

A study of allergens in celery with cross-sensitivity to mugwort and birch pollens

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Summary

Sixty-one sera with positive RAST to mugwort pollen (*Artemisia vulgaris*) were submitted to RASTs for birch pollen (*Betula verrucosa*) and celery (*Apium graveolens*). In 36 cases RAST results were positive for celery. In addition, 23 sera presented specific IgE to birch pollen. The binding of specific IgE to individual allergens in celery, mugwort pollen and birch pollen was studied by the immunoblotting technique. This involved electrophoretic separation of allergenic extracts, electrotransfer of proteins onto nitrocellulose sheets and sensitive immunoenzymatic detection. Eighteen sera had specific IgE binding to two celery components of molecular weight around 15 kD. All these sera also detected a 15 kD allergen in mugwort and two allergens in birch of 14 kD and 16 kD molecular weight. The sera that did not detect the 15 kD bands in celery failed to react with both the 15 kD mugwort component and the 14 and 16 kD birch components. Specific cross-inhibitions of the detection of these allergens on immunoblots were obtained by pre-incubation of the sera with crude extract of the three species. These results strongly suggest that such allergens display some structural identity and that they could be at the origin of some cases of crossed hypersensitivity to celery, mugwort pollen and birch pollen.

Introduction

Celery is recognized as a rather frequent food allergen that can cause severe allergic manifestations [1–4]. More recently, association of celery-induced allergic reactions

with sensitivity to other vegetables, fruits, spices, and pollens from birch and mugwort has been reported by several groups [3-8]. These findings were documented by clinical observations, skin-test results and by the presence of cross-reacting IgE antibodies in these patient sera. Inhibition of birch and mugwort radio-allergosorbent tests (RAST) by celery extract suggested the existence of allergenic epitopes common to celery and to mugwort and birch pollens [6, 9]. The aim of this study was to compare the IgE binding components in celery, mugwort pollen and birch pollen in order to identify some allergens shared by the members of this cluster of hypersensitivity.

Materials and methods

Selection of sera reacting with celery, mugwort pollen and birch pollen

Hypersensitivity to mugwort pollen is frequent in the Lyon area whereas that to celery or birch pollen is more uncommon. For these reasons, selection of sera with IgE antibodies reacting with mugwort, celery and birch was done by testing all the sera with positive RAST to mugwort pollen for their positivity to celery and birch pollen. Sera were collected at Louis Pradel Hospital, Lyon, France. No attempt was made to make a selection on a clinical basis. All sera were kept frozen until use.

Allergen extracts

Extraction of birch pollen (Sigma, St Louis, MO, U.S.A.) was done by overnight agitation of the pollen grains in distilled water, using a magnetic stirrer. The $100\,000\times g$ for 60 min supernatants were lyophilized and used as crude extracts. Extract from mugwort pollen was obtained from Laboratoires des Stallergènes, Paris, France. Celery extract was made by grinding piece-cut celery in a Warring Blendor homogenizer in 50 mM Tris/HCl, pH 7.6 (1.5 ml/g). After filtration on gauze and centrifugation ($4000\times g$ for 15 min), solid ammonium sulphate was added to the supernatant up to 75% saturation (at 4°C). Proteins were resolubilized in, and dialysed extensively against, 10 mM Tris/HCl, pH 7.6. Extract was further defatted with ethyl ether. The aqueous phase, referred to as celery crude extract, was stored frozen.

One-dimensional electrophoresis

Allergen extracts were separated by polyacrylamide slab-gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE), according to Laemmli [10]. The sample buffer contained 62.5 mM Tris/HCl, pH 6.8, 1% (w/v) SDS, 100 mM dithiothreitol, 10% (w/v) glycerol and 0.001% (w/v) bromophenol blue. Samples were heated at 95°C for 5 min. Electrophoretic migrations were performed in a temperature-controlled apparatus (SEBIA, Issy les Moulineaux, France) allowing simultaneous runs in eight slab gels under the same conditions [11].

Electrophoretic transfer of proteins

Transfer of proteins from the gel onto the nitrocellulose membrane (0.45 μ m, Schleicher and Schuell, Dassel, FRG) was performed with a semi-dry electroblotting apparatus (Ancos, Denmark). Up to six gels could be blotted simultaneously in 2 hr. The quality of the transfer was checked by nitrocellulose staining with Ponceau S in 5% TCA. Membranes were stored at 4°C in water until use.

