ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/236014256

Latex B-Serum p-1,3-Glucanase (Hev b II) and a Component of the Microhelix (Rev b IV) are Major Latex Allergens

Article *in* Journal of Rubber Research · January 1995

CITATIONS
57

READS
55

7 authors, including:

Sunderasan Elumalai
Malaysian Rubber Board
47 PUBLICATIONS **489** CITATIONS

SEE PROFILE

Sundus Hamzah
University of Al-Qadisiyah
16 PUBLICATIONS **419** CITATIONS

SEE PROFILE

Malcolm A Ward
Proteome Sciences plc London
178 PUBLICATIONS **4,762** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project protein aggregates isolation View project

Project Continue with research in the same areas, as reflected in publications in recent years. View project

All content following this page was uploaded by Sunderasan Elumalai on 31 May 2014.

The user has requested enhancement of the downloaded file.

J nat Rubb Res, **10(2)**, 82–99

*Latex B-Serum β-1,3-Glucanase (*Hev b *II) and a Component of the Microhelix (*Hev b *IV) are Major Latex Allergens*

E. SUNDERASAN*, SAMSIDAR HAMZAH*, SHARIFAH HAMID**, M.A. WARD***, H.Y. YEANG*# AND M.J. CARDOSA****

Two allergenic (IgE-binding) proteins have been isolated from the B-serum of natural rubber latex by dialysis-induced protein precipitation followed by gel filtration. One of the allergens, Hev b *II, that is specific to the monoclonal antibody USM/RB4, commonly appears as a doublet of molecular weights 34 and 36 kDa under reducing conditions of SDS-polyacrylamide gel electrophoresis. In the absence of 2-mercaptoethanol, a 70 kDa band can also be seen in addition to the doublet. The allergen has a pI of* circa *9.5 and is identified as β-1,3-glucanase. The second allergen,* Hev b *IV, has a pI at* circa *4.5 and appears under reducing conditions as a band of 50–57 kDa. In its unreduced form, a principal band of approximately 100 kDa is discernible Immuno-gold labelling with the monoclonal antibody USM/RB3 shows that* Hev b *IV is a component of the microhelix protein complex of B-serum.*

Allergenic latex proteins that are retained in end-use manufactured products (*e.g.* latex examination and surgical gloves) pose a serious concern to a small proportion of users and patients sensitised to these allergens. Existing evidences point to the water-extractable proteins from the finished products as the causal allergens[1]. Despite the fact that a large number of allergenic proteins have been reported, they have seldom been characterised beyond their molecular weights. Even this attribute could be equivocal since proteins extracted from latex products would have undergone harsh physical and chemical treatments that might alter their properties during product manufacture and thence could account for the wide range of allergens detected. Attempts to characterise allergens from preserved latex concentrate or frozen field latex might be similarly problematic as such latices are also prone to destabilisation and modification arising from the preservatives (commonly ammonia) added and/or storage conditions. In attempting to identify and characterise latex allergens, therefore, there are obvious advantages in working with fresh latex.

Latex of the rubber tree (*Hevea brasiliensis*) is the cytoplasm of specialised cells known as laticifers. Upon high speed centrifugation, latex is separated into the rubber cream, the latex serum (C-serum) and the 'bottom fraction' which consists mainly of vacuole-like organelles called lutoids[2,3]. The lutoids contain

*Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia
**Venture Technologies (M) Sdn. Bhd., Penang, Malaysia
***Structural Chemistry Department, Glaxo Research and Development Ltd., Stevenage, Herts SG1 2NY, United Kingdom
****School of Pharmaceutical Sciences, University Sains Malaysia, 11800 Penang, Malaysia.
#Corresponding author

a fluid, the B-serum[4], that has long been of interest to biochemists and physiologists as it contains several hydrolases[5] and some pathogenesis-related proteins[6]. B-serum also has considerable influence on the latex flow characteristics[7]. Some highly confined proteins of lutoid B-serum form complexes – the microfibrils[8] and the microhelices[9-11], that are large enough to be visible under the electron microscope.

Latex B-serum has been examined as a source of latex allergens[12-14]. While a greater number of individual proteins in latex are to be found in the C-serum[12], it has been established that B-serum is more immunogenic[14]. B-serum appears, therefore, to be a major origin of latex allergens.

The present study is aimed at isolating, identifying and characterising some major allergenic proteins in B-serum. Monoclonal antibodies specific for these proteins are used in the elucidation of the parental and breakdown fragments and sub-units of the allergens. The allergens are further characterised by immunogold labelling using electron microscopy.

MATERIALS AND METHODS

Preparation of B-serum

Latex B-serum was prepared as previously described[4]. Briefly, fresh latex from *Hevea brasiliensis* clone RRIM 600 was collected into chilled containers and centrifuged at 19 000 r.p.m. (43 000 g) for 1 h at 4°C–7°C. The rubber cream and the C-serum were discarded while the bottom fraction was collected and resuspended in 0.4 *M* mannitol to aid the removal of contaminant C-serum while retaining the lutoids intact. The cleansed bottom fraction was recovered after another centrifugation and subjected to alternate freezing and thawing (4 times) to rupture the lutoids. The fluid from the lutoids, the B-serum was recovered by centrifugation for subsequent analyses.

Precipitation of B-serum Proteins by Dialysis

Aliquots of 20 ml were dialysed using cellulose acetate tubings (molecular weight cut-off 2500 Da) against 5 litres of distilled water for 48 h at about 5°C. The resulting proteinaceous precipitate was recovered by centrifugation at 15 000 r.p.m. (20 000 g) for 30 min. The precipitate was redissolved in 20 ml of 0.35 *M* NaCl with the solution kept in ice water bath throughout this operation.

Column Chromatography

The solution (5 ml) of proteins redissolved in sodium chloride was chromatographed on a column (77 cm × 1.6 cm) of Sephacryl S-200 (Pharmacia, Uppsala, Sweden) which was equilibrated with 0.35 *M* NaCl. Elution was carried out in the same solvent with the flow rate maintained at 20 ml h^{-1}. Sequential fractions of 5 ml were collected and the optical density of each fraction was measured at 280 nm. Several such column chromatography runs were made to recover substantial quantities of the components eluted from the column for analysis.

Concentration of the Eluted Components

The fractions under the major UV absorption peaks obtained from several runs were pooled and dialysed overnight against 3 litres of 1% glycine at approximately 5°C essentially to eliminate the salt while retaining the proteins in their soluble state. The dialysed fractions were concentrated up to 10 × by partial freeze-drying.

Amino Acid Sequence Analysis

N-terminal sequencing. 10 µl of the protein sample (8 mg ml^{-1}) were loaded onto hydro-

COPYRIGHT © MALAYSIAN RUBBER BOARD

phobic sequencing columns using 2% trifluoroacetic acid (TFA). All sequence analyses were performed using the HP G1000A protein sequencer and following the manufacturer's instructions.

