ORIGINAL ARTICLE

Evaluation and Comparison of Commercially Available Latex Extracts for Skin Prick Tests

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Abstract

Background: Crude latex extracts are commonly used in skin prick tests (SPT) for the diagnosis of natural rubber latex (NRL) allergy. Nevertheless, variations in protein and allergen composition between latex extracts from different manufacturers can hamper a correct diagnosis.
Objectives: To analyze the heterogeneity of proteins and allergens in latex extracts from 7 different manufacturers and to assess its relevance in the diagnosis of latex allergy.
Methods: Seven latex SPT extracts were analyzed for protein content using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The 4 major allergens Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 were also quantified using enzyme immunoassay. All commercial extracts were tested for their in vitro allergenic capacity using microarray inhibition assays and for their ability to induce biological reactivity in latex-allergic patients undergoing SPT.
Results: The protein content of the extracts varied widely from 8.0 µg/mL to 526.5 µg/mL. SDS-PAGE revealed broad differences in protein profiles between the extracts. Marked variability in the contents of all 4 major allergens was observed, and Hev b 3 and Hev b 5 were undetectable in some extracts. Microarray inhibition assays and SPT demonstrated relevant differences in allergenic capacity between the extracts.
Conclusions: The marked heterogeneity in protein and allergen content of latex extracts from different manufacturers could explain the broad spectrum of SPT results recorded. Our findings suggest that the extracts used for the diagnosis of latex allergy should be improved and standardized.

Key words: Allergen extracts. Latex allergy. Microarrays. Skin prick test.

Resumen

Antecedentes: En el diagnóstico de la alergia a látex natural se utilizan habitualmente extractos crudos de látex en técnica de puntura. No obstante la variación que existe en el contenido proteico y de los distintos alérgenos entre los extractos comerciales procedentes de distintos fabricantes podría afectar al correcto diagnóstico de la alergia.
Objetivos: Analizar la heterogeneidad proteica y de alérgenos entre siete extractos de látex de distintos fabricantes y comprobar las posibles implicaciones clínicas en el diagnóstico de la alergia al látex.
Métodos: Se analizó el contenido proteico de siete extractos de látex y también el perfil mediante la técnica de electroforesis en gel de poliacrilamida (SDS-PAGE). Además, se cuantificaron mediante enzimo-inmunoensayo (EIA), los cuatro alérgenos principales de látex Hev b 1, Hev b 3, Hev b 5 y Hev b 6.02. También se estudió en los siete extractos comerciales su capacidad de inhibición "in vitro", del ensayo de micromatrices y su capacidad para inducir respuestas biológicas "in vivo" en pacientes con alergia al látex, mediante pruebas cutáneas en puntura (SPT) .

Resultados: Los extractos presentaban una amplia variación en el contenido proteico que oscilaba entre 8.0 y 526.5 µg/mL de extracto. También se observaron importantes diferencias en el perfil proteico mediante la técnica de SDS-PAGE. El contenido de los principales cuatro alérgenos fue también muy variable, de forma que en algunos extractos los contenidos de Hev b 3 y Hev b 5 fueron prácticamente indetectables. Tanto la técnica de inhibición de micromatrices como las pruebas de puntura mostraron diferencias notables en la capacidad alergénica de los distintos extractos.
Conclusiones: Los extractos de látex provenientes de distintos fabricantes presentan una importante heterogeneidad en contenido proteico y de alérgenos que podría claramente explicar las notables diferencias observadas en los resultados de las pruebas cutáneas en puntura que presentan los pacientes. Nuestros resultados apoyan la necesidad de mejora de la estandarización de los extractos de látex habitualmente utilizados en el diagnóstico clínico de la alergia al látex.

Palabras clave: Extracto alergénico. Alergia al látex. Micromatriz. Pruebas cutáneas en puntura.

Introduction

Natural rubber latex (NRL) is widely used for the manufacture of medical devices, particularly medical gloves, and in a variety of everyday articles [1,2]. The International Union of Immunological Societies recognizes 14 latex allergens that bind to human immunoglobulin (Ig) E (Hev b 1 to Hev b 14) [3]. The clinically relevant allergens in products made from NRL are Hev b 1 (a rubber elongation factor), Hev b 3 (the small rubber particle protein), Hev b 5 (a structural acidic protein), and Hev b 6 (a prohevein) [4,5]. A capture enzyme immunoassay based on specific monoclonal antibodies to these 4 major NRL allergens is available commercially, with separate assays providing individual results for each allergen [5].