Table 1. RAST scores and results of immunodetection for 36 sera with specific IgE to mugwort pollen and celery

	RAST (PRU/ml)			Immunodetection on blots			
	f85	w6	t3	Celery		Mugwort 15 kD	Birch 14-16 kD
				15 kD	HMW		
Group I							
A	15.1	> 17.5	> 17.5	+	+	+	+
B	6.7	3.1	0.4	+	+	+	+
C	4.2	> 17.5	> 17.5	+	+	+	+
D	2.8	3.8	> 17.5	+	+	+	+
E	10.7	12.5	2.1	+	+	+	+
F	> 17.5	4.5	> 17.5	+	+	+	+
G	3.0	8.6	11.7	+	+	+	+
H	0.7	0.9	13.1	+	+	+	+
I	1.3	0.6	Neg	+	+	+	+
J	1.5	0.6	0.7	+	+	+	+
K	1.3	1.3	Neg	+	+	+	+
Group II							
L	1.7	> 17.5	1.3	+	-	+	+
M	2.6	15.6	1.3	+	-	+	+
N	1.3	2.3	0.4	+	-	+	+
O	0.4	17.5	Neg	+	-	+	+
P	0.6	5.4	8.5	+	-	+	+
Q	0.7	0.4	0.8	+	-	+	+
R	0.9	1.6	1.1	+	-	+	+
S	0.4	1.4	1.3	+	-	+	+
T	0.4	1.0	0.9	+	-	+	+
U	2.4	1.7	1.8	+	-	+	+
Group III							
a	0.5	> 17.5	> 17.5	-	+	-	-
b	0.8	> 17.5	Neg	-	+	-	-
c	15.5	1.5	0.8	-	+	-	-
d	6.0	1.5	Neg	-	+	-	-
e	16.3	> 17.5	Neg	-	+	-	-
f	0.7	1.9	Neg	-	+	-	-
g	10.8	8.7	2.6	-	+	+	-
h	10.5	8.2	Neg	-	+	-	-
i	1.0	2.8	11.0	-	+	-	-
j	1.1	1.3	8.8	-	+	-	-
k	0.4	15.7	Neg	-	+	-	-
l	0.6	1.9	Neg	-	+	-	-
Group IV							
m	2.8	17.5	Neg	-	-	-	-
n	1.9	> 17.5	Neg	-	-	-	-
o	0.4	15.3	Neg	-	-	-	-

The RAST scores are indicated in Phadebas RAST Units (PRU) (f85: celery, w6: mugwort, t3: birch). (+) and (-) indicate the presence or the absence of the indicated bands on the immunoblot, respectively. HMW: high molecular weight bands.

Immunodetection on blots

The apparatus designed by Alric *et al.* [12] and produced by SEBIA was used to perform simultaneous immunodetections with different sera on uncut nitrocellulose membranes. The membrane was incubated for 30 min in phosphate buffered pH 7.3 saline (PBS), containing 0.2% Tween 20 and 1% non-fatty milk, in order to block free protein binding sites. Half-diluted sera in PST solution (PBS containing 10% newborn calf serum and 0.2% Tween 20) were incubated for 2 hr under agitation. After five washes with PST, the membrane was incubated sequentially for 60 min with anti-human IgE (ϵ specific raised in rabbit, from Dako, Copenhagen, Denmark) diluted 1 : 500, biotinylated anti-rabbit IgG (from Vector supplied by Biosys, Compi gne, France) diluted 1 : 1000 and streptavidin-biotinylated horseradish peroxidase complex (from Amersham, Les Ulis, France) diluted 1 : 1000. After five washes (three with PST and the last two with PBS), the membrane was immersed for 2–5 min in a solution containing 10 mg of diaminobenzidine and 20 μ l of 33 M H_2O_2 in 50 ml PBS.

Results*Immunodetection of IgE binding components in celery extract*

Among 61 positive sera in RAST mugwort, 36 (59%) and 26 (41%) also had positive RAST to celery and birch, respectively. Twenty-three sera were positive for the three allergens.

