

Specific monoclonal antibodies and human immunoglobulin E show that Hev b 5 is an abundant allergen in high protein powdered latex gloves

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Summary

Background Hev b 5 is a major latex allergen recognized predominantly by latex-allergic health care workers (HCWs). Recombinant Hev b 5 (rHev b 5) was previously expressed as a fusion protein with maltose binding protein (MBP), itself an immunogenic molecule; therefore non-fusion rHev b 5 is desirable. Moreover, standardized immunological assays for the detection of Hev b 5 are currently lacking and may have important implications for both allergen avoidance and diagnosis in latex allergy.

Objectives To generate and use Hev b 5-specific mAbs to determine the relative abundance of Hev b 5 in different latex extracts, correlating this with the IgE reactivity of latex-allergic HCWs and to produce non-fusion rHev b 5.

Methods For the production of mAbs, mice were immunized with rHev b 5/MBP fusion protein and mAbs selected with rHev b 5/MBP but not MBP reactivity. The mAb reactivity was compared with polyclonal IgE from latex-allergic HCWs using direct and inhibition ELISA and immunoblot assays. Recombinant Hev b 5 was expressed and purified in the pPROEX-HTa bacterial expression system.

Results Four Hev b 5-specific mAbs were produced. Immunoblotting and ELISA using the mAbs indicate abundant Hev b 5 in high protein powdered latex glove extracts as compared with crude latex sap extracts. High quality surgical gloves with no detectable protein have no detectable Hev b 5. Inhibition ELISAs using serum IgE from latex-allergic HCWs and Hev b 5-specific mAbs gave strong correlation. Non-fusion recombinant Hev b 5 was successfully expressed and purified, showing reactivity with both the Hev b 5-specific mAbs and serum IgE of latex-allergic HCWs.

Conclusion Hev b 5-specific mAbs and human IgE from latex-allergic HCWs demonstrate the greater content of Hev b 5 in high protein powdered glove extracts. This may explain the observed higher frequency of sensitization to this allergen in HCWs.

Keywords Hev b 5, latex allergy, latex gloves, monoclonal antibodies, recombinant proteins.

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Introduction

Latex allergy is characterized by sensitivity to the protein fraction of natural rubber latex (NRL) [1] used in the production of gloves and other medical products. The major groups affected include health care workers (HCWs) [2], spina bifida children [3] and others undergoing multiple operations [4]. The predominant cause of sensitization and symptoms amongst HCWs is the use of latex gloves [5], but much previous research has focused on crude latex sap extracts such as non-ammoniated latex (NAL) and low-ammoniated latex (LAL). Hev b 5 is a major latex allergen, isolated and characterized by recombinant DNA technology [6,7] and initially expressed as a fusion protein with

maltose binding protein (MBP). The majority of latex-allergic HCWs show reactivity to the recombinant Hev b 5/MBP fusion protein (rHev b 5/MBP) by serum IgE [6], T cell function [8] and skin testing assays [9]. The presence of immunoreactive natural Hev b 5 (nHev b 5) in NRL, however, has been difficult to clearly demonstrate [10], in part because of the physico-chemical properties of the protein (highly negatively charged, proline rich) and the complexity of latex extracts, and in part because of the lack of specific probes. Hev b 5 may also be in low abundance in commercially available capture assays [11], reducing their diagnostic sensitivity. Moreover, the effects of the glove manufacturing process on the immunogenicity of NRL and nHev b 5 are poorly understood. Previous monoclonal antibodies (mAbs) to Hev b 5/MBP were not mono-specific for Hev b 5, with reactivity reported to MBP alone in addition to NAL and rHev b 5/MBP fusion protein [6]. In this study, we aimed to generate rHev b 5-specific mAbs to characterize and determine the relative amount of immunoreactive Hev b 5 in

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different latex preparations and compare this with the rHev b 5-specific serum IgE reactivity of latex-allergic patients. Since MBP is itself a large (42 kDa) and immunogenic molecule, we also aimed to express rHev b 5 without a MBP fusion partner and evaluate its IgE and mAb reactivity. Production of highly specific mAbs is mandatory for the determination of the relative abundance of Hev b 5 in different glove extracts. Such novel information is pivotal for allergen avoidance while the availability of IgE-reactive rHev b 5 is important for allergen standardization to permit accurate diagnosis of latex allergy.

