

Several manifestations have been reported in LAD patients, the severity of which appears to be related to the degree of surface expression of CD18 and CD11. However, to the best of our knowledge, NHL has not been reported in LAD. However, unfortunately, the lymphoma itself was not tested for CD11 or CD18. NHL, which is malignant proliferation of lymphocytes, seems to be one of the most common childhood malignancies. As genetic abnormalities are risk factors for NHL, such abnormalities in integrin molecules could predispose patients to malignancy in lower ages. As $\beta 2$ integrins are essential for the regulation of antigen-dependent activation threshold, low expression of the molecule could cause deficiency in the quantitative and qualitative of CD4⁺ T-cells and lymphocytes that normally regulate immune function and suppress malignant clone. There is some evidence showing association of primary immunodeficiency disorders and cancers.⁴ Although predisposition to viral infections as well as susceptibility to DNA breakage were considered as specific aetiologies of a number of cancers in certain immunodeficiencies, the exact biological pathway and mechanisms for such association have not been completely recognised. Development of malignancy in LAD patients could provide insight into the possible role of $\beta 2$ integrins in cancers.

Ethical disclosures

Protection of human subjects and animals in research. Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Patients' data protection. Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects mentioned in the article. The author for correspondence is in possession of this document.

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Separation of the four most important latex allergens from latex gloves: A potential tool for diagnosis and immunotherapy purposes

To the Editor,

Due to a more wide-spread use of latex products, in particular medical gloves, allergy to natural rubber latex (NRL) had posed serious concerns during the 1980s and 90s. Currently, it is known that this epidemic of allergic reactions is especially prevalent in certain occupational groups repeatedly exposed to latex products, such as health care workers (HCW) and patients with spina bifida (SBP).¹

The identification of the patients who become sensitised and are likely to suffer from symptoms upon repeated exposure to latex products is a major goal for prevention of the allergic reactions. It is generally accepted that the diagnosis must be based on the clinical history and on a confirmative assay which may include *in vivo* tests such as skin prick tests (SPT), provocation tests and/or laboratory-based *in vitro* analyses.² Among these, SPT are described as the most reliable *in vivo* method for diagnosis of sensitisation to latex proteins.³ However, a potential low diagnostic sensitivity of latex SPT is associated to poorly represented and/or denatured allergens, such as Hev b 3 and Hev b 5.^{4,5} In fact, to perform an appropriate diagnosis, it is critical that the applied extract contains an adequate amount of all clinically relevant allergen components.⁴

In the management of latex allergy, specific immunotherapy (SIT) represents the most promising alternative for latex allergy treatment. Nevertheless, SIT is currently associated with a high risk of adverse events and is not available in routine clinical practice. Refined allergen preparations and administration regimens must be developed to allow widespread use. In immunotherapy extracts, unlike diagnostic extracts, the inclusion of allergens that are not clinically relevant can dilute the essential allergen components of extract and decrease its effectiveness.

Nowadays, immunotherapy and diagnosis of latex allergy are performed using NRL extracts that are obtained directly from the tree *Hevea brasiliensis* sap without any purification steps. This crude extract contains several hundred proteins from which 14 (Hev b 1 to Hev b 14) have been recognized by the International Union of Immunological Societies (IUIS) as latex allergens (www.allergen.org). Among the latex allergens, Hev b 1, Hev b 3, Hev b 5 and Hev b 6 are regarded as the most crucial allergens in natural rubber products.⁶ Here we describe a new approach to obtain and separate the four most relevant latex allergens from the major sensitisation source, the latex gloves.

Since the proteins of gloves and other NRL products are responsible for latex sensitisation, we consider that they are the ideal allergen sources of reagents for clinical purposes. Indeed, Yeang et al. reported that the latex allergens for research and clinical applications should theoretically be proteins isolated and purified from latex gloves. These researchers also noted that there are considerable problems in sourcing latex allergens from gloves because they vary widely in their protein content, both qualitatively and quantitatively. However, these problems also exist in crude extracts because there is an inherent variation in allergen content due to genetic environmental and agronomic factors.⁷

As a first step of the present method, six different glove brands were collected from health institutions in Portugal, aiming to reduce the effect of inherent protein variation that results from glove manufacture and processing. Latex proteins were extracted from the gloves and lyophilized as described previously.⁸ Then, 300 mg of lyophilized latex glove extract were dissolved in 20 mM Tris-HCl, pH 7.5 and a salt precipitation was applied for both enrichment and concentration of latex allergens. This step was first performed by using 25% ammonium sulphate. After being stirred for 30 min, at 4 °C, the precipitated extract (denoted P1) was centrifuged at $12,000 \times g$ for 15 min, at 4 °C. The same process was repeated adding 50% and 75% of ammonium sulphate (extracts denoted P2 and P3, respectively). The three precipitated latex extracts (P1, P2 and P3) were then fractionated by Hydrophobic Interaction Chromatography (HIC). This technique was employed to exploit the differences in protein hydrophobicity between the four major latex allergens and to accomplish their separation. In fact, it is known that Hev b 5 and Hev b 6 have mainly hydrophilic characteristics while Hev b 1 and Hev b 3 are hydrophobic allergens.^{9,10} HIC was applied using a FPLC system (GE Healthcare Biosciences, Uppsala, Sweden) on a Sepharose CL-6B column modified with butyl-1,4-bis-(2,3-epoxy-propoxyd). The gel was packed in a XK column 16/20 (GE Healthcare Biosciences, Uppsala, Sweden) and equilibrated in a series of experiments using

as eluent PBS 0.01 M, pH 7.4 with different ammonium sulphate concentrations. The best separation conditions were achieved using 0.5 M ammonium sulphate for precipitate P1, 1.0 M for P2 and 1.5 M for P3 (Fig. 1). At these conditions, two resolved peaks corresponding to unbound proteins were eluted (peaks 1 and 2). Bound proteins were then eluted (peak 3) by subsequently decreasing of ammonium sulphate concentration in eluent buffer to 0 M, in a stepwise mode. Results showed different chromatographic profiles which suggest qualitative and quantitative differences in protein content of precipitated extracts (Fig. 1A–C).

Quantification of Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 in each HIC fraction was performed by capture enzyme immunoassay (EIA) using a commercial kit (FITkit™, Icosagen, Estonia) according to the manufacturer's instructions. FITkit™ tests are based on the use of specific monoclonal antibodies developed against the four major NRL allergens, providing individual results for each NRL allergen. All assays were performed in triplicate and when allergen levels were below the detection limit they were denoted as zero (Table 1). In this quantitative analysis was observed that HIC fractionation of P1 (Fig. 1A) promoted a selective separation of hydrophobic allergens, Hev b 3 (2.53 µg/L) and Hev b 1 (1.40 µg/L), in peaks 1 and 3 respectively (Table 1). Moreover, HIC of P2 (Fig. 1B) showed a separation of Hev b 6.02 (3.43 µg/L) in peak 1 (Table 1), whereas in peak 2 a mixture of Hev b 5 and Hev b 6.02 was obtained. It was also interesting to observe a high concentration of Hev b 1 in peak 3, despite the slight contamination of Hev b 6.02. On the other hand, separation of Hev b 5 (0.53 and 0.50 µg/L) was achieved in peaks 2 and 3 (Table 1) of the HIC assay applied to the P3 precipitate (Fig. 1C). It is known that although latex sensitisation is mainly acquired at health institutions for both SBP and HCW, in general, these risk groups present different sensitisation profiles, being that Hev b 1 and Hev b 3 are considered major allergens for SBP, while Hev b 5 and Hev b 6.02 are the major allergens for HCW.¹ Although the sensitisation profile of these risk groups may be more complex than this general rule, since it is common that SBP are also sensitised to Hev b 5 and Hev b 6, it is interesting to note that two different fractions containing specific allergens for each risk group were also obtained with this methodology: Peak 2 of P1 with Hev b 1 (1.60 µg/L) and Hev b 3 (4.40 µg/L), and Peak 2 of P2 containing Hev b 5 (0.68 µg/L), Hev b 6.02 (2.80 µg/L) (Table 1). These extracts and the separated allergens can be used to adjust the content of each major allergen that is present in the diagnosis reagents and also can provide a specific extracts for a more effective immunotherapy. In fact, the success of allergen-specific diagnosis and treatment is dependent on the allergen composition of the used material. It is recognized that the commercially available latex extracts vary significantly in their allergen composition and concentration and this heterogeneity could strongly affect not only the diagnosis but also the immunotherapy, due to the lack of correct identification of patient sensitisation profile and to the poor quality of the therapeutic preparations. In order to achieve balanced allergen content, commercially available diagnosis reagents should be enriched in trace latex allergens. Thus, the improvement of NRL reagents using allergen preparation such our extracts could favour the correct diagnosis of clinical allergy that is crucial to

