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Artemisia and Ambrosia hypersensitivity: co-sensitization or co-recognition?

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Clinical and Experimental Allergy

Summary

Background Ragweed and mugwort have nearly identical flowering periods. Clinical and serological studies showed that ragweed and mugwort sensitization are often associated and this poses relevant clinical problems in patients for whom specific immunotherapy is warranted.

Objective To establish whether the concurrent ragweed and mugwort pollen hypersensitivity is the result of co-sensitization or of co-recognition by using purified recombinant allergens.

Methods Sensitization to ragweed and mugwort pollen was assessed by skin prick test (SPT) in all patients reporting allergic symptoms in August and September. IgE reactivity of sera from 42 patients (26 Amb+/Art+, 14 Amb+/Art–, and two Amb–/Art+) to ragweed and mugwort pollen extract as well as to several recombinant ragweed (rAmb a 1, rAmb a 5, rAmb a 6, rAmb a 8, rAmb a 9, and Amb a 10) and mugwort (rArt v 1, rArt v 4, rArt v 5, rArt v 6, and three EF-hand calcium-binding protein) allergens was detected by dot-blot and ELISA analyses.

Results IgE reactivity of 372 weed pollen-allergic patients was studied. Mugwort reactivity was strongly associated with ragweed hypersensitivity: only 10/147 (7%) mugwort-hypersensitive patients were not sensitized to ragweed, whereas 225/362 (62%) ragweed-hypersensitive patients were not sensitized to mugwort. *In vitro*, 90% of ragweed-allergic patients reacted with rAmb a 1. Reactivity to other ragweed allergens ranged between 20% and 35%. Forty-six percent of the mugwort-sensitized patients recognized rArt v 1%, 25% reacted to Art v 4, Art v 5, and Art v 6, and 7% recognized the three-EF hand calcium-binding protein. Immunoblot inhibition experiments showed that pre-incubation with ragweed pollen extract only weakly decreased IgE reactivity to mugwort allergens.

Conclusion Patients showing both ragweed- and mugwort-positive SPT and/or RAST are co-sensitized. Future studies will establish whether IgE reactivity translates into clinical symptoms and, hence, if co-sensitized patients should undergo specific immunotherapy with extracts of both mugwort and ragweed pollen.

Keywords cross-reactivity, immunotherapy, mugwort, ragweed, recombinant allergens, weed pollen allergy

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Introduction

As few as 15 years after its first appearance in the area north of Milan [1], ragweed pollen has become the second cause of respiratory allergy after grass pollen [2]. One interesting aspect that has been rather overlooked so far is

that the impressive increase in ragweed hypersensitivity has been paralleled by a similar increase in the prevalence of positive skin prick test (SPT) with mugwort pollen. Mugwort has always been present in this area, but few patients were found as being mugwort-sensitive before the appearance of ragweed and even less reported mugwort-induced symptoms. The association between sensitivity to ragweed and mugwort pollen has been observed

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also by other researchers. In an Austrian survey about 50% of patients hypersensitive to Compositae pollen reacted against both ragweed and mugwort in SPTs and RAST [3], and similar findings were reported in other studies as well [4, 5]. In accordance with this observation, a study carried out at the allergy centre of the hospital of Bollate, a town 5 km north of Milan, at the beginning of the 90s and including 81 subjects with a history of seasonal respiratory allergy from mid-August to the end of September, showed that 28% of the patients were sensitized to *Artemisia* pollen only, 26% to *Ambrosia* pollen only, and 46% were sensitized to both [6]. The concomitant sensitization to *Artemisia* and *Ambrosia* poses relevant clinical problems in patients for whom specific immunotherapy is warranted because ragweed and mugwort show nearly identical flowering periods in this area [7, 8] and both belong to the great botanical family of Compositae. Possible cross-reactivity between these two pollens has been considered in the past with conflicting results. In their study, Hirschwehr et al. [9] concluded that in addition to profilin, mugwort and ragweed pollen contain a number of cross-reactive allergens, including the major mugwort allergen Art v 1 and a cross-reacting 27–28 kDa ragweed allergen, which was also recently described by Mutschlechner et al. [10]. In contrast, other studies concluded that *Ambrosia* and *Artemisia* pollen allergens show little or no cross-reactivity [11, 12]. The main clinical question remains whether patients hypersensitive to both ragweed and mugwort should undergo specific immunotherapy with extracts from one particular pollen or from both.

