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Hev b 1, Hev b 2 and Hev b 3 Contents in Natural Rubber Latex and Powdered Latex Gloves

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The contents of three latex allergens, Hev b 1, Hev b 2 and Hev b 3 were compared in 24 latex examination gloves and in fresh natural rubber latex using immunoassays that employed a two-site ELISA format. Hev b 1, Hev b 2 and Hev b 3 that were extractable from the powdered latex gloves were found to represent only a very small proportion of their contents in fresh latex and a very small proportion of the total extractable glove proteins. Of the three allergens studied, only Hev b 2 content was significantly correlated with total protein content. Hence, total protein content did not always reflect the contents of the individual latex allergens. Of the three allergens studied, Hev b 2 and Hev b 3 contents were significantly correlated with binding to IgE pooled from latex-allergic patients. One brand of glove had undetectable levels of the three individual latex allergens, indicating that it was feasible to remove a large proportion of the allergens during glove manufacture.

Key words: allergen; allergy; ELISA; glove; Hev b 1; Hev b 2; Hev b 3; *Hevea*; IgE; immunoassay; latex; RAST-inhibition

Contact with latex gloves or powder from the gloves can induce an allergic reaction in sensitised persons. Although the prevalence of latex allergy remains low — about one in a thousand in the general population in Europe[1-3] — incidence of latex allergy is between 2.4 and 17% in healthcare workers who habitually don latex gloves in the course of their work[4,5]. Reports of latex allergy increased rapidly in the early 1990s with the advent of AIDs and the concomitant introduction of poor quality gloves supplied by inexperienced manufacturers to meet the sudden demand. These gloves had a high content of latex proteins that included a number of allergenic proteins. With more and more attention drawn to the problem of latex allergy arising from latex glove usage, better gloves with low residual proteins gradually became available in the market. Extractable protein[6] and allergen[7] contents of latex gloves have declined in recent years as manufacturers refined their factory procedures to produce better gloves.

The allergenicity of latex gloves is commonly estimated from the amount of proteins

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extractable from them[6,8,9] While a relationship does exist between total extrac- table proteins and allergenicity, this correlation is not infallible Discrepant results are sometimes encountered[6,10] because the determination of total proteins does not discriminate between proteins that are allergenic and those that are innocuous While natural rubber latex contains hundreds of proteins, only a handful are major allergens Hence, immunoassays that detect the major allergens directly may provide a better assessment of the allergenicity of latex gloves

Several formats for immunoassays to assess latex glove allergenicity are available ELISA-based assays can employ antibodies that were developed against latex The disadvantage here would be that the antibodies would pick up a large number of antigenic proteins Since not all antigenic proteins are allergenic in nature and the technique would still be picking up a large number of non-allergenic proteins A more direct assay of allergenicity would be an IgE inhibition assay based on RAST or ELISA

Although 13 latex allergens (Hev b 1 to Hev b 13) have been identified, there have been few reports on the levels of the specific allergens present in latex gloves The pre-sence of three latex proteins Hev b 1, Hev b 2 and Hev b 3 in latex gloves are examined in this paper Hev b 1 and Hev b 3 are the two major proteins located on the surface of the rubber particles Hev b 1, also called the rubber elongation factor, is a 14 6 kDa protein found mainly on the large rubber particles (generally above 0 4 μm in diameter) The 22 kDa Hev b 3, known as the small rubber particle protein, is more abundant in the smaller rubber particles[11] Together with Hev b 3, sensitivity to Hev b 1 has been particularly associated with the spina bifida condition[11,12] with latex-sensitive adults far less commonly sensitive to this protein [11,13,14] Although rubber particle proteins may be regarded as cytosolic peptides[15], Hev b 1 and Hev b 3 are insoluble because they are tightly bound to the rubber particles, although a small amount of Hev b 3 protein solubilises when latex is ammoniated to stabilise it [16,17] This property is similarly expected of Hev b 1 although it has not been demonstrated Hev b 1 and Hev b 3 show a high similarity to each other and to the stress-related protein, PvSRP, of the French bean, *Phaseolus vulgaris*