Cyanogen bromide digests. An aliquot of 0.25 *M* cyanogen bromide in 70% formic acid (50 μl) was added to 100 μl protein sample in an eppendorf tube. The digest was incubated for 3 h at 50°C after which, it was frozen using dry ice. The digest solution was then loaded onto a PCS mini column using 1 ml of 2% TFA. The column was placed in line to a 2.1 mm Aquapore RP-300 C18 reversed phase column. Peptide fragments were separated by gradient elution at a flow rate of 200 μl min^{-1} beginning at 80% A and 20% B and rising to 60% B in 40 min, where solvent A = 0.1% TFA/ H_2O and solvent B = 0.1% TFA/MeCN. Detection was at 215 nm 0.500AUFS. The peptide fragments were collected and sequenced.

Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on Laemmli[15] was carried out on 15% gels at pH 8.9 on a Mini Protean II Cell (Bio-Rad, USA) according to the manufacturer's instructions. With some protein samples that were to be run under non-reducing conditions, β-mercapto-ethanol was excluded from the sample buffer. Native cathodic electrophoresis was carried out on Mini Protean II Cell with 7.5% gels at pH 4.3 based on Reisfeld *et al.*[16] using β-alanine buffer. Separated proteins were stained with Coomassie Brilliant Blue R250. Molecular weights of the proteins were estimated from their relative mobility by interpolation against protein standard markers. Two sets of markers were used (Integrated Separation Systems, USA and BioRad, USA) as slightly different molecular weight readings were obtained depending on the markers employed.

Western blotting of the separated proteins onto nitrocellulose membranes (Hybond-C, 0.45 μm) and subsequent incubation with patient serum/plasma or with a monoclonal antibody was as previously described[12]. Detection of protein-antibody binding was achieved using an appropriate secondary antibody conjugated with horseradish peroxidase to generate a coloured product. Dot enzyme immuno-assays were processed in a similar manner except that the test protein was pipetted (dotted) directly on to the nitrocellulose membrane.

Visualisation of β -1,3-glucanase Activity on Native Cathodic Polyacrylamide Gel

The gel was incubated at 37°C in 0.05 *M* sodium acetate buffer, pH 5.2 containing 1.5 mg/ml laminarin (Sigma). After 3 h, the solution was dicarded and the gel was placed in 0.05 *M* K_2HPO_4, pH 8.6 containing 0.05% aniline blue for 1 h to visualise dark blue bands indicating the enzyme activity[17].

Isoelectric Focusing (IEF)

The isoelectric point (pI) of the test proteins was determined using the LKB Multiphor Model 2117 electrofocusing apparatus equipped with precast Ampholine PAG-plate of pH range 3.5 to 9.5 following the manufacturer's instructions (Pharmacia LKB, Sweden). About 15 μl of test sample concentrated in 1% glycine was applied to the polyacrylamide gel.

Preparation of Antibodies

To prepare monoclonal antibodies against B-serum proteins, spleen cells from Balb/c mice immunised with B-serum were fused with mouse myeloma cells following protocols previously described by Kohler and Milstein[18,19]. The resulting hybridoma cells were screened for antibodies specific to the identified

allergenic latex proteins using several immunoassays[20,21]. Selected hybridomas were recloned twice and monoclonal antibodies secreted were used either in unpurified form in hybridoma cell supernatants or as preparations purified by affinity chromatography.

Polyclonal antibodies against proteins eluted from latex examination gloves were prepared as previously described[12].

Immuno-gold Labelling and Electron Microscopy

B-serum was dialysed against distilled water at 4°C to generate a precipitate containing the microhelix protein complex as described previously[11]. The precipitate was diluted (1:1) in distilled water and deposited on to formvar-carbon coated nickel grids which were blocked for 30 min with phosphate buffered saline, pH 7.2, containing 1% bovine serum albumin (PBS-BSA). Incubation for 15 min was carried out separately with monoclonal antibodies USM/RB4 and USM/RB3 that are specific for *Hev b* II and *Hev b* IV, respectively. After washing, antibodies bound to the microhelices were detected by 15 min incubation with 10 nm goat anti-mouse immunoglobulin (IgG) gold conjugate (the secondary antibody), contrasted with negative stain 2% phosphotungstic acid (pH 6.8) and examined with the Philips CM 12 electron microscope. Control samples were also prepared where the primary antibody was omitted or substituted with an irrelevant antibody.

Hev b IV was also incubated with a latex-allergic patient plasma which showed selective binding to the allergen in the immunoblot carried out earlier. In this particular case, goat anti-human IgE served as the secondary antibody and gold conjugate of rabbit anti-goat IgG was used for immuno-detection.

IgE Patients Plasma and Sera

Samples of blood plasma from adult patients allergic to latex were sourced from PlasmaLab International, USA. Other sera were kindly provided by Dr. Ercan Unver of Diagnostics Products Corporation, USA and Dr Robert Hamilton of John Hopkins University School of Medicine, USA. All patients had clinical symptoms of latex allergy and the plasma/sera samples had anti-latex IgE confirmed by radioallergosorbant test or enzyme-immuno assay. Control plasma were obtained from Kuala Lumpur General Hospital, Malaysia.

RESULTS

Latex B-serum has been shown to contain several allergenic polypeptides[13]. When B-serum was subjected to prolonged ultracentrifugation (4 h) at 65 000 g, the gelatinous pellet that was sedimented at the bottom of the centrifuge tube was found to be significantly more allergenic than the supernatant or the whole (uncentrifuged) B-serum *(Table 1)*. As prolonged ultracentrifugation is known to sediment a B-serum component called the 'Microhelix assembly factor'[11], further investigations into the microhelix, a protein complex in B-serum, were pursued to evaluate its significance as an allergen.

Gel Filtration of Dialysis-induced B-serum Proteinaceous Precipitate

The microhelix protein complex that are present in B-serum can be precipitated out by dialysing the serum against water to lower the ambient ionic strength[11] as described in *Materials and Methods*. When B-serum was precipitated in this manner and the precipitate redissolved in 0.35 *M* sodium chloride, dot immuno-assays showed that the precipitate was much more allergenic than the supernatant of

TABLE 1. ALLERGENICITY OF PELLET AND SUPERNATANT FROM ULTRACENTRIFUGED B-SERUM COMPARED WITH THE UNCENTRIFUGED B-SERUM

Plasma sample	Uncentrifuged B-serum	Centrifuged B-serum	
		Pellet	Supernatant
1	+++	++++	++
2	+	++	+
3	+++	++	+

Binding of plasma IgE in dot enzyme immuno-assay: + = low binding; ++++ = high binding

the dialysate. From this observation, it appeared therefore that the dialysis-induced precipitate was a major source of allergenic proteins in B-serum.

