

Original article

Assessment of profilin as an allergen for latex-sensitized patients

Background: The presence of the actin-binding protein, profilin, has been demonstrated in natural latex extracts; but the clinical significance of this molecule as an allergen for latex-allergic patients is not clear. We studied the allergenic relevance of isolated latex natural and recombinant profilin, by in vivo and in vitro techniques, in two populations of spina bifida children (SB) and adults allergic to latex (AL).

Methods: Profilin is present in small amounts in latex extracts obtained from low ammoniated (LA) natural latex. Its purification by affinity chromatography resulted difficult due to Hev b 1 unspecific binding. Therefore a method was developed to obtain natural profilin from natural latex, combining affinity chromatography (PLP, poly-L-proline Sepharose column) and previous ammonium sulfate fractionation. Alternatively, latex c-serum containing a low amount of Hev b 1 and a relatively higher profilin content could be used. Recombinant latex profilin isoform (rHev b 8) was cloned by PCR amplification. The entire coding region of Hev b 8 was subcloned into the expression vector pKN172 and a non-fusion form of Hev b 8 was expressed in *Escherichia coli* BL21 (DE3). Purified recombinant protein was obtained after a single passage through PLP-Sepharose column.

Results: Natural and recombinant purified Hev b 8 were tested cutaneously by intradermoreaction (ID) in 17 SB and 14 AL patients. They were positive in 15 SB and 14 AL patients. No wheals were produced when tested in nonatopic control patients. Only 42% of sera from latex-allergic patients revealed specific IgE titers of class 1 or higher by enzyme immunoassay and only 39% of them exhibited IgE binding by SDS-PAGE immunoblotting with any natural or recombinant Hev b 8 forms.

Conclusion: It seems that profilin is a relevant allergen for both groups of patients from a frequency point of view, but with scarce presence in natural latex extracts and raw sources, with a subsequent low IgE induction capacity.

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IgE mediated hypersensitivity to latex from the commercial rubber tree, *Hevea brasiliensis*, has received great attention in the last decade with the increase of documented reports of allergic reactions.

In adults, the main groups at risk for sensitisation to latex are those of rubber industry and of healthcare workers (1, 2). In children with spina bifida, the high level of exposure to latex due to repeated surgical procedures confers on them the maximal potential risk to develop allergy to natural rubber latex (3).

A number of proteins from natural rubber latex products and latex from *Hevea brasiliensis* have been identified as reacting with IgE antibodies. Most of them have been cloned and sequenced, and twelve of them, fully characterized, have been included in the official list from the IUIS Allergen Nomenclature Subcommittee (4).

The actin binding protein, profilin, has been found as an allergen in a large variety of pollens, fruit and vegetables, thus being defined as panallergen (5). Profilin has been demonstrated to be involved in allergenic cross-reactions, in some cases with associated clinical expression (6).

In most sources, profilin has been detected as a minor allergen showing IgE binding prevalences of around 20% (7), with only a few exceptions (8, 9).

Profilin (Hev b 8) was first identified in latex by Vallier et al. (10), but it has been considered of

Abbreviations: AL: adults allergic to latex; CNBr: cyanogen bromide; ID: skin intradermal test; mAb: mouse monoclonal antibody; PBS: phosphate buffered saline; PLP: poly-L-proline; RAST: radioallergosorbent test; SB: spina bifida patients allergic to latex; SDS-PAGE: polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; TBS: Tris-buffered saline solution.

negligible importance in natural rubber latex extracts as a significant allergen (10, 11). Recently, two studies reported different Hev b 8 prevalences among latex-allergic subjects. Yip et al. (12) reported a proportion of latex-allergic adult subjects reacting with a recombinant profilin isoform (rHev b 8) of 3%. Rhis et al. (13) detected, by the CAP System (Pharmacia Diagnostics, Uppsala, Sweden), the presence of rHev b 8-specific IgE antibodies in two out of 17 sera from spina bifida (SB) patients and in five out of 25 sera (20%) from healthcare workers. However, in preliminary studies with isolated natural latex profilin, we observed a high frequency of sensitisation, by cutaneous test, in a sample population of children with spina bifida and adults allergic to latex (14).

Therefore, we undertook the present study with the aim of evaluating the allergenic relevance of latex profilin, by *in vivo* and *in vitro* means, using isolated natural and recombinant Hev b 8 and two sample populations of children with spina bifida (SB) and adults (AL) allergic to latex.

Material and methods

Study subjects

Two separate groups of patients were used in the study: a first group (SB) of 17 children with spina bifida sensitized and with clinical reactions to latex ranging from urticaria to anaphylactic shock. A second group (AL) of 14 adults suffering from asthma and/or rhinitis caused by latex allergy, nine of them healthcare workers. Ten patients from AL group showed allergy to aero-inhalants, only two of them to birch or grass pollen (sera 6 and 14 in tables and blot figures).

