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Latex Allergy Studies: B-serum from the Latex Bottom Fraction as a Major Source of Immunogenic Glove Proteins

E. SUNDERASAN* AND H.Y. YEANG*

A study was carried out to elucidate the source of potential allergenic proteins in fresh Hevea latex that are retained through the glove manufacturing process and appear in the finished product. By identifying the proteins of various latex component(s) that are responsible for the allergenicity, it might be possible to eliminate, or at least greatly reduce, these proteins at source. The origin of the potential allergens were traced by interacting latex proteins derived from Hevea *latex C-serum, B-serum, rubber particle membranes and leaves with antibodies raised in rabbits against latex glove proteins. The strongest antigenic responses to the antibodies were from the B-serum proteins which arise from the lutoids, the main constituents of the latex bottom fraction.*

Latex from the tree *Hevea brasiliensis* is the source material used in the manufacture of latex gloves for examination and surgical use. In the medical field especially, the rising concerns for protection from blood-borne pathogens and the inherent barrier characteristics of the latex products has precipitated a massive demand for latex examination and surgical gloves. Recently, there have been reports that natural latex gloves and other surgical aids can cause hypersensitivity reactions[1-4]. There is evidence suggesting that the water-extractable proteins in latex is the cause of the immediate allergy (hypersensitivity Type I reactions)[5]. Allergic reactions might arise from more than one protein[2,5]. For example, Charous[6] suggested the possibility that latex allergy could involve more than one antigen since at least six distinct latex antigens have been found to bind IgE antibodies from sera of latex-allergic patients.

Natural rubber latex contains approximately 1% protein found in the rubber particles, the serum (C-serum) and the bottom fraction[7]; these are the three main latex fractions separated by high speed centrifugation (*Figure1*). In the present study, we used two dimensional (2D) immunoelectrophoresis and Western-immunoblot to investigate whether

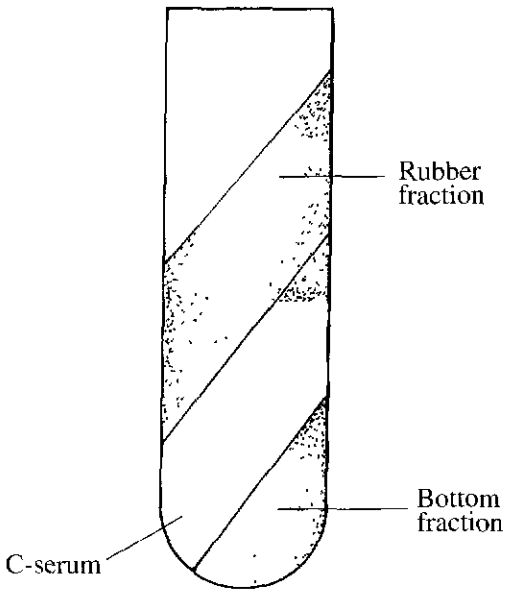

Figure 1. Centrifuged fresh Hevea brasiliensis *latex*

* Rubber Research Institute of Malaysia, P O Box 10150, 50908 Kuala Lumpur, Malaysia

the water soluble antigens from latex examination gloves originated from the rubber particle membrane protein, the C-serum or the bottom fraction. A check was also made on *Hevea* leaf protein which has been reported to be also allergenic[8]. By identifying the source of the proteins of various latex component(s) that are responsible for the allergenicity, it might be possible to eliminate, or at least greatly reduce, these proteins at source prior to the use of the latex for glove manufacture.

MATERIALS AND METHODS

Preparation of Glove Protein

Glove extract was prepared from seven brands of non-sterile examination gloves. The latex gloves were cut into pieces (1 × 1 cm) and incubated overnight in distilled water at room temperature. The eluate was centrifuged in a Sorvall RC5C centrifuge for 15 min at 19 000 r.p.m. (43 000 g) to remove insoluble matter. It was then desalted by dialysing with small pore (1.5 kDa molecular weight cut-off) tubing against distilled water for two days with water changed twice daily and then concentrated by partial freeze-drying. Eluates of different gloves were adjusted to obtain parity in concentration and a cocktail of eluates was used to immunise rabbits. Protein concentration was determined by a modified Lowry method[9] with bovine serum albumin (BSA) as the calibration standard.

