

Latex allergy: allergen identification in *Hevea brasiliensis* fractions by immunoblotting

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Summary

Background Latex is the cause of several clinical symptoms of allergy, but the identification of allergens is not completely known.

Objective The aim of this report was to study the immunoreactivity of purified stable latex fractions from *Hevea brasiliensis*.

Methods We purified the cytoplasm of *Hevea brasiliensis* and obtained three fractions: latex particles (LP), lutoids (L) and cytosolic serum (CS). Using Western blot, specific IgE directed to latex allergens was found in 80 patients with latex allergy.

Results Five major groups of allergens migrating as 14, 25, 29, 37–45 and 50 kDa were recognized. They were unequally distributed within the latex fractions: 37–45 kDa proteins were essentially recognized in CS and LP, whereas 14 and 29 kDa proteins were mainly labelled in the L fraction. As a control, aqueous glove extracts exhibited a more restricted pattern of reactivity, because only 14 and 29 kDa proteins were recognized by patient sera. The pattern of reactivity was not correlated with specific IgE levels, but sera from patients suffering from spina bifida reacted specifically with the minor protein of 25 kDa located in LP.

Conclusions The present results show that latex allergic patients recognize several allergens which are differently distributed in subcellular fractions extracted from *H. brasiliensis* and aqueous GE. The L fraction and GE were enriched in low molecular weight proteins and apparently contained the same allergens.

Keywords: latex, *Hevea brasiliensis*, allergens, spina bifida

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Introduction

Since the first clinical description of latex allergy [1], a high incidence and a large range of symptoms, from discomfort associated with glove wearing to life-threatening shocks, have been reported [2,3]. The reasons for a such a high incidence are still unclear, although it is likely that frequent latex contacts is one condition for sensitization [4]. In

addition, patients suffering from spina bifida are apparently greatly exposed to the risk of latex allergy [5].

Different latex allergens have been characterized using varied techniques and heterogeneous latex materials [6–15]. Latex allergens have been reported in supernates of ammoniated [6–8] and non-treated [9–12] latex, aqueous extracts from gloves [13] and condoms [14], and glove powder [13,15]. Therefore, the use of preparations from *Hevea brasiliensis* latex might allow the standardization of latex extracts, and then the better definition of allergens involved in latex allergy. *Hevea brasiliensis* latex is a cytoplasm characterized by three main fractions: latex particles (LP)

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consisting of 1 μm diameter spheres of cis 1-4 polyisoprene, lutoids (L) consisting of 5–6 μm diameter vesicles involved in homeostatic and coagulation processes, and cytosolic serum (CS) containing numerous proteins [16].

The aim of this report was to study the immunoreactivity of purified stable latex fractions from *H. brasiliensis*. In addition, we took advantage of a large cohort of patients with latex allergy to analyse the relationship between the immunoreactivity of latex and the clinical status of the patients. We found that five main groups of proteins were recognized by patient sera and were specifically distributed in latex fractions. We suggest that these proteins may constitute major allergens involved in latex allergy.

Materials and methods

Subjects

Eighty patients with latex allergy were included in this study. They consisted of 41 males and 39 females, with a mean age of 22 years ranging from 4 to 65 years. They exhibited different degrees of contact urticaria: mild (62 patients), moderate (10 patients) and severe (eight patients). Two patients suffered from latex asthma and in six anaphylactic shocks were observed (one severe and five moderate). Thirty-four patients had spina bifida. All patients displayed a positive skin prick test (Laboratoire des Stallergènes, France) and increased levels of IgE specific for latex. The mean IgE levels to include patients were class 2, as determined by CAP-system RAST (Pharmacia, Uppsala, Sweden). The patient IgE levels were distributed as follows: 45 were class 2, 13 class 3, 10 class 4, eight class 5 and four class 6. No discrepancy between skin test and specific IgE measurements was noted in each latex allergic patient. All patients were devoid of anti-allergic treatment. Twenty healthy controls were selected. They consisted of 11 males and nine females, with a mean age of 31 years ranging from 7 to 61 years. All controls were negative for the skin prick test and did not contain detectable specific IgE as measured by CAP-system RAST.

