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Microarrays of Recombinant *Hevea brasiliensis* Proteins: A Novel Tool for the Component-Resolved Diagnosis of Natural Rubber Latex Allergy

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Abstract

Background: Component-resolved diagnosis using microarray technology has recently been introduced in clinical allergology, but its applicability in patients with natural rubber latex (NRL) allergy has not been investigated.
Objectives: To evaluate the utility of microarray-based immunoglobulin (Ig) E detection in the diagnostic workup of NRL allergy and to compare this new diagnostic tool with established methods of NRL-specific IgE detection.
Methods: We investigated 52 adults with immediate-type NRL allergy and 50 control patients. Determination of specific serum IgE against 8 recombinant *Hevea brasiliensis* allergen components was performed using a customized allergen microarray and a conventional fluorescence enzyme immunoassay (FEIA).
Results: The panel of microarrayed allergen components was shown to represent a comprehensive repertoire of clinically relevant NRL proteins. NRL-specific IgE recognition patterns and sensitization rates determined by microarray analysis were similar to those obtained by conventional FEIA. The diagnostic sensitivity rates of combined single-component data were not significantly different for the respective recombinant test system, whereas the sensitivity level of extract-based FEIA analysis was markedly higher.
Conclusion: The current study provides evidence that microarrays of recombinant NRL allergen components are a suitable new tool for the diagnosis of NRL-specific sensitization. They show performance characteristics comparable to those of current diagnostic tests and could be indicated in small children in whom only limited blood volumes are obtainable. Further large-scale studies in unselected patient populations and in high-risk groups are warranted before the microarray can be introduced into routine management of patients with NRL allergy.

Key words: Latex allergy. *Hevea brasiliensis*. Microarray. Component-resolved diagnosis.

Resumen

Antecedentes: El diagnóstico por componentes mediante la tecnología de microarrays ha sido introducido recientemente en la alergología clínica, si bien su aplicación en pacientes con alergia al látex de caucho natural no ha sido investigada.
Objetivos: Evaluar la utilidad de la detección de la inmunoglobulina (Ig) E basada en microarrays en el diagnóstico de la alergia al látex de caucho natural, y comparar esta nueva herramienta diagnóstica con los métodos establecidos para la detección de IgE específica al látex de caucho natural.
Métodos: Se estudiaron a 52 adultos con alergia inmediata al látex de caucho natural y 50 pacientes control. Se determinó la IgE sérica específica frente a 8 componentes alergénicos de *Hevea brasiliensis* mediante microarrays de alérgenos personalizada y un fluoroenzimoinmunoanálisis (FEIA) convencional.
Resultados: El panel de componentes alergénicos demostró representar un amplio repertorio de proteínas clínicamente relevantes de látex de caucho natural. Los patrones de reconocimiento de IgE específica al látex de caucho natural y los índices de sensibilización determinados

mediante el análisis de microarrays fueron similares a los obtenidos con el FEIA convencional. Los índices de sensibilidad diagnósticos de los datos combinados de un único componente no fueron significativamente diferentes para el sistema analítico recombinante correspondiente, mientras que el nivel de sensibilidad del FEIA basado en extractos fue notablemente superior.
Conclusión: El estudio actual aporta datos que muestran que los microarrays de componentes alergénicos recombinantes de látex de caucho natural constituyen una herramienta nueva adecuada para diagnosticar la sensibilización específica al látex de caucho natural. Esta herramienta presenta características de rendimiento comparables a las de las pruebas diagnósticas actuales, y podría estar indicada para su uso en niños pequeños de los que solo se pueden obtener volúmenes de sangre reducidos. Es necesario realizar más estudios a gran escala en poblaciones de pacientes no seleccionadas y en grupos de alto riesgo antes de poder introducir las micromatrices en el control rutinario de los pacientes con alergia al látex de caucho natural.

Palabras clave: Alergia a látex. *Hevea brasiliensis*. Microarray. Diagnóstico por componentes.

Introduction

Despite considerable prevention efforts, natural rubber latex (NRL) allergy remains an important medical issue, affecting nearly 1.5% of the general population, more than 4% of health care workers, and up to one-third of patients undergoing multiple surgical procedures [1-3].

As a natural product of the tropical *Hevea brasiliensis* tree, NRL is an aqueous elastomer emulsion containing mainly cis-1,4-polyisoprene (30%-40%) and water (55%-65%), but also more than 240 polypeptides. Of these, 13 proteins have been identified, characterized, and officially accepted as allergen components by the International Union of Immunological Societies. Most of these allergenic proteins, designated as Hev b 1-13, have been recombinantly synthesized and used for component-resolved in vitro diagnosis of NRL sensitization by our group and other investigators [4-7].

Furthermore, protein microarrays have recently been introduced into allergological research as promising tools for the simultaneous assessment of specific immunoglobulin (Ig) E antibodies (sIgE) against multiple recombinant or purified natural allergen components [8-10]. We previously demonstrated in a proof-of-principle study with NRL-sensitized BALB/c mice that detection of sIgE against NRL proteins is feasible using a customized allergen microarray [11].

The clinical implications of component-resolved diagnosis (CRD) including microarrayed NRL allergens have not been investigated. Thus, our main objective was to assess the performance of an allergen microarray containing recombinant NRL allergen components for sIgE detection in adults with latex allergy. We also compared clinically relevant performance parameters of this allergen microarray with those of an established extract-based and component-based fluorescence enzyme immunoassay (FEIA).

Patients and Methods

Study Population

We analyzed serum samples from 52 adult patients who had been referred to our dermatology and allergology outpatient departments for evaluation of NRL hypersensitivity. All patients had a conclusive history of IgE-mediated allergy occurring immediately after NRL exposure and at least 1 positive reaction in skin prick tests performed with NRL allergen extracts. The severity of the clinical reactions was classified according to von Krogh and Maibach [12] as follows: contact urticaria (grade 1), generalized urticaria with or without angioedema (grade 2), bronchial asthma with or without rhinoconjunctivitis (grade 3), and anaphylactic shock (grade 4). Serum samples of 50 age-matched and sex-matched insect venom–allergic adults with no history of latex allergy served as negative controls.

Data processing was performed in accordance with ethical standards on human experimentation and with the Declaration of Helsinki (1975), as revised in 1983. The study was approved by the local ethics committee of the Medical Faculty of the RWTH Aachen.

Protein Microarray Test Procedure

We used a commercially available allergen microarray platform (ISAC, VBC Genomics Bioscience Research, Vienna, Austria) that has been shown to yield reliable analytical results when compared to fluorescence enzyme immunoassays in other clinical settings and with different sets of implemented inhalant or food allergen components [8-10].

This ambient analyte assay consists of a microscopy glass slide modified with a Teflon mask in order to create 4 individual reaction sites. These were coated with amine-reactive polymers allowing covalent immobilization of allergenic molecules. In the present study, we used a customized version of the microarray containing recombinant birch pollen profilin (rBet v 2) and a series of 8 recombinant NRL allergen components—rubber elongation factor (rHev b 1), small rubber particle protein (rHev b 3), acidic protein (rHev b 5), hevein (rHev b 6.02), profilin (rHev b 8), enolase (rHev b 9), Mn-superoxide dismutase (rHev b 10), and class I chitinase (rHev b 11) (Biomay, Vienna, Austria)—that were spotted onto the microarray in vertical triplicates. As indicated by the manufacturer, the degree of purity of the respective allergenic proteins was as follows: ≥90% (rHev b 1), ≥97% (rHev b 6.02), >98% (rHev b 3, rHev b 5, rHev b 9, rHev b10, rHev b 11), and >99% (rHev b 8). Serum samples that proved positive for the *H brasiliensis* profilin rHev b 8 were additionally screened for sIgE against rBet v 2, the recombinant profilin of white birch (*Betula verrucosa*) (Biomay, Vienna, Austria) at a purity of >98%.

Microarray immunoassays were performed according to

the manufacturer's recommendations as recently published by Deinhofer et al [13]. Briefly, each microarray reaction site was incubated with 20 µL of undiluted patient serum for 180 minutes in order to capture allergen-specific serum IgE antibodies using their corresponding allergen molecules. In a second step, the microarray slides were washed twice with a conventional Tris-buffered saline/Tween (TBS-T) buffer solution for 5 minutes, rinsed with deionized water, and dried under a nitrogen flow. Microarray-bound IgE was then marked with a secondary fluorescence-tagged antihuman IgE antibody for 60 minutes at room temperature. After a second washing procedure with TBS-T, the corresponding fluorescence signals were scanned at a 10-µm resolution using a conventional biochip reader (Scan Array Express, Perkin Elmer Life Sciences, Boston, Massachusetts, USA). Based on calibration sera of known specific IgE content, raw data of the corresponding digitized microarray images were analyzed with the QuantArray 3.1 software (Perkin Elmer Life Sciences, Boston, Massachusetts, USA) by transforming image information into numerical data quantified as dimensionless fluorescence intensity (FI) values.

Fluorescence Enzyme Immunoassays

Determinations of allergen-specific IgE (sIgE) and serum total IgE (tIgE) levels were performed with a widely used, commercially available FEIA, as proposed by the manufacturer (UniCAP, Phadia, Uppsala, Sweden). We assessed sIgE titers against latex allergen extract (manufacturer's code, k72) and the recombinant latex allergen components Hev b 1 (k215), Hev b 3 (k217), Hev b 5 (k218), Hev b 6.02 (k220), Hev b 8 (k221), Hev b 9 (k222), Hev b 10 (k223), and Hev b 11 (k224) (Phadia). Total and specific IgE levels were quantified in protein units designated as kU_A/L according to the product manual.

Statistics

The obtained data were expressed as mean (SD, range), unless otherwise indicated. The serum IgE levels were used as response variables during linear regression analysis. The association between microarray and FEIA data was assessed using the Pearson correlation coefficient. Two-tailed unpaired *t* tests were performed for group comparisons and results were considered significant at $P<.05$.

Receiver operating characteristic (ROC) curve analysis was performed to study the effect of varying sIgE thresholds on the test performance of microarray-based and FEIA-based IgE detection. The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of both test systems were calculated using the sIgE cutoff leading to the highest accuracy, that is, the maximum value for the sum of sensitivity and specificity. The corresponding 95% confidence intervals (CI) of these diagnostic measures were also calculated.

All statistical analyses were performed using the S Plus 6.1 statistical software package (Insightful Corp., Seattle, Washington, USA). SigmaPlot 2004 Version 9.01 (Systat Software, Erkrath, Germany) was used to graph scatter plots and box and whisker plots.

Results

The study population comprised 52 latex-allergic adults (27 male, 25 female) with a mean (SD [range]) age of 48 years (9 [23-68 years]). None of these patients suffered from spina bifida or had undergone multiple surgical procedures. Twenty-two (42%) displayed only cutaneous symptoms (contact urticaria, generalized urticaria with[out] angioedema), while 28 patients (54%) reported respiratory and or mucosal reactions (asthma with[out] rhinoconjunctivitis). Only 2 cases (4%) of severe anaphylactic shock were identified. The control group consisted of 50 insect venom–allergic adults (23 male, 27 female) with a mean age of 52 years (13 [20-73] years) who had never experienced NRL-associated symptoms. Conventional FEIA analysis detected sIgE against the NRL extract in 51 patients (98%) at a mean level of 15.6 kU_A/L (24.0 [0.35-100 kUA/L]), whereas none of the controls revealed extract-specific sIgE antibodies (Table 1).

Table 1. Demographic and Clinical Characteristics of Recruited Patients and Healthy Controls (n=102)

	Patients (n=52)	Controls (n=50)
Age, mean (SD [range]), y	48 (9 [23-68])	52 (13 [20-73])
Gender, No. (%)		
Male	27 (51)	23 (46)
Female	25 (49)	27 (54)
Severity of reaction, No. (%)		
I: Contact urticaria	16 (31)	0
II: Generalized urticaria with(out) angioedema	6 (11)	0
III: Asthma with(out) rhinoconjunctivitis	28 (54)	0
IV: Anaphylactic shock	2 (4)	0
sIgE latex[a] [kU_A/L], mean (SD [range])	15.6 (24, [0.35-100])	0

Abbreviation: Ig, immunoglobulin.
[a]sIgE latex: allergen-specific IgE antibodies against latex extract (k82) as determined by fluorescence enzyme immunoassay.

Microarray-based assessment of sIgE recognition patterns in all 52 patients detected a mean of 2 sensitizations (range, 0-6 sensitizations) per individual. The NRL proteins rHev b 6.02 (n=36, 69%), rHev b 5 (n=23, 44%), and rHev b 11 (n=17, 33%) elicited the highest rates of positive results, whereas sIgE against microarrayed rHev b 9 and rHev b 10 could not be detected at all. IgE against the latex profilin Hev b 8 was detected in 16 patients (31%), of whom 6 (12%) were also sensitized to the birch pollen profilin Bet v 2.

At a mean of 1.8 sensitizations (range, 0-7 sensitizations) per individual, FEIA analysis yielded similar results with regard to the high prevalence of sIgE antibodies against rHev b 6.02 (n=27, 52%), rHev b 5 (n=26, 50%), and rHev b 11 (n=15, 29%), while lower results were observed for rHev b 8 (Table 2). The association between FEIA and microarray data is illustrated in Figure 1, the respective Pearson correlation coefficient was calculated at r=0.63 (95% CI, 0.57-0.68). Single correlations of FEIA and microarray results obtained with the corresponding allergen components are indicated in Table 2. In the control group, only 1 patient (2%) revealed sIgE in both assays, and this was directed against Hev b 11.

ROC curve analysis yielded an optimal diagnostic threshold of >0 kU_A/L and an FI value >0 for FEIA and microarray testing, respectively. At this threshold, sensitivity rates determined with the sum of all single-component data were not significantly different for the respective recombinant test system (FEIA, 87% [95% CI, 74%-94%] vs microarray, 81% [95% CI, 67%-90%], whereas the sensitivity level of extract-based FEIA analysis was markedly higher (98%; 95% CI, 88%-100%) (Table 3). With regard to single components, the highest sensitivity was reached with rHev b 6.02 (71% [95% CI, 57%-82%] vs 69% [95% CI, 55%-81%]). As positive FEIA and microarray results were virtually absent in the control group, specificity was very high (>98%) for both recombinant test systems and the extract-based FEIA system.

Group comparison of nonsensitized or monosensitized patients with polysensitized patients revealed that the number of recognized allergen components was significantly correlated with the concentration of sIgE against NRL extract as determined by Kruskal-Wallis-testing (Figure 2A, B). In contrast, the number of sensitizations or the extract-specific IgE serum levels were not significantly different (P>.05) in patients with a history of low-grade cutaneous reactions (grade I, II) compared to patients who had suffered from more severe respiratory or cardiocirculatory symptoms (grade III, IV). Similarly, no significant group differences were observed for component-specific IgE serum levels with the exception of the NRL profilin rHev b 8 and the birch pollen profilin Bet v 2, which yielded significantly more positive results in patients with cutaneous reactions only (Table 4).

Table 2. Specific Immunoglobulin E Recognition Patterns to Recombinant Hevea brasiliensis (rHev b) Allergen Components of All Included Patients Suffering From Immediate Type Natural Rubber Latex Allergy (n=52)

	FEIA		Microarray		FEIA vs Microarray
Allergen Component	No. (%)	Mean (SD [range]) sIgE, kU_A/L	No. (%)	Mean (SD [range]) sIgE, kU_A/L	Pearson Correlation Coefficient, r (95% CI)
rHev b 1	8 (15)	0.3 (0.9 [0.39-5.19])	12 (23)	974.9 (3364.9 [1218-22 401])	0.82 (0.7-0.89)[a]
rHev b 3	4 (8)	0.1 (0.5 [0.5-2.86])	1 (2)	8704	0.64 (0.44-0.78)[a]
rHev b 5	26 (50)	6.1 (14.1 [0.55-80.5])	23 (44)	28 259.9 (57 028.3 [1226-256 482])	0.56 (0.33-0.72)[a]
rHev b 6.02	27 (52)	5.4 (27.6 [0.48-67.9])	36 (69)	54 260.3 (84 668 [1329-275 360])	0.59 (0.34-0.74)[a]
rHev b 8	8 (15)	0.3 (1 [0.4-5.24])	16 (31)	3124.6 (9906.7 [1077-45 590])	0.35 (0.08-0.57)[b]
rHev b 9	5 (10)	0.2 (0.7 [0.43-4.02])	0	–	–
rHev b 10	1 (2)	4.02	0	–	–
rHev b 11	15 (29)	0.1 (0.6 [0.36-36.5])	17 (33)	8163.1 (32 602.2 [1058-225 055])	0.98 (0.97-0.99)[a]

Abbreviations: CI, confidence interval; FEIA, fluorescence enzyme immunoassay; sIgE, specific immunoglobulin E.
[a]P<.0001
[b]P<.05

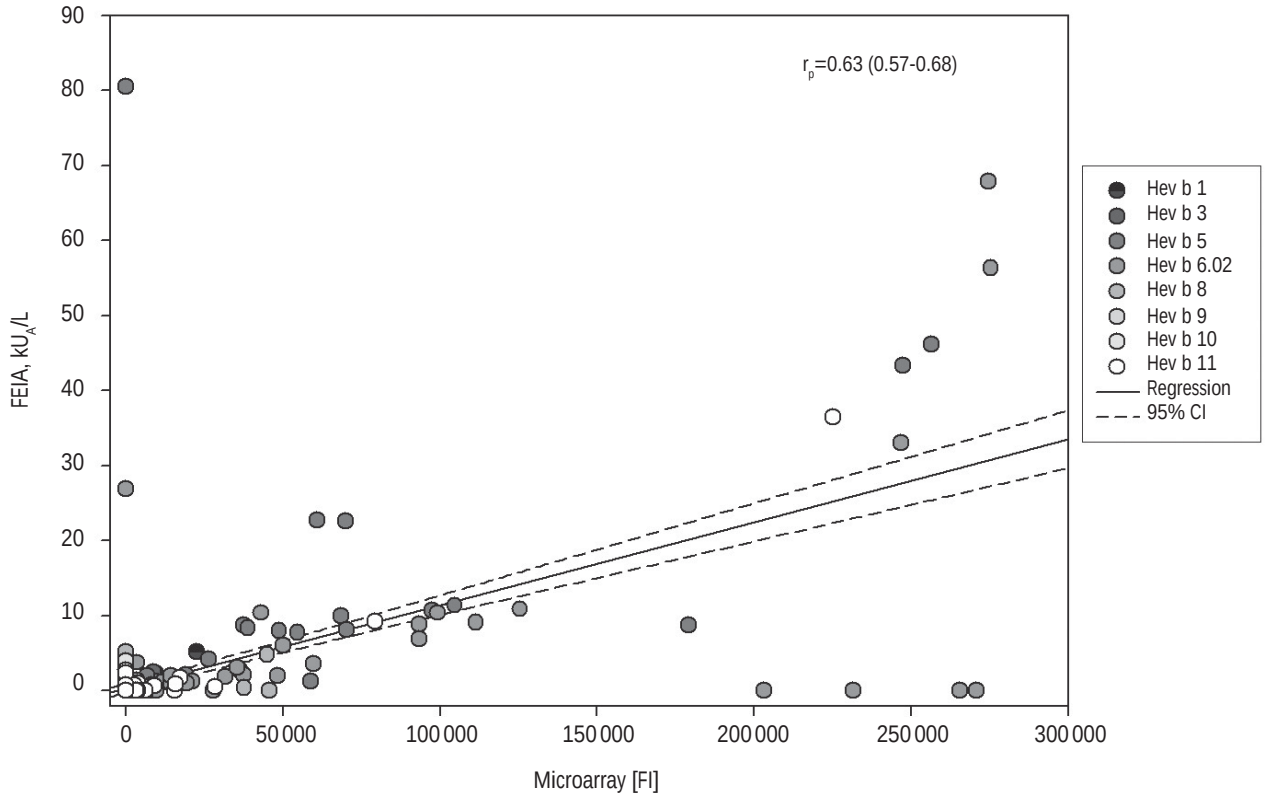

Figure 1. Correlation of all specific IgE serum levels obtained with FEIA and microarray analysis in 52 patients with natural rubber latex allergy. rp: Pearson correlation coefficient (95% confidence interval).

Table 3. Test Performance of FEIA and Microarray-Based Detection of Component-Specific Immunoglobulin E Antibodies in Patients With Immediate-Type Natural Rubber Latex Allergy

	FEIA				Microarray			
Allergen	Sensitivity,% (95% CI)	Specificity, % (95% CI)	Positive Predictive Value, % (95% CI)	Negative Predictive Value, % (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive Predictive Value, % (95% CI)	Negative Predictive Value, % (95% CI)
rHev b 1	15 (7-29)	100 (91-100)	100 (60-100)	53 (43-63)	23 (13-37)	100 (91-100)	100 (70-100)	56 (45-66)
rHev b 3	8 (2-19)	100 (90-100)	100 (40-100)	51 (41-61)	2 (0.1-12)	100 (91-100)	100 (5-100)	50 (39-60)
rHev b 5	50 (36-64)	100 (91-100)	100 (84-100)	66 (54-76)	44 (31-59)	100 (91-100)	100 (82-100)	63 (52-74)
rHev b 6.02	71 (57-82)	100 (91-100)	100 (88-100)	77 (65-86)	69 (55-81)	100 (91-100)	100 (88-100)	76 (63-85)
rHev b 8	15 (7-29)	100 (91-100)	100 (60-100)	53 (43-63)	31 (19-45)	100 (91-100)	100 (76-100)	58 (47-68)
rHev b 9	10 (4-22)	100 (91-100)	100 (46-100)	52 (41-62)	–	–	–	–
rHev b 10	2 (0.1-12)	100 (91-100)	100 (5-100)	50 (39-60)	–	–	–	–
rHev b 11	29 (18-43)	98 (88-100)	94 (68-100)	57 (46-67)	33 (21-47)	98 (88-100)	94 (71-100)	58 (47-69)
Combination[a]	87 (74-94)	98 (88-100)	98 (87-100)	87 (75-94)	81 (67-90)	98 (88-100)	98 (86-100)	83 (71-91)
Latex extract	98 (88-100)	100 (91-100)	100 (91-100)	98 (88-100)	ND	ND	ND	ND

Abreviations: CI, confidence interval; FEIA, fluorescence enzyme immunoassay; ND, not done.
[a]Sum of all test results obtained by single-component analysis.

Table 4. Component-Resolved Specific Immunoglobulin E Recognition Patterns Detected by FEIA and Microarray Analysis in Natural Rubber Latex–Allergic Patients With a History of Cutaneous Reactions (Grade I, II) or Extracutaneous Reactions (Grade III, IV)

Allergen Component	FEIA [kU_A/L][a]		*P* Value[b]	Microarray [FI][a]		*P* Value[b]
	Cutaneous Symptoms (Severity Grade I + II)	Extracutaneous Symptoms (Severity Grade III + IV)		Cutaneous Symptoms (Severity Grade I + II)	Extracutaneous Symptoms (Severity Grade III + IV)	
rHev b 1	0.3 (0.7 [0.48-2.73])	0.3 (1.0 [0.39-5.19])	.90	415 (913.8 [1256-3921])	1383.8 (314.6 [1218-22 401])	.31
rHev b 3	0.2 (0.6 [0.5-2.86])	2.52	.52	–	8704	.40
rHev b 5	5.6 (16.7 [0.55-80.5])	6.5 (11.8 [0.7-46.2])	.82	13 081.7 (31 252 [8121-104 596])	39390.5 (68030.3 [1226-256482])	.10
rHev b 6.02	5.2 (8.5 [0.48-26.9])	5.4 (15.4 [0.57-67.9])	.96	37 039.9 (59 880.7 [1690-246 826])	66888.5 (97052.7 [1329-275360])	.21
rHev b 8	0.4 (1.1 [0.41-4.84])	0.3 (1.0 [0.4-5.24])	.64	6570.6 (14 477.8 [1077-45 590])	597.5 (1144 [1653-3630])	.03
rHev b 9	0.2 (0.7 [0.67-3.48])	0.1 (0.7 [0.43-4.02])	.69	–	–	–
rHev b 10	–	4.02	.40	–	–	–
rHev b 11	0.3 (0.7 [0.36-2.4])	1.8 (6.7 [0.5-36.5])	.31	1511.3 (3511.5 [1384-15 607])	13041 (42 156.4 [2406-225 055])	.22
rBet v 2		ND	ND	ND (5801.7 [1077-22 459])	2821.5 (501.2 [1198-1792])	194.2 .02 .02

Abbreviations: FEIA, fluorescence enzyme immunoassay; FI, fluorescence intensity; ND, not done.
[a]FEIA and microarray results are expressed as mean (SD [range]).
[b]Two-tailed unpaired *t* test performed for group comparisons (*P*<.5)

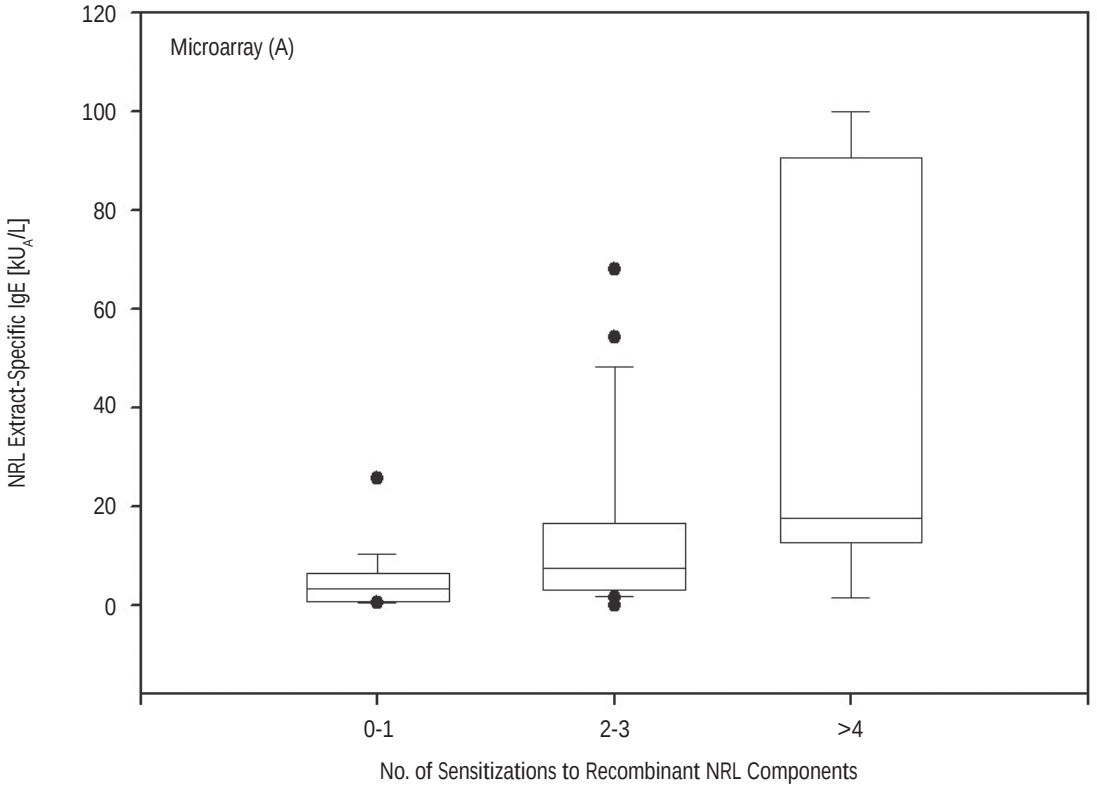

Figure 2. A

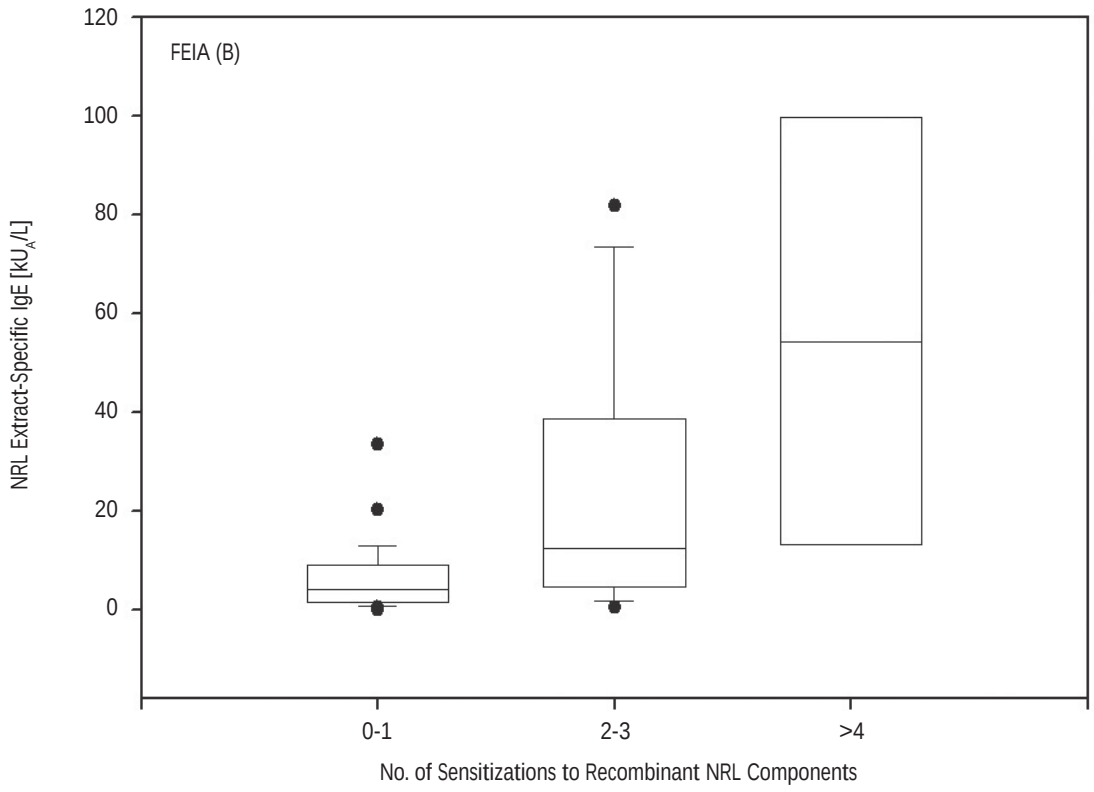

Figure 2. Group comparison of NRL extract-specific Immunoglobulin E serum levels in patients with no or 1 sensitization, 2 to 3 sensitizations and >4 sensitizations as determined by microarray (A) and FEIA (B) analysis with 8 recombinant NRL allergen components.

Discussion

CRD has been reported to be clinically useful in patients with immediate-type allergic reactions to a variety of food allergens and aeroallergens including recombinant *H brasiliensis* proteins [4,14-17]. Furthermore, proteomic microarrays of recombinant and natural allergen components have been described as potentially good diagnostic tools in the context of atopic diseases such as food allergy or allergic rhinoconjunctivitis [8-10,18,19]. However, it is not clear whether microarrays of single recombinant *H brasiliensis* proteins represent a viable mode of sIgE determination in humans with NRL allergy. Therefore, we analyzed serum samples from 102 adults to evaluate the performance of an allergen microarray containing 8 recombinant *H brasiliensis* allergen components.

The microarray identified rHev b 5 and rHev b 6.02 as the most relevant allergen components eliciting positive sIgE results in 44% and 69% of patients, respectively. This is consistent with previous reports describing sensitization rates of up to 70% for Hev b 5 and 40%-80% for Hev b 6.02 in both adult patients and children who had undergone multiple surgical procedures [4,5,20]. Moreover, sIgE antibodies against rHev b 11 could only be detected in patients who were also sensitized to rHev b 6.02, probably because of an amino acid sequence homology of nearly 60% between rHev b 6.02 and the chitin-binding domain of rHev b 11 [21].

In contrast, microarrayed rHev b 1 and rHev b 3 elicited sIgE responses in only a minority of our study population (<25%). This supports the results of earlier publications that defined these components as specific biomarkers of NRL sensitization in children suffering from spina bifida, but not in NRL-allergic adults [2,4,22]. The lack of detectable sIgE against rHev b 9 and rHev b 10 in our population is consistent with previous enzyme-linked immunosorbent assay–based and FEIA-based studies identifying these NRL components as basically irrelevant in the clinical diagnosis of NRL allergy [4,5,23].

Intriguingly, FEIA and microarray results revealed good levels of correlation with most of the components analyzed. These findings are consistent with data recently published by Wöhrl et al [10], who assessed the clinical utility of a similar protein microarray containing inhalant allergen components. By matching combinations of microarrayed recombinant birch pollen and grass pollen components with corresponding FEIA allergens, the authors demonstrated that assay performance did not differ fundamentally between both diagnostic methods.

However, the rate of sensitization against microarrayed NRL profilin rHev b 8 (31%) was clearly high compared to the FEIA results obtained in our population (15%) and previous studies of American (16%) and German (14%) health care workers [4]. This discrepancy might be attributable to a higher analytical sensitivity of the allergen microarray as compared to the FEIA method, which has already been demonstrated in

standardized dilution assays, particularly for the birch pollen profilin rBet v 2 [24]. Interestingly, 38% of rHev b 8–positive sera also recognized rBet v 2, thus implying sensitization to cross-reactive plant panallergens [25,26]. Still, 2 of these patients were monosensitized to microarrayed rHev b 8 and presented with a clear-cut history of contact urticaria immediately after NRL exposure. This suggests a possible role of rHev b 8 as a clinically relevant NRL allergen, albeit in a small number of affected individuals. However, this could not be investigated more exhaustively, since further profilins from other plant allergen sources such as timothy grass (Phl p 12), tomato (Lyc e 1), or melon (Cuc m 2) were not implemented on the microarray used in the present study.

Of note, the panel of allergen components investigated in our study can be regarded as a comprehensive repertoire of clinically relevant NRL proteins, since the number of sensitizations to single microarray components was significantly associated with the NRL extract–specific IgE level (Figure 2A). This is also highlighted by the high rates of sensitivity obtained with combined CRD data in both FEIA (87%) and microarray (81%) assays. Nevertheless, component-resolved sIgE detection did not reach the diagnostic sensitivity of extract-based FEIA analysis (98%), irrespective of the component-based test method used. This might be due to the fact that linear epitope sequences of recombinant proteins that have not undergone complete posttranslational modification potentially interfere with the recombinant molecule's IgE-binding capacity [27]. In addition, 2 further NRL components (Hev b 2, Hev b 13) were not included in the allergen microarray, because they were not available in recombinant form at the time of this investigation. However, a prior allergenicity study in populations from 3 distinct geographical areas revealed maximum sensitization rates of 15% for purified natural glycosylated Hev b 2 (nHev b 2), while nHev b 13 yielded markedly higher sensitization rates of up to 30% [28]. Thus, we cannot rule out the possibility that the addition of these 2 components would have significantly enhanced the microarray's diagnostic sensitivity.

It is also noteworthy that the very high degrees of specificity observed for single allergen components and combined CRD data in our study are consistent with the results of another research group who report specificities of 100% using either rHev b 6 or a combination of all 13 NRL components [5]. Nevertheless, the specificity data for our patients should be interpreted with caution, since the pretest probability could have been directly influenced by a study population selection bias in favor of seronegative controls. This might hamper the transferability of our specificity data to other, nonselected patient populations.

Another crucial area of allergic disease management is the capacity to predict whether the affected individual will develop severe reactions after exposure to the culprit allergen. In this context, CRD has recently identified characteristic sensitization patterns that were closely related to the clinical reaction level of patients with food allergy [29,30]. On the contrary, sIgE profiles associated with a more severe course of NRL allergy have not been described to date and could not be demonstrated in our patients either. While significantly higher levels of rHev b 8–specific IgE in patients with mild to moderate clinical reactions could be traced back to rBet v 2–associated profilin cross-sensitization, sIgE to the other NRL components was not differentially expressed within the investigated patient groups. Therefore, NRL-specific IgE recognition patterns cannot be regarded as reliable biomarkers of severity of reaction in patients with latex allergy.

The present study demonstrates that detection of sIgE antibodies against multiple microarrayed allergen components can be performed with minimal volumes of patient serum that have been shown to be easily obtained by capillary blood sampling [31]. Therefore, allergen microarrays might serve as minimally invasive tools for NRL-specific IgE detection, thus avoiding peripheral venipuncture, which is somewhat problematic in the case of infants and small children. Perhaps even more importantly, the investigated microarray represents a diagnostic platform that can be loaded with virtually all allergen components of relevance in the context of NRL allergy. This would not only drastically empower future epidemiological studies, but also clearly enhance the clinical management of NRL-allergic patients with symptomatic cross-reactivity to plant foods. For instance, natural or recombinant marker allergens for the latex-fruit syndrome such as Hev b 6–homologous class-I-chitinases from chestnut (Cas s 5) and banana (Mus a 1), the Hev b 7–homologous patatin-like protein from potato (Sol t 1), or the Hev b 2–homologous ß-glucanase from olive pollen (Ole e 9) could be implemented using the microarray for simultaneous sIgE profiling [32-35]. Finally, trials of NRL-specific immunotherapy could be improved by the establishment of comprehensive pretreatment sensitization patterns that have previously been shown to be relevant, as in birch pollen–specific immunotherapy [36,37].

In conclusion, allergen microarrays are a novel tool to diagnose NRL-specific sensitization. They show performance characteristics comparable to the current CRD-based sIgE detection method and may be particularly indicated in young children from whom only small blood volumes are obtainable. Additionally, they could significantly broaden our knowledge of sensitization patterns in different geographical areas and in patient groups with varying NRL allergy risk profiles. Further large-scale evaluation studies in unselected patient populations and in high-risk groups such as children with spina bifida are warranted before the allergen microarray can be introduced into the routine management of patients with NRL allergy.

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