Materials and methods

Antigens

rHev b 5/MBP The cDNA encoding Hev b 5 was expressed as a fusion protein with MBP in the pMAL/c-2 vector and purified on an amylose column (New England Biolabs, Beverly, MA, USA) as described previously [6]. An MBP-LacZ α fusion protein produced by expressing the pMAL/c-2 vector alone was used as the negative control protein.

rHev b 5 The sequence encoding the Hev b 5 protein was amplified by the polymerase chain reaction (PCR) using plasmid pMAL/Hev b 5 DNA as the template [6]. In detail, 20 ng of plasmid DNA was amplified using the primers: HEVB5F (5'-GCGGAATTCATGGCCAGTGTGAGGTTG-3') and HEVB5R (5'-GCGGTCGACTTATTCCTCTGTTTTTCCACC-3'). Thirty cycles of amplification were conducted with each cycle consisting of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C, followed by incubation at 72 °C for 10 min. The pProX-HTa vector (Life Technologies, Carlsbad, CA, USA; possessing a hexahistidine tag to facilitate purification) and the Hev b 5 PCR product were cleaved with *Eco*RI and *Sal*I and purified by agarose gel electrophoresis followed by extraction of the DNAs from the gel (QIAGEN Gel purification kit; Germantown, MD, USA). Vector DNA and the PCR product were ligated using T4 DNA ligase and transformed into DH5 α competent cells. *E. coli* DH5 α cells were grown to logarithmic stage and expression of rHev b 5 induced by the addition of isopropyl-1-thio- β -D-galactoside to a final concentration of 0.6 mmol/L. Following induction of expression the cells were grown for 4 h at 37 °C. The bacteria were harvested by centrifugation for 15 min at 4000 g at 4 °C and the pellets frozen overnight at -20 °C. The pellets were resuspended in 20 mL of native lysis buffer (50 mM Na₂H₂PO₄, 300 mM NaCl, 10 mM imidazole) per litre of original culture. The cell suspension was sonicated 6 $\times$ 20 s on ice, using a Branson sonifier model 250 and then centrifuged for 30 min at 16000 g at 4 °C. The supernatant was applied to a column containing Ni-NTA agarose (QIAGEN) with a bed volume of 5 mL. The column was washed with native lysis buffer and then eluted (50 mM Na₂H₂PO₄, 300 mM NaCl, 250 mM imidazole). Recombinant Hev b 5 was soluble with a yield of 10 mg per litre of broth culture. The concentration of rHev b 5 was determined by the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA) with bovine gamma globulin (Bio-Rad, Hercules, CA, USA) as the standard. SDS-PAGE (under reducing and denaturing conditions) and Western analysis was used to assess purity and integrity of rHev b 5 (see Fig. 1a).

