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Different *in vivo* reactivity profile in health care workers and patients with spina bifida to internal and external latex glove surface-derived allergen extracts

C.M. Peixinho,* P. Tavares-Ratado,*†‡ M.F. Gabriel,* A.M. Romeira,§ C. Lozoya-Ibanez,¶ L. Taborda-Barata,*†** and C.T. Tomaz*

*CICS-UBI, Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
†Department of Health Sciences, University of Beira Interior, 6201-001 Covilhã, Portugal
‡Laboratory of Clinical Pathology, Sousa Martins Hospital, ULS Guarda, Portugal
§Department of Allergy and Clinical Immunology, Dona Estefânia Hospital, Lisbon, Portugal
¶Department of Allergy and Clinical Immunology, Amato Lusitano Hospital, Castelo Branco, Portugal
**Department of Allergy and Clinical Immunology, Cova da Beira Hospital, Covilhã, Portugal

Correspondence
Cândida T. Tomaz.
E-mail: ctomaz@ubi.pt

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Summary

Background Allergy to natural rubber latex is a well-recognized health problem, especially among health care workers and patients with spina bifida. Despite latex sensitization being acquired in health institutions in both health care workers and patients with spina bifida, differences in allergen sensitization profiles have been described between these two risk groups.

Objective To investigate the *in vivo* reactivity of health care workers and patients with spina bifida to extracts of internal and external surfaces of latex gloves and also to specific extracts enriched in major allergens for these risk groups.

Methods Gloves from different manufacturers were used for protein extraction, and salt precipitation and hydrophobic interaction chromatography (HIC) were applied to obtain the enriched latex extracts. The major latex allergens were quantified by an enzyme immunoassay. The extracts obtained were tested in 14 volunteers using skin prick tests (SPT).

Results Latex glove extracts enriched in the hydrophobic allergens that are most often seen in patients with spina bifida were obtained by selective precipitation, whereas HIC produced extracts enriched in the hydrophilic allergens commonly found in health care workers. The health care workers had positive SPTs to glove extracts from internal surfaces and to the hydrophilic allergen-enriched extracts. By contrast, patients with spina bifida had larger skin reactions both to external glove extracts and to the extracts enriched with the hydrophobic major allergens for this risk group. Despite the protein concentration of these extracts being less than half the concentration of the commercial extract, the weal-and-flare reactions were of similar magnitude.

Conclusion Using novel latex extracts, our study showed a different *in vivo* reactivity pattern in health care workers and in patients with spina bifida to extracts of the internal and external surfaces of gloves, which suggests that sensitization may occur by different routes of exposure, and that this influences the allergen reactivity profiles of these risk groups.

Allergy to natural rubber latex (NRL) is a significant medical and occupational public health problem, especially for health care workers and patients with spina bifida, with an exponential increase in prevalence in the 1980s and 1990s, possibly due to the drastic increase in glove usage in response to concerns about AIDS and other infectious diseases.[1–3] Over the past 10 years, latex sensitization and allergy in health care workers have decreased because of avoidance practices that include a latex-reduced environment by wearing powder-free latex gloves and synthetic latex-free gloves in health institu-

tions.[4–7] However, despite significant progress in the management of the disease, the prevalence values of NRL allergy in health care workers and patients undergoing multiple surgery, such as patients with spina bifida, are 3–17% and 40–50%, respectively.[3,8]

Although latex sensitization is mainly acquired in health institutions by both patients with spina bifida and health care workers, these risk groups may have different sensitization profiles to latex allergens. In fact, whereas major allergens for patients with spina bifida are hydrophobic (Hev b 1 and Hev b 3), major allergens for health care workers are hydrophilic (Hev b 2, Hev b 5, Hev b 6·01 and Hev b 13).[9–11] Although genetic factors may be involved,[12,13] environmental exposure to natural rubber latex products is necessary for sensitization in both risk groups.[14]

In this context, the different reactivity profiles observed between health care workers and patients with spina bifida may be partly due to different direct environmental exposures, namely the different allergen composition of the internal and external surfaces of latex gloves.[15–17]