The B-serum precipitate reconstituted in 0.35 *M* sodium chloride was subjected to column chromatography to separate its constituents by their molecular weights. The elution profile of the proteins after gel filtration on Sephacryl S-200 is shown in *Figure 1*. Four peaks, designated A to D, were obtained. In initial experiments, recovery of Peak D was erratic and it was subsequently found that Peak D could be recovered in sizable amounts only when B-serum was dialysed for more extended periods (more than 48 h).

All four peaks were assessed for allergenicity by dot enzyme immuno-assays. As shown in *Table 2*, Protein in Peak A was recognised by plasma of 11 out of 13 latex-allergic patients while 4 out of 13 recognised the protein in Peak D. In a subsequent screen of sera from seven more adult latex-allergic patients, 6 out of 7 were reactive to the protein in Peak A while 2 were reactive to Peak D. Combining the figures for the two assessments, therefore, 17 out of 20 sera/plasma (85%) were positive for Peak A while 6 out of 20 (30%) were positive for Peak D. Proteins in Peak B and Peak C showed very weak reactions with a small number of plasma samples but were non-allergenic in most cases. They were not investigated further in this study.

From a panel of monoclonal antibodies developed against B-serum, USM/RB3 and USM/RB4 were respectively found to bind specifically to the allergens in Peak A (designated *Hev b* IV) and in Peak D (designated *Hev b* II) *(Table 2)*. Proteins from all four elution peaks reacted strongly with polyclonal antibodies developed against latex examination gloves.

Characterisation of the B-serum Allergens, *Hev b* II and *Hev b* IV

Hev b *II*. *Hev b* II obtained from Peak D of the Sephacryl S-200 column was recovered in the elution fractions where the elution volume was greater than the total volume. The protein must therefore have interacted with the column material. Under denaturing conditions of SDS-PAGE, *Hev b* II resolved into a doublet at 34 and 36 kDa *(Figure 2)*. Both peptides were recognised by the monoclonal antibody USM/RC4. The later elution fractions constituting Peak D tended to show the 34 kDa moiety more prominently. In addition to the doublet, a band of approximately 70 kDa was also detected in the unreduced protein sample (untreated with β-mercaptoethanol) as shown in *Figure 2* (Lane 3). The immunoblot in *Figure 3* shows the allergenicity of *Hev b* II by

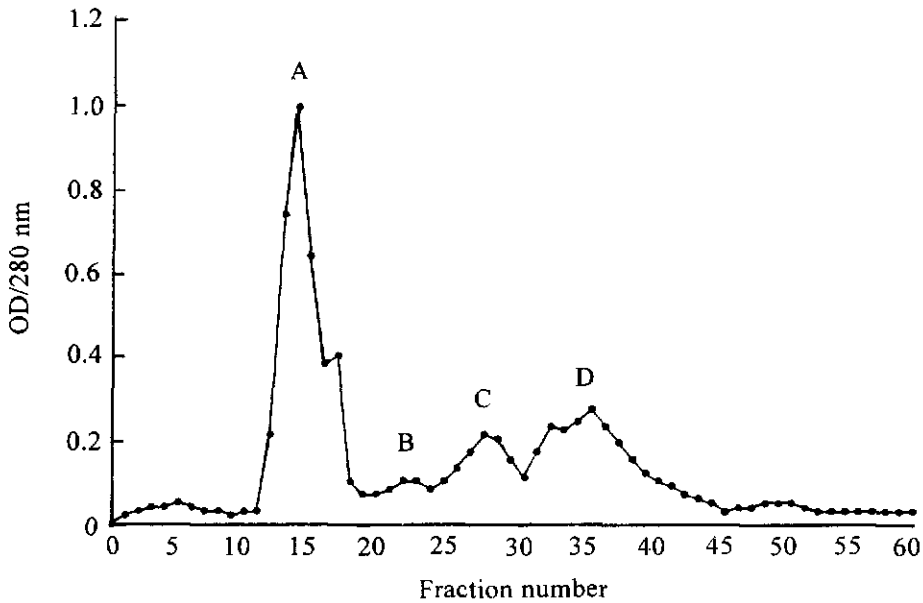


Figure 1. Sequential protein fractions obtained by Sephacryl S-200 gel filtration of the precipitate obtained from dialysed B-serum. The precipitate was redissolved in 0.35 M *sodium chloride solution before gel filtration. The main fractions A to D are indicated.*

TABLE 2. BINDING OF PROTEINS IN FRACTIONS A TO D OBTAINED BY GEL COLUMN CHROMATOGRAPHY TO IgE IN PLASMA SAMPLES (a – m) AND TO MONOCLONAL ANTIBODIES USM/RB3 AND USM/RB4 (n and o)

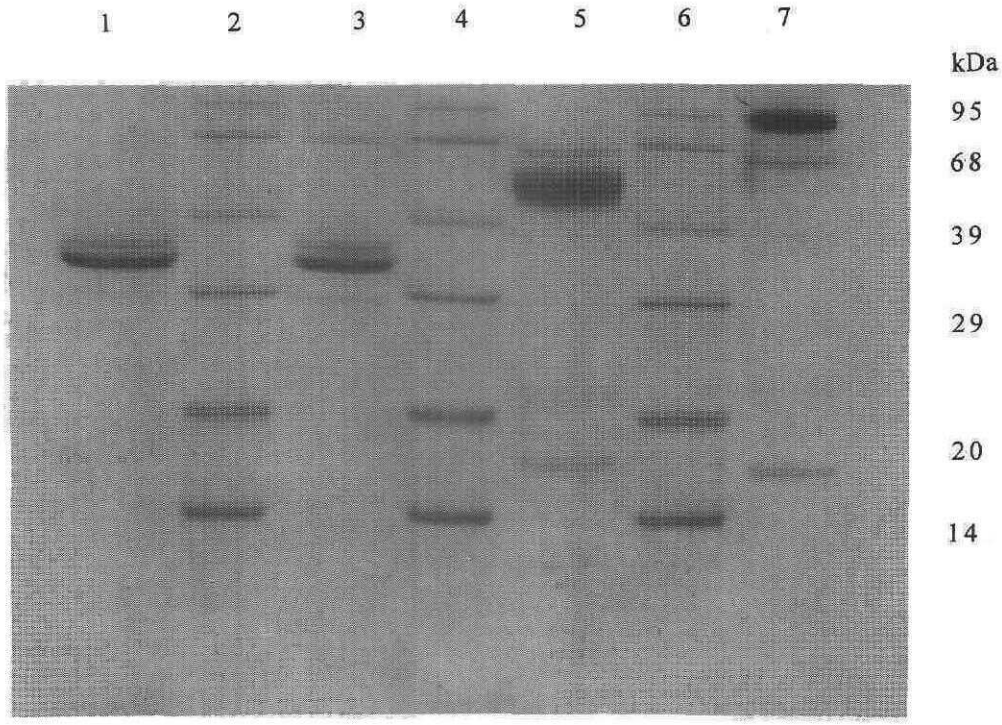
Antibody/antibody source	A	B	C	D
a. 9706	++	+	+	
b. 1012	++++			++++
c. OB1	++	+	+	
d. A190	++++			+++
e. 9586	++	++	++	
f. 3510-28	++			
g. ASP	+			
h. 4414				
i. 4398	++			+++
j. 4376	++			
k. 4265	++			
l. 4396				
m. 4393	+++			+
n. USM/RB3	++++	+	w	+
o. USM/RB4	+	w	w	++++

++++ = Strong protein-antibody binding
\+ = Weak protein-antibody binding
w = Very weak protein-antibody binding

its binding with IgE from blood plasma of a latex-allergic patient. Isoelectric focusing of *Hev b* II re-vealed a single band at pI approximately 9.5 *(Figure 4)*.

