Differential expression of allergens on the internal and external surfaces of latex surgical gloves

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ABSTRACT

Background: Differences in latex allergen sensitization profiles have been described between children undergoing repeated surgical interventions and health care workers. The purpose of this study was to determine whether such sensitization profiles are associated with differences in the expression of latex allergen between the internal and external surfaces of surgical gloves.

Methods: Extracts were obtained from whole surgical gloves as well as from their external and internal surfaces. The extracts were centrifuged, filtered, concentrated, dialyzed and lyophilized. The protein profile of the extracts was analyzed using hydrophobic interaction chromatography (HIC) and *s*odium *d*odecyl *s*ulfate *p*oly*a*crylamide *g*el *e*lectrophoresis (SDS-PAGE). Immunoblotting was performed using sera from two patients with confirmed latex allergy. Latex recombinant allergen-specific IgE in these two patients was determined using a fluorescence enzyme immunoassay (FEIA) method. Latex allergen quantification was determined on both glove surfaces using an ELISA method.

Results: HIC and SDS-PAGE showed qualitative and quantitative differences in proteins between the internal and external glove surfaces, with the former being much richer in proteins. Immunoblotting of glove extracts using sera from two latex-allergic health workers showed differences between glove surface extracts. ELISA quantification of latex allergens demonstrated that the internal glove surface had high amounts of Hev b 5 and Hev b 6.02 whereas the external surface showed Hev b 1, Hev b 3, and Hev b 6.02.

Conclusions: Our results reveal substantial differences in the composition of latex allergen profiles between the internal and external surfaces of surgical latex gloves, which may suggest a relationship between latex allergen localization and sensitization routes in different risk groups.

Key words: Healthcare workers. Latex allergens. Latex gloves. Spina bifida.

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INTRODUCTION

Latex sensitization is recognized as a serious health problem since latex products are increasingly used, especially among health care workers and patients undergoing multiple surgical interventions, such as *spina bifida* patients[1-5]. Some epidemiological studies have reported that the incidence and prevalence of latex allergy has increased during the past 10 years, and demonstrated a prevalence ranging between 2.9 % and 17 % in health care workers[6,7]. For the latter risk group there are numerous routes of exposure

such as the skin, from direct contact with latex products, and/or the nose and bronchi, from inhalation of aerosolized glove powder coated with natural rubber latex allergens. Various studies[8-10] have reported several risk factors for the development of latex allergy, such as having *spina bifida*, being subjected to multiple surgical interventions, having a history of atopy and/or food allergies namely to kiwi fruit, banana, avocado, tomato, papaya and chestnut.

Natural rubber latex is a complex mixture of several substances such as rubber particles (30-40 %), proteins (2,3 %) and water (55-65 %). In manufactured products, proteins can comprise up to 3 % of the contents[11,12].

Latex sensitization, especially among health care workers, is linked to proteins from natural rubber latex gloves. Several hundred proteins have been described in latex, of which 13 (Hev b 1 to Hev b 13) have been recognized by the International Union of Immunological Societies (IUIS) as latex allergens[13]. From these proteins, Hev b 1, Hev b 3 and Hev b 7 are major allergens for spina bifida patients, and Hev b 2, Hev b 5, Hev b 6.01 and Hev b 13 are major allergens for healthcare workers. The reason for this discrepancy in the allergen sensitisation profiles is currently unknown[14-17]. However, although genetic factors may influence this differential pattern of allergen recognition in these risk groups, the different reactivity patterns may also be due to differences in the allergen make-up of internal and external glove surfaces. Therefore, the purpose of the present study was to characterise and identify latex allergens in the internal and external surfaces of natural rubber latex surgical gloves.

MATERIALS & METHODS

Characterization of latex-allergic patients

Two health care workers with a clinical history of latex allergy were recruited. One (A) had a history of rhinoconjunctivitis and bronchial asthma symptoms on exposure to latex-rich environments whereas the second patient (B) had a history of anaphylactic shock upon contact with latex allergens. Sensitisation to latex allergens was confirmed by positive skin prick tests (Bial-Aristegui), as well as positive latex-specific IgE results. One of the patients had positive latex-specific IgE to Hev b 5 (12.4 kUA/l), Hev b 6.01 (9.17 kUA/l), and Hev b 6.02 (9.14 kUA/l), whereas the second patient had a reactivity of 1.70 kUA/l, 1.02 kUA/l and 1.12 kUA/l, respectively to the same allergens. Sera was obtained from both of these patients.

Latex allergen extraction

Latex allergens were extracted separately from the internal and external surfaces of two commercial brands and batches of latex surgical gloves. Extracts were separately obtained from both surfaces according to the method of Tomazic et al[23], and recommended by the American Society for Testing and Materials (ASTM D 5712-95, 1995). Briefly, each glove was sealed with a string in the proximal wrist end, covered with 200 mL of phosphate-buffered saline (PBS), in a 1L Erlenmeyer flask under agitation (260 r.p.m.) at 37 °C for 24 h. Extracts of the internal glove surface were prepared in the same way after turning the internal surface out. Glove extracts were centrifuged at 4000 g for 30 min to remove insoluble particles, concentrated (AMICON 8050 unit, Bedford, U.S.A.) with a regenerated cellulose membrane (NMWL 1000 Da, Millipore, Bedford, U.S.A.), dialyzed (snakeskin pleated tube, Pierce, Rockford, U.S.A., with NMWL 3500 Da), lyophilized and stored at –18 °C.

SDS-PAGE assays

Thirty-microlitre aliquots of each extract were separated by analytical 15 % or 4-20 % gradient SDS-PAGE. A Rainbow Marker (Amersham, UK) was used as a molecular weight standard. Proteins were visualized by Coomassie Blue staining.

IgE immunoblotting

Thirty microlitre aliquots of each glove extract were separated by preparative 15 % SDS-PAGE. Proteins were blotted onto PVDF membrane (Hybond-P- Pharmacia, Buckinghamshire, England). PVDF strips of 5 mm were blocked twice for 5 min and once for 30 min in buffer (50 mmol/L sodium phosphate, pH 7.5, 0.5 % v/v TWEEN-20, 0,5 % w/v BSA, 0.05 % w/v NaN_3). PVDF strips containing blotted latex extracts were incubated with sera diluted 1:10 in buffer or with buffer alone, overnight at 4 °C. After two washes in buffer for 5 min and one for 30 min, bound IgE was detected using alkaline phosphatase-labelled antihuman IgE antibodies (Sigma, Steinheim, Germany) diluted 1:1000 in buffer, for 2 h, at room temperature. The reaction was visualised using enhanced chemifluorescence analysis, ECF (Amersham Biosciences, Buckinghamshire, England). Strips were washed as above, dried and exposed to Molecular Imager Fx Pro System (Bio-Rad, Hercules, U.S.A.)

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