Isolation of rubber fractions

The sap from *H. brasiliensis*, harvested in the Ivory Coast, was obtained from only one clone, GT2, and frozen before used. It corresponds to cell cytoplasm, i.e. whole cell content without nucleus and mitochondria [16]. The total protein content of the cytoplasm was 1.8–2.5% of the raw material. We used the following sequential procedure to separate latex fractions. After adjusting raw latex pH to 9.0 with ammonia, the latex sap was centrifuged at 10 000 r.p.m. for 20 min, leading to a solution containing L and LP. LP were washed three times in 10% ammonia solution and spun

down at 10 000 r.p.m. for 20 min to discard any component promoting coagulation. After withdrawing solution with LP, the remaining solution was centrifugated at 100 000 r.p.m. for 90 min. The pellet consisting mainly of L and Frey–Wyssling particles (chloroplasts) was filtrated on Whatman paper and then washed with phosphate-buffered saline (PBS). The preparation contained 90–95% L. After removal of L, the remaining sap was filtrated to discard any precipitate. The final extract contained CS. The three fractions were stable for several months when they were saved as aliquots and stored at -20°C before use. Glove extracts used as controls in this study were obtained by a different procedure. Briefly, gloves (Ansell Bioser latex surgical gloves and Baxter examination gloves) were cut into small pieces and incubated overnight in PBS at room temperature. After centrifugation at 10 000 r.p.m. for 20 min, supernates were collected and concentrated by heat-drying centrifugation (concentrator-centrifugator, Jouan, St-Nazaire, France) overnight at 40°C . Glove extracts from the two sources were pooled and concentrated at 1 mg/mL proteins before being frozen at -20°C .

Immunoblotting

Samples of each fraction were diluted 1:3 in solution consisting of 0.05% bromophenol blue, 15% glycerol, 86 mM Tris-HCl pH 8.6, 9% sodium dodecylsulphate (SDS) and 6% β -mercaptoethanol, and then boiled for 10 min. As previously described [17], 7 μg proteins (determined by Bradford's method) were applied to 12.5% SDS–polyacrylamide gels (PAGE) and electrophoresed for 60 min at a constant voltage of 200 V. Proteins were then transferred to nitrocellulose sheets (Schleicher & Schuell, Dassel, Germany) in buffer containing 50 mM Tris-HCl, pH 8.30, 70 mM glycine and 20% methanol for 1 h at 50 V (Mini Trans-blot cell; Biorad, Richmond, CA, USA). Membranes were then washed three times for 5 min with blocking buffer containing 5% bovine serum albumin (BSA) in 0.3% Tween 20–Tris-buffered saline (TBS) and incubated for 1 h in blocking buffer at 37°C . Control and patient sera at 1:5 dilution were added to strips in blocking buffer and incubated for 1 h at room temperature under shaking. The 1:5 serum dilution was used in all experiments and corresponds to dilutions reported elsewhere [11]. Membranes were washed six times for 5 min blocking buffer and incubated for 1 h at room temperature with 1:500 dilution rabbit anti-human IgE antibody (Dako, Glostrup, Denmark). After washing, 1:5000 dilution horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Amersham) was added to strips for 1 h. The strips were then washed six times in blocking buffer and finally in PBS. The reaction was revealed using peroxidase ECL substrate and exposure to ECL–Hyperfilm (Amersham).

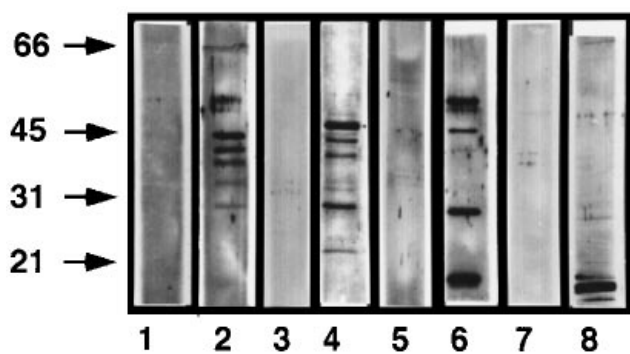


Fig. 1. Immunoblot analysis of binding of control and patient sera to latex fractions and glove extracts. Latex fractions, CS (lanes 1, 2), LP (lanes 3, 4), L (lanes 5, 6) and GE (lanes 7,8) were immunoblotted with control serum (lanes 1, 3, 5, 7) and allergic patient serum (lanes 2, 4, 6, 8). Immunoblots were revealed using an ECL detection kit. Molecular weight (kDa) standards are presented in the left margin.