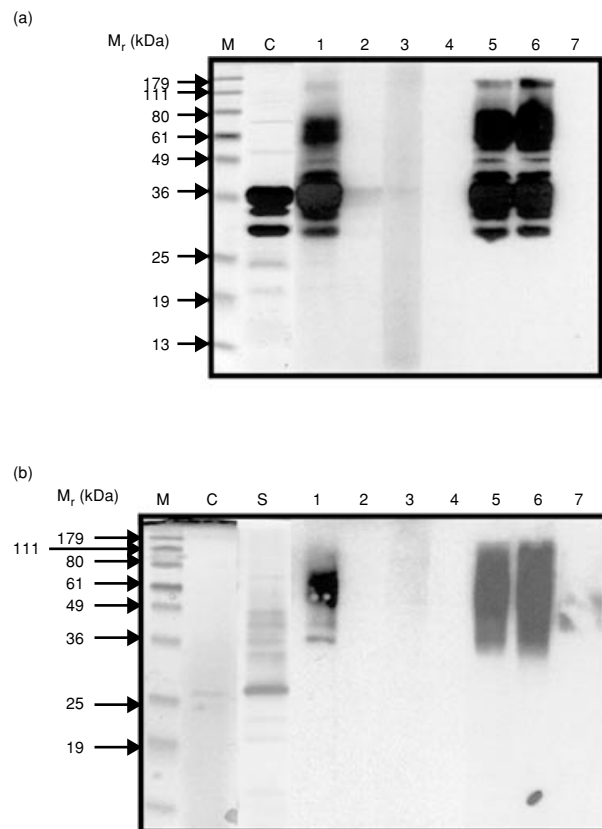


Fig. 1. Western blot and inhibition analysis of rHev b 5 and glove extract with human IgE and mAbs. Recombinant Hev b 5 (a) and glove extract (b) were resolved by 12% SDS-PAGE and transferred onto NC for Western analysis. NC blots were probed for antibody binding with pooled sera from latex-allergic patients (lane 1), pooled sera from latex-allergic patients pre-incubated with 20 μ g rHev b 5 per mL of diluted (1:5) serum pool (lane 2), pooled sera from non-allergic patients (lane 3), no serum control (lane 4), mAb 6A10 (lane 5), mAb 6F6 (lane 6), and mAb A1 isotype control (lane 7). Molecular mass markers, coomassie brilliant blue-stained gel slices and silver-stained gel slices are denoted by M, C and S, respectively.

Non-ammoniated latex (NAL) This was obtained from the Rubber Research Institute of Malaysia. The sap was centrifuged for 1 h at 50 000 g and the C-serum removed for protein estimation using the BCA assay described above. It was then aliquotted and stored at -20 °C. The concentration of the undiluted NAL extract was 7.3 mg/mL. The NAL extract was diluted in PBS, as appropriate, for the immunoassays described below.

Low-ammoniated latex (LAL) This was a gift of Ansell Australia and was processed as described above. The concentration of the undiluted LAL extract was 7.6 mg/mL.

Glove Extracts (GEs) These were obtained by adding phosphate buffered saline (PBS; 1 mL per gram of glove) to the interior of intact, commercially available latex gloves and incubating for 1 h at room temperature (20 °C) on an orbital shaker. The resultant glove eluate was then removed, centrifuged at 5000 g for 10 min to remove all glove donning powder, then filter-sterilized through a 0.2- μ m filter. Protein concentration was estimated with the BCA assay as described above (assay sensitivity 20 μ g/mL). Five different commercially available gloves were used. For convenience these were designated G1 to G5. Gloves G1 and G2 were powdered, non-sterile latex

gloves: G1 a laboratory utility glove, G2 a non-medical grade household disposable glove available from supermarkets in Australia. Gloves G3, G4 and G5 were all sterile surgical gloves currently in use in our hospital: G4 was non-powdered and G3 and G5 were powdered.

Human subjects

Latex-allergic subjects were recruited from the Allergy and Asthma Clinic at the Alfred Hospital and all patients gave informed consent with the study being approved by the Alfred Hospital Ethics Committee. Five out of six subjects have been previously shown to recognize rHev b 5/MBP by immunoblotting and T cell proliferation assays [8]. The strongest responder was used for the IgE inhibition ELISA experiments, while the sera were pooled for the IgE immunoblots. A serum pool of latex-exposed, non-atopic HCWs was used as a negative control serum in the IgE immunoblots.

