

Exercise with latex sport bands represents a risk for latex allergic patients

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Abstract

Based on two clinical observations of adverse reactions during exercise with latex sport bands, we aimed to assess the possible risk for allergic patients posed by this equipment by investigating allergen content and IgE binding potential. Protein extracts of three different latex sport bands were characterized with sera of latex allergic patients. The IgE recognition profile of the allergic patients was identified by component resolved diagnosis and the allergen composition of the extracts was characterized by inhibition assays with the recombinant latex allergens Hev b 1, 3, 5, 6.02, and 8. The sera showed pronounced IgE binding to all three blotted extracts, however with diverse patterns. Inhibition assays revealed the presence of Hev b 1, 3, 5, and 8 in latex sport band extracts. The clinical relevance of contained allergens was demonstrated by strong skin reactions when testing with latex sport bands. From our results we conclude that latex sport bands contain clinically relevant allergens and may cause latex allergic individuals to experience allergic symptoms, potentially amplified by exercise-induced mechanisms. Even though latex is labeled on products, it is important that patients as well as athletic trainers and physical therapists recognize the risk of adverse reactions with these bands. © 2007 Elsevier B.V. All rights reserved.

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1. Introduction

Due to its functional properties, natural rubber latex (NRL) from *Hevea brasiliensis* is the basis for manufacturing a large variety of products and, thus, plays an important role in modern life. Nevertheless, NRL also triggers adverse reactions and IgE mediated latex allergy is potentially hazardous for the allergic patient.

In the health care environment, latex allergy is a well-recognized problem. On the one hand, accidental exposition to latex may lead to life threatening complications in latex allergic patients [1,2]. On the other hand, health care workers themselves

have a high risk of sensitization to latex due to the increased level of latex exposure in their occupational environment [3–6]. The prevalence of latex allergy in health care workers was shown to range between 7 and 10% [3], whereas only 1% of the general population is estimated to be affected with main relevance for subjects with respiratory symptoms [7,8]. So far, almost exclusively latex products used in health care have been studied for their allergen content and composition [9]. The second group of persons having an increased risk of latex sensitization are spina bifida patients or patients born with uro-genital defects where the estimated prevalence of latex allergy varies between 15 and 72% [10,11]. In both at risk groups, sensitization through skin contact with latex products and mucosal contact with latex allergens bound to powder like cornstarch were demonstrated [12].

Nevertheless, the danger of encountering latex products is not confined to these conditions. Due to its physicochemical properties, latex is an important material for sport equipment. Indeed, sporadically delayed type contact dermatitis was observed in students using latex sport equipment [13]. Only recently athletic trainers were advised of the possibility of immediate type allergic reactions upon latex exposure during exercise, and the usage

Abbreviations: SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; NRL, natural rubber latex; kUA/l, kilo units antigen-specific per liter; Tris, tris-(hydroxymethyl)aminomethane.

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of latex-free alternatives was suggested [14]. Thus, the potential danger associated with latex products is today not sufficiently recognized in this environment.

Recently, two patients with a prior history of latex hypersensitivity reported severe adverse reactions during exercise with latex sport bands. These bands have never been examined for their allergen content to the best of our knowledge. Thus, the aim of this study was to examine whether rubber bands could be a source of latex allergens by testing sera of “regular” latex allergic patients in comparison with these two cases.

2. Materials and methods

2.1. Study Participants

Two patients previously diagnosed as latex allergic experienced allergic symptoms during exercise with latex sport bands. Patient A, a 43-year old female health care worker (RAST-class 4.7, 42.0 kUA/l, total IgE 770 kU/l, skin prick test ++++) complained of shortness of breath after her first contact with latex sport bands during work-out.

Patient B was a 14-year old boy having undergone surgery due to a testicular tumor at the age of 8 months. He was diagnosed as latex allergic at age 9 years by a local dermatologist (skin prick test ++++) and experienced an episode of allergic rhinoconjunctivitis when performing sport with a latex band for the first time. Furthermore, 10 sera of randomly selected latex allergic subjects reporting adverse reactions after exposure to latex products, or after ingestion of latex associated foods were included in this study (patient 1–10).

