

Celery allergens in patients with positive double-blind placebo-controlled food challenge

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Background: Recently, for the first time, allergy to celery was confirmed by double-blind placebo-controlled food challenge (DBPCFC). Api g 1, Api g 4, cross-reactive carbohydrate determinants (CCD), and a 60 kDa allergen have been described as celery allergens.

Objective: To get insights in IgE responses of patients with a positive DBPCFC to celery tuber (celeriac) compared with patients with a negative challenge test.

Methods: Specific IgE to native and heated celery tuber and to recombinant Api g 1, the major celery allergen, were determined by enzyme allergosorbent test and immunoblotting. IgE binding to Api g 1, Api g 4, and CCD was confirmed by inhibition experiments that used recombinant Api g 1, recombinant Api g 4, pure N-glycans, and extracts of celeriac, lychee fruit, and pollens of birch, mugwort, and timothy grass as inhibitors.

Results: Immunoblotting with sera from 22 patients with a positive DBPCFC to celeriac confirmed the presence of known allergenic structures: The major allergen Api g 1 (16 kDa) was recognized by IgE from 13 of 22 patients (59%). Another major allergen was CCD, determined by IgE reactivity in 12 of 22 patients (55%). Celery profilin, Api g 4, was recognized by IgE from 5 of 22 patients (23%).

Conclusion: Our DBPCFC-positive patients exclusively presented IgE to known celery allergens, although the prevalences were slightly different than were previously reported. No obvious differences were found in patients with positive IgE antibody but negative challenge test. IgE binding to all 3 structures in celeriac extract was inhibited by birch pollen extract, whereas mugwort pollen extract could only inhibit IgE reactivity to Api g 4 and CCD. Inhibition experiments with a purified carbohydrate moiety clearly showed that the IgE epitope mannose-xylose-fucose-glycan (Man α 1-6[Xyl β 1-2]Man β 1-4GlcNAc β 1-4[Fuc α 1-3]GlcNAc) or a closely related structure is present in celeriac extract and is important in patients with clinical allergy to celery. (*J Allergy Clin Immunol* 2000;106:390-9.)

Key words: Celery allergy, double-blind placebo-controlled food challenge, Api g 1, Api g 4, carbohydrate epitopes, cross-reactive carbohydrate determinants, IgE, enzyme allergosorbent test, immunoblotting

Abbreviations used

DBPCFC:	Double-blind placebo-controlled food challenge
SPT:	Skin prick test
CCD:	Cross-reactive carbohydrate determinants
EAST:	Enzyme allergosorbent test
MXF:	Mannose-xylose-fucose-glycan
MM:	Mannose-mannose-glycan
mAb:	Mouse monoclonal antibody
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Celery allergy is known to be associated with birch pollen and mugwort pollen sensitization.¹ The clinical features of celery allergy have been summarized and discussed in a recent publication.² In that article, we presented clinical data of 22 out of 32 patients given challenge tests who had a positive DBPCFC to celery.² This study was undertaken to identify the allergens recognized by IgE from these patients, to compare them with patients with a negative challenge test, and to determine cross-reactivities to pollen allergens.

Four allergenic structures in celery have been characterized so far. A 15-kDa to 16-kDa protein has been identified as a Bet v 1-related celery allergen³⁻⁶ and cloned.⁷

Vallier et al⁸ have shown that the panallergen profilin^{9,10} is involved in celery allergy. Profilins seem to be particularly important in patients allergic to celery with a birch-mugwort-celery sensitization^{4,11,12} and have been identified as IgE-binding and cross-reacting proteins in many kinds of plant foods.^{3,13} Recently we have cloned and expressed celery profilin in *Escherichia coli*.¹⁴

It has been known for many years that some distinct carbohydrate structures containing α 1,3-fucose and β 1,2-xylose attached to proteins via N-glycosidic linkages are highly immunogenic in mammals. These structures can induce specific IgE in atopic subjects and are termed "cross-reactive carbohydrate determinants" (CCD).¹⁵⁻²² When investigating plant food extracts by IgE immunoblotting, recognition of these determinants usually causes staining of multiple bands >45 kDa, indicating the presence of CCD on many plant proteins. Some patients allergic to celery exclusively display CCD-specific IgE.²⁰ However, the clinical significance of CCD-specific IgE is still a matter of controversial discussion.²⁰⁻²⁴ Moreover, 40-kDa to 60-kDa bands cross reacting with IgE against mugwort pollen extract have been described in celery^{12,25} and N-terminal sequenced.²⁶

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In this study, we show that, in general, 3 of the 4 celery allergens are recognized by IgE from DBPCFC-positive patients but with allergen prevalences differing from those previously reported and that no obvious differences were found in subjects who display specific IgE antibodies but exhibit a negative challenge test.

MATERIALS AND METHODS

Human sera

Twenty-two patients selected for this study showed clinical symptoms in DBPCFC to raw celeriac as described (sera No. 1-22).² All patients were sensitized to various kinds of pollens. The sera of another 4 patients with suspected celery allergy did not react in DBPCFC, but they did react in open challenge (sera No. 23-26), and the sera in 4 patients were negative in both blinded and open challenge (sera No. 27-30) (Table I).

Control serum from a nonallergic donor (N) was taken from the serum collection of the Paul-Ehrlich-Institut, Langen, Germany.

Rabbit antiserum

A rabbit polyclonal antiserum raised against celery profilin⁸ was supplied by Dr P. Deviller, Laboratoire de Biochimie, Hôpital Louis Pradel, Lyon, France. A rabbit normal serum (Riedel de Haen, Seelze, Germany) was used as negative control.

