

Appraisal of latex glove proteins in the induction of sensitivity to multiple latex allergens

H.Y. Yeang, K.S. Chow, F. Yusof, S.A.M. Arif, N.P. Chew and Y.H. Loke

Biotechnology and Strategic Research Unit, Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia

SUMMARY

Six *Hevea brasiliensis* latex protein allergens, Hev b 1, Hev b 2, Hev b 3, Hev b 4, and two variants of Hev b 7 (7b and 7c), were purified from Hevea latex, while a seventh protein, Hev b 5, was prepared in recombinant form. The presence of these proteins in glove extracts was indicated by their respective antibodies in the serum of rabbits immunized against the extracts. The relative propensities of IgE binding to the individual latex allergens were compared using sera from latex-allergic patients. IgE recognition of Hev b 4, Hev b 7b, Hev b 5 and Hev b 2 was most frequently encountered, with 75, 61, 31 and 28%, respectively, of the patient sera reacting. Sensitivity to multiple latex proteins was common, and out of the 31 seropositive patients, 23 (74%) had IgE against at least two latex allergens, while 12 (39%) had IgE specific for at least three allergens. Statistical analysis of the data suggested that many patients might have acquired sensitivity to Hev b 2, Hev b 4 and Hev b 7b from a common source, (e.g., from latex products). On the other hand, sensitivity to Hev b 5 and to Hev b 7c were interrelated. It is plausible that sensitivity to these two proteins might have been acquired from sources other than latex products (e.g., from certain foods).

Key words: Latex allergens - Latex allergy - Latex proteins - Latex gloves - *Hevea brasiliensis* - IgE antibodies

INTRODUCTION

Serious reaction to natural rubber latex from *Hevea brasiliensis* did not surface in the literature until Nutter's publication (1) in 1979. By the late 1980s and into the 1990s, reports began to be received with increasing frequency in Europe and America of allergic reactions occurring among users of surgical and examination gloves

made of latex and among spina bifida patients [see the reviews by Hamann (2) and Turjanmaa *et al.* (3)]. There has been much interest in identifying the proteins in *Hevea* latex responsible for latex allergy and considerable effort was expended on isolating and purifying the allergenic proteins from *Hevea* latex or latex products (4, 5). Today, the International Union of Immunological Societies (IUIS) recognizes ten latex allergens, Hev b 1 to Hev b 10.

The significant increase in the number of reports of latex allergy in the last decade has been attributed to increased usage of latex gloves in healthcare in tandem with the rising cases of AIDS (2). Turjanmaa and Reunala (6) concluded that sensitization to latex among healthcare workers was clearly work-related, the main cause being latex gloves. Nevertheless, numerous cross-sensitivities between latex protein allergens and various food and pollen allergens are known (3-5). It is therefore not improbable that many latex-allergic patients may have been initially sensitized not only by proteins from latex products, but also by proteins from other sources.

This paper reports on a study that was undertaken with the following objectives: i) to assess commercial latex gloves as a source of the protein allergens Hev b 1, 2, 3, 4, 5, and 7 (Table 1), and ii) to study IgE binding to multiple latex allergens in order to assess which combinations of allergens, either from latex gloves or other sources, a patient might likely have been exposed to that had resulted in latex sensitization.

MATERIALS AND METHODS

Latex collection

Fresh latex from *Hevea brasiliensis* trees (clone RRIM 600) was collected in chilled containers at the

Table 1
Common names and molecular weights of latex allergens.

Hev b 1	Rubber elongation factor	14.6 kDa
Hev b 2	β 1,3 glucanase	36 kD doublet
Hev b 3	Small rubber particle protein	22 kDa
Hev b 4	Microhelix component	110 kD triplet*
Hev b 5	kDa	16 kDa
Hev b 7	Patatin-like protein	43 kDa
	Rubber biosynthesis inhibitor	

*Appears as 55 kDa triplet on electrophoretic separation under reducing conditions.

