

Detection of immunoglobulin antibodies in the sera of patients using purified latex allergens

V. P. KURUP*, H. Y. YEANG†, G. L. SUSSMAN‡, N. K. BANSAL§, D. H. BEEZHOLD¶, K. J. KELLY*, D. R. HOFFMAN**, B. WILLIAMS†† and J. N. FINK*

*Department of Medicine, Allergy-Immunology Division, Medical College of Wisconsin and Research Service, Veterans Affairs Medical Center, Milwaukee, WI, USA, †Biotechnology and Strategic Research Division, Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia, ‡University of Toronto, Ontario, Canada §Department of Mathematics, Statistics, and Computer Science, Marquette University, Milwaukee, WI, ¶Laboratory of Macrophage Biology, Guthrie Research Institute, Sayre, PA, **The Department of Pathology and Laboratory Medicine, East Carolina University, Greenville, NC and ††IBT Reference Laboratory, Lenexa, KS, USA

Summary

Background Latex allergy is largely an occupational allergy due to sensitization to natural rubber latex allergens present in a number of health care and household products. Although several purified allergens are currently available for study, information on the usefulness of these purified, native or recombinant allergens in the demonstration of specific immunoglobulin (Ig) E in the sera of patients is lacking.

Objective To evaluate the purified latex allergens and to demonstrate specific IgE antibody in the sera of health care workers and spina bifida patients with clinical latex allergy.

Methods Two radioallergosorbent and an enzyme-linked immunosorbent assay (ELISA) using latex proteins Hev b 1, 2, 3, 4, 6 and 7 along with two glove extracts and Malaysian nonammoniated latex (MNA) were evaluated to demonstrate IgE in the sera of health care workers and spina bifida with latex allergy and controls with no history of latex allergy.

Results ELISA using the purified latex allergens demonstrated specific IgE in 32–65% health care workers and 54–100% of spina bifida patients with latex allergy. The corresponding figures for RAST were 13–48 and 23–85 for RAST-1 and 19–61 and 36–57 for RAST-2. These results were comparable with the results obtained with glove extracts and crude rubber latex proteins.

Conclusions When used simultaneously, latex proteins Hev b 2 and Hev b 7 reacted significantly with specific serum IgE in 80% of health care workers and 92% of spina bifida patients with latex allergy by ELISA technique, while this combination gave lower positivity when the RASTs were used. By the addition of Hev b 3, specific IgE was detected in all spina bifida patients with latex allergy. Both RASTs failed to show specific IgE in the control subjects, while the ELISA showed significant latex-specific IgE in 22% of controls.

Keywords: ELISA, health care worker, latex allergy, latex proteins, RAST, recombinant allergen, spina bifida

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Introduction

In recent years, allergy to natural rubber latex has been reported in individuals frequently exposed to latex allergens [1–7]. Health care workers especially have shown a dramatic increase in latex allergy [1,6,8–11]. These workers

Correspondence: V. P. Kurup, V A Medical Center, Research Service 151-I, 5000 West National Avenue, Milwaukee, WI 53295, USA (e-mail: vkurup@post.its.mcw.edu).

are subjected to frequent exposure to proteins present in natural rubber latex gloves and a number of other latex medical products used in patient care and up to 10% may develop symptoms of immunoglobulin (Ig) E mast cell-mediated latex hypersensitivity [8–12]. In order to demonstrate latex specific IgE in such patients, a number of antigen preparations have been used *in vitro* and *in vivo* [12,13]. These antigens include proteins extracted from the latex sap of the rubber tree *Hevea brasiliensis*, as well as from finished products such as gloves. Furthermore, several commercial latex antibody assays are available for use in diagnosis. None of these extracts have been well characterized or standardized, and over 250 polypeptides have been reported from the crude preparation [1]. Because of the heterogeneity and variability of these antigens, the results may be less reproducible and may vary in their comparability among different laboratories. Hence, it is essential to have reliable, consistent, and reproducible antigens for *in vitro* testing in the diagnosis of latex allergy as life threatening anaphylaxis and other adverse reactions related to exposure to latex proteins occur in such individuals.

Although a number of proteins and peptides from natural rubber latex products and latex from *H. brasiliensis* have been identified as reacting with IgE antibodies from latex-sensitive patients, only a few of these purified allergens have been evaluated immunologically [14–22]. Crude antigens contain relevant and non-relevant proteins and their use may produce variable results and interpretations. In order to identify the relevant latex proteins, in the present study, we have evaluated several purified native or recombinant latex proteins and studied their binding to specific IgE antibodies in patient sera using several *in vitro* methods. The studies were blinded and carried out in four different centres. The results indicate that sera from a majority of the latex-allergic patients demonstrated significant levels of IgE antibody to most of the purified latex allergens.

Materials and methods

Latex antigens

Six purified latex proteins along with two glove extracts and an nonammoniated natural rubber latex preparation were used in the present study. The purified allergens used in the study namely, Hev b 1, 2, 3, 4, 6 and 7 are shown in Table 1. Hev b 5 was not available for study. Of the six antigens used, four have been purified by conventional methods, such as preparative isoelectric focusing and by ion exchange chromatography, while Hev b 6 and 7 were recombinant proteins expressed in bacterial vectors. All antigens have been characterized previously, but had not been tested simultaneously against sera from patients with latex allergy. The two glove extracts were prepared from Perry X-am and Baxter Triflex gloves as previously described [6,12,23]. Non-ammoniated latex (MNA) was obtained from Malaysia and has been characterized previously [24].