Monoclonal antibody production, isotyping and purification

Monoclonal antibody production was carried out according to established protocols [12,13] and all experiments were approved by the Monash University Animal Ethics Committee with animal care conducted according to National Health and Medical Research Guidelines. Briefly, six-week-old-female BALB/c mice received six injections intraperitoneally (IP) at 2–4 week intervals with 20 µg rHev b 5/MBP initially with, subsequently without, Complete Freund's adjuvant. Immune mouse splenocytes were fused with cells from the murine myeloma cell line X63-Ag8.653 and hybridomas screened by duplicate ELISA for each well against rHev b 5/MBP and MBP. ELISA plates (Costar, Acton, MA, USA) were coated overnight (O/N) at 4 °C with 50 µL per well of rHev b 5/MBP or MBP alone at 0.2 µg/mL in ELISA coating buffer (0.03 M NaHCO₃/0.02 M Na₂CO₃, pH 9.5). The plates were then washed eight times in PBS with 0.05% Tween 20 (BDH, Poole, UK; PBST) and then blocked with 5% w/v skim milk powder (SMP) in PBST (SMP-PBST) O/N at 4 °C before being washed again eight times in PBST. Hybridoma supernatants were then incubated (50 µL/well) for 1 h at 37 °C. The plates then washed eight times in PBST then 50 µL per well of horseradish peroxidase (HRP) labelled sheep anti-mouse antibody (Silenius, Melbourne, Australia) diluted 1 : 5000 in 1% SMP-PBST was added and incubated for 1 h at 37 °C. The plates were then washed four times with PBST and then four times with PBS alone before the addition of 50 µL/well of a solution of 5 mg O-phenylenediamine dihydrochloride (OPD; Sigma, St Louis, MO, USA) in 12.5 mL of 0.05 M phosphate-citrate buffer with 0.03% sodium perborate (Sigma). This was incubated for 15 min at 37 °C and the reaction stopped with 4 M HCl. Colour development was then observed and the plate read at 490 nm. In all cases, one well of the ELISA plate had cell culture medium alone as a negative control and the terminal bleed of the fusion mouse at a 1 : 5000 dilution in 1% SMP-PBST as positive control. After expansion and cloning of hybridomas by limiting dilution, supernatants were assayed by ELISA for reactivity to NAL. The NAL ELISA was performed as above but the antigen coating concentration was 10 µg/mL.

Once monoclonality of hybridomas was achieved, supernatants were isotyped by ELISA (Pharmingen, San Diego, CA, USA) and mAbs purified on protein A (Biospra©, Life Technologies)

or protein G (Pharmacia, Uppsala, Sweden) columns as appropriate, following the manufacturer's instructions.

Immunoblotting

For SDS-PAGE immunoblots, extracts of NAL and LAL (50 µg/lane) or GE (G1; 5 µg/lane) or recombinant proteins (5 µg/lane) were resolved on 12% SDS-PAGE mini gels (Novex, Carlsbad, CA, USA) for 90 min at 125 V DC with Benchmark© prestained protein ladder (Life Technologies) as the molecular mass standards. Gels were then stained with Coomassie Brilliant Blue (CBB) or silver (Novex) for total protein visualization or, for Western blotting transferred to nitrocellulose (NC) membranes (Schleicher and Schuell, Dassel, Germany) for 60 min at 25 V DC. After transfer, NC membranes were dried then cut into strips. They were then blocked with 5% SMP-PBST for 1 h at room temperature, washed twice in PBST and then incubated with hybridoma supernatants (1 : 100), purified mAb (0.1–1 µg/mL) or pooled human serum (1 : 5) for 1–2 h at room temperature. After washing and incubation with horseradish peroxidase (HRP) labelled detection antibodies, blots were washed and developed with 4-chloro-1-naphthol as previously described [14] or enhanced chemiluminescence (ECL) reagent (NEN Life Sciences, Boston, MA, USA) with luminescence detected by autoradiography (Kodak, Melbourne, Australia). Inhibition immunoblot analysis was essentially the same as described above, except the serum pool was preincubated with 20 µg of rHev b 5 per mL of sera (diluted 1 : 5) for 1 h at 37 °C before incubation of the NC strips.