Adverse reactions caused by repeated exposure to NRL allergens include nonallergic contact dermatitis, delayed type IV hypersensitivity (allergic contact dermatitis), and immediate type I hypersensitivity [6]. Adverse reactions to NRL are especially prevalent in specific occupational groups, such as health care workers (HCW), and in spina bifida patients (SBP) [7,8].

Identifying individuals who have become sensitized and are likely to experience symptoms upon repeated exposure to latex products is a major goal in the prevention of latex-associated allergic reactions. It is now generally accepted that the diagnosis of type I allergy (IgE-mediated) must be based on a clinical history of symptoms and on a confirmatory assay including in vivo tests such as skin prick tests (SPTs) and challenge tests, as well as in vitro analyses [9-11]. SPTs are the most reliable method for diagnosis of sensitization to latex proteins. However, the accuracy of the skin test is affected by several variables [12]. In fact, the diagnostic performance and reproducibility of these assays are highly dependent on allergen composition, the concentration of the reagents used, and, in particular, the raw material used in their preparation [13,14]. At present, SPTs in latex-allergic individuals are frequently performed with commercial crude NRL extracts that are complex and variable in composition because of their biological source [15,16]. Ideally, allergen extracts from different manufacturers should be qualitatively similar, with appropriate proportions of relevant allergenic components. Nevertheless, ensuring such homogeneity is a complex task because of the difficulty in obtaining well-characterized extracts [17]. Therefore, the complexity of allergen extracts and their standardization continue to be major challenges in the optimization of diagnostic assays [18]. Standardization of allergen extracts is an essential part of any attempt to control variability and achieve consistency and reproducibility in the clinical setting [19]. Moreover, both safety and efficacy are dependent on the relative amounts of individual major allergens, and to a lesser extent, minor allergens [20]. During recent years, regulatory agencies in several European countries have started asking allergen manufacturers to provide the levels of major allergens used in their products, and it is expected that compliance with this request will soon become a requirement for registration [21].

Since the allergenic profile of an allergen-containing reagent can vary in its protein composition, allergenic potency, and immunoreactivity [22], the aims of this study were to compare the heterogeneity of protein and allergen composition between different manufacturers and to investigate the role of this heterogeneity in the diagnosis of latex allergy.

Materials and Methods

Commercial Latex Allergen Extracts

We invited 7 European manufacturers of latex allergen extracts to participate in this study. Each was informed about the objectives, and participation was voluntary. All companies whose products are distributed in the Portuguese market agreed to participate. These companies were Alk-Abelló, Allergopharma, Bial-Aristegui, Leti, Lofarma, Q-Pharma, and Stallergènes. Each manufacturer sent its in vivo diagnosis products. As stated in the material transfer agreement, all products were assigned a code. The results are presented in a random order with a code (ie, manufacturers A to G). Three were nonammoniated NRL extracts (B, C, and F), and the remainder were low-ammoniated.

Total Protein Content

The total protein concentration of the extracts was estimated using the Bradford method (Bio-Rad) [23]. Two standard curves ranging from 50 µg/mL to 500 µg/mL and 8 µg/mL to 80 µg/mL of bovine serum albumin were constructed. Each experiment was carried out at least 3 times, and each point was tested in duplicate. In order to make direct comparisons of the protein

concentrations between the extracts and given the very small amount of protein present in some of these, all 7 were analyzed in their undiluted form. The statistical analysis was performed using SPSS, version 17.0 (SPSS Inc).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Latex extract proteins were separated by electrophoresis in 4-20% polyacrylamide gels (Bio-Rad), which were loaded with equal volumes (35 μL/lane) of each commercial latex extract. A protein molecular weight marker (Bio-Rad) was used as a standard. Proteins were visualized using Coomassie Blue staining.