Preparation of Antibodies

To raise polyclonal antibodies against glove proteins, 0.5 ml of 0.8 mg/ml glove eluate in 0.5 ml phosphate buffered saline (PBS) was mixed with an equal volume of complete Freunds' adjuvant and injected subcutaneously in the back of four rabbits. Seven booster doses, each containing 0.5 ml of 0.8 mg/ml glove protein in 0.5 ml PBS and a equal volume of incomplete Freunds' adjuvant were administered at two-week intervals. Blood was drawn from the rabbits and the anti-serum was collected pre- and post-immune.

Preparation of Latex and Leaf Proteins

Fresh *Hevea* latex from the clone RRIM 600 was collected into chilled containers and centrifuged at 19 000 r.p.m. (43 000 g) for 1 h at 4–7°C. The rubber cream was scooped off while the latex serum (C-serum) was pipetted from the centrifuge tubes. The rubber cream obtained from centrifuged latex was re-suspended in 30% sucrose and re-centrifuged. The rubber cream was recovered and proteins of the membranes of rubber particles were solubilised and extracted by adding an equal volume of detergent comprising 0.1% Triton-X 100 and 1% sodium dodecyl sulphate (SDS). The mixture was vortexed and then centrifuged. The protein was recovered in the liquid phase. The bottom fraction of the centrifuged latex was subjected to alternate freezing and thawing (4 times) to rupture its constituent lutoids[10] and the lutoid serum (B-serum) was recovered by centrifugation. *Hevea* leaf juice was obtained by squeezing the leaves with a pinch-roller and the protein extract (supernatant) recovered by centrifugation.

Immunodetection Methods

The immunospecificity of the anti-serum towards glove protein and the other proteins was tested by Ouchterlony immunodiffusion, 2D-immunoelectrophoresis and immunoblotting at 1:800 dilution of the serum in PBS performed basically according to the methods described by Walker[11]. Various protein (antigens) preparation (about 300 μg) and post-immune rabbit serum (22 μl sq. cm) were used in each 2D-immunoelectrophoresis. The first dimension were run at constant current (20 mA) until bromophenol blue from the tracker well stained the anode wicks. The antiserum was incorporated in the upper gels and the second dimension were run at constant current (2 mA), overnight and stained with Coomassie Blue. To carry out immunoblots on latex proteins, the proteins were separated by SDS polyacrylamide gel electrophoresis (SDS–PAGE) and were then transferred on to a nitrocellulose membrane by Western blotting. The blotted

proteins were reacted with antibodies to latex glove proteins after washing in Tween 80 to reduce non-specific binding. In later experiments, blocking of the protein-free sites was by washing in a 5% solution of low-fat powdered milk in Tris buffered saline. Alkaline phosphatase conjugated goat anti-rabbit IgG was used as the secondary antibody.

High ammonia (HA) and high ammoniated pre-vulcanised (PV) latex concentrates, which are commonly used in glove manufacture, were obtained from commercial sources.

RESULTS AND DISCUSSION

The soluble proteins that were extracted into water at room temperature from latex examination gloves represent only a portion of the proteins present in the gloves. Indeed, it has been estimated that insoluble proteins make up at least 94% of the total glove proteins[12]. However, the proteins obtained as such would be essentially those that elute from latex gloves when they are used, and hence are the most relevant proteins for the purpose of immunogenic studies.

The post-immune rabbit antisera were tested by the Ouchterlony double immunodiffusion with latex glove eluate. Well defined precipitin lines were obtained in every case (results not presented). The immunospecifity of the anti-serum was tested by 2D-immunoelectrophoresis which demonstrated the presence of at least 10 different antigenic constituents in the glove eluate (*Figure 2a*). Similar analyses on sera from high ammoniated (HA) and prevulcanised (PV) latex (the source materials used in latex glove production) revealed a reduction in the precipitin lines; only about four and six constituents were perceived respectively (*Figures 2b and 2c*). The larger number of precipitin lines from the glove eluate might be attributed to the fact that it was derived from a combination of gloves from different sources. Nevertheless, it was possible also that the protein degraded into smaller units while sustaining antigenicity. The antiserum also reacted with