Inhibition ELISA for mAbs and human IgE

Inhibition ELISAs were performed as above but coating of ELISA plates with rHev b 5 was at 0.5 µg/mL and for the detection of human IgE, rabbit anti-human IgE (Dako, Carpinteria, CA, USA) was used at a dilution of 1 : 500 in 1% SMP-PBST. Dilution curves with purified Hev b 5 specific mAbs and latex-allergic HCW IgE were performed to determine the optimal concentrations (data not shown); 0.017 µg/mL for mAb 6F6, and 1 : 20 for HCW IgE. Inhibitors were added and incubated with antibody/sera at 37 °C for 1 h prior to addition to the assay plate. The GE inhibitors were used neat or diluted 1 : 10 in PBS before addition to an equal volume of the diluted human sera or mAb, giving a final dilution of 1 : 2 or 1 : 20 of the extract. Final effective protein concentrations therefore were for G1 274 µg/mL and 27.4 µg/mL, G2 370 µg/mL and 37 µg/mL or, where the protein concentration was beneath the levels of detection, GE inhibitors were expressed as dilutions of 1 : 2 and 1 : 20 (Fig. 3). The NAL and LAL extracts were similarly diluted in PBS before addition to the sera or mAb, giving final effective concentrations for both of 100 µg/mL and 10 µg/mL. Rye grass pollen extract was used as a negative control protein at a final effective concentration of 100 µg/mL.

In addition, to ensure that observed inhibitions were specific, a control ELISA was performed using rye grass pollen extract to coat the plates (10 µg/mL) and mAb A1, a Lol p 1-specific mAb of isotype IgG1, for detection [15].

Results

Four hybridomas were produced which showed strong reactivity to rHev b 5 fusion protein by ELISA and immunoblot, no

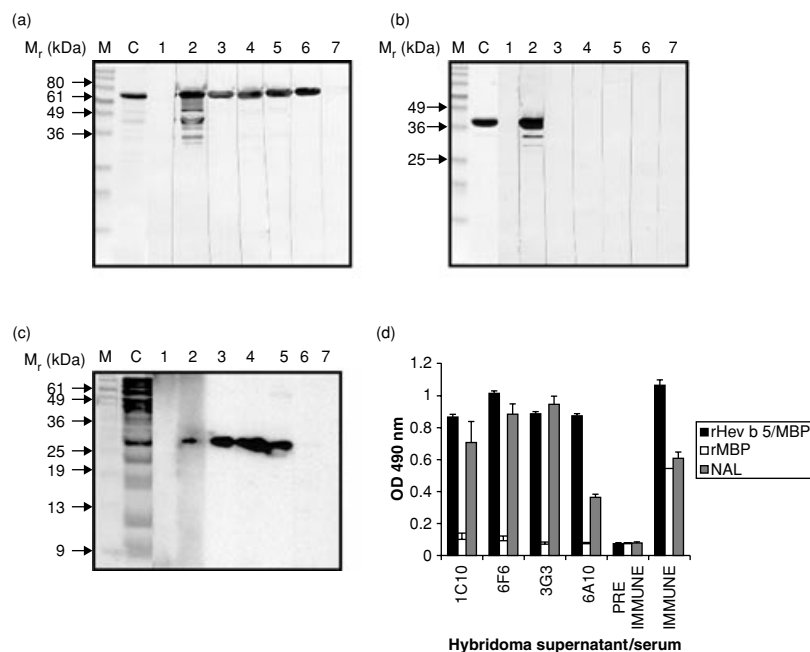


Fig. 2. Immunological analysis of recombinant and natural latex proteins. Recombinant Hev b 5/MBP fusion protein (a), rMBP (b) and NAL (c) were resolved by 12% SDS-PAGE and transferred onto nitrocellulose (NC) for Western analysis. NC blots were probed for antibody binding with pre-immune mouse serum (lane 1), polyclonal immune mouse serum (lane 2), mAb 6A10 (lane 3), mAb 6F6 (lane 4), mAb 3G3 (lane 5), mAb 1C10 (lane 6) and mAb A1 isotype control (lane 7). Molecular mass markers and Coomassie brilliant blue-stained gel slices are denoted by M and C, respectively. Monoclonal antibody binding to rHev b 5/MBP, rMBP and NAL was also assessed by ELISA (d).

reactivity to MBP alone and also reactivity to NAL (Fig 2). The immunoblot reactivity to NAL was maximal to a band at around 26 kDa on a 12% SDS-PAGE gel and was strongest for mAbs 6F6, 6A10, 3G3 and weak for 1C10. Isotyping showed that three of the mAbs were IgG1 subclass (6A10, 1C10 and 3G3), the other subclass IgG2b (6F6).

