

Original article

Array-based profiling of ragweed and mugwort pollen allergens

Background: Ragweed (*Ambrosia artemisiifolia*) and mugwort (*Artemisia vulgaris*) pollen is the main cause of allergic reactions in late summer and autumn. The differential diagnosis between ragweed and mugwort pollen allergy is a frequent problem encountered by allergologists in areas where both plants are present due to shared antigenic structures and overlapping flowering seasons.

Objective: To evaluate the sensitization pattern of weed allergic patients towards a large panel of purified allergens in the microarray format and by enzyme-linked immunosorbent assay (ELISA).

Methods: Eight ragweed and six mugwort pollen allergens were purified from natural source or expressed as recombinant proteins in *Escherichia coli*. Allergens were spotted on protein microarray slides or coated onto ELISA plates. Sera from 19 ragweed and/or mugwort allergic individuals were used to determine the reactivity towards single molecules in both assays.

Results: All ragweed allergic individuals were sensitized to Amb a 1, among them 30% were monosensitized to the major ragweed allergen. Art v 1 and Art v 3 were recognized by 89% of mugwort pollen-allergic patients. Extensive cross-reactivity was observed for both patient groups mainly involving the pan-allergens profilin and nonspecific lipid transfer proteins. Comparable IgE profiles were obtained with both allergen microarray and ELISA methods.

Conclusions: Molecule-based diagnosis provides essential information for the differential diagnosis between ragweed and mugwort pollen allergy and for the selection of the appropriate allergen source for specific immunotherapy.

G. Gadermaier^{1*}, N. Wopfner^{1*},
M. Wallner^{1*}, M. Egger¹,
A. Didierlaurent², G. Regl³,
F. Aberger³, R. Lang⁴, F. Ferreira¹,
T. Hawranek⁴

¹Christian Doppler laboratory for allergy diagnosis and therapy, Department of Molecular Biology, University of Salzburg, Salzburg, Austria; ²Research Laboratory, Stallergenes S.A., Antony, France; ³Department of Molecular Biology, Division of Genomics, University of Salzburg, Salzburg, Austria; ⁴Department of Dermatology, Paracelsus Private Medical University, Salzburg, Austria

Key words: allergen microarray; molecule-based allergy diagnosis; mugwort; ragweed.

Dr Gabriele Gadermaier
Department of Molecular Biology
University of Salzburg
Hellbrunnerstraße 34
A-5020 Salzburg
Austria

*The authors contributed equally.

Accepted for publication 5 April 2008

Ragweed (*Ambrosia artemisiifolia*) and mugwort (*Artemisia vulgaris*) belong to the *Asteraceae* plant family and represent the most important allergenic sources within this genus (1). The *Ambrosia* sp. are native to eastern and central North America, but are also persistently spreading in Central and Southern Europe. In the USA and Canada 45% of atopic individuals suffer from ragweed pollen allergy. In Europe, atopic sensitization in ragweed-populated areas (e.g. France, Italy, Austria, Hungary, Croatia and Bulgaria) is constantly increasing.

Mugwort is spread in the temperate and humid zones of the northern hemisphere, the Mediterranean basin and parts of Asia. The incidence of allergic reactions caused by *A. vulgaris* pollen is between 10% and 14% among pollinosis patients. The expansion of ragweed in formerly mugwort-dominated areas is concomitant with an increase of weed pollen allergies in those regions. Several studies showed that the allergens from ragweed and mugwort share antigenic structures; however, the respective major allergens Amb a 1 and Art v 1 are structurally and antigenically not related (2, 3). Sensitivity to both

plant species was demonstrated to be caused by co-recognition of the major allergens rather than to cross-reactivity of common antigens (4). As there is an extensive overlap in the flowering seasons of ragweed and mugwort, differential diagnosis is crucial for successful allergen-specific immunotherapy.

In recent years, the microarray technology based on proteins did arise and thus a number of purified allergens were used to dissect patient's IgE reactivity profiles (5–7). Regarding weed pollen allergens, solely rArt v 1 and nArt v 3 were tested in the microarray format. However, no clear correlation was found between the used allergens in microarray and mugwort extract in the ImmunoCAP system (7).

This is the first study investigating the pattern of IgE reactivity of weed pollen-allergic individuals using a panel of purified proteins comprising all mugwort and ragweed pollen allergens described so far. Furthermore, the panel of weed pollen allergens was used to compare and verify the sensitivity of component-resolved diagnosis using protein microarray and enzyme-linked immunosorbent assay (ELISA).

Methods

Patients

Sera from 19 ragweed and/or mugwort pollen allergic patients from three different centres were included in this study. Mugwort pollen sensitized patients' sera were derived from Salzburg ($n = 5$) and Vienna (Dr Christof Ebner, Allergieambulatorium, Vienna, Austria, $n = 5$), ragweed allergic patients' sera from Canada ($n = 9$). Patients were selected based on typical case history, positive skin prick test, and increased specific IgE serum levels to ragweed and/or mugwort (RAST class 2–6). Informed written consent was obtained from all subjects included in the study.

Pollen extracts and purified allergens

Extracts from ragweed (*A. artemisiifolia*) and mugwort (*A. vulgaris*) pollen (Allergon AB, Ångelholm, Sweden) were prepared by suspending 150 mg of pollen in 1 ml distilled water and shaking at room temperature for 15 min. After centrifugation at 14 000 *g*, the supernatant was collected and stored at -20°C until use. Natural allergens from ragweed and mugwort (Amb a 1, Amb a 8, Art v 1 and Art v 3) were purified from natural pollen sources as previously described (8–11). Production in *Escherichia coli* of all recombinant proteins (rAmb a 5, rAmb a 6, rAmb a 8, rAmb a 9, rAmb a 10, rArt v 1, rArt v 4, rArt v 5 and rArt v 6) was described elsewhere (4, 10–12) (Table 1). Recombinant Amb a 1.3 was purchased from Biomay AG (Vienna, Austria).

Protein microarray

Purified allergens were used at 1.000, 0.500, 0.250 and 0.125 mg/ml in 300 mM sodium phosphate pH 8.5, 0.005% (v/v) CHAPS, 1 mg/ml human serum albumin (HSA) and spotted in quadruplicates onto Nexterion Slide H (Schott, Jena, Germany) with a MicroGridII arrayer (Genomic Solutions, Ann Arbor, MI, USA) using Stealth SMP3 Microarray Spotting Pins (TeleChem International, Inc., Sunnyvale, CA, USA) with a delivery volume of 600 pL. Purified human IgE (Serotec, Raleigh, NC, USA) was diluted to 0.500, 0.250, 0.125 and 0.063 mg/ml and used as reference for quantitative determination of specific IgE. Spotting buffer and HSA (1 mg/ml) were used for background subtraction and to assure specificity respectively. Stable coupling of proteins to the chip surface was achieved by incubating the slides in a humidity chamber for 1 h at room temperature using 0.5× phosphate buffer saline (PBS). Slides were briefly washed with double distilled H_2O followed by 3× washing steps with 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 , 1.4 mM KH_2PO_4 , pH 7.5 with 0.5% (v/v) Tween 20 (PBST). Free binding sites were blocked at room temperature for 1 h with 50 mM ethanolamine in 50 mM sodium borate buffer pH 8.0 with gentle agitation. Slides were rinsed three times with PBS and double distilled H_2O and dried by centrifugation at 200 *g* for 5 min. Twenty microlitre of human serum was used for each array and slides were incubated overnight at 25°C in a hybridization chamber in the dark. Slides were washed with PBST and double distilled H_2O and dried as described above. Anti human IgE (BD Biosciences, Franklin Lakes, NJ, USA) was labelled with Alexa Fluor 647 Monoclonal Antibody Labeling Kit (Invitrogen, Carlsbad, CA, USA) according to

Table 1. Purification of natural and recombinant weed allergens

Allergen	Source	Purification method	Additional characterization
Amb a 1 Pectate lyase family	Natural (ragweed pollen) Recombinant (<i>E. coli</i>)	Gel filtration, ion exchange, IMAC, gel filtration	Protein obtained and method developed by King T.P. Method developed by Biomay AG from inclusion bodies (solubilized in urea)
Amb a 5 Group 5 allergen	Recombinant (<i>E. coli</i>)	IMAC	Purified from inclusion bodies using C-terminal His-tag, followed by refolding
Amb a 6 nsLTP family	Recombinant (<i>E. coli</i>)	IMAC, TEV cleavage, IMAC	Purified from soluble fraction, His-tag cleaved by His-tagged TEV
Amb a 8 Profilin	Natural (ragweed pollen) Recombinant (<i>E. coli</i>)	Immunoaffinity chromatography with poly-(L-proline) IMAC, TEV cleavage, IMAC	Produced under denaturing conditions (urea), followed by refolding using dialysis Purified from soluble fraction, His-tag cleaved by His-tagged TEV
Amb a 9 Polcalcin	Recombinant (<i>E. coli</i>)	IMAC, TEV cleavage, IMAC	Purified from soluble fraction, His-tag cleaved by His-tagged TEV
Amb a 10 Calcium-binding protein	Recombinant (<i>E. coli</i>)	IMAC, TEV cleavage, IMAC	Purified from soluble fraction, His-tag cleaved by His-tagged TEV
Art v 1 PR-12 protein, defensin-like	Natural (mugwort pollen) Recombinant (<i>E. coli</i>)	Ultrafiltration, ion exchange, gel filtration Ultrafiltration, ion exchange	Glycoprotein migrating as double-band at 24–28 kDa in SDS-PAGE Purified from soluble fraction
Art v 3 nsLTP family	Natural (mugwort pollen)	Ultrafiltration, ion exchange, gel filtration	Purified under the same conditions as nArt v 1
Art v 4 Profilin	Recombinant (<i>E. coli</i>)	Immunoaffinity chromatography with poly-(L-proline)	Produced under denaturing conditions (urea), followed by refolding using dialysis
Art v 5 Polcalcin	Recombinant (<i>E. coli</i>)	IMAC, TEV cleavage, IMAC	Purified from soluble fraction, His-tag cleaved by His-tagged TEV
Art v 6 Pectate lyase family	Recombinant (<i>E. coli</i>)	IMAC	Purified from inclusion bodies (solubilized in urea); refolding using dialysis

TEV, tobacco etch virus protease; IMAC, immobilized metal ion affinity chromatography; nsLTP, nonspecific lipid transfer protein.

manufacturer's instructions. Labelled antibody was diluted 1 : 1000 in PBST and slides were incubated in a humidity chamber for 2 h at 25°C in the dark. After washing, slides were dried and subsequently scanned on a ScanArray 4000 confocal microarray scanner (Perkin Elmer, Waltham, MA, USA).

Data acquisition and analysis

Images were acquired with 100% laser power with optimum gains ranging from 60 to 95. Array readouts were regarded positive when they exceeded two times the background values obtained with buffer and HSA. Calculated values represent the mean of quadruplicates and were baseline subtracted. Bound-specific IgE (ng/ml) for each allergen was calculated based on the spotted IgE standards.

ELISA

Pollen extracts (16 µg/ml) and purified proteins (5 µg/ml) were coated overnight at 4°C to Maxisorp ELISA plates (Nalge Nunc, Rochester, NY, USA). Plates were washed twice with 137 mM NaCl, 10 mM Tris, pH 7.5, 0.5% (v/v) Tween 20 (TBST) and blocked with TBST, 1% (w/v) BSA, for 4 h at room temperature. Patients' sera were diluted 1 : 4 in TBST, 0.5% (w/v) BSA and incubated overnight at 4°C.

After five TBST buffer washes, plates were incubated (1 h at 37°C and 1 h at 4°C) with alkaline phosphatase-conjugated monoclonal anti-human IgE antibodies (BD Biosciences, San Diego, CA, USA) and developed with the substrate 4-Nitrophenyl-phosphate (Sigma-Aldrich, St Louis, MO, USA). The optical density was measured at 405 nm, reference wavelength 492 nm, using a microplate reader (SLT Spectra 1.1; TECAN, Salzburg, Austria). Measurements were performed in duplicates. Readings exceeding three times the background values were considered positive.

Statistical analysis

Correlation efficiency of microarray and ELISA for single allergens was performed based on reactivity/nonreactivity and calculated by the Spearman's rank test (SPSS, Chicago, IL, USA).

Results

Allergens

This study aimed to evaluate the diagnostic value of a large panel of *Ambrosia* and *Artemisia* pollen allergens purified from the natural source and/or produced as recombinant proteins in *E. coli* (Table 1). All proteins have been biochemically and immunologically characterized. Allergens with limited sequence information (Amb a 3/7 family and a 60 kDa protein from mugwort pollen), Art v 2, which was only recently described (13) and Amb a 2, an isoallergen of Amb a 1, were all not included in our working set.

Comparison microarray/ELISA and natural/recombinant allergens

For microarray analyses, increasing amounts of purified proteins were spotted onto protein slides and the immunoreactivity of 19 sera from ragweed and mugwort pollen-allergic individuals was evaluated. Specificity of the signals was assured using HSA, buffer alone and nonatopic human serum (data not shown). Spots with concentrations in the linear range of the microarray system were used to calculate the IgE concentration based on the purified IgE standard spotted in parallel (Fig. 1). IgE-reactivity was additionally evaluated using ELISA experiments. Proteins were coated onto ELISA plates and incubated with patients' IgE from mugwort and ragweed allergic individuals and reactivity was measured at OD₄₀₅.

With the exception of natural and recombinant Amb a 8, we found good correlation (correlation coefficient 0.896–0.889) for the IgE binding activity of the allergens in both microarray and ELISA systems. For example, natural and recombinant forms of Art v 1 showed similar

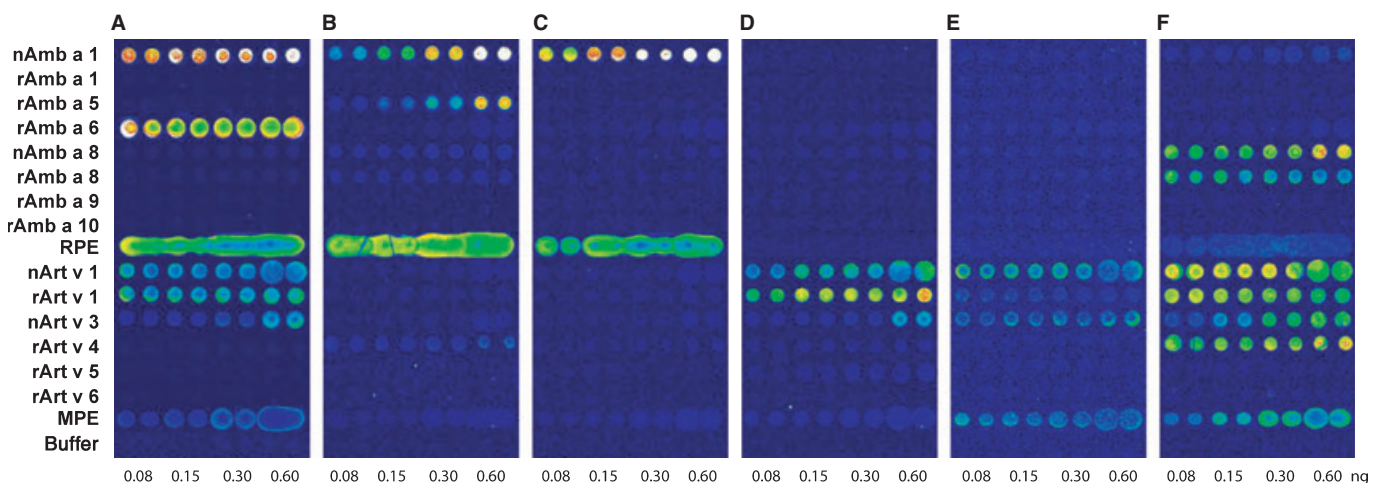


Figure 1. Microarray with pollen allergic patients' sera. Allergens were spotted as duplicates in serial dilutions (0.08, 0.15, 0.30 and 0.60 ng protein from left to right). Slides were incubated with sera derived from ragweed pollen allergic patients A, B and C (corresponding to patient number 3, 5 and 7) and mugwort pollen allergic patients D, E and F (corresponding to patient number 1, 4 and 6). RPE, ragweed pollen extract; MPE, mugwort pollen extract.

IgE binding in ELISA and microarrays. One patient (Nr. M4) only reacted with nArt v 1, but showed no reactivity with the recombinant protein in the microarray assay, whereas another patient (Nr. R9) demonstrated almost 10 times stronger reactivity with rArt v 1 compared to the natural protein. In the ragweed pollen-sensitized group of patients, rAmb a 8 showed lower IgE reactivity compared to the natural protein in the microarray system, whereas the ELISA showed comparable results for both natural and recombinant forms. Thus, the microarray system seemed not to be suitable for rAmb a 8 which might be attributed to problems in allergen and microarray surface material interaction. Recombinant Amb a 10 was only reactive in the ELISA system and also Art v 4 showed patient-specific differences in both systems tested. The same problem could be observed with nArt v 3, which did react with only two patients in the array system and three in the ELISA. The recombinant form of Amb a 1 was essentially nonreactive in both assays, whereas Amb a 1 purified from natural source showed good IgE binding activity in both systems. Similar results were obtained for

recombinant Art v 6, the pectate lyase from mugwort pollen homologous to ragweed Amb a 1.

IgE reactivity pattern

Sensitization pattern towards single ragweed and mugwort allergens was found to be heterogeneous and differed substantially among allergic individuals (Table 2). Signals obtained in either microarray system or ELISA were counted positive. All ragweed allergic patients' sera displayed IgE reactivity to Amb a 1, the major ragweed pollen allergen. Three patients were monosensitized to Amb a 1, while all other ragweed patients reacted with at least two allergens (predominantly Amb a 8 and Amb a 6) and 40% showed reactivity to three ragweed pollen allergens (Fig. 2). Interestingly four ragweed sensitized patients also reacted with Art v 1, the major allergen from mugwort pollen, suggesting that these patients are *Ambrosia* and *Artemisia* co-sensitized (4). Three patients showed reactivity with nArt v 3, a nonspecific lipid-transfer protein (nsLTP) from mugwort

Table 2. IgE reactivity of ragweed (R1–R10) and mugwort (M1–M9) pollen-allergic patient's sera towards purified allergens and extracts

	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	M1	M2	M3	M4	M5	M6	M7	M8	M9
A. Allergen-specific IgE (ng/ml) at 0.6 ng spotted allergen calculated based on intensities obtained from spotted purified IgE																			
nAmb a 1	95.4	9.0	283.8	138.9	39.9	77.4	95.7	35.7	1075.2	87.3						3.6	2.7		
rAmb a 1																			
rAmb a 5	27.0				9.6														
rAmb a 6		5.7	148.5			14.4						2.7							
nAmb a 8		11.4		76.5	0.9								49.5			34.2	87.9	10.2	48.9
rAmb a 8		7.2											14.4			18.3	51.3	4.5	
rAmb a 9				67.5															
rAmb a 10																			
RPE	103.8	44.1	198.9	336.3	99.3	89.4	91.2	57.9	718.5	71.7						9.3	48.6	16.8	
nArt v 1		28.8	42.9						33.0		22.5	1.5		42.6	44.7	69.3	160.5	89.1	70.2
rArt v 1		16.8	39.6						291.3		44.7	2.4			35.1	44.7	113.7	70.2	43.5
nArt v 3		23.1	10.2								3.9	1.5		9.6	40.2	18.9	40.5	79.2	26.7
rArt v 4		72.0		12.0	2.1								32.1			32.7	114.6	15.9	20.1
rArt v 5				9.6															
rArt v 6																			
MPE		49.8	14.7	10.8							1.5	4.5	6.6	25.8	53.7	30.6	75.6	70.8	74.7
B. IgE levels measured as OD 405 nm in ELISA																			
nAmb a 1	2.47	1.80	>2.80	>2.80	>2.80	>2.80	>2.80	>2.80	>2.80	>2.80						2.73	0.61	0.32	
rAmb a 1																			
rAmb a 5	0.54				0.59														
rAmb a 6		1.33	>2.80			0.85													
nAmb a 8		2.42		nd	0.73	0.53		0.60			0.54		1.47				2.61	1.80	>2.80
rAmb a 8		2.56		1.91	0.71	0.46		0.66			0.30		0.90				2.21	1.65	1.62
rAmb a 9				2.07															
rAmb a 10				0.46															
RPE	0.60	1.82	>2.80	nd	1.27	2.05	0.37	0.59	>2.80	0.45					0.34	0.50	1.24	0.32	0.55
nArt v 1		1.86	>2.80	0.22					>2.80		>2.80	0.33		0.42	2.30	1.15	2.67	>2.80	>2.80
rArt v 1		0.68	>2.80	0.38					>2.80		>2.80	0.31			1.88	0.73	2.42	>2.80	>2.80
nArt v 3		1.97	>2.80						2.09		0.95	0.32		0.63	2.14	1.51	1.50	>2.80	>2.80
rArt v 4		2.58		>2.80	0.77			0.73			0.31		1.16				2.47	2.03	2.49
rArt v 5				2.40															
rArt v 6																			
MPE		2.17	>2.80	nd					2.26	0.46	1.05			0.38	1.76	1.12	2.05	>2.80	>2.80

RPE, ragweed pollen extract; MPE, mugwort pollen extract; nd, not determined.

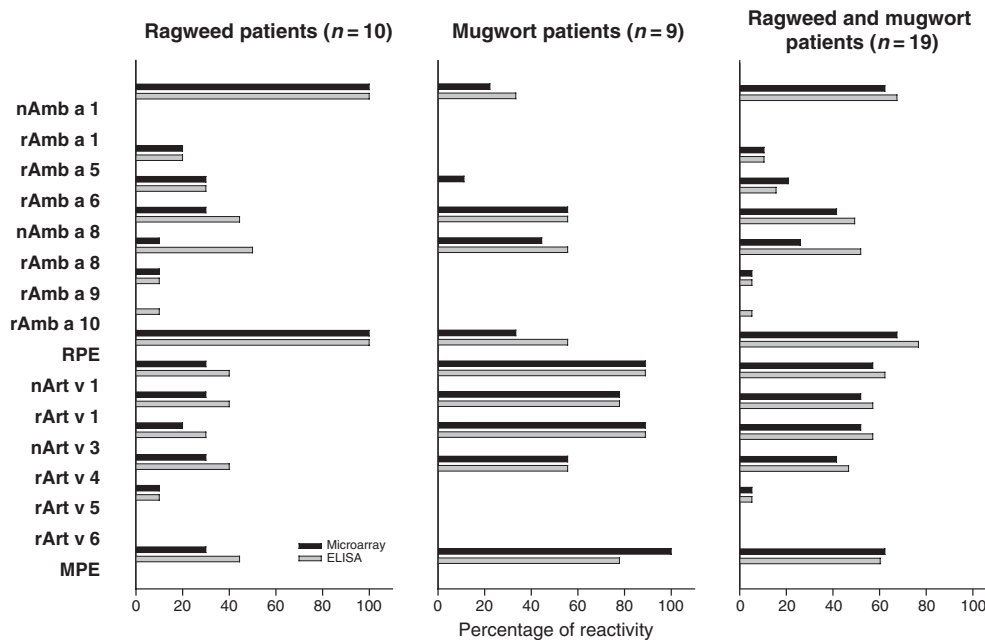


Figure 2. IgE reactivity (%) of patients' sera with ragweed and mugwort pollen allergens in microarray (black bars) and ELISA (grey bars). RPE, ragweed pollen extract; MPE, mugwort pollen extract.

with cross-reactivity to the homologous protein in ragweed (Amb a 6). Besides, cross-reactivity between the pan-allergens profilin and calcium-binding proteins could be observed for four of 10 and one of 10 patients respectively.

Among the mugwort pollen-sensitized patients, Art v 1, the major mugwort pollen allergen was recognized by eight of the nine sera. The nsLTP of mugwort pollen, Artv 3 was recognized with the same frequency as Art v 1, although lower IgE reactivity was observed. Eighty-nine per cent of the mugwort allergic individuals were sensitized to at least two mugwort pollen allergens and 40% showed reactivity to three different allergens. Mugwort profilin was recognized with a frequency of about 60% (Fig. 2). Six of nine mugwort pollen-allergic patients also displayed IgE reactivity with ragweed pollen allergens Amb a 1, Amb a 6 and Amb a 8 (Table 2). Almost no cross-reactivity between Art v 3 and Amb a 6 was observed for patients' sensitized to mugwort, suggesting a possible unidirectional IgE cross-recognition of these two LTP.

Extract vs molecule-based diagnosis

Patients' sera used in this study were designated ragweed or mugwort pollen sensitized based on the results of the routine clinical setup tested. Our data demonstrate that patients often react with both extracts; four of 10 and five of nine of ragweed and mugwort pollen suspected individuals respectively. Using Amb a 1 and Art v 1 as marker molecules for diagnosis of ragweed and mugwort pollen allergy, patients R2, R3 and R9 as well as M6, M7

and M8 can be considered co-sensitized and allergy treatment should therefore be accordingly. Patient M9 also shows reactivity with both extracts, but clearly lacks reactivity to Amb a 1. In fact, IgE reactivity to the ragweed extract can be solely attributed to the cross-reactive profilin Amb a 8, and therefore the patient might not be sensitized to ragweed. In this particular case, component-based diagnosis can identify the eliciting molecules and thus allows to discriminate between cross-reactivity and co-sensitization.

Discussion

Pollen extracts currently used for allergy diagnosis and therapy pose the problem of poorly defined allergenic components and great variability between different manufacturers and batches (14). Some allergens are either under-represented in extracts, difficult to extract or the yields of allergen purified from natural pollen sources are poor. Because extracts are used for diagnosis and treatment of allergic diseases, these differences in allergen content and concentration (e.g. major allergens) can be significant and therefore clearly demonstrate the need for well-characterized allergenic molecules (15, 16). In the case of Art v 1, the yields and cost-efficacy of the recombinant production (15 mg/l bacterial culture) was superior to purification from natural source (0.25–0.5 mg/g pollen).

In general allergen microarrays are a promising technique, not only for research purpose but also for routine diagnostic settings. Advantages are the simultaneous

measurement of many allergen samples and the use of minute amounts of serum, which might be of great benefit, particularly for paediatric patients. The parallel spotting of different allergen concentrations facilitates measurement in a linear range and thus provides more accurate results regarding the quantification of specific IgE. Computer-based evaluation of the gathered data not only facilitates diagnosis of allergic diseases but also provides much more comprehensive information on patients' sensitization patterns. Furthermore, spotted protein microarray slides can be stored up to at least 6 months at -20°C , which would make them ideal for large-scale production and use in clinical routine diagnostics.

The only study so far available comparing microarray and CAP system in clinical practice showed that rArt v 1 was not appropriate for diagnosis of mugwort pollen allergy (7). Because no information was given about the production of the used rArt v 1, it was not clear if the poor performance was associated with the quality of the protein preparation or the lack of carbohydrate chains, which are crucial for recognition by IgE antibodies from certain Art v 1-allergic patients. With exception of one patient, the IgE reactivity of natural and recombinant Art v 1 was comparable in both our test systems.

The results obtained in this study clearly demonstrate that Art v 3, the mugwort ns-LTP, can be considered as a new major mugwort pollen allergen, with a prevalence of 85% reactivity among mugwort pollen-sensitized individuals. Therefore, adequate molecule-based diagnosis and therapy of mugwort pollen allergy should also include the Art v 3 allergen. As all sera from the mugwort group were obtained from Austrian donors, the high frequency of IgE reactivity of Art v 3 was rather unexpected. In the Mediterranean area, patients are mainly sensitized to nsLTP from *Rosaceae*-derived foods (e.g. peach and chestnut) and within this plant family extensive cross-reactivity has been described (9, 17, 18). In addition, it has been reported that mugwort pollen-allergic patients in Mediterranean countries frequently react with Art v 3. Whether Art v 3 from mugwort pollen could act as primary sensitizing agent and elicit adverse reactions in *Rosacea* food-allergic patients in the Mediterranean area still needs to be evaluated. Interestingly, limited cross-reactivity between Art v 3 and ragweed LTP Amb a 6 was observed, which could be explained by the low sequence identity (32%) between the two proteins.

All ragweed-sensitized patients in this study displayed IgE antibodies against natural Amb a 1, the major ragweed pollen allergen, whereas mugwort-sensitized patients showed very low levels of IgE reactivity with nAmb a 1. Unfortunately, recombinant Amb a 1 and Art v 6 (Amb a 1-homologue in mugwort) produced in *E. coli*

failed to bind IgE antibodies under native conditions in both test systems, and hence recombinant production of allergenic pectate lyases is still a major obstacle for their use in molecule-based approaches.

Ambrosia and *Artemisia* are flowering nearly at the same time of the year, which poses problems for patients reacting to both plants and for those whom specific immunotherapy is warranted. In this study, a group of mugwort patients (Nr. M1, M3, M9) was identified to react to the pan-allergen profilin from ragweed Amb a 8 in addition to various mugwort allergens. As extensive IgE cross-reactivity has been reported among plant profilins (19), it could be possible that this patient is not primarily sensitized by mugwort pollen but by profilins from other sources (e.g. ragweed or grass pollen). Extract-based diagnosis would identify these individuals as ragweed and mugwort allergic subjects, although this reactivity could be simply regarded as cross-reactivity. In such cases, treatment with both extracts might not be recommended, to avoid sensitization with other pollen allergens as shown for birch pollen extract-based immunotherapy (20). A previous study conducted by Asero et al. (4) showed that allergic individuals reacting to the major allergens Amb a 1 and Art v 1 from ragweed and mugwort pollen, respectively, are co-sensitized and treatment should be performed with both plant extracts, which could be regarded as a general guideline for clinical practice. However, allergen profiling is not usually carried out in routine setups. The use of single allergen molecules for diagnosis could fully address these problems and would allow a patients' customization of specific immunotherapy. As an increasing number of recombinant allergens become available, molecule-based diagnosis could improve the decision-making process in immunotherapy for any allergy. The microarray platform would allow parallel testing of numerous allergens using little amounts of sera. High-throughput procedures would help studying the sensitization and cross-reactivity patterns of large and representative samples of the population. Despite all the exciting possibilities of this new technology, optimization of recombinant production and validation of allergen arrays on an allergen-by-allergen basis should be performed before a routine application in clinical diagnosis of allergies.

Acknowledgments

This work was supported by the grant NFN S88 (Projects S8802 and S8816) from the 'Fonds zur Förderung der Wissenschaftlichen Forschung, FWF' Vienna, Austria. The authors are thankful to Annemarie Frischau for providing the Microarray Facilities.

References

1. Gadermaier G, Dedic A, Obermeyer G, Frank S, Himly M, Ferreira F. Biology of weed pollen allergens. *Curr Allergy Asthma Rep* 2004;**4**:391–400.
2. Wopfner N, Gadermaier G, Egger M, Asero R, Ebner C, Jahn-Schmid B et al. The spectrum of allergens in ragweed and mugwort pollen. *Int Arch Allergy Immunol* 2005;**138**:337–346.
3. Oberhuber C, Ma Y, Wopfner N, Gadermaier G, Dedic A, Niggemann B et al. Prevalence of IgE-binding to Art v 1, Art v 4 and Amb a 1 in mugwort-allergic patients. *Int Arch Allergy Immunol* 2008;**145**:94–101.
4. Asero R, Wopfner N, Gruber P, Gadermaier G, Ferreira F. *Artemisia* and *Ambrosia* hypersensitivity: co-sensitization or co-recognition? *Clin Exp Allergy* 2006;**36**:658–665.
5. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;**16**:414–416.
6. Jahn-Schmid B, Harwanegg C, Hiller R, Bohle B, Ebner C, Scheiner O et al. Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin Exp Allergy* 2003;**33**:1443–1449.
7. Wohrl S, Vigl K, Zehetmayer S, Hiller R, Jarisch R, Prinz M et al. The performance of a component-based allergen-microarray in clinical practice. *Allergy* 2006;**61**:633–639.
8. King TP, Norman PS, Connell JT. Isolation and characterization of allergens from ragweed pollen II. *Biochemistry* 1964;**3**:458–468.
9. Diaz-Perales A, Lombardero M, Sanchez-Monge R, Garcia-Selles FJ, Pernas M, Fernandez-Rivas M et al. Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, Castanea nut and Rosaceae fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000;**30**:1403–1410.
10. Himly M, Jahn-Schmid B, Dedic A, Kelemen P, Wopfner N, Altmann F et al. Art v 1, the major allergen of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain. *FASEB J* 2003;**17**:106–108.
11. Wopfner N, Gruber P, Wallner M, Briza P, Ebner C, Mari A et al. Molecular and immunological characterization of novel weed pollen pan-allergens. *Allergy* 2008;**63**:872–881.
12. Wopfner N, Willeroidee M, Hebenstreit D, van Ree R, Aalbers M, Briza P et al. Molecular and immunological characterization of profilin from mugwort pollen. *Biol Chem* 2002;**383**:1779–1789.
13. Arilla MC, Ibarrola I, Puente Y, Daza JC, Martinez A, Asturias JA. Cloning, expression and characterization of mugwort pollen allergen Art v 2, a pathogenesis-related protein from family group 1. *Mol Immunol* 2007;**44**:3653–3660.
14. Akkerdaas JH, Wensing M, Knulst AC, Krebitz M, Breiteneder H, de Vries S et al. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? *Int Arch Allergy Immunol* 2003;**132**:132–140.
15. Ferreira F, Wallner M, Gadermaier G, Erler A, Fritz G, Glatter O et al. Physico-chemical characterization of candidate reference materials. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M* 2006;**95**:75–82; discussion 82–83, 100–104.
16. van Ree R, Chapman M, Ferreira F, Vieths S, Bryan D, Cromwell O. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. *Allergy* 2008;**63**:310–326.
17. Garcia-Selles FJ, Diaz-Perales A, Sanchez-Monge R, Alcantara M, Lombardero M, Barber D et al. Patterns of reactivity to lipid transfer proteins of plant foods and *Artemisia* pollen: an in vivo study. *Int Arch Allergy Immunol* 2002;**128**:115–122.
18. Lombardero M, Garcia-Selles FJ, Polo F, Jimeno L, Chamorro MJ, Garcia-Casado G et al. Prevalence of sensitization to *Artemisia* allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy* 2004;**34**:1415–1421.
19. Radauer C, Willeroidee M, Fuchs H, Hoffmann-Sommergruber K, Thalhammer J, Ferreira F et al. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006;**36**:920–929.
20. Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. *Allergy* 2002;**57**:423–430.