

Mugwort-Sensitized Individuals from North Europe, South Europe and North America Show Different IgE Reactivity Patterns

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Key Words

Amb a 1 • Art v 1 • Art v 3 • Cross-reactive carbohydrate determinants • Mugwort pollen • Par j 2 • Polcalcin • Profilin • Sal k 1

Abstract

Background: Sensitization to weed pollen allergens at the molecular level is not fully understood. We studied IgE reactivity to the major mugwort allergen Art v 1 in relation to allergens from other weed pollen and cross-reactive components in mugwort-sensitized subjects. **Methods:** Art v 1 and Amb a 1 were affinity purified and coupled to experimental ImmunoCAP® tests. Samples from North Europe (n = 50), South Europe (n = 19) and North America (n = 41) were analyzed for IgE against mugwort pollen, weed allergen components, pan-allergens and cross-reactive carbohydrate determinants (CCDs). **Results:** The prevalence of IgE reactivity (>0.35 kU_A/l) to Art v 1 was significantly higher in samples from North Europe than in those from North America. IgE to Amb a 1 was more common in North America than in North and South Europe, while IgE to Par j 2 was common in South Europe, less common in North America, and absent in North Europe. IgE to Art v 3 in mugwort-allergic patients was more common in North Europe than in South Europe and North America, while IgE to Sal k 1 was similar between

the areas. Subjects with an Art v 1/mugwort-specific IgE ratio <0.5 had more often IgE to Amb a 1, profilin, polcalcin and CCDs than subjects with a ratio >0.5. **Conclusions:** Mugwort-sensitized subjects have different IgE reactivity profiles to weed allergens, reflecting their exposure to various pollens. Subjects with a low ratio between the IgE levels to Art v 1 and mugwort have a diverse IgE reactivity profile, indicating a role for cross-reactive allergens in their mugwort sensitization.

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Introduction

Allergy to weeds is a serious health problem for pollen-allergic individuals. Mugwort is common in most parts of Europe and present in parts of North America. It is the major cause of weed allergy in Northern Europe, where its pollination season is in late summer [1]. Wall pellitory (*Parietaria judaica*) is a common weed in Southern Europe (characterized by a Mediterranean climate) and causes rhinitis and asthma during almost the whole year in allergic individuals due to its extensive pollination season [1]. For many years, ragweed has been one of the major allergen sources causing allergy in North America. However, due to spread, it has become a serious problem

also in many European countries in which it was previously absent [1].

The most important allergen in mugwort pollen is Art v 1. Up to 95% of symptomatic patients have IgE antibodies to this major allergen, which belongs to the defensin family [2]. It is composed of a globular cysteine-rich defensin domain and a heavily glycosylated proline-rich domain with a predicted extended left-helical structure [2]. Recent data indicate that the amino acid sequence of the defensin domain is highly conserved between Art v 1 isoforms and contains all relevant IgE epitopes [3]. Its O-linked glycans are composed of galactoses and arabinoses and differ in structure from the common cross-reactive carbohydrate determinants (CCDs) often found on other plant allergens [2, 4]. Common CCDs are responsible for a great deal of IgE cross-reactivity between pollen and plant-derived foods, often without clinical relevance [4]. However, a similar role has not yet been shown for the so-called type III arabinogalactan on Art v 1.

Two major allergens have been described in pollen from the wall pellitory, Par j 1 and Par j 2. Both belong to the nonspecific lipid transfer protein (LTP) family, and there is extended IgE cross-reactivity between the two allergens, but not between Par j 1/2 and other plant LTPs [5, 6]. The most important allergen in ragweed pollen is Amb a 1, a pectate lyase that is recognized by IgE antibodies in more than 95% of ragweed-allergic patients [7, 8]. Sal k 1, a pectin methylesterase, is considered as a major allergen in saltwort, a weed also called Russian thistle and found in most arid and semi-arid regions of the world [9].

Even if Art v 1 seems to be the most important allergen in mugwort, other mugwort allergens have been described that might contribute to IgE reactivity to mugwort [10]. A pectate lyase with homology to Amb a 1 and an LTP with only limited homology to Par j 1 and Par j 2 have been found in mugwort extract. These mugwort allergens are called Art v 6 and Art v 3, respectively [10]. Cross-reactivity of IgE antibodies between several weeds has been described [11]. However, the cross-reactivity between the pectate lyases and LTPs in weeds might be of minor importance, even if it cannot be ruled out [6, 12]. Cross-reactivity between pollen allergens from grasses, trees and weeds is common as well. The profilins and polcalcins (named Art v 4 and Art v 5 in mugwort) are important cross-reactive pan-allergens [10]. Furthermore, CCDs might also be involved in mugwort sensitization.