Purification of Hev b 1, 2, 3, 4

Fresh latex obtained by tapping *Hevea brasiliensis* trees was collected into chilled containers at the Rubber Research Institute of Malaysia Experimental Station, Sungei Buloh, Selangor. The latex was centrifuged at 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 containing lutoids, and the C-serum located in between [25,26]. Hev b 1 and Hev b 3 were extracted from the rubber cream as described previously [27]. Briefly, the rubber cream was sampled at the intersection between Zones 1 and 2 so as to obtain rubber particles belonging to both the large and small size classes. The rubber particles were then washed by resuspension in 30% sucrose and proteins on the rubber particle surfaces were solubilized and extracted using a detergent mixture of 0.1% Triton-X 100 and 1% sodium dodecylsulphate (SDS). The main detergent-extracted rubber particle

*IUIS allergen nomenclature	Biochemical and other characteristics	Molecular size (kDa)
Hev b 1	Rubber elongation factor; rubber cream	14
Hev b 2	β -1,3-glucanase; latex B-serum	35
Hev b 3	Small rubber particle protein; rubber cream	23
Hev b 4	Microhelic protein; latex B-serum	55
Hev b 6	Prohevein cloned and expressed	20
Hev b 7	Patatin-like protein; cloned and expressed	43

Table 1. Purified latex allergens used in this study

* International Union of Immunological Societies.

proteins, Hev b 1 and Hev b 3, were purified by preparative electrophoresis on 15% polyacrylamide gel using the Prep Cell (Bio Rad, Hercules, CA, USA) according to the manufacturer's instructions.

Hev b 2 and Hev b 4 were prepared from the bottom fraction of centrifuged latex as previously described [18]. Briefly, lutoids in the bottom fraction of centrifuged latex were washed by resuspension in 0.4 mol/L mannitol. The lutoids were then ruptured by repeated freezing and thawing [28] to recover the B-serum contained within. The recovered B-serum was dialysed (2.5 kDa cutoff) against distilled water for 48 h at 5 °C to induce formation of a proteinaceous precipitate. The precipitate was redissolved in 0.35 mol/L NaCl and the solution loaded onto a Sephacryl S-200 (Pharmacia, Uppsala, Sweden) gel filtration column. Hev b 2 and Hev b 4 were found in the two major peaks obtained by eluting with 0.35 mol/L NaCl [18].

Cloning and expression of Hev b 6

The gene encoding prohevein was amplified by means of PCR from a cDNA library generously supplied by Dr Anil Kush of the University of Singapore. A sense primer (5' GAGCAATGTGGTCGGCAAGCA 3') and an antisense primer (5' ATTATTACTGATGATTT 3') were used to obtain the full-length prohevein gene [29]. The sense and antisense primers had EcoRI and Hind III at their 5' ends. The amplified PCR product was purified and sub-cloned in the EcoRI/Hind III sites of expression vector pMALc2 (New England BioLabs, Beverly, MA, USA). *Escherichia coli* (TB1) was transformed with pMALc2 vectors with and without insert of prohevein gene. The fusion protein was expressed along with maltose binding protein (MBP) [30]. Maltose binding protein expressed from vectors without prohevein inserts was used as a control in enzyme-linked immunosorbent assay (ELISA) and radioallergosorbent test (RAST).

Cloning and expression of Hev b 7

The gene encoding Hev b 7 was cloned as described previously [22]. The GST Gene Fusion System (Pharmacia Biotech Inc., Piscataway, NJ, USA) was used to express rHev b 7. An overnight culture of BL21/pGEX2T/Hev b 7 was diluted 1/10, grown at 37 °C to an OD 600 of 1.0, and induced with 0.1 mmol/L IPTG for 3 h at 37 °C. The culture was centrifuged and the pellet resuspended in STE with 100 µg/mL lysozyme. Following 15 min incubation on ice, dithiothreitol and Sarkosyl were added to 5 mmol/L and 1.5% final volumes, respectively. The sample was sonicated for 2 min on ice in a water bath sonicator, centrifuged, and Triton-X (2% final volume) was added to the supernatant. Glutathione agarose beads were added to the supernatant

and incubated for 20 min at 4 °C. The beads were washed five times with cold phosphate-buffered saline (PBS). The fusion protein (Hev b 7/GST), was obtained by digesting the beads with thrombin incubating overnight at room temperature. After centrifugation, the supernatant containing rHev b 7 was dialysed against water, lyophilized and resuspended in 100 mmol/L TRIS (pH 9).

Characterization of the antigens

The protein content in the various extracts and recombinant proteins were determined using Lowry [31] and Coomassie blue protein assays [32] or the bis-cinchoninic acid method (Pierce Chemical Company, Rockford, IL, USA). All the fractionated antigens and recombinant proteins expressed as fusion proteins pMal 2 vector or GST fusion system were studied for their SDS-polyacrylamide gel electrophoresis (SDS-PAGE) profile. The molecular weight and reactivity with inhouse standard sera by Western blot were ascertained. Those antigens complying with the expected size and reactivity only have been selected for the study.