Twenty atopic patients nonallergic to latex and 20 nonatopic subjects, according to the EAACI revised allergy nomenclature (15), were used as negative controls for skin tests and as serum donors. Informed written consent was obtained from each participant for the study.

Latex extract

In order to obtain the aqueous allergenic latex extract, a sample of commercial LA-TZ (low ammoniated) centrifuged latex was used as raw material. This type of latex showed an ammonia content limited to 0.29% (of the weight of the latex) and 0.025% of TZ (tetramethylthiurden-disulfide/ZnO).

Latex LA lattices were first frozen and then ground in a wearing blender. Afterwards, latex proteins were extracted by mechanical stirring in PBS, pH 7.3 at 37°C for 10 h. Then, the solubilized proteins were isolated from the solid matrix by filtration through glass fiber filters, and dialyzed in visking tubing (10 kDa cut-off). Finally the extract was sterilized by filtration through 0.22 µm pore diameter membranes, frozen and lyophilized.

Alternatively, latex c-serum was used to obtain latex extract enriched in profilin content and with reduced Hev b 1 presence. This sample was extracted in PBS, as described above. (Latex c-serum was kindly provided by Dr. H. Y. Yeang, Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia; it is the supernatant fluid obtained when rubber particles are removed by centrifugation.)

Profilin purification

Natural profilin was isolated either from ammoniated latex or from latex c-serum extracts. In the first case, the high Hev b 1 (rubber

elongation factor) content affected the profilin purification by affinity chromatography by unspecific adsorption, therefore a previous step of salt fractionation was required to separate fractions containing Hev b 1 (precipitated at 20–60% ammonium sulfate saturation) from fractions containing profilin (70–90% saturation).

The resolutive step in profilin purification was accomplished by affinity chromatography in PLP–CNBr-activated Sepharose column, as previously described (16).

Isolation of poly(A+) mRNA and first-strand cDNA synthesis

Leaves of *H. brasiliensis* clone RRIC 133 (kindly donated by Dr. Attanayaka from the Rubber Research Institute of Sri Lanka, Sri Lanka) were snap frozen, ground in a mortar, and conserved at –20 °C until used. Poly(A+) enriched RNA was isolated from 100 mg of ground leaves using the Quick Prep MicroRNA Purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). First-strand cDNA was synthesized from 1 mg poly(A+) enriched RNA using random hexadeoxynucleotide primers and Moloney murine leukemia virus reverse transcriptase (First-Strand Synthesis kit, Amersham Pharmacia Biotech) as recommended by the manufacturer.

Amplification of *H. brasiliensis* profilin cDNA by polymerase chain reaction (PCR)

Degenerate oligodeoxynucleotide primers (Genosys Biotechnologies Inc., Cambridge UK) for cDNA amplification were designed according to previously reported sequences (17–20). The primers used were: sense primer, 5'-AGAGAATTCCATATGTCGTGGCA-(A/G)(A/G)CGTACGT-3' and antisense primer 5'-CCAAGCTTGGATCC (C/T)TACA(G/T)GCC(C/T)TGTTT(G/A/T)A(G/T/C)-GAGGTAGTCACCGAGCC-3'. The slash between bases means that they were added at that particular position in equimolecular amounts during the synthesis of the primer. The regions corresponding to the 5'- and 3'-sequences, respectively, of the full-length cDNA encoding profilin are underlined, and the EcoRI, NdeI, HindIII, and BamHI restriction sites are in italics. Products of the first-strand cDNA synthesis (5 ml) and primers were mixed in PCR standard mixture, and subjected to amplification with Taq DNA polymerase (Bioline, London, UK) in a Robocycler 40 (Stratagene, La Jolla CA). After denaturation at 94°C for 4 min, the sample was subjected to 5 cycles of denaturation at 94°C (1 min), annealing at 45°C (2 min), and extension at 72°C (2 min), followed by 30 cycles at 94°C (1 min), 56°C (2 min), and 72°C (2 min), and a final incubation at 72°C (6 min). cDNA was isolated from the gel by GeneClean (Bio101, La Jolla CA) and after cutting the resulting cDNA fragment with EcoRI and HindIII, cloned into pBluescript II SK (Stratagene, La Jolla, CA). The ligation mixture was used to transform competent cells of *E. coli* DH5a by the rubidium chloride method (21). Restriction analysis and recombinant DNA techniques were performed by standard methods or as recommended by the manufacturer.

