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Binding Patterns of IgE Antibodies in Sera of Rubber Tappers to Fresh Hevea *Latex Serum Proteins*†

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The binding patterns of IgE antibodies to fresh natural rubber latex B- and C-serum proteins were determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis immunoblotting technique All the IgE samples were from Malaysian rubber tappers who had been skin-prick tested with extracts of gloves and food avocado, potato, tomato and watermelon

Two of the five IgE samples from tappers reacting to gloves bound to latex proteins, specifically to 35, 38 and 40 kD B-serum proteins and to 30 kD and 75 kD C-serum proteins The remaining three either did not bind or bound faintly to the latex proteins Similar binding pattern to only a few latex proteins was shown in one of the three sera of tappers reacting to both gloves and food. The other two, however, exhibited multiple bindings to a wide variety of B-serum proteins of molecular weights less than 20 kD to greater than 202 kD and to a number of C-serum proteins between 30 kD to 75 kD The heterogeneous binding pattern was also demonstrated by eleven of the twenty IgE serum samples of tappers reacting to food and by twenty-five of the hundred and thirty-six serum samples of tappers reacting negatively to both gloves and food. The fact that only two of the thirty-eight serum samples that showed strong multiple binding pattern corresponded to a positive skin-prick test to gloves, indicated that in vitro *immunoblotting using IgE antibodies in human blood is an unreliable indicator of latex allergy*

A number of natural rubber latex proteins has been shown to be 'allergenic' as they can interact with the IgE antibodies present in the blood sera and on the surface of the mast cells of latex sensitised individuals[1–3] The reaction of these allergenic proteins with the IgE on the mast cells is normally assessed by *in vivo* skin-prick test (SPT) The identity of the allergenic proteins is however, usually characterised by the binding of the proteins to the IgE antibodies present in the individuals' sera This is frequently determined by *in vitro* Western blotting To date, about 57 latex allergenic proteins/polypeptides have been reported[4] with the 14, 20, 27, 29/30, 35/36, 45/46 and 75 kD proteins being the most commonly cited[5–9] Many of these latex allergens particularly the 20[10], 36[9], 46 and 110 kD[11] have sequence homology to plant proteins which could

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partly explain the extensive cross-reactivity observed between latex and various food such as avocado, banana, chestnuts and potato[11-14] This cross-reactivity between latex and food allergens has been suggested to result in predisposition to latex allergy in patients allergic to food and *vice versa*[11] Thus caution should be exercised when evaluating latex allergy as the clinical response may not be directed to primary sensitising agent but to cross-reacting allergens[14]

Proteins derived from *Hevea brasiliensis* latex constitute about 1% of the latex A quarter of the proteins is associated with the rubber particles while the rest are present in the non-rubber fractions, particularly the B-serum (contained in the lutoids) and the C-serum An immunoblotting study with the IgE from the Finnish patients[8] shows that majority of the latex allergens are in fact found in the two latex sera, especially the B-serum There is so far no immunoblotting study of the latex proteins with the IgE antibodies of the Malaysian individuals although there is a paper[15] on the low prevalence of latex allergy among Malaysians frequently exposed to latex and latex products It is thus the aim of this paper to describe the binding pattern of the fresh latex B- and C-serum proteins with the IgE antibodies from the sera of the Malaysian rubber tappers skin-prick tested positive to gloves, to both gloves and food and to food only The binding pattern with the IgE of tappers reacting negatively to both gloves and food will also be demonstrated Some reference would be made as to the reliability of the *in vitro* immunoblotting test in predicting latex allergy

EXPERIMENTS

IgE Serum Samples

Sera were obtained from a hundred and sixty-four Malaysian rubber tappers who were all skin-prick tested with extracts of NR gloves and food avocado, potato, tomato and watermelon Five serum samples were from tappers reacting to gloves, three from those reacting to gloves and food, twenty from those responding to food and a hundred and thirty-six from those reacting negatively to both gloves and food