Rubber Research Institute of Malaysia Experiment Station, Sungei Buloh, Selangor. The latex was centrifuged at 19,000 rpm (43,000 g) on a Sorvall RC 5C high-speed centrifuge for 1 h at 4–7 °C to separate it into three main fractions: the top fraction which was the rubber cream, the heavy bottom fraction and the C-serum located in-between (7). These three fractions were obtained for the preparation of the native protein allergens Hev b 1, 2, 3, 4 and 7. Tapped latex for the purpose of mRNA preparation was collected directly into an equal volume of chilled 2 x RNA extraction buffer [100 mM TrisCl, 300 mM LiCl, 10 mM ethylenediaminetetraacetic acid (EDTA), 10% sodium dodecyl sulfate (SDS), pH 9.0]. The latex-buffer mixture was kept in ice and brought back to the laboratory for RNA extraction and subsequent preparation of recombinant Hev b 5.

Preparation of latex B-serum

Latex B-serum was prepared based on the method of Hsia (8). The latex bottom fraction from centrifuged latex was washed by resuspension in 0.4 M mannitol and recovered by centrifugation. The washed bottom fraction was then subjected to repeated freezing and thawing to rupture its main components, the luteoids. The luteoid fluid, the B-serum, was recovered as the supernatant after recentrifugation.

Preparation of Hev b 1 and Hev b 3

To extract the rubber particle proteins, Hev b 1 and Hev b 3, the rubber cream was sampled at the intersection between Zone 1 and Zone 2 so as to obtain rubber particles belonging to both the large and small size classes (9). The rubber particles were washed by resuspension in 30% sucrose. Proteins on the rubber particle surfaces were then solubilized and extracted using a detergent mixture comprising 0.1% Triton-X 100 and 1% SDS. The two main proteins obtained, Hev b 1 and Hev b 3, were separated and purified by preparative electrophoresis on 15% SDS-polyacrylamide gel using the Prep Cell (BioRad, USA) according to the manufacturer's instructions.

Preparation of Hev b 2 and Hev b 4

Hev b 2 and Hev b 4 were prepared from the bottom fraction of centrifuged latex as described previously (10). Briefly, latex B-serum was dialyzed (2.5 kDa molecular weight cut-off tubing) against distilled water for 48 h at 8 °C. This induced the formation of a proteinaceous precipitate which was then re-dissolved in 0.35 M NaCl and loaded on to a Sephacryl S-200 (Pharmacia, Sweden) gel filtration column. Hev b 2 and Hev b 4 were recovered in the two major peaks in the eluate.

Preparation of Hev b 7

Hev b 7 was prepared from both the latex C-serum and latex B-serum following the method described previously (11). Briefly, C-serum or B-serum was fractionated on a Sephadex G-150 (Pharmacia, Sweden) column (2.6 x 81 cm) equilibrated in 250 mM Tris-HCl, pH 8.0 with 5 mM 2-mercaptoethanol and 1% Tween-20. The active fractions, identified using a polyclonal antibody against the protein, were pooled and dialyzed against 50 mM Tris-HCl, pH 8.0 before being subjected to anion-exchange chromatography. The dialysate was loaded on to a DEAE-Cellulose (Whatman, UK) column (1.6 x 20 cm) equilibrated in the same buffer with 5 mM 2-mercaptoethanol added. After flushing out unbound protein with the Tris-HCl buffer, active fractions were recovered using a linear gradient of NaCl (0–0.3 M) in buffer. The active fractions were pooled and dialyzed against a buffer comprising 50 mM Tris-HCl, pH 8.0, 5 mM 2-mercaptoethanol and 0.5 M NaCl for further purification by hydrophobic interaction chromatography. The dialysate was then loaded on to a column of Phenyl Sepharose (Pharmacia, Sweden) (1.6 x 15 cm) equilibrated in this buffer. After washing out unbound protein with the buffer, the active protein was eluted using the same buffer, but with NaCl omitted. Hev b 7 from the B-serum and from the C-serum are referred to in this report as Hev b 7b and Hev b 7c, respectively.