As might be expected of a basic protein, *Hev b* II could be electrophoresed on a cathodic native PAGE system. The protein appeared as a single band after the electrophoresis at acidic pH. β-1,3-glucanase activity was detected at the corresponding band after a similar gel was incubated with laminarin as substrate *(Figure 5)*.

Amino acid sequencing was carried out on *Hev b* II. The N-terminal of *Hev b* II was found to be blocked, but cyanogen bromide digest gave a peptide with an 11 residue sequence: FDENNXQPEVE and another peptide with a 13 residue sequence: RNIHDAIRSAGLQ *(Figure 6)*. Both sequences show good similarity to the published sequence of endo-1,3 β-glucanase from *Nicotiana plumbaginifolia*[22] (Leadwort-leaved tobacco), *Nicotiana tabacum*[23] (tobacco), and *Lycopersicon esculentum*[24] (tomato) while the latter



Lane 1: Peak D, reduced; Lane 3: Peak D, unreduced;
Lane 5: Peak A, reduced; Lane 7: Peak A, unreduced.
The remaining lanes (2,4 and 6) are reference molecular weight reference markers. Coomassie Blue staining.

Figure 2. Electrophoretic separation (15% gel) of proteins from Peak A and Peak D obtained by Sephacryl S-200 gel filtration of the precipitate obtained from dialysed B-serum.

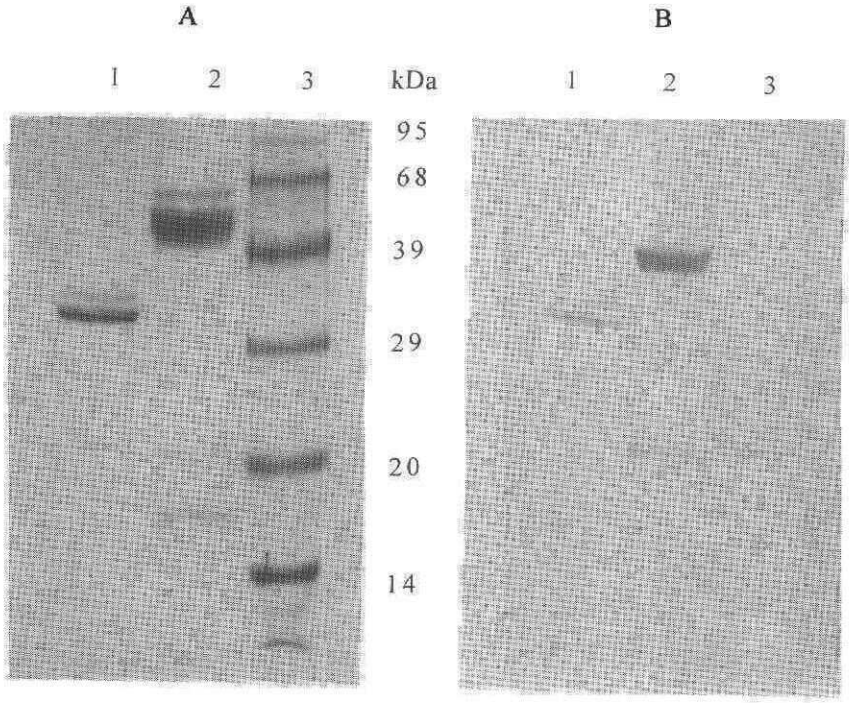


Figure 3. Electrophoretic separation (15% gel) and immuno-detection of allergens from Peak A (Lane 2) and Peak D (Lane 1) obtained by Sephacryl S-200 gel filtration of the precipitate obtained from dialysed B-serum. Panel A: Coomassie Blue staining. Panel B: Western blot of a matching gel to show binding of the allergens with IgE in plasma from a latex-allergic patient. Molecular weight standards are shown in Lane 3.

sequence also bears a similarity to the paraflagellar rod protein of a protozoan, *Trypanosoma brucei*[25].

Hev b *IV*. When electrophoresed under reducing conditions, *Hev b* IV that was recovered from peak A after gel filtration showed a band of about 50–57 kDa (*Figure 2*, Lane 5). This broad band, which is recognised by the monoclonal antibody USM/RB3, might represent more than one protein or protein sub-unit. In its unreduced form, a band of about 100 kDa was discernible *(Figure 2,* Lane 7*)*. (Using another brand of protein standard markers, its molecular weight was estimated at 110 kDa.) USM/RB3 also recognised a 30 kDa peptide in whole B-serum electrophoretically separated under non-reducing conditions, but this peptide was not prominent in Peak A. In both reduced and unreduced states, minor bands of 65, 22, and a 18 kDa from Peak A were also seen, but it is unclear if they were derived from *Hev b* IV. The immunoblot in *Figure 3* shows *Hev b* IV to be allergenic, as are two minor proteins of 22 and 65 kDa from Peak A. The 18 kDa polypeptide from Peak A is apparently non-allergenic. Isoelectric focusing revealed *Hev b* IV to be an acidic protein with pI in the region of pH 4.5 *(Figure 4)*.

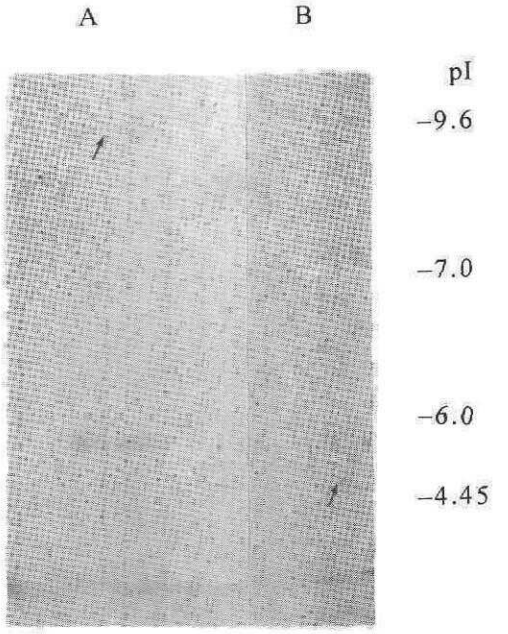


Figure 4. Isoelectric focussing of Hev b *II (Lane A) and* Hev b *IV (Lane B), indicated by arrows. IEF standard markers positions are shown. Coomassie Blue staining.*

Amino acid sequence analysis of *Hev b* IV showed an N terminus sequence of ELDEYLFSGDGLYDAGNA which does not match any previoulsy published amino acid sequence.