Fresh NR Latex Serum Proteins

The B- and C-sera of fresh *Hevea brasiliensis* latex were obtained by the procedures described by Moir[16] Fresh latex was centrifuged on a Beckman L8-70 ultracentrifuge at 19 000 r p m using rotor 21 for about an hour The C-serum and the bottom non-rubber particle fraction containing mainly lutoids were isolated The bottom fraction was freeze-thawed three times, centrifuged at 10 000 r p m using rotor 21 for 45 min before the clear B-serum was obtained

Polyacrylamide Gel Electrophoresis and Immunoblotting

The B- and C-sera, solubilised in the solubilising buffer at a ratio of 1 4 were first subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for 35 min at a constant voltage of 200 volts (Mini Protean 11 Cell, Bio-Rad, Richmond, Calif , USA) The proteins were then electroblotted onto 0 2 µm pore size nitrocellulose membrane (Trans-Blot, Bio-Rad) and those proteins which bound to the IgE antibodies in the sera, classified as allergenic proteins, were identified following the protocols established by Alenius *et al* [17]

RESULTS AND DISCUSSION

The latex allergic response is usually assessed by skin-prick testing individuals with proteins

extracted from gloves The glove proteins of normally low molecular weights (< 14 kD)[18], however, could not be used in the SDS-PAGE immunoblotting study due to their poor binding affinity to the blotting membrane and their variable composition arising from different ways of producing gloves The B- and C-serum proteins were thus used as the allergen source in this study They not only had a good binding affinity to the blotting membrane but also showed a more consistent composition

Binding Pattern of IgE from Rubber Tappers with Positive SPT to Gloves

Figure 1 shows the binding pattern of B- and C-serum latex proteins with IgE antibodies from tappers skin-prick tested positive to glove but not to food extracts Out of five samples, one showed strong binding to B-serum proteins of approximate molecular weights of 35, 38, and 40 kD and to a 30 kD C-serum protein while another sample bound only to 75 kD C-serum protein The rest either did not react with any or reacted very faintly to the latex proteins This however, is not uncommon as there have been reports of negative serologic tests with sera of patients skin-prick tested positive to glove proteins[7,19] One of the possible reasons to this could be that fresh latex serum proteins used in the immunoblotting study did not contain the specific allergen(s) present in the gloves which evoke positive reaction on SPT A study by Alenius *et al* [17] showed that certain allergenic proteins in the gloves were not present in fresh latex These allergens could result from unfolding or exposure of relevant internal epitopes in the native protein molecules when the latex is processed into gloves[5,11]

It is interesting to note that the IgE of this particular group of Malaysians who are in constant contact with fresh latex did not only bind to a few latex proteins but also showed no response to 14 kD and 20 kD proteins commonly identified by the Finnish NR latex allergic patients[6,7] However, a larger number of serum samples are needed to confirm this

Binding Pattern of IgE from Rubber Tappers with Positive SPT to Gloves and Food

The selective binding of IgE to a few latex proteins was also observed with one of the three sera of the tappers reacting to both gloves and food extracts (*Figure 2*) The IgE of this individual recognised three allergenic proteins of molecular weights between 40 kD to 75 kD in the C-serum but none in the B-serum The IgE of the other two tappers however, bound to a wide variety of B- and C-serum proteins Their IgE bound to B-serum proteins of molecular weight less than 20 kD to greater than 202 kD and to C-serum proteins of molecular weights between 30 kD to 75 kD

Binding Pattern of IgE from Rubber Tappers with Positive SPT to Food

The multiple binding pattern of IgE to latex proteins was observed to be more common with IgE from tappers reacting to avocado, potato, tomato and watermelon (*Figure 3*) Eleven of twenty (55%) IgE samples from this group showed multiple bindings to B- and C-serum proteins, more with the B- than the C-serum proteins The remainder either did not show any binding or bound to only a few proteins This clearly demonstrated that IgE from individuals reacting to avocado, potato, tomato and watermelon can cross-react with latex proteins on immunoblotting