SDS-PAGE and Western blot analysis

The proteins were separated by SDS-PAGE on 15% gels, after denaturation in electrophoresis sample buffer. The proteins were then transferred to nitrocellulose membrane (Protran, 0.1 mm; Schleicher and Schuell, Keene, NH, USA) according to the method of Towbin *et al.* [33] and stained with 0.5% Ponceau S to visualize the protein standards (Pharmacia Biotech Inc.) and latex proteins. The membrane was blocked for 1 h in 5% bovine serum albumin (BSA) in PBS, and incubated overnight in *E. coli*-adsorbed patient sera pooled from five skin test positive latex allergic health care workers (1 : 10, v/v in PBS) [22]. The membrane was washed, incubated for 1 h in monoclonal antihuman IgE (clone GE-1, 1 : 1000; Sigma, St Louis, MO, USA), washed, and incubated for 1 h in antimouse IgG conjugated to alkaline phosphatase (1 : 5000; Sigma). After washing, the membrane was developed in the alkaline phosphatase substrate NBT/BCIP (Promega Corp., Madison, WI, USA). Each antisera was pre-adsorbed for 30 min with 0.1 volume of 0.2 mg/mL *E. coli* extract (Promega Corp.).

Sera from patients with latex allergy and normal controls

Health care worker sera Sera from 31 health care workers who had clinically diagnosed latex allergy were used in the study. Latex allergy in health care workers was diagnosed by (a) a history of skin and respiratory symptoms often progressing from contact dermatitis through urticaria to asthma and anaphylaxis on latex contact, usually with latex glove powder inhalation, or (b) immediate weal and flare

skin reaction to latex glove antigens, (c) a history of reaction to cross-reactive latex antigens such as bananas or other foods, and/or (d) IgE antibodies to latex glove extract carried out by ELISA or RAST utilized before commercial tests were available [1–5]. The patients included physicians, technologists, nurses, and medical students. All patients showed skin test reactivity to ammoniated latex extracts (Bencard Latex) and to glove extracts used in day to day screening. Latex specific IgE was also demonstrated against these antigens by ELISA, RAST or Western blots.

Spina bifida patient sera A total of 24 spina bifida patients was studied. Thirteen sera were obtained from patients who had sensitivity to latex as indicated by clinical history. Latex allergy in spina bifida patients was diagnosed by a history of perioperative anaphylaxis and/or the demonstration of respiratory symptoms on latex glove powder contact, and/or the demonstration of skin or serological reaction to latex antigens and cross-reaction to food allergens. Skin test reactivity to latex antigen was detected in eight of the 13 patients. Five of the 13 patients demonstrated anaphylaxis on exposure to natural rubber products and hence, skin test was not carried out. All 13 patients showed elevated levels of latex-specific IgE by previously determined ELISA and RAST [5,9,12,13].

Control sera Eleven sera from patients with spina bifida, without clinical evidence of latex allergy and 23 healthy laboratory or health care workers who frequently use gloves but showed no evidence of clinical latex allergy were also studied. All 23 health care worker subjects had no immediate weal and flare skin test reactivity to latex extracts. No ELISA or RAST were performed in this group. None of the 11 spina bifida controls had symptoms on exposure to latex products. No skin tests were done in this group, but ELISA showed low IgE levels. All sera and antigens used were blinded, numbered and distributed to the four centres for the study. Institutional review committees approved the study and informed consent from all patients were obtained.

Antibody assays

Two RASTs and one ELISA were used to detect latex specific IgE antibody in the sera. These methods are described below:

Enzyme-linked immunosorbent assay The concentrations of various latex antigens to coat ELISA plates (Immulon II, Fisher Scientific, Itasca, IL, USA) were determined by checkerboard titration of the latex proteins and sera from patients with latex allergy [34]. Latex proteins (100 µL) diluted in PBS were used to coat the wells of polystyrene

microwell plates. The plates were incubated at room temperature for 3 h, followed by a further incubation at 4 °C for overnight. The wells were washed with PBS-Tween20 (PBS-T) and blocked with 0.5% BSA in PBS-T. The wells were again washed and 100 µL of human serum dilutions (doubling dilutions of 1:25 to 1:400) added and incubated at room temperature for 3 h. After washing the wells, 100 µL of 1:500 dilution of biotinylated goat antihuman IgE (Vector Labs, Burlingame, CA, USA) was added and incubated for 1 h at room temperature. The plates were again washed and 1:1000 dilution of streptavidin peroxidase (Sigma) added and incubated for 30 min. Finally, the wells were washed and the peroxidase reactivity was detected by the addition of orthophenylene diamine [34]. The substrate colour was developed for 15 min in a dark chamber and the reaction stopped by the addition of 2 mol/L H₂SO₄. The colour was read in an automated ELISA plate reader using a 490-nm filter (Molecular Devices, Sunnyvale, CA, USA). The data analysed by the aid of a computerized comparison and the log₁₀ titre calculated from the OD of four or more dilutions of the sera using a predetermined cutoff value [34]. A cutoff value of 0.25 (mean + 2 SD) was used based on the latex-specific IgE ELISA values of normal subjects. The latex-specific IgE log₁₀ titres of the different groups of subjects were compared.

