

IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome

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Summary

Background Allergy to celery is often associated with sensitization to birch and/or mugwort pollen.

Objective and methods In a multi-centre study, sera from 23 patients suffering from type I allergy to celery and 15 patients with positive celery RAST but no clinical sensitization were compared. To examine whether cross-reactivity between celery and mugwort pollen includes cross-sensitization to birch pollen allergens, we determined cross-reacting structures in birch pollen, mugwort pollen and celery by means of immunoblotting. Inhibition studies were performed by preincubation of sera with extracts of birch pollen, mugwort pollen, and celery.

Results We identified three groups of proteins—homologues of Bet v 1 and birch profilin (Bet v 2) as well as a group of proteins with a molecular range of 46 to 60 kD—displaying IgE-cross-reactivity, which were shared by birch pollen and celery. Two of these groups of allergens (profilin and the 46 to 60 kD proteins) were also present in mugwort pollen. In this paper we demonstrate that most cross-reacting allergens present in mugwort pollen and celery can also be detected in birch pollen extract.

Conclusion Therefore we propose, from a serological point of view, to extend the mugwort-celery syndrome to the birch-mugwort-celery syndrome.

Keywords: celery, birch pollen, mugwort pollen, allergy, cross-reactivity

Clinical and Experimental Allergy, Vol. 26, pp. 1161–1170. Submitted 19 September; revised 18 March 1996; accepted 10 June 1996.

Introduction

Patients with type I allergies to pollens frequently produce IgE to a wide range of food allergens [1–26]. It is well known that patients suffering from allergy to birch pollen often exhibit adverse reactions after ingestion of various fruits of the family *Rosaceae* (apple, pear, cherry, peach), nuts and vegetables (celery, carrot, potato) [2–4,6,9,12,17,20,22,26]. Recently, a 'birch pollen-kiwi fruit'

cluster was described [27]. The major birch pollen allergens Bet v 1 and Bet v 2 (birch pollen profilin) and their homologues could be identified as cross-reacting structures in birch pollen, fresh fruits, nuts and vegetables [22,26].

The observation that the ingestion of celery can induce hypersensitivity reactions has been made for 70 years [28]. In Central Europe celery tuber (or celeriac) is an important food allergen [29]. In contrast to the fruits which mainly cause the so-called 'oral allergy syndrome' [30,31], it is responsible, not only raw, but also cooked, and as a spice, for various IgE-mediated reactions, from local symptoms in the oral mucosa to life-threatening

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anaphylactic shock [11,13,15]. Celery-allergic patients are frequently sensitized to mugwort pollen [11]. A coincident allergy to several spices is quite common, therefore the term 'celery-mugwort-spice-syndrome' has been proposed [11,24]. Since an allergy to carrot is also sometimes associated (celery and carrot both belong to the *Apiaceae* family), this term was expanded to 'celery-carrot-mugwort-spice-syndrome' [15]. Several groups demonstrated additional sensitization of celery-mugwort patients with allergens in birch pollen [13,18,19].

In order to examine whether cross-reactivity between celery and mugwort pollen includes cross-sensitization to birch pollen allergens, we investigated IgE-binding to extracts of celery, birch pollen and mugwort pollen in celery-allergic patients by means of immunoblotting and IgE-inhibition experiments.

Materials and methods

Sera

Serum samples from 23 patients suffering from allergic symptoms after ingestion of celery (group I; Table 1) and

15 patients with a positive RAST to celery but no clinical sensitization have been selected (group II).

Protein extracts

Birch pollen and mugwort pollen were obtained from Allergon AB (Engelholm, Sweden) and extracted as described [32]. Celery tuber (from now on referred to as celery) was purchased from a local retail shop. Proteins from celery were extracted in 10% (w/v) by overnight shaking at 4°C in extraction buffer: 10 mmol/L potassium phosphate buffer containing 2% (w/v) suspended solid polyvinylpyrrolidone (PVPP), 2 mmol/L ethylene diamine tetraacetic acid (EDTA), 10 mmol/L sodiumdiethyldithiocarbamate (DIECA), and 3 mmol/L sodium azide, pH 7.0. After centrifugation at 40 000 g for 60 min at 4°C, the supernatant was filtered and subsequently dialysed (Spectra/Por 1, Molecularporous Dialysis Membrane, mwco: 6–8000; Spectrum, Houston, Texas) against 5 L of 10 mmol/L potassium phosphate buffer containing 3 mmol/L sodium azide, pH 7.0, for 48 h with two changes of buffer. The dialysates were lyophilized and subsequently stored at –20°C.