Immuno-gold Labelling of *Hev b* II and *Hev b* IV

While the microhelix protein complex is a prominent component of the precipitate arising from the dialysis of B-serum, the precipitate may contain proteins other than the microhelices as well. *Figure 7* shows bundles of microhelices from the precipitate of dialysed B-serum under the electron microscope. After incubation with the monoclonal antibody USM/RB3 that is specific for *Hev b* IV, the antibody-antigen binding sites (as indicated by the gold particles) were seen to be concentrated on the microhelices. Concentration of gold particles on the microhelix bundles were similarly observed when incubation was carried out with plasma from a patient allergic to *Hev b* IV, but not to *Hev b* II *(Figure 8)*. This shows that *Hev b* IV is an allergenic protein that is a component of the microhelix complex.

The technique of immuno-gold labelling was also used to examine if *Hev b* II is a component of the microhelix complex. As shown in *Figure 9*, there was no association of gold particles with microhelices in B-serum precipitate that had been incubated with USM/RB4, a monoclonal antibody specific for *Hev b* II. This allergen, which co-precipitates after prolonged dialysis of B-serum is therefore not a component of the microhelix complex.

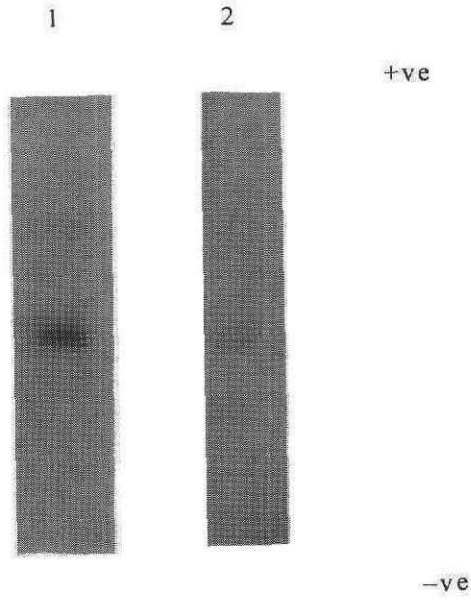


Figure 5. Polyacrylamide gel electrophoresis of Hev b *II on a cathodic native PAGE system. Lane 1: Coomassie blue staining. Lane 2: β-1,3-glucanase activity indicated by reaction with laminarin.*

H. brasiliensis	F	D	E	N	N	X	Q	P	E	V	–	E			
N. plumbaginifolia	F	D	E	N	N	K	N	P	E	L	–	E	K	H	F
N. tabacum	F	D	E	N	N	K	N	P	E	L	–	E	K	H	F
L. esculentum	F	D	E	N	R	K	D	G	K	P	S	E	Q	H	F

H. brasiliensis	R	N	I	H	D	A	I	R	S	A	G	L	Q
N. plumbaginifolia	R	N	I	R	N	A	I	S	S	A	G	L	G
N. tabacum	V	N	I	Y	K	A	I	G	E	A	G	L	G
L. esculentum	E	N	I	Y	N	A	L	S	S	A	G	L	Q
T. brucei	R	N	L	H	D	A	I	Q	K	A	D	L	E

Figure 6. Comparison of related amino acid sequence from H. brasiliensis, N. plumbaginifolia, N. tabacum, L. esculentum *and* T. brucei.

DISCUSSION

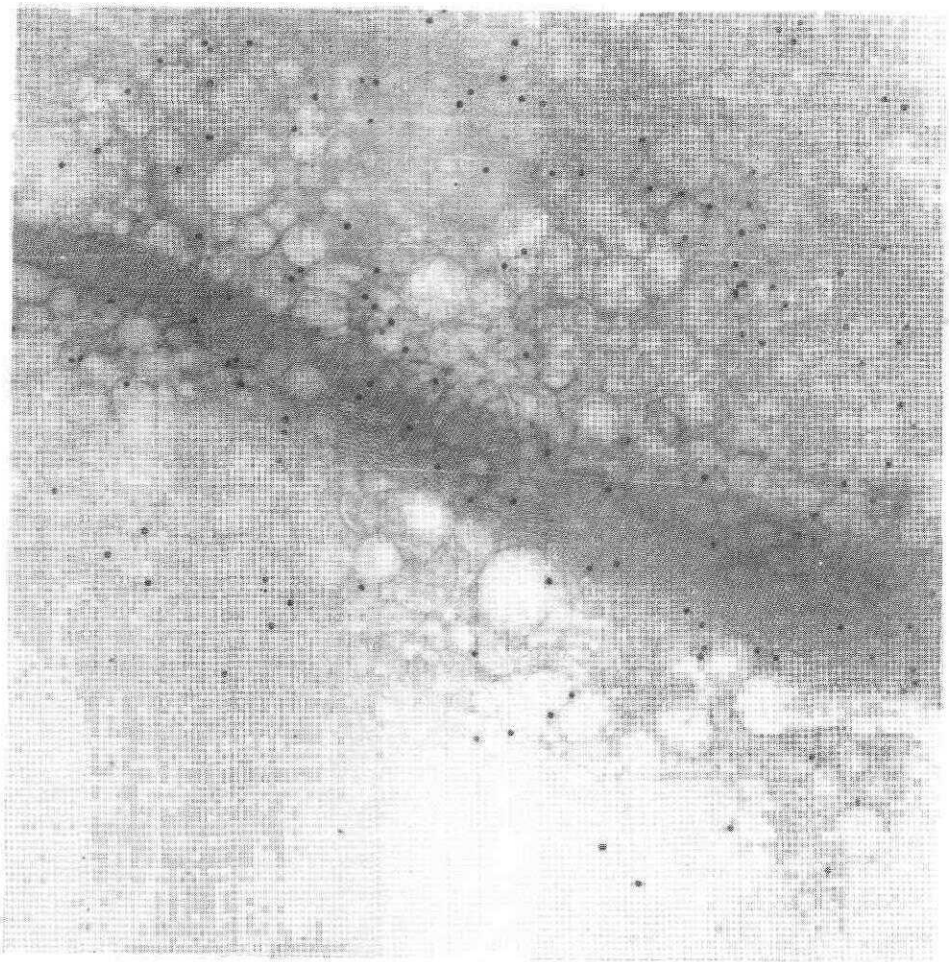
Latex allergens are commonly described as water-soluble proteins. While *Hev b* II and *Hev b* IV are indeed soluble in saline or sweat, they precipitate out when the ambient ionic concentration is reduced[11]. Because of this tendency, the allergens can be lost if they are diluted with or dialysed against water or a dilute buffer, unless steps are taken to recover the precipitated proteins. A major part of the dialysis-induced precipitate is a protein complex known as the microhelix. Immunogold labelling with the monoclonal antibodies USM/RB3 showed that *Hev b* IV is indeed a component of the microhelix. This glycoprotein complex that assumes a filamentous helical structure has been observed under the electron microscope and characterised in some detail previously[9-11]. As summarised by Gomez and Yip[9], microhelices prepared from dialysed B-serum are 1 μm or more in length with a diameter of 20 nm. The fibre width is approximately 5 nm and the pitch of the helix, which is open and hollow, is about 30 nm. Individual microhelices are often associated into bundles (as in *Figure 7)*. At high resolution under the electron microscope, microhelices are seen to have a repeating beaded structure consisting of spherical particles 3 nm – 3.5 nm in diameter, arranged in a helical manner with three to four particles per turn.