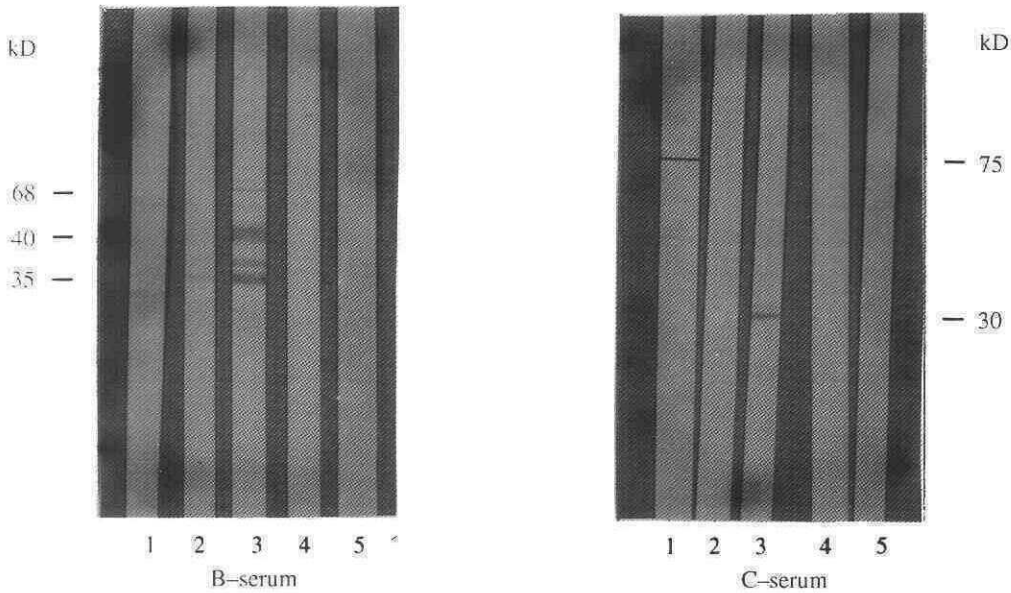


Figure 1. Immunoblots of B– and C– serum proteins with IgE antibodies of rubber tappers skin-prick tested positive to gloves.

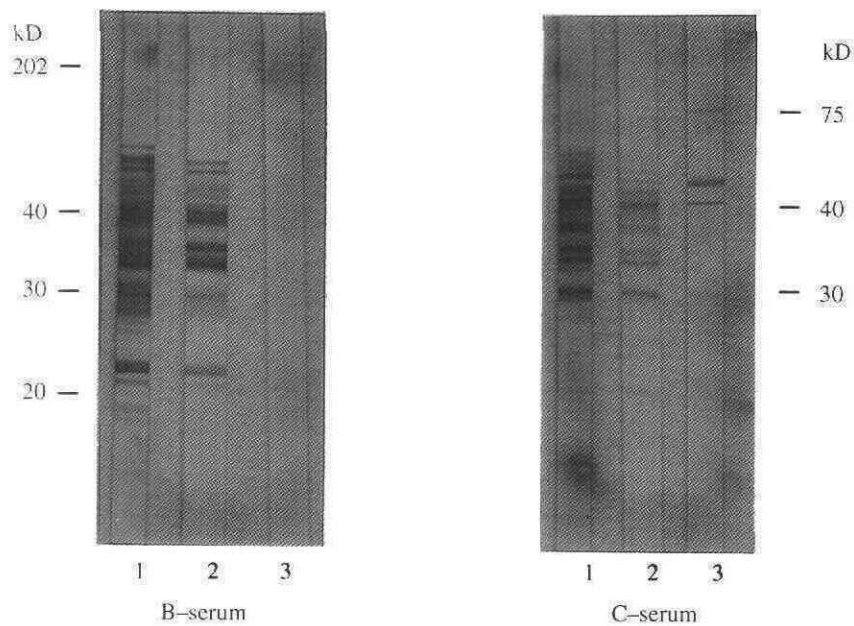


Figure 2. Immunoblots of B– and C– serum proteins with IgE antibodies of rubber tappers skin-prick tested positive to gloves and food.