Preparation of recombinant Hev b 5

Recombinant Hev b 5 was prepared using the same approach as that adopted by Slater *et al.* (12). Total RNA from latex was isolated essentially according to the method of Prescott and Martin (13). Two oligonucleotides flanking the published Hev b 5 protein coding sequences (12) were synthesized (Genosys, USA) for reverse-transcription polymerase chain reaction (RT-PCR) of the corresponding cDNA. To facilitate directional cloning, the forward (5'-GATGAATTCCAAATGGCCAGT-GTTGAG3') and reverse (5'-GATGTCGACTATGCATAA TAGACCTATTTATTC3') primers incorporated sequences of the restriction sites, Eco R I and Sal I, respectively.

RT-PCR reactions were performed using the Access RT-PCR kit (Promega, USA) with 100 ng of latex total RNA per reaction. The RT reaction was carried out at 48 °C for 45 min, followed by sample denaturation at 94 °C for 2 min. The reactions for PCR were programmed for 40 cycles on a DNA thermal cycler (Perkin-Elmer Corp, USA), each cycle being performed at a denaturing temperature of 94 °C (30 sec), annealing temperature of 60 °C (1 min), and extension at 68 °C (2 min). A single RT-PCR product of 500 bp was obtained. This product was purified from agarose gel and digested with the restriction enzymes, Eco R I and Sal I. The Hev b 5 PCR product was ligated as an Eco R I-Sal I insert into the pMAL-c2 expression vector (New England Biolabs, USA) such that the Hev b 5 cDNA was fused to the maltose-binding protein (MBP) upstream in the same translational frame. This plasmid was isolated and transformed into *Escherichia coli* DH5 α (Clontech, USA) host cells. DNA sequencing confirmed that the cloned cDNA encoded the complete Hev b 5 protein. Expression and affinity purification of recombinant Hev b 5 protein in pMAL-c2 was performed according to the manufacturer's instructions (New England Biolabs, USA) as described previously (9). Cleavage of the MBP-Hev b 5 fusion protein was by Factor Xa protease in the presence of 0.04% (weight/vol) SDS.

Preparation of polyclonal antibodies against latex glove proteins

Pieces (1 x 1 cm) cut from each of nine brands of latex examination gloves were incubated separately in distilled water overnight at room temperature. The extracts were centrifuged to remove powder and other insoluble matter. They were then desalinated by dialyzing with small pore (2.5 kDa molecular weight cut-off) tubing against distilled water for 2 days with water changed twice daily and then concentrated by partial freeze-drying. Extracts of different brands of gloves were adjusted to obtain parity in concentration and a blend of the nine extracts was used to immunize rabbits.

To raise polyclonal antibodies against glove proteins, 0.5 ml of 0.8 mg ml⁻¹ glove extract in 0.5 ml phosphate buffered saline (PBS) was mixed with an equal volume of complete Freund's adjuvant. The mixture was injected subcutaneously in the back of four rabbits. Seven booster doses, each containing 0.5 ml of 0.8 mg ml⁻¹ glove protein in 0.5 ml PBS and an equal volume of incomplete Freund's were administered at 2-week intervals before bleeding for antiserum.

Western blot and dot blot analyses

Purified proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% gels

according to the method of Laemmli (14) and stained with Coomassie blue or silver nitrate. For Western blots, proteins in the gel were transferred electrophoretically to a nitrocellulose membrane. To detect protein-rabbit IgG binding, the nitrocellulose membrane was blocked with 5% non-fat milk in PBS and then incubated for 90 min with rabbit antiserum (diluted 1:800 in PBS and milk) as the primary antibody. After three cycles of washing with PBS and milk, the nitrocellulose membrane was incubated for 1 h with the secondary antibody, anti-rabbit IgG conjugated to alkaline phosphatase. After a further three cycles of washing with PBS and milk, the nitrocellulose membrane was incubated for 10 min in Tris-buffered saline (TBS) before being immersed in 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) substrate to generate the colored alkaline phosphatase reaction product. To detect protein-IgE binding in Western blots, a similar procedure was followed, except that the nitrocellulose membrane was incubated overnight with patient serum (diluted 1:5.25 in PBS and milk and 0.05% sodium azide) as the primary antibody. Anti-human IgE conjugated to alkaline phosphatase served as the secondary antibody.