Immunodetection after SDS-PAGE and electrotransfer was used to map the proteins that bind IgE antibodies contained in celery-RAST-positive sera. The specificity of the technique was established by different ways. Firstly, the detection was abolished by treatment of the extract with pronase prior to electrophoresis. The same result was obtained when one of the antibodies (anti-human IgE or biotinylated anti-

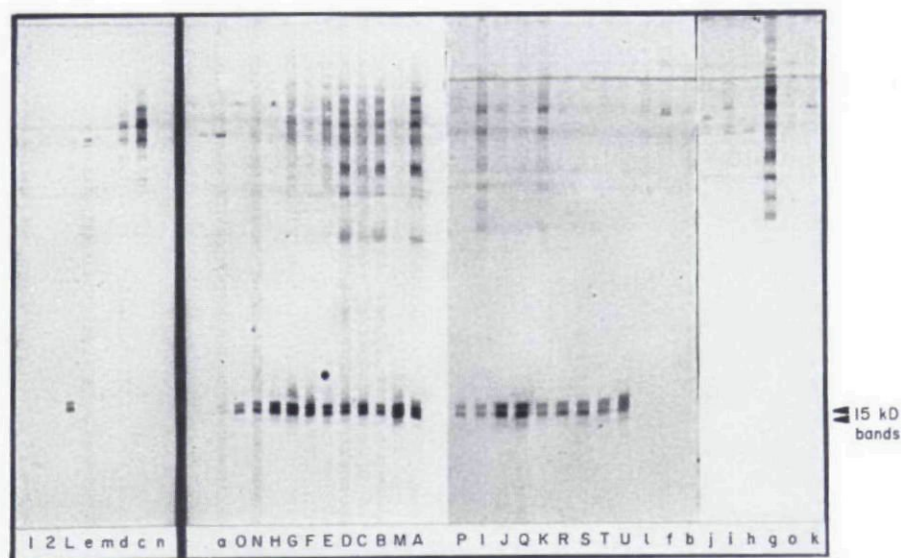


Fig. 1. Detection of specific IgE binding to celery proteins. The 36 sera with positive RAST to celery and mugwort pollen were used as a source of IgE. The sera were diluted two-fold except for serum L (five-fold dilution). Lanes 1 and 2: PBS.

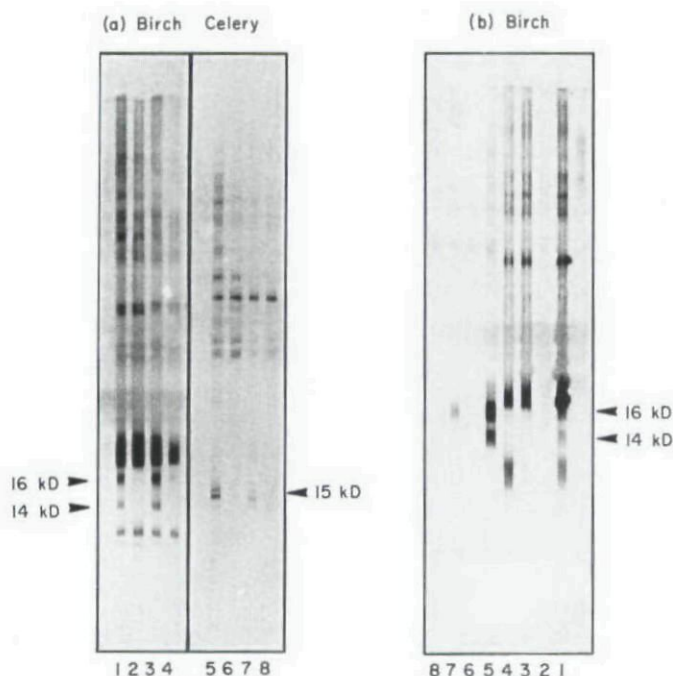


Fig. 2. Effects of sera pre-incubation with celery, birch pollen or mugwort pollen extracts on immunodetection of celery and birch proteins. Sera (200 μ l) were incubated for 1 hr at room temperature with PBS or 50 μ g protein of allergen extracts. The sera were then incubated on celery and birch blots as described in the Materials and methods section. Two experiments are shown. (a) Lanes 1, 5: serum D+PBS, lane 2: serum D+celery, lanes 3, 7: serum F+PBS, lane 4: serum F+celery, lane 6: serum D+birch, lane 8: serum F+birch. (b) Lane 1: serum D+PBS, lane 2: serum D+birch, lane 3: serum D+mugwort, lane 4: serum D+celery, lane 5: serum Q+PBS, lane 6: serum Q+birch, lane 7: serum Q+mugwort, lane 8: serum Q+celery.

