Carrot allergy: Double-blinded, placebo-controlled food challenge and identification of allergens

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Background: Allergic reactions to carrot affect up to 25% of food-allergic subjects. Clinical manifestations of carrot allergy and IgE responses to carrot proteins, however, have never been studied in subjects with carrot allergy confirmed by means of double-blinded, placebo-controlled food challenge (DBPCFC).
Objective: The purposes of this investigation were to confirm clinically relevant sensitizations to carrot by means of DBPCFC, to validate current diagnostic methods, and to identify IgE-reactive carrot proteins in patients with true allergy.
Methods: DBPCFCs were performed in 26 subjects with histories of allergic reactions to carrot. Patients underwent skin prick tests with carrot extract, fresh carrot, and various pollen extracts. Specific IgE to carrot, celery, birch, and mugwort pollen and to rBet v 1, rBet v 2, and rBet v 6 were measured through use of the CAP method. Carrot allergens were identified by means of immunoblotting and blotting inhibition.
Results: Twenty of 26 patients had positive DBPCFC results. The sensitivity of the determination of carrot-specific IgE antibodies through use of the CAP method (≥0.7 kU/L) was 90%, the sensitivity for skin prick testing with commercial extracts was 26%, and the sensitivity for prick-to-prick tests with raw carrot was 100%. The Bet v 1–related major carrot allergen Dau c 1 was recognized by IgE from 85% of patients; 45% were sensitized to cross-reactive carbohydrate determinants and 20% to carrot profilin. In 1 subject, a Bet v 6–related carrot allergen was recognized. In 4 patients, IgE binding to Dau c 1 was not inhibited or was weakly inhibited by rBet v 1 or birch pollen extract.
Conclusion: This study confirmed the allergenicity of carrot by means of DBPCFC. DBPCFC-positive patients had exclusively specific IgE antibodies to birch pollen–related carrot allergens, Dau c 1 being the major allergen. The lack of inhibition of IgE binding to Dau c 1 by birch allergens in a subgroup of patients might indicate an secondary immune response to new epitopes on the food allergen that are not cross-reactive with Bet v 1. (J Allergy Clin Immunol 2001;108:301-7.)

Key words: *Food allergy; double-blinded, placebo-controlled food challenge; DBPCFC; carrot allergy; Dau c 1; carrot profilin; carbohydrate epitopes; cross-reactive carbohydrate determinants; immunoblotting*

Vegetables belonging to the Apiaceae family are frequent causes of pollen-related food allergy, particularly in European countries.[1-4] Research has thus far focused mainly on celery (*Apium graveolens*) and has neglected carrot (*Daucus carota*); the latter is an important allergenic food, inasmuch as up to 25% of food-allergic subjects in Central Europe have carrot allergy.[5] Carrot allergy is associated with a sensitization to celery, spices, mugwort, and birch pollen; this is referred to as *celery-carrot-birch-mugwort-spice syndrome*.[6,7] This clustering of hypersensitivity to botanically related and unrelated families is explained by cross-reactions between homologous allergens present in these plant materials.[7-9] In tree pollen–allergic patients, food allergy is mediated mainly by cross-reactivity of Bet v 1, the major allergen of birch pollen, and its homologous proteins in foods of plant origin belonging to the pathogenesis-related protein-10 family.[10] Recently, the allergen Dau c 1 from carrot has been identified as a Bet v 1 homologous protein.[11]

In addition, profilin, first identified as the birch pollen allergen Bet v 2, is now recognized as a ubiquitous cross-reacting plant allergen that is also present in carrots.[12,13] Further allergenic structures have not yet been identified in carrots.

The purposes of this study were (1) to perform double-blinded, placebo-controlled food challenges (DBPCFCs) in subjects with histories of allergic reaction to carrot, (2) to validate current diagnostic procedures in subjects with confirmed carrot allergy, (3) to identify the allergens recognized by IgE from these patients, and (4) to determine cross-reactivities to pollen allergens.

METHODS

Patients

Patients with histories of allergic reactions to carrot were recruited at the Allergy Unit of University Hospital Zürich from September 1999 to February 2000. Pregnancy, history of a severe, life-threatening anaphylactic reaction after carrot consumption, significant concurrent disease, and medication with glucocorticosteroids, H_1-receptor antagonists, angiotensin-converting enzyme inhibitor, or β-blocking agents were exclusion criteria. Symptoms and time course of pollinosis were assessed in each patient.