For dot blots, the purified latex allergens were each adjusted to concentration of 100 μ g ml⁻¹. Modifications were made to the PBS used to dilute Hev b 1, Hev b 2, Hev b 3 and Hev b 4. For the rubber particle membrane proteins Hev b 1 and Hev b 3, 0.1% SDS was added to the PBS to ensure that the proteins (which are insoluble in their native state) (15) remained soluble. To dilute Hev b 2 and Hev b 4, additional sodium chloride was added to PBS to attain 0.2 M since these proteins separate partially when the atmospheric ionic concentration is reduced (10). Protein samples (2 μ l) were dotted on to nitrocellulose membrane and tested for binding with patient serum IgE using the same experimental protocol as for Western blotting.

Protein quantitation

Concentrations of the glove extracts and purified latex protein allergens were determined by the modified Lowry procedure after removal of interfering buffer salts by precipitation with trichloroacetic acid and phosphotungstic acid (16, 17).

Patient sera

Patients were identified as being allergic to latex through a history of allergic reactions, skin prick tests and the detection of latex-specific IgE in the blood. Thirty-six serum samples obtained from adult latex-sensitive patients were used in this study. Twenty-eight samples were sourced from the United States, while

eight samples originated from Finland. A serum pool comprising a mixture of 101 samples from American patients with confirmed and suspected spina bifida served as a positive control as they were known to have IgE against all the allergens tested. For the nonallergic control patients, sera were obtained from 29 randomly selected blood donors from Malaysia. Skin tests were not conducted, but latex allergy in the general Malaysian population could be expected to be even rarer than the approximately one in 1,000 recorded for the general population in Europe (18-20). Even among high risk Malaysian groups [rubber product factory workers: 2% (21), rubber tappers: 3% (22)], prevalence of latex allergy in Malaysia is at the low end of the range of 2.4-17% recorded for healthcare workers in the West (23, 24).

RESULTS

Purified latex allergens and their presence on latex gloves

The purified native latex proteins were checked for purity by SDS-PAGE followed by silver staining (Fig. 1).

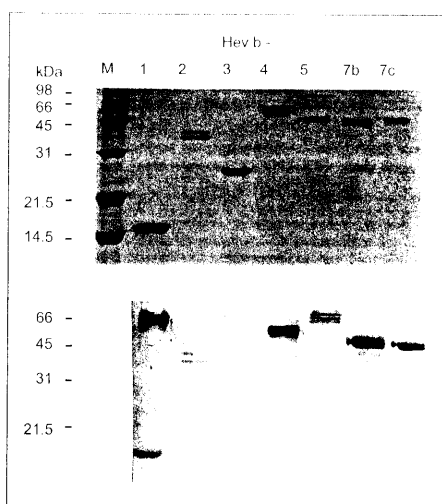


Fig. 1. SDS-PAGE-Western blot of the latex allergens Hev b 1, 2, 3, 4, 5, 7b and 7c. Upper panel: silver staining of the proteins after SDS-PAGE. Lower panel: immunostaining of the Western blot after incubation with rabbit anti-glove IgG. All are native proteins except for the recombinant fusion protein of Hev b 5 linked to the maltose-binding protein (MBP). Some of the MBP (about 46 kDa) that dissociated from the fusion protein appears on the silver-stained gel. Hev b 7b and 7c are Hev b 7 proteins isolated from the latex B-serum and C-serum, respectively. M = Molecular weight markers. The blotted markers were stained with naphthalene black.

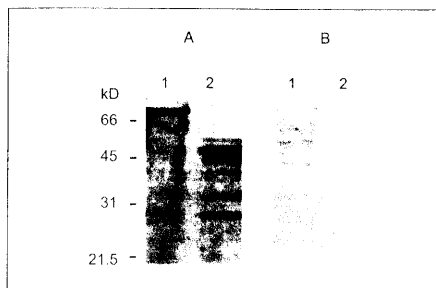


Fig. 2. Effect of cleaving recombinant Hev b 5 from the fusion protein. Panel A: Coomassie blue staining. Panel B: Western blot incubated with serum of patients sensitive to Hev b 5 to show IgE binding. Lane 1: uncut fusion protein. Lane 2: fusion protein cleaved with Factor Xa protease. In Panel B, IgE binding to the fusion protein is observed in lane 1, but this interaction is lost when Hev b 5 is cleaved from the fusion protein. A trace amount of IgE binding to residual uncut fusion protein is seen in lane 2.