Radioallergosorbent test

Two different RAST techniques were used to demonstrate the specific IgE antibody in the sera. In one, antigens were coupled to activated filter paper discs while in the other, latex was bound to polystyrene microwell plates.

Filter paper disc coupled RAST (RAST-1) A batch of 28.7 g of 7-mm disks punched from Whatman (Bridewell, NJ, USA) #50 filter paper was washed in 500 mL of 1 mol/L sodium carbonate. The disks were suspended in 700 mL of 2 mol/L sodium carbonate with rapid stirring, while 25 g of cyanogen bromide dissolved in 50 mL of dimethyl formamide was added. The pH was maintained between 10.5 and 11.5 by addition of 50% sodium hydroxide. After 10 min the disks were filtered and extensively washed in cold 0.1 mol/L sodium bicarbonate (5 L), cold ultrapure water (4 L), 50% acetone (1 L), and acetone (2 L). The disks were air dried and stored at –20 °C.

Batches of 200 disks were coupled to allergens in borate saline buffer, pH 8.0 [35]. The amounts of allergens used for coupling were 0.4 mg for Hev b 1, Hev b 2, Hev b 3 and Hev b 6; 0.5 mg for Hev b 4 and Hev b 7; and 3 mg for nonammoniated latex and about 0.4 mg of each glove extract. After overnight coupling the disks were extensively washed with borate-buffered saline, pH 8.0.

Specifically purified horse antihuman IgE antibody (100 µg) was labelled with 1 millicurie of Iodine-125 using a Pierce Chemical Company (Rockford, IL, USA) precoated Iodogen tube for 12 min [35]. After decanting and standing for 2 min 50 µL of saturated tyrosine in 0.1 mol/L TRIS-HCl, pH 7.4 was added. After standing 5 min the labelled antibody was purified using a 1-mL capacity Sephadex G-25 (Pharmacia Inc.) column. Radioactive antibody was diluted to 7 mL and then further diluted to 1:200 just before use.

The RAST was performed as previously described [35] using a single disk per 12 × 75 mm tube with 25 µL of serum plus 50 µL of borate-buffered saline, pH 8.0. Incubations with serum and with 100 µL of diluted labelled antibody were both stored overnight at room temperature, each followed by three washes with 2.5 mL each of 0.9% saline. Tubes containing the disks were counted for 1 min in a Micromedic 4/600 PLUS automatic gamma counter (Titertek Inc., Huntsville, AL, USA). Controls included normal horse serum as well as negative sera for each allergen and performance controls [35].

RAST with antigen bound to microtitre plates Individual wells of Immulon-4 microwell strips (Dynatech, Chantilly, VA) were coated with 70 µL antigen dissolved in sodium carbonate buffer (0.1 mol/L, pH 9.6) containing NaN₃ (0.01%) overnight at 4 °C. All antigens were coupled at a concentration of 5 µg/mL except for MNA, which was coupled at 7 µg/mL. Following coupling wells were washed three times in 0.01 mol/L TRIS-buffered saline containing 0.1% Tween20 and 0.01% NaN₃ (pH 7.9).

Sera were assayed by incubating coated wells for 3 h with 50-µL aliquots of diluted serum/well. Dilutions used were neat, 1/3, 1/5, 1/10, 1/50. Wells were washed four times with TRIS-buffered saline with Tween (TBS-T) washing solution. Fifty microlitres of radiolabelled (¹²⁵I) rabbit antihuman IgE (Kallestad, Chaska, MN, USA) was then added and allowed to incubate overnight in a humidified chamber. Wells were again washed four times with TBS-T and individual wells were counted for 5 min. Normal rabbit sera was used in eight replicates for each allergen as a negative control and dilutions of a positive latex serum pool (*n* = 42) were used as a positive control. It was determined that the 1/3 dilution of serum contained the least background and that most results in this range were not saturated. The positive cutoff value was selected as 2SD above the mean of normal controls for both RASTs and was used in discriminating latex-allergic patients.

Statistical analysis

The mean values of ELISA log titres and RAST values were calculated and the results were analysed. The multivariate analysis of variance (MANOVA) was performed to determine

the differences, if any existed, among groups of subjects studied; namely, normal subjects, spina bifida patients with no evidence of latex allergy, health care workers and spina bifida patients with clinical evidence of latex allergy. A *P*-value of 0.05 was considered significant. If a significant difference was found, then the discriminant analysis was performed. Discriminant analysis gives canonical discriminant functions and Fischer discriminant functions. Canonical discriminant functions are new linear scores calculated from the two dimensional scores for placing the individuals in the different groups [36]. This method was used to determine whether specific serum IgE determined against purified latex antigen by ELISA and RASTs agreed with the clinical diagnosis of latex allergy. The stepwise discriminant analysis was then performed to select the most significant antigen/antigens demonstrating IgE binding activity. Fisher discriminant scores were obtained using the selected antigen/antigens and the usefulness in detecting specific IgE using ELISA and RAST were evaluated. All the computations were carried out using SPSS statistical software (SPSS, Chicago, IL, USA).