The last decade has seen a tremendous progress in molecular biology techniques, which gives us now the possibility to address these crucial questions. In the present study we aimed to establish whether the contemporary presence of hypersensitivity to ragweed and mugwort pollen is the result of co-sensitization (i.e. parallel sensitization to distinct allergens) or of co-recognition (i.e. sensitization to allergenic molecules that are shared by distinct allergen sources). For this purpose, a panel of recombinant mugwort and ragweed allergens was used in *in vitro* IgE assays to define the sensitization patterns of SPT-positive patients.

Materials and methods

Patients and skin tests

All patients reporting seasonal rhino-conjunctivitis with or without asthma from mid August to the end of September [7, 8], first seen at the allergy centre of the Clinica San Carlo in Paderno Dugnano (10 km north of Milan) from the beginning of January 2003 to the end of March 2005, were considered eligible for the study.

All these subjects underwent SPT with a large spectrum of commercial pollen extracts (50 000 SBU/mL; Allergopharma, Reinbeck, Germany), including grass, birch, ragweed, mugwort, plantain, pellitory, cypress, and olive. Readings were taken after 15 min, and reactions were expressed as mean weal diameter (adding the longest diameter to the orthogonal diameter and dividing it by 2). A weal diameter of 3 mm or more was considered positive [13]. Histamine (10 mg/mL) and saline were used as positive and negative controls, respectively.

In order to avoid the interference by cross-reacting plant pan-allergens, such as profilin or calcium-binding proteins [14], only patients sensitized to < 3 pollens other than mugwort and/or ragweed were finally included.

Based on SPT with ragweed and mugwort extracts, three groups of patients were distinguished:

- sensitized to ragweed but not to mugwort (Amb+/Art-);
- sensitized to mugwort but not to ragweed (Amb-/Art+); and
- sensitized to both mugwort and ragweed (Amb+/Art+).

Allergen preparations

Aqueous protein extracts of ragweed (*Ambrosia artemisiifolia*) and mugwort (*Artemisia vulgaris*) pollen (Allergon AB, Ängelholm, Sweden) were prepared by re-suspending 150 mg of pollen in 1 mL of distilled water and shaking at room temperature for 15 min. After centrifugation at 14 000 g, the supernatants were collected and stored at -20 °C until use.

Natural Amb a 1 (nAmb a 1) was purified from ragweed pollen extract by affinity chromatography using rabbit anti-Amb a 1 antibodies [15]. Natural Art v 1 was purified according to a published procedure [16].

The Amb a 5 and Amb a 6 cDNAs were obtained by RT-PCR using mRNA purified from ragweed pollen (Allergon AB). The oligonucleotides for the cDNA synthesis were designed based on the published sequences of Amb a 5 and Amb a 6, respectively [17, 18]. The cDNA clones coding for mugwort and ragweed calcium-binding proteins (two EF-hand and three EF-hand) and for ragweed profilin (Amb a 8, GenBank Accession AY894 660) were obtained by screening mugwort and ragweed cDNA libraries, respectively, using patients' sera from Austrian weed pollen-allergic individuals. Ragweed Amb a 8 shows 89% sequence identity to mugwort profilin Art v 4 [19]. The two EF-hand CBPs (ragweed Amb a 9, GenBank Accession AY894 657, and mugwort Art v 5, GenBank Accession AY904 434) are homologous to Bet v 4, a two EF-hand calcium-binding protein from birch pollen [20]. Amb a 10, the three EF-hand CBP from ragweed shows 55% sequence identity to Ole e 8, a four EF-hand binding

protein from *Olea europea* [21]. The mugwort CBP with three EF-hands shows 85% sequence identity to a putative calmodulin from *Solanum demissium*. The Art v 6 (mugwort homologue of Amb a 1) cDNA (GenBank Accession AY904 433) was isolated from a mugwort cDNA library by immunoscreening, using purified rabbit anti-Amb a 1 antibodies. All recombinant proteins were purified as 6 × histidine-tagged fusion proteins, except for Art v 1 [16] and Art v 4 [19]. rAmb a 1 was purchased from Biomay AG (Vienna, Austria). Details of cloning, recombinant production, and purification of Amb a 5, Amb a 6, Amb a 8, Amb a 9, Art v 5, and Art v 6 will be published elsewhere. The calculated molecular weights of the allergens used in this study were as follows: Amb a 1, 40 136.48 Da; Amb a 5, 4 978.80 Da; Amb a 6, 9 857.50 Da; Amb a 8, 14 245.35 Da; Amb a 9, 9 311.19 Da; Amb a 10, 17 798.64 Da; Art v 1, 10 800.02 Da; Art v 4, 14 207.30 Da; Art v 5, 8 997.90 Da; Art v 6, 40 910.69 Da.

Enzyme-linked immunosorbent assay, immunoblot and dot-blot analyses with recombinant allergens

In order to detect whether in Amb+/Art+ patients mugwort sensitivity is the result of cross-reactivity with ragweed allergens or if these patients have a double allergy, the IgE reactivity of sera from 42 patients (26 Amb+/Art+, 14 Amb+/Art−, and two Amb−/Art+) to several recombinant and natural ragweed and mugwort allergens was detected both by dot-blot analysis and ELISA. These included rAmb a 1, nAmb a 1, rAmb a 5, rAmb a 6, rAmb a 8 (profilin), rAmb a 9 (two EF-hand calcium-binding protein), rAmb a 10 (three EF-hand calcium-binding protein), rArt v 1, nArt v 1, rArt v 4 (profilin), rArt v 5 (two EF-hand calcium-binding protein), rArt v 6 (Amb a 1 homologue from mugwort), and mugwort three EF-hand calcium-binding protein. In addition, purified nAmb a 1, rAmb a 1, and whole ragweed and mugwort pollen extracts were tested in immunoblots.

For immunoblot analysis, ragweed and mugwort pollen extracts (25 µg total protein/cm) were separated by denaturing SDS-PAGE according to the method of Laemmli [22] and electroblotted [23] onto nitrocellulose membranes (Protran, Schleicher & Schuell, Dassel, Germany). For the dot-blot analysis equal amounts of purified recombinant proteins (2.5 µg/dot) were dotted onto nitrocellulose membrane using the Bio-Dot Microfiltration apparatus (BioRad, Hercules, CA, USA). Sera from atopic individuals were diluted 1 : 10 in 50 mM sodium phosphate buffer, pH 7.4%, 0.5% (v/v) Tween-20%, 5% (w/v) bovine serum albumin (BSA), and 0.05% (w/v) sodium azide. Immunoblots and dot-blots were incubated with diluted patients' sera overnight at 4 °C. Bound IgE antibodies were detected using ¹²⁵I-rabbit anti-human IgE (MALT Allergy system, IBL GmbH, Hamburg, Germany) on a phosphor-imager screen (Fujifilm BAS-1800 II, Tokyo, Japan).

Maxisorp ELISA plates (NUNC, Roskilde, Denmark) were coated with purified recombinant allergens (5 µg/mL) and aqueous pollen extracts (10 µg/mL) overnight at 4 °C. Plates were washed three times with Tris-buffered saline (TBS), pH 7.4%, 0.05% Tween (TT buffer) and remaining binding sites were saturated by incubation with TBS, pH 7.4%, 1% BSA, 0.05% Tween for 4 h at room temperature. Coated plates were incubated with patients' sera overnight at 4 °C. All sera were diluted 1 : 3 in TBS, pH 7.4%, 0.5% BSA, 0.05% Tween (TBT buffer). After five TT 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 Pharmingen, 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). Adequate ELISA controls, including leaving out primary and/or secondary antibodies, were performed. For the calcium-binding allergens, either 10 mM CaCl₂ or 10 mM EGTA was added to the TBS and TBT buffers. The negative cut-off values were 0.2.

Immunoblot and enzyme-linked immunosorbent assay inhibition

Immunoblot-inhibition experiments were carried out on sera from five Amb+/Art+ patients using mugwort and ragweed pollen extracts as solid-phase allergen. IgE reactivity of sera was assessed before and after absorption of sera with different amounts of ragweed and mugwort pollen extract (50 and 100 µg of total protein). For negative control inhibition, sera were pre-incubated with 20 µg/mL of recombinant Bet v 1a (Biomay AG). For ELISA inhibitions, plates were coated overnight at 4 °C with mugwort pollen extract (800 ng/mL total protein). Sera from two Amb+/Art+ patients (diluted 1 : 5 in TBT buffer) were pre-incubated with increasing amounts (0–5 µg total protein/50 µL diluted serum) of mugwort pollen extract. Bound IgE was detected as described above.