For control purposes, we used serum of a nonallergic, nonatopic subject.

2.2. Protein extracts of latex sport bands

Three different latex sport bands, Theraband™ yellow (The Hygienic Cooperation, Akron, Ohio), Theraband® blue, and Body-Band™ red (Dittmann, Fuchsstadt, Germany) were included in the testing.

The sport bands were cut into 1 cm² pieces and incubated in 20 mmol/l Tris-buffer with 1 mmol/l sodium azide at room temperature under continuous agitation for 7 h. Supernatants were filled into dialysis membrane with a cutoff of 1000 Da and dialyzed against distilled water. Thereafter, extracts were lyophilized and stored at −20 °C.

Protein content was measured by the Bradford method [15] using the Bradford protein assay Bio-Rad® quick start (Bio-Rad, Hercules, Canada).

2.3. SDS-PAGE and immunoblot

Latex sport band extracts were separated by SDS-PAGE according to Laemmli [16] using 12% separation and 8% stacking gels. Protein bands were made visible by the silver stain procedure [17] and electroblotted onto nitrocellulose (Protran®, Whatman Inc., Florham Park, NJ) [18]. After drying, unspecific binding sites on the membranes were blocked

with buffer (0.75% di-sodium hydrogenphosphate, 0.1% sodium hydrogenphosphate, 0.1% powdered milk, 0.5% TWEEN 20) for 1 h at room temperature. Patient and control sera were diluted fivefold in the blocking buffer and incubated with blotted extracts overnight at 4 °C. After intensive washing, bound patient antibody was detected by I¹²⁵ labeled antihuman antibody (IBL, Hamburg, Germany) and visualized by autoradiography.

2.4. Component resolved diagnosis

The comprehensive sensitization profile of patients A and B were investigated using the ISAC CRD79® allergen chip (VBC Genomics Bioscience Research LLC, Vienna, Austria) representing a panel of 79 common allergens immobilized onto solid phase on a chemically modified microscopy slide. The allergens included in the testing were the latex allergens rHev b 1, rHev b 3, rHev b 5–10, and rHev b 11, pollen allergens, allergens from animal sources like dog or cat and common food allergens [19].

The testing was performed according to the manufacturers assay protocols [20]. In short, 20 µl of patients' sera were incubated on the chip for 2 h in a humid chamber and allergen specific IgE bound to the individual allergens were detected with fluorescence-labeled detection antibodies. Fluorescence was measured using a commercial biochip reader (ScanArray Gx, Perkin Elmer, Wellesley, MA), and the IgE titer of each allergen calculated with a custom software package [20].

In immunodot assays, we performed component resolved diagnosis with the recombinant latex allergens rHev b 1, rHev b 3, rHev b 5, rHev b 6.02, and rHev b 8 (Biomay, Vienna, Austria). The allergens were dotted on a nitrocellulose filter (pore size 0.45 µm; Millipore, Billerica, MA) in triplicates. Dots were blocked and incubated with diluted patient's serum (1:5) at 4 °C overnight. After repeated washing, bound antibodies were detected as described above for IgE immunoblot.

2.5. Inhibition assay

Sera of latex allergic patients diluted in blocking buffer were preincubated with 10 µg of recombinant major latex allergens (rHev b 1, 3, 5, 6.02, and 8) overnight at 4 °C. Single patient's sera were diluted 1:5 in buffer, serum pools 1:7. Blotted latex extracts were incubated with the preincubated sera overnight at 4 °C. Based on component resolved diagnosis serum of patient B was used for detection of Hev b 1, serum of patient A for Hev b 5 and 6.02. Hev b 3 or Hev b 8 content in latex sport bands were evaluated using a serum pool of patients 5, 7, and 8 or serum from patient 9, respectively. Bound IgE antibodies were detected by autoradiography as described above.

2.6. Prick-to-prick testing

The potential of latex sport bands to elicit positive skin reactions were examined by prick-to-prick testing in patient A.

Pieces of the three latex sport bands were applied in duplicates on the inside of the lower forearm and skin was punctured by a lancet before removing the sport band. Additionally, latex, banana, kiwi, and fig skin test substances were used for skin prick testing. Histamin-dihydrochloride (10 mg/ml) served as a positive, sodium saline as a negative control. Reactions were read after 20 min and a diameter >3 mm was defined as positive (+) [21].

3. Results

3.1. Characteristics of latex sport band extract

We extracted protein from $2.5 \times 0.14 \text{ m}^2$ (35 dm²) of latex sport bands, corresponding to the size usually used for exercise. From the Therabands® 0.01‰ protein could be extracted

(0.6 mg in the case of Theraband® yellow, 1 mg in the case of Theraband® blue) each, while the extractable protein of the Body-Band® amounted to 0.06‰ (4.4 mg) of its weight, corresponding to 17 µg/dm² (Theraband® yellow), 28 µg/dm² (Theraband® blue), and 126 µg/dm² (BodyBand®).

The protein patterns analyzed by silver staining showed strong protein bands at 66, 45, and 30 kDa in the extracts originating from the Therabands®. In both extracts, a diffuse protein area below 20 kDa of molecular mass was seen. The Body-Band® extract revealed prominent 20 and 14 kDa protein bands and a diffuse area of proteins below 50 kDa (Fig. 1, lanes 1).

3.2. IgE reactivity of patients' sera with latex sport bands

When incubating sera of “sport patients” A and B with the blotted extracts we observed pronounced IgE reactivity to all

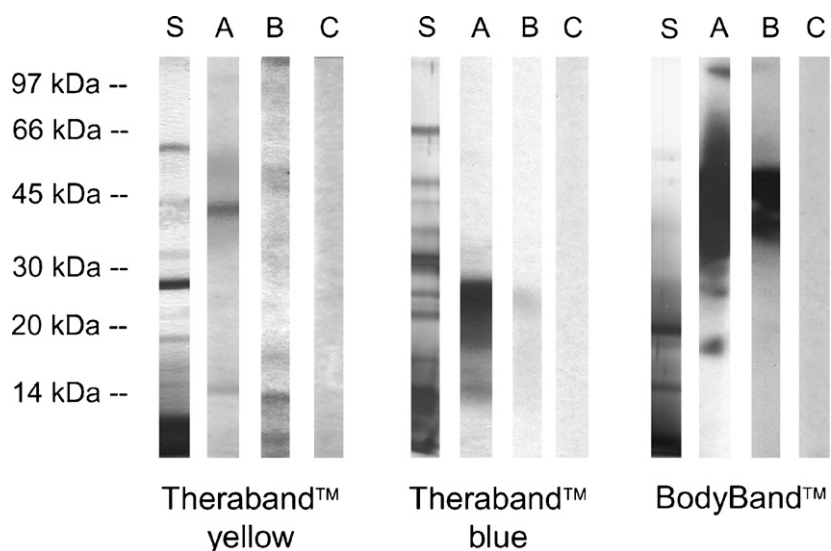


Fig. 1. Specific IgE recognition of latex sport band extracts. Aqueous extracts from latex sport band contained various proteins as shown on silver-stained SDS page. Patients with a history of allergic reactions during exercise showed pronounced IgE binding to the blotted extracts both to high and low molecular latex proteins with distinct recognition patterns. Lanes S: silver stained SDS Page; lanes A: serum patient A; lanes B: serum patient B; lanes C: control serum of a nonatopic subject.

Table 1
IgE reactivity of latex allergic patients' sera

Serum	Total IgE (kU/l)	Latex RAST class (kUA/l)	Blotted extracts			Immunodot assays				
			Extract 1	Extract 2	Extract 3	rHev b 1	rHev b 3	rHev b 5	rHev b 6.02	rHev b 8
A	770	4.7 (42.0)	+	++	+++	+/-	—	+++	++	—
B	n.d.	n.d.	+	+/-	++	—	—	+++	++	—
1	60	3.1 (5.6)	+	++	+++	—	—	++	—	—
2	120	4.0 (19.1)	+	+	+++	n.d.	n.d.	n.d.	n.d.	n.d.
3	100	2.2 (1.4)	+/-	+/-	+	—	—	—	—	+
4	1530	3.0 (4.2)	++	++	++	—	—	—	—	—
5	1030	3.2 (6.9)	+	+	++	+/-	+	—	—	+
6	110	2.4 (1.9)	++	++	++	—	—	—	—	—
7	120	2.4 (2.0)	+	++	+++	+/-	+	—	—	—
8	560	3.4 (9.8)	+	+	+	+	+	—	—	—
9	2000	4.4 (32.1)	+	+	—	—	—	—	—	++
10	170	3.3 (8.4)	+/-	+	++	—	—	++	—	—
Control	—	—	—	—	—	—	—	—	—	—

n.d. = not determined.

Table 2
Reactivities of patient A and B on the ISAC CRD79 chip

Source	Allergen denomination	Patient A (kUA/l)	Patient B (kUA/l)
Cow milk	Cow milk (Bos d 7)	0.56	0.13
Mites	Dermatophagoides farinae (Der f 1)	1.24	
	Dermatophagoides pteronyssinus (Der p 1)	0.58	
Honey bee venom	Apis mellifera (Api m 1)	0.38	
Mugwort	Artemisa vulgares (rArt v 1)	3.31	
Perennial ryegrass	Lolium perenne (Lol p 1)	0.73	
Bermuda grass	Cynodon dactylon (Cyn d 12)	0.17	
Timothy grass	Phleum pratense (rPhl p 2)	0.78	
	Phleum pratense (rPhl p 5)	0.27	
Olive tree	Olea europaea (Ole e 1)	0.22	
Latex	Hevea brasiliensis (rHev b 5)	35.84	2.74
	Hevea brasiliensis (rHev b 6)	11.38	6.72
Wheat	Triticum aestivum (Tri a 18)		0.41

extracts, however, with differing patterns of recognition. Patient A showed an overall stronger IgE reactivity to the extracts as compared to patient B, which was most obvious in the case of Theraband® blue. The pattern of all proteins separated by SDS-PAGE and stained by the silver stain procedure did not show strong similarity to the observed IgE binding pattern (Fig. 1, lanes 2 and 3).

Moreover, the 10 additional sera from previously diagnosed latex allergic patients included in the testing (sera 1–10) showed reactivity to the three extracts, with the exception of serum from patient 9 who failed to react to BodyBand®. They showed diverse recognition patterns and binding intensities to the different extracts (Table 1), but reaction to BodyBand® was the overall strongest.

3.3. Component resolved diagnosis of patients

For component resolved diagnosis, the ISAC®CRD79 chip as well as an in-house dot assays were used, both allowing testing on latex allergens rHev b 1, 3, 5, 6, and 8. Out of the array of latex allergens, for both patients (A and B), reactivity to rHev b 5 and 6 could be shown in both assays. In the in-house dot assay, additionally a weak IgE binding to rHev b 1 was discovered. Moreover, when evaluating the molecular sensitization profile of patients A and B on the ISAC® CRD79 chip we could diagnose specific IgE reactivities against mites, honey bee venom, grass pollen, mugwort, olive and cow milk for patient A, and against cow milk and wheat for patient B (Table 2, Fig. 2).

Of the nine latex allergic patients tested on the dot-assay seven patients recognized one or more of the recombinant allergens. Interestingly, sera from patients 4 and 6 did not reveal any IgE binding to the dotted allergens even though reactivity to blotted latex sport band extracts could be proven (Table 1).

3.4. Characterization of allergen composition of latex sport bands by inhibition assays

To characterize the allergen composition of the latex band extracts, inhibition experiments were performed. For this, single patients' sera or pools of different sera were preincubated

with recombinant allergens, and the IgE binding patterns were compared to untreated serum on blotted extract. As depicted in Fig. 3 for Theraband® yellow recombinant Hev b 1 inhibited a band at 14 kDa (Fig. 3A). Preincubation with rHev b 3 (Fig. 3B) impeded IgE binding to a protein of 23 kDa and to a diffuse area of 14 kDa and below. Incubation with recombinant Hev b 5 (Fig. 3C) resulted in reduced IgE binding to an area between 35 and 65 kDa. Recombinant Hev b 8 (Fig. 3E) inhibited binding to a protein band at 14 kDa. Inhibition exper-

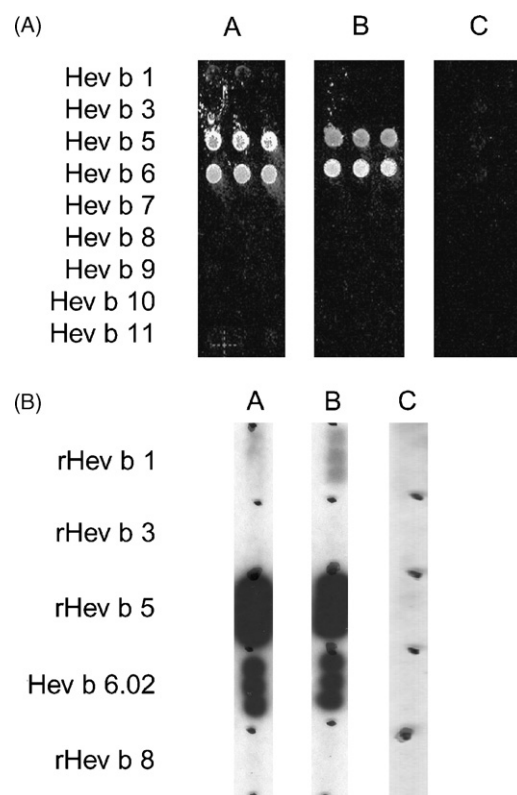


Fig. 2. Component resolved diagnosis of sera from patients A and B. Using the ISAC CRD79® (A) and an in-house immunodot assay (B), the sensitization profile of patient A and B was assessed. In both assays strong reactivity to rHev b 5 and rHev b 6 can be seen, additionally the immunodot revealed IgE binding to rHev b 1 in patient B. Lane A: patient A; lane B: patient B; lane C: control serum of a nonatopic subject.

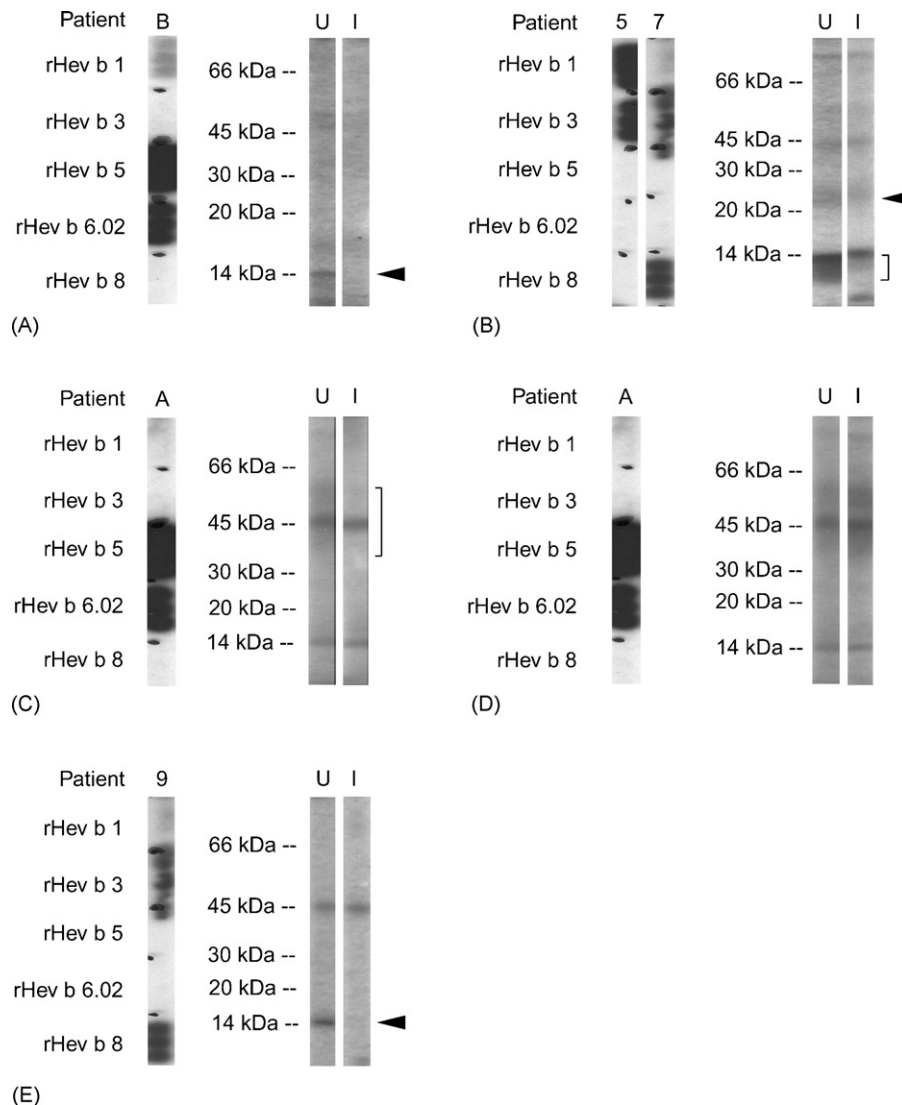


Fig. 3. Characterization of allergen content by inhibition assays. Serum samples were preincubated with rHev b 1 (A), rHev b 3 (B), rHev b 5 (C), rHev v 6.02 (D), and rHev b 8 (E). IgE binding reactivity before (lanes 1) and after (lanes 2) preincubation on Theraband[®] yellow is shown as an example, inhibition of IgE binding is indicated by arrows or brackets. Dot blot strips depict specific allergen recognition of serum samples used for inhibition experiments (panel A, patient B; panel B, serum pool of patients 5, 7, and 8; panels C and D, patient A; panel E, patient 9).

iments with Theraband[®] blue revealed similar results. Thus, in the Therabands[®] the presence of allergens Hev b 1, 3, 5, and 8 could be confirmed. With the BodyBand[®] extract no inhibition with rHev b 8 could be observed, allowing the conclusion that this sport band does not contain profilin. Also, no inhibition was seen in all three extracts when preincubating sera with recombinant Hev b 6.02 (Fig. 3D).

3.5. Clinical relevance of reactions with latex sport bands evidenced by positive skin testing

To examine the clinical relevance of the allergen content of latex sport bands, prick-to-prick test was performed in patient A and compared with reactions to latex prick solution. To elucidate the possible presence of cosensitization to food allergens often associated with latex allergy, kiwi, banana, and fig were included in the skin testing. All three sport bands elicited strong wheal

and flare reactions, comparable to reactivity with latex prick test solution, while fruit allergens elicited only weak reactions (Fig. 4).

4. Discussion

Since its discovery, rubber originating from latex of *H. brasiliensis* has been used for the production of sport equipment and toys due to its favorable mechanical properties [22]. However, regarding IgE mediated allergies, rubber also represents a health threat. When being present in bound form, e.g., in scuba diving gear, latex allergens themselves or rubber processing additives may cause hypersensitivity contact dermatitis [13,23]. Since the 1980s, latex is increasingly used in gymnastic bands. Due to extensive mechanical stretching of the powdered bands, latex allergens may get aerosolized especially when bound to starch particles in the powder. Being inhaled deeply, these aller-