Results

Antigen characterization

The purified latex antigens showed homogeneity when subjected to SDS-PAGE analysis (Fig. 1). The molecular

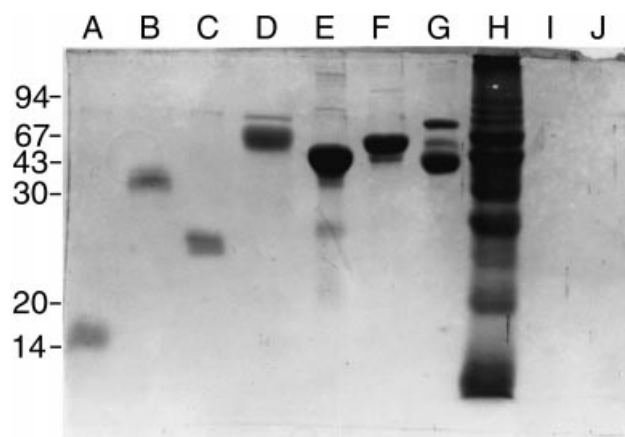


Fig. 1. SDS-PAGE analysis of latex extracts and purified proteins. SDS-PAGE was performed using a 15% polyacrylamide gel and protein was visualized using 0.1% Coomassie blue staining. Molecular weight was estimated using molecular weight markers (Pharmacia). The gel was loaded as follows: A – 8.5 µg Hev b 1; B – 4.5 µg Hev b 2; C – 4.5 µg Hev b 3; D – 6.5 µg Hev b 4; E – 20 µg rHev b 7; F – 20 µg recombinant maltose binding protein (rMBP, control); G – 10 µg rHev b 6; H – 300 µg MNA; I – 3 µg glove extract 1; and J – 3.5 µg glove extract 2 (see 'Methods' for details)

Table 2. The binding of individual latex proteins with IgE in the sera of various groups by the three assays

Antigens	ELISA				RAST-1				RAST-2			
	Latex allergy				Latex allergy				Latex allergy			
	HCW	SB	Both	Normals	HCW	SB	Both	Normals	HCW	SB	Both	Normals
	31*	13	44	34	31	13	44	34	31	13	44	34
Hev b 1	10 (32)**	13 (100)	23 (52)	4 (12)	4 (13)	7 (54)	11 (25)	1 (3)	6 (19)	11 (85)	17 (38)	2 (6)
Hev b 2	20 (65)	7 (54)	27 (61)	4 (12)	15 (48)	5 (38)	20 (45)	1 (3)	19 (61)	6 (46)	25 (57)	2 (6)
Hev b 3	10 (32)	13 (100)	23 (52)	1 (3)	6 (19)	11 (85)	17 (38)	0	6 (19)	10 (77)	16 (36)	2 (6)
Hev b 4	20 (65)	10 (77)	30 (68)	7 (20)	7 (23)	4 (30)	11 (25)	1 (3)	19 (61)	6 (46)	25 (57)	2 (6)
Hev b 6	17 (55)	9 (69)	26 (59)	2 (6)	14 (45)	5 (38)	19 (43)	2 (6)	14 (45)	4 (30)	18 (41)	1 (3)
Hev b 7	13 (42)	10 (77)	23 (52)	2 (6)	7 (23)	3 (23)	10 (22)	2 (6)	14 (45)	2 (15)	16 (36)	2 (6)
MNA	24 (77)	13 (100)	37 (84)	2 (6)	24 (77)	9 (69)	33 (75)	3 (9)	19 (61)	7 (54)	26 (59)	1 (3)
GI-1	21 (68)	12 (92)	33 (75)	1 (3)	17 (55)	6 (46)	23 (52)	2 (6)	8 (25)	3 (23)	11 (25)	1 (3)
GI-2	25 (80)	9 (69)	34 (77)	3 (9)	20 (65)	10 (77)	30 (68)	1 (3)	14 (45)	3 (23)	17 (38)	1 (3)

*Number studied. **Number positive, those showing 2 SD over the mean values of normals, () % positive.

weights and the reactivity of the proteins with positive sera in Western blots were comparable with the inhouse standards. The Hev b 6 expressed with MBP and Hev b 7 showed a few additional minor bands, however, the relevant fusion protein bands were readily discernible. Both glove extracts showed only a few bands. However, when higher concentrations of the glove extracts were used in SDS-PAGE, considerable streaking and background staining was noticed.

All the latex proteins included in the present study reacted in Western blots with pooled patient sera, but not with normal control sera (data not shown). In Western blots of MNA, multiple bands demonstrated reactivity with IgE antibody from latex-allergic patients.

IgE binding assays

IgE binding to the crude individual latex extracts and individual latex proteins showed considerable variability (Table 2). In general, the MNA and glove extracts detected higher levels of reactivity than did single purified protein. Spina bifida patients reacted more commonly with Hev b 1 and 3, while Hev b 2 and 6 were generally more reactive with health care worker sera. Although Hev b 4 showed reactivity with more patients than Hev b 6 and 7, this antigen also reacted with more normal controls.