Table 1. Summary of the clinical symptomatology, the kind of celery preparation which provoked the allergic symptoms, and the results of the skin prick tests of patients suffering from allergic symptoms after ingestion of celery; nd = not done

Patient #	Symptoms		Celery preparation	Skin prick test	
	Local	Systemic		Raw celery	Cooked celery
1	+	+	Canned	++	–
2	+	–	Raw	++	–
3	+	–	Raw	+	+/-
4	+	–	Raw	++	–
5	+	–	Raw	++	+/-
6	+	–	Raw, cooked	++	+/-
7	+	–	Raw	++	+/-
8	+	+	Raw	++	+
9	+	–	Raw	++	–
10	+	–	Raw	+	–
11	+	–	Raw	–	–
12	+	+	Raw	+	nd
13	+	+	Raw	++	+
14	+	+	Raw	+/-	–
15	+	+	Raw	+/-	–
16	+	–	Raw	++	–
17	+	–	Raw	++	–
18	+	–	Raw	+	–
19	+	–	Raw	+/-	–
20	+	–	Raw	++	nd
21	+	+	Raw	++	+
22	+	+	Raw	+	+
23	+	+	Raw	+	+/-

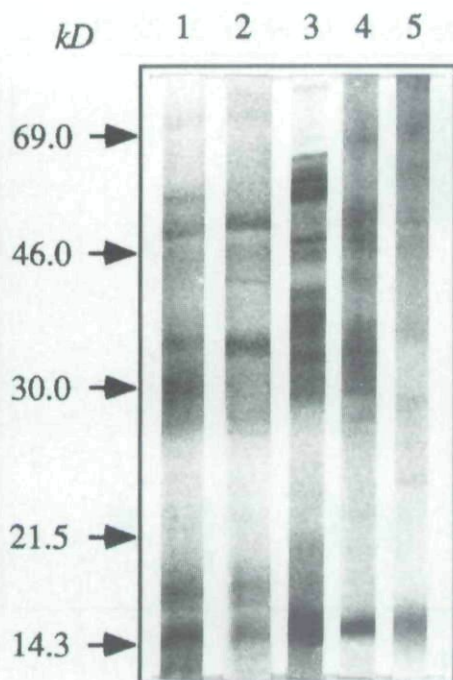


Fig. 1. Coomassie brilliant blue R stained 12% polyacrylamide gels show the separation of celery proteins under five different conditions: uncooked (lane 1) and cooked celery (lane 2), celery extract in SDS-PAGE (lane 3), celery extract in SDS-PAGE without 2-ME (lane 4), and celery extract by leaving out both the 10% SDS and 2-ME in the Laemmli procedure (lane 5).

In addition, uncooked (Fig. 1; lane 1) and cooked (30 min, 95°C) (Fig. 1; lane 2) celery was prepared for loading onto gels: 3 g of sample was added to 10 mL of Laemmli buffer [33], homogenized in an Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany), and centrifuged (10 min $\times$ 11 000 rpm). The supernatants were stored at -20°C. Protein concentrations were determined according to Bradford [34].

Deglycosylation of celery extract was performed with 0.01 M NaIO₄ in acetate buffer (pH 5.0) at room temperature in the dark. After 2 h the reaction was stopped with sorbitol.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), protein transfer and immunoblotting

SDS-PAGE (12% homogenous gels, 140 $\times$ 115 $\times$ 1 mm, 5% stacking gels) was performed according to Laemmli [33] in a vertical slab gel apparatus (Hoefer, San Francisco, USA) with modifications as previously described [32]. Samples were diluted (4:1) in Laemmli buffer prior to heating at 95°C for 10 min and subsequently loaded onto gels (celery: Fig. 1; lane 3). In the case of celery extracts, proteins were also separated under non-reducing

conditions by omitting 2-mercaptoethanol (2-ME) from the Laemmli buffer (Fig. 1; lane 4), and by leaving out both the 10% SDS and 2-ME in the Laemmli procedure (Fig. 1; lane 5). In the latter, samples were not heated before being loaded onto gels. Transfer of separated proteins to nitrocellulose sheets was done according to Towbin *et al.* [35]. IgE-immunoblots (IB) were performed as previously described [32].

IgE-inhibition experiments

Inhibition with extracts of birch pollen, mugwort pollen, and celery: sera from three patients (Fig 2; lanes 11, 12, 15) displaying IgE-binding to proteins at a molecular mass ranging from 46 kD to 60 kD in extracts of birch pollen, mugwort pollen and celery were pooled (pool A), diluted 1:10 in test buffer (50 mmol/L of sodium phosphate buffer (pH 7.5), containing 0.5% (vol/vol) of bovine serum albumin, 0.05% (wt/vol) of sodium azide) and preincubated with 0 μ g, 3 μ g, 10 μ g, 30 μ g, 100 μ g, respectively, of birch pollen as well as mugwort pollen extract. Thereafter, nitrocellulose blotted celery proteins were incubated with this dilution (1 mL/nitrocellulose strip), and bound IgE was detected as described [32].