Monoclonal antibodies

A mouse monoclonal antibody (mAb) 12B2 directed against Api g 1 was used as hybridoma supernatant; mAb 14G10 against the birch pollen allergen Bet v 6²⁷ served as negative control. They were supplied by Dr K. Fötisch, Paul-Ehrlich-Institut, Langen, Germany.

Protein extracts and recombinant allergens

Celeriac extracts were prepared from raw and heated (30 minutes, 100°C) celery tuber (*Apium graveolens* var *prinz*) as described.²

Extracts from lychee fruit and low-fat milk were prepared as described for apple extract.²⁸ These extracts and commercially available pollen extracts from birch (MAST diagnostica, Reinfeld, Germany), mugwort, and timothy grass (both: Allergopharma, Reinbek, Germany) were used for enzyme allergosorbent test (EAST) inhibition experiments.

Recombinant major celery allergen Api g 1,⁷ recombinant major birch pollen allergen Bet v 1,²⁹ and recombinant birch profilin (Bet v 2)⁹ were purchased from BIOMAY (Linz, Austria). Recombinant major timothy grass pollen allergen Phl p 1³⁰ was provided by Dr A. Petersen, Research Center Borstel, Borstel, Germany, and was used as negative control. Recombinant celery profilin Api g 1¹⁴ was supplied by Dr S. Scheurer, Paul-Ehrlich-Institut, Langen, Germany.

In all extracts, the amount of protein was estimated by a commercial dye binding assay (Pierce, Rockford, Ill). All antigens were stored freeze-dried at -20°C until used.

Total and specific IgE

Diagnostic measurements of allergen-specific IgE were performed with the CAP System FEIA (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

For additional IgE-determinations, recombinant (r) Api g 1 and native and cooked celeriac (100°C, 30 minutes) were coupled to cyanogen bromide-activated filter paper disks (Hycor, Kassel, Germany) at optimized concentrations depending on the source (5 µg per disk of native or heated celeriac protein and 0.25 µg per disk of rApi g 1) according to the method originally described by Ceska and Lundkvist.³¹ EAST was performed with Allergopharma Spez. IgE ELISA according to the instructions of the manufacturer (Allergopharma, Reinbek, Germany).

The results of CAP and EAST graded in arbitrary units (units per milliliter) by the reference systems of the suppliers were expressed in EAST classes 1 through 4 and CAP classes 1 through 6.

Dose-related EAST inhibition studies on native celeriac extract immobilized on paper disks were performed as described.^{20,32} Concentrations of inhibitors and dilutions of sera are given in the legends of corresponding figures.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of recombinant proteins was carried out according to the method of Laemmli³³ performed as previously described.^{32,34} Celeriac extracts were separated by tricine-SDS-PAGE according to Schägger and von Jagow.³⁵ The 16.5% T (T = total concentration [wt/wt] of acrylamide plus bis-acrylamide), 6% C (C = percentage [wt/wt] of the cross-linker relative to T) separating gel was attached to a 10% T, 3% C spacer gel overlaid by a 4% T, 3% C stacking gel. Proteins were reduced by 1,4-dithiothreitol (DTT) (Sigma-Aldrich, Deisenhofen, Germany) and loaded onto the gel at concentrations of 26 µg celeriac extract/cm and 0.5 µg recombinant protein/cm.

Immunoblotting

The proteins were transferred to nitrocellulose membranes by semidry blotting³⁶ as described³⁴ and blocked twice in 0.05 mol/L TRIS/hydrogen chloride buffer (pH 7.4), which contains 0.15 mol/L sodium chloride and 0.3% polysorbate 20 (TBST buffer). All sera and immunoreagents were diluted in TBST buffer, which contains 0.1% BSA. Cut nitrocellulose strips were probed with human sera overnight, which were applied in a final dilution of 1:6.7 on blots with celeriac extract and 1:10 with recombinant proteins. Immunostaining of bound IgE antibodies was performed with alkaline phosphatase conjugated mouse anti-human IgE (1:1000, 4 hours) (PharMingen, San Diego, Calif.) and an alkaline phosphatase-staining kit (Biorad, Munich, Germany). Rabbit sera (1:10000, 1 hour) and mAb (hybridoma supernatants 1:5, 1 hour) were detected as described.³⁷

Immunoblot inhibitions were carried out by simultaneous incubation of patients' sera (diluted 1:10) with the following inhibitors or buffer as a control: 100 µg celeriac extract protein or 100 µg ovalbumin or 100 µg low-fat milk extract as control for nonspecific inhibition or 15 µg recombinant protein or 15 µg of different glycopeptides, respectively. Bound IgE was detected as above.

N-glycans

N-linked glycopeptides with the glycan structure Man α 1-6(Xyl β 1-2)Man β 1-4GlcNAc β 1-4(Fuc α 1-3)GlcNAc (mannose-xylose-fucose-glycan; MXF) and only 2 to 4 amino acid residues were prepared from pineapple stem bromelain by Professor F. Altmann, Vienna, Austria, as previously described.³⁸ Briefly, glycopeptide was isolated from extensively digested bromelain by gel filtration and ion-exchange chromatography. The purity of the glycopeptides was checked by matrix-assisted laser-desorption ionization time-of-flight mass spectrometry²⁰; amino acids and monosaccharides were analyzed.³⁹⁻⁴¹ A glycopeptide with the common pentasaccharide core Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc (mannose-mannose-glycan; MM) prepared from bovine fibrin was used as control.^{20,42} Moreover, the purity of the samples was controlled by SDS-PAGE,³³ followed by silver staining with 10 µg of the bromelain and fibrin glycopeptide per lane. No contaminations by protein or larger peptides were detectable in the glycan preparations (data not shown). The glycopeptides were stored freeze-dried at -20°C until used.