The purpose of this work is to study, for the first time, the *in vivo* reactivity of health care workers and patients with spina bifida to extracts from the internal and external surfaces of gloves to determine whether the sensitization profiles are related to their different exposures to the latex glove surfaces. Bearing in mind the paradigm of hydrophobic major allergens in patients with spina bifida *vs.* hydrophilic major allergens in health care workers, the effectiveness of selective precipitation and hydrophobic interaction chromatography (HIC) to produce enriched allergen fractions from latex was also explored. In order to obtain the real allergome, closer to the source of sensitization of health care workers and patients with spina bifida, we aimed to develop a process for the generation of extracts enriched in the major latex allergens from latex gloves and to assess *in vivo* the sensitization to these specific extracts. It was also our goal to assess the adequacy of different latex glove extracts produced in the study (internal, external and extracts enriched in the major latex allergens) for *in vivo* skin tests.

Materials and methods

Glove extracts

To obtain the NRL glove extracts, six different glove brands were collected from health institutions in Portugal, during 2009. Extraction of proteins from the gloves was performed on a bath, for 24 h at 37 °C, using 200 mL of phosphate-buffered saline (PBS) and 20 g of glove. After extraction, the rubber products were removed and the extracts were centrifuged at 4000 *g* for 10 min, filtered through 0·45-μm membranes and ultra-concentrated on an AMICON unit with membranes NMWL 1000 Da (Millipore, Billerica, MA, U.S.A.). Dialysis against water was performed on a Pierce Snakeskin Pleated NMWL 3500 Da membrane (Pierce, Rockford, IL, U.S.A.). The product was then lyophilized, and stored frozen (−20 °C) for further analysis.

External glove surface extracts were prepared by sealing the proximal wrist end. Extracts of internal surfaces of the gloves were prepared in the same way after turning internal surfaces out. Extracts for selective precipitation and HIC experiments were obtained from whole gloves cut into pieces. To avoid cross-contamination of NRL allergens, vinyl gloves were worn for all laboratory procedures.

Fractionation of glove extracts by ammonium sulphate precipitation

Three hundred milligrams of lyophilized latex glove extract were dissolved in 20 mmol L^{-1} tris-HCl, pH 7·5. A precipitation step was 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 *g* for 15 min, at 4 °C. The same process was repeated after adding 50% and 75% ammonium sulphate (precipitated extracts were denoted P2 and P3, respectively). The extracts obtained from the three precipitation steps were dialysed against water, lyophilized and were stored at −20 °C.

Hydrophobic interaction chromatography

HIC was performed using a fast protein liquid chromatography (FPLC) system (GE Healthcare Biosciences, Uppsala, Sweden) on a Sepharose CL-6B column modified with butyl-1,4-bis-(2,3-epoxy-propoxyd), prepared in our laboratory according to Queiroz *et al.*[18] The gel was packed in an XK column 16/20 (GE Healthcare Biosciences) and equilibrated with PBS 0·01 mol L^{-1}, with ammonium sulphate, 1·0 mol L^{-1}, pH 7·4. An injection of latex glove extract (100 μL) was loaded onto the column at a flow rate of 1 mL min^{-1} and the absorbance of the eluate was continuously monitored at 280 nm. Elution was carried out with PBS 0·01 mol L^{-1}, pH 7·4. Extracts obtained previously by selective precipitation, P1, P2 and P3, were also loaded onto the column in a series of experiments with different eluent composition. The column was equilibrated with PBS 0·01 mol L^{-1}, pH 7·4, with concentrations of ammonium sulphate in the eluent varying between 0·5 and 1·5 mol L^{-1}. Elution was carried out using PBS 0·01 mol L^{-1}, pH 7·4. Fractions were concentrated, dialysed against water and lyophilized as previously described.[16] All assays were performed at room temperature.

Capture enzyme immunoassay for quantification of allergens

Quantification of Hev b 1, Hev b 3, Hev b 5 and Hev b 6·02 was performed using a commercial Kit (FITkit™, IcoSagen, Tartu, Estonia) according to the manufacturer's instructions. The detection limit levels of allergens on FITkit™ were: Hev b 1, 1·2 μg L^{-1}; Hev b 3, 2·3 μg L^{-1}; Hev b 5, 0·5 μg L^{-1}; Hev b 6·02, 0·1 μg L^{-1}. When allergen readings were below the detection limit levels were denoted as zero. All assays were performed in triplicate.