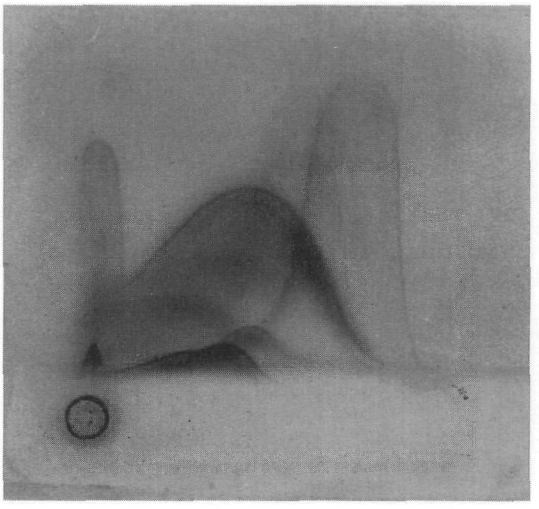

Figure 2a. 2D-Immunoelectrophoresis of glove eluate.

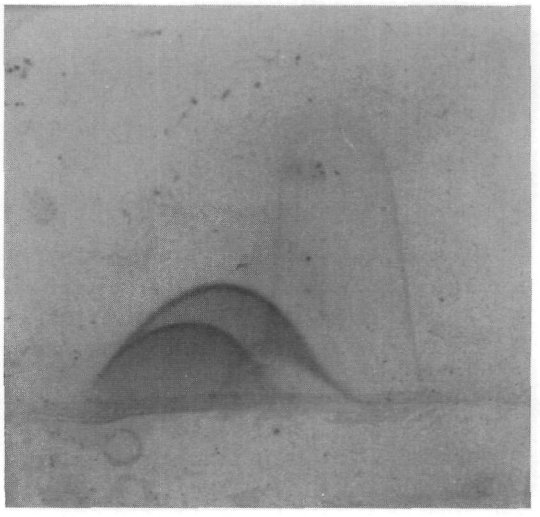

Figure 2b. 2D-Immunoelectrophoresis of high ammoniated (HA) latex serum.

proteins from various fractions of centrifuged latex and *Hevea* leaf proteins (*Figures 2d – 2g*). The antiserum showed high cross reactivity with proteins from the B-serum (which arise from lutoids, the main constituents of the latex bottom fraction). A moderate level of cross-reactivity was observed with C-serum proteins and the

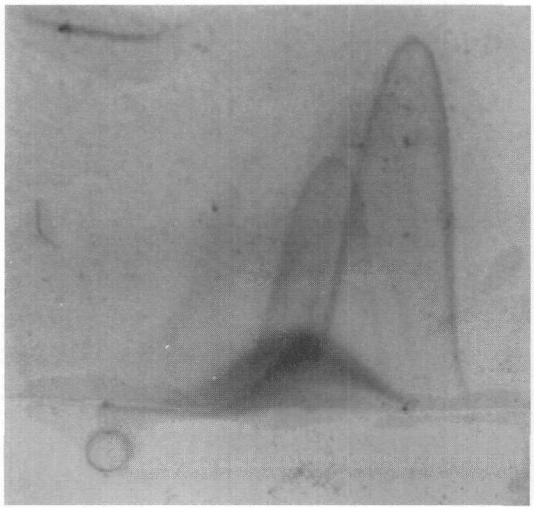

Figure 2c. 2D-Immunoelectrophoresis of pre-vulcanised latex serum.

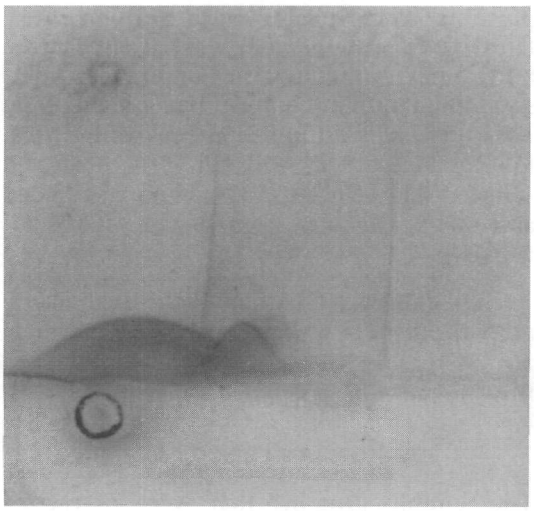

Figure 2e. 2D-Immunoelectrophoresis of C-serum.