Results

Skin tests

In total, 372 patients (age 8–72; M/F 172/200) fulfilled the clinical criteria and were included in the study. Sensitization patterns recorded in the three subgroups of patients (Amb+/Art−; Amb−/Art+; Amb+/Art+) are described in Table 1. Mugwort reactivity on SPT was strongly associated with ragweed hypersensitivity: only 10/147 (7%) mugwort-hypersensitive patients were not sensitized to ragweed as well. In contrast, of 362 patients positive on SPT with ragweed pollen, 225 (62%) were not sensitized to mugwort. Apart

Table 1. Sensitization patterns of the three subgroups of study patients

	Amb+/Art+	Amb+/Art-	Amb-/Art+
No.	137	225	10
M/F	63/74	103/122	6/4
Age range (mean)	8–72 (34)	10–70 (35)	13–71 (37)
Other pollen allergies			
None	56 (41%)	97 (43%)	6 (60%)
Grass	42 (31%)	71 (32%)	4 (40%)
Birch	24 (18%)	67 (30%)	1 (10%)
Pellitory	10 (7%)	8 (4%)	1 (10%)
Cypress	17 (12%)	31 (14%)	
Plantain	3 (2%)	13 (6%)	
Olive	14 (10%)	5 (2%)	

from a higher prevalence of sensitization to birch pollen among Amb+/Art- subjects ($P < 0.05$), the three subgroups did not show any other relevant differences.

In vitro experiments

Amb+/Art+ patients. In immunoblot experiments, sera from all 26 patients showed IgE reactivity against various ragweed pollen proteins (Fig. 1a). In ELISA, sera from 24/26 (92%) strongly reacted to nAmb a 1. Among the two Amb a 1-negative sera, one reacted to both Amb a 5 and Amb a 8 and one did not react to any recombinant ragweed allergen. In total, 10 and seven sera reacted to rAmb a 6 and rAmb a 8, respectively. Only three sera reacted to rAmb a 5. Sera from 5/26 (19%) and 6/26 (23%) reacted to the calcium-binding proteins Amb a 9 and Amb a 10, respectively. Similar results were obtained with dot- and immunoblotted purified proteins. These results are summarized in Table 2.

17/26 (65%) sera from these patients reacted to proteins in whole mugwort pollen extract, as determined by IgE immunoblots (Fig. 1b). Using blotted or ELISA-coated proteins, 12/17 (71%) recognized both rArt v 1 and nArt v 1; 7 reacted to both Art v 4 and Art v 6 (Amb a 1 homologue). Sera from two patients reacted to the mugwort three EF-hand calcium-binding protein, and 6/26 (23%) reacted to the two EF-hand calcium-binding protein Art v 5. Sera from six subjects did not show any reactivity to recombinant or to natural mugwort allergens. Table 2 summarizes these results.

Amb+/Art- patients. The combined results from ELISA, IgE dot- and immunoblots (Fig. 1a) showed that 13/14 (93%) sera reacted to whole ragweed pollen extract, and all of them showed Amb a 1 reactivity (Table 3). Five, one, and one sera reacted to Amb a 6, Amb a 5, and Amb a 8, respectively. Sera from 3/14 recognized Amb a 9, and 4/14 reacted to Amb a 10. Serum from one patient did not show reactivity to any natural or recombinant ragweed allergens.

Although patients in this group were tested negative for mugwort in SPT, seven sera (50%) reacted with mugwort allergens. Sera from 5/14 (36%) recognized Art v 5, 1 serum reacted with Art v 4, and 2 with Art v 6. However, no patient in this group displayed IgE against natural or recombinant Art v 1 (Fig. 1b, Table 3).

Amb-/Art+ patients. Sera from these two patients showed weak reactivity to whole mugwort pollen extract (Fig. 1b), and one of them reacted to natural and rArt v 1, whereas one did not react with any of the recombinant mugwort allergens tested (Table 3).

