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Allergenic Proteins of Hevea brasiliensis *Latex Fractions*

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A spectrum of proteins derived from the rubber tree, Hevea brasiliensis, *reacted with IgE in the plasma from persons allergic to natural rubber latex. Latex B-serum, C-serum and rubber particle membrane proteins were all found to contain allergenic proteins. Variations were observed in the reactivity of the latex IgE positive plasma to the different proteins. Monoclonal antibodies have been developed against some of these allergens.*

Studies on natural rubber latex hypersensitivity have steadily increased since the report of latex-induced contact urticaria in 1979[1]. Many laboratories, are focused on the identification of proteins in unprocessed latex and latex products, especially those responsible for the elicitation of allergic responses. Differences in the manufacturing conditions of latex products have been shown to have a bearing on the composition of allergenic proteins in the finished product and may have contributed to variations in allergenicity, as evidenced by varying rates of reaction in sensitised subjects to a variety of brands of latex gloves[2]. The differences in IgE immune responses towards latex antigens among various populations could also be related to the mode of exposure or sometimes been attributed to genetic factors[3]. Apparent cross-reactivity has been reported between *Hevea* latex allergens and allergens from other latex producing plants[4,5] and some fruits[6,7,8].

Earlier we elucidated the potential latex allergens by interacting the proteins derived from *Hevea* leaves, C-serum, B-serum and rubber particle membrane with rabbit polyclonal antibody (IgG) raised against proteins eluted from latex gloves[9]. Although there have been a number of studies on the possible allergenic proteins from natural rubber 'sap', there is still a lack of substantial information available on the allergenic proteins of the different latex fractions. In the present study, we have employed immunoblot techniques to compare IgE antibody responses in latex allergic patients to proteins of C-serum, B-serum and rubber particle membranes.

MATERIALS AND METHODS

IgE Patients' Plasma

Latex IgE positive plasma of 11 latex allergic patients were sourced from the United States of America. In addition, plasma from two pools of latex IgE-positive patients were obtained. Control plasma were obtained from Kuala Lumpur General Hospital, Malaysia.

Antigens

Preparation of latex proteins were carried out as described earlier[9].

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Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) and electroblotting were carried out basically according to Mayer and Walker[10]. Electrophoresis was carried out with 15% gels for 50 min at constant current of 30 mA (Mini Protean II Cell; Bio-Rad Richmond Calif., USA). Separated proteins were detected by staining with Coomassie Brilliant Blue R250. After electrophoresis, the proteins were transferred from the gel to 0.45 m pore size nitrocellulose membrane in blotting buffer containing 22 mM Tris, 150 mM glycine and 20% methanol, pH 8.3, for 3 h at constant voltage 8 V/cm. After blotting, the membrane was stained with Ponceau-S red to demonstrate successful transfer of proteins. The membrane was then incubated for 1 h in a blocking buffer containing 5% non-fat milk powder in phosphate buffered saline (PBS).

Plasma diluted 1:5 in the blocking buffer containing 0.01 *M* sodium azide were added, and the nitrocellulose sheets were incubated overnight at room temperature with continuous shaking. The nitrocellulose membranes were then washed four times for 10 min with PBS-milk. Peroxidase conjugated goat anti-human IgE antibody diluted 1:500 in PBS-milk was then added and the nitrocellulose sheets were incubated for 30 min at room temperature. After washing five times for 10 min with PBS-milk (with the milk omitted in the last wash), the sheets were placed in a colour development solution (Sigma DAB fast system). The reaction was stopped after 1 min by rinsing in tap water and finally with distilled water.

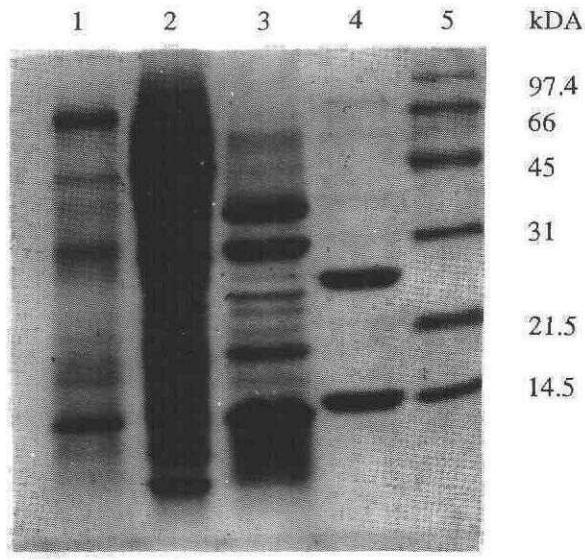

Figure 1. SDS-PAGE of proteins from various fractions of latex (15% gel). Lane 1: leaf extract, 2: C-serum, 3: B-serum, 4: rubber particle extract, 5: molecular weight markers.

RESULTS AND DISCUSSION

Electrophoresis (SDS PAGE) of the B-serum, C-serum and rubber particle membrane extract revealed the differences in protein composition of the various extracts *(Figure 1)* C-serum showed the most numerous bands ranging from *circa* 5 kD up to 100 kD. B-serum has fewer in number but discernible bands appeared at 14, 20, 25, 30, 35 kD and less predominant bands at 43 and 57 kD Two major components of rubber particles membrane extract were detected at 14 kD and 24 kD respectively

Immunoblotting of the SDS PAGE with eleven individual plasma and two pool plasma IgE positive to latex protein demonstrated the presence of allergens in all the *Hevea* extracts examined although there were notable differences in each individual's response to the extracts *(Table 1)*

Nine out of the eleven patients were found to be positive to B-serum while positive bands were also observed in C-serum from eight patients Rubber particle membrane proteins have the least allergenicity with only three patients found to be positive Both the pool plasma tested were positive to B-serum protein(s) while only one of them reacted to a C-serum protein

The immunoblots also revealed the presence of some allergens that were recognised by IgE in many of the plasma samples tested The most frequent bands observed in B-serum blots were in the region of 43 kD to 65 kD with a thick smear at 58 kD. The majority of the C-serum positive patients had IgE binding to a thick band in the region of 14 kD Slater and Chabra[11] described a 14 kD protein to be a major allergen among US spina bifida patients Antibodies against a 14 kD protein were also found frequently among the Finnish patients with congenital anomalies and also among adult latex allergic patients[12] In view of the numerous low molecular weight (14 kDa and below) polypeptides that have been reported to be allergenic[13], the possibility exists that some of these could be break-down fragments of larger proteins

TABLE 1 IMMUNOBLOT OF *HEVEA* PROTEINS WITH IgE AFFINITY

Latex positive plasma	Rubber particles	B-serum	C serum	Total IgE (kU/l)	Specific IgE (kU/l)
3510-28		20 35 46 58	14 18 22 31 40 47 60	>500	7 0
9706		14 31 46 58		>500	>30 0
9586	10	14	14	>500	>30 0
0B1[a]		29 35–60	67	>500	2 78
ASP[a]		20		>500	1 58
4414			60 67 75	>500	7 57
1012		46 58	14	>500	6 12
A190		46 58		457	10 0
4265		42 47 55	66	800	2 0
4376		40 42 47 55	12	1 100	13 7
4393	>50	47 55	12 14	10 000	21 6
4396	>50		40	1 500	12 4
4398		42 47		600	16 8

[a]Plasma pool of atopic patients

Latex specific IgE results were provided by Diagnostic Products Corporation

Monoclonal antibodies have been developed against proteins of B-serum and C-serum. Some of the proteins for which monoclonal antibodies are available have been shown to be allergens. They would be used in the development of an immunoassay for latex allergens.

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