Table 1 Demographic and clinical characterization of health care worker participants and patients with spina bifida

Patients	Sex/Age	Clinical symptoms triggered by latex-H rich environments	[a]Phadiatop™ (kUa L^{-1})	[b]k82™ (kUa L^{-1})	rHev b reactivity (kUa L^{-1})
1. HCW	Female 31	Nasal and ocular pruritus, wheezing, occasional dyspnoea, cough and increased bronchial secretions	33·4	1·24	rHev b 8 (4·82)
2. HCW	Female 40	Acute urticaria	20·3	6·53	rHev b 5 (4·55) rHev b 6·01 (2·32) rHev b 6·02 (2·32)
3. HCW	Female 20	Nasal and ocular pruritus, urticaria	1·0	0·65	rHev b 8 (1·08)
4. HCW	Female 45	Rhinoconjunctivitis, hand dermatitis, pruritus, urticarial bouts	< 0·35	< 0·35	No serum reactivity was detected
5. HCW	Female 33	Nasal and ocular pruritus, sneezing bouts, urticarial rashes, anaphylaxis	1·51	0·49	rHev b 8 (1·01)
6. HCW	Female 34	Hand dermatitis, ocular and nasal pruritus	n.d.	1·65	rHev b 5 (1·01) rHev b 6·02 (0·63)
7. HCW	Female 40	Nasal pruritus, hand oedema with pruritus	n.d.	1·92	rHev b 5 (0·97) rHev b 6·01 (0·75) rHev b 6·02 (1·02)
8. PSB	Female 20	Urticarial rashes, ocular and nasal pruritus, sneezing, rhinoconjunctivitis	30·6	9·79	rHev b 1 (2·79) rHev b 3 (2·47) rHev b 5 (6·65)
9. PSB	Female 17	Ocular and nasal pruritus	0·11	1·81	rHev b 1 (1·06) rHev b 5 (0·37) rHev b 6·01 (0·54)
10. PSB	Female 22	Urticaria, thoracic discomfort	1·58	0·57	rHev b 6·01 (0·91)
11. PSB	Male 15	Hands and face erythema, urticarial rashes, ocular angio-oedema, ocular pruritus, pruritus hands	16·4	1·43	rHev b 3 (1·18)
12. PSB	Female 23	Ocular pruritus with eyelid oedema, nasal pruritus, thoracic discomfort, wheezing	n.d.	17·9	rHev b 1 (0·92) rHev b 5 (61·7) rHev b 6·01 (17)
13. PSB	Male 18	Lips angio-oedema, ocular pruritus, urticaria	< 0·35	< 0·35	No serum reactivity detected
14. PSB	Female 19	Asymptomatic	79·9	15·1	rHev b 5 (9·29)

[a]Multiallergen IgE antibody screen, cutoff 0·35 kUa L^{-1}. [b]Allergen-specific IgE antibody test, cutoff 0·35 kUa L^{-1}. n.d., not determined; HCW, health care workers; PBS, patient with spina bifida

Characterization of patients allergic to latex

Fourteen volunteers (seven health care workers and seven patients with spina bifida) diagnosed as having latex allergy, with a consistent clinical history, were recruited for the study. The participants' demographic and clinical characterizations are described in Table 1.

Determination of allergen-specific IgE

Latex-specific IgE levels in the sera of the 14 patients were measured using total latex extract k82 and recombinant Hev b allergens (ImmunoCAPS™, Phadia, Uppsala, Sweden).

Skin prick tests

Having obtained written informed consent from all patients and subjects, skin prick tests (SPT) were performed in accordance with recommendations of the European Academy of Allergy and Clinical Immunology. The Ethics Committee of the collaborating hospitals approved this study.