Sera from two patients exhibiting IgE-binding to a 60 kD band in extracts of mugwort pollen with no correlated band in extracts of birch pollen and celery (Fig. 2b; lanes 21, 22), were pooled (pool B), diluted 1:10 in test buffer and preincubated with 0 μ g and 100 μ g, respectively, of celery extract. Nitrocellulose blotted mugwort pollen proteins were incubated with this dilution (1 mL/nitrocellulose strip), and bound IgE was detected as described [32].

Results

Characterization of celery extracts

Coomassie brilliant blue R stained polyacrylamide gels were produced under five different conditions: multiple protein bands ranging from 13 kD to 70 kD were apparent (Fig. 1). The preparation of lane 3 (Fig. 1) was chosen for the IBs shown.

Immunoblot experiments

Figure 2 shows IgE-IBs of 23 patients with clinical symptoms after ingestion of celery (group I; Table 1). Patients 1-10, 14, 16-18, 20, 21 and 23 reacted with Bet v 1 (17 kD), the major birch pollen allergen, in the birch pollen IB (Fig. 2a), and displayed IgE-binding to a 15 kD protein in the celery extract (Fig. 2c). No

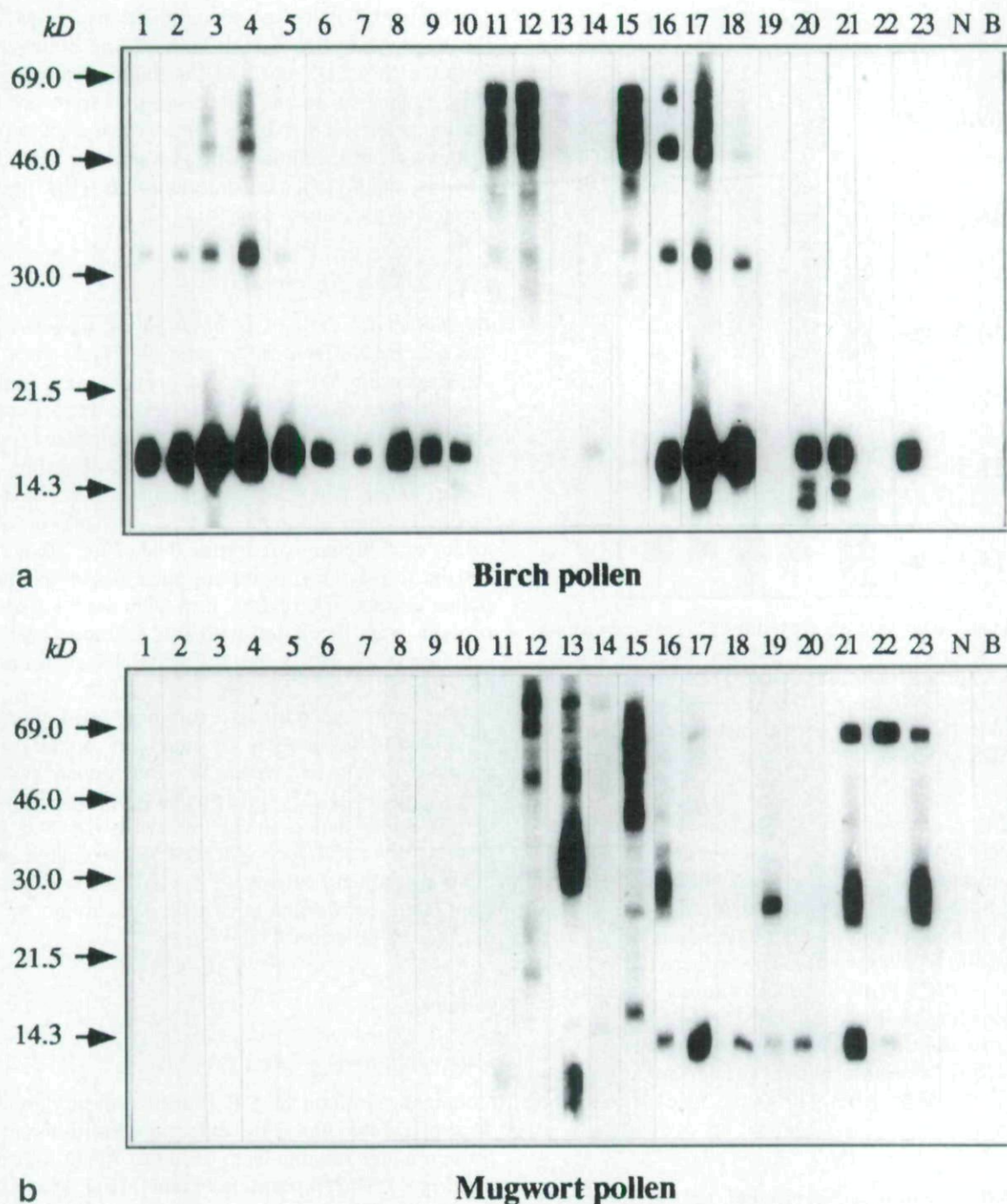


Fig. 2. Autoradiograph of IgE-immunoblot analysis of extracts of birch pollen (Fig. 2a), mugwort pollen (Fig. 2b) and celery (Fig. 2c) with sera from 23 patients suffering from type I allergy to celery (group I). N = normal human serum pool, B = buffer control.

IgE-binding was found in patients 1–10 in mugwort IB (Fig. 2b). Patients 11–17 exhibited IgE-binding to molecules at a molecular mass ranging from 46 kD to 60 kD in extracts of birch pollen and celery (Fig 2a, c). In this molecular range patients 12–17 showed IgE-binding in

the mugwort IB as well (Fig. 2b). A mugwort specific protein at 28 kD was recognized by patients 12, 13, 15, 16, 19, 21 and 23 in the mugwort pollen extract (Fig. 2b). Patients 21–23 displayed IgE-binding to a 60 kD molecule which was apparent in the mugwort extract,

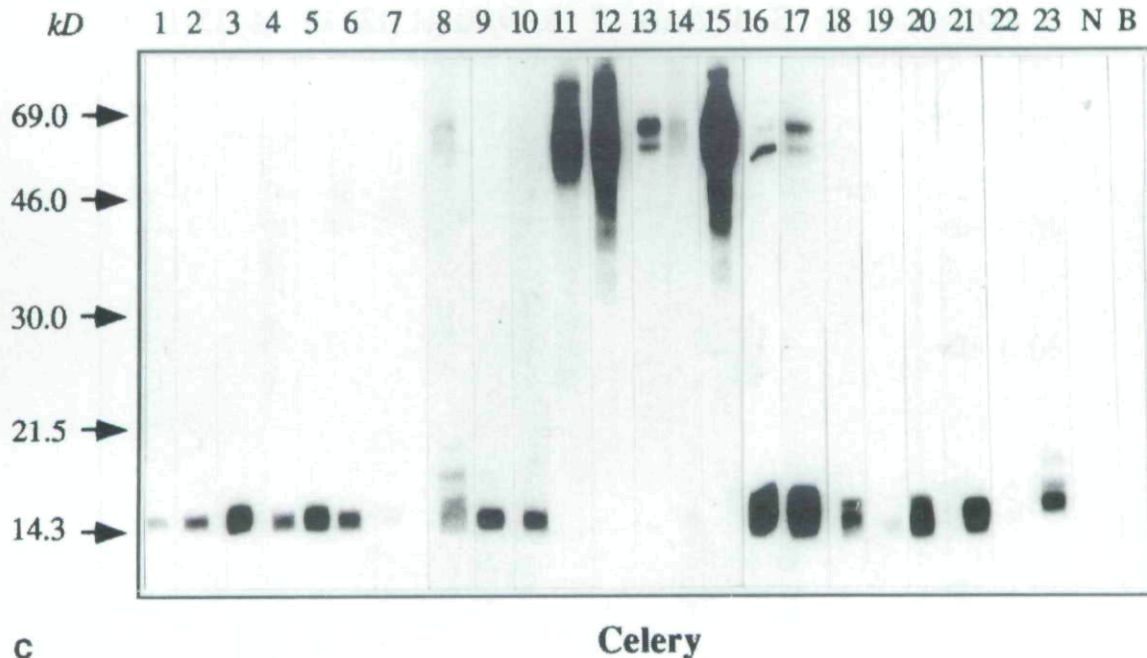


Fig. 2. Continued.

exclusively (Fig. 2b). Patients 16–22 bound IgE to a 14 kD protein in all extracts (Fig. 2).