All the mAbs also recognized the rHev b 5 expressed without MBP. A representative Western blot with two of the antibodies is shown (Fig. 1a). The pattern of reactivity was almost identical to that of the pooled sera of latex-allergic individuals. The HCW IgE reactivity was also Hev b 5 specific with almost complete inhibition by preincubation with 20 µg/mL rHev b 5 (Fig. 1a). Although the majority of the protein was monomeric rHev b 5, breakdown products and aggregates were evident (lanes C, 1, 5 and 6 of Fig. 1a). However, the integrity of rHev b 5 (i.e. IgE and mAb 6F6 reactivity) was maintained (Fig. 1a). Western blotting of GE by HCW IgE and Hev b 5-specific mAbs showed strong reactivity to glove extract with a principal band at approximately 36 kDa and a smear of reactivity extending from 36 to 80 kDa. This IgE reactivity was Hev b 5-specific (inhibitable) and a similar pattern of smeared reactivity was shown by the Hev b 5-specific mAbs (Fig. 1b) although the smear extended to a higher molecular mass of around 110 kDa. Strong reactivity to the glove extract was observed despite a low protein amount (5 µg per lane) of the glove extract being loaded on the gel and almost no protein being visualized on CBB stain, though some protein was observed with silver staining (Fig. 1b).

The inhibition ELISAs showed that rHev b 5 gave a strong dose-dependent inhibition of antibody reactivity (Fig. 3a) with a strong correlation between the two standard curves (IgE and mAb6F6) $R^2 = 0.94$ ($P < 0.001$). The IgE ELISA was more sensitive with a limit of sensitivity (statistically significant difference from 0 inhibitor) of 0.2 µg/mL rHev b 5; the mAb inhibition ELISA sensitivity was 0.8 µg/mL rHev b 5. The high protein powdered glove extracts gave relatively more inhibition even after adjusting for protein content than NAL or LAL

(Fig. 3b and Table 1). Rye grass pollen extract used as a negative control gave no inhibition. Similarly, the high quality surgical gloves with undetectable total protein, both powdered and non-powdered also gave little or no inhibition, though there was a trend towards G5 having a greater Hev b 5 content than G3 or G4. Natural and recombinant latex proteins gave no inhibition of a rye grass pollen specific mAb in a control rye grass pollen-specific ELISA (data not shown).

Discussion

Hev b 5-specific mAbs have important implications for allergen avoidance and diagnosis of latex allergy, but are currently lacking. In this study, we have produced and immunologically characterized Hev b 5-specific mAbs. With the use of these, we have shown that immunoreactive rHev b 5 equivalents are more abundant in some latex GEs than in crude latex sap extracts. The predominant immunoreactivity in GE is in the higher molecular mass range (approximately 36–110 kDa) as assessed by immunoblotting, compared with NAL at around 26 kDa. These proteins also stain poorly with CBB, consistent with Hev b 5 [7]. The predominant reactivity in GE is observed as a smear, indicating that Hev b 5 may be degraded following glove manufacture, form aggregates and/or interact with other latex proteins. The rHev b 5 also appeared to form higher molecular mass aggregates seen on our Western blotting. The smeared pattern of rHev b 5-specific IgE reactivity observed in this study is also seen in IgE immunoblots of latex glove extracts reported by other investigators [16–18] but has not previously been attributed to nHev b 5. Interestingly, this smear is also similar to that noted by Swanson et al. in their IgE immunoblots of latex aeroallergen samples [18]. This suggests an important role for Hev b 5 as a latex aeroallergen bound to glove donning powder and may explain its noted higher frequency of sensitization amongst HCWs compared with spina bifida children [6].