All three methods used to detect latex-specific IgE showed overlap among subjects belonging to health care worker and spina bifida groups with latex allergy. These two groups of patients showed differences in the IgE binding with different antigens (Fig. 2a,b). The results demonstrate only fewer normal sera reacting with the two RASTs (3–9%), while ELISA showed more reactivity with control sera (3–20%). The normal controls were discriminated with an accuracy of 88% by the ELISA and 97–100% by the RAST techniques. Specific IgE was detected in 100, 61 and 58% of spina bifida patients by ELISA, RAST-1 and RAST-2, respectively. The corresponding figures for health care workers with latex allergy were 77.4, 58 and 48%. The sensitivity and specificity of the three assays using purified latex allergens for demonstrating specific IgE in the sera are shown in Table 3. High specificity was shown by all six allergens in the three assays used. However, sensitivity varied among antigens, patients and assays used. The reactivity of spina bifida patients with purified antigens vary from 100% by ELISA, while with the RASTs the percentage was never more than 85% with the different antigens. The reactivity of health care worker by ELISA and RAST-2 were more comparable, while RAST-1 showed fewer reactors among health care worker patients.

The IgE binding of the purified antigens studied individually demonstrated significant differences ($P \leq 0.05$) in the normals and latex-allergic patients (Fig. 2a,b). Normal

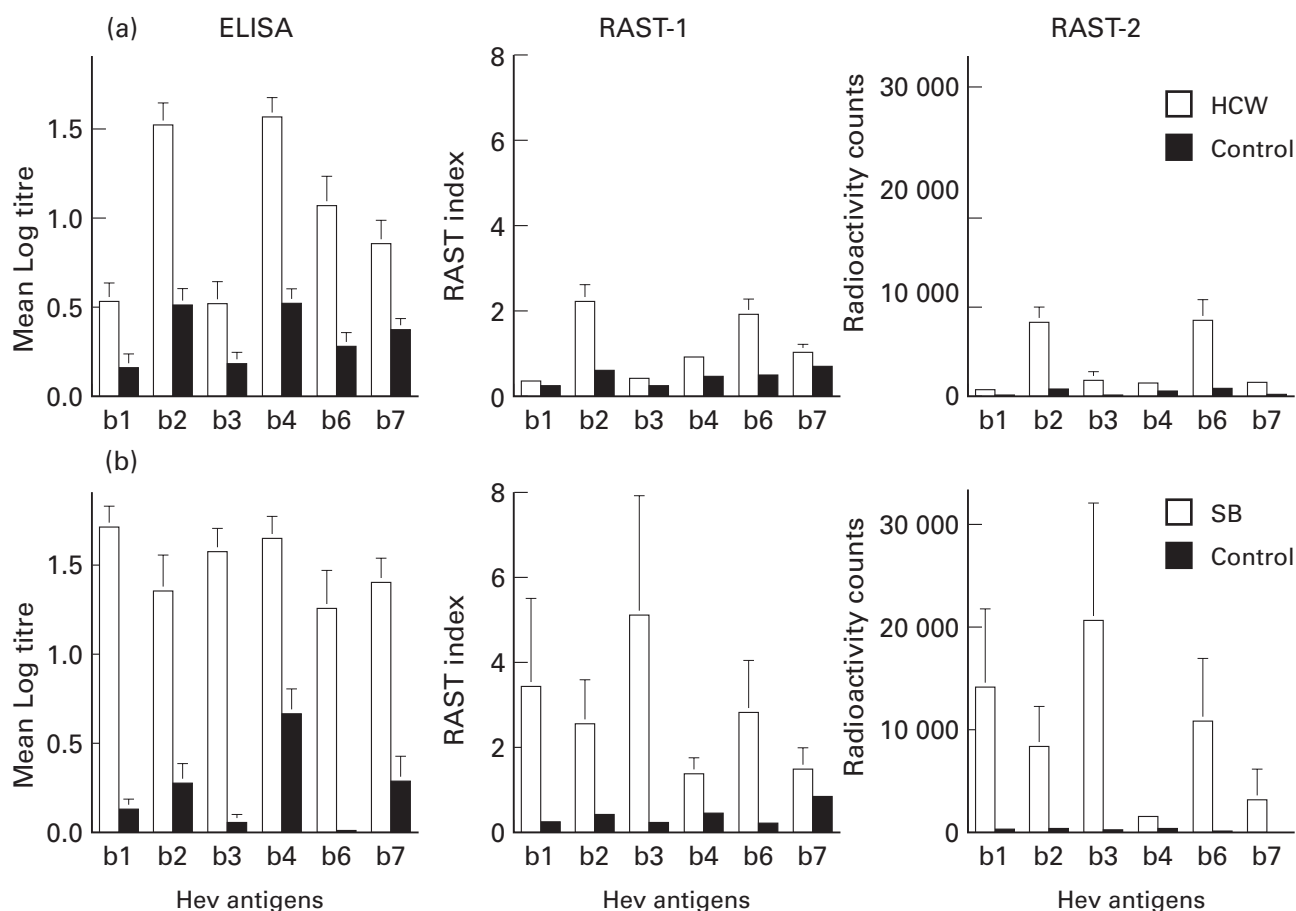


Fig. 2. The IgE binding of Hev b 1, 2, 3, 4, 6 and 7 assayed by ELISA and two RAST assays. Mean IgE values are presented; the bars indicate SE of the means. a – HCW (health care worker) patients with latex allergy and control; b – SB (spina bifida) with and without latex allergy. The values above the mean and two standard deviations of the non-latex-allergic subjects were taken as positive

controls invariably showed less positivity when tested by RAST, while ELISA showed some IgE binding in normal controls. Hev b 4 failed to show high titres with both RAST techniques, but showed similar titres with both normals and patients by ELISA. MANOVA using Hev b 1 to Hev b 7 showed a significant difference ($P < 0.05$) between normals and latex-allergic patients tested by both ELISA as well as RAST.