Verification that the isolated proteins were the latex allergens Hev b 1 to Hev b 7 was by molecular weight as determined by SDS-PAGE and by positive binding with polyclonal antibodies (against Hev b 1, 2, 3, 4, 7b and 7c) and monoclonal antibodies (against Hev b 2, 3, and 4). Recombinant Hev b 5 was produced as a fusion protein with Hev b 5 adjoined to the maltose binding protein (MBP). Under reducing conditions, the fusion protein showed a molecular weight of about 72 kDa (Fig. 2). When Hev b 5 was cleaved away from the MBP with Factor Xa protease, the MBP (apparent molecular weight about 46 kDa) was observed together with peptide bands of about 28, 32 and 37 kDa, representing Hev b 5 or its derivatives (Fig. 2). While the recombinant fusion protein was recognized by IgE from patients sensitive to Hev b 5, this property was lost when Hev b 5 was cleaved from the MBP (Fig. 2). Hence, the uncut fusion protein was used in the study (but only the Hev b 5 moiety of the fusion protein was taken into consideration in calculating the protein concentration).

The production of IgG antibodies against the different glove proteins varied quantitatively between four immunized rabbits. Taken as a whole, positive binding of the antibodies to all the seven latex allergen proteins tested was observed in the Western blot of the proteins after incubating with rabbit antiserum (Fig. 1). There were two main discrepancies between the silver-stained gel and the Western blot. With the Hev b 1 protein, there was additional strong IgG binding around 60 kDa even though no protein of that size could be detected by silver staining (Fig. 1). There was also a discrepancy between the silver staining and the immunoreaction in the case of Hev b 7b. Prominent binding of anti-glove IgG

on the Western blot was not at exactly the position indicated by silver staining, but at a slightly higher molecular weight (Fig. 1).

Binding of latex allergens to IgE in patient sera

Dot blots of the individual latex allergens were incubated with sera from latex-allergic patients. The positive binding of serum IgE to the individual latex protein dots (100 µl, 200 ng) were scored in at least two experiments, except for the eight Finnish samples and four of the American samples where availability of the sera was limited. Nevertheless, there was good agreement between scores in different experiments (approx. 90%, the discrepancies being mainly in borderline cases) and so errors arising from unreplicated scores were not serious.

Binding of IgE to at least one of the seven latex allergens was observed in 31 out of 36 (86%) adult sera. Results for the individual allergens are presented in Table 2. The seven latex allergens were not equally represented in terms of the prevalence of their specific IgE in the serum samples studied. Three-quarters of the patients were sensitive to the most commonly recognized allergen, Hev b 4. Among the latex-allergic Americans, 89% had IgE recognizing this protein, although only 25% out of the eight Finns were similarly sensitized. The next most important latex allergen was Hev b 7b (Hev b 7 isolated from the B-serum). IgE specific to the protein was comparably represented among the Finnish (50%) and the American sera (64%), giving an overall prevalence of 61%. Unlike Hev b 7b, Hev b 7 from the C-serum (Hev b 7c) was seropositive in only three samples, all from the American group. About 36% of the American patients were sensitized to Hev b 5, and a similar proportion (32%) sensitized to Hev b 2. IgE to these two allergens were, however, poorly represented in the Finnish serum samples. For both the populations combined, prevalence of IgE specific to Hev b 5 and Hev b 2 was 31% and 28%, respectively.