Hev b 2, a soluble protein located in latex organelles called lutoids, has been identified as β-1,3-glucanase (EC 3 2 1 39)[18] It is one of the most allergenic among latex proteins, both in IgE-binding[14,19,20] and skin reactivity[21] Latex glucanase has been detected as several isoforms[22-24] When prepared from fresh latex, the protein appears on a SDS-polyacrylamide gel electrophoresis as a doublet of 34-36 kDa [8], 32-35 kDa[23] or 35-38 kDa[24] the variation perhaps due to differences in the laboratory technique Unlike all the other recognised latex allergens that are acidic proteins, Hev b 2 is a basic protein with a pI of about 9 5[18] This protein solubilises only in high ionic (salt) content, and precipitates out when the protein solution is diluted or dialysed[18] The allergen therefore resists being washed away with water during the manufacture of latex gloves, but subsequently dissolves in the sweat of the glove user

In the present study, the contents of three latex allergens, Hev b 1, Hev b 2 and Hev b 3 were compared in latex examination gloves and in fresh natural rubber latex using immunoassays that employ a two-site ELISA format

MATERIALS AND METHODS

Preparation of Fresh Latex for Protein and Allergen Assay

Fresh natural rubber latex was treated with detergents to solubilise membrane proteins and to release organelle-bound proteins. One part of latex was mixed with two parts of a detergent mixture comprising 1% sodium dodecyl sulphate and 0.1% Triton-X 100. The treated latex was centrifuged at 1°C – 4°C at 43 000 g to recover the serum phase for allergen and total protein determination.

Extraction and Assay of Latex Glove Proteins

Twenty-four brands of powdered latex examination gloves were extracted with phosphate buffered saline (PBS) with the sodium chloride content increased to assist recovery of some latex proteins. To prepare the extractant for proteins from latex gloves, additional sodium chloride was dissolved in PBS to attain a concentration of 0.2 M sodium chloride. Pieces of latex gloves approximately 2 cm x 2 cm were cut from the palm portion. Pieces making up about 3 grams of each test sample were leached in 9 mL of extractant in a 50 mL polypropylene tube at RT with intermittent 2-minute-soaking and agitating followed by a 3-minutes-soaking. This stir-soak procedure was repeated thrice resulting in a total of 20 min duration per test sample. The glove pieces were then removed and the extractant centrifuged at 2060 g for 15 min to remove powder and other insoluble matter. The glove protein extracts were then decanted into labelled tubes and stored at 4°C for use the following day. The extracts were assayed for total proteins by the Lowry microassay[25] according to *ASTM D 5712-99*.

Assay of Hev b 1, Hev b 2 and Hev b 3 by ELISA

Quantitation of Hev b 1, Hev b 2 and Hev b 3 was by 2-site ELISA that employed a mouse monoclonal antibody and a biotinylated polyclonal antibody raised in rabbits. The monoclonal antibodies, J9221, USM RB4 and USM RC2, specific to Hev b 1, Hev b 2 and Hev b 3 respectively, were used as the capture antibodies. Carbonate-bicarbonate coating buffer (100 µL, 50 mM, pH 9.6) containing the antibodies was pipetted into wells of the ELISA plate and incubated at room temperature for three hours followed by a further incubation at 4°C overnight. Blocking was carried out the following day at room temperature for 1 h using 1% bovine serum albumin (BSA) in PBS. The plates were then washed thrice with PBS containing 0.05% Tween 20 (PBS-T). Test samples were then pipetted into the wells of the ELISA plate in triplicate and incubated at room temperature for three hours, and then at 4°C overnight. The following day, the ELISA plates were again washed thrice with PBS-T to remove all unbound material. The absorbed protein was labelled by the addition of the respective biotinylated polyclonal secondary antibodies for Hev b 1, Hev b 2 and Hev b 3 that were diluted with 1% BSA in PBS. After an incubation of 2 h at room temperature, the plates were washed once with PBS-T and then twice with Tris buffered saline containing 0.05% Tween 20, pH 8.0 (TBS-T). Following this, 100 µL of streptavidin-conjugated alkaline phosphatase in TBS containing 0.2 mM magnesium chloride was added. The plates were incubated at room temperature in the dark for 1 h. The plates were then washed twice with TBS-T and once with TBS, pH 9.5, containing 50 mM magnesium chloride. Colour development was initiated by adding p-nitrophenyl phosphate in 10% diethanolamine buffer and the absorbance read at 405 nm using an ELISA

plate reader Readings of calibration standards comprising purified Hev b 1, Hev b 2 and Hev b 3 were obtained in a similar manner The calibration standards (6, 10 and 1 μg mL^{-1} for Hev b 1, Hev b 2 and Hev b 3, respectively) were serially diluted four-fold (Hev b 2) and five-fold (Hev 1, Hev b 3) to obtain seven concentrations of each protein Test readings that differed from the blank readings by less than 2 times the standard deviation of the latter were deemed to be below detection Glove photometric readings read against the calibration curves were then converted to ng allergen per g glove The lowest concentration of the latex allergens Hev b 1, Hev b 2 and Hev b 3 detectable was 5 8, 62 0 and 1 0 ng per g glove, respectively For the purpose of computing statistical correlations, values below detection were taken as the mean between 0 and the lowest detectable level