Sequencing of *H. brasiliensis* profilin cDNA

The nucleotide sequence of cDNA inserts was determined by the dideoxy chain-termination method (22). Standard protocols of the manufacturer for ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer, Foster City, CA) were used. The sequencing reactions were analyzed on an ABI 373A DNA sequencer (Perkin-Elmer). Commercial or vector-specific primers were used for sequencing both strands. Sequence analyses were done using the GCG Computer Package (Computer Group Inc., Madison, WI) and the BLAST program.

Production of recombinant latex profilin (rHev b 8)

E. coli BL21 (DE3 23), cells containing the pKN172-derived

recombinant plasmids were grown in LB with 200 (g/ml ampicillin. When cultures reached an OD 600 of 0.6, 0.6 mM IPTG was added to the culture and incubation continued for another 3 h. Cells were harvested by centrifugation and the pellet, resuspended in 0.1 M NaCl, 0.05 M Tris-HCl, pH 7.5, was lysed by lysozyme treatment (100 mg/ml, 15 min, 37°C) and mild sonication. Insoluble material was removed by centrifugation and profilin in the supernatant was purified by PLP-Sepharose chromatography.

Skin tests

Cutaneous reactivity with purified natural and recombinant Hev b 8 were assayed by intradermal tests at 14 and 7 µg/ml of protein estimated by Lowry method (24). They were performed in duplicate on the upper outer arm by the injection of 0.02 ml of extract with a 1 ml disposable syringe. Control skin tests were performed with 0.03% human serum albumin phenol-saline and with histamine phosphate 0.001 mg/ml.

After 15 min, translucent tape was applied over the resulting wheals and erythemas, and these were transferred to a record sheet. Areas were expressed in square millimetres and estimated by digitalization with a computer-aided design program (AUTOCAD version 11). A positive intradermal skin test result was defined as a wheal of 7 mm² or larger with definite erythema.

Radioallergosorbent assay (RAST)

Solid-phase antigen was obtained by coupling the extract solution to the 6 mm diameter CNBr-activated paper disks. RAST was performed according to standard procedures using the chromogenic β-galactosidase method (Pharmacia Upjohn, Sweden). Purified natural and recombinant latex profilin were used as solid phases (at 0.05 mg/ml coupling protein concentrations, respectively). Samples with specific IgE concentrations of ≥ 0.35 kU/l were regarded as positive.

SDS-PAGE immunoblotting

SDS-PAGE was carried out by standard procedures. Polyacrylamide concentrations of 12.5% and 3% were used for separating and stacking gels, respectively. Proteins dissolved in 0.125 M, Tris-HCl buffer (pH 6.8) were dissociated with 0.1% SDS and 5% (v/v) β-mercaptoethanol by treatment at 100°C for 5 min. Twenty micrograms of protein estimated by the Bradford's method (25) were applied per well.

Separated protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Milford, MA). Western blots were incubated with undiluted human serum (16 h at 4°C), washed and incubated with 2.5 ml of 1:10 000 diluted rabbit immunoglobulins to human ε-chains (DAKO, Glostrup, Denmark) conjugated to peroxidase.

Profilin and Hev b 1 were identified in western blots by first incubating with 1/1000 dilutions of rabbit polyclonal antiserum anti-sunflower pollen profilin and monoclonal antibody (mAb) II4F9 anti-Hev b 1 (a kind gift from Dr. Z. Chen, BGFA Bochum, Germany), respectively. Secondary antibodies were rabbit immunoglobulins to rabbit and mice IgG (DAKO), also diluted 1/1000. Bound peroxidase was detected by the addition of 3 ml of freshly prepared chromogenic substrate of 0.06% w/v of 4-chloro-1-naphtol solution (Bio-Rad Laboratory, Richmond, CA) and 0.01% (v/v) H₂O₂ in TBS. Analysis of electrophoretic components was performed by image analysis in the Bio-Rad GS-700 Densitometer.

Alternatively, IgE Western blots were visualized by the chemiluminescence method (ECL, Amersham Pharmacia Biotech), as recommended by the manufacturer.

Results

Two different groups of patients allergic to latex were covered in the present study: 17 spina bifida children and 14 adults occupationally sensitized to latex. They were randomly included in the study once latex hypersensitivity was ascertained by their clinical history (all the patients suffered from cutaneous and/or respiratory symptoms on exposition to latex) and positive cutaneous test and specific IgE assay to latex complete extract.

Natural profilin

Natural profilin was satisfactorily isolated from latex serum or from natural ammoniated serum by affinity chromatography (PLP-Sepharose), in the latter case after prior removal of Hev b 1 excess by salt fractionation. Hev b 1 precipitated between 20 and 60% ammonium sulfate saturation and profilin between 70 and 90% sat. as checked by immunoblotting using anti Hev b 1 mAb and anti-profilin polyclonal antiserum.

