binding to latex C-serum by 20 μ g of purified rHev b 7 isoform D2. Same amounts of latex C-serum proteins were blotted on all strips. **B**, Inhibition of IgE binding to potato proteins by rHev b 7. *Lane 1*, Serum pool of 9 patients with latex and potato allergy and sensitized to Hev b 7 tested on potato extracts without inhibition; *lane 2*, same serum pool as lane 1 preincubated with 20 μ g of purified rHev b 7/S2; *lane 3*, serum pool of 6 patients with latex allergy and sensitized to Hev b 7 but no adverse reactions to potato proteins tested on potato extracts; *lane 4*, the same serum pool as lane 3 preincubated with 20 μ g of purified rHev b 7/S2. Controls are a nonallergic human serum pool (N) and buffer instead of serum (B).

ing to latex proteins in IgE immunoblots, only reacted to the positive histamine control and did not react to the other solutions (Table I). All participants had negative SPT reactions to saline solution and positive reactions to histamine and NRL. Of the 9 individuals identified as latex and potato allergic, 8 reacted to fresh potato extracts and 6 to commercial potato extracts in SPT. Thus, with the combination of both potato extracts, all patients were covered.

In a second set of experiments we tested sera from 29 patients with NRL allergy and positive RAST and SPTs to potato. Fourteen of these potato-sensitized patients displayed allergic symptoms to potato. All 29 patients had no IgE specific for Hev b 7 as judged by IgE immunoblots. Seven of these 29 patients (24%) reacted to the recombinant potato patatin, 2 of which were only sensitized and 5 allergic to potato (data not shown). In an ELISA assay no inhibition of IgE binding to potato patatin could be detected in a pool of these 5 latex- and potato-allergic patients after preincubation with 60 μ g/mL rHev b 7/S2.

No IgE cross-reactivity was observed for Hev b 7 and avocado and banana proteins in immunoblot inhibitions with use of both individual sera or a serum pool.

DISCUSSION

In this study we examined the degree of sequence polymorphism of the NRL allergen Hev b 7 by charac-

terizing 32 cDNA clones containing the full-length coding regions. We were able to identify and characterize 2 new Hev b 7 isoforms, S2 and D2. The isoforms S1 and S2 occurred most frequently (17/32 and 8/32 cDNA clones, respectively). All 4 isoforms were produced in recombinant form, purified to homogeneity, and shown to be fully equivalent in their IgE binding capacities in both IgE immunoblots and SPTs (Fig 2, B and C for S2 and D2, Table I). All isoforms were able to completely abolish IgE binding to natural Hev b 7 (nHev b 7) present in NRL in IgE immunoblot inhibition experiments (Fig 3, A, data shown for S2 and D2). Similarly, rHev b 7/S2 as well as rHev b 7/D2 were able to abrogate IgE binding to nHev b 7 in an ELISA inhibition assay. Purified recombinant isoforms S2 and D2 and nHev b 7 led to equivalent reactions in SPTs. The purified isoforms displayed esterase activity, indicating that the recombinant proteins were correctly folded. Thus these recombinant isoforms are equivalent to nHev 7 and represent suitable reagents for the development of diagnostic and effective therapeutic reagents in NRL allergy.

Different immunologic properties have been described for naturally occurring or engineered isoforms of the major birch pollen allergen Bet v 1, which served as a basis for new immunotherapy concepts.^{8,9} In contrast, the Hev b 7 isoforms discovered to date display similar immunologic characteristics. As demonstrated by IgE immunoblot inhibitions and ELISA inhibition assays, IgE binding to nHev b 7 was completely abolished by

TABLE I. Clinical data and laboratory results of 15 Hev b 7-allergic patients

Patient No.	Age (y)/sex	Anti-Hev b 7 IgE blot	Potato extract IgE blot	Recombinant patatin IgE blot	Occupation	Symptoms to NRL	Latex RAST (kU/L)	Potato RAST (kU/L)	SPT rHev b 7/D2	SPT rHev b 7/S2	SPT Potato	SPT Latex
1	32/F	+	+	—	Nurse	CU, E, FE, GU, BA	1.5	2.9	+	+	—	+
2	35/F	+	+	—	Physician	CU, AR	9.0	9.1	+	+	+	+
3	29/M	+	+	—	Medical student	AR, CT	7.8	1.7	+	+	+	+
4	37/F	+	+	—	Nurse	CU, E	15.1	3.2	+	+	—	+
5	42/F	+	+	—	Nurse	AN	2.8	2.1	+	+	+	+
6	47/M	+	+	—	Physician	AR, CT, BA	4.3	3.9	+	+	+	+
7	40/F	+	+	—	Dental assistant	AR, CT, BA	2.8	2.4	+	+	+	+
8	31/F	+	+	—	Housewife	CU, AN	27.8	1.5	+	+	—	+
9	55/F	+	+	—	Nurse	AN	20.8	1.9	+	+	—	+
10	43/F	+	+	—	Dental assistant	CU	2.1	0.7	+	+	—	+
11	38/F	+	+	—	Housewife	AN	3.4	0.9	+	+	—	+
12	43/M	+	+	—	Physician	CU, E	2.0	7.1	+	+	+	+
13	44/F	+	+	—	Nurse	CU, E	8.9	2.4	+	+	+	+
14	40/M	+	+	—	Physician	AR	7.3	2.2	+	+	+	+
15	38/M	+	+	—	Physician	CU, AR, CT	3.0	1.7	+	+	+	+
N1	27/M	—	—	—	Computer programmer	—	0	0	—	—	—	—
N2	31/M	—	—	—	Talent agent	—	0	0	—	—	—	—
N3	37/M	—	—	—	Homemaker	—	0	0	—	—	—	—
N4	35/M	—	—	—	Homemaker	—	0	0	—	—	—	—
N5	58/F	—	—	—	Teacher	—	0	0	—	—	—	—