The allergenic components used for SPT included a commercial latex extract (Laboratorios LETI SL, Tres Cantos, Madrid, Spain; 1000 μg protein mL^{-1}) and specially prepared extracts from the interior and exterior surfaces of latex gloves, and preparations enriched in the major allergens (400 μg protein mL^{-1}) fractionated by selective precipitation of extracts from patients' gloves and by HIC from health care workers' gloves. Tests were performed in duplicate on the volar surface of both forearms. Histamine (0·1%; 10 μg mL^{-1}) and PBS were used, respectively, as positive and negative control reagents. SPT sites were wiped clean after 15 min and the weal and flare reactions were carefully recorded. Positive weal and flare reactions (≥ 3 mm, in the presence of a negative control) were outlined by a marker, transferred onto transparent tape and kept as a permanent record, after having measured the mean weal diameter.

Results

Selective precipitation with ammonium sulphate

Proteins of the total glove extract were first fractionated by selective precipitation using 25%, 50% and 75% ammonium

Table 2 Quantification of allergens Hev b 1, Hev b 3, Hev b 5, Hev b 6·02 by enzyme immunoassay of latex glove extracts. Precipitates, P1, P2 and P3, were obtained by selective precipitation with 25%, 50% and 75% ammonium sulphate. Peak 1, peak 2 and peak 3 were recovered by hydrophobic interaction chromatography

	Hev b 1 (μg L^{-1})[a]	Hev b 3 (μg L^{-1})[a]	Hev b 5 (μg L^{-1})[a]	Hev b 6·02 (μg L^{-1})[a]
Internal extract	28·2	14·1	283·1	250·0
External extract	55·7	54·0	29·0	317·8
Selective precipitation				
P1 (pPSB)[b]	49·16	26·41	0	5·10
P2[b]	8·26	0	1·22	6·65
P3[b]	7·41	0	1·03	7·44
HIC				
Peak 1 (pHCW)	0	0	3·11	7·65
Peak 2	12·50	0	0	6·09
Peak 3	6·05	6·05	0	6·76

[a]FITKit™. [b]Precipitation steps 1, 2 and 3.

sulphate in tris-HCl. The extract obtained with 25% of ammonium sulphate (P1), denominated pPSB, showed enrichment in the hydrophobic allergens Hev b 1 and Hev b 3 that are major allergens for patients with spina bifida (Table 2), despite the presence of Hev b 6·02 (5·10 μg L^{-1}). Enzyme immunoassay (EIA) quantification of Hev b 1 and Hev b 3 was 49·16 μg L^{-1} and 26·41 μg L^{-1}, respectively. On the other hand, extracts obtained using 50% and 75% of ammonium sulphate (P2 and P3) had a mixture of hydrophilic allergens (Hev b 5 and Hev b 6·02) and Hev b 1 (Table 2).

Hydrophobic interaction chromatography

HIC was applied to samples of total extracts from gloves, loaded onto an epoxy support. Under the conditions used, two peaks were resolved corresponding to unbound proteins and were eluted with 1·0 mol L^{-1} ammonium sulphate in PBS (peaks 1 and 2). Bound proteins were eluted (peak 3) by subsequently decreasing ammonium sulphate concentration in eluent buffer to 0 mol L^{-1}, in a stepwise mode. Quantification of the four major allergens Hev b 1, Hev b 3, Hev b 5 and Hev b 6·02 by EIA revealed that the first peak (denominated pHCW) contained only the hydrophilic allergens most important in health care workers, Hev b 5 and Hev b 6·02 (Table 2). Analysis of peak 2 showed a latex fraction with Hev b 1 at a two-fold concentration (12·50 μg L^{-1}), when compared with Hev b 6·02 (6·09 μg L^{-1}), whereas peak 3 showed a mixture of Hev b 1, Hev b 3 and Hev b 6·02. Briefly, HIC assays of total glove extracts promoted a selective separation of a mixture of hydrophilic allergens (major for health care workers) in pHCW fractions, whereas a mixture of hydrophobic allergens was found in peaks 2 and 3 (Table 2).

Skin testing

The ability of latex glove extracts to induce reactions *in vivo* was demonstrated by SPT in 14 participants with a clinical diagnosis of latex allergy (seven health care workers and seven patients with spina bifida), using extracts from internal and external glove surfaces and also extracts enriched in the major allergens for patients with spina bifida (pPSB) and health care workers (pHCW).