Amplification of *H. brasiliensis* profilin cDNA and sequence analysis

PCR following synthesis of the first cDNA strand using reverse transcriptase PCR (RT-PCR) and degenerate primers produced a main fragment of 400 base pairs corresponding in size with the full-length profilin gene. The fragment was subcloned in the vector pBluescript II SK and sequenced (Fig. 1). cDNA encoded a polypeptide of 131 amino acids with a predicted molecular mass of 14 194 Da and an isoelectric point (pI) value of 4.66. No sequences containing putative N-glycosylation sites (N_xT/S) were found, but a protein kinase phosphorylation site (S/T_xR/K) was found at residue 93. Comparison of the deduced amino acid sequence of the *H. brasiliensis* profilin using the BLAST program revealed high homology with other described plant profilins. Highest homologies were found with profilins from *Phaseolus vulgaris* (kidney bean) with a 83% identical residues and *Zea mays* (maize) with 81% identity. The lowest identity among plant profilins was detected with *Nicotiana tabacum* with a 75% of identical residues. Much lower sequence identities were found with profilins from non-plant organisms such as slime moulds, 44%; insects, 43%; yeasts, 34% and protists, 26%. Vertebrate profilins have an average of 30% identical amino acids. All of the 18 residues identical in 80 % of sequenced profilins (26) are conserved in the *H. brasiliensis* profilin sequence (Fig. 1).

Expression of recombinant profilin

The expression of full-length profilin region was carried out using the T7 system, in which the gene of interest is expressed under the control of the φ10 promoter specifically activated by the T7 RNA polymerase produced from the lac promoter. A nonfusion form of profilin was expressed in *E. coli* BL 21 (DE3). The

ATG	TCG	TGG	CAG	ACG	TAC	GTG	GAT	GAG	CGT	CTT	ATG	TGC	GAA	ATC	GAG	GGC	AAT	CAT	CTC	60
M	S	W	Q	T	Y	V	D	E	R	L	M	C	E	I	E	G	N	H	L	20
ACT	GCT	GCA	GCC	ATC	ATC	GGC	CAA	GAC	GGC	AGC	GTC	TGG	GCC	CAG	AGC	TCC	AAC	TTC	CCT	120
T	A	A	A	I	I	G	Q	D	G	S	V	W	A	Q	S	S	N	F	P	40
CAG	TTC	AAG	TCT	GAA	GAA	ATC	ACT	GCC	ATT	ATG	AGT	GAC	TTT	GAC	GAA	CCT	GGA	ACA	CTA	180
Q	F	K	S	E	E	I	T	A	I	M	S	D	F	D	E	P	G	T	L	60
GCT	CCA	ACT	GGT	TTG	CAC	CTG	GGT	GGC	ACA	AAA	TAC	ATG	GTG	ATC	CAA	GGA	GAG	GCA	GGA	240
A	P	T	G	L	H	L	G	G	T	K	Y	M	V	I	Q	G	E	A	G	80
GCT	GTC	ATT	CGG	GGG	AAG	AAG	GGA	CCT	GGT	GGT	GTT	ACT	GTT	AGG	AAG	ACA	AAT	CAG	GCC	300
A	V	I	R	G	K	K	G	P	G	G	V	T	V	R	K	T	N	Q	A	100
CTA	ATA	ATT	GGT	ATC	TAT	GAT	GAG	CCA	ATG	ACT	CCA	GGG	CAG	TGC	AAC	ATG	ATT	GTT	GAA	360
L	I	I	G	I	Y	D	E	P	M	T	P	G	Q	C	N	M	I	V	E	120
AGG	CTC	GGT	GAC	TAC	CTC	CTA	GAA	CAA	GGC	ATG	TAA									396
R	L	G	D	Y	L	L	E	Q	G	M	*									132

Figure 1. Complete nucleotide and amino acid deduced sequence (in bold) of *Hevea brasiliensis* profilin. Primers used for cDNA amplification are underlined. Black boxes indicated conserved residues in 80% profilins sequenced (26). Putative phosphorylation site is boxed. The nucleotide sequence of *H. brasiliensis* profilin was submitted to GenBank/European Molecular Biology Laboratory (EMBL) databases under Accession Number Y15042.

soluble material was chromatographed over a PLP-Sepharose affinity column. After elution with 8 M urea in PBS rHev b 8 was purified to electrophoretic homogeneity (Fig. 2). rHev b 8 showed an apparent molecular size of 14.7 kDa by SPS-PAGE, similar to the deduced molecular mass of 14,194 Da.

In vivo and *in vitro* assays of natural and recombinant latex profilins

Table 1 shows the results found with the 17 SB latex-allergic children. Specific IgE was determined by RAST. A control pool of sera from patients allergic to sunflower pollen profilin was used as positive control. Solid phases containing recombinant and natural profilin from olive pollen, *Olea europaea* (16) were comparatively assayed. Table 2 shows the results encountered with the 14 AL allergic patients.

Both natural and recombinant purified Hev b 8 were positive, by cutaneous intradermoreaction (ID) tests, at 7 µg/ml, in 15 and 14 SB and AL allergic patients. No wheals were produced when tested in nonatopic and atopic patients nonallergic to latex.

Overall, only 42% of sera from ID test-positive patients revealed specific IgE titers, RAST class 1 or higher to any latex profilin forms (six in the SB and seven in the AL patients). In any case, IgE titer was higher than class 3.

SDS-PAGE immunoblotting performed with sera from both group of patients is shown in Fig. 3. Sera from three SB patients (numbers 7, 15 and 17) recognized natural profilin bands at 14 kDa (Fig. 3A), in some cases also reacting to aggregative profilin forms of higher molecular masses, as evidenced by blot incubated with antiprofilin polyclonal antiserum. Sera from six SB

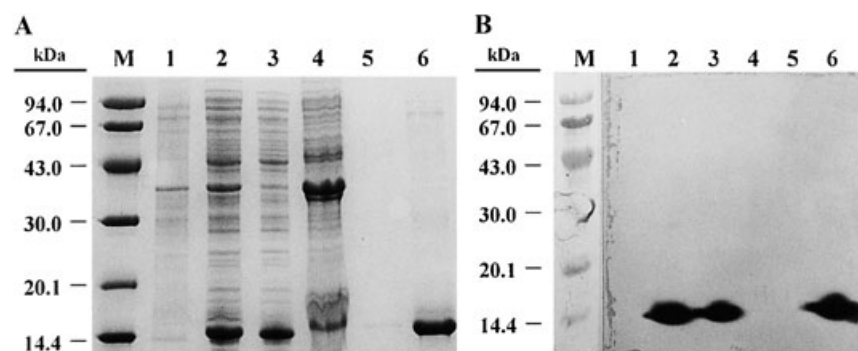


Figure 2. Purification of recombinant Hev b 8 expressed in *Escherichia coli*. A: 12.5% SDS-PAGE analysis of samples from the purification steps. B: Western blotting of (A) using rabbit antiprofilin serum. Lane M: molecular mass markers; lanes 1 and 2: total cell lysate from *E. coli* BL21 (DE3) containing pKN172 alone and pKN172 with Hev b 8 DNA insert, respectively; lanes 3 and 4: soluble and insoluble fractions after centrifugation of sample on lane 2; lane 5: PLP-Sepharose column non-retained fraction; lane 6: 6 M urea elution of recombinant Hev b 8 from PLP-Sepharose column.

Table 1. *In vivo* and *in vitro* analysis of spina bifida (SB) patients

Serum number	RAST class (radioallergosorbent assay)				Western blot		Wheal area (mm ²) intradermal test		
	rHev b 8	nHev b 8	rOle e 2	nOle e 2	rHev b 8	nHev b 8	rHev b 8	nHev b 8	Histamine
1	0	0	0	0	-	-	0	0	180
2	0	1	0	0	-	-	91	83	77
3	0	0	0	0	-	-	0	0	120
4	0	0	1	1	+	-	195	210	266
5	1	2	0	0	+	-	151	176	162
6	0	0	0	0	-	-	208	202	236
7	2	3	2	2	+	+	191	252	252
8	0	0	0	0	-	-	74	95	218
9	1	3	1	0	+	-	87	118	108
10	0	0	0	0	-	-	45	78	111
11	0	0	0	1	-	-	235	201	202
12	1	2	1	0	+	-	183	291	243
13	0	0	0	0	-	-	90	115	108
14	0	0	0	0	-	-	69	100	92
15	2	2	0	1	+	+	171	170	167
16	0	0	0	0	-	-	36	103	188
17	0	0	0	3	-	+	84	45	108
Control pool	2	3	3	4	+	-			
Control (-)	0	0	0	0	-	-			
Conjugated control	0	0	0	0	-	-			
Area (mean $\pm$ SEM)							123.3 $\pm$ 16.9	149.3 $\pm$ 18.4	166.9 $\pm$ 15.0

r: recombinant; n: natural.

patients (numbers 4, 5, 7, 12, 15) reacted against recombinant latex profilin (Fig. 3B). Regarding sera from AL patients, also three of them reacted to natural profilin (#2, 13, 14; Fig. 3 C) and four to recombinant profilin (numbers 9, 11, 13, 14; Fig. 3C). Therefore 12 patients out of 31 (39%) showed a detectable IgE serum level to any recombinant or natural Hev b 8 forms by this technique.

Discussion

The identification of profilin as an IgE-binding component in latex from *H. brasiliensis* was already reported by Vallier et al. (10); they demonstrated its presence in the soluble phase of latex by probing latex blots with rabbit profilin antisera and with human sera containing IgE antibodies to ragweed profilin. They also preliminary passed a cytosolic fraction of latex through a poly-L-proline affinity column, finding specifically retained proteins in the 15 kDa range. However they strongly questioned the clinical relevance of latex profilin, based on the result that only two sera out of 19 tested displayed detectable IgE antibodies to profilin by immunoassay, as well as by immunodetection of blots from latex cytosolic phase.

Later, the clinical relevance of profilin in ammoniated latex milk extract was evaluated by Fuchs et al. (11), by studying the IgE-reactivity profiles of a representative number ($n = 56$) of patients with latex allergens, before and after preadsorption of their sera with pollen allergens and recombinant birch pollen allergens (rBet v 1 and rBet v 2). They found no significant

inhibition of IgE binding when rBet v 2 (birch profilin) was used for preadsorption suggesting the explanation of the low amount of profilin in latex extracts. They indicated that only two sera, out of the 56 tested ones, contained profilin-specific IgE by RAST, and that both patients belonged to the group of patients originally sensitized to latex; meanwhile the evaluated sera showing high molecular mass (30–60 kDa) cross-reacting components with pollen extracts belonged to patients preferentially sensitized to pollen.

In spite of these preliminary findings, isolated samples of profilin had not been used in combined *in vitro* and *in vivo* assays in order to elucidate the allergenic clinical significance of this molecule for latex allergic patients.

The purification of natural profilin from ammoniated natural latex extracts faced two main problems, one related to the low yield of the process, probably due to the low presence of profilin in latex milk and to its solubility in the conventional aqueous extraction buffers. The other was derived from the interference of Hev b 1 protein (rubber elongation factor) in the purification of profilin by affinity chromatography in PLP–Sephadex columns. Eluates of profilin retained by the resin contained Hev b 1 traces, as demonstrated by Western blotting using anti-Hev b 1 mAb (figure not shown), due to unspecific interaction of this protein with the column matrix, probably because of its sticky properties derived from its physiological functions (27). Moreover, the molecular mass of Hev b 1 (14.6 kDa), in the same range as profilin (14 kDa), could complicate the detection of contaminant traces by the SDS–PAGE

Table 2. *In vivo* and *in vitro* analysis of latex-allergic (AL) patients

Serum number	RAST class (radioallergosorbent assay)				Western blot		Wheal area (mm ²) intradermal test		
	rHev b 8	nHev b 8	rOle e 2	nOle e 2	rHev b 8	nHev b 8	rHev b 8	nHev b 8	Histamine
1	0	1	0	0	-	-	54	111	114
2	0	1	0	0	-	+	146	175	65
3	0	0	0	0	-	-	55	76	66
4	0	0	0	0	-	-	85	106	120
5	0	0	0	0	-	-	70	142	156
6	0	0	0	0	-	-	103	357	156
7	0	1	0	0	-	-	126	181	136
8	0	1	0	0	-	-	102	128	88
9	0	0	0	0	+	-	17	15	59
10	1	0	0	0	-	-	48	67	126
11	0	0	0	0	+	-	86	135	99
12	0	0	0	0	-	-	57	103	247
13	0	1	1	2	+	+	19	132	104
14	1	2	0	3	+	+	222	214	202
Control pool	2	3	3	4	+	+			
Control (-)	0	0	0	0	-	-			
Conjugated control	0	0	0	0	-	-			
Area (mean $\pm$ SEM)							85.0 $\pm$ 14.5	138.7 $\pm$ 21.5	124.1 $\pm$ 14.2

r: recombinant; n: natural.

technique or size exclusion chromatography. This should be borne in mind when evaluating apparently pure profilin separations by PLP–Sephacryl chromatography from other biological sources, reinforcing the need for purity assays by two-dimensional analytical techniques and mAb based identification tests. In the case of profilin purification from latex, this drawback was solved either by previous fractionation of the extract, recovering the fraction precipitated between 70 and 90% ammonium sulfate saturation, or by using c-serum latex (the supernatant fluid obtained when rubber particles are removed by centrifugation), in which Hev b 1 is practically absent. In the latter source, the relative content of profilin is higher, although not so easily obtained commercially.

In order to make a more complete evaluation of the allergenic relevance of latex profilin, a recombinant form was produced to be comparatively tested with its natural counterpart.