rabbit IgG) was omitted. Secondly, neither the presence of bands on the membrane nor their intensities were linked to the total IgE level in the sera. Sera highly positive (> 17.5 PRU) in ragweed or timothy RAST but negative in celery RAST detected no band on celery blots (Fig. 5). Finally, results given by this method correlated quite well with the corresponding RAST scores. However, some exceptions were observed (Table 1). They will be discussed below. Figure 1 shows patterns of IgE binding to celery proteins revealed by the positive sera in celery RAST. Many sera strongly detected two bands of 15 kD molecular weight. Different patterns were observed from one serum to another. Sera may tentatively be distributed into the four following groups (Table 1): group I, presence of both 15 kD and high molecular weight bands (sera A–K); group II, presence of 15 kD bands only (sera L–U); group III, presence of high molecular weight bands only (sera a–l); group IV, no detectable bands (sera m–o). The 15 kD bands were detected by 21 out of 36 (58%) positive sera in celery RAST. Twenty-three sera revealed some high molecular weight bands (63%). Thirty-three out of the 36 sera (92%) detected some bands on blots. On the 25 sera that were mugwort pollen positive but celery negative, only three reacted with the 15 kD components. With some exceptions, sera of groups I and III had higher celery-specific IgE levels than sera of group II (Table 1).

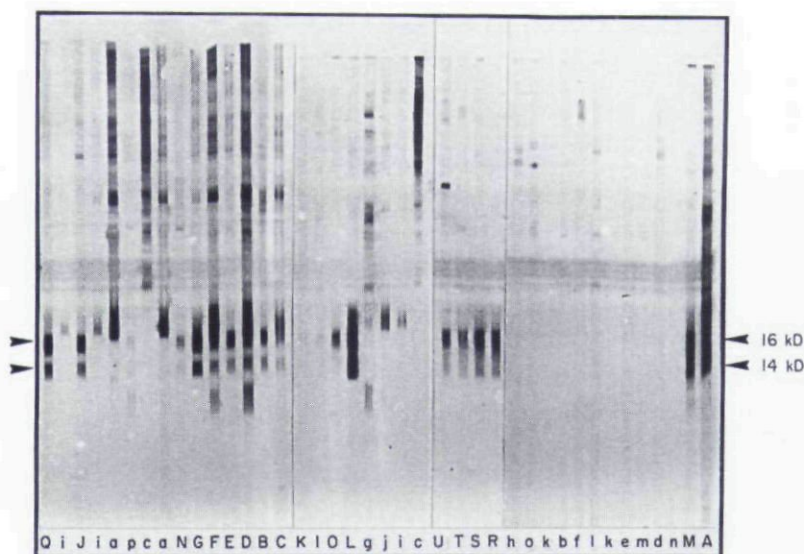


Fig. 3. Detection of specific IgE binding to birch pollen proteins. Sera with positive RAST to mugwort and celery were incubated on birch blots. All the sera were diluted two-fold. Arrowheads indicate the position of the 14 and 16 kD birch pollen allergens.

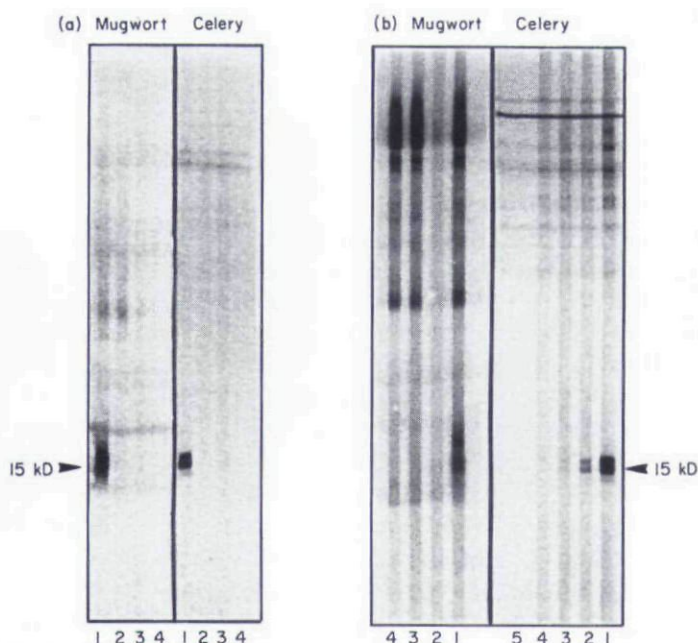


Fig. 4. Effects of sera pre-incubation with celery, birch pollen or mugwort pollen extracts on immunodetection of celery and mugwort proteins. Pre-incubations and immunodetections were done as indicated in the legend of Fig. 2. Two experiments are shown. (a) Lane 1: serum L + PBS, lane 2: serum L + celery, lane 3: serum L + mugwort, lane 4: PBS. (b) Lane 1: pool of serum B, J, O and Q + PBS, lane 2: pool + mugwort, lane 3: pool + birch, lane 4: pool + celery, lane 5: PBS.

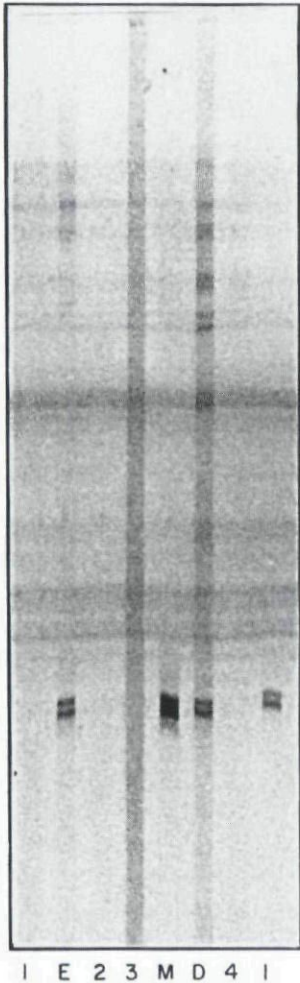


Fig. 5. Influence of total and specific IgE levels on immunodetection on celery blots. Immunodetection was conducted as indicated in the Materials and methods section. Sera E, M and D are those indicated in Table 1. Total IgE level (kU/l): E: 2040, M: 1950, D: 2600. Sera 1, 2 and 3 are negative in celery RAST. Serum 1: total IgE: 1190, ragweed RAST > 17.5 PRU; serum 2: total IgE: 2600, *Candida albicans* RAST = 3.4 PRU; serum 3: total IgE: 4860, timothy RAST > 17.5 PRU, house dust RAST = 5 PRU; lane 4: PBS.

15 kD bands in celery as cross-reacting material

Eighteen out of the 23 sera (78%) that reacted with celery, mugwort pollen and birch pollen also detected the 15 kD bands on the celery blot. The possibility that these components represent cross-reacting material present in the three allergens was further investigated. Birch or mugwort pollen extracts and celery extract were separated on the same gel. The whole gel was transferred onto nitrocellulose. This method provided homogeneity in the separation of the two extracts, allowing direct comparison of the allergen molecular weights. Pre-incubation of sera with birch pollen extract completely inhibited the detection of the 15 kD bands on celery blot, whereas some high molecular weight bands remained (Fig. 2a). Pre-incubation of the same sera with celery

specifically suppressed IgE binding to two birch pollen components of about 16 and 14 kD (Fig. 2a). In addition to these two bands, mugwort pollen also prevented the binding of IgE to a lower molecular weight band (Fig. 2b). A strongly detected band of about 17 kD was not inhibited and might correspond to the major thermostable allergen of birch pollen. The 16 and 14 kD birch pollen components were revealed by all the sera that detected the 15 kD bands in celery (Fig. 3 and Table 1). Some of these sera were negative in birch pollen RAST (sera O, I, K). The sera that failed to recognize the 15 kD bands on the celery blot also failed to detect the two birch pollen components, despite RAST positivity for celery and birch pollen (sera a, c, g, i, j). Sera of group II that only reacted with the 15 kD components on the celery blot exclusively detected the 16 and 14 kD bands on the birch blot (sera L, M, Q, R, S, T, U; Fig. 3).

The same study was done by immunodetection of celery and mugwort pollen proteins by serum L and by a pool of serum. Serum L, which mainly detected the 15 kD allergens of celery, reacted almost exclusively with a 15 kD component on the mugwort blot (Fig. 4a). Pre-incubation of serum L with celery extract suppressed the 15 kD bands on both blots. For the pool of serum (Fig. 4b), pre-incubation with celery or birch pollen inhibited the binding of IgE to the 15 kD band and to another band of slightly higher molecular weight, but not to other mugwort pollen allergens. A correlation exists for all but one serum between the detection of the 15 kD band of celery and that of the 15 kD band of mugwort pollen (Table 1).