Table 3 Skin prick test results of 14 participants using a commercial extract and specially prepared latex glove extracts (internal surface, external surface and extracts enriched with the major allergens for health care workers and for patients with spina bifida)

Patients	Internal glove extract	External glove extract	pHCW (enriched with major allergens for HCW) (400 μg mL^{-1})	pPSB (enriched with major allergens for spina bifida) (400 μg mL^{-1})	Commercial extract (1000 μg mL^{-1})	Positive control	Negative control
1 (HCW)	+	−	+	−	+	+	−
2 (HCW)	+++	+	+++	++	+++	+	−
3 (HCW)	+	−	−	−	+	+	−
4 (HCW)	+	−	+	−	+	+	−
5 (HCW)	+	−	+	−	+	+	−
6 (HCW)	+	−	++	+	+	+	−
7 (HCW)	+	−	++	+	+	+	−
8 (PSB)	−	+	+	++	+	+	−
9 (PSB)	+	++	+	+++	+	+	−
10 (PSB)	+	+	−	++	++	+	−
11 (PSB)	−	++	+	++	+	+	−
12 (PSB)	+	++	−	++	++	+	−
13 (PSB)	−	−	−	−	−	+	−
14 (PSB)	−	−	−	−	−	+	−

+, Positive reaction: weal diameter ≥ 3 mm; ++, positive reaction: weal diameter ≥ 5 mm; +++, highly positive reaction: weal diameter > 15 mm; −, negative reaction: weal diameter < 2 mm; HCW, health care worker; PSB, patient with spina bifida.

The results showed that all the health care workers had positive SPT to extracts from the internal surfaces of the gloves but had no detectable skin reactivity to the external surfaces, except subject 2, who had a small reaction to this extract (Table 3).

By contrast, among patients with spina bifida who showed positive SPT, all had higher reactivity to extracts from the external surfaces of the gloves than to the internal ones, except for patient 10, who had an equally positive reaction for both extracts. Patients with spina bifida 9, 10 and 12, who have serum Hev 6·01-specific IgE (Table 1), also showed slight reactivity to internal extracts. Two patients with spina bifida (patients 13 and 14) did not show any skin reactivity to the extracts used for SPT. In fact, patient 13 had negative results for all serum tests performed (Phadiatop™, k82™ and rHev b allergens), but had positive clinical symptoms to latex-enriched environments and to provocation tests with latex gloves (Table 1). On the other hand, patient 14 is presently asymptomatic, despite having high latex-specific IgE to total latex extract k82 and to latex recombinant allergens.

Interestingly, all health care workers had positive SPT to pHCW extract (enriched in the major allergens for health care workers), with the exception of participant 3 who did not show reactivity to either of the enriched extracts, pHCW or pPSB (Table 3). Additionally, health care workers who only had serum-specific IgE to Hev b 8 (participants 1, 3 and 5) showed a lower degree of skin reactivity to the extracts used, being always negative for the external glove extract and for pPSB. It is noteworthy that health care workers who had serum-specific IgE to Hev b 6·02 (participants 2, 6 and 7) also showed reactivity to pPSB (Tables 1 and 3). Patients with spina bifida showed a strongly positive reaction to this enriched extract, confirming the results obtained for latex glove extracts from external surfaces.

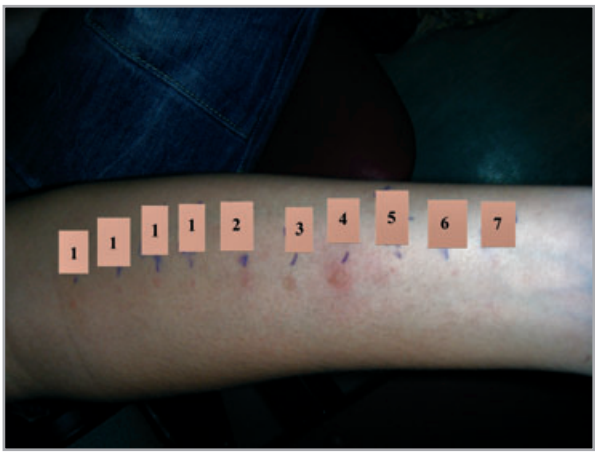

Fig 1. Skin prick test results of a patient with spina bifida (patient 8): 1 Commercial latex extract; 2 External glove surface extract; 3 Positive control; 4 Glove extract enriched with hydrophobic major allergens (pPSB) for patients with spina bifida; 5 Glove extract enriched with hydrophilic major allergens (pHCW) for health care workers; 6 Internal glove surface extract; 7 Negative control.