Component-resolved diagnostics (CRD) offers the possibility to study IgE reactivity to individual allergenic components. The aims of this study were to develop nov-

el Art v 1 and Amb a 1 experimental ImmunoCAP® tests, to investigate the IgE reactivity pattern to Art v 1, Amb a 1, Par j 2, profilin, polcalcin and CCDs in mugwort-sensitized subjects from three geographically and botanically different areas using CRD, and to study the relationship between IgE reactivity to Art v 1 and IgE reactivity to the other allergenic components. IgE to Art v 3 and Sal k 1 was measured using recently launched ImmunoCAP tests in a subgroup of patients with a medical diagnosis of mugwort allergy.

Material and Methods

Samples

Plasma (citrate) samples (n = 110) from mugwort pollen-sensitized subjects (serological analysis) with a documented specific IgE concentration >0.35 kU_A/l were obtained from the internal sample collection of Phadia (Uppsala, Sweden). The samples were grouped into three regions, characterized by their climate and botanical flora. The North European group consisted of samples from Sweden (n = 48) and Estonia (n = 2). The South European group consisted of samples from southern Switzerland (n = 3), Spain (n = 15) and Greece (n = 1). The Swiss subjects were from Locarno, which is close to the Italian boarder. The region (Ticino) has a Mediterranean climate and the corresponding flora [13]. The North American group consisted of samples from the United States (n = 23) and Canada (n = 18). Sixty-four of these 110 samples were from patients with a medical diagnosis of mugwort allergy.

Development of Monoclonal Antibodies to Art v 1 and Amb a 1

Balb/c mice were hyperimmunized with purified native Art v 1 or Amb a 1. Art v 1 was purified from mugwort pollen extract (*Artemisia vulgaris*; Allergon, Ängelholm, Sweden) using cation exchange chromatography (SP Sepharose FF) and gel filtration following a previously published method [2]. Amb a 1 was purified from ragweed pollen extract (*Ambrosia artemisiifolia*; Allergon) using ion exchange chromatography followed by gel filtration and hydrophobic interaction chromatography, in principle following methods previously described [14–16]. The mice were sacrificed and the spleen cells were used for the development of hybridoma cells. Hybridoma cells producing antibodies against the allergens, as measured in ELISA, were cloned and their antibody reactivity was confirmed using immunoblotting with mugwort and ragweed pollen extract. Amb a 1-binding was also confirmed in ELISA using recombinant Amb a 1.3 (Indoor Biotechnologies, Charlottesville, Va., USA). Two cell lines were selected that produced monoclonal antibodies against Art v 1 (clone 6C8) and Amb a 1 (clone 2F3). The allergen-specific monoclonal antibodies were produced in roller bottle cultures and purified using recombinant protein A affinity chromatography.

Affinity Purification of Art v 1 and Amb a 1 Using Monoclonal Antibodies

Monoclonal antibodies against Art v 1 (6C8) or Amb a 1 (2F3) were coupled to NHS-activated Sepharose HP (GE Healthcare, Uppsala, Sweden) according to the manufacturer's instructions.

Proteins from mugwort or ragweed pollen were extracted for 2 h in 0.1 M Na₂KPO₄, pH 7.6 at 5°C, followed by filtration (0.45 µm) and buffer exchange to 20 mM Na₂HPO₄, 150 mM NaCl, pH 7.4 using a G-25 desalting column. The pollen extracts were added to the corresponding affinity column. Bound allergen was eluted in 0.1 M glycine-HCl, pH 3.0 and immediately neutralized with 1 M K₃PO₄. The protein concentration was assessed using A₂₈₀ determination and the extinction coefficients (ε_{mg/ml}) of 0.81 for Art v 1 and 1.1 for Amb a 1. The purified allergens were frozen at -70°C until use.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on NuPAGE 4–12% Bis Tris Gel (Invitrogen, Carlsbad, Calif., USA). Purified Art v 1 and Amb a 1 were treated with NuPAGE LDS Sample Buffer and heated at 97°C for 5 min. For reducing conditions, 1 µl 100 mM dithiothreitol was added before heating, followed by alkylation with 2 µl 135 mM iodoacetamide. The gel was loaded with 0.20–0.33 µg Art v 1 and Amb a 1 (reduced and nonreduced). The weakly stained Art v 1 was also loaded at 2.0 µg (reduced). The molecular weight calibration of the gel was achieved using Mark 12 Unstained Standard (Invitrogen). Electrophoresis was performed in MES running buffer following the manufacturer's instruction. The gel was silver-stained according to Blum et al. [17] in a Hoefer Processor Plus (GE Healthcare).

Mass Spectrometry Analysis

In-gel digestion of SDS-PAGE-separated proteins was performed with 5 ng/µl Trypsin Gold (Promega, Madison, Wisc., USA) overnight following a method adapted from Shevchenko et al. [18].

In-solution digestion of 5 µg protein solution (about 10 µl) was performed by reduction with 1 µl 100 mM dithiothreitol, heating at 97°C for 5 min and alkylation with 2 µl 135 mM iodoacetamide. The protein was then digested with 100 ng trypsin overnight. The digest was mixed with an equal volume of matrix solution containing saturated α-cyano-4-hydroxy cinnamic acid, 50% acetonitrile, 0.1% TFA and 10 mM NH₄H₂PO₄ for suppression of matrix signals and applied onto a MALDI target plate [19]. All MS analyses were recorded in positive reflector ion mode using a Bruker Autoflex II (Bruker Daltonic, Bremen, Germany).

Amino Acid Sequencing

N-terminal protein sequencing of Art v 1 was performed using the Edman degradation method in a G1000A sequencer (Hewlett-Packard, Palo Alto, Calif., USA). The obtained sequence was compared with known sequences using NCBI protein BLAST. Amb a 1 is known to be N-terminally blocked for Edman degradation and was therefore not sequenced [20].

Development of Experimental Art v 1 and Amb a 1 ImmunoCAP Tests

Experimental ImmunoCAP tests with purified Art v 1 and Amb a 1 were developed according to the standard method of Phadia. Optimal coupling concentration was determined using samples with various concentrations of Art v 1- and Amb a 1-specific IgE antibodies (previously characterized by IgE immunoblotting) and essentially as described elsewhere [21]. No difference was observed between affinity-purified Amb a 1 and convention-

ally purified Amb a 1 regarding the capacity to bind specific IgE antibodies (data not shown). Only affinity-purified Art v 1 was coupled to ImmunoCAP. The absence of contaminating allergens in the tests was confirmed using patient sera with defined IgE reactivity, and a mouse monoclonal antibody against Bet v 2 (profilin) using an in-house method for mouse IgG antibodies developed for the ImmunoCAP 100 instrument (Phadia).

Component-Resolved Diagnostics

The main set of 110 samples from mugwort pollen-sensitized subjects was analyzed for IgE antibodies to mugwort pollen extract (code w6), native Art v 1 (experimental ImmunoCAP) and Amb a 1 (experimental ImmunoCAP), recombinant Par j 2 (w211), Bet v 2 (profilin, t216) and Bet v 4 (polcalcin, t221), and CCD/MUXF³ (Ro214) using ImmunoCAP. Of the 64 samples from patients with a medical diagnosis of mugwort allergy, the volume of 60 samples allowed further analysis of IgE reactivity to native Art v 3 (w233) and Sal k 1 (w232). The measuring range was 0.1–100 kU_A/l. Results <0.1 kU_A/l were given the value of 0.05 kU_A/l before data evaluation. All tests, including ImmunoCAP equivalents to the experimental Art v 1 and Amb a 1 tests (code w231 and w230, respectively), are now commercially available from Phadia.

Statistics

The Mann-Whitney U test and Fisher's exact test (both two-tailed) were used for pairwise comparisons of continuous parameters (specific IgE concentrations) and categorical data (prevalence of IgE reactivity) between groups, respectively. *p* < 0.05 was considered significant.

Results

Purification of Art v 1

MALDI-TOF analysis identified the purified protein as Art v 1 (data not shown). This was also confirmed by N-terminal sequencing, which revealed the N-terminal sequence of the first 10 amino acid residues of the allergen (AGSKLCEKTS). SDS-PAGE with reduced affinity-purified Art v 1 showed a weak double band at about 28–36 kDa after silver staining (fig. 1). Nonreduced Art v 1 migrated to a slightly higher position on the gel. The purity was estimated to be more than 95% since no other bands were observed under reducing conditions. When the gel was overloaded with Art v 1 (2.0 µg/ml), the double band was very intense, and a weak, broad band at around 55 kDa appeared. It was defined as a dimer form of Art v 1 as the Art v 1-specific monoclonal antibody 6C8 bound to a 55-kDa band in Western blotting (data not shown).

Purification of Amb a 1

Affinity-purified Amb a 1 resulted in 4 major bands on nonreduced SDS-PAGE (fig. 1). The strongest band was at about 44 kDa (identified as isoform Amb a 1.1 and Amb a 1.4 using MALDI-TOF), the other 3 were at about

Table 1. Prevalence of IgE reactivity (number and percentage) to mugwort pollen and individual allergenic components in mugwort-sensitized subjects from North Europe (n = 50), South Europe (n = 19) and North America (n = 41)

	ImmunoCAP® test						
	mugwort	Art v 1	Amb a 1	Par j 2	Bet v 2	Bet v 4	CCDs
>0.35 kU _A /l							
North Europe	50 (100%)	42 (84%) ^a	10 (20%) ^a	0 (0%) ^{a, c}	16 (32%)	3 (6%) ^c	11 (22%)
South Europe	19 (100%)	14 (74%)	3 (16%) ^d	8 (42%) ^{a, e}	3 (16%)	2 (10.5%)	3 (16%)
North America	40 (98%)	19 (46%) ^a	28 (68%) ^{a, d}	4 (10%) ^{e, c}	13 (32%)	10 (24%) ^c	17 (41%)
>3.5 kU _A /l							
North Europe	26 (52%) ^b	18 (36%) ^a	3 (6%) ^a	0 (0%) ^a	7 (14%)	1 (2%)	0 (0%) ^c
South Europe	7 (37%)	4 (21%)	1 (5%) ^e	8 (42%) ^{a, d}	2 (11%)	1 (5%)	0 (0%)
North America	10 (24%) ^b	2 (5%) ^a	19 (46%) ^{a, e}	0 (0%) ^d	5 (12%)	4 (10%)	4 (10%) ^c

Two cut-off levels, 0.35 and 3.5 kU_A/l, were used in the evaluation. Significant differences between North Europe and South Europe or North America: ^a p < 0.001, ^b p < 0.01, ^c p < 0.05. Significant differences between South Europe and North America: ^d p < 0.001, ^e p < 0.01.

29 kDa (Amb a 1.1), 24 kDa (Amb a 1.4) and 15 kDa (Amb a 1.1). Reduced Amb a 1 migrated to a slightly higher position on the gel. The Amb a 1 preparation was considered as more than 95% pure, since no other protein was noticed under reducing conditions.

Also conventional purification of Amb a 1 using ion exchange chromatography, gel filtration and hydrophobic interaction chromatography resulted in pure allergen as revealed by SDS-PAGE and silver staining (fig. 1). MALDI-TOF analysis identified the Amb a 1.1 isoform in the conventionally purified Amb a 1 preparation.

Analysis of Mugwort-Sensitized Subjects from Different Geographic Areas Using CRD

All but one sample (from North America; 0.28 kU_A/l) in the main set had IgE antibodies to mugwort pollen >0.35 kU_A/l. The North European samples had higher IgE levels to mugwort compared to the North American samples, shown as a larger fraction of samples with IgE antibody concentrations >3.5 kU_A/l (table 1) and a higher median specific IgE concentration (fig. 2a).

Significantly fewer North American samples had Art v 1-specific IgE antibodies >0.35 kU_A/l compared to samples from North Europe (table 1). Furthermore, the North American samples had lower IgE levels to Art v 1 than the samples from North and South Europe (fig. 2b).

IgE antibodies to Amb a 1 were significantly more common in the North American samples than in the samples from North and South Europe, and the Amb a 1-specific IgE levels were higher in the North American group (fig. 2c).

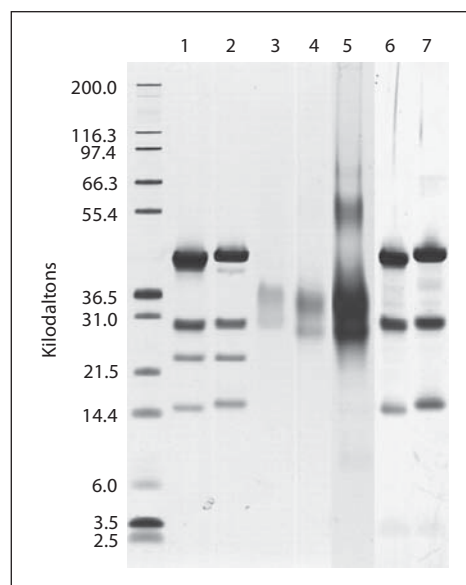


Fig. 1. SDS-PAGE analysis of Amb a 1 purified by affinity chromatography (0.33 µg: lanes 1 and 2) and conventional chromatography (0.20 µg: lanes 6 and 7), and Art v 1 purified by affinity chromatography (0.33 µg: lanes 3 and 4, and 2.0 µg: lane 5) under nonreduced (lanes 1, 3 and 6) and reduced (lanes 2, 4, 5, and 7) conditions.

Par j 2-specific IgE antibodies were significantly more common in the samples from South Europe compared to North Europe and North America (table 1). The IgE antibody concentrations to Par j 2 were high (>3.5 kU_A/l) in all 8 positive South European samples (table 1, fig. 2d).

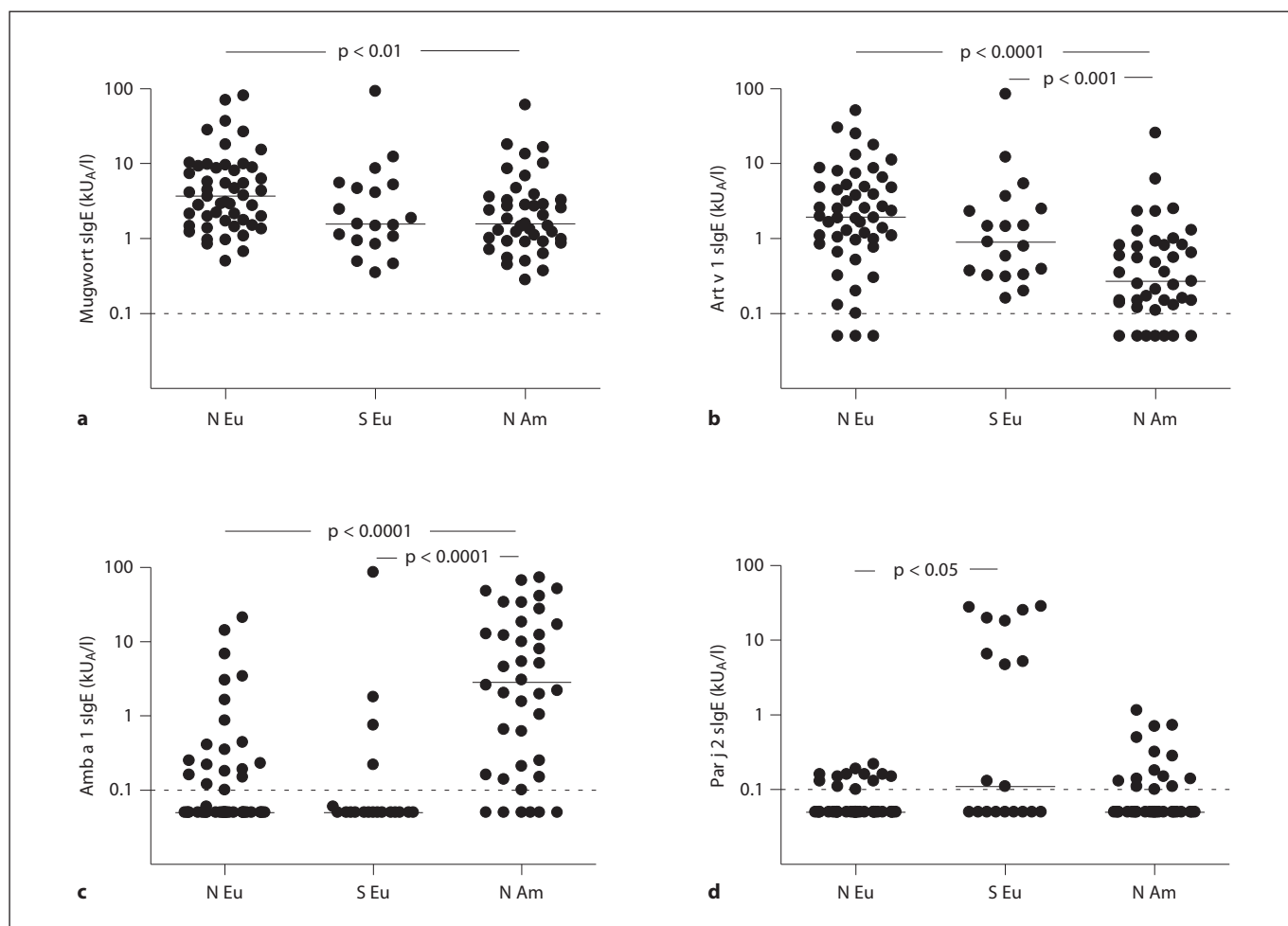


Fig. 2. IgE antibody levels to mugwort pollen (a), Art v 1 (b), Amb a 1 (c) and Par j 2 (d) in mugwort-sensitized subjects from North Europe (N Eu), South Europe (S Eu) and North America (N Am). The dashed line represents the lower level of quantitation of 0.1 kU_A/l.

There were no significant differences between the three study groups regarding the prevalence of IgE antibodies to profilin (Bet v 2) and the profilin-specific IgE antibody levels (table 1). IgE reactivity to polcalcin (Bet v 4) was more common in the North American samples compared to the North European samples (table 1), but no significant difference was seen in the polcalcin-specific IgE concentrations (data not shown).

There was a trend towards more IgE-reactivity to CCDs in the North American samples than in the samples from North ($p = 0.067$) and South Europe ($p = 0.077$). Four samples, all from North America, had specific IgE levels >3.5 kU_A/l (table 1).

Differences between Samples with High and Low Relative IgE Levels to Art v 1

All samples were split into two groups as a function of their relative IgE levels to Art v 1, i.e. having a ratio between specific IgE to Art v 1 and mugwort pollen higher or lower than 0.5 (50%). The *High-Art v 1 group* with a median ratio of 84% (range: 52–130%) was compared with the *Low-Art v 1 group* with a median ratio of 14% (range: 0–49%) for IgE reactivity to individual allergens.

There was no significant difference between the High-Art v 1 group and the Low-Art v 1 group regarding the IgE concentrations to mugwort pollen. However, there was significantly less IgE reactivity (>0.35 kU_A/l) to Art v 1 in the Low-Art v 1 group (42%) than in the High-Art v 1 group (96%) (table 2). In contrast, IgE reactivity to

Table 2. Prevalence (number and percentage) of IgE reactivity to mugwort pollen and individual allergenic components in mugwort-sensitized subjects and the subgroup of individuals with a medical diagnosis of mugwort allergy

	ImmunoCAP® test (>0.35 kU _A /l)								
	mugwort	Art v 1	Art v 3	Amb a 1	Par j 2	Sal k 1	Bet v 2	Bet v 4	CCDs
Sensitized subjects									
High-Art v 1 group (n = 53)	53 (100%)	51 (96%) ^a	ND	9 (17%) ^a	4 (8%)	ND	9 (17%) ^c	1 (2%) ^a	8 (15%) ^b
Low-Art v 1 group (n = 57)	56 (98%)	24 (42%) ^a	ND	32 (56%) ^a	8 (14%)	ND	23 (40%) ^c	14 (25%) ^a	23 (40%) ^b
Doctor's diagnosis									
High-Art v 1 group (n = 29)	29 (100%)	27 (93%) ^a	3 (10%)	7 (24%) ^b	3 (10%)	2 (7%)	5 (17%)	1 (3%)	5 (17%)
Low-Art v 1 group (n = 31)	31 (100%)	11 (36%) ^a	6 (19%)	18 (58%) ^b	2 (6%)	5 (16%)	9 (29%)	2 (6%)	5 (16%)

The individuals were grouped into either the High-Art v 1 or Low-Art v 1 group based on the ratio of their specific IgE levels to Art v 1 and mugwort pollen (i.e. higher or lower ratio than 0.5, respectively). Significant differences: ^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$. ND = Not determined.

Amb a 1, profilin (Bet v 2), polcalcin (Bet v 4) and CCDs was significantly more common in the Low-Art v 1 group compared to the High-Art v 1 group (table 2). The prevalence of IgE reactivity to Par j 2 was statistically similar in the two groups. When comparing the specific IgE levels to the allergenic components, they were found to be significantly raised, except to Art v 1, in the Low-Art v 1 group compared to the High-Art v 1 group (data not shown).

The High-Art v 1 group was mostly composed of samples from North Europe (62%), followed by South Europe (23%) and North America (15%), while the Low-Art v 1 group was dominated by samples from North America (58%), followed by those from North Europe (30%) and South Europe (12%).

IgE Reactivity Pattern in Patients with a Medical Diagnosis of Mugwort Allergy

The subgroup of 60 patients with a medical diagnosis of mugwort pollen allergy did not significantly differ from the main set of 110 mugwort-sensitized subjects regarding the prevalence for IgE reactivity to individual allergens (data not shown). Similarly, the geographic differences in the IgE reactivity pattern observed for the whole study group were in general similar for the patients with a diagnosis of mugwort pollen allergy. Interestingly, IgE reactivity to Art v 3 was significantly more common in mugwort-allergic patients from North Europe (32%) than in patients from North America (4.3%, $p < 0.05$), but the difference was not significant regarding South Europe (6.7%, $p = 0.11$). No correlation was observed between the IgE levels to the two pollen LTPs Art v 3 and Par j 2 (data

not shown). No significant differences were found regarding the prevalence of IgE reactivity to Sal k 1 (North Europe: 9.1%, South Europe: 13% and North America: 13%).

Twenty-nine (48%) of the samples from the mugwort-allergic patients had an Art v 1/mugwort-specific IgE ratio higher than 0.5, and a similar IgE reactivity profile as the High-Art v 1 group in the main set of mugwort-sensitized individuals (table 2). However, the significant differences in IgE reactivity to profilin, polcalcin and CCDs observed between the High- and Low-Art v 1 group in the main set were not observed in the mugwort-allergic patients (table 2).

Discussion

The present study shows that individuals from different geographic areas with specific IgE test results >0.35 kU_A/l to mugwort pollen have IgE antibodies against different major weed pollen allergens. At the beginning of the study, ImmunoCAP tests were not available for the major mugwort and ragweed allergens Art v 1 and Amb a 1, which were therefore purified from pollen extract. We found that affinity purification with monoclonal antibodies was efficient and resulted in >95% pure allergen preparations. In fact, no other protein was observed using SDS-PAGE and sensitive silver staining in combination with MALDI-TOF analysis. The rather diffuse double band at 28–36 kDa is typical for native Art v 1 and is most likely explained by various glycosylation patterns on different isoforms of the allergen [2, 3]. Purified Amb a 1 is

known to exist in an intact form that was represented by the approximately 44-kDa band in the present SDS-PAGE, and as α - and β -chains represented by the approximately 29- and 15-kDa bands, respectively [15, 20]. The fourth band at approximately 24 kDa in the affinity-purified Amb a 1 preparation was identified by MALDI-TOF as Amb a 1.4, an isoform not observed in the conventionally purified Amb a 1. The purified Art v 1 and Amb a 1, which both lack the CCDs commonly found among other plant allergens [10], were coupled to experimental ImmunoCAP tests that showed high binding of specific IgE antibodies. Thus, the Art v 1 and Amb a 1 ImmunoCAP tests were found to be suitable for use in CRD.

While IgE reactivity to the major mugwort pollen allergen Art v 1 was common in North and South Europe, it was less common in the samples from North America. On the contrary, IgE reactivity to the major ragweed allergen Amb a 1 was common in samples from mugwort-sensitized individuals from North America (prevalence 68%) while only a minority of the samples from North and South Europe contained IgE antibodies against Amb a 1. Sensitization was defined as having a positive mugwort pollen-specific IgE test $>0.35 \text{ kU}_A/\text{l}$. Furthermore, IgE antibodies to the major wall pellitory allergen Par j 2 were rather common in samples from mugwort-sensitized individuals from South Europe. In a recent study using microarray-based CRD, 10 ragweed-allergic patients from Canada all had IgE reactivity to Amb a 1, while only 3 had IgE to Art v 1. Eight out of 9 European patients with mugwort allergy had IgE reactivity to Art v 1, and only 2 had IgE to Amb a 1. However, no information about sensitization to whole mugwort pollen extract was given [8]. The prevalence of IgE reactivity to Art v 1 of 84% in mugwort-sensitized subjects from North Europe found in the present study is similar to the prevalence of 79% recently reported for mugwort-allergic subjects in Germany [22]. In the German study, 14% of the subjects were sensitized to Amb a 1; this percentage is also similar to the corresponding prevalence figures obtained for the European subjects in our study. While no significant differences in profilin sensitization was observed in our study, IgE reactivity to polcalcin (a pan-allergen family present in pollen) was more common in the samples from North America than in those from North Europe. There was also a trend towards more IgE to CCDs in the North American samples.

The differences in sensitization to individual allergen components most probably reflect geographical variations in pollen exposure. Ragweed is originally a North

American weed and its pollen is a major health problem in infested areas. However, in the last decades, ragweed has started to spread to new habitats in Europe [1]. In Southern Europe, wall pellitory, olive tree, Cupressaceae and grass pollens cause great problems to allergic individuals [1, 23, 24], many of whom are also sensitized to mugwort pollen [25].