Molecular weights were determined by using Bio-Rad standards.

Statistical analysis

The comparison of immunoreactivity and clinical parameters was performed by analysis of variance (ANOVA) and chi-square analysis.

Results

The fractionation of *H. brasiliensis* sap yielded three distinct fractions. The analysis by SDS-PAGE showed that the CS and LP fractions had the richest protein content, whereas

the L fraction specifically lacked proteins migrating above 40 kDa (J. L. Jacob, personal communication). Each fraction was then immunoblotted and probed with patient and control sera. As shown in Fig. 1, non-allergic control serum did not label CS (lane 1), LP (lane 3) and L (lane 5) fractions. Patient serum recognized several proteins in the three fractions, i.e. 14, 25, 29, 33, 37–39, 42, 45–47, 50 and 66 kDa proteins (lanes 2, 4 and 6). These immunoreactive bands were differently distributed in the latex fractions. In the CS fraction, proteins migrating at 29, 33, 37, 42, 45, 50 and 66 kDa were recognized by an allergic serum (lane 2). In the LP fraction, a similar pattern was found (lane 4). In contrast, an allergic serum recognized only 14, 29, 45 and 50 kDa proteins in the L fraction (lane 6). It is noteworthy that the immunoreactive 14 kDa protein was found almost exclusively in this latter fraction.

When the pattern of immunoreactivity of the allergic patient cohort was analysed, we noted marked inter-individual variations. As summarized in Table 1, 37, 42 and 45 kDa proteins were recognized in CS by most allergic patients (89%), whereas 29 and 50 kDa proteins were labelled by 23% and 25% of patients, respectively. In LP, 89% of allergic patients recognized the group of proteins migrating between 39 and 47 kDa. Nevertheless, a protein of 14 kDa that was labelled by 21% of patients in CS was recognized by 45% of allergic patients in the LP fraction. Although CS and LP displayed a close pattern of immunoreactivity, the results obtained in LP were not the consequence of LP contamination by the CS fraction. In the L fraction, two prominent proteins of 14 and 29 kDa specifically bound IgE present in almost all allergic patient sera.

Because the allergens possibly involved in latex allergy are mainly derived from gloves, we prepared and immunoblotted aqueous glove extracts (GE) with serum from allergic patients and non-allergic controls. GE exhibited a pattern of proteins similar to that of the L fraction (data not shown). As shown in Fig. 1, serum from an allergic patient recognized a restricted pattern of proteins compared with the pattern found in latex fractions (lane 8 vs lanes 2, 4 and 6). A protein of 14 kDa was recognized by 65% of patients (Table 1). The protein of 29 kDa was recognized by all patient sera studied (Table 1) but was less intensively labelled than the 14 kDa protein (Fig. 1, lane 8). It is noteworthy that the immunoreactive patterns of aqueous GE and the L fraction were close but the proteins of 46 and 50 kDa were not detected in aqueous GE (compare lanes 6 and 8).