TABLE I. Summary of patients' data

Patient No.	Clinical symptoms to celery	SPT celery	CAP extracts			
		Native	Celery	Mugwort	Timothy	Birch
DBPCFC positive						
1-22	Positive results:		77%	73%	67%	91%
1	OAS	++	3	2	5	5
2	OAS	++	0	0	5	4
3	OAS	++	3	0	0	4
4	OAS	++	3	2	6	6
5	F, RC	+	1	2	1	4
6	OAS	+	0	1	4	3
7	OAS	++	0	4	2	3
8	GIT	++	2	0	0	3
9	U, RC, Dy	+++	0	4	0	0
10	OAS	+/-	2	2	4	4
11	OAS	++	3	0	0	5
12	OAS	++	3	1	2	6
13	OAS	++	3	2	nd	4
14	OAS, AE	++	2	3	3	4
15	Dy, Nau	+++	3	3	6	6
16	AE	+	2	3	6	5
17	OAS	+	4	2	5	4
18	U	+	2	2	3	2
19	Nau, Em	++	0	2	0	0
20	OAS	++	6	3	6	6
21	OAS, Dy, RC	++	3	0	0	4
22	RC, Inh	++	3	0	0	2
DBPCFC negative, open challenge positive						
23	OAS	++	3	2	3	4
24	OAS	+++	2	2	2	4
25	OAS	++	3	0	3	6
26	OAS	++	2	3	3	4
DBPCFC negative, open challenge negative						
27	None	+++	2	5	4	4
28	None	+/-	0	2	2	0
29	None	0	0	0	0	3
30	None	++	0	3	0	0

OAS, Oral allergy syndrome; F, flush; RC, rhinoconjunctivitis; GIT, gastrointestinal pain; U, urticaria; Dy, dyspnea; AE, angioedema; Nau, nausea; Em, emesis; Inh, symptom occurs after inhalation; nd, not done; Al, almond; Ap, apple; As, asparagus; At, apricot; Ba, banana; Ca, carrot; Cu, curry; Fi, fish; Ha, hazelnut; Ki, kiwi; Ko, kohlrabi; Ma, mango; Nu, nuts; Pa, parsley; Pe, pea; Pf, pomaceous fruits (as apple, pear, quince); Ph, peach; Pn, peanut; Po, potato; Pp, paprika; Pr, pear; Sa, salads; Sf, sunflower-seed; Sh, shrimp; So, soy bean; To, tomato.

*Values in parentheses indicate weak binding to immunoblots and are not included in the sum of positive results.

RESULTS

Allergen profile of patients with positive DBPCFC determined by CAP, EAST, and immunoblotting

A properly performed DBPCFC is the only conclusive confirmation of the diagnosis of a food allergy.⁴³ Sera from 22 responders to our DBPCFC with celeriac were selected for this study.² The symptoms of the patients with confirmed celery allergy are summarized in Table I. All these patients (1 weakly) were positive in skin prick test (SPT) with our self-prepared celeriac extract.

CAP

Sera from 17 of 22 patients (77%) were positive in the cel-

ery CAP (classes 1-6). All of the 22 patients either had a CAP positive for mugwort (73%), birch pollen (91%), or both (64%). Twenty patients had a positive IgE-dependent sensitization to Bet v 1 with CAP classes ranging from 2 to 6; 6 patients were also sensitized to Bet v 2. Sera from 14 of 21 patients (67%) were positive to grass pollen CAP (Table I).

EAST

For the determination of specific IgE against extract from native and cooked celeriac and against rApi g 1, solid phases were prepared in our laboratory. IgE antibodies from 15 patients resulted in EAST classes from 1 to 3 on native celeriac extract paper disks. Antibodies against the heated protein were found in the sera of only 6 patients. IgE specific for rApi g 1 was detected in 13 sera (Table I).

CAP allergens		EAST celery			Blot celeriac-extract			Blot allergens		Other food allergies (due to anamnesis)
Bet v 1	Bet v 2	Native	Cooked	rApi g 1	14 kDa	16 kDa	CCD	rApi g 4	rApi g 1	
91%	27%	68%	27%	59%	23%	45%	55%	23%	59%	
5	3	3	0	3	+	+++	++	++	+++	Nu, Ha, Ca, Ap
5	0	0	0	0	0	0	0	0	(+)	Ap, Ca, Po, So
5	0	2	0	4	0	++	0	0	++	Ap, Pr, Ca, Ha
6	3	3	0	3	+	++	++	+++	++	Po, Ca, Ha, Ap, Ba, Pp
4	0	0	0	0	0	0	++	0	0	None
3	2	0	0	0	+	0	+	++	0	To, Ma
3	0	0	0	0	0	0	0	0	0	As, Ha
3	0	0	0	2	0	(+)	0	0	+	Ap, Ph, Ha
0	0	0	0	0	0	0	0	0	0	None
4	0	1	1	0	0	0	+++	0	0	Ap, At, Ha
4	0	2	0	3	0	+	0	0	++	Ca
5	0	2	0	3	0	(+)	++	0	+	Ca, Ha, Cu
4	0	3	0	3	0	+	++	0	++	None
5	0	1	0	2	0	(+)	0	0	+	None
4	4	2	1	3	++	+	++	+++	+	Ap, Ki, Nu, Ba
5	3	2	1	0	++	0	+	+++	0	None
5	0	3	2	2	0	+++	++	0	+++	Sa, Fi, Pa, Ca, Ko
2	0	1	1	0	0	0	+	0	0	Ap, Ca, Nu
0	0	0	0	0	0	0	0	0	0	None
6	1	3	2	3	0	++	+++	0	++	Ca
4	0	2	0	2	0	+	0	0	+	Ap
2	0	2	0	2	0	++	0	0	+	Pa, Ca, Po, As
4	1	2	1	2	+	+	++	+	+	Ca, Po, Pe, Pn, Al, Pf
4	0	1	0	2	0	(+)	(+)	0	+	Nu, Ap, Ca, Po
6	0	2	0	0	0	0	0	0	0	Ap, Ha
4	2	1	0	1	(+)	+	(+)	+	+	Unknown
0	5	2	2	0	+	0	++	++	0	Ca, Sf
0	0	0	0	0	0	0	0	0	0	None
3	0	0	0	0	0	0	0	0	0	Sh
0	0	0	0	0	0	0	0	0	0	None

Immunoblotting

Immunoblotting with celeriac extract was performed to receive a general view over the entire allergen spectrum (Fig 1).

An mAb against Api g 1 recognized a strong single band at 16 kDa (lane A). Blots probed with mAb against Bet v 6 as control remained blank (lane A_N).

The polyclonal rabbit anti-celery profilin antiserum showed an intensive band at 14 kDa (lane P), whereas a weaker band about 25 kDa also occurred in the controls for unspecific binding of both rabbit (lane P_N) and human normal serum (lane N).

Strong IgE binding to one or more proteins from native celeriac extract on immunoblots was observed

with 16 of 22 human sera. In addition, 2 sera (lanes 2, 4) had considerably weaker detectable IgE binding, and 4 sera did not recognize any bands other than the nonspecific binding control (N) (ie, especially proteins of about 25 kDa to 30 kDa and around 40 kDa). Sera of 13 patients showed bands at 16 kDa of which 3 sera (lanes 8, 12, and 14) showed weak bands poorly visible in the figure as a result of technical problems. Bands were detected at 14 kDa in the sera of 5 patients, with 1 serum (lane 1) that faded after drying but that is better preserved on an immunoblot strip from the inhibition experiment (see Fig 3, B, serum No. 1, lane Ø = no inhibitor). IgE to multiple bands >45 kDa were detected in 12 patients.

Immunoblotting with rApi g 1 and rApi g 4 and the same sera showed higher sensitivity. The 13 sera recog-

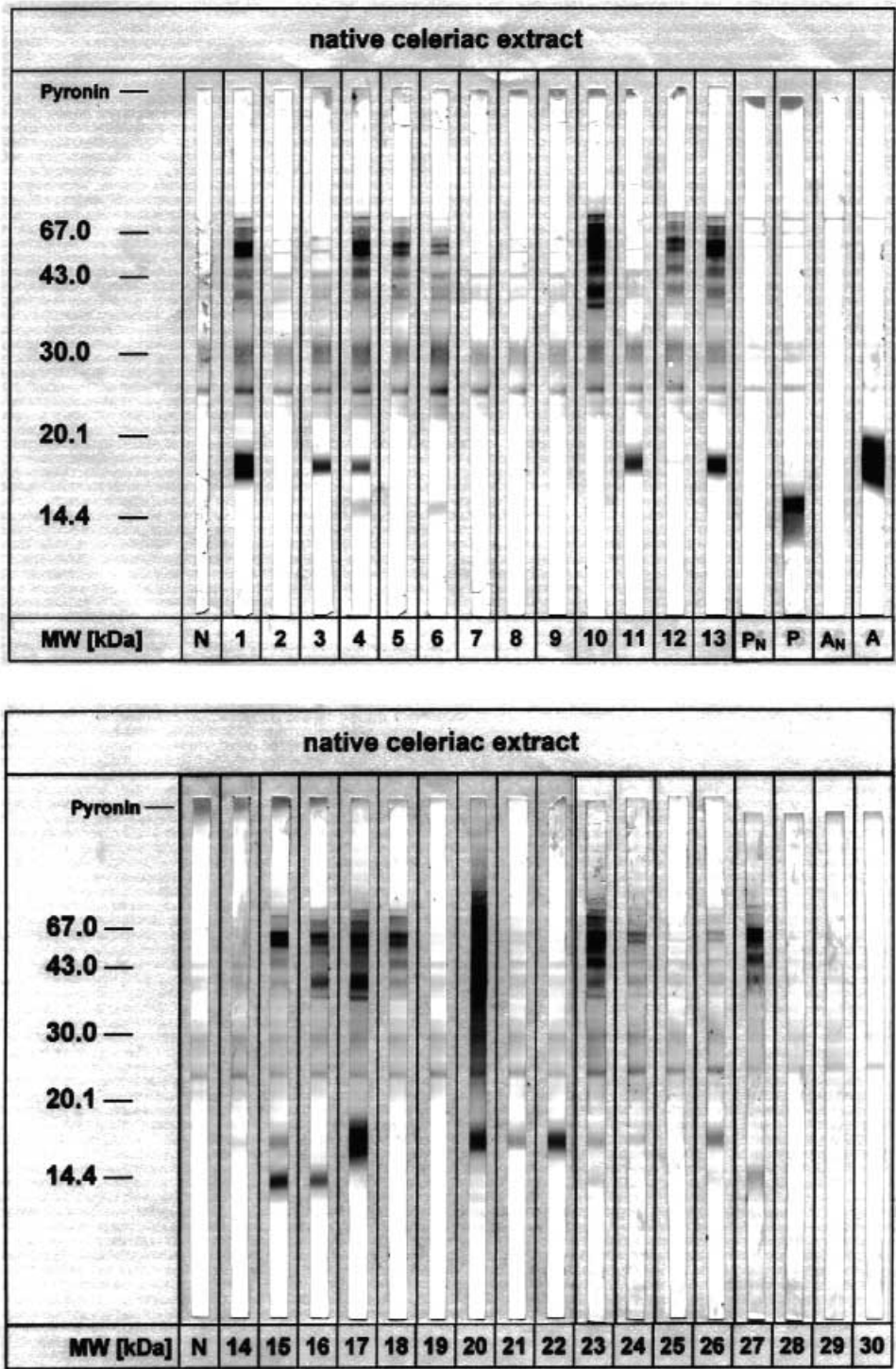


FIG 1. Immunoblot of patients' sera (DBPCFC positive: 1-22; nonresponders with a positive open challenge: 23-26; negative open challenge: 27-30). Nonallergic human control serum (*N*), polyclonal rabbit antiserum against celery-profilin (*P*), control rabbit normal serum (*P_N*), mAb against Api g 1 (*A*), and mAb against Bet v 6 as control (*A_N*) tested with native celeriac extract.

nizing rApi g 1 (Fig 2, A) were identical to those that reacted with the 16-kDa protein on the celeriac extract blots (the 3 sera with low IgE included). They all showed elevated specific IgE in the EAST with rApi g 1 (classes ≥ 2).

Besides this, they were all sensitized to Bet v 1 (Table I). IgE against rApi g 4 was clearly present in 5 patients (Fig 2, B), which confirms the weaker results of the extract blots. They were all positive in CAP with Bet v 2.

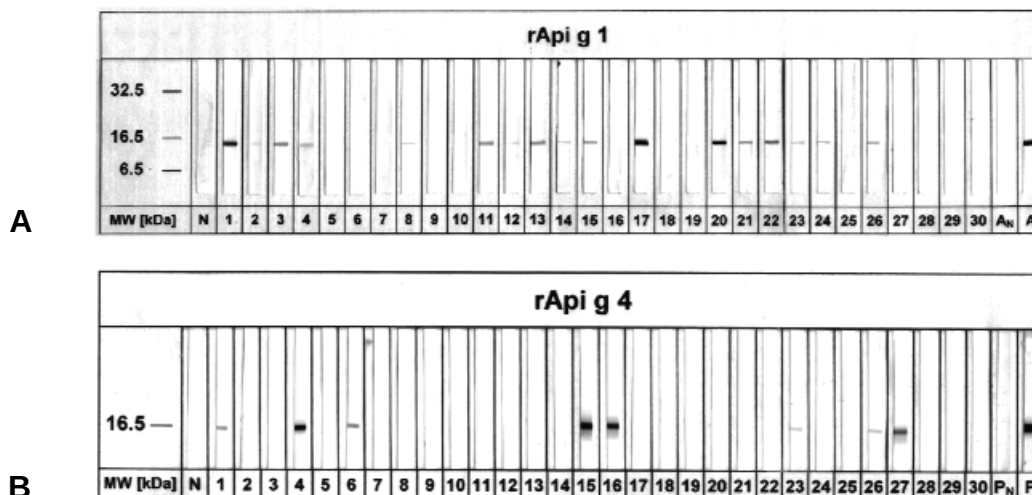


FIG 2. Immunoblot of patients' sera (DBPCFC positive: 1-22; nonresponders with a positive open challenge: 23-26; negative open challenge: 27-30), nonallergic human control serum (*N*) tested with rApi g 1: mAb against Api g 1 (*A*) and mAb against Bet v 6 as control (*A_N*) (*A*) and tested with rApi g 4: polyclonal rabbit antiserum against celery-profilin (*P*) and control rabbit normal serum (*P_N*) (*B*).

IgE reactivity of patients with negative DBPCFC

Eight patients with suspected celery allergy were not responding to DBPCFC² (No. 23-30; Table I, Figs 1 and 2). Four of the patients (No. 23-26) showed symptoms of oral allergy syndrome in an open challenge to celeriac. No obvious differences in their sensitization patterns were observed compared with patients with positive DBPCFC, as IgE antibodies against Api g 1, Api g 4, and CCD were detected. Four patients (No. 27-30) did not show symptoms in the open challenge. Only one serum (No. 27) had a positive celery CAP and EAST. It showed specific IgE for Api g 4 and CCD on immunoblots, further CAP class 5 to Bet v 2. Among the other 3 patients, specific IgE was detected against pollen of mugwort, timothy grass, or birch, but not against celery.

Recombinant and natural celery allergens display similar IgE-binding activity

Blot-inhibition experiments were performed to confirm the specificity of the detection and to demonstrate epitope identity of natural and recombinant celery allergens.

In one experiment, rApi g 1 was blotted on nitrocellulose membranes and incubated with patient serum No. 15 (recognizing all of the 3 previously described allergens) and different inhibitors on each strip, respectively. Recombinant Api g 1 and native celeriac extract as inhibitors quenched IgE binding to rApi g 1 on solid phase completely, whereas rApi g 4 and ovalbumin caused no inhibition (results not shown). With the same serum and blot strips loaded with rApi g 4, specific IgE binding was inhibited by rApi g 4 and by extract from raw celeriac; rApi g 1 and ovalbumin did not inhibit IgE binding (results not shown).

The reverse experiment performed on blots of native celeriac extract showed that IgE binding to the natural

Api g 1 and Api g 4 could specifically be inhibited only by the corresponding recombinant allergens, respectively (Fig 3, A). Binding to high-molecular-weight components was not affected by the recombinant allergens.

Glycopeptide preparations without detectable protein strongly inhibit IgE binding to celery extract

IgE binding to high-molecular-weight components was suspected to be caused by CCD on glycoproteins. Inhibition experiments with highly pure N-linked glycopeptides MXF and MM²⁰ were carried out to prove this hypothesis.

Exemplary for 2 sera, No. 1 and No. 4, the blots are depicted in Fig 3, B. The bromelain glycopeptide MXF as inhibitor quenched IgE binding to high-molecular-weight components completely (lane B), and specifically because no other bands were affected. Celeriac extract as inhibitor erased any binding patterns to the blot (lane C), whereas the fibrin glycopeptide MM (lane F) and ovalbumin (lane O) did not show any influence on IgE binding.

All 3 celery allergens can bind a high amount of celery-specific IgE

The contribution of different celery components to the total IgE-binding capacity of the whole extract was tested by dose-dependent EAST inhibitions. Serial dilutions of inhibitors were prepared from native celeriac extract, bromelain, or fibrin glycopeptides (MXF or MM), rApi g 1, rApi g 4, rPhl p 1, and milk protein, respectively (Fig 4). Sera were selected from patients with characteristic differences in sensitization profiles (see legend of Fig 4). For all sera tested, specific inhibition of IgE binding >90% was obtained with native celeriac extract. With serum No. 3, a complete inhibition was achieved with rApi g 1 at an inhibitor concentration about tenfold lower

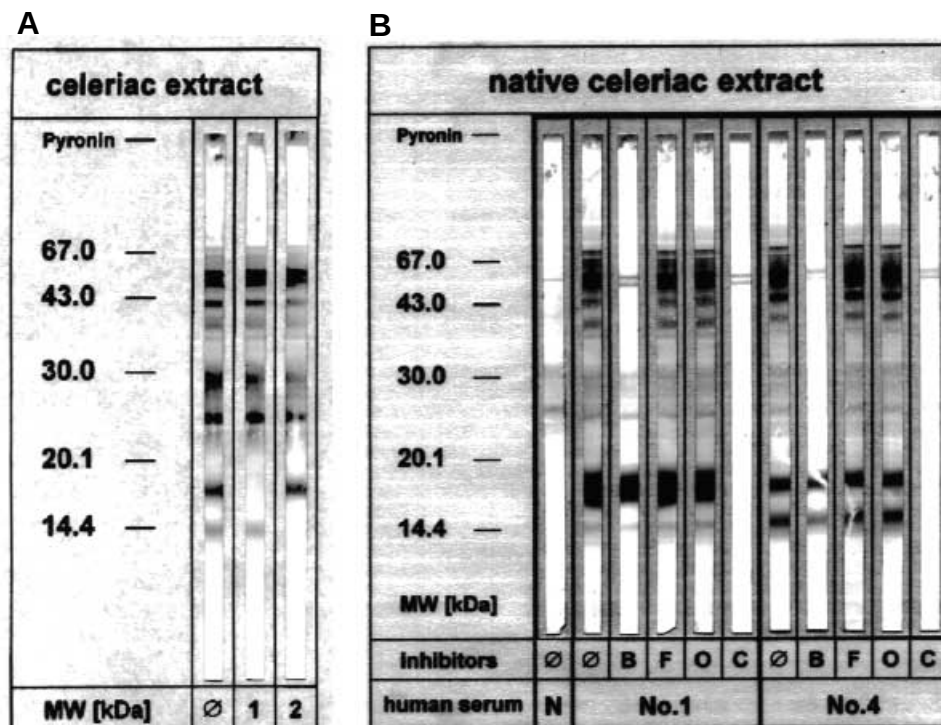


FIG 3. Immunoblot inhibition with native celeriac extract tested with patient's serum No. 4 (A) and patients' sera No. 1 and No. 4 (B). Lanes: No inhibitor ($\emptyset$); inhibitors: rApi g 1 (1), rApi g 4 (2), bromelain-glycopeptide MXF (B), fibrin-glycopeptide MM (F), ovalbumin (O), native celeriac extract (C).

compared with celeriac extract. Recombinant Api g 4, the bromelain glycopeptide MXF, and rPhl p 1 showed no inhibitory effects. With serum No. 10, MXF as inhibitor reached 97% inhibition, whereas the fibrin glycopeptide MM, rApi g 1, and rPhl p 1 showed no influence at all. Because in our group of patients no serum had IgE exclusively against Api g 4, serum No. 16 with IgE against Api g 4 and CCD was used. IgE binding of this patient was about equally inhibited by rApi g 4 and MXF with a maximal inhibition of 42% and 37%, respectively. Interestingly, at the maximal inhibitor concentration of 100 μ g/ml, MM led to 35% inhibition. Recombinant Api g 1 and Phl p 1 did not quench IgE binding (Fig 4, A).

Moreover, cross-inhibitions with extracts of birch pollen, mugwort pollen, timothy grass pollen, lychee fruit, and cooked celeriac were carried out to show the ubiquitous occurrence of CCD and profilin. For serum No. 3 with an IgE response restricted to Api g 1, complete inhibition (>90%) was achieved even with low inhibitor concentrations of birch pollen extract, because 0.03 μ g/mL led to 50% inhibition. None of the other 4 extracts inhibited IgE binding. With serum No. 10, birch pollen extract reached 100% inhibition, lychee fruit extract about 90%, and the other 3 extracts of mugwort, timothy grass, and cooked celeriac around 75% inhibition each. Maximal inhibition of IgE binding of serum No. 16 ranged from 83% to 94% with all 5 extracts (Fig 4, B).

DISCUSSION

Here we present the first in vitro data of 22 patients whose celery allergy had been confirmed by DBPCFC² (Table I). Specific IgE in celery CAP was present in only 17 patients with a positive DBPCFC, indicating a relatively low sensitivity of the CAP. Four patients were IgE negative to celery in all serologic assays. The reason for these negative results remains unknown. However, we can exclude allergen extract quality because all participants presented a positive SPT to the extract used in serologic assays. All of the 22 patients were positive in the CAP either for mugwort pollen (73%) or birch pollen (91%); for the latter all these 20 patients were sensitized to Bet v 1 and 6 of them also to Bet v 2 (23%).