Figure 3 displays IgE-IBs of 15 patients with positive celery RAST, but no clinical sensitization (group II). Patients 1–5, 9–12, 14 and 15 reacted with Bet v 1 (17 kD) in the birch pollen IB (Fig. 3a), and displayed IgE-binding to a 15 kD protein in the celery extract (Fig. 3c). No IgE-binding was found in patients 1, 4, 5 and 15 in mugwort IB (Fig. 3b). Patients 6–14 exhibited IgE-binding to molecules at a molecular mass ranging from 46 kD to 60 kD in the celery extract (Fig. 3c). In this molecular range patients 3–15 showed IgE-binding in the birch pollen IB as well, although reactivity in lanes 3, 4 and 10 were weak or very weak (Fig. 3a). Comparable IgE-reactivity was detected in the mugwort pollen IB in patients 3, 6–8 and 11–14 (Fig. 3b). A mugwort specific protein at 28 kD was recognized by patients 2, 3, 6–8 and 10–14 in the mugwort pollen extract (Fig. 3b). Patients 3, 9 and 11–14 bound IgE to a 14 kD protein in all extracts (Fig. 3).

IgE-binding to deglycosylated celery extract

Three celery-allergic patients and a serum pool P were used to investigate IgE-reactivity to a deglycosylated celery extract. Allergens at a molecular mass of 14 kD, 15 kD and 46–60 kD were detected both in the aqueous extract of celery (panel A) and this extract after periodate treatment (panel B, Fig. 4).

IgE-inhibition experiments

IgE-binding to proteins of 46 kD to 60 kD in celery extract was significantly inhibited by preincubation of serum pool A (patients 11, 12, 15) with 3 µg, 10 µg, 30 µg, 100 µg of birch pollen and mugwort pollen extract, respectively (Fig. 3; lanes 2–5, 7–10).

Preincubation of serum pool B (patients 21, 22) with 100 µg of celery extract had no influence on IgE-binding to a 60 kD protein present in the mugwort pollen extract (Fig. 5; lanes 11, 12).

Discussion

Almost every celery-allergic patient exhibits sensitization to birch and/or mugwort pollen. According to a study by Vallier *et al.* [18], 88% of birch pollen positive sera displayed IgE-binding in the celery RAST, and 59% of mugwort pollen RAST-positive sera were also found positive in the celery RAST. Halmepuro *et al.* [12] evaluated 71% and 51% in this respect. Clinical symptoms range from contact urticaria and angioedema, to severe reactions such as generalized urticaria, bronchial asthma and anaphylactic shock.

We asked whether allergy to celery includes cross-sensitization to birch pollen and mugwort pollen, and revealed the cross-reacting structures in birch pollen, mugwort pollen and celery by means of immunoblotting. We selected two groups of patients: 23 patients suffering