IgE binding to Hev b 1 and Hev b 3 was rare in the study sample. Only one patient was seropositive to the rubber particle protein, Hev b 1 and none to the second rubber particle protein, Hev b 3. Sensitivity to Hev b 1 and Hev b 3 is known to be more commonly observed

in spina bifida patients than in adult patients (9). To confirm that negative IgE binding to these proteins was not the result of the dot blot assay being defective, a positive control dot blot of all the purified proteins was made using serum from spina bifida patients. All the allergens, including Hev b 1 and Hev b 3, were recognized by IgE from this spina bifida serum pool (results not presented).

While most of the control sera (24 samples or 83%) did not react with any of the latex proteins tested, Hev b 4 and Hev b 7b were each recognized by IgE in three serum samples (10%), whereas IgE against Hev b 2 was detected in one sample (3%).

Sensitivity to multiple latex allergens

Most of the latex-allergic patients were sensitized to more than one latex allergen. Thus, out of the 31 seropositive patients, 23 (74%) had IgE against at least two latex allergens, while 12 (39%) were IgE-specific for at least three allergens. In view of the many patients being sensitized to multiple latex allergens, an analysis was carried out to determine if sensitivity to the individual latex proteins had been acquired randomly and independently of one another. A series of 2 x 2 contingency analyses with Yates' continuity correction (25) was carried out pair-wise with all possible combinations of the latex proteins to answer the following question: given the observed frequencies of sensitivity to allergens X and Y taken separately in the study population, what is the probability of encountering the number of patients in the study that have acquired sensitivity to both allergen X and allergen Y through random chance? The objective of the analysis was essentially to determine if any two allergens were associated with one another with sufficient consistency for the link to be deemed over and above what might be expected out of coincidence and chance.

The results in Table 3 show that sensitivity to Hev b 4 tended to be closely associated with sensitivity to Hev b 7b. This association was statistically significant ($p = 0.018$). The link between Hev b 2 and Hev b 4 ($p = 0.086$) and between Hev b 2 and Hev b 7b ($p = 0.068$) were also noteworthy as the probabilities of such oc-

Table 2
Prevalence of IgE from adult patient sera binding to purified latex allergens.

Origin	Sample size	Hev b 1	Hev b 2	Hev b 3	Hev b 4	Hev b 5	Hev b 7b	Hev b 7c
Finland	8	1 (13)	1 (13)	0	2 (25)	1 (13)	4 (50)	0
USA	28	0	9 (32)	0	25 (89)	10 (36)	18 (64)	3 (11)
Total	36	1 (3)	10 (28)	0	27 (75)	11 (31)	22 (61)	3 (8)

Percentages are given in brackets.

Table 3

Probability of chance occurrence for the observed number of patients being sensitized to any two particular allergens at the same time.

	Hev b 1	Hev b 2	Hev b 4	Hev b 5	Hev b 7b
Hev b 2	1.000				
Hev b 4	0.558	0.086*			
Hev b 5	1.000	0.208	1.000		
Hev b 7b	1.000	0.068*	0.018*	0.364	
Hev b 7c	1.000	0.654	0.728	0.038*	0.680

* $p < 0.1$; ** $p < 0.05$.

currences being explained by coincidence were below $p = 0.1$. Table 3 also shows a separate relationship between sensitivity to Hev b 7 c and to Hev b 5 beyond what might be expected out of chance ($p = 0.038$).

DISCUSSION

While immunoassays such as ELISAs are often preferred research tools because of their high sensitivity, the dot blot assay was selected in the present study to exploit its inherent advantages in assaying multiple proteins. The binding of proteins to the ELISA plate can be optimized for a given protein by selecting a coating buffer pH appropriate for its pI. However, as the seven different proteins being studied had varying pIs ranging from highly acidic [Hev b 5; pI = 3.5 (26)] to highly basic [Hev b 2; pI = 9.5 (10)], it was not possible to select a common coating buffer optimized for all the proteins being compared. A further problem with the ELISA lay in the fact that the Hev b 1 and Hev b 3 that were purified by preparative electrophoresis contained SDS that interfere with the binding of the proteins to the plate. With dot blots, on the other hand, the nitrocellulose membrane absorbed all the dispensed proteins and the assay results were not influenced by how well a protein could bind to the solid support.