The results of ELISA and RASTs using the purified latex allergens were studied by a stepwise discriminant analysis. Variables with a P -value of < 0.05 were entered and variables with $P > 0.10$ were removed at each step of the stepwise analysis with each allergen. The results of the analysis for Hev b 1, 2, 3, 4, 6 and 7 for ELISA and RASTs are given in Fig. 3. This resulted in selecting Hev b 2 and Hev b 7 for use in IgE binding assay by ELISA. Canonical discriminant scores obtained for Hev b 2 and 7 for ELISA are shown in Table 4. The results indicate that Hev b 2 and 7 discriminated more patients by ELISA, although with the

RASTs this was not true. We have shown a comparison of all three assays in Table 4. The nonallergic subjects studied by all three assays merged to form one group (Fig. 3c,d). The results presented in Table 4 for ELISA demonstrated 77.4% of health care worker patients and 84% of spina bifida patients with latex allergy and 84% normals and 100% of spina bifida without latex allergy agreed with the clinical findings. However, with the two RAST techniques, these percentages were much lower for latex-allergic patients although almost all subjects without latex allergy failed to show significant IgE to latex allergens.

Discussion

In the present multicentre study, we have used three different immunological tests to examine the IgE binding and diagnostic potential of six purified latex proteins and compared the results with three crude latex extracts. The IgE binding of crude extracts from *Hevea brasiliensis* latex and

Table 3. Specificity and sensitivity of the three IgE binding assays using six purified latex allergens

Latex allergen	Patient groups*	ELISA		RAST 1		RAST 2	
		Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity
Hev b 1	HCW*	77.7	52.7	63.6	46.8	85.7	46.8
	SB	80.0	100.0	70.5	85.7	91.6	83.3
Hev b 2	HCW	74.2	73.6	100.0	56.0	100.0	57.5
	SB	90.0	71.4	100.0	57.8	100.0	55.0
Hev b 3	HCW	75.0	53.0	81.8	48.8	100.0	45.0
	SB	92.8	100.0	84.6	81.8	100.0	68.7
Hev b 4	HCW	85.7	56.2	72.7	53.1	100.0	65.7
	SB	75.0	87.5	75.0	66.6	100.0	61.1
Hev b 6	HCW	77.2	56.2	87.5	55.2	92.8	55.0
	SB	100.0	73.3	100.0	57.8	80.0	55.0
Hev b 7	HCW	69.2	53.5	72.7	53.1	100.0	50.0
	SB	80.0	88.8	63.6	53.8	100.0	47.8

* HCW—Health care worker, SB—spina bifida.

glove extracts gave interesting results. When patient sera was tested, the ELISA agreed maximally with the clinical diagnosis with fewer negative reactions when compared with the results of the two RASTs. However, more control sera showed reactivity with latex protein by ELISA when compared with RAST. The RASTs discriminated the non-latex-allergic subjects more clearly than ELISA, and the specific IgE levels more often agreed with the clinical diagnosis. The differences in the results between the three assays may be due to differences in the reagents used, the conditions of the tests or the differential binding of antigens to the solid phases.

Eight natural rubber latex allergens have been highly purified and have been reported as major allergens, but their availability is limited [1,2,14–16,18–22]. When the reactivity of six individual antigens were compared in the

present study, the three different assays yielded similar discriminatory results. The RASTs distinguished 100 and 97% of the control subjects without latex allergy. The ELISA had more difficulty in distinguishing the controls (84%), perhaps because Hev b 4 showed reactivity in more than 20% of the controls. Analysis of individual reactivity showed that Hev b 1 and 3 reacted with all 13 spina bifida sera by ELISA (Table 2). These two antigens also reacted with a maximum number of spina bifida sera by RASTs. Hev b 1 and 3 have previously been identified as important allergens for spina bifida patients [27]. However, when Hev b 2 and 7 were used together, IgE could also be detected in 12 out of 13 patients studied. Unfortunately, we were unable to obtain the newly reported Hev b 5 while this multicentre investigation was carried out [19,37]. However, subsequently it was cloned and the protein expressed by one of

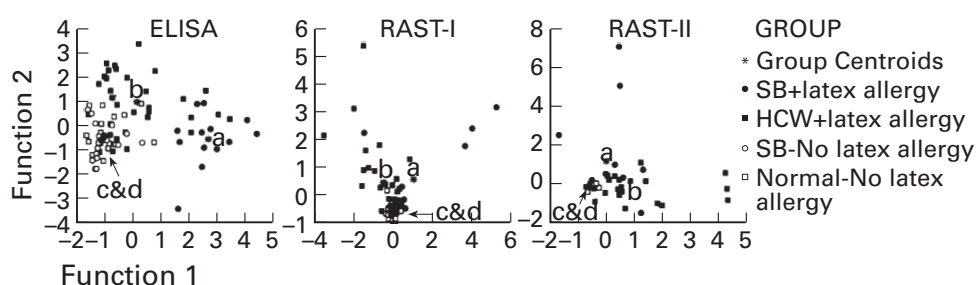


Fig. 3. The ELISA, RAST-1 and RAST-2-values were analysed by discriminant functions, and Canonical discriminant scores were calculated for Hev b 1, 2, 3, 4, 6 and 7. The values linearized from the two dimensional scores for classifying the individuals in the four different groups. Functions 1 and 2 are the linearized Canonical scores; (a) centroid of spina bifida with latex allergy; (b) centroid for health care worker with latex allergy; (c & d) centroid of normal controls and spina bifida with no latex allergy