Capture Enzyme Immunoassay

The major allergen content of the extracts was analyzed using a commercial kit (FITkit, Icosagen AS). Briefly, a 25-μL sample was added to 100 μL of assay buffer in the precoated microwell plate. After incubation for 1 hour, the plate was washed and the enzyme conjugate (100 μL) added to each well. The plate was incubated for 30 minutes and washed, and 100 μL of horseradish peroxidase substrate solution was added. After 15 minutes, the plate was washed, and 100 μL of stop solution added to each well. Absorbance was measured at 414 nm. Allergen content was measured for Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 in individual assays. The extracts of Hev b 1, Hev b 3, and Hev b 5 were analyzed in undiluted form with the FITkit assay. Given the expected high amounts of Hev b 6.02, the extract was analyzed with 1:10 and 1:100 dilutions. All assays were performed in triplicate. For each extract, the mean of the 3 points in the linear range of the curve was calculated.

Microarray Inhibition Assays

A serum sample that had been characterized using ImmunoCAP ISAC (Phadia) (specific IgE as ISAC standardized units [ISU/L]: Hev b 1, 3 ISU/L; Hev b 3, 6.6 ISU/L; Hev b 5, 1.8 ISU/L; Hev b 6, 1.2 ISU/L; and Hev b 8, 3 ISU/L) was selected from the serum bank of the Department of Immunology and Parasitology of the University of the Basque Country, Vitoria, Spain. A serum sample with specific IgE against Der p 1 (11 ISU/L) was used as a control. After centrifugation of each latex extract, and using commercially available *Dermatophagoides pteronyssinus* extract as a control, 10 μL of supernatant was mixed with serum in a 1:1 ratio and incubated overnight at 4ºC with shaking. Samples were then centrifuged and the supernatants analyzed using ImmunoCAP ISAC for Hev b 1, Hev b 3, Hev b 5, Hev b 6, Hev b 8, and Der p 1 (ImmunoCAP ISAC, Phadia).

Table 1. Demographic and Clinical Characteristics of the Study Patients

Patients	Gender Age	Clinical Symptoms Triggered by Latex-Rich Environments	rHev b Reactivity, kU_A/L	NRL Glove Use Test
SBP1	Female 17	Ocular and nasal pruritus	rHev b 5 (0.37) rHev b 6.01 (0.54) rHev b 6.02 (0.72) rHev b 1 (1.06)	Not performed
SBP2	Female 22	Urticaria and chest tightness	rHev b 6.02 (0.90)	Positive
SBP3	Female 23	Ocular pruritus with eyelid edema, nasal pruritus, chest tightness, and wheezing	rHev b 1(0.92) rHev b 5 (61.7) rHev b 6.01 (17)	Not performed
SBP4	Male 15	Erythema on hands and face, urticaria, ocular angioedema, ocular pruritus, pruritus on hands	rHev b 3 (1.18)	Positive
SBP5	Female 19	History of nasal pruritus, currently asymptomatic	rHev b 5 (9.29)	Not performed
HCW1	Female 31	Nasal and ocular pruritus, wheezing, occasional dyspnea, cough, and increased bronchial secretions	rHev b 8 (4.82)	Not performed
HCW2	Female 34	Dermatitis of the hands, ocular and nasal pruritus	rHev b 5 (1.01) rHev b 6.02 (0.63)	Positive
HCW3	Female 40	Nasal pruritus, edema of the hands with pruritus	rHev b 5 (0.97) rHev b 6.01 (0.75) rHev b 6.02 (1.02)	Positive
HCW4	Female 45	Rhinoconjunctivitis, dermatitis of the hands, pruritus, urticaria, latex-fruit syndrome	No serum reactivity	Positive
HCW5	Female 33	Nasal and ocular pruritus, sneezing attacks, urticarial rash, anaphylaxis, latex-fruit syndrome	rHev b 8 (1.01)	Positive
HCW6	Female 20	Nasal and ocular pruritus, urticaria, latex-fruit syndrome	rHev b 8 (1.08)	Positive

Abbreviations: SBP, spina bifida patient; HCW, health care worker; NRL, natural rubber latex.

Latex-Allergic Patients

Eleven latex-allergic patients (5 SBPs and 6 HCWs) were studied. Three HCWs had clinical and laboratory features of latex-fruit syndrome. They were selected from patients attending the Allergy Units at Amato Lusitano Hospital (Castelo Branco, Portugal), Dona Estefânia Hospital (Lisboa, Portugal), and Cova da Beira Hospital (Covilhã, Portugal). The inclusion criteria were a previous positive SPT result with latex allergen solution and a clinical history consistent with symptoms related to glove use or environmental latex exposure (Table 1). Patients presenting clinical symptoms with a cutaneous component underwent a latex glove challenge test; all results were positive. Patients were not receiving allergen-specific immunotherapy, corticosteroids, or antihistamines. The study was reviewed and approved by the ethics committees of the participating hospitals. Written informed consent to participate was obtained from all volunteers. Serum samples were collected, aliquoted, and stored at –20ºC.

Determination of Allergen-Specific IgE

Serum latex-specific IgE levels were measured using total latex extract k82 and recombinant Hev b allergens (ImmunoCAP, Phadia).

Skin Prick Tests

SPTs were performed with NRL extracts from the 7 manufacturers in all 11 patients in accordance with recommendations of the European Academy of Allergy and Clinical Immunology. The tests were performed in duplicate on the patient's forearms using disposable lancets. Histamine (0.1%; 10 mg/mL) was used as a positive control and saline solution as a negative control. Each test was carried out single-blind, that is, the product identities were unknown to the allergist. Reactions were recorded 15 minutes after testing by transferring the ballpoint pen–surrounded wheal area to paper with scotch tape. According to guidelines, skin wheal size was measured and recorded [24]. Each result was the mean of 2 values, and a wheal diameter 3 mm greater than that of the negative control was considered the positivity cutoff.

Table 2. Quantification of Total Protein by the Bradford Method[a] in Latex Extracts From Manufacturers A to G

Manufacturer	Raw Material	Protein, mg/mL, Mean (SD)[b]
A	LAL	526.5 (2.4)
B	NAL	13.0 (0.7)
C	NAL	143.2 (3.9)
D	LAL	217.8 (0.9)
E	NAL	61.5 (4.4)
F	LAL	BDL
G	NAL	281.5 (5.3)

Abbreviations BDL, below detection limit (<8 µg/mL); LAL, low-ammoniated latex; NAL, nonammoniated latex.
[a]See reference 23.
[b]Results are the mean of 3 different determinations.

Results

Quantitative and Qualitative Protein Analysis

We observed high variability in total protein amounts between the 7 extracts (Table 2). The lowest total protein concentration was found in the extract from company F (below detection limit, 8 µg/mL), and the highest mean (SD) concentration was found in the extract from company A (526.5 ([2.4] µg/mL), thus demonstrating an approximately 65-fold variation. We also analyzed the total protein content in 2 different batches from 4 manufacturers (A, C, D, and F) and observed significant batch-to-batch variability (data not shown).

The pattern and intensity of protein bands obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis

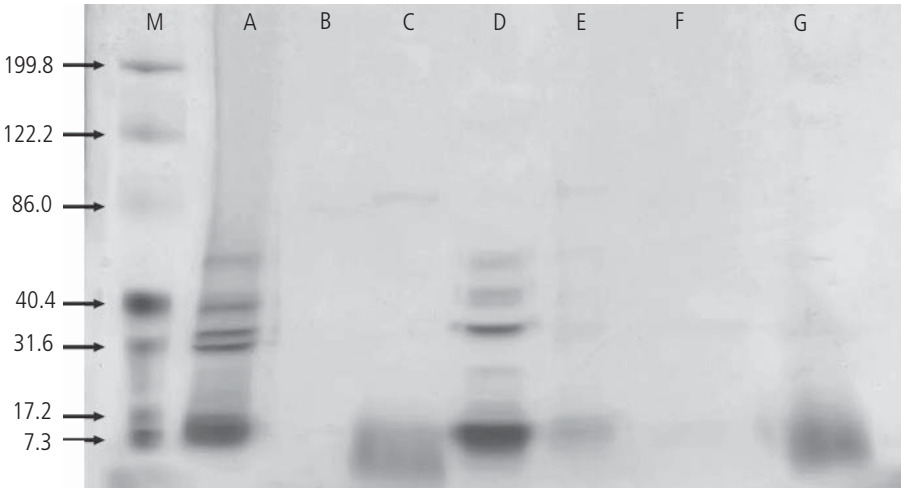

Figure 1. Band A, sodium dodecyl sulfate polyacrylamide gel electrophoresis 4-20% analysis of latex commercial extracts (manufacturers A to G). Band M, molecular weight marker (kDa).

(SDS-PAGE) were highly heterogeneous, since all electrophoretic profiles were different (Figure 1). The pattern was highly heterogeneous in terms of the number and intensity of the bands between 4 kDa and 60 kDa (molecular weight of the major latex allergens). Extracts from manufacturers B and F had no visible bands at all. In the extracts from the remaining manufacturers, an expanded protein band was observed in the low-molecular-weight area (<20 kDa). However, the corresponding intensities were very variable. In fact, most latex allergens have apparent molecular weights within this range, as follows: Hev b 6.02, 4.7 kDa; Hev b 12, 9.3 kDa; Hev 6.03, 14.0 kDa; Hev b 1, 14.6 kDa; Hev b 8, 10.2-15.7 kDa; and Hev b 6.01, 20 kDa). The appearance of a band with an apparent molecular weight greater than 60 kDa (approximately 85 kDa) in extracts C and E indicated that some latex proteins form aggregates that resist disaggregation by SDS, fail to denature completely, or are another kind of latex protein. Simultaneously, the 4 extracts from different batches were also analyzed using SDS-PAGE. The electrophoretic profiles varied, especially in band intensity (data not shown).

Major NRL Allergen Quantification by EIA

The levels of the 4 latex allergens studied in the extracts ranged from below detection limit (BDL) to above detection limit (ADL) (Table 3). The results varied from 141.0 to over 1000 μg/L (ADL) for Hev b 1, from 10 μg/L (BDL) to more than 1000 μg/L (ADL) for Hev b 3, from 5 μg/L (BDL) to more than 100 μg/L (ADL) for Hev b 5, and from 105.5 μg/L to more than 20 000 (ADL) for Hev b 6.02. Thus, Hev b 1 and Hev b 6.02 were the only allergens detected in all commercial latex extracts. On the other hand, the most difficult NRL allergen to detect was Hev b 3, since it was not quantified in 4 of the 7 extracts studied. Moreover, the extract from manufacturer A was the SPT reagent in which all major NRL allergens were best represented. By contrast, the extract from manufacturer D showed the poorest content in major allergens, since 2 of the 4 major allergens were not detected. Only 2 extracts (from companies A and E) presented detectable amounts of all 4 major NRL allergens.

Microarray Inhibition Assays

IgE microarray inhibition experiments revealed a visible variation between the 7 commercial extracts, especially with respect to the allergenic activity of Hev b 8 (Figure 2). In fact, different inhibition percentages were observed for this latex allergen. On the other hand, inhibition of Hev b 1 and Hev b 6

Table 3. Quantification of Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 by Enzyme Immunoassay in Latex Extracts From 7 Manufacturers[a]

	Manufacturer						
Allergen	A	B	C	D	E	F	G
Hev b 1	>1000	199.00	148.34	141.00	>1000	280.34	181.67
Hev b 3	>1000	<10	<10	<10	218.20	<10	13.20
Hev b 5	>100	17.61	6.18	<5	49.80	78.73	<5
Hev b 6.02	>20 000	393.3	3125	>20 000	9216	4631	105.5

[a]Results are the mean of triplicates. Values are expressed as micrograms of allergen per liter of extract.

Table 4. Mean Wheal Diameters After Skin Prick Testing With the Study Extracts in 11 Patients With Confirmed NRL Allergy and Latex-Specific Immunoglobulin E (k82) Levels

Extract	Spina Bifida Patients					Health Care Workers					
									Latex-Fruit Syndrome		
	SBP1	SBP2	SBP3	SBP4	SBP5	HCW1	HCW2	HCW3	HCW4	HCW5	HCW6
A	4	5	5.5	7.5	0[a]	9	4.5	5	4	3	3
B	2.5[a]	0[a]	2.5[a]	3.5	0[a]	4	1.5[a]	1[a]	0[a]	0[a]	0[a]
C	2.5[a]	2[a]	4.5	4.5	5	4	2.5[a]	2[a]	0[a]	0[a]	0[a]
D	4.5	8	10	5.5	0[a]	4	3.5	3	3	0[a]	0[a]
E	2.5[a]	3.5	6.5	5	0[a]	4	4	3.5	0[a]	3	0[a]
F	2a	0[a]	2.5[a]	3.5	0[a]	0[a]	1.5[a]	1.5[a]	0[a]	2[a]	4
G	2.5[a]	4	4.5	4	4	3	2[a]	2.5[a]	0[a]	0[a]	0[a]
Histamine	4.5	5	4.5	5	4.5	12	5	5.5	8	9	6
k82 kU_A/L	1.74	0.52	17.9	1.37	15.3	1.24	1.65	1.92	0.10[a]	0.49	0.62

Abbreviations: HCW, health care worker; NRL, natural rubber latex; SBP, spina bifida patient.
[a]Negative values according to cutoff <3 mm above the negative control for skin prick test results and <0.35 kU_A/L for detection of latex-specific immunoglobulin E by k82.

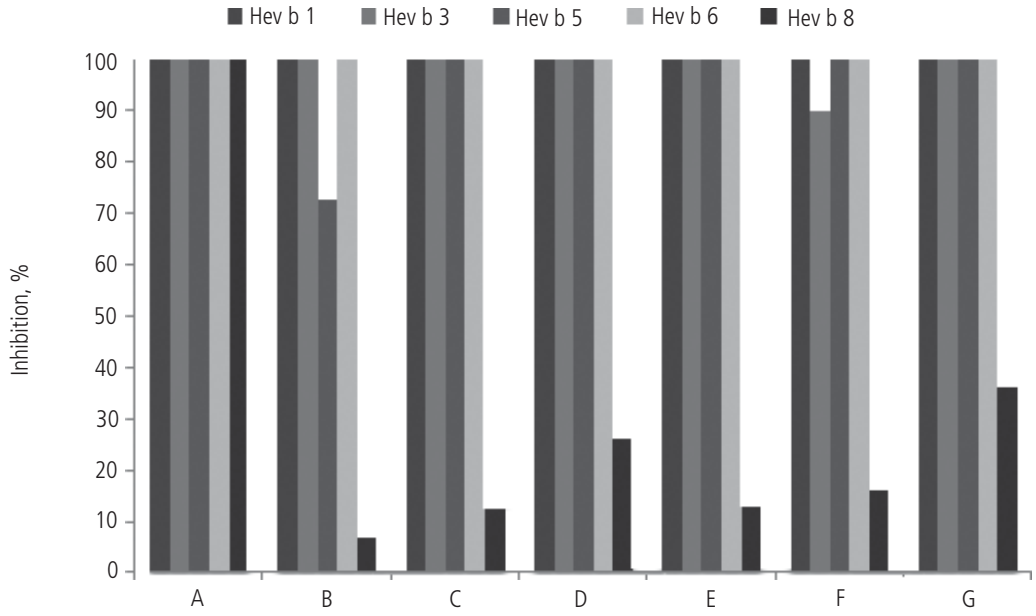

Figure 2. Microarray inhibition: percentages of IgE binding to Hev b 1, Hev b 3, Hev b 5, Hev b 6, and Hev b 8 in the 7 extracts (manufacturers A-G).

was 100% for all extracts. Similar results were observed in the Hev b 3 and Hev b 5 assays, in which only extracts from manufacturers F and B presented an inhibition capacity below 100%. Among all the extracts analyzed, that of manufacturer A was the only one with total allergenic activity for Hev b 1, Hev b 3, Hev b 5, Hev b 6, and Hev b 8. Microarray inhibition control data revealed that no inhibitory effect was detected with the control serum or *D pteronyssinus* extract in the NRL allergen assays. In contrast, for Der p 1 assays, no inhibition was observed using latex extracts and sera. Inhibition was 100% when the *D pteronyssinus* extract was mixed with Der p 1 serum (data not shown).