Latex allergens extractable from commercial latex gloves

The fact that proteins from latex gloves can induce allergy in sensitized patients is extensively documented. Less well investigated is the presence or absence on gloves of the various known latex allergens that can potentially trigger the allergic reaction. Although all the recognized latex allergens are found in raw natural rubber latex, comprehensive studies are lacking to establish whether some of them are removed in the course of glove manufacture, or if they are all still to be found in the finished commercial product. The preliminary finding from the present study is that all the allergens

examined appear to be present in the latex extracted from commercial gloves as indicated by their respective antibodies developed in immunized rabbits. Polyclonal antibodies to Hev b 2 and Hev b 4 cross-react with their antigenic proteins (results not presented), but the individual presence of the two proteins in glove extracts has previously been demonstrated using more specific monoclonal antibodies (27). Nevertheless, further study should be carried out to determine if some of the positive immunoreactions observed in this study had resulted from other cross-reactions with other allergenic and nonallergenic latex proteins.

The approximately 60 kDa protein in the Hev b 1 sample that was detected by anti-glove IgG was possibly the 58 kDa tetramer of Hev b 1 reported by Czuppon *et al.* (28). Under reducing conditions of SDS-PAGE, formation of the tetramer was not favored (28) and accordingly, the protein was not detected by silver staining (Fig. 1). The fact that the glove antiserum bound so strongly to it despite its meager presence suggests that Hev b 1 may be present as a tetramer in latex gloves, and that Hev b 1 in its tetrameric form is highly antigenic.

Propensity of IgE binding with individual latex allergens

Diagnosis of allergy is commonly determined by challenging with a preparation made from whole latex. Hence, a person diagnosed with allergy to latex could be sensitive to any one or any combination of the different proteins in latex. In this study, the large majority of patients were sensitive to more than one latex allergen. Multiple sensitization could have been induced by frequent exposure to a source (such as latex gloves) that harbored several allergens. The finding that sera from five out of the 36 patients did not react with any of the proteins tested might be due to the fact that there were other latex allergens which were not included in the present investigation. Possibly also, the dot blots might not have been sufficiently sensitive, but this was not considered critical since the main aim of the study was to deduce the interrelationships between allergens in sensitizing patients to latex allergy. The finding in the present investigation that 17% of sera sourced from the general population was IgE-positive for at least one latex allergen was also unsurprising. A significant gap exists between seropositive results and actual symptomatic allergy, especially among samples drawn from the general low-risk population (29).

The rubber particle proteins, Hev b 1 and Hev b 3, were found to be generally unreactive with IgE from latex-allergic adults, although a strong reaction was observed with the spina bifida patient serum that was used as a positive control. This finding is in accordance

with similar observations (9, 30, 31) reported previously [although other reports indicate sensitivity to Hev b 1 to be prevalent also among latex-allergic adults (28, 32)]. This characteristic has been attributed to the fact that the rubber particle proteins are insoluble or only sparingly soluble in water (15) and so are not readily released from latex products such as gloves used by healthcare workers. Some of our patients undergo regular catheterization and repeated surgical operations in which latex products (gloves, tubings and catheters) come into direct contact with the body membranes of the patients. The rubber particle proteins may thus elicit immunogenesis without their being soluble in water (9).

Besides Hev b 1 and Hev b 3, other latex allergens also display special solubility characteristics that affect their effectiveness as sensitizing agents. The B-serum proteins, Hev b 2 and Hev b 4, are only very sparingly soluble in pure water but dissolve in an ionic ambience in the presence of salt (10). Hence, leaching in water during the manufacture of latex gloves does not easily wash off these proteins, but the presence of salt in sweat increases their solubility. Among the latex allergens, sensitivity to Hev b 4 was the most frequently encountered (75%) (Table 2).

The recombinant protein was used in place of native Hev b 5, which has been reported to be a major latex allergen (12, 26). The three-dimensional structure of the protein appears to be important in specifying the IgE-specific epitope. IgE binding ability was lost when the MBP moiety was detached from the recombinant fusion protein using the Factor Xa protease (Fig. 2), although Hev b 5 itself does not contain recognition sites for factor Xa.