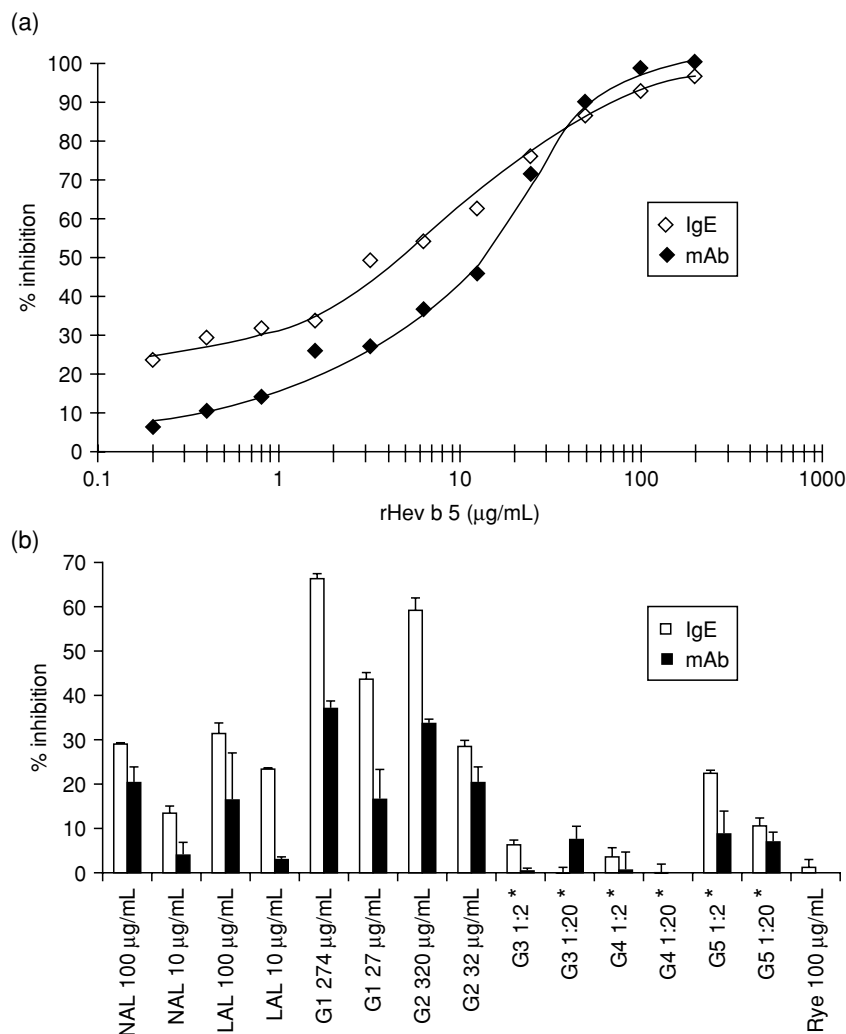


Fig. 3. Inhibition ELISA. analysis of rHev b 5 with serum IgE of latex-allergic HCWs and mAb 6F6. Recombinant Hev b 5 was immobilised on an ELISA plate and human IgE/mAb 6F6 binding was inhibited with different amounts of rHev b 5 to generate standard curves (a) for the determination of rHev b 5 equivalents in the different latex extracts (b). Latex extracts used (as potential inhibitors of antibody binding to rHev b 5) included NAL, LAL, different glove extracts (GE; G1 and G2 are powdered non-sterile utility gloves, G3 and G5 are powdered sterile surgical gloves and G4 is a non-powdered sterile surgical glove) and rye grass pollen extract (irrelevant allergen negative control). Measurements are means ($\pm$ SEM) of triplicates. The sensitivity of the human IgE assay was, 0.2 µg/ml and of the mAb assay was, 0.8 µg/ml. For glove extracts (GE) where protein was undetectable, concentration is expressed as an extract dilution (*).