It should be noted that SPT using enriched latex glove extracts, with a total protein concentration of 400 μg mL^{-1}, had similar or, in five participants, higher skin reactivity than that obtained with the commercial latex extract although this had a higher protein concentration, 1000 μg mL^{-1} (Table 3). An illustrative photograph of the forearm of patient 8 after SPT with the latex glove extracts produced in this study is shown in Fig. 1.

Discussion

On the basis of the evidence of environmental latex sensitization, our data indicates that differential contact with latex gloves may be associated with different *in vivo* allergen reactivity profiles in health care workers and in patients with spina bifida. Despite the great variability that is usually found between SPT and serum latex-specific IgE in patients and others, we observed a different pattern of reactivity for each latex risk group in this work.

This study has novel *in vivo* data showing that, in general, health care workers have a higher SPT reactivity to allergens from the internal surfaces of latex gloves and to the enriched hydrophilic extracts pHCW, whereas patients with spina bifida showed stronger positive skin reactions to allergens from external surfaces and to the enriched hydrophobic extracts pPSB (Table 3). Patient 13, who did not have skin reactivity to latex extracts, did not have serum reactivity to any recombinant latex allergens. These results may suggest that his clinical symptoms were triggered by nonlatex components of the gloves. On the other hand, serological and SPT results from patient 14 could indicate the presence of sensitization to latex allergens in the absence of clinically relevant allergic symptoms.

It is interesting to observe that patients with spina bifida with elevated serum levels of specific IgE to Hev b 5 and/or Hev b 6·01 (patients 8, 9, 10 and 12) also showed mild skin reactivity to extracts from internal glove surfaces and/or to the enriched hydrophilic pHCW extracts, as these allergens are expected to be present mainly in these extracts.

The high concentration of Hev b 6·02 in all the extracts studied (Table 2) confirmed the widespread presence of this allergen in latex products,[19,20] and may explain the reactivity of health care workers 2, 6 and 7 to the hydrophobic pPSB extract (Table 3). In fact, all these subjects showed the presence of serum Hev b 6·02 specific IgE (Table 1).

Although some participants had a clinical history of latex allergy associated with positive SPT to latex extracts they did not show any positive latex extract-specific IgE levels (subject 4). In fact, it is known that some patients with allergic disease are classified as having false-negative IgE antibody test results, and, in addition, the presence of IgE antibodies is necessary but not sufficient for the expression of allergic disease.[21] In fact, the immunological mechanisms underlying biological reactivity to latex glove allergens in subject 4 may be non-IgE mediated.

Moreover, some allergic patients are known to respond differently to recombinant extracts with different combinations of allergens that are identical except for minor differences in their primary amino acid composition.[22] In fact, recombinant allergens that do not represent all isoallergen forms might not be able to detect all clinically relevant IgE antibodies.[21] On the other hand, allergens extracted from their natural sources may be heterogeneous and often contain nonallergic proteins. It was reported that natural extracts also vary in their allergen composition and potency.[23] Overall, SPT results suggest that preferential contact with the internal surfaces of latex gloves by health care workers may represent the main route of sensitization in this risk group. In fact, the previous work of our research group showed a different allergen composition in the inner and outer surfaces of latex gloves, suggesting a relationship between latex allergen location and sensitization routes in latex risk groups.[16,17] It is known that health care workers make direct contact with the internal surface of latex gloves and with aerosolized glove powder and have clinical manifestations such as contact dermatitis, nasal and ocular pruritus, wheezing, dyspnoea, cough and increased bronchial secretions.[24] By contrast, the main route of senzitization of patients with spina bifida is the direct contact with the external surfaces of latex gloves during surgical procedures.