Hev b 7, which has a molecular resemblance to patatin (Sol t 1), the major storage protein in potato tubers (33), is also known to inhibit rubber biosynthesis activity in *Hevea* latex (11). There appear to be two forms of Hev b 7 in *Hevea* latex. Both Hev b 7 from the latex B-serum (Hev b 7b) and from the C-serum (Hev b 7c) are of about 43 kDa and both show homology to patatin (11, 34). A polyclonal antibody made against Hev b 7c also recognizes Hev b 7b, suggesting similarities in the two variants of the protein (unpublished results). Notwithstanding these similarities, the fact that the two proteins can bind to IgE from different patients indicates the existence of two variants with different IgE recognition epitopes, the Hev b 7b variant being far more commonly encountered (Table 2). This is the first report of the existence of two molecular variants of Hev b 7.

Disparate routes of sensitization to latex allergens

The statistical analyses denoted a significant relationship between sensitivity to Hev b 4 and sensitivity to Hev b 7b (Table 3). There were similar links between Hev b 4

and Hev b 2 and between Hev b 2 and Hev b 7b. This interlinkage between Hev b 2, 4 and 7b suggests that many patients could have been exposed to all three allergens *en bloc* resulting in sensitivity to the proteins having been acquired together. Since all these proteins are present in latex gloves (Fig. 1), it is plausible that the three proteins were found on latex products (such as gloves) that the patients had been in contact with.

Despite their molecular similarities, Hev b 7b and 7c do not generally elicit a positive response in the same patients. While Hev b 7b was linked to Hev b 2 and Hev b 4, patients who were sensitive to Hev b 7c tended to be also sensitive especially to Hev b 5 (Table 3). If sensitivity to Hev b 2, 4 and 7b had been acquired *en bloc* from contact with latex products as suggested above, it is plausible that sensitivity to Hev b 5 and Hev b 7c might have been acquired from other sources, notwithstanding the fact that both these proteins are also found on latex gloves. In this connection, reports of IgEs that bind to Hev b 5 and Hev b 7 cross-reacting with various food proteins such as those from kiwi fruit and vegetables of the *Solanaceae* family are noteworthy (4, 2, 26, 33).

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RESUMEN

Seis proteínas alergénicas del látex *Hevea brasiliensis*, Hev b1, Hev b2, Hev b3, Hev b4, y dos variantes de Hev b7 (b7b y b7c), fueron purificadas de látex *Hevea*, mientras que siete proteínas de Hev b5 fueron preparadas en forma de recombinantes. Estas proteínas fueron detectadas mediante sus respectivos anticuerpos en el suero de ratas inmunizadas frente a los extractos. La relativa capacidad de fijar IgE con los alérgenos individuales del látex fue comparada empleando suero de pacientes alérgicos al látex. Se observó con mayor frecuencia una fijación de IgE en el suero de los pacientes hacia Hev b4, Hev b7b, Hev b5 y Hev b2, respectivamente, con los siguientes porcentajes 75%, 61%, 31% y 28%. La sensibilización frente a varias proteínas del látex fue común, y así de los 31 pacientes seropositivos, 23 (74%) tenían IgE frente al menos dos alérgenos del látex, mientras que 12 (39%) tenían IgE específica frente al menos tres de los alérgenos del látex. El análisis estadístico de los datos sugiere que muchos pacientes pudieron haber adquirido una sensibilización frente a Hev b2, Hev b4 y Hev b7b a través de una

fuente común (por ejemplo productos del látex). Por otra parte, la sensibilización frente a Hev b5 y Hev b7c se encuentra interrelacionada. Es posible que la sensibilización frente a estas dos proteínas pueda adquirirse a través de otras fuentes diferentes de productos del látex.

Palabras clave: Alérgenos del látex - Alergia al látex - Proteínas del látex - Guantes de látex - Anticuerpos IgE - Hevea brasiliensis

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Correspondence: H.Y. Yeang, Biotechnology and Strategic Research Unit, Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia.