

Diagnostic value of the ISAC[®] allergy chip in detecting latex sensitizations

Florian Seyfarth · Sibylle Schliemann ·
Cornelia Wiegand · Uta-Christina Hipler ·
Peter Elsner

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Abstract

Purpose Latex allergy can be diagnosed by different test methods such as IgE quantification, Western blot, cellular antigen stimulation test (CAST), and in vivo methods [e.g. skin prick test (SPT)]. Phadia provides two modern methods using recombinant latex allergens: ImmunoCAP[®] and the Immuno Solid-phase Allergen Chip (ISAC[®]), which enables simultaneous determination of specific IgE against five latex allergens. We compared the diagnostic sensitivity of the ISAC[®] test kit and the conventional Hev b 5—spiked ImmunoCAP[®] latex extract.

Methods Forty sera were sampled from subjects with suspected natural rubber latex (NRL) allergy. These patients had positive SPT to NRL extract, positive NRL Western blots, and positive results in the CAST. All sera were analysed using the ISAC[®] and recombinant NRL ImmunoCAP[®] allergens and compared to the results of 20 negative control sera.

Results Only 22 of the 40 subjects (55 %) showed positivity to at least one latex allergen on the ISAC[®] (sensitivity ISAC[®] 55 %). The sensitivity of the ImmunoCAP[®] latex extract was 70 %. The most frequently detected sensitization was against Hev b 6.01 ($n = 12$). When the serum samples were tested with all recombinant ImmunoCAP[®] allergens, three additional sensitizations against latex could be detected compared to the ISAC[®].

Conclusions Microarrays do offer many potential benefits such as elegant simultaneous determination of sensitizations against different NRL allergens with minimal amounts of serum. However, a negative NRL test result should be regarded with caution and at least be confirmed by other in vitro methods.

Keywords Natural rubber latex · Microarray · Sensitivity · Recombinant allergens

Introduction

Natural rubber latex (NRL) allergy is usually an immediate-type hypersensitivity reaction and presents with allergic rhinoconjunctivitis, bronchial asthma, contact urticaria, or anaphylaxis (Cullinan et al. 2003; Taylor and Erkek 2004). In addition, eczematous skin reactions associated with both immediate and delayed hypersensitivity may randomly occur (Guin 2004). A risk factor for developing latex allergy is occupational use of powdered rubber gloves (Toraason et al. 2000).

Clinicians may use a number of diagnostic tools, such as specific IgE measurement (e.g. ImmunoCAP[®] k82) and different in vivo methods (skin prick test (SPT), glove use test) to confirm immediate-type allergies (Taylor and Erkek 2004). The published test specificity of the latex SPT was reported to be as high as 96 % (Hamilton et al. 2002). Other authors reported sensitivity rates of 93 % (non-ammoniated latex puncture skin test reagent, manufactured by Stallergenes). However, these authors used latex allergic patients with previously positive SPT reactions to a different extracts (Turjanmaa et al. 1997). In a very recent evaluation of commercial SPT solutions, the sensitivity of a SPT with latex solutions from several manufacturers was

Florian Seyfarth and S. Schliemann have contributed equally to this manuscript.

F. Seyfarth · S. Schliemann (✉) · C. Wiegand · U.-C. Hipler ·
P. Elsner
Department of Dermatology, Jena University Hospital,
Erfurter Straße 35, 07740 Jena, Germany
e-mail: schliemann@derma-jena.de

reported to be as high as 89 %, for 4 out of 5 solutions investigated, in latex-exposed healthcare workers who reported skin problems due to NRL in 84.8 % (van Kampen et al. 2013). Additional in vitro methods are the Western blot and the cellular antigen stimulation test (CAST), which are also characterized by a high specificity in diagnosing clinically relevant latex allergy (97–100 %) (de Weck and Sanz 2004). A sophisticated method is the determination of specific IgE antibodies against different single protein components found in NRL.

Up to now, 16 different allergens with wide-ranging biological functions in *Hevea brasiliensis* are known (Hev b 1, 2, 3, 4, 5, 6.01, 6.02, 6.03, 7.01, 7.02, 8, 9, 10, 11, 12, 13) (Sussman et al. 2002; Yeang 2004). Hev b 1 and 3 are involved in the synthesis of polyisoprene (the basic matrix of latex), whereas Hev b 6 and 7 facilitate coagulation of latex. Hev b 2 and 11 function as defence proteins in the plant, and Hev b 8 is a part of the cytoskeleton (Sussman et al. 2002). Many allergens are similar to those found in other allergen sources derived from foodstuffs and may therefore cause cross sensitizations. Hev b 6 is the reason for cross sensitization to banana, avocado, or kiwi, whereas Hev b 7.01 causes cross sensitization to tomato and potato (Rihs and Raulf-Heimsoth 2003). Hev b 8 belongs to the profilins, a protein family of ubiquitous pan-allergens found in dozens of plants and fruits, e.g. birch, olive, mercury, timothy grass, peach, and celery (Radauer et al. 2006; Rihs and Raulf-Heimsoth 2003; Wagner and Breiteneder 2002). Mono-sensitizations to Hev b 8 are assumed to be clinically not relevant (Nieto et al. 2002; Quercia et al. 2009). Along with the growing insight into the function of different antigens comprised in natural products and methods to synthesize single recombinant allergens, new sophisticated diagnostic tools have emerged.

Phadia AB (Uppsala, Sweden) offers 8 single recombinant allergens for the detection of specific IgE with the UniCAP® system (Hev b 1, 3, 5, 6.01, 6.02, 8, 9, and 11). Another possibility of identifying specific IgE against latex allergens (Hev b 1, 3, 5, 6.01, 8) is offered by the Immuno Solid-phase Allergen Chip (ISAC®) by the same company. This allergen chip contains 103 recombinant allergens as well as allergen extracts derived from 47 different allergen sources (food allergens, aeroallergens, bee venom, and latex). These allergens have been fixed on an object slide and can be incubated with 20 µL patient serum only. The binding of the specific IgE to the corresponding allergens is quantified fluorometrically.

The present study aimed at comparing the sensitivity of the recently introduced in vitro methods, the ISAC® chip with the recombinant ImmunoCAP® test system, to the conventional extract-based method in the UniCAP® (k 82), in detecting latex sensitizations. In addition, potential cross

allergens of NRL were addressed by analysing the occurrence of different sensitizations to food and pollen related to the frequency of Hev b 8 (profilin) sensitizations.

Methods

Description of the study population

In this retrospective study, sera of 40 patients from the Department of Dermatology in Jena, Germany, were evaluated ($n_{\text{♂}} = 15$, $n_{\text{♀}} = 25$, mean age 34 years, median age 36 years, range 15–61 years). The sera were sampled from subjects with suspected NRL allergy due to private or occupational NRL exposure, caused by NRL glove use, between 1999 and 2001. All patients had positive skin prick tests (SPT) to NRL extract, positive NRL Western blots (WB), and positive results in the cellular antigen stimulation test (CAST). No spina bifida patients were included (Scheiner et al. 1999). Twenty negative control sera were sampled from subjects without suspected NRL allergy and with negative results in SPT, WB, and CAST. All serum samples had been stored at -20°C . For the present study, the samples were unfrozen and retested for specific IgE (k82) in order to reconfirm the former results and then tested with the new methods (ISAC® and single recombinant allergens in ImmunoCAP®). The Institutional Ethics Committee of the University Hospital Jena approved the protocol (vote 2990-12/10).

Skin prick test and CAST

The latex extract used is manufactured from native latex milk with a protein content of 2–5 mg/mL (*Hevea brasiliensis* clon PRIM 600, Malayan Rubber Research Institute, Malaysia, Stallergenes, France). The extract is non-ammoniated and was also used for the CAST.

The CAST was performed as described by the manufacturer (CAST-2000, BÜHLMANN Laboratories AG, Schönenbuch, Switzerland). The CAST was assessed positive in case a release of sulfido-leukotrienes of at least 200 pg/mL was observed (Hipler et al. 2002; Karamfilov et al. 1999). Additionally, the positive control (monoclonal anti-IgE antibody derived from mice) had to release above 300 pg/mL sulfido-leukotrienes, whereas the negative control remained below 200 pg/mL.

Western blot

The WBs were performed with the Alablot® test system (DPC Biermann GmbH, Bad Nauheim, Germany) which is no longer available. The respective latex test stripe (AlaSTAT® AlaBLOT® Allergen Strip Module Latex) was

developed for quality control of specific IgE determination (AlaSTAT™). WBs were assessed positive whenever antibodies against at least a single NRL allergen were detected.

Determination of specific IgE against ImmunoCAP® k 82 and recombinant allergens

The Phadia ImmunoCAP® test system (an enzyme-linked immunosorbent assay) was used to measure specific IgE in the sera according to the instructions of the manufacturer. Values above 0.35 kU/L were considered positive. Hev b 5-amplified and the former non-amplified ImmunoCAP® latex extract both were used (k82). The non-amplified k 82 was in use between 1999 and 2001, while for the current investigations, the current Hev b 5-amplified extract was used. The sensitivity of the obtained results was compared with the results obtained by ISAC® and the recombinant ImmunoCAP® preparations (Hev b 1 = k 215, Hev b 3 = k 217, Hev b 5 = k 218, Hev b 6.01 = k 219, Hev b 6.02 = k 220, Hev b 8 = k 221, Hev b 9 = k 222, and Hev b 11 k 224).

For detection, the UniCAP 100® (Phadia AB, Uppsala, Sweden) was used.

ISAC® (Phadia AB, Uppsala, Sweden; formerly VBC Genomics, Vienna, Austria)

Measurements were taken as recommended by the manufacturer (Phadia AB, Uppsala, Sweden). The microarray was incubated with 20 µL serum for 2 h. Afterwards, the microarray slides were rinsed with buffer, left to dry, and then incubated with fluorescence-labelled anti-human IgE (1 h, room temperature). After washing the stained slides, fluorescence measurements were taken (Lux Scan—10 K/A, Capital Bio, Beijing, China).

The ISAC® quantifies IgE specific to Hev b 1, 3, 5, 6.01, and 8. Hev b 8 is a profilin, which is involved in certain latex-fruit syndromes. Among others, the ISAC® contains the potentially cross-reactive profilins Bet v2 (birch), Mer a 1 (annual mercury), Phl p 12 (timothy grass), and Ole e 2 (olive tree). The sera with sensitization to Hev b 8 were therefore analysed with respect to their cross-sensitivity profiles to profilins and in addition to bromelain (Ana c 2), a marker for cross sensitizations due to “cross-reactive carbohydrate determinants” (CCDs).

Statistics

All data were entered into Microsoft Excel for Windows 2003 and analysed by SPSS for Windows (version 16.0) using Fisher's exact test.

Results

Hev b 5-amplified and non-amplified k82 latex extract

Both extracts did not detect all of the 40 positive samples (SPT-, WB- and CAST-positive). However, the performance of the Hev b 5-amplified test kit was slightly better compared to the non-amplified extract (test sensitivity 70 vs. 62.5 %). The amplified extract disclosed 28 out of 40 sera, whereas the non-amplified test kit revealed only 25 (see Fig. 1). Sensitizations against Hev b 5 were not detected in the 3 discrepant samples when tested with the ISAC® and recombinant ImmunoCAP® test kit. In 8 cases, higher IgE levels were noted with the Hev b 5-amplified extract compared to the non-amplified one (increase by 1 CAP class $n = 7$, by 3 CAP classes: $n = 1$), and in 4 out of 8 cases, Hev b 5 sensitizations were detected. Only 2 samples showed a decrease of 1 CAP class when tested with the amplified k 82, and 1 of them was Hev b 5-positive.

Sensitivity of the ISAC® test kit (Fig. 1)

ISAC® results were assessed positive in case one or more latex allergens were detected. In total, only 22 out of 40 sera showed positive results (sensitivity = 55 %).

Detected allergens in the ISAC® test kit (Table 1)

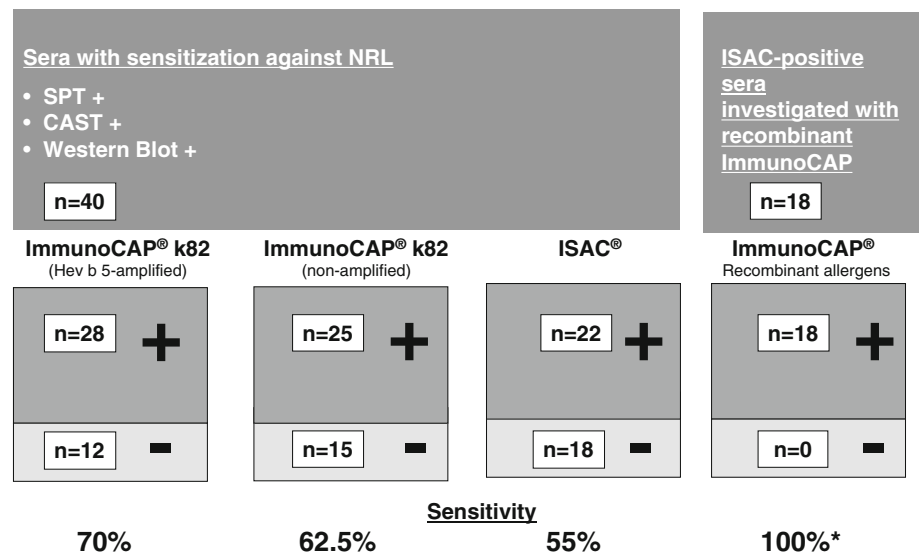
For the 22 ISAC®-positive sera, the most frequently detected allergen was Hev b 6.01 ($n = 12$), followed by Hev b 8 ($n = 9$), Hev b 5 ($n = 5$), and Hev b 1 ($n = 3$). Sensitizations against Hev b 3 were not found. Sensitization to Hev b 5 occurred only in Hev b 8-negative sera.

Only two Hev b 8-positive serum samples showed co-sensitizations to other latex allergens. In 7 sera, mono-sensitization to Hev b 8 was detected.

The ISAC software categorizes the intensity of the signal into groups 1, 2, or 3. The intensity was 2 or 3 (strong signal) in the majority of the samples. Only 2 sera showed low-intensity signals. One of them was mono-sensitized to Hev b 1 (corresponding k82, CAP class 2), and the other was mono-sensitized to Hev b 6.01 (k82: CAP class 3).

Sensitizations against bromelain (Ana c 2), a marker for cross sensitizations due to “cross-reactive carbohydrate determinants” (CCDs), were found in 6 out of 40 sera of the latex-sensitized samples (signal intensities 1–3 ISU-E). Three of them belonged to the samples that were false negative in the ISAC®. Bromelain sensitizations were not found in Hev b 8 mono-sensitized sera. In the negative control group ($n = 20$), no sensitizations against bromelain were detected.

Fig. 1 Numbers of sensitizations detected with the different methods and resulting test sensitivities. Forty positive control sera (SPT-, WB-, and CAST-positive) were investigated using the conventional ImmunoCAP k82 with and without Hev b 5-amplification, and ISAC. Due to limited amount of remaining serum, the sensitization rates against recombinant ImmunoCAP allergens were only investigated in 18 out of 22 ISAC-positive sera



Recombinant ImmunoCAP® (Table 1)

Due to limitations of the amount of serum available, not all samples could be measured with the recombinant ImmunoCAP® test kit ($n = 30$). Eighteen samples, which had been tested ISAC®-positive for latex, and 12 samples falsely identified as negative were included in this analysis and compared with the results obtained by the conventional ImmunoCAP® system.

Seventeen out of 18 were found to be positive for latex using the k82. The remaining subject showed Hev b 8 mono-sensitization, which could be detected using both the ImmuoCAP® components and the ISAC®. The recombinant system was able to detect all ISAC®-positive serum probes. In two cases, the recombinant ImmunoCap® identified sensitizations against Hev b 3 that were not detected by the ISAC® system. Sensitizations to two allergens that are not available on the ISAC® kit, Hev b 9 and Hev b 11, were discovered in 4 cases with the recombinant ImmunoCAP® (see Table 1). Analysis of the samples with false-negative results in the ISAC® but proven latex sensitization by SPT, WB, and CAST was performed in order to evaluate whether the allergen spectrum on the ISAC® test slide would benefit from adding more components. Using the recombinant test kit revealed that three additional samples with proven latex sensitization would have been overlooked using the ISAC® system alone. One case was detected to be positive for Hev b 9 (CAP class 1), which is not included in the ISAC®. The IgE against k82 (Hev b 5-spiked) in this sample amounted CAP class 2. The other two samples had sensitizations to allergens that can be found on the ISAC® but were missed in the test (Hev b 1, 3, 5, 6.01, and 8, see Table 2).

Cross sensitization against profilins (Table 2)

Besides Hev b 8, the ISAC® contains 4 other profilins: Bet v 2 (birch), Mer a 1 (annual mercury), Phl p 12 (timothy grass), and Ole e 2 (olive tree). Nine of 22 sera that were found to be latex-sensitized in the ISAC® test, showed sensitization to Hev b 8, and in 7 out of 9 cases, this was the only positive allergen detected. All 9 sera were also positive to the profilins Mer a 1 and Ole e 2, and 8 out of 9 were sensitized to Bet v 2 and Phl p 12 (see Table 2). In contrast, only 1 subject out of 13 without Hev b 8 sensitization had a solitary sensitization to Mer a 1.

Discussion

Comparison of Hev b 5: amplified and non-amplified latex extract (ImmunoCAP® k82)

Lundberg et al. demonstrated that Hev b 5-amplified k82 increased the amount of specific IgE measured in 16 % of the latex-sensitized sera (Lundberg et al. 2001). In 16 cases (7 %), the amplified test kit detected latex sensitization which had been overlooked using conventional k82 in their study. In our study population, 11 samples (from 40 sensitized sera, 27.5 %) showed higher IgE, although this increase comprised only one CAP class in most cases. In contrast, the number of detected sensitizations that would have been overlooked with the non-amplified extract was lower ($n = 2$; i.e. 5 % of the 40 latex-sensitized serum samples). The data are consistent with the results found by Lundberg et al. and suggest the use of Hev b 5-amplified latex extracts. The sensitivity of the amplified k82 was higher than the non-amplified k82 (sensitivity “old” k82 = 62.5 %, “new” k82 = 70 %). The results of the non-

Table 1 Sensitizations against different latex allergens detected by the ISAC® and the ImmunoCAP®—components

Tested with	Any allergen	Hev b 1	Hev b 3	Hev b 5	Hev b 6.01	Hev b 6.02	Hev b 8	Hev b 9	Hev b 11
<i>Latex-sensitized subjects (n = 40)</i>									
ISAC® positive									
ISAC® (n = 22 tested)	n = 22 (100 %)	n = 3 14 %	n = 0	n = 5 23 %	n = 12 55 %	n.t.	n = 9 41 %	n.t.	n.t.
Mean [ISU-E]		4.80		25.09	14.72		6.58		
Median [ISU-E]		5.48		29.94	7.47		2.75		
Recombinant ImmunoCAP® (n = 18 tested)	n = 18 100 %	n = 3 17 %	n = 2 11 %	n = 6 33 %	n = 8 44 %	n = 8 44 %	n = 10 56 %	n = 4 22 %	n = 4 22 %
Mean [kU/L]		4.36	4.01	39.20	12.23	13.67	7.48	2.44	1.30
Median [kU/L]		5.33	4.01	16.70	2.96	3.08	1.16	2.17	1.28
ISAC® negative									
Recombinant ImmunoCAP® (n = 12 tested)	n = 3 (25 %)	n = 1 (8 %)	n = 1 (8 %)	n = 1 (8 %)	n = 2 (17 %)	n = 1 (8 %)	n = 2 (17 %)	n = 2 (17 %)	n = 2 (17 %)
Mean [kU/L]		0.76	0.38	0.49	0.91	0.62	1.04	1.16	0.67
Median [kU/L]		0.76	0.38	0.49	0.91	0.62	1.04	1.16	0.67
<i>nt not tested, since the allergen is not available on the ISAC® chip</i>									

amplified k82 were inferior to those from other studies dealing with non-amplified k82 (sensitivity 75.2–97 % (Hamilton et al. 2002; Ownby et al. 2000)).

Sensitivity of the ISAC®

Both major allergens present on the ISAC® (Hev b 6.01 and Hev b 5) were detected frequently (Hev b 6.01: 12 of 22 ISAC latex-positive samples, ≈55 %; Hev b 5: 5 of 22 samples, = 20 %). However, the test sensitivity of the ISAC® was lower compared to other authors. Only 22 out of 40 sensitized subjects were detected, resulting in a sensitivity of 55 %, whereas the sensitivity was 100 % in a study performed by Ebo et al. (Ebo et al. 2009). Ott et al. found a slightly lower test sensitivity of 81 % (Ott et al.). When comparing these results, it has to be taken into consideration that sensitivity calculations in such studies are significantly influenced by the definition criteria of the positive control groups and by the selection of patients. In our study, the results obtained were compared to SPT-, WB-, and CAST-positive sera, while other authors used sera from patients with both positive results in the SPT and in the UniCAP® (Ebo et al. 2009; Ott et al. 2010). We therefore cannot exclude influence of the selected subjects and solutions that were used for the SPT, WB, and CAST analyses.

In clinical routine, the diagnosis of clinically suspected latex allergy is usually based on the skin tests and measurement of specific IgE (ImmunoCAP® k82). However, it is known that neither the SPT nor the specific IgE assay has 100 % diagnostic accuracy (Hamilton et al. 2002; Ownby et al. 2000). In a previous prospective study, the sensitivity was found to be 86 % for the UniCAP® system, in correspondence with our findings (Blanco et al. 1998). Related to the subgroup of both positive SPT and IgE results (plus WB and CAST positivity) in our material, the calculated sensitivity of the ISAC® would have been similar to that of Ott et al. (2010). In clinical routine, subjects with clinically relevant latex allergy and positive SPT but negative specific IgE do occur, and our results indicate that in these patients, usage of the ISAC® does not offer diagnostic benefit. Furthermore, a considerable percentage of patients with positive IgE in the ImmunoCAP® system will be false negative in the ISAC®. The results presented here therefore put the diagnostic value of the ISAC® chip into the real clinical perspective of an ongoing challenge of diagnosing latex allergy.

Our study is limited by the fact of a retrospective design. We were not able to characterize the proportions of patients with genuine clinical relevant allergy or just inapparent sensitization, since the individuals did not undergo standardized provocation tests. Furthermore, the potential influence of using frozen sera has to be addressed. We

Table 2 Co-sensitizations of latex-sensitized subjects to profilins ($n = 22$), provided separately for Hev b 8-positive and Hev b 8-negative sera

Allergen source	Allergen	Occurrence of positivity in latex-sensitized samples (ISAC)		
		Hev b 8-sensitized ($n = 9$)	Hev b 8-negative ($n = 13$)	Fisher's exact test
Birch	Bet v 2	8 (88.9 %)	0	$p < 0.0001$
Annual mercury	Mer a 1	9 (100 %)	1 (0.1 %)	$p < 0.0001$
Olive	Ole e 2	9 (100 %)	0	$p < 0.0001$
Timothy grass	Phl p 12	8 (88.9 %)	0	$p < 0.0001$

determined the specific IgE from the stored sera with good reproducibility, thus indicating a sustained quality. Accordingly, there was no indication of a decreased biological activity of the material.

It was of interest to compare the results obtained by the ISAC[®] with the recombinant ImmunoCAP[®] components. The ISAC[®] chip does not cover all known NRL antigens. Hev b 9 and Hev b 11 are not included. By testing the serum samples with recombinant ImmunoCAP[®] latex allergens, 3 new subjects could be identified, who would have been overlooked if tested with the ISAC[®] alone. One of them was only sensitized to Hev b 9. Thus, including Hev b 9 on the ISAC[®] would have increased its sensitivity from 55 to 57.5 % in our data. However, this is only marginal and raises the question, whether it would be beneficial to add further allergens (Kurup et al. 2005; Ott et al.; Raulf-Heimsoth et al. 2007). Kurup et al. demonstrated an increased sensitivity of 80 % in the system when measuring specific IgE against native Hev b 2 and 13 (together with Hev b 3, 5, and 6) in healthcare workers (Kurup et al. 2005). New data suggest sensitizations to the glycoprotein Hev b 13 as being frequent in latex-sensitized subjects (prevalence in NRL-sensitized HCWs 78 %, in NRL-sensitized children with spina bifida 77 %) (Ebo et al. 2009; Kurup et al. 2005; Raulf-Heimsoth et al. 2007; Rouge et al. 2010; Yeang et al. 2004). However, some conflicting results were reported showing a low prevalence of IgE to the highly purified protein among Finnish, Spanish, and American patients (Palosuo et al. 2007). Unfortunately, recombinant Hev b 13 is commercially not available. Experiments with the recombinant allergen produced in *E. coli* revealed loss of the IgE-binding capacity of the allergen due to missing glycosylation (Arif et al. 2004), although recent publications propose that the allergenicity of Hev b 13 does not primarily depend on its carbohydrate moiety (Rouge et al. 2010). Hev b 2 is also a major allergen that does not show IgE-binding epitopes when produced as recombinant allergen (Raulf-Heimsoth et al. 2007).

Although the results of the ImmunoCAP[®] components were more or less similar to the ISAC[®] outcome, a better test sensitivity could be shown for Hev b 3 (k217) with ImmunoCAP[®] components compared to the ISAC[®]. Hev b

3 is mainly found in spina bifida patients but only rarely in those patients with occupational latex exposition (health-care workers, etc.) (Raulf-Heimsoth et al. 2007). The investigation presented here did not include spina bifida patients, a circumstance that sufficiently explains the low prevalence of Hev b 3, which is also reported in other publications (Ott et al. 2010). Altogether, further extension of the recombinant allergen spectrum can be expected to improve the sensitivity only to a limited degree, since all remaining allergens are minor allergens. However, in the individual case, these reported minor allergens maybe of predominant clinical relevance.

Interpretation of Hev b 8 sensitizations

Nieto et al. and Quercia et al. showed that Hev b 8 mono-sensitized subjects often lack any clinical symptoms (Nieto et al. 2002; Quercia et al. 2009). In a latex glove use test, none of their 7 Hev b 8 mono-sensitized subjects showed any pathologic result. All of them underwent an uneventful surgical procedure (use of latex gloves, etc.) without any complications (Quercia et al. 2009). In another study, only subjects with clinically relevant allergy were included (e.g. immediate-type allergy symptoms). Only 1 of 22 subjects with latex allergy had a sensitization to Hev b 8, whereas the group of latex-sensitized subjects without clinical symptoms ($n = 20$) comprised 15 subjects with Hev b 8 sensitizations (Ebo et al. 2009). Thus, Hev b 8 could either be clinically irrelevant or not present in most latex products (Quercia et al. 2009). In a recent publication, a minor clinical relevance of Hev b 8 is postulated (Ott et al.).

Also, in the study presented, a considerable proportion, namely 9 out of 22 ISAC[®]-positive subjects, showed Hev b 8 sensitizations, with 7 of them being mono-sensitized. All showed additional sensitizations to other profilins, while this was the case only in 1 of the remaining subjects without Hev b 8 sensitization. In the majority of positive sera, only low levels of specific IgE against this component were detected (CAP class 1). The corresponding k82 IgE level was higher in some cases (CAP 3: $n = 1$, CAP 2: $n = 3$), in some identical, or even lower (CAP 1: $n = 2$, CAP 0: $n = 1$), which might indicate that not all Hev b 8 mono-sensitized persons were really mono-sensitized.

Since the main challenge in clinical routine is the discrimination of mere sensitization from clinically relevant allergy, one of the main advantages of the component-resolved IgE tests may be the detection of cross-reactive IgE binding to allergens that appear to be indicators of clinically irrelevant sensitization. Hev b 8 and CCDs are examples of such allergens.

In conclusion, the sensitivity of the ISAC[®] with respect to detection of NRL sensitization was lower compared to findings of other authors, as far as a positive control group of SPT, WB, and CAST-positives, but not necessarily IgE-positive sera was considered. Microarrays do offer many potential benefits such as elegant simultaneous determination of a multitude of sensitizations and cross reactions with minimal amounts of serum. Detecting mono-sensitizations to selective allergens such as Hev b 8 and CCDs may be helpful to identify clinically irrelevant sensitization as compared to true allergy. However, negative results should be regarded with caution and at least be confirmed by other in vitro methods. The conventional ImmunoCAP[®] k82 showed a superior sensitivity. The results presented here are limited by the fact of a retrospective study design and cannot be transferred to the other allergens incorporated on the ISAC[®] chip. More investigations using prospective study designs are necessary.

Conflict of interest The authors declare that they have no conflict of interest. The study was investigator-driven and was performed without external funding.

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