Immunoblot and enzyme-linked immunosorbent assay inhibition

Pre-incubation with ragweed pollen extract did not cause any reduction in mugwort IgE reactivity, with the exception of reactivity to low molecular weight proteins (most probably profilin), thus showing that all five sera contained IgE specific for mugwort pollen proteins (Fig. 1c). Complete inhibition of IgE binding to immobilized mugwort proteins was observed after pre-absorption of sera with mugwort pollen extract (data not shown).

Discussion

The differential diagnosis between ragweed and mugwort pollen allergy represents one of the most frequent problems that allergologists have to face in areas where both plants are present. A distinction based uniquely on seasonal and/or clinical criteria is a virtually impossible task. Routinely available *in vitro* assays, such as the RAST, also do not provide additional information to SPT results, as both tests are based on the use of whole pollen extracts. In the large series of Compositae-allergic subjects studied here, nearly all patients sensitized to mugwort pollen were sensitized to ragweed pollen as well, whereas a large proportion of patients sensitized to ragweed pollen did not show any mugwort sensitization. These findings strongly suggested that patients sensitized to both pollens were possibly ragweed-allergic subjects showing co-recognition of mugwort allergens. However, the results of this study ruled out this hypothesis. A high percentage (46%) of Amb+/Art+ patients reacted to Art v 1, the major mugwort pollen allergen. Cross-reactivity to an Art v 1-homologous protein in ragweed seems not to be the case as immunoblot inhibition experiments showed a persistent IgE reactivity to mugwort allergens after absorption of sera with whole ragweed pollen extract. Furthermore, of the 24/26 patients reacting to Amb a 1, only six recognized Art v 6, the Amb a 1-homologue in mugwort. Taken together, these results indicate that (i) there is very limited cross-reactivity between Amb a 1 and