IgE to known celery allergens was exclusively present in our DBPCFC-positive patients, although the prevalences were slightly different than were previously reported: IgE against Api g 1 was less abundant with 59% in our patient group compared with (1) 80% of 30 patients with pollen allergy reporting allergy of immediate type after ingestion of raw celeriac from a study of Jankiewicz et al,¹¹ and (2) 74% in a group of 23 patients with type I celery-allergy from a study of Bauer et al.¹² The sensitization rate to profilin remained largely unchanged with 23% in our group related to (1) 23% and (2) 30%, with the first corresponding well to the occur-

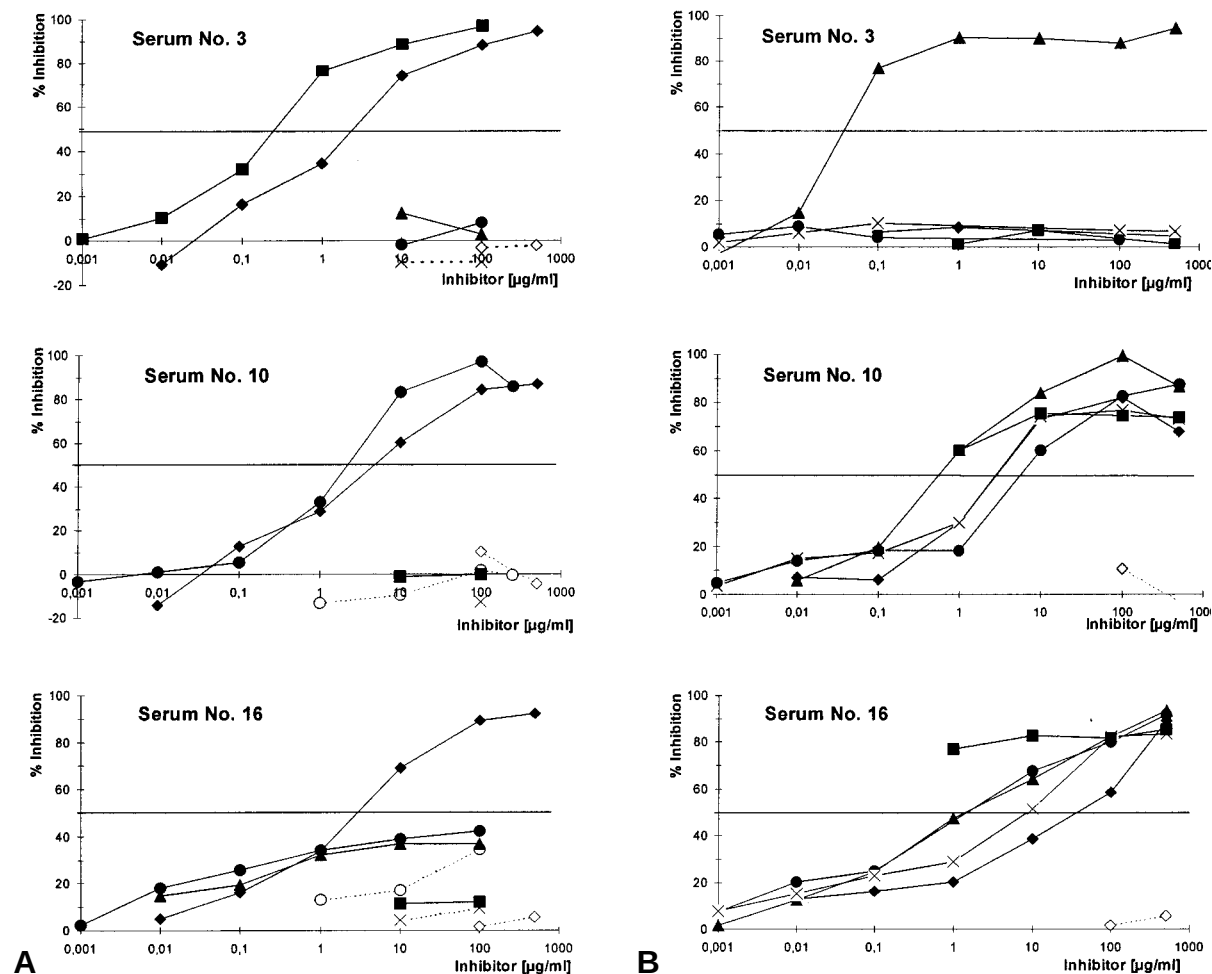


FIG 4. Dose-related EAST-inhibition with patients' sera No. 3 monosensitized to Api g 1 (final serum dilution 1:5), No. 10 monosensitized to CCD (1:4), No. 16 sensitized to Api g 4 and CCD (1:5), and native celeriac extract immobilized on paper disks. Inhibitor concentrations are given on the abscissa. **A**, Tested with inhibitors; native celeriac extract (closed diamond), Api g 1 (closed box), Api g 4 (closed triangle), MXF (closed circle), MM (open circle), milk extract (open diamond), Phl p 1 (x). **B**, Tested with inhibitors: birch pollen extract (closed triangle), mugwort pollen extract (x), timothy grass pollen extract (closed box), lychee fruit extract (closed circle), cooked celeriac extract (closed diamond), milk extract (open diamond).

rence of 20% in all patients with pollen allergy described by Valenta et al.¹⁰ IgE to CCD was more abundant with 55% in our group and thus identified as another major allergen versus (1) 27%.¹¹

The sensitization pattern to Api g 1 and Api g 4 was confirmed by immunoblotting with the recombinant allergens, which showed a higher sensitivity than did the natural allergens. Thus 3 sera with low IgE binding on celeriac blots could clearly be detected with rApi g 1 by both immunoblot and EAST. All patients with IgE against Api g 1 were also sensitized to Bet v 1, and the 5 patients positive for Api g 4 were also positive in the CAP with Bet v 2. Generally, 16 of 22 patients (73%) were IgE positive in the celery immunoblot. Six patients, including 1 participant who had exclusively respiratory allergy to celery, were monosensitized to Api g 1, 3 to CCD, and none to profilin alone.