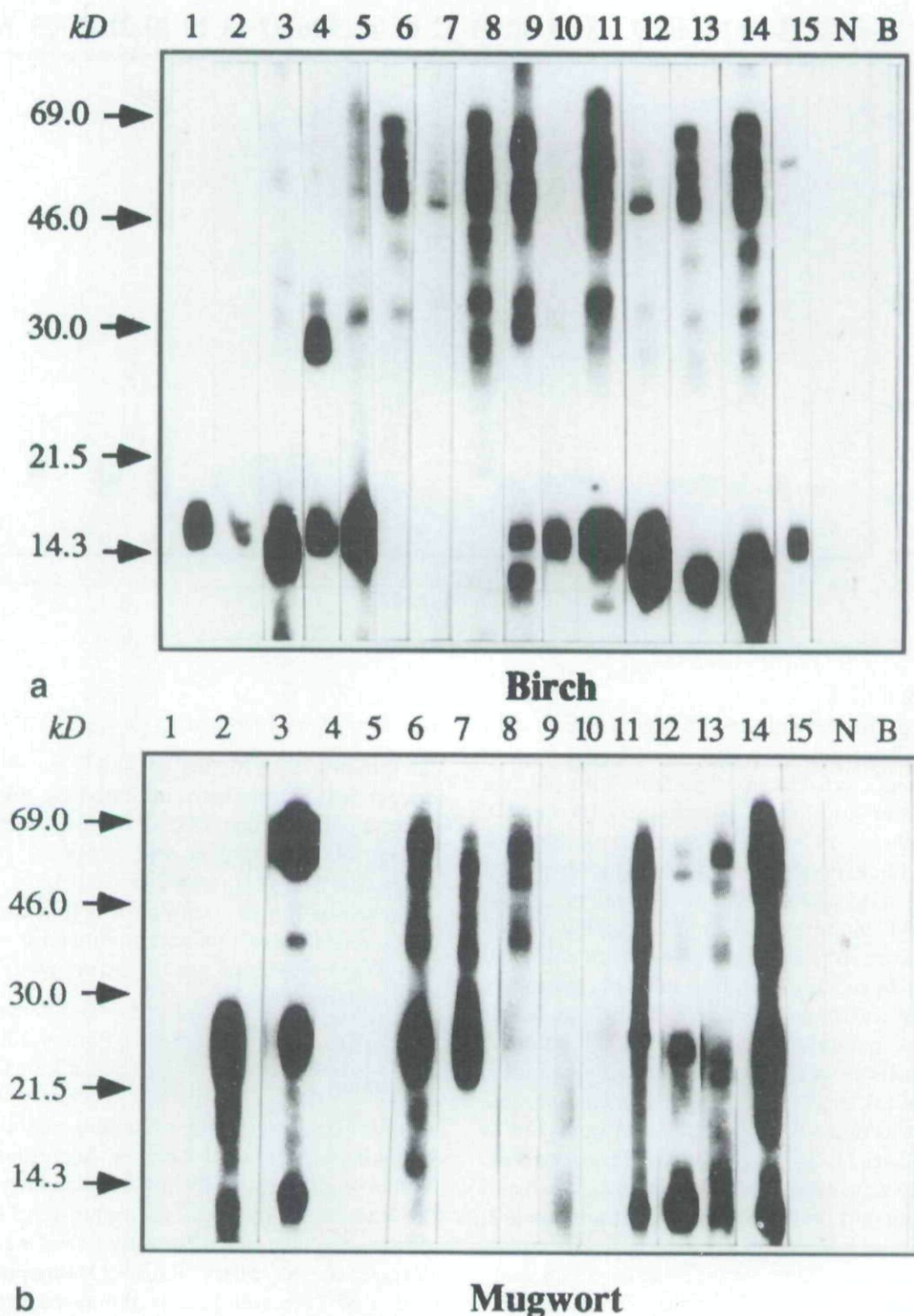


Fig. 3. Autoradiograph of IgE-immunoblot analysis of extracts of birch pollen (Fig. 3a), mugwort pollen (Fig. 3b) and celery (Fig. 3c) with sera from 15 patients with positive celery RAST, but no clinical sensitization (group II).

from type I allergy to celery (group I) and 15 patients with positive celery RAST but no clinical sensitization (group II) were compared. Thirteen out of 23 were sensitized to mugwort and birch pollen, all of them

reacted with proteins in birch pollen (patients 19 and 22 displayed only very weak IgE-binding to a 14kD molecule). We separated celery proteins under five different conditions (Fig. 1) to ensure we did not miss any

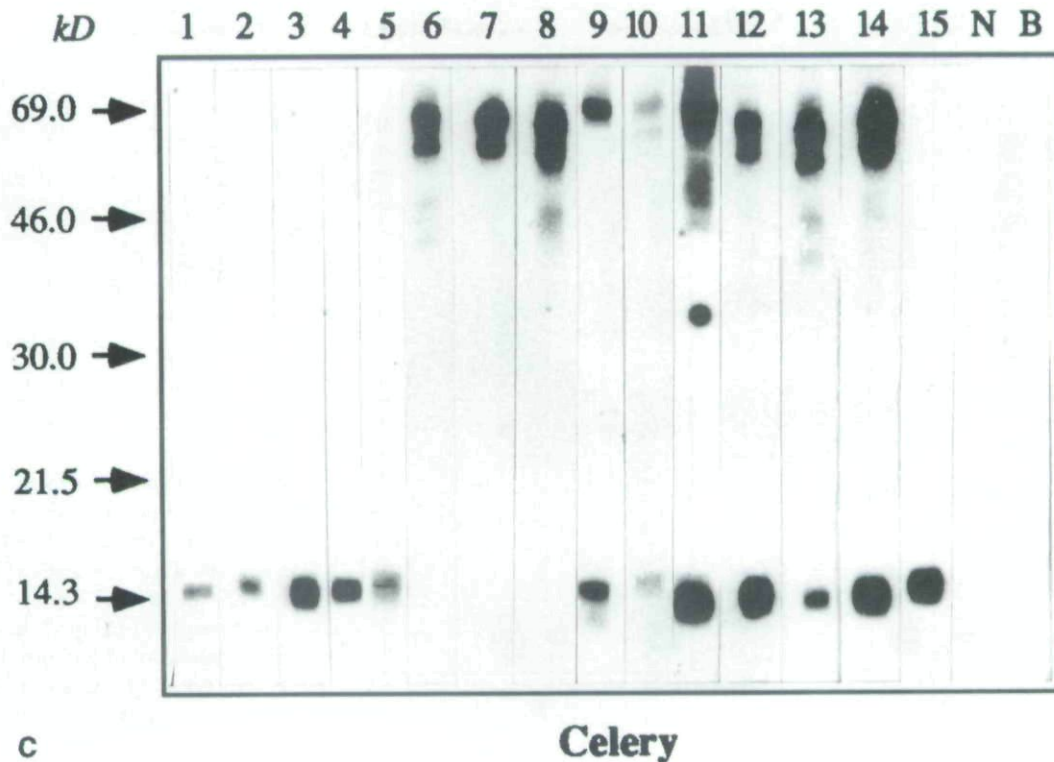


Fig. 3. Continued.

proteins in celery that could not be detected under denaturing conditions or certain thermolabile molecules that might degrade during the cooking process. Wüthrich *et al.* [19] described celery-birch associated thermolabile and celery-mugwort associated thermostable allergens in celery tuber. In IBs with three different serum pools, however, no difference in the IgE-binding pattern could be observed with five extract-preparations using SDS-PAGE, nor in IBs performed under non-denaturing conditions (data not shown).