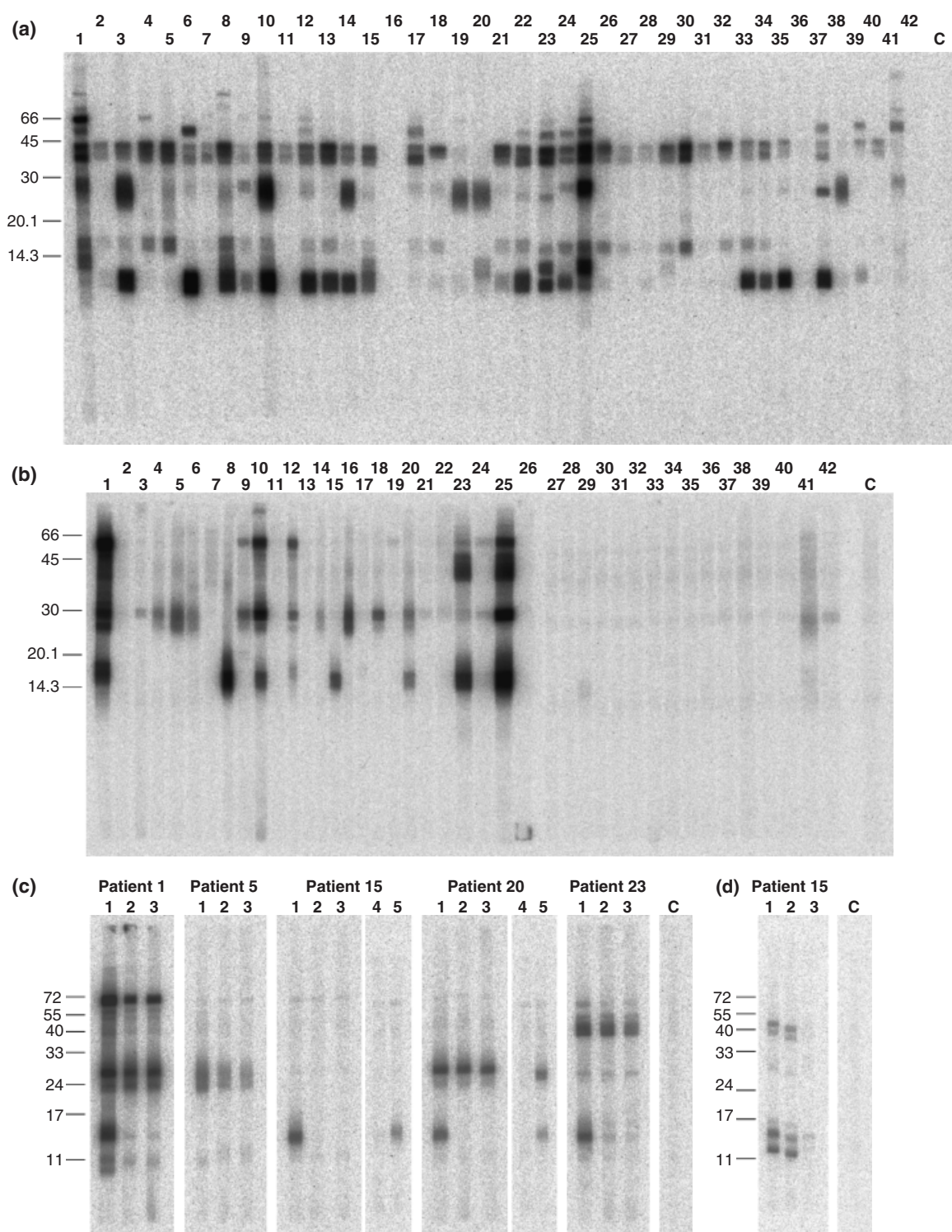


Fig. 1. IgE immunoblot and immunoblot inhibition. Crude ragweed (a) and mugwort (b) pollen extracts were separated by 15% SDS-PAGE, transferred to nitrocellulose, and developed with serum IgE from Amb+/Art+ (lanes 1–26), Amb+/Art– (lanes 27–40), and Amb–/Art+ (lanes 41 and 42) patients. (c) Crude mugwort pollen extract was separated by 15% SDS-PAGE, transferred to nitrocellulose, and developed with serum IgE from five Amb+/Art+ patients (lanes 1). Sera were pre-incubated overnight with 50 µg (lanes 2) and 100 µg (lanes 3) of ragweed or mugwort (lanes 4) pollen proteins. (d) Crude ragweed pollen extract was separated by 15% SDS-PAGE, transferred to nitrocellulose, and developed with serum IgE from an Amb+/Art+ patient (lane 1). Serum was pre-incubated overnight with 100 µg mugwort (lanes 2) and ragweed (lanes 3) pollen proteins. Negative controls included leaving out the serum incubation step (lanes C) and pre-incubation with the unrelated allergen Bet v 1 (lanes 5; 20 µg rBet v 1a/mL serum).

Table 2. IgE reactivity of Art+/Amb+ patients to ragweed and mugwort allergens

Ragweed allergens								Mugwort allergens					
Nr	1	5	6	8	9	10	RPE	1	4	5	CBP	6	MPE
1	++	—	—	—	—	—	++	++	—	—	++	—	++
2	++	—	—	—	—	—	+	—	—	—	—	—	—
3	++	—	++	—	+	+	++	++	—	+	—	—	+
4	++	++	—	—	—	—	++	++	—	—	—	—	+
5	++	—	—	—	—	—	++	++	—	—	—	—	++
6	++	—	++	—	—	—	++	++	—	—	—	—	+
7	++	—	—	—	++	+	+	—	—	+	—	—	—
8	++	—	—	++	—	—	++	—	++	—	—	++	++
9	+	—	++	—	—	—	++	++	—	—	—	—	++
10	++	—	++	++	—	—	++	++	++	—	—	++	++
11	++	—	—	—	—	—	+	—	—	—	—	—	—
12	++	—	++	—	—	—	++	—	—	—	—	—	++
13	++	—	++	—	—	—	++	—	—	—	—	—	—
14	++	—	++	—	—	—	++	+	—	—	—	—	+
15	++	—	—	++	—	—	++	—	++	—	—	++	+
16	++	—	—	—	+	+	++*	++	—	+	—	—	++
17	++	—	—	—	—	—	++	—	—	—	—	—	—
18	++	—	—	—	—	—	+	++	—	—	—	—	+
19	—	—	—	—	—	—	++	—	—	—	—	—	—
20	—	++	—	++	—	+	++	++	++	—	—	++	+
21	++	—	+	—	—	+	++	—	—	+	—	—	—
22	++	—	++	—	—	—	++	—	—	—	—	—	—
23	++	—	++	++	—	—	++	+	++	—	—	++	++
24	++	—	—	++	—	—	++	—	+	—	—	+	+
25	++	++	—	++	+	++	++	—	++	+	+	++	++
26	++	—	—	—	+	—	+	—	—	+	—	—	—