Discussion

This study confirms the frequency of associated sensitivity to celery, mugwort pollen and birch pollen, as reflected by specific IgE antibodies. We found that 59% of mugwort-RAST-positive sera were also positive for celery, and that 88% of birch-positive sera had IgE reacting in celery RAST. In a previous study, Halmepuro *et al.* [9] have found 51% and 71%, respectively. Among a population with positive RAST to mugwort pollen, Kremser and Lindermayer [13] have observed 56% of positive results in RAST to celery.

Of the sera that had positive RAST to celery and mugwort, 58% displayed specific IgE binding to two components of 15 kD molecular weight. Three out of 25 sera with negative RAST for celery detected the 15 kD allergens on the celery blot. The assumption that these 15 kD components could be quantitatively minor allergens, present in relatively low amount on the commercial RAST disc, may explain the apparent discrepancy between RAST and immunodetection on blot for these three sera. The rather low scores of celery RAST for the sera that reacted only with the 15 kD allergens (group II) support this view. Of all the mugwort- and celery-positive sera, 42% did not detect the 15 kD bands (groups III and IV). This may correspond to cases of concomitant allergy. Alternatively, and more probably, other cross-reacting allergens, not detected by this study, could be involved to explain these cases. Evidence that other components act as cross-reacting allergens in celery, mugwort pollen and birch pollen is brought by the inhibition of immunodetection of several bands on the celery blot after pre-incubation of sera with mugwort or birch pollens. Halmepuro *et al.* [9] have observed that birch pollen was able to inhibit completely the IgE binding to celery tuber discs, suggesting that all epitopes present in celery were present in birch pollen. Due to the sample preparation used in this study, temperature and/or SDS and dithiothreitol-sensitive cross-reacting allergens were possibly lost. More generally, this

may explain some unusual cases with no band or only faint bands on celery blots, whereas the corresponding scores in RAST were rather high (sera d, e, h, i, m, n).

The arguments to implicate the 15 kD allergens in celery and mugwort pollen, and the 14 and 16 kD allergens in birch pollen, as cross-reacting material between these three species, are the following: (i) the high percentage (82%) of sera with positive RAST for the three allergens that detected the 15 kD allergens on celery blots; (ii) the inhibition of IgE binding to the 15 kD allergens in celery by pre-incubation of sera with birch or mugwort pollens; (iii) the inhibition of IgE binding to the 15 kD allergens in mugwort by birch and to the 14 and 16 kD allergens in birch by mugwort; (iv) the rather high selectivity of the inhibition by celery of IgE binding to mugwort and birch pollen components with a molecular weight of around 15 kD; (v) the close correlation between the detection of the 15 kD allergens of celery and the detection of the allergens of similar molecular weight in birch and mugwort pollens.

Halmepuro and Lowenstein [14] have shown by crossed-line immunoelectrophoresis that one minor allergen of birch pollen (Bv ag 19) was affected by celery extract. In non-denaturing conditions, the molecular weight of this allergen was 29 kD [14, 15]. The 14 and 16 kD allergens in birch pollen whose IgE binding capacity was specifically inhibited by celery, might correspond to subunits of Bv ag 19 produced under denaturing conditions. In the same study these authors did not find any structural similarity between allergens in celery and mugwort pollen. Biochemical studies on mugwort allergens have been limited and no data on molecular weight of allergens in mugwort pollen are available. Nilsen and Smestad Paulsen [16] have found seven components acting as allergens in mugwort pollen, three of them being resistant to heat treatment. In an other species, *Artemisia scoparia*, Jaggi and Gangal [17] have described a heat stable and acidic allergenic component of 14.3 kD molecular weight. The relation with the heat stable 15 kD allergen we found in *Artemisia vulgaris* pollen has to be investigated further.

The biochemical nature of these cross-reacting allergens remains unknown. It has been suggested [18] that cross-reacting material in plants may be carbohydrates. In the present case, the fact that these allergens were thermostable was in agreement with this assumption. Further work is currently in progress to verify this hypothesis.

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