From a serological point of view celery-allergic patients can be divided into two groups: the birch-celery group (patients 1–10, Fig. 2; patients 1, 4, 5, 15 Fig. 3) and the birch-mugwort-celery group (patients 11–23, Fig. 2; patients 2, 3, 6–14, Fig. 3). The cross-reacting structure in the birch-celery group is Bet v 1. A corresponding allergen with high homology is present in celery (Api g 1 [36]), but not in mugwort pollen (Figs 2 and 3). Since two of the birch-celery group patients reacted locally and even systemically with cooked or canned celery (Table 1), Api g 1 could represent a heat-stable, anaphylactic allergen. Three cross-reacting molecules can be found in the birch-mugwort-celery group in the birch pollen IB: Bet v 1, Bet v 2 (birch pollen profilin), and bands in the range of 46 kD to 60 kD (Figs 2a and 3a). Homologues of these molecules can be found in

celery (Figs 2c and 3c) [18,26]. In mugwort pollen IBs, however, no Bet v 1 homologue can be detected, while profilins and bands at 46–60 kD were present (Figs 2b and 3b). According to the literature these allergens seem to represent homologues of Art v 1 [37]. IgE-inhibition revealed cross-reactivity of these molecules (Fig. 5). Patients with an exclusive IgE binding pattern to profilin, Api g 1 or the cross-reacting 60 kD band structure, respectively, can exhibit a systemic allergic reaction after the ingestion of celery. Thus, all of these molecules must be considered as candidates for inducing systemic reactions in celery-allergic patients. In a recent study by Vieths *et al.* [38] structures at comparable molecular mass appeared to contain cross-reacting carbohydrate determinants. However, we could not observe any difference in terms of IgE-binding with deglycosylated celery extracts (Figure 4). Comparing the two groups of patients, one could not find major differences in their allergen recognition pattern.

Patients 21–23 (group I) and patient 3 (group II) exhibit IgE-binding to a 60 kD band in the mugwort pollen IB (Figs 2b and 3b). Preincubation of pooled serum from patients 21 and 22 with 100 µg of celery extract did not influence IgE-binding to this 60 kD molecule in mugwort pollen extract (Fig. 5; lane 12). Therefore, this particular 60 kD protein seems to represent a

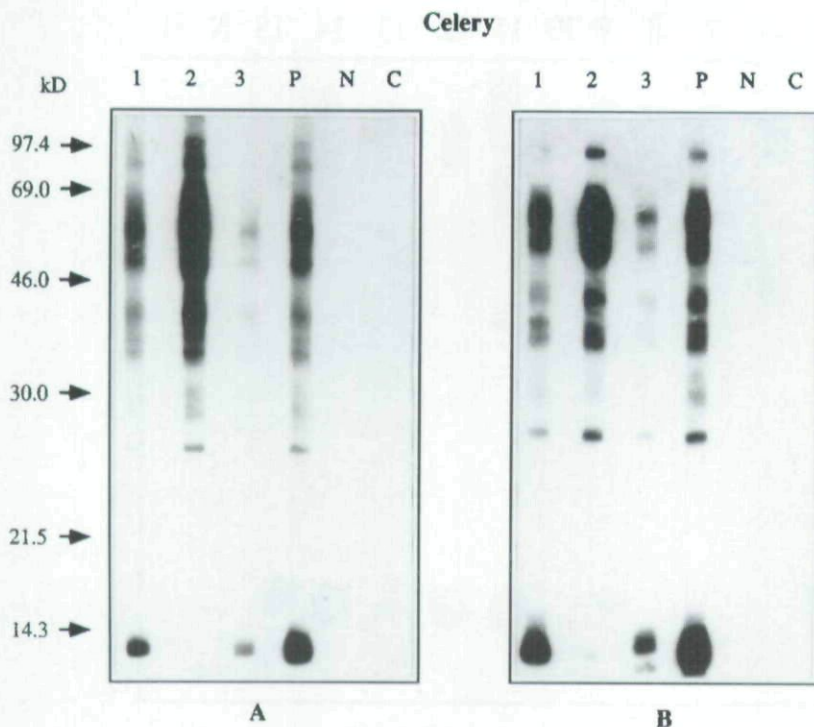


Fig. 4. Comparison of IgE-binding to aqueous extract of celery (panel A) to this extract after periodate treatment (panel B). Lane 1 = patient 14, group II; lane 2 = patient 12, group I; lane 3 = patient 10, group II; lane P = serum pool consisting of patients 15–17, group I; N = normal human serum pool; C = buffer control.

non-cross-reacting, mugwort-specific allergen. Other mugwort pollen-specific allergens are the 28 kD allergen (according to literature possibly Art v 2 [39]) and proteins with 12 kD and lower.