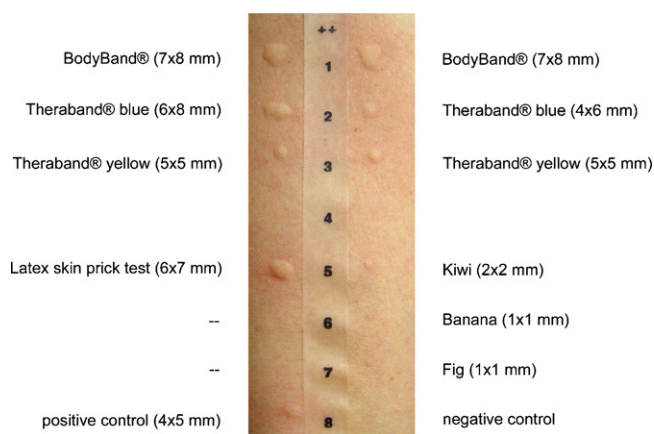


Fig. 4. Skin testing with latex sport bands. Prick-to-prick testing on the lower forearm of patient A with the three different latex sport bands (applied in duplicates) elicited strong wheal and flare reactions. Similar responses were observed when skin-prick testing with commercially available latex skin test solution. Kiwi, banana, and fig revealed only weak skin reactions. The diameters of wheals are given in brackets.

gens represent a risk for IgE-mediated immediate type allergic reactions, in already sensitized patients and possibly also a risk of sensitization by inhalation [24].

In fact, our study was triggered by two latex allergic patients (A and B), who independently experienced allergic complaints during first-time exercise with latex sport bands. Therefore, we aimed to examine the presence and composition of latex proteins in these bands. We could detect pronounced IgE binding to blotted extracts of three different sport bands using both sport patients' sera as well as randomly selected sera of latex allergic patients.

Interestingly, serum of patient A, being a health care worker, showed strong IgE binding to Hev b 5 and 6 both in dot assays as well as on the ISAC® CRD79 chip. Both allergens belong to the most important triggers for this risk group [25]. Serum of patient B revealed pronounced IgE binding to Hev b 5 and 6, additionally weaker binding reactivity to Hev b 1 was observed only in the more sensitive dot assay. Especially Hev b 1 is an allergen frequently recognized by patients having undergone multiple surgical procedures [25,26], as patient B also did.

However, when using sera of the other latex allergic patients for inhibition assays Hev b 1, 3, 5, and 8 could be detected in the latex sport band extracts. Hev b 1 (14.6 kDa), or rubber elongation factor, and Hev b 3 (22 kDa), or small rubber particle protein, are needed for rubber biosynthesis in trees [27]. Hev b 3 is known to get dissociated into various smaller fragments during storage [28]. This might be an explanation why in the assay Hev b 3 inhibited IgE binding to a band at 22 kDa and to an area below 14 kDa. Hev b 5 (17.4 kDa), the so-called acidic protein, associates to multiple protein bands between 50 and 100 kDa on SDS page due to its high proline content [29]. Together with Hev b 13, it was proposed as a marker for estimation of the allergic potency of latex gloves [30]. Furthermore, the latex profilin Hev b 8 was revealed to be present in at least two extracts.

By skin testing of patient A with latex sport bands, we could furthermore confirm the presence of clinically relevant amounts

of allergens. The FDA approves labeling of latex products containing less than 50 µg/g (50 µg/dm²) extractable protein as being of low protein content, even though also these latex products have been shown to be potent elicitors of IgE responses [31]. Accordingly, prick-to-prick testing with the Theraband® yellow of low protein content (17 µg/dm²) was revealed to be a potent trigger for skin reaction in the allergic patient A.

Taking our data together, we could confirm the presence of clinically important latex allergens in rubber sport bands, which may have triggered clinical reactions in patients through their IgE binding capacity. Additionally, it is well known that exercise lowers the threshold levels for allergic reactions [32]. The observed increased intensity of allergic symptoms during exercise is caused by enhanced allergen uptake and distribution due to accelerated blood circulation or an altered inflammatory homeostasis during physical stress [33].

In conclusion, training with latex sport bands represents a risk for latex allergic patients to experience severe allergic symptoms during exercise. Although allergen amount may be low in latex sport bands, they are of clinical relevance for sensitized persons. As neither allergic patients nor trainers are sufficiently aware of this health threat, it is of importance that patients as well as sports instructors and physical therapists are informed accordingly and use latex-free alternatives.

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