Recombinant Hev b 8 was efficiently purified by affinity chromatography on a PLP–Sephacryl column (Fig. 2). It exhibited immunochemical properties equivalent to its natural counterpart.

Both natural and recombinant Hev b 8 were recognized by a great majority of latex allergic patients (88% of SB and 100% of AL) when evaluated by ID skin tests. Around 40% of patients from both the latex-sensitized groups of patients exhibited positive (but not elevated) IgE serum titres to any of the purified natural Hev b 8 or rHev b 8 proteins, as determined by enzyme immunoassay or western blot techniques (Tables 1, 2 and Fig. 3). These findings are not in agreement with previous data from Vallier et al. (10) and Fuchs et al. (11), nor with the recent results reported by Yip et al. (12). These authors found that only one out of 29 (3%) adults allergic to latex reacted positively to rHev b 8 by prick test.

It is not the first time that this type of controversy has been reported concerning individual latex allergens, thus Hev b 1 was considered a major allergen by Czuppon et al. (28) and Chen et al. (29), while another studies from Alenius et al. (30) and Kurup et al. (31) demonstrated the low frequency of reactivity for Hev b 1 among the various investigated latex-allergic patients. The discrepancies have been attributed to the different patient populations and assays utilized (31). Differential reactivity profiles due to different populations have been ascertained for profilin from birch pollen (32). It was found that the frequency of sensitisation to rBet v 2 in Swedish and Finnish groups was 5–7% compared to 20–38% in the central/south European groups.

rHev b 8 used in the present study was obtained as a nonfusion protein, and the results found evidenced similar behaviour, both *in vivo* and *in vitro*, to the corresponding natural profilin. On the contrary, the recombinant Hev b 8 used in skin prick tests (SPTs) by Yip et al. (12) was obtained and remained as a fusion protein to the bacterial maltose binding protein (MBP) a much bigger protein than profilin, that could lead to the masking of epitope regions. No data is provided concerning the comparative immunological properties of recombinant and natural Hev b 8.

On the other hand, Rihs et al. (13) reported a similar low prevalence of profilin reactivity within SB patients (2/17), but a higher level in healthcare adult workers (20%), by CAP analysis of serum samples using rHev b 8 obtained also as a MBP fusion protein that was cleaved for immunoblotting assays. However, they did not perform cutaneous tests or *in vitro* comparison with purified natural latex profilin.

Overall, results presented in Tables 1 and 2 show a higher reactivity of latex allergic patients to natural Hev b 8 than to rHev b 8 (as in the case of Ole e 2).