The 35, 38 and 40 kD B-serum proteins and the 30 kD C-serum proteins were again among the major proteins identified by the IgE of tappers reacting to food. Other prominent B-serum proteins were the three proteins around 20 kD to 30 kD, a 30 kD protein, two proteins of 68 kD to 75 kD and a high molecular weight protein of greater than 202 kD. The 14 kD and 20 kD proteins were not significant allergens. As a high proportion of latex proteins reacted with this group of IgE antibodies, a large number of the latex proteins may have sequence homology to plant proteins. The 35 kD B-serum protein could be the 36 kD protein characterised by Alenius *et al.*[9] to have a considerable structure homologies to several plant 1,3-β-glucosidases.

Although some tappers reacted specifically to certain food on SPT, their IgE binding pattern did not show any characteristic features to distinguish them from the rest (*Figure 3*). Based on the twenty serum samples it was generally observed that the IgE from tappers responding to watermelon alone bound to more proteins than the IgE of tappers reacting to potato only. The least was with the IgE from tappers reacting to only avocado. There was no IgE sample specific to tomato as the tappers' reaction to tomato was always associated with reaction to other food.

Binding Pattern of IgE from Rubber Tappers with Negative SPT to Gloves and Food

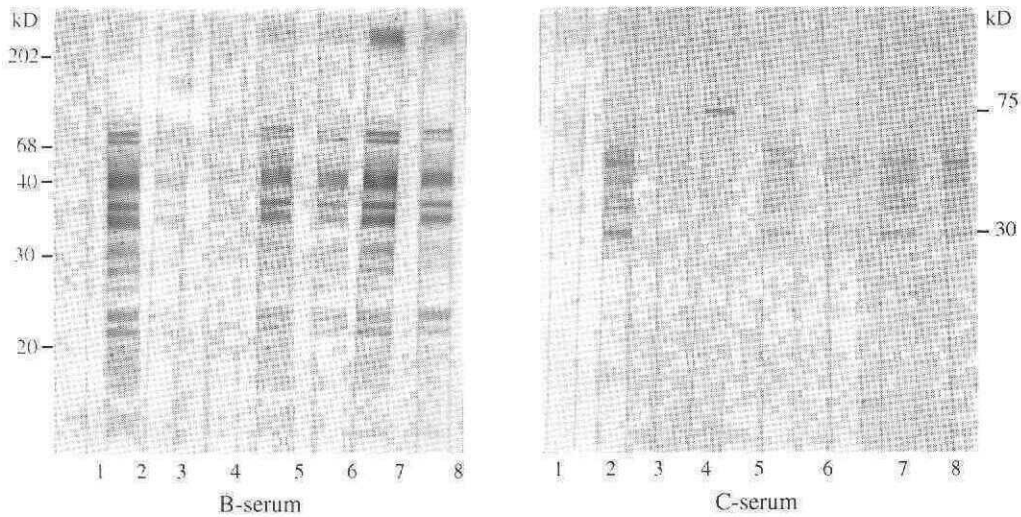
A similar multiple binding pattern was observed with twenty-five of a hundred and thirty-six IgE serum samples from tappers with negative response to both food and glove extracts (*Figure 4*). The remaining samples showed none to faint binding to the latex proteins. This indicated that some tappers contained IgE which could be induced by allergens other than gloves, avocado, potatoes, tomatoes and watermelons which could cross-react with fresh latex serum proteins.

Multiple binding pattern with fresh latex proteins seemed to occur mainly with IgE antibodies of rubber tappers reacting to food and probably to other non-latex allergens. The fact that these IgE could bind to latex proteins, implied that there are other non-latex sensitising agents to latex allergy. Since twenty of the twenty-three rubber tappers who reacted to food responded negatively on SPT with glove extracts, it showed that reaction to food need not necessarily result in adverse reaction to latex gloves. This is in agreement with Levy[19], who reported that majority of the patients who were primarily allergic to food were less likely to be clinically allergic to latex goods.