Tata and Gomez[11] have previously carried out column chromatography of dialysed B-serum precipitate redissolved in sodium chloride. While their results are not directly comparable to those in the present study because of differences in the chromatographic systems employed, it is possible that *Hev b* IV is similar as an acidic protein that they isolated and described as the 'microhelix assembly factor'.

Although many latex proteins are denatured and lost in the course of latex product (*e.g.* glove) manufacture, *Hev b* II and *Hev b* IV conserve their epitiopic sites even in the finished product. Dot enzyme immuno-assays showed that the two proteins were readily recognised by polyclonal antibodies developed against latex glove leachate (results not presented).

While the microhelix has been studied previously, the results presented here represent the first report of its being immunogenic and allergenic. Recently, the presence of β-1,3-glucanase in B-serum was observed by Churngchow *et al.*[26] and by Beintema[27]. This protein is shown in the present study to be a latex allergen. The allergenicity of these two proteins might have been encountered in the past, but the identities of the proteins have not been well established. Numerous latex allergens have been reported in the literature, most of them characterised on the basis of their molecular weights only. Among these, several of them have molecular weights in the region of 55 kDa or 110 kDa. For example, Alenius *et. al.*[28] showed a 53 kDa allergen in immunoblots from both ammoniated and frozen latex while Beezhold *et al.*[29] identified an allergen of 110 kDa from the similar source materials. Kurup *et al.*[30] have described a monoclonal antibody against an antigen with molecular weight of 55 to 65 kDa. It is possible that some of these proteins could be similar to *Hev b* IV. Other reports of latex allergens with molecular weight of approximately 36 kDa (for example Alenius *et al.*[28], Yagami *et al.*[31] and Unver *et al.*[32]) could be references to proteins similar to *Hev b* II.

The identification and preparation of the major latex allergens, *Hev b* II and *Hev b* IV will be useful in the development of quantitative immunological assays for the diagnosis of latex allergy and to quantitate latex allergens. At present, many of such assays make use of crude (unpurified) latex serum as the allergen source. These are not entirely satisfactory because of



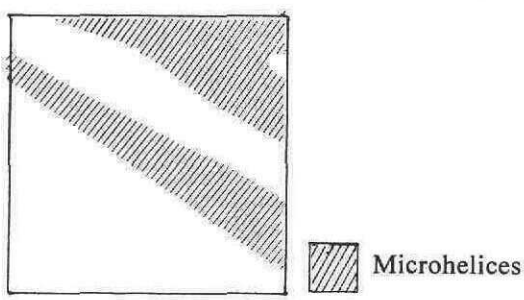


Figure 7. Immuno-gold detection of Hev b *IV on microhelices prepared from dialysed B-serum. Gold particles conjugated to goat anti-mouse IgG indicate the presence of* Hev b *IV to which the monoclonal antibody USM/RB3 binds specifically. Microhelices are readily seen occurring in bundles (see inset for reference), but single strands of microhelices are not as easily discernible.*