Ragweed allergens: 1, 5, 6, 8, 9, 10 = Amb a 1, 5, 6, 8, 9, and 10, respectively. RPE = whole ragweed pollen extract. Mugwort allergens: 1, 4, 5, 6 = Art v 1, 4, 5, and 6, respectively. CBP, three EF-hand calcium-binding protein; MPE, whole mugwort pollen extract; ++, strong IgE reactivity; +, weak IgE reactivity. —, no IgE reactivity.

*Positive results only in ELISA and dot-blot.

Table 3. IgE reactivity of Art—/Amb+ and Art+/Amb— patients to ragweed and mugwort allergens

Ragweed allergens								Mugwort allergens					
Nr	1	5	6	8	9	10	RPE	1	4	5	CBP	6	MPE
27	+	—	—	—	—	—	+	—	—	—	—	—	—
28	+	—	+	—	—	—	+	—	—	+	—	—	—
29	++	—	—	+	—	+	+	—	++	—	—	+	+
30	++	++	—	—	++	++	++	—	—	++	—	+	+
31	++	—	—	—	—	—	+	—	—	—	—	—	—
32	++	—	—	—	—	—	+	—	—	—	—	—	—
33	++	—	++	—	—	—	++	—	—	—	—	—	—
34	++	—	++	—	—	—	++	—	—	—	—	—	+
35	++	—	++	—	—	—	++	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—	—	+	—	—	—
37	+	—	++	—	—	—	++	—	—	—	—	—	—
38	+	—	—	—	+	+	++	—	—	++	—	—	—
39	++	—	—	—	—	—	+	—	—	—	—	—	—
40	++	—	—	—	+	+	+	—	—	++	—	—	—
41	—	—	—	—	+	+	+	+	—	+	—	—	+
42	—	—	—	—	—	—	—	—	—	—	—	—	+

See Table 2 legend.

Patients 27–40 = Art—/Amb+. Patients 41–42 = Art+/Amb—.

Art v 6 and between Art v 1 and its homologue in ragweed; (ii) the major allergens Amb a 1 and Art v 1 can thus be considered *bona fide* indicators of ragweed and mugwort co-sensitization.

As reported in several studies [9, 19, 24, 25], between 20% and 35% of the mugwort- and ragweed-sensitized patients displayed IgE antibodies to the panallergens profilin (Art v 4 and Amb a 8) and calcium-binding proteins (Art v 5, Amb a 9, Amb a 10). It is worth noting that all patients recognizing Art v 4 ($n=8$, 20%) cross-reacted with ragweed profilin (Amb a 8). In contrast, cross-reactivity between the ragweed and mugwort calcium-binding allergens was not complete. These results are in accordance with the fact that sequence similarity between calcium-binding allergens is variable and mostly confined to the EF-hand calcium-binding motifs. Thus, it is possible that IgE antibodies directed to regions outside the EF-hands of one calcium-binding allergen might not bind to homologous proteins found in other pollen.

So far only allergen extracts are routinely applied for diagnostic and therapeutic purposes. These extracts are difficult to standardize regarding their allergen content: several allergens might be under-represented because of degradation, and other non-allergenic components may be present. Extract-based diagnosis allows the identification of allergenic sources only. Therefore, a discrimination between co-sensitization and cross-sensitization in patients showing adverse reactions to more than one allergenic source is not possible [26]. Developments in this area were brought by the concept of recombinant allergen-based component-resolved diagnosis and therapy [27]. The advantages of using recombinant allergens for diagnosis have been documented in several studies [28–32]. In this study, recombinant weed pollen allergens were used to dissect sensitization patterns of mugwort and ragweed allergic patients.

In conclusion, the results of the present study demonstrate that patients showing both ragweed and mugwort IgE reactivity on SPT and/or RAST are in-effect co-sensitized. The next step will be to assess the clinical relevance of IgE reactivity in these subjects by nasal provocation tests with ragweed and mugwort pollen extracts in order to establish whether they should undergo specific immunotherapy with only one pollen extract or both.

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