Table 4. IgE binding to Hev b 2 and Hev b 7 by sera from latex-allergic patients and normal controls by discriminant analysis

Groups	IgE Detection					
	ELISA		RAST 1		RAST 2	
	Negative	Positive	Negative	Positive	Negative	Positive
Latex allergic patients						
HCW (<i>n</i> = 31)	6 (19.4)	25 (80.6)	18 (58.1)	13 (41.9)	17 (54.8)	14 (45.2)
SB (<i>n</i> = 13)	1 (7.7)	12 (92.3)	8 (61.5)	5 (38.5)	9 (69.2)	4 (30.8)
Subjects with no latex allergy						
Normal (<i>n</i> = 23)	18 (78.2)	5 (21.7)	22 (95.7)	1 (4.3)	23 (100)	0 (0)
SB (<i>n</i> = 11)	11 (100)	0 (0)	11 (100)	0 (0)	11 (100)	0 (0)

* Number of subjects, the number in parenthesis indicates percent.

us (Yeang) and our preliminary studies indicate that Hev b 5 will not be advantageous over Hev b 2 and Hev b 7 in the diagnosis of latex allergy (unpublished results). Hev b 2 demonstrated reactivity with IgE antibody with more latex-allergic patients in all three assays compared with Hev b 7, which showed less reactivity with the patients. However, the reactivity of Hev b 7 was seen predominantly with Hev b 2-negative sera and hence, when used together these two allergens reacted with more latex-allergic patients. When these two allergens were used to test latex-specific IgE, a specificity of 100 and 83% and sensitivity of 91 and 75% were detected for spina bifida and health care worker patients by ELISA. The specificity of the two RASTs were comparable, but the sensitivity was low compared with ELISA. It is likely that other allergens with additional specific reactivity may still be present in the native rubber latex, which may increase the diagnostic accuracy.

It is well known that latex-allergic patients react to multiple latex allergens [1,6]. When purified allergens are used in diagnostic tests, multiple antigens are necessary to demonstrate latex-specific IgE. Our study indicates that additional antigens may be needed to reach the diagnostic accuracy obtained with crude latex. Using discriminant analysis, two purified allergens, Hev b 2 and 7, demonstrated specific IgE titres in only slightly lower than that achieved using the crude latex extract MNA. However, one needs to be aware of the potential limitation of this approach. If the appropriate allergens are not used in the assays, antibody to the particular purified antigen may be lacking in the sera resulting in a false-negative reaction. On the other hand, purified antigens can be better characterized and hence, the results obtained with the purified allergens may be more comparable and reproducible from one laboratory to the other.

Studies using purified latex allergens for IgE-binding

reactivity with patients' sera have been few. Furthermore, those studies available from different laboratories are difficult to compare due to the different patient populations and assays utilized [14,16,18,20,21,27,30,38]. Hev b 1 was considered to be a major allergen by Czuppon and coworkers [14]. Chen and coworkers reported that 80% of spina bifida and 50% of health care worker patients showed IgE-binding to Hev b 1 [38]. However, Yeang *et al.* concluded that Hev b 1 is not a major allergen [27]. Alenius and colleagues found only a low frequency of reactivity for Hev b 1 among various latex-allergic patients investigated [15]. In the present study, we have demonstrated moderate to low reactivity among latex-allergic patients with this allergen by the different *in vitro* methods (Table 2).

In the present study, we have been able to compare reactivity to several purified latex allergens using a common set of sera. Hev b 2 (β -1,3-glucanase) was not considered as an important allergen and has not been widely evaluated. Sunderasan and coworkers found some binding with IgE by this antigen [18]. However, in the present study, Hev b 2 showed strong IgE-binding reactivity in all three assays. In the previous reports, Hev b 3 has been shown to react with a majority of spina bifida patients comparable with the results obtained in the present study [16,27,30]. However, our study showed only very few health care worker patients reacting to this antigen (Table 2). Beezhold *et al.* reported that patatin-like protein (Hev b 7) reacted with 23% of health care worker patients and considered this protein a major allergen associated with latex allergy [20,22]. In the present study, this allergen showed significant IgE-binding with both health care worker and spina bifida patients by ELISA and by the two RASTs (Table 2, Fig. 2). Hev b 6 has been reported as a major allergen, was also supported from the results of the present study [15]. In conclusion, the use of Hev b 2 and Hev b 7 simultaneously,

latex-specific IgE in both spina bifida and adult health care workers with latex allergy patients can be demonstrated by ELISA, while the specificity and sensitivity can be enhanced further by the addition of more purified latex allergens in the panel for a reliable laboratory diagnosis of latex allergy.

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