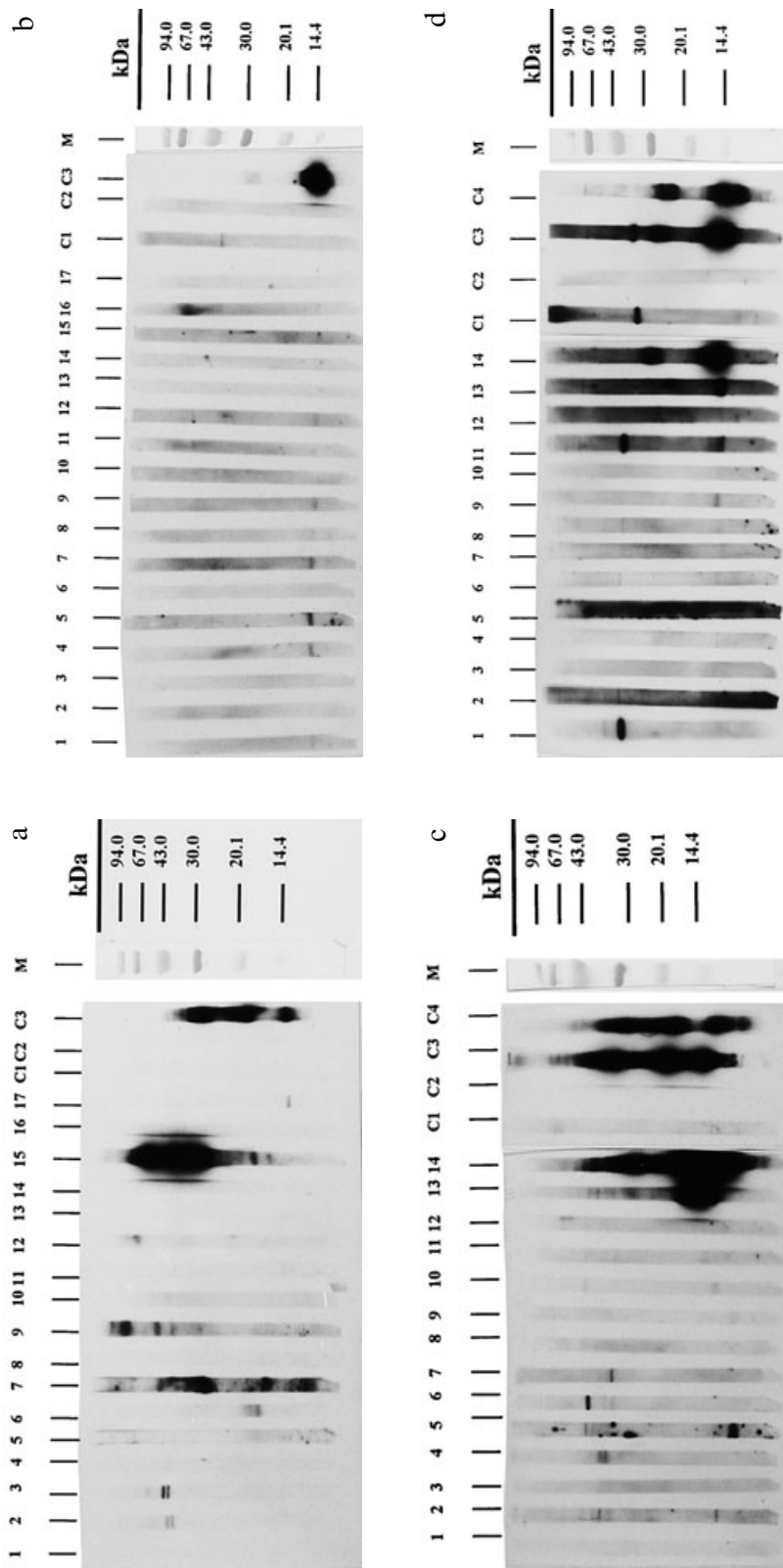


Figure 3. SDS-PAGE immunoblotting of natural (A and C) and recombinant (B and D) profilins incubated with individual human sera from SB (A and B) and AL (C and D) latex-allergic patients. C1: negative control; C2: conjugate control; C3: antiprofilin polyclonal antisera; C4: pool of sunflower profilin sensitized patients.

Taking into account the checked purity of natural Hev b 8 preparations, a suggested explanation of these findings is that IgE reactivity directed only against a specific isoform can be lower than the one directed against the polymorphic nHev b 8 (six variants and two isoforms are currently registered in the IUIS Allergen Nomenclature database). As it has been demonstrated (33), the absence of some amino-acid residues in the epitopes of expressed isoforms can change its immunochemical properties, giving rise to a decreased reactivity.

Intradermal tests were used in the present study to assay cutaneous reactivity due to reasons of sample availability and to the advantage of its greater sensitivity as a diagnostic test. Some studies, it could be argued, have shown that for certain aeroallergens, such as cat dander and timothy grass pollen, the presence of positive intradermal skin test response does not indicate the presence of clinically significant sensitivity (34, 35); this should be considered when interpreting the present results. However it has also been documented that in different clinical situations, e.g. drug and insect venom allergies, SPTs and RASTs may not be sufficiently sensitive, leading to potential false-negative results if intradermal tests are not used (36, 37). In the present study, allergen sensitizations have been produced by a route other than inhalation in most of the patients (and in all SB children), therefore the usefulness and reliability of the intradermal skin test should be comparable to the latter situations.

Although a lack of correlation between positive IgE and cutaneous responses has been observed, since only around 40% of the patient sera showed specific IgE, similar findings have been observed with natural latex

extracts. Thus Kurup et al. (31) reported that only 61% of 31 healthcare workers, and 54% of 13 SB children with positive SPT had positive specific IgE by RAST.

The specificity of positive responses was also supported by the negative results obtained in the cutaneous test and serological assays of control subjects, both nonatopic individuals and atopic ones nonallergic to latex. The estimated 100% (in SB) or 88% (AL) frequency of profilin sensitizations, as seen by cutaneous tests, are far higher than the one reported from profilin in pollen-allergic patients, which was estimated at 20% overall by Valenta et al. (7). Only in recent studies has profilin been considered as a major allergen, at least from an *in vitro* point of view, being detected as an IgE-binding component in more than 50% of the sera evaluated by immunoblotting of allergenic extracts from *Mercurialis annua* pollen (8) and soybean (9).

It can be concluded, in terms of frequency of cutaneous reactivity, that Hev b 8 seems to be a relevant allergen for these two groups of latex-allergic patients. However, the low intensity of positive *in vivo* and *in vitro* responses, and the lack of correlation between them, somewhat diminish its allergenic significance; together with a low capacity for IgE induction, probably due to the extremely low profilin content of latex sources, it is therefore bordering on the threshold of allergen exposure levels.

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