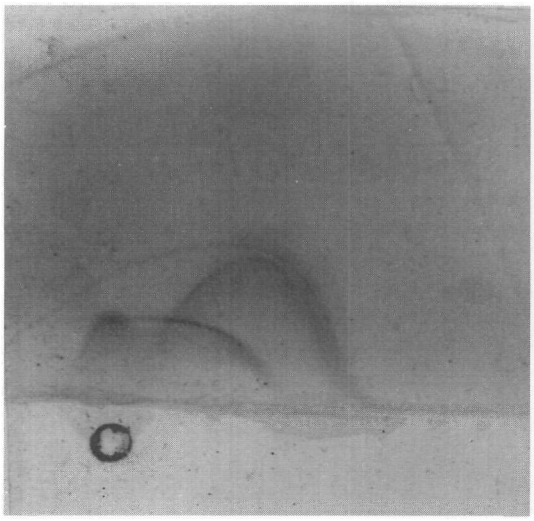

Figure 2d. 2D-Immunoelectrophoresis of B-serum.

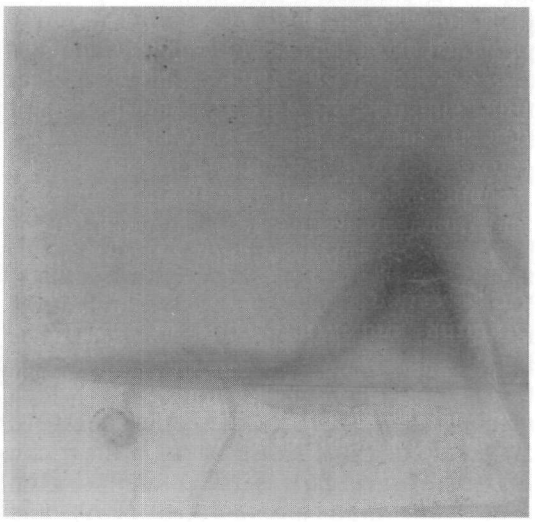

Figure 2f. 2D-Immunoelectrophoresis of rubber particle membrane proteins.

leaf proteins whereas the rubber particles protein elucidated the least antigenicity. Some of the precipitin lines obtained from the C-serum were similar to those found in the B-serum. Cross-reaction found in the leaf proteins might indicate the occurrence of epitopes similar to glove proteins.

Figure 3 shows a gel stained for protein by Coomassie Blue and an immunoblot of a replicate gel stained with alkaline phosphatase conjugated goat anti-rabbit antibody. As expected, glove proteins were high antigenic to anti-glove IgG and distinct bands were seen even when the amount of glove proteins loaded on the gel was very low (undetected