In Vivo Analysis of Allergenic Activity

All latex-allergic patients except HCW4 had detectable serum latex-specific IgE and a positive SPT result to at least 1 of the 7 extracts (Table 4). Even though HCW4 had negative latex-specific IgE, she was included in the study because she had a consistent clinical history of latex-fruit syndrome with relevant clinical symptoms when exposed to latex products. Moreover, HCW4 had positive SPT results with 2 of the 7 commercial extracts (manufacturers A and D). The wheal reactions induced with the extracts studied were highly variable for all the patients (Table 4). For example, the extract from manufacturer D induced a wheal diameter of 8 mm in patient SBP2, whereas extracts from manufacturers B and F did not induce any visible skin reaction in the same patient. In fact, in almost all individuals, the skin response was negative (<3 mm) to at least 1 of the 7 available extracts. Among the 11 allergic patients, only 1 had a positive skin reaction to all the commercial extracts applied (SBP4). Curiously, latex-allergic patients who also had clinically relevant latex-fruit syndrome (HCW4-6) did not react to most of the latex SPT extracts. In fact, each of these patients only had a positive in vivo reaction to 2 of the commercial latex extracts. Another interesting case is patient SBP5, who, despite having one of the highest values of serum latex-specific IgE levels, showed skin reactivity by SPT to only 2 of the extracts (manufacturers C and G).

Discussion

Early and accurate diagnosis of latex allergy can facilitate appropriate treatment [25]. However, the success of an allergen-specific diagnosis depends on the composition of the allergen used. Although an allergic patient might be exposed to allergens with different compositions on different occasions, the material used for clinical evaluation with SPT should contain all the relevant allergens in appropriate concentrations [26]. Moreover, batch-to-batch consistency in allergen extracts from a single manufacturer and homogeneity in the same extract from different manufacturers should be ensured, so that reproducible and reliable in vivo results can be obtained [19]. Several SPT extracts produced by different companies are commercially available in Europe. Crude NRL is the raw material preferred by manufacturers for the development of the reagents used in SPT [27]. However, the resultant allergen extracts are complex mixtures, whose heterogeneous protein content and effect on allergen potency are not well understood. Therefore, we compared the protein content and the effectiveness of the extracts. These extracts were from 7 different European manufacturers and are commercially available in Portugal.

We first showed that the extracts differed approximately 65-fold between manufacturers in terms of total protein content (Table 2). This value is the highest published to date in the

assessment of total protein content of commercial allergen extracts [17,28,29]. The heterogeneity in protein content we observed was confirmed qualitatively using SDS-PAGE (Figure 1), which revealed different band patterns across extracts from different manufacturers and, in some extracts, equivalent bands with different intensities. These results are an indication of the heterogeneous protein content of the allergen extracts currently used in the diagnosis of NRL allergy.

Second, we detected marked variability in the most important latex allergens (Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02), with different amounts of allergen in all the extracts. Hev b 6.02 was the most widely represented allergen in all cases (Table 3). In fact, the widespread presence of Hev b 6.02 in crude latex has been described elsewhere [30,31]. Hev b 3 and Hev b 5, on the other hand, were not detected in some extracts, in which they are apparently underrepresented. This finding is not surprising, since it has been reported that easily degraded allergens, such as Hev b 3 and Hev b 5, may be entirely absent in diagnostic solutions [26,32]. However, both are major latex allergens. Therefore, as with all clinically relevant latex allergens, Hev b 3 and Hev b 5 should be present in all of the allergen SPT extracts, thus stressing the need for improvement in the method of obtaining NRL extract in order to ensure homogeneous allergen content.

Several explanations can be posited for the variations observed. Differences in allergen content may be due to variability in agricultural practice, storage, and laboratory handling of allergen sources [27]. Additionally, different methods of extraction and treatment of extracts used by individual manufacturers will have a major influence on the presence of allergens and their possible degradation [33].

Finally, we found remarkable variations in in vitro and in vivo allergenic activity between different SPT solutions. Microarray inhibition assays showed that all extracts contained the allergens Hev b 1, Hev b 3, Hev b 5, and Hev b 6, which were able to bind to specific IgE. However, this was not the case for the minor allergen Hev b 8. In fact, all of the extracts presented different allergenic capacity for Hev b 8, with the highest inhibition percentage being observed in the extract from manufacturer A. Hev b 3 and Hev b 5 microarray inhibition results were quite surprising, because, although these allergens were undetectable in some extracts using enzyme immunoassay, their residual content, specifically in solutions from manufacturers B, C, D, and F for Hev b 3 and D and G for Hev b 5, was sufficient to bind to significant amounts of specific IgE. In vivo assays were performed in patients selected from 2 important risk groups, SBP and HCW, in which the prevalence of latex sensitization varies between 5% and 40% [34,35] and 2.7% and 36% [36,37], respectively. All SPTs were performed under routine conditions in order to replicate daily clinical practice. The intensity of the skin reaction varied considerably in all 11 patients (Table 4). In general, a higher number of false-negative SPT results were recorded for the extracts from manufacturers C, B, and F, which were nonammoniated. Indeed, some studies report higher in vivo efficiency and sensitivity for ammoniated latex extracts than for nonammoniated products [13,25].