Wheal-and-flare reactions at least half the size of the histamine control were considered positive in SPTs. *F*, Female; +, positive; —, negative; *CU*, contact urticaria; *E*, eczema; *FE*, facial edema; *GU*, generalized urticaria; *BA*, bronchial asthma; *AR*, allergic rhinitis; *M*, male; *CT*, allergic conjunctivitis; *AN*, anaphylaxis; *N1-N5*, nonallergic individuals.

each Hev b 7 isoform tested (Fig 3, A). Therefore the discovery of additional Hev b 7 isoforms with significantly differing IgE reactivity seems highly unlikely.

Patients with latex allergy are often reported to be allergic to certain fruits, particularly avocado, banana, chestnut, and kiwi,^{27,34-37} leading to the term latex-fruit syndrome to describe this phenomenon.³⁸ Since the first report suggesting cross-reactivity between NRL and banana proteins,³⁹ a number of studies dealing with possible cross-reactivity between NRL and various food allergens have been published.^{27,35-37,39,40} Cross-reacting IgE antibodies were demonstrated by RAST and immunoblotting inhibition assays, to recognize both latex allergens and proteins in different foods.^{37,41,42}

An especially high degree of association between latex and food allergy was observed for latex and banana or avocado^{20,27,36,37,43,44} and is based on the presence of homologous proteins.⁴⁵ The production of purified recombinant allergens allows a more precise definition of such cross-reactivities. We applied IgE immunoblot inhibitions to test for cross-reactivity between Hev b 7 and avocado or banana proteins with sera from individuals with latex and avocado or latex and banana allergy. The results of these experiments suggested a lack of cross-reactivity between Hev b 7 and avocado or banana proteins, indicating that Hev b 7 and proteins from avocado and banana do not share cross-reactive IgE epitopes. The association between latex and avocado or banana allergy appears to be the result of cross-reactivity

of Hev b 6 with class I endochitinases present in these foods.⁴⁵

The association of latex and potato allergy is documented.^{20,25,26} Hev b 7 was first defined as a patatin-like protein on the basis of its sequence similarity to the well-described potato tuber storage protein family of the patatins.^{6,7} Recently, potato patatin was identified as an allergen in 20 of 27 (74%) children with positive SPT responses to potato.²⁴ Hev b 7 and potato patatins share 39% to 42% amino acid identities and were considered to be potential cross-reactive allergens. In a recent short note, Sepällä et al²⁶ reported cross-reactivity between the patatin of potato tuber (tentatively termed Sol t 1 by the authors) and Hev b 7. However, we have been unable to confirm their findings. We tested 15 sera from Hev b 7-sensitized latex-allergic patients (IgE immunoblot and SPT positive), 9 of whom also had potato allergy, for their IgE binding to recombinant potato patatin in immunoblots. None of these sera showed any antipatatin reactivity. In IgE immunoblot inhibitions using sera from patients allergic to latex and potato (Fig 3, B, lanes 1 and 2) or latex alone (Fig 3, B, lanes 3 and 4) and sensitized to Hev b 7, the pattern of IgE binding to potato proteins did not change after preincubation of sera with rHev b 7. We also did the reverse experiments with a pool of 5 sera from patients with latex and potato allergy and sensitized to potato patatin. The IgE binding to potato patatin could not be inhibited by rHev b 7 in an ELISA assay.

Taken together, these findings indicate that there is no

cross-reactivity between Hev b 7 and potato patatin and suggest that cross-reactivity between latex and potato must be the result of other cross-reactive allergens. The lack of cross-reactivity between Hev b 7 and patatin is not surprising if the low sequence identities of 40% to 42% between these 2 allergens are considered. Recent studies on the major shrimp allergen tropomyosin, Pen a 1, show that, although it shares 60% to 62% amino acid identities with tropomyosins from mammalian species in a region of sequence that binds IgE, shrimp-allergic individuals obviously do not show clinical sensitivity to these mammalian tropomyosins.⁴⁶ This demonstrates that amino acid sequence similarity alone is not sufficient to predict cross-reactivities.

Our IgE immunoblot inhibitions and ELISA inhibition assays with serum pools from patients displaying both latex and potato allergy indicate that there actually is no cross-reactivity between Hev b 7 and potato patatin. However, these results must be taken with caution because food challenge results may not correlate completely with in vitro results.⁴⁷ Although Hev b 7 may not be responsible for the cross-reactivity with potato, clearly some latex-allergic individuals also react to potato patatin.

In the current study we have characterized 4 recombinant isoforms of the NRL allergen Hev b 7. The recombinant isoforms displayed esterase activity and were equivalent in their IgE binding capacity in both in vitro (IgE immunoblots) and in vivo tests (SPT) to its natural counterpart. Thus recombinant Hev b 7 isoforms produced in *P pastoris* or *E coli* represent a diagnostic tool equivalent to nHev b 7. It can be used as a component of a panel of recombinant latex allergens to diagnose latex allergy. Among the Hev b 7-sensitized population of latex-allergic patients, Hev b 7 represents the only latex allergen for 7 of 15 (47%) and the major allergen for 12 of 15 (80%) of the patients (Fig 2). Therefore Hev b 7 will need to be included in a diagnostic panel to improve reliability of allergen formulations in the diagnosis of NRL allergy.

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