Table 1. Relative Hev b 5 content of latex extracts

	Total protein concentration (ug/mL)	rHev b 5 equivalents by mAb ELISA (ug/mL)	rHev b 5 equivalents by IgE ELISA (ug/mL)	Mean % rHev b 5 equivalents by mAb ELISA #	Mean % rHev b 5 equivalents by IgE ELISA#
G1	274	6.5	12.5	3.0	7.5
	27.4	1.0	2.8		
G2	320	5.0	8.0	1.6	2.2
	32	†	0.6		
G3	*	†	†	†	†
G4	*	†	†	†	†
G5	*	†	†	†	†
NAL	100	1.8	0.6	1.8	0.6
LAL	100	1.0	1.0	1.0	1.0

G1, G2: powdered non-sterile utility gloves; G3, G4, G5: sterile surgical gloves. Levels of total protein (*) or rHev b 5 equivalents (†) below limits of detection. Mean percentage rHev b 5 equivalents (#) were obtained by calculating the mean of [(rHev b 5 equivalent/total protein concentration) $\times$ 100] for two concentrations of extract where rHev b 5 equivalent values were detectable, or one value only where lower dilution had undetectable rHev b 5 levels (e.g. NAL or LAL).

Both our Hev b 5-specific mAb and human IgE ELISAs successfully determined rHev b 5 equivalents in the different latex extracts. However, the human IgE ELISA gave higher

values. This may be due to the higher sensitivity of the human IgE ELISA (i.e. 0.2 µg/mL) than the mAb ELISA (i.e. 0.8 µg/mL). In addition, unlike mAbs which may recognize only one

rHev b 5 epitope, due to its polyclonal nature, human IgE may recognize more rHev b 5 epitopes, therefore contributing to the observed higher detection levels. Nevertheless, there was a strong correlation between the rHev b 5-specific mAb and IgE ELISAs. The advantage of standardization available with the mAb make it a potentially useful tool in assays of Hev b 5 content of latex extracts and aeroallergen samples. Considering that a number of different mAbs were produced, we attempted to establish a two-site ELISA for Hev b 5 detection using three of the mAbs: 6A10, 3G3 and 6F6 (1C10 not yet evaluated). However, no sandwich formation was observed using these antibodies suggesting that these mAbs may recognize the same region on the allergen molecule. Nevertheless, we did successfully develop a sensitive one-site mAb ELISA, as presented here, to determine relative Hev b 5 content of different latex extracts.

Our method of glove protein elution (short incubation time, PBS alone on interior of glove) may have favoured the elution of Hev b 5 over other latex allergens, however, it may represent a more 'physiological' method compared with cutting gloves up or using detergents such as SDS to elute particle bound proteins. Recent publications have shown a higher level of protein on the interior of gloves and in powdered latex gloves [16]. We speculate that Hev b 5 proteins are altered during the glove manufacturing process (high temperatures, addition of cornstarch donning powder) resulting in greater immunoreactivity through exposure of IgE binding cryptopes [19]. Other investigators have found some allergens more IgE reactive after roasting [20]. Unfortunately, glove-manufacturing processes are non-standardized and often considered proprietary, making systematic evaluation difficult. Unlike the latex allergen Hev b 1 which is more abundant in crude latex extracts (7% of NAL) compared with glove extracts (2–4%) [21], our results indicate that Hev b 5 may be enriched by the glove manufacturing process in some high protein powdered gloves.

The high quality surgical gloves assayed showed levels of both protein and rHev b 5 equivalents that were beneath the levels of detection. It is likely that the modern processes of extensive high temperature washing and leaching used in the manufacture of such gloves and other medical rubber products [22,23] have been highly effective with regard to reduction in immunoreactive nHev b 5 levels. This study is in agreement with other studies showing a reduction in surgical glove protein levels and allergenicity over the last decade [5]. It cautions however, that lower quality, non-medical grade powdered latex gloves are a rich source of immunoreactive nHev b 5 and represent a potential hazard if inappropriately used (e.g. repeated high level use by atopic individuals or food handlers) in the community. Such gloves may be also high in endotoxin [24] and this has been shown to exert a Th2-adjuvant effect in a mouse model of rHev b 5/MBP immunization [25] adding to their potential hazard. This information is an important reminder that as medical glove technology and awareness in the health care setting improves, we must be vigilant to ensure that inappropriate high allergen glove use by non-HCWs does not expand the latex allergy epidemic unnecessarily into the wider community. The availability of highly specific mAbs offers the potential to monitor the Hev b 5 content of different latex extracts to assist in allergen avoidance strategies and together with purified and IgE reactive rHev b 5 offers hope for improved diagnosis of latex allergy.

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