The relationship between the patterns of immunoreactivity, clinical and biological parameters of patients was then investigated. We did not find any significant correlation between the proteins recognized by patient sera and the levels of specific IgE, whatever the latex fraction. It is likely that latex allergens present in the CAP system were quite distinct from those obtained by latex

Table 1. Distribution of immunoreactive allergens in allergic patients

	14 kDa	25 kDa	29 kDa	37–45 kDa	50 kDa
CS	17 (21%)	10 (13%)	18 (23%)	71 (89%)	20 (25%)
LP	36 (45%)	18 (23%)	31 (39%)	71 (89%)	0 (0%)
L	31 (97%)	8 (25%)	32 (100%)	0 (0%)	0 (0%)
GE	13 (65%)	0 (0%)	20 (100%)	0 (0%)	0 (0%)

Latex fractions (CS, LP, L) and aqueous glove extract (GE) were immunoblotted with serum from allergic patients: 80 patient sera were used with CS and LP, 32 patient sera with L, and 20 patient sera with GE. Results are expressed as number of positive patient per given immunoreactive band, and the percentage is presented in parentheses. Immunoreactive bands migrating as 37, 39, 42 and 45 kDa are included in the table as a 37–45 kDa group.

fractionation or glove preparations. In addition, we did not find any correlation between the pattern of immunoreactivity and the type of allergic symptoms, the severity of the disease and the age of patients. However, we noted that 13 of 34 spina bifida patients and only five of 46 allergic patients without neurological defects recognized the protein of 25 kDa in the LP fraction ($P < 0.03$). This protein was not recognized by spina bifida patient sera in GE.

Discussion

In this study, we analysed the allergenic components of latex by using a specific procedure of *H. brasiliensis* fractionation. We provided evidence that several proteins or groups of proteins are specifically recognized by allergic serum in latex fractions. A group of allergens migrating as 37–45 kDa proteins was predominantly detected in CS and LP fractions. Alenius *et al.* also described allergens of 40–46 kDa and 50 kDa but did not characterize them [12]. Proteins of 14 and 29 kDa were the main allergens present in the L fraction from raw latex. This finding deserves several comments. First, the L fraction and GE were enriched in low molecular weight proteins and apparently contained the same allergens. Although a great heterogeneity in protein content of the GE from different sources was found, it has been reported that some proteins eluted from latex gloves are also present in the L fraction and are immunogenic [18]. These allergens are probably involved in latex allergy.

Second, the two immunoreactive proteins of 14 and 29 kDa have also been found by several authors but their molecular identity is still debated [8,10,12,15]. Indeed, the 14 kDa allergen had been related to rubber elongation factor (REF), known to be present in cytosolic serum from latex extract [16], or profilin [19,20]. Although we found an allergen migrating as a 14 kDa protein, its location in the L fraction did not support an identity with REF present in the CS fraction. It is likely that the main 14 kDa allergen in the L fraction and GE was distinct from the minor 14 kDa allergen present in CS. Indeed, some patient sera recognized a 14 kDa protein in CS but not in LP and, conversely, others only labelled a 14 kDa protein in LP.

Third, several allergens migrating as 29–33 kDa proteins have been reported [8,11,12,21]. Nevertheless, their molecular characterization is just at the beginning. Jekel *et al.* purified a 30 kDa protein from rubber tree related to heveamine [22]. The similarity in molecular size between our 29 kDa allergen and heveamine suggests that this 29 kDa protein may be one of the major allergens of latex. In contrast, few data concerning the molecular identity of major allergens present in CS and LP fractions are available so far.

On the other hand, we found that the pattern of recognition of allergens in latex fractions and aqueous GE was not

clearly related to several parameters of latex allergy disease. These latter included the type of symptoms, the severity of the disease and the levels of specific IgE. Surprisingly, we found that a minor immunoreactive protein of 25 kDa was observed in allergic patients with spina bifida. Alenius *et al.* reported spina bifida patients with a history of anaphylaxis specifically recognizing a 27 kDa allergen [23]. Each allergic patient displayed a specific pattern of allergen recognition in latex and GE. The pattern of specificity of each allergic patient might be influenced by the genetic background and the route of sensitization.

This study clearly shows that latex allergic patients recognize several allergens which are differently distributed in subcellular fractions extracted from *H. brasiliensis* and aqueous GE and confirms that the luteoid fraction is a potent source of allergens as was recently shown (24). The fractionation associated with the purification and characterization of allergens will allow the determination of specificity for each patient and the development of specific immunotherapy. It would be also useful for the realization of specific skin tests and immunoassays, and would constitute the basis for industrial strategies leading to hypoallergenic latex material engineering.

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