Genetic factors may also be related to latex allergy in health care workers.[14] According to Brown *et al.*,[12] the significant association of IL-13 and IL-18 promoter polymorphisms with latex allergy may suggest a potential location for genetic control in the induction of latex allergy in health care workers. Rihs *et al.*[13] also showed that HLA-DQ8 and HLA-DQ8-DR4 haplotypes are positively associated with specific immune responses to Hev b 6·02 in health care workers with latex allergy, but not in patients with spina bifida.

In patients with spina bifida, the number of surgical procedures rather than the spina bifida *per se*, is related to sensitization to latex.[15,25] In fact, currently, latex allergy in children with spina bifida is regarded as a multifactorial situation associated with a propensity towards latex sensitization, early exposure and number of surgical procedures.[26] Overall, apart from possible genetic factors, environmental factors play a crucial role in the development of NRL sensitization, for both health care workers and patients with spina bifida.[14] In this regard, our data suggest that the key question concerns the different routes of exposure for different risk groups for latex allergy.

Another interesting and important feature of this study was the preparation of enriched extracts with major allergens for the latex risk groups using selective precipitation and HIC. SPT results showed that our extracts exhibit good allergenic capacity and good specificity for *in vivo* diagnosis, even though they contain less than half of the protein concentration in the commercial latex extract (Table 3). This is very important as we were able to detect sensitization to latex allergens in all the participants who had latex-related symptoms, which suggests that our extracts are sensitive enough for diagnosis and also decrease the exposure of different risk groups to allergens that are less relevant for the risk group-oriented diagnosis (e.g. health care workers can be sufficiently diagnosed as latex sensitive using only the extracts from the inner glove surface, thereby avoiding unnecessary exposure to extracts from the outer surface). These novel findings will be further evaluated with a larger number of patients.

Our study also emphasized the potential of selective precipitation and HIC in the separation of latex allergens by performing breakthrough experiments in order to produce latex extracts that are specific to the latex risk groups and which were obtained directly from the source of allergens, latex gloves. Selective precipitation and HIC were applied with the purpose of taking advantage of the different hydrophobicities shown by the major latex allergens in health care workers, Hev b 2, Hev b 5, Hev b 6·01 and Hev b 13, which have mainly hydrophilic characteristics, and in patients with spina bifida, Hev b 1 and Hev b 3, which are hydrophobic.[9–11] Although this technique is not highly specific, in this study it was applied for both the enrichment and concentration of latex allergens. Thus, salt precipitation of glove latex extract promoted a selective separation and enrichment in the hydrophobic major allergens seen in patients with spina bifida in the pPSB fraction (Table 2). As discussed above, the presence of Hev b 6·02 in almost all fractions is justified by its abundance and widespread presence in latex,[19,20] which made the purification process difficult. HIC results showed a selective separation of hydrophilic major allergens in the pHCW fraction (Table 2), which holds potential for the health care workers risk group.

Based upon the differential latex-sensitization profiles in both health care workers and patients with spina bifida, specific latex glove extracts could represent an alternative approach not only to a more targeted and effective diagnosis of latex allergy but also to a more reliable and possibly safer assessment than with conventional latex extracts.

In conclusion, our study showed, for the first time, *in vivo* a differential skin reactivity of health care workers and patients with spina bifida to the internal and external surfaces of rubber latex gloves and to extracts enriched in the major latex allergens. This suggests that differential contact with latex glove surface allergens may be associated with different reactivity profiles to latex allergens in health care workers and patients with spina bifida.

What is already known about this topic?

- We have previously shown that there are substantial differences in the composition of latex allergen profiles between the internal and external surfaces of natural rubber latex gloves.
- Concentrations of Hev b 1 and Hev b 3, major allergens for patients with spina bifida, were found to be significantly higher on external surfaces, while internal surfaces had higher levels of the major allergens for health care workers, Hev b 5 and Hev b 6·02.

What does this study add?

- Novel data on a differential *in vivo* reactivity pattern in health care workers and patients with spina bifida to internal and external glove extracts, which suggests that sensitization by different routes of exposure may clearly influence the reactivity profiles in these risk groups.

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