Patient 11 (group I) appears to represent a case of

coincident birch-celery syndrome with mugwort pollen allergy (Fig. 2): serum from patient 11 detects on the one hand a 11 kD band in mugwort pollen extract, which is not found in birch pollen or celery. On the other hand, it is the only serum in group I that binds to the 46 kD to

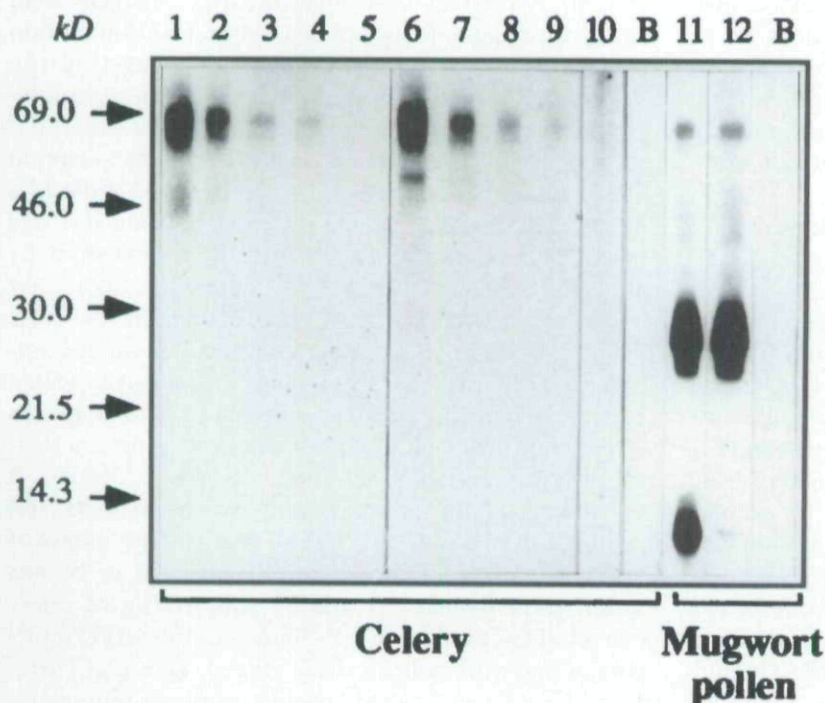


Fig. 5. Autoradiograph of IgE-inhibition experiments performed with extracts of birch pollen, mugwort pollen and celery, respectively. Serum pool A was preincubated with 0 µg, 3 µg, 10 µg, 30 µg, 100 µg, respectively, of birch pollen (lanes 1–5) as well as of mugwort pollen extract (lanes 6–10), nitrocellulose blotted celery proteins were incubated with this solution and immunoblotting was performed. Serum pool B was preincubated with 0 µg and 100 µg, respectively, of celery extract (lanes 11, 12), nitrocellulose blotted mugwort pollen proteins were incubated with this solution and immunoblotting was performed.

60 kD allergens only in extracts of birch pollen and celery. In group II (Fig. 3a, b, c), IgE-binding at this molecular range appeared to be even more diverse: serum from patient 3 reacted with mugwort and birch pollen, serum from patients 4 and 5 reacted with birch pollen, exclusively, and IgE antibodies from patient 9 bound to birch pollen and celery. This reactivity pattern at the 46 kD to 60 kD allergens provides evidence for epitopes on this/these protein(s) which are shared by celery and birch pollen or shared by birch and mugwort pollen. In conclusion, we could not detect one single patient displaying IgE-binding to proteins in celery and mugwort pollen IB, but not to allergens in birch pollen IB.

Taken together these results present us with the following picture: extracts of birch pollen and celery share three groups of molecules; mugwort pollen and celery have two of these molecules in common. Therefore, we propose, from a serological point of view, to extend the mugwort-celery syndrome to the birch-mugwort-celery syndrome.

Acknowledgements

This work was supported by grants SO 6704-MED and 6707-MED of the 'Fonds zur Förderung der Wissenschaftlichen Forschung', Austria.

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