Assay of Allergenic Proteins by RAST Inhibition

The radioallergosorbent test (RAST), a solid-phase radioimmunoassay for detecting IgE antibody specific for particular allergens, was used to evaluate allergenicity of the latex gloves In the RAST inhibition format, allergens eluted from latex gloves competed with proteins present in a standardised latex preparation for binding with IgE from latex-allergic patients The RAST-inhibition assay was carried out as described previously[26] The assays employed a non-ammoniated latex allergosorbent and a human serum pool (n=100 subjects) blended to contain IgE specific for the latex allergens Hev b 1 to Hev b 7 The E8 non-ammoniated latex from the US Food and Drug Administration Center for Biologics Evaluation and Research (FDA-CBER) was used as the reference preparation (100 000 AU/mL with a total protein estimate of 3 89 mg/mL)

RESULTS AND DISCUSSION

Hev b 1, Hev b 2, Hev b 3 and Total Protein Content in Latex Gloves

Of the 24 brands tested, Hevb 1 was detected in 22, Hevb 2 in 23, and Hevb 3 in 15 brands The mean, median and range of Hev b 1, Hev b 2 and Hev b 3 and total protein contents in 24 brands of latex gloves are given in *Table 1* Only a small proportion of the latex allergens Hev b 1 and Hev b 3 originating from fresh latex was present in the manufactured products, the latex gloves The median Hev b 1 and Hev b 3 contents in the gloves represented only 3 x 10^{-5} and 4 x 10^{-7} that of their respective original content in fresh latex This was not surprising since Hev b 1 and Hev b 3 are membrane proteins, most of which would remain insoluble on the surface of the rubber particles On the other hand, Hev b 2 is a soluble protein, and although its content in gloves was also very low (median = 0 017 of total fresh latex protein), a larger proportion of it was extractable from gloves as compared with Hev b 1 or Hev b 3

Correlation between Hev b 1, Hev b 2, Hev b 3 and Total Protein

Cross-correlations were carried out between contents of Hev b 1, Hev b 2, Hev b 3 and total proteins in the gloves (*Figures 1–3*) Only Hev b 2 content was found to be significantly correlated with the total protein content (*Figure 2*) This result was not surprising since Hev b 1 and Hev b 3 constituted less than 0 1 and 0 001% respectively, of the total extractable glove proteins (comparing median values) and therefore had limited bearing on the total protein content While Hev b 2 content was also low, making

TABLE 1. PROTEIN CONTENT OF LATEX AND LATEX GLOVES

Allergen	Fresh Latex	24 powdered latex gloves		
		Mean	Median	Range
Total protein	48 000	464.2	334.0	86 – 1288
Hev b 1	7 600	0.254	0.211	<0.006 – 0.750
Hev b 2	280	9.908	4.630	<0.062 – 46.108
Hev b 3	5 100	0.018	0.002	<0.001 – 0.111

Values expressed as μg/g glove or rubber

up only 1.3 % of the total glove proteins, its range was much wider than in the other two allergens (*Table 1*), rendering a significant correlation more easily attainable. These results show, nevertheless, that total protein content did not always reflect the contents of the individual latex allergens.

Hev b 2 was correlated with neither Hev b 1 nor Hev b 3, but the latter two were inter-related (*Figure 4*). As Hev b 1 and Hev b 3 were both rubber particle proteins, they would be expected to share common molecular characteristics. Glove factory processes that were varyingly effective in removing Hev b 1 from the finished product were just as effective in removing Hev b 3.

IgE-binding of Latex Glove Extracts in Relation to Hev b 1, Hev b 2, Hev b 3 and Total Protein Contents

IgE binding of proteins in glove extracts, reflecting the overall allergenicity of the gloves, was determined by RAST-inhibition assay. Of the three allergens, Hev b 2 and Hev b 3 were found to be significantly correlated with IgE binding (*Figures 5–7*), but Hev b 1 was not. Hence, RAST IgE-inhibition assays could not always determine the allergenicity of latex gloves with respect to specific allergenic proteins.

A good relationship was found between total protein and IgE-binding of the glove extracts (*Figure 8*), although this might have been partly the result of the IgE pool used in the assay having been carefully blended to contain IgE specific to a wide array of latex allergens.