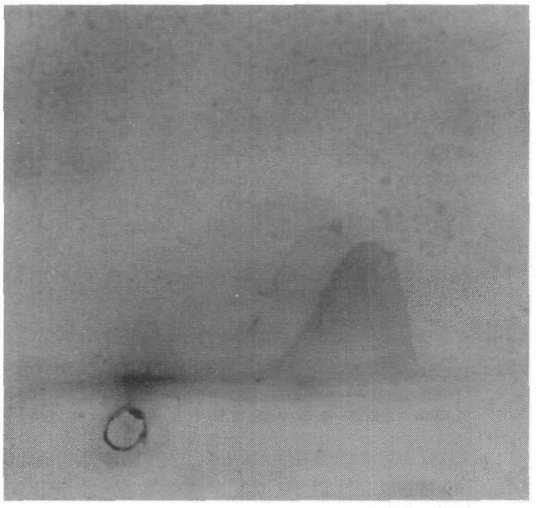

Figure 2g. 2D-Immunoelectrophoresis of Hevea *leaf proteins.*

with Coomassie Blue staining). Heavily stained bands were observed from the rubber particles and C-serum proteins in the gel but these were poorly represented in the immunoblot. On the other hand, several bands of B-serum proteins of a wide range of molecular weights appeared prominent on the immunoblot. The immunoblot of C-serum proteins showed a single prominent band of about 45 kDa while the other proteins stained by Coomassie Blue in the gel were very faint or absent in the immunoblot. Some of these faint C-serum bands corresponded with bands of the B-serum with similar electrophoretic migration. In agreement with the observation in the 2D-immunoelectrophoresis, the cross reaction in C-serum proteins could have been partly attributed to the presence of B-serum in the C-serum. Hence, both 2D-immunoelectrophoresis and immunoblot analyses implicated B-serum proteins in the induction of the immune response in rabbits. Our study therefore provides evidence that the bottom fraction proteins are a major source of antigens in the glove eluate.

The leaf proteins also cross-reacted with the glove protein antibodies where bands were detected in the immunoblot and may be associated with the allergy response reported

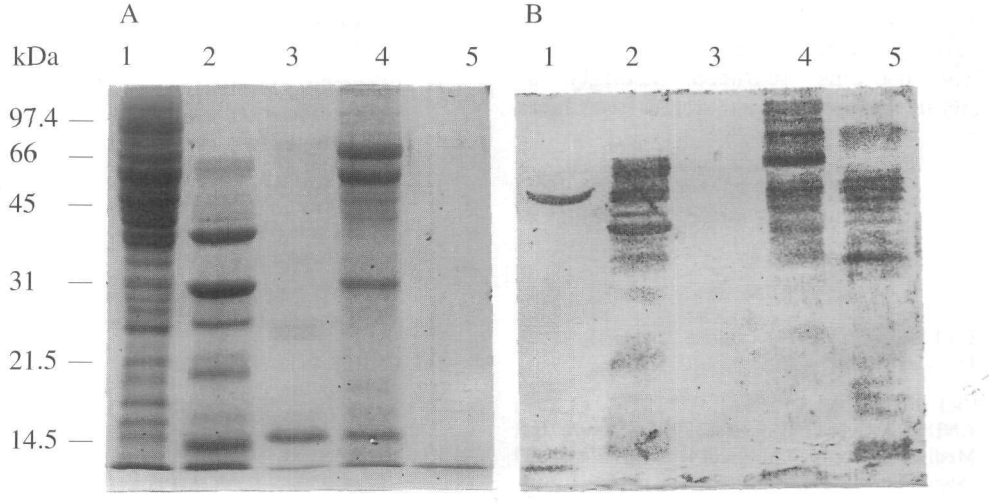

Figure 3. SDS-PAGE of proteins from Hevea *leaves and various fractions of latex (15% gel). Lane 1: C-serum, 2: B-serum, 3: rubber particle extract, 4: leaf extract, 5. latex glove extract.*
A: Coomassie Blue staining
B: Nitrocellulose blot of a replicate gel incubated in rabbit anti-glove IgG and stained with goat anti-rabbit IgG conjugated with alkaline phosphatase.

by workers[8] elsewhere. It is not clear whether the antigenic proteins arise from the small amount of latex present in the *Hevea* leaves

A 14kD latex protein has been reported to be an important allergen in the Type 1 latex-induced allergic reaction[13]. The major rubber particle membrane protein is a 14 kDa polypeptide referred to as the 'rubber elongation factor' (REF)[14] and this gave a faint but distinct band on the immunoblot (*Figure 3*). Although the REF (and the other major rubber particle protein of 24 kD[14]) are normally insoluble, they would have been partially solubilised by the added ammonia[15] in the latex concentrate. However, the immunoblots showed that these rubber particle proteins were not as immunogenic as might be expected, at least in the present study based on rabbit immunoglobulin G

CONCLUSION

The employment of various immunodetection techniques with polyclonal rabbit antibodies to latex gloves proteins enabled the detection and revelation of the origin of water- soluble antigenic glove proteins Latex B-serum proteins featured strongly as antigens in the proteins extracted from latex gloves.

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