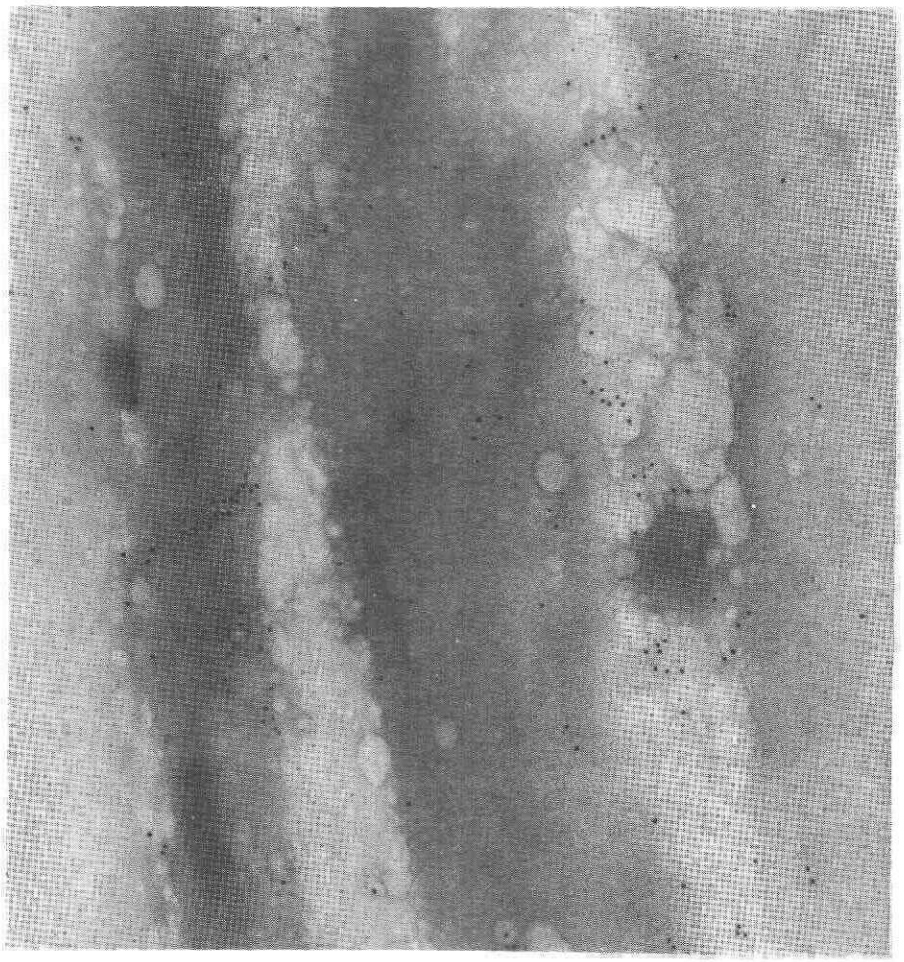
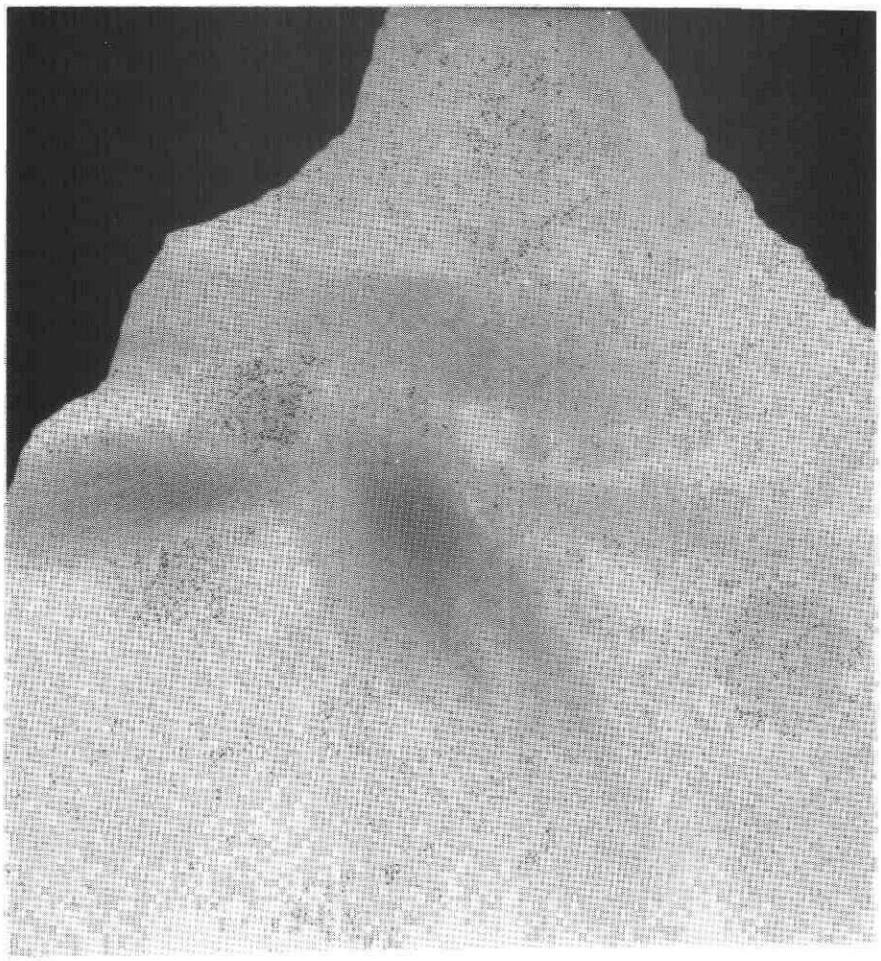
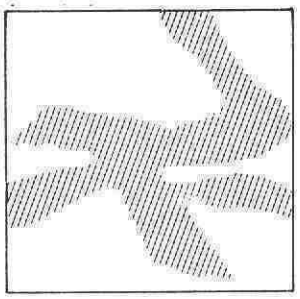


Figure 8. Immuno-gold labelling to show allergenicity of Hev b *IV located on microhelixes (occurring in bundles, see inset). Gold particles conjugated to anti-goat IgG indicate the presence of goat anti-human IgE bound to IgE that recognises the microhelices.*





270 nm

Microhelices

Figure 9. Immuno-gold detection of Hev b *II in the proteinaceous precipitate prepared from dialysed B-serum. The precipitate was incubated with the monoclonal antibody USM/RB4 which is specific for* Hev b *II. Microhelices (shown occurring in bundles, see inset) are generally free of gold particles, indicating that* Hev b *II is not a component of the microhelix complex.*

the variability of the allergen constituents in the latex. For example, the microhelix content in latex has been reported to be variable and sometimes undetectable. This variation occurs between clones and also between samples taken at different times from the same group of trees[10,33]. Microhelices have also been observed to increase as a result of yield stimulation by ethephon[33]. Commercial latex gloves have also been used as the protein antigen component of immunological assays. A drawback of this approach is that different brands of gloves (or even different batches of the same brand) show qualitative and quantitative differences in their allergen composition[34].

CONCLUSION

Two B-serum proteins, β-1,3-glucanase (*Hev b* II) and a component of the microhelix (*Hev b* IV), have been shown to be allergenic by virtue of their binding to IgE from the blood serum or plasma of latex-allergic patients. The identity of the allergens, information on their preparation and the availability of their specific monoclonal antibodies should contribute to the development of immunological assays for latex allergy and latex allergens.

ACKNOWLEDGEMENTS

The authors would like to thank Messrs. Abd. Karim bin Ishak and Alphons Ambrose for their excellent technical assistance.

Post-submission Note

Breton *et al.*[35] recently reported the presence of five isoforms of β-1,3-glucanase in *Hevea brasiliensis* latex B-serum.

Date of receipt: May 1995
Date of acceptance: November 1995

REFERENCES

1. TURJANMAA, K., LAUIRILA, K., MAKINEN-KILJUNEN, S. AND REUNALA, T. (1988) Rubber Contact Urticaria: Allergenic Properties of 19 Brands of Gloves. *Contact Derma -titis*, **19**, 362.
2. MOIR, G.F.J. (1959) Ultracentrifugation and Staining of *Hevea* Latex. *Nature, Lond*, **184**, 1626.
3. HOMANS, L.N.S., VAN DALFSEN, J.W. AND VAN GILS, G.E. (1948) Complexity of Fresh *Hevea* Latex. *Nature, Lond*, **161**, 177.
4. HSIA, R.C.H. (1958) Oxygen Absorption by *Hevea brasiliensis* Latex. *Trans Instn Rubb Ind*, **34(6)**, 267.
5. PUJARNISCLE, S. (1971) Etude Biochimique des Lutoides du Latex d'*Hevea brasiliensis*. Mull. Arg. Memoire O.R.S.T.O.M. No. 48. Paris: Office de la Recherche Scientifique et Technique Outremer.
6. MEINS, F, Jr., NEUHAUS, J-M., SPERISEN, C. AND RYALS, J. (1992) The Primary Structure of Plant Pathogenesis-related Glucanohydrolases and Their Genes, *Genes Involved in Plant Defence* (Boller, T., Meins, F., Jr., eds.) 245 Vienna/New York: Springer-Verlag.
7. SOUTHORN, W.A. AND YIP, E. (1968) Latex Flow Studies III. Electrostatic Considerations in the Colloidal Stability of Fresh Latex. *J Rubb. Res Inst Malaya*, **20**, 201.
8. AUDLEY, B.G. (1965) Studies of an Organelle in *Hevea* Latex Containing Helical Protein Microfibrils. *Proc nat Rubb Res Ass Jubilee Conf Cambridge*, 1964, 67. Maclaren & Sons Ltd.
9. GOMEZ, J.B. AND YIP, E. (1975) Microhelices in *Hevea* Latex. *J. Utrastruct Res*, **52**, 76.
10. GOMEZ, J.B. AND TATA, S.J. (1977) Further Studies on the Occurrence and Distribution of Microhelices in Clones of *Hevea*. *J. Rubb Res Inst. Malaya*, **25(3)**, 120.