LTP sensitization is connected with allergy, especially in Mediterranean countries [6]. It was therefore surprising that IgE reactivity to the LTP from mugwort pollen (Art v 3), in contrast to IgE to Par j 2, was most common in mugwort pollen-allergic patients from North Europe. The reason is not clear, but Art v 3 has been shown to act as a primary sensitizer to LTP, i.e. not via the peach LTP (Pru p 3) pathway commonly observed for LTP sensitizations in the Mediterranean area [6]. Thus, it might be more common with Art v 3 sensitizations than other LTP sensitizations in Northern Europe. The absence of a correlation between the IgE levels to Art v 3 and Par j 2, that has also been shown by others [6], points to different sensitization pathways, and is a consequence of the low structural homology between the two pollen LTPs [26, 27].

Saltwort is a weed that grows in arid and semi-arid areas, and its major allergen is Sal k 1 [9]. In the present study, only a few mugwort-allergic patients had IgE to Sal k 1, which probably reflects their exposure to saltwort pollen [23, 24]. However, 2 of them were from North Europe where saltwort is uncommon. Perhaps these 2 patients have been sensitized to Sal k 1 abroad, or to a structurally related domestic weed allergen.

Exposure to various plant pollens makes an accurate diagnosis difficult. A positive test result to mugwort pollen could be due to either primary sensitization to mugwort allergens or to cross-reactive IgE antibodies originally induced to pollen allergens from other plants. In a study by Stumvoll et al. [25], it was shown that 83% of wall pellitory pollen-sensitized subjects from the Mediterranean area, but only 7% of sensitized subjects from other geographic areas, including North Europe, displayed IgE antibodies to Par j 2. Profilin and polcalcin were suggested to be responsible for cross-reactivity between wall pellitory and other weed species in non-Mediterranean regions. An analogous situation seems plausible for sensitization to other weeds. Thus, while IgE reactivity to Art v 1 suggests primary sensitization to mugwort pollen and associated allergy, IgE reactivity to Amb a 1 and Par j 2 suggest primary sensitization to ragweed and wall pellitory, respectively [12, 25].

In the present study, all samples were grouped according to their relative ratio of specific IgE to Art v 1 and mugwort pollen. The prevalence of IgE reactivity to Amb a 1, profilin, polcalcin and CCDs was significantly higher in the samples with a low Art v 1/mugwort ratio (Low-Art v 1 group, i.e. less than 50% IgE reactivity to Art v 1) compared to the High-Art v 1 group. This indicates that mugwort sensitization in the Low-Art v 1 group (dominated by samples from North America) could be a secondary phenomenon due to cross-reactive IgE antibodies to other plant pollens, i.e. ragweed and grass. IgE antibodies to Amb a 1 has been shown to express limited cross-reactivity with the mugwort pollen homologue Art v 6 [12]. However, IgE epitopes on profilin, polcalcin and CCDs are highly cross-reactive between plant species [4, 10, 11]. This has implications when interpreting the test results using in vitro or in vivo tests based on mugwort pollen extract. Mugwort pollen allergy might be overdiagnosed since positive test results may evolve due to cross-reacting IgE antibodies to other plants, especially other weeds. Interestingly, in the subgroup of patients with a medical diagnosis of mugwort pollen allergy, the prevalence of IgE reactivity to profilin, polcalcin or CCDs was not significantly different in patients with low and high Art v 1/mugwort-specific IgE ratios, indicating a somewhat more restricted sensitization profile in mugwort pollen-allergic patients than in the whole group of mugwort pollen-sensitized patients.

The IgE reactivity pattern to individual allergenic components is of importance when recommending avoidance, and when treatment with specific immunotherapy (SIT) is considered. It could be a challenge to doc-

tors to choose the appropriate extract for SIT to achieve sufficient clinical efficacy [23]. Studies have shown that new IgE reactivities could develop during SIT [28], and it is recommended to use extracts standardized for the allergen components to which the patient is reactive [29]. Furthermore, new SIT strategies using recombinant allergens are being developed [30], and component-resolved immunotherapy may be the future [31]. All this speaks for the need for more precise analysis of the sensitization profile than extract-based tests can offer, i.e. CRD will be more important.

To sum up, we have shown that mugwort pollen sensitization could be dominated by IgE reactivity to the major mugwort allergen Art v 1, especially in European subjects. Some sensitized subjects, however, might have little or no IgE to Art v 1, while they have prominent IgE reactivity to major allergens from other weeds, to cross-reactive pan-allergens or to CCDs. This indicates a role for cross-reactive IgE antibodies in positive test results for mugwort in these subjects. CRD using purified native or recombinant pollen allergens in clinical practice leads to more detailed information on IgE reactivity at the molecular level that could be helpful in the diagnosis of pollen allergy and might help the clinician to choose appropriate pollen extracts for SIT.

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