Variation in Latex Proteins and Allergens in Different Brands of Gloves

Despite significant strides having been made in the production of better, low protein latex gloves, considerable variation in extractable protein still existed between different manufacturers. In tandem with the variation in total extractable proteins, considerable differences in the specific latex allergens, Hev b 1, Hev b 2 and Hev b 3, was also encountered. Variation in latex sources and, especially, in factory practices might have contributed to the differences in residual latex protein and allergen content between different brands of gloves. The best brands of gloves tested had undetectable levels of individual latex allergens showing that it is possible to produce very good powdered latex gloves containing undetectable or close to undetectable of latex allergens.

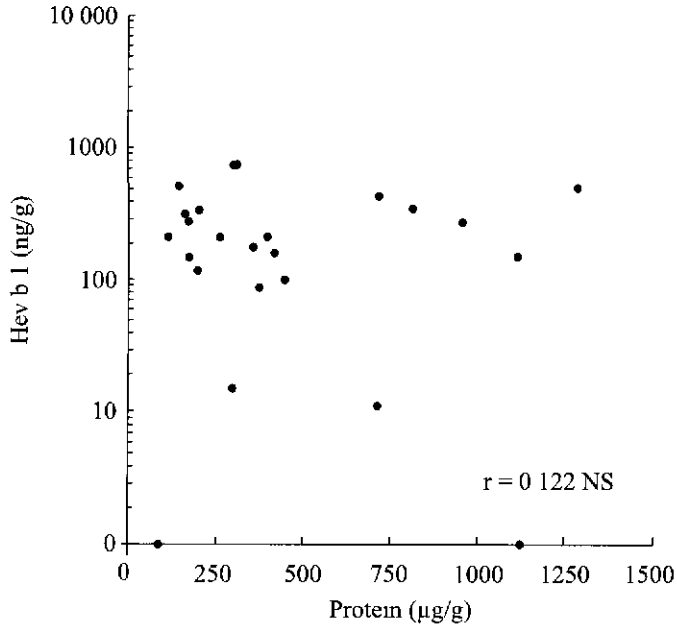

Figure 1 Correlation between total protein and Hev b 1 content in gloves

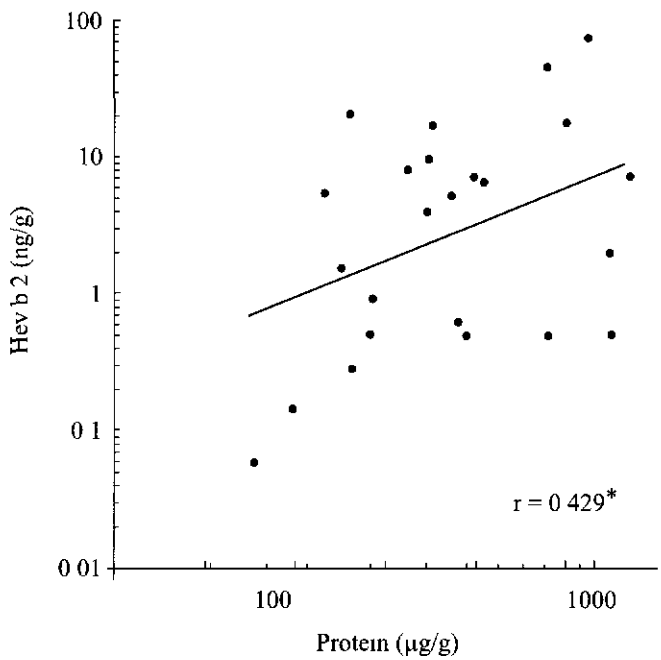

Figure 2 Correlation between total protein and Hev b 2 content in gloves

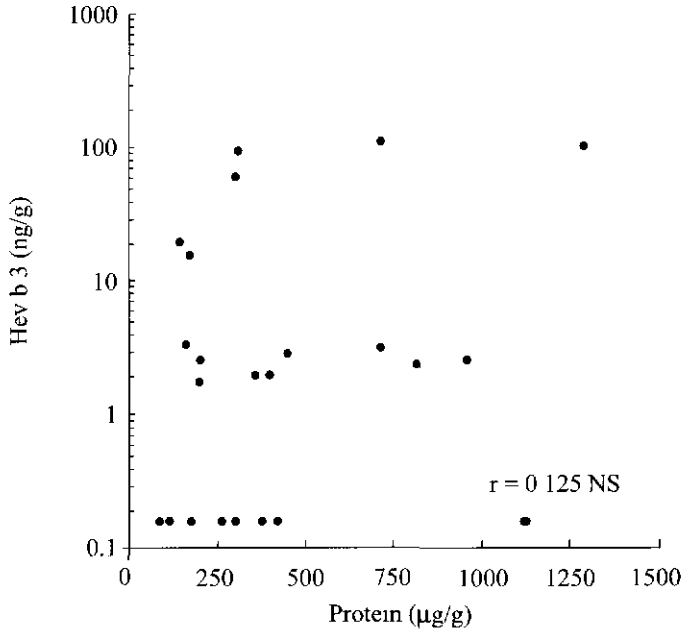

Figure 3 Correlation between total protein and Hev b 3 content in gloves

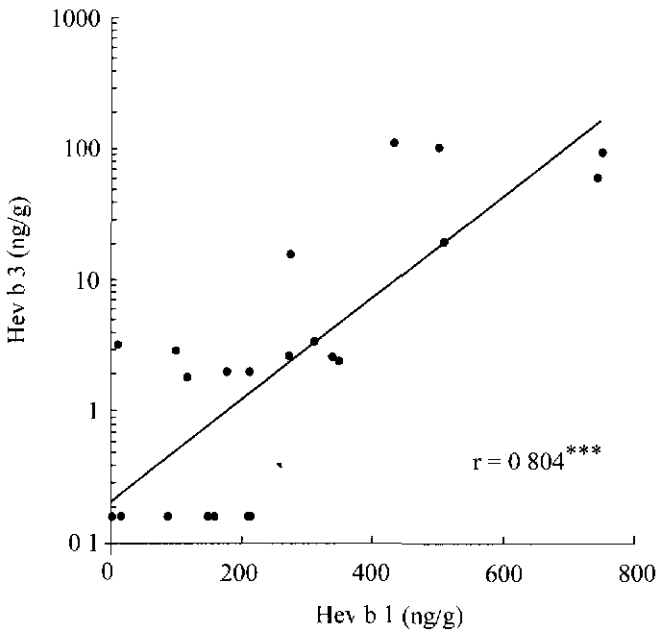

Figure 4 Correlation between Hev b 1 and Hev b 3 content in gloves

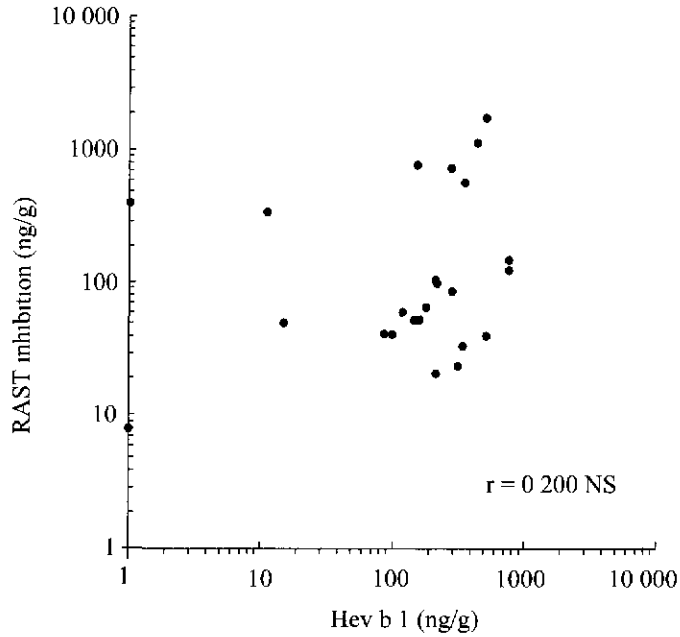

Figure 5 Correlation between Hev b 1 and IgE RAST inhibition

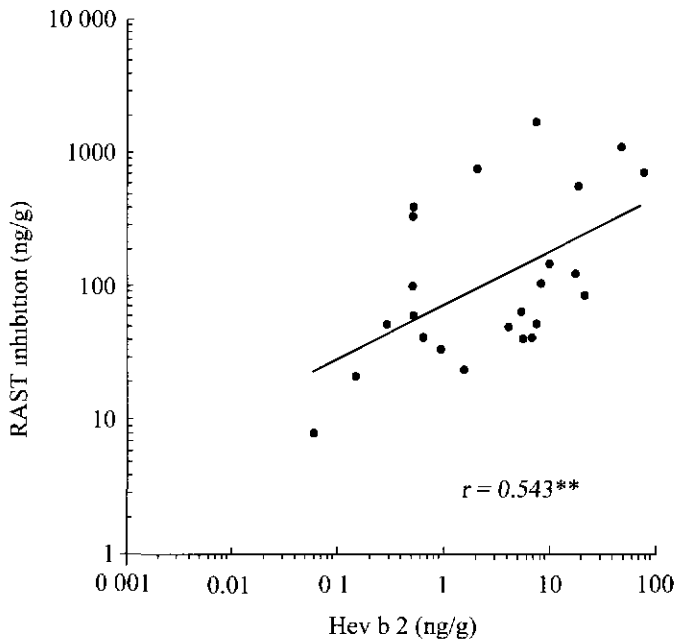

Figure 6 Correlation between Hev b 2 and IgE RAST inhibition

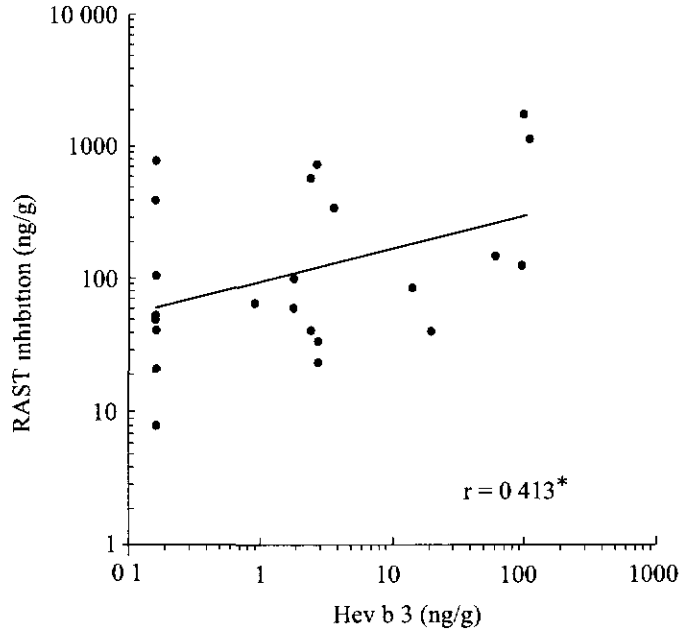

Figure 7 Correlation between Hev b 3 and IgE RAST inhibition

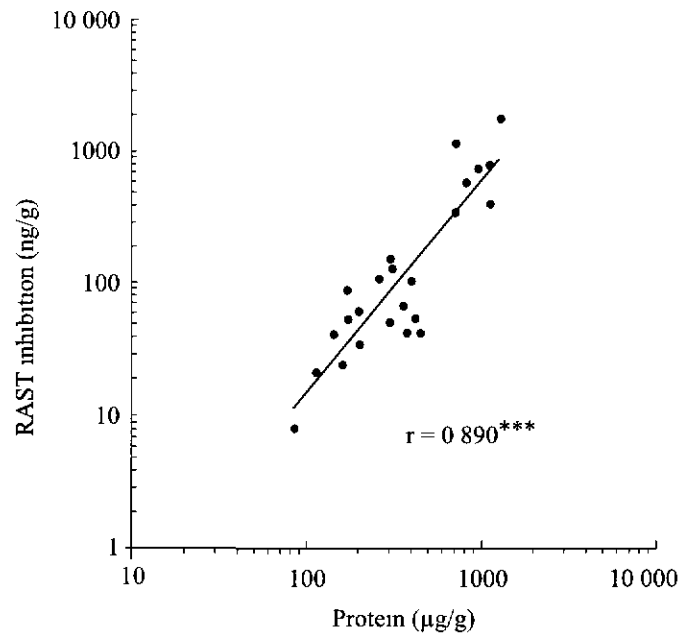

Figure 8 Correlation between glove protein content and IgE RAST inhibition

CONCLUSION

It was shown from this study that Hev b 1, Hev b 2 and Hev b 3 proteins that were extractable from the latex gloves represented only a very small proportion of their content in fresh latex and a very small proportion of the total extractable glove proteins Of the three allergens studied, only Hev b 2 content was significantly correlated with total protein content Hence, total protein content did not always reflect the contents of the individual latex allergens Of the three allergens studied, Hev b 2 and Hev b 3 contents were significantly correlated with capacity to bind IgE pooled from latex-allergic patients. The fact that one brand of glove had undetectable levels of three individual latex allergens showed that it was feasible to remove a large proportion of the allergens during glove manufacture

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