The study also showed that screening IgE antibodies in human sera by immunoblotting technique is not a good means of predicting latex allergy as out of thirty-eight cases of positive immunoblots with multiple binding pattern to latex proteins only two or 5.3% corresponded to positive SPT to gloves. On the other hand six cases of few to no bands showed positive SPT to gloves. This evidence supports the general view that medical history and good clinical allergic reactions, such as that of SPT, provide more reliable predictions for latex protein allergy.

CONCLUSION

The binding pattern of IgE antibodies of Malaysian rubber tappers with fresh latex B- and C-serum proteins depended strongly on the source of the IgE. It was observed that IgE from tappers who were skin-prick tested positive to gloves showed negative to limited binding to a few latex proteins particularly to 35, 38 and



1, 3, 5 - tappers reacting to potato (P)
2 - tappers reacting to watermalon (W)
4 - tappers reacting to avocado
6,7 - tappers reacting to P & W
8 - tappers reacting to tomato & W

Figure 3. Immunoblots of B - and C - serum proteins with IgE antibodies of rubber tappers skin- prick tested positive to food.

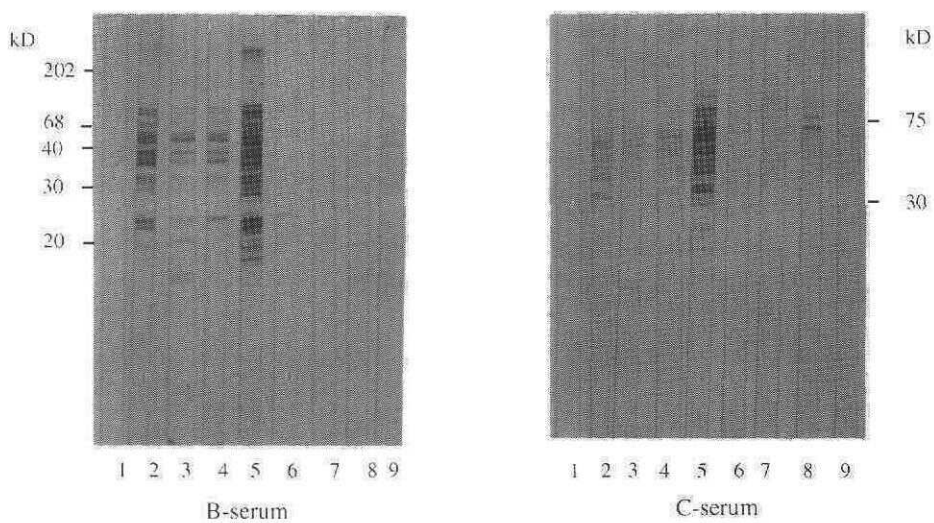


Figure 4 . Immunoblots of B - and C - serum proteins with IgE antibodies of rubber tappers skin- prick tested negative to gloves and food.

40 kD B-serum proteins and to 30 and 75 kD C-serum proteins. On the other hand IgE from tappers who did not react to gloves showed negative to either few bindings or multiple bindings to latex proteins. The variety of proteins recognised by the latter group of IgE range from less than 20 kD to greater than 202 kD B-serum proteins and 30 kD to 75 kD C-serum proteins. It is noteworthy that the 14 kD and 20 kD common latex allergens were not significantly recognised by all the IgE of the rubber tappers under study. As the IgE from tappers reacting to avocado, potato, tomato and watermelon could bind to latex proteins, these food could be another sensitising agent to latex allergy. However, majority of the rubber tappers who reacted to these food did not respond to latex gloves

Although immunoblotting is known to be a good technique to identify allergenic proteins it was found to be an unreliable test to predict latex allergy as its results did not correspond well with the results of SPT.

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