11. TATA, S.J. AND GOMEZ, J.B. (1980) Isolation and Characterisation of Microhelices from *Hevea* Latex *J Rubb Res Inst Malaysia*, **28(2)**, 67

12 SUNDERASAN, E AND YEANG, H.Y. (1993) Latex Allergy Studies: B-serum from Latex Bottom Fraction as a Major Source of Immunogenic Glove Proteins *J nat Rubb Res*, **8(4)**, 293.

13. SUNDERASAN, E., SHARIFAH HAMID, CARDOSA, M.J. AND YEANG, H.Y (1994) Allergenic Proteins of *Hevea brasiliensis* Latex Fractions *J nat Rubb Res* **9(2)**, 127.

14 CARDOSA, M.J., SHARIFAH HAMID, SUNDERASAN, E. AND YEANG, H.Y. (1994) B-Serum is Highly Immunogenic When Compared to C-serum Using Immunoassays *J nat Rubb Res*, **9(3)**, 205.

15. LAEMMLI, U.K. (1970) Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T_4. *Nature*, **227**, 680

16 REISFELD, R.A. LEWIS, U.J. AND WILLIAMS, D.E. (1962) Disk Electrophoresis of Basic Proteins and Peptides in Polyacrylamide Gels. *Nature, Lond*, **195**, 281.

17. COTE, F., LETARTE, J., GRENIER, J, TRUDEL, J. AND ASSELIN, A. (1989) Detection of β-1,3-Glucanase Activity After Native Polyacrylamide Gel Electrophoresis: Application to Tobacco Pathogenesis-related Proteins. *Electrophoresis*, **10**, 527

18. KOHLER, G. AND MILSTEIN, C. (1975) Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity *Nature*, **256**, 495

19. KOHLER, G AND MILSTEIN, C. (1976) Derivation of Specific Antidody Producing Tissue Culture and Tumor Lines by Cell Fusion *Eur J Immunol*, **6**, 292.

20 SHEKARCHI, I.C. AND SEVER, J.L. (1986) Enzyme Immunoassay, *Clinical Virology Manual (Specter, S and Lancz, G J eds)*, 133. N Y.: Elsevier.

21 CARDOSA, M.J, TIO, P.H AND NOOR SHAM SHAARI (1988) Development of a Dot Enzyme Immunoassay for Dengue 3 A Sensitive Method for the Detection of Anti-dengue Antibodies, *J Virol Methods*, **22**, 81

22. CASTRESANA, C., DE CARVALHO, F, GHEYSEN, G, HABETS, M., INZE, D. AND VAN MONTAGU, M. (1990) Tissue-specific and Pathogen-induced Regulation of a *Nicotiana plumbaginifolia* Beta-1,3- Glucanase Gene. *Pl Cell*, **2(12)**, 1131.

23. SHINSHI, H, WENZLER, H., NEUHAUS, J.-M., FELIX, G., HOFSTEENGE AND MEINS, F. JR. (1988) Evidence for N- and C- Terminal Processing of a Plant Defence-related Enzyme Primary Structure of Tobacco prepro-β-1,3-glucanase. *Proc Natl Acad Sci USA*, **85**, 5541.

24 VAN KAN, J A, JOOSTEN, H H, WAGEMAKERS, C.A., VAN DEN BERG-VELTHUIS, G.C. AND DE WIT, P J. (1992) Differential Accumulation of mRNAs Encoding Extracellular and Intracellular PR Proteins in Tomato Induced by Virulent and Avirulent Races of *Cladosporium fulvum Plant Mol Biol*, **20(3)**, 513.

25. SCHLAEPPI, K. DEFLORIN, J. AND SEEBECK, T. (1989) The Major Component of the Paraflagellar Rod of *Trypanosoma brucei* is a Helical Protein that is encoded by Two Identical, Tandemly Linked Genes. *J Cell Biol*, **109(4)**, 1695.

26 CHURNGCHOW, N., SUNTARO, A AND WITITSUWANNAKUL, R. (1994) Two β -1, 3-Glucanase Isozymes from Rubber Latex. *11th FAOBMB Symposium, Bangkok, Thailand, 1994*

27. BEINTEMA, J.J. (1993) Private Communication.

28 ALENIUS, H., (1994) Distribution of Allergenic Proteins between Fractions of Natural Rubber Latex Separated by Ultracentrifugation. *Latex Protein Allergy Wkshop, Kuala Lumpur, 1994*

29. BEEZHOLD, D.H., SUSSMAN, M.D. AND CHANG N-S. (1994) Identification of Latex Protein Allergens in Health Care Workers *Latex Allergy Symposium, Toronto, Ontario, Canada, 1994*

30. KURUP, W.P , KUMAR, A., KELLY, K.J AND FINK, J.N (1993) Characterisation of a Monoclonal Antibody Against Latex Protein Associated With Latex Allergy *J Allergy Clin Immunol*, **92(5)**, 638

31. YAGAMI, T., SATO, M., NAKAMURA, A. AND SHONO, M. (1994) One of the Rubber Latex Allergens Has Lysozyme Activity. *Int Arch Allergy Immonol*, **104**, 307.

32 UNVER, E., JAGGI, K., ORDONEZ, M. AND HOVANEC-BURNS, D. (1994) Comparison of Allergenic Extract Preparations from Latex Gloves by *In vitro* Methods. Poster presented at *Latex Allergy Symposium, Toronto, Canada*

33. GOMEZ, J B. AND MOIR, G.F.J. (1979) *The Ultracytology of Latex Vessels in* Hevea brasiliensis. M.R.R D B. Monograph No.4, Malaysian Rubber Research and Development Board, 37.

34. CARDOSA, M.J., SHARIFAH HAMID, SAMUEL-VERGHESE, S., AND YEANG, H.Y. (1994) Monoclonal Antibodies Identify Different Antigens in Proteins Eluted fromNatural Rubber Latex Glvoes Obtained from Different Sources. *J nat Rubb Res*, **9(4)**, 270.

35. BRETON, F., COUPE, M., SANIER, C. AND D'AUZAC, J. (1995) Demonstration of β-1,3-glucanase Activities in Lutoids of *Hevea brasiliensis* Latex. *J nat Rubb Res*, **10(1)**, 37.