Although the number of latex-allergic patients in this study is relatively low, we were still able to detect significant differences in the SPT results between the various extracts used. These patients had consistent latex-induced clinical symptoms and signs, and all but 1 had elevated latex-specific IgE levels. Finally, each patient also served as his/her own control in terms of response to the allergen extracts. Thus, we believe that the results for biological variability we found in these patients are reliable. Such variability may be related to differences in the content of the extracts used. In fact, the association we observed between allergen content and SPT reactivity values was based upon the individual sensitization profile in most cases. For example, patients HCW2 and HCW3, who had similar sensitization profiles (Table 1), showed greater skin reactivity to extract from manufacturer A (Table 4), which had the highest sum of sensitizing allergen content (Hev b 5 and Hev b 6.02) (Table 3) and highest total protein content (Table 2). By contrast, in these same patients, extracts B, C, F, and G induced the lowest reactivity, which corresponded to allergen solutions with a lower sum of Hev b 5 and Hev b 6.02 content. As another example, Hev b 8–sensitized patients (HCW1, HCW5, and HCW6) showed more intense skin reactivity to extract from manufacturer A (Table 4), which had the highest Hev b 8 activity in the microarray inhibition experiments (Figure 2).

Hev b 8 is considered a clinically irrelevant allergen that is present in minimal amounts (if any) in medical gloves, suggesting that sensitization to Hev b 8 only is unlikely to result in allergic reaction to latex and that sensitization to latex profilin probably occurs via pollen or food profilins. In this work, 2 of the patients monosensitized to Hev b 8 (HCW5 and HCW6) presented fruit-specific IgE (data not shown) and positive glove use test results, thus demonstrating that the glove used for the challenge tests contained Hev b 8. This finding leads us to question the appropriateness of this allergen in diagnostic extracts. In fact, although the presence of Hev b 8 could induce false-positive results, the extracts should contain the various allergens to which most patients are naturally exposed, including Hev b 8. Therefore, NRL-positive results in allergological tests in subjects with an unrelated history of allergy should be interpreted with caution.

As occurs with other allergen extracts, there may have been a certain degree of nonspecific positivity in response to the extracts we used. Despite a positive result in the latex glove test and a consistent clinical history, patient HCW4 did not have detectable levels of latex-specific IgE and only had positive SPT reactions to the 2 extracts that induced a positive response in most patients. However, all of the remaining patients had positive latex-specific IgE levels and reacted to most of the commercial extracts (albeit with varying intensities), thereby suggesting consistency of response and low probability of nonspecific responses.

Our SPT results showed that, for most patients, at least 1 of the extracts was not able to elicit a positive result. Although the functional significance of these findings should be further investigated, in practice, depending on the extract used by a particular allergist, the results for latex-allergic patients may be either negative or positive depending on the absence or presence of latex allergy. Thus, the use of SPT solutions with poor sensitivity could prove disastrous if clinical decisions are made solely on the basis of these results. This finding confirmed that the absence of important components in SPT reagents can

affect the diagnosis of latex allergy and suggests that SPT-based diagnosis of latex allergy should be carried out using at least 2 extracts from different companies in order to minimize the possibility of false-negative results. Furthermore, some of the allergen extracts used for the preparation of skin test reagents are also used for the preparation of allergen-specific immunotherapy treatments. Differences in the presence of allergens may have even more important implications for such therapeutic extracts, because allergens that are missing or present in small amounts will fail to induce protective immune responses against these components [29].

In summary, commercially available latex extracts from different manufacturers show considerable heterogeneity in their protein and major allergen composition, and this may negatively affect the accuracy of SPT testing. Our findings should alert physicians to the variable skin reactivity of NRL extracts from different manufacturers. In addition, similar questions about commercial extracts such as therapeutic reagents should also be raised. Our results reveal considerable room for improvement and standardization of the reagents currently used for clinical purposes.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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