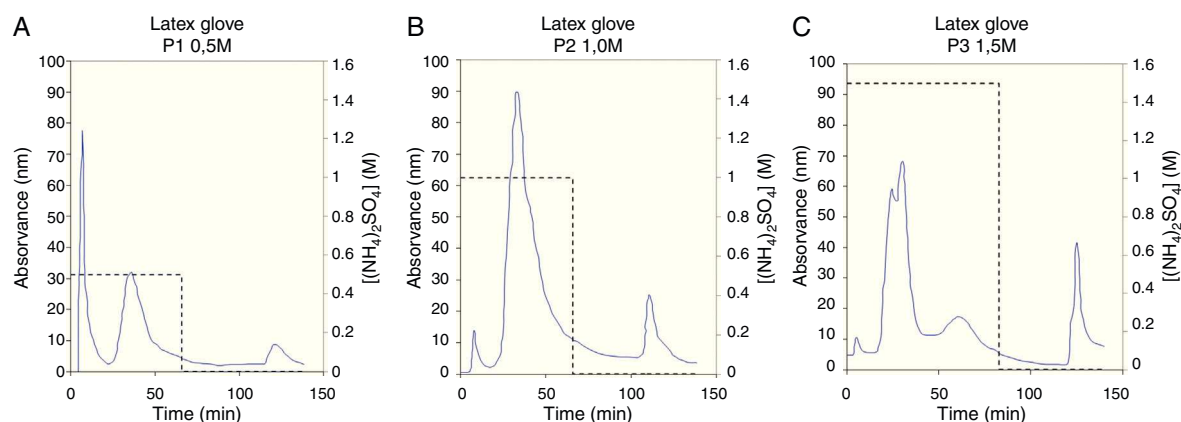


Figure 1 HIC on Epoxy-Sepharose CL-6B column. Sample: 100 μ L, 0.4 mg/mL of latex glove extract obtained by selective precipitation with ammonium sulphate: A: 25% (P1), B: 50% (P2), C: 75% (P3). Eluent: PBS 0.01 M, pH 7.4 containing 0.5 M (A); 1.0 M (B) and 1.5 M ammonium sulphate (C).

Table 1 Quantification of Hev b 1, Hev b 3, Hev b 5, Hev b 6.02 by EIA in latex glove fractions obtained by HIC of P1, P2 and P3 (precipitation with 25%, 50% and 75% ammonium sulphate, respectively).

	P1			P2			P3		
	Peaks (μ g/L)			Peaks (μ g/L)			Peaks (μ g/L)		
	1	2	3	1	2	3	1	2	3
Hev b 1	0	1.60	1.40	0	0	5.01	1.30	0	0
Hev b 3	2.53	4.40	0	0	0	0	0	0	0
Hev b 5	0	0	0	0	0.68	0	0.57	0.53	0.50
Hev b 6.02	0	0	0	3.43	2.80	0.50	3.13	0	0

The detection limit levels of allergens on FITkitTM were: Hev b 1, 1.2 μ g/L; Hev b 3, 2.3 μ g/L; Hev b 5, 0.5 μ g/L; Hev b 6.02, 0.1 μ g/L. Values below these detection limits were denoted as zero.

guide subsequent investigations and treatment. On the other hand, in terms of immunotherapy purposes, the complex NRL extracts should be replaced by refined protein preparations, containing the clinically relevant allergens for the sensitised patient to treat, with minimal protein contamination. In this regard, we showed that the different hydrophobicity presented by the most clinically relevant latex allergens can be exploited to obtain allergen preparations which can be useful to formulate specific immunotherapy reagents, based on the individual reactivity profile of each latex-allergic patient to treat. It is also important to stress that the minor NRL allergen content of our fractions is unknown and a high concentration of some minor allergens in each fraction cannot be excluded. For this reason, the use of such extracts in immunotherapy trials should raise safety concerns, especially in patients presenting co-sensitisation to minor NRL allergens.

To summarize, in this work it was described a reproducible procedure that allowed the separation of the four most important latex allergens directly from rubber gloves and its enrichment in specific fractions which can potentially be applied for diagnostic or clinical purposes.

Ethical disclosures

Protection of human subjects and animals in research. Protection of human and animal subjects. The authors

declare that no experiments were performed on humans or animals for this investigation.

Patients' data protection. Confidentiality of data. The authors declare that no patient data appears in this article.

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