The carbohydrate epitope recognized by 12 patients with celery allergy on celeriac immunoblots (Fig 1) was identified as an N-glycan containing α 1,3-fucose and β 1,2-xylose (MXF). However, in patients with a weaker response to CCD, the control glycan MM was able to inhibit the celery EAST as much as 35% (Fig 4, A), indicating that the mannose core could be involved in IgE binding. IgE binding to the short peptide sequences present in the N-glycans cannot be completely excluded, but is unlikely because restricted sequence variation is possible in N-glycosylation sites. Bearing in mind the controversial discussion on the clinical relevance of IgE against CCD,²⁰⁻²⁴ it is remarkable that CCD are the unique structure in celery recognized by IgE from 3 patients with confirmed allergy to this vegetable.

Among the group of patients with a negative DBPCFC but a positive open challenge, symptoms occurred when

chewing 5 g of celery, so the amounts of celery were lower compared with the last 2 doses in DBPCFC.² IgE antibodies of these patients were directed against the same allergens as in DBPCFC-positive patients, indicating that these patients might also be allergic to celery and that the negative blinded challenge might be related to inherent problems of the challenge procedure. Four patients with a negative DBPCFC and a negative open challenge² tolerated 20 g of celery without having symptoms, though 2 of them (and 1 slightly) were positive in SPT. Interestingly, serum No. 27 was highly reactive to profilin (CAP class 5 to Bet v 2) and showed CCD-specific IgE on immunoblots. With the other 3 sera, no celery-specific IgE was detectable in CAP, EAST, and immunoblotting. The positive SPT in 2 patients serologically negative might be explained by cross-reacting mugwort or timothy grass pollen-specific IgE. Moreover, we conclude that the IgE response to profilin and CCD in patient No. 27 is clinically insignificant.

EAST inhibition experiments were undertaken to determine the proportion of IgE directed against specific allergenic structures in celery extract. Sera representative for the 3 major binding patterns were selected according to immunoblotting and EAST experiments. Taken together, these experiments confirmed the allergen profiles of the selected patients and demonstrated that all 3 allergen structures of this vegetable can bind a relevant proportion of celery-specific IgE (Fig 4, A).

Cross-inhibitions with extracts of birch pollen, mugwort pollen, timothy grass pollen, and lychee demonstrated the ubiquitous occurrence of CCD and profilin. Further, extract of cooked celeriac (100°C, 30 minutes) was included to examine the heat-resistance of celery allergens (Fig 4, B). As expected, with serum No. 3, which was exclusively positive to the major allergen Api g 1, complete inhibition was obtained with birch pollen extract as inhibitor because Bet v 1 is thought to initiate sensitization to Api g 1.^{7,44} Thus this major allergenic structure is exclusively shared with birch pollen, but has no homologue in mugwort pollen, grass pollen, lychee fruit, and cooked celeriac, as shown by the lack of inhibition with these extracts. Moreover, these data confirm that Api g 1 is a heat-labile allergenic component in celery. In contrast, CCD and homologues of Api g 4 are also present in tree pollen and pollens from weeds, Gramineae, and other plant families. This high presence is reflected by the high maximal inhibition values (at least 70%) with all inhibitor extracts (birch pollen, mugwort pollen, grass pollen, and lychee fruit) that were achieved with the other 2 sera containing IgE against profilin or CCD. These data also show that sera from our patients did not recognize a cross-reactive structure exclusively shared by mugwort and celery.

High inhibitions with extract of cooked celeriac were obtained with the latter 2 sera, demonstrating that at least CCD represents a thermostable component of celeriac extract. This finding confirms earlier studies examining the thermostable allergenic compounds of celeriac.^{11,20,32}

Because our patients with celery allergy were all sensitized against cross-reactive allergens, the clinical relevance of these cross-reactions has to be discussed. All the other foods that the 4 food-allergic patients monosensitized to

Api g 1 claimed to be "allergic" to contained allergens belonging to the Bet v 1-family. In contrast, the spectrum of "other food allergies" is enlarged when sensitization to profilin or CCD is involved. These findings indicate that cross-reactions to certain allergen sources can be clinically relevant in some patients but may be irrelevant in others (Table I).

Five patients with positive DBPCFC were negative in celery CAP and EAST. Two of them (No. 9 and 19) did not say they had other food allergies, were negative with any celery-specific tests performed, and exhibited only mugwort pollen-specific IgE with CAP. This finding could perhaps give rise to speculation about an unknown cross-reactive structure exclusively shared by mugwort and celery (Table I).

Heiss et al²⁵ identified an allergen of approximately 60 kDa in pollens, fruits, and vegetables such as celery. Recently, Api g 5 was included in the WHO/IUIS allergen list as a protein of 55 kDa to 58 kDa (P81943).²⁶ Whether both of them are identical or similar remains unclear. They possibly are involved in CCD-reactivity because we obtained IgE binding in the same molecular weight region with our sera.

However, contrasting data on the clinical relevance of CCD still exist.^{20-24,45-47} In our study, 3 DBPCFC-positive patients were monosensitized to CCD. Our current view is that depending on the structure of the CCD-containing glycoproteins, CCD are indeed important epitopes for IgE and may be clinically relevant allergens in certain patients and irrelevant in others.

Further work will be focused on the identification of allergens recognized by IgE of patients with confirmed allergy to cooked celery.

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