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Supported by the Food Agricultural Industrial Research (FAIR) service (DGXII) of the European Commission, CT97-3224, and by the Swiss Federal Office for Education and Science, BBW 97.0334.

Received for publication February 12, 2001; revised April 10, 2001; accepted for publication April 11, 2001.

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0091-6749/2001 $35.00 + 0 **1/81/116430**
doi:10.1067/mai.2001.116430

Abbreviations used
CCD: Cross-reactive carbohydrate determinant
DBPCFC: Double-blinded, placebo-controlled food challenge
MM: Mannose-mannose glycan
MUXF^3: Mannose-xylose-fucose glycan
OAS: Oral allergy syndrome
SE: Sensitivity
SP: Specificity
SPT: Skin prick test

Ethical considerations

The study was reviewed and approved by the local ethics committee. All subjects provided written informed consent before enrollment in the study.

Skin tests

Skin prick tests (SPTs) were performed on the flexor aspect of each patient's forearm with a standardized prick needle (Stallerpoint, Stallergènes, Antony Cedex, France). Histamine dihydrochloride (10 mg/mL) was used as a positive control, and the glycerol-containing diluent of the prick solution (Soluprick, ALK, Hørsholm, Denmark) was used as a negative control. Patients were tested with pollen extracts from alder, birch, hazel, ash, grass, rye, and mugwort (Soluprick) as well as with carrot extracts (Stallergènes). Reaction to raw native carrot was tested through use of the prick-to-prick technique.[1,2] Reactions were recorded after 15 minutes. A wheal with a diameter of at least 3 mm was considered positive.[14]

In vitro diagnosis

Specific IgE to carrot, celery, birch pollen, recombinant (r) Bet v 1, rBet v 2, rBet v 6, and mugwort pollen were measured through use of the CAP FEIA system (Pharmacia & Upjohn, Uppsala, Sweden). ImmunoCaps coupled with rBet v 6 were produced and kindly provided by Dr J. Lidholm of Pharmacia & Upjohn.[15] Specific IgE of at least 0.7 kU/L (class 2) were considered positive.

DBPCFC with carrot

DBPCFC was performed through use of the 2-step spit (local mucosal challenge) and swallow procedure, as described previously for celery.[4] Two different drinks, identical in color, texture, and taste, were prepared. The active drink contained 70 g of carrot, 100 g of cooked pumpkin, 20 g of cream, 60 g of yogurt without flavoring, 35 g of water, 2 pinches of saffron, and salt and pepper, which were mixed in a blender. With the exception of carrot, the placebo drink and the active drink contained the same ingredients. Apart from carrot, all ingredients were known to be tolerated by all patients.

Open provocation with carrot

In each patient with a negative DBPCFC result, an open challenge was performed. The patient had to chew 5 g of raw carrot and spit it out; if no reaction was experienced, he or she chewed and swallowed 5 g, then 10 g, and then 20 g.

Protein extracts and recombinant allergens

Carrot extract was prepared from raw carrot, according to a method described elsewhere for celery.[4] Skim milk extract was prepared as described elsewhere.[16]

Birch pollen extract was obtained from MAST Diagnostica (Reinfeld, Germany) and mugwort pollen extract from Allergopharma (Reinbeck, Germany). rApi g 1, rBet v 1, and rBet v 2 were purchased from BIOMAY (Linz, Austria); rApi g 4 was supplied by Dr S. Scheurer[17] of the Paul-Ehrlich-Institut, Langen, Germany. Freeze-dried and redissolved extracts were kept at –20°C until used.

Electrophoresis

Carrot extract was separated with Tricine SDS-PAGE according to the method of Schägger and von Jagow[18] through use of a Mini-Protean cell (Bio-Rad, Munich, Germany). The 14% T, 2.6% C separating gel was overlaid by a 4% T, 2.6% C stacking gel. Proteins were reduced by 1,4-Dithiothreitol (Sigma-Aldrich, Deisenhofen, Germany) and loaded onto the gel at a concentration of 25 μg of carrot protein per cm.

Immunoblotting

The proteins were transferred onto nitrocellulose membranes by semidry blotting and blocked twice in 50 mmol/L Tris(hydroxymethyl)aminomethane/HCl buffer (pH 7.4) containing 0.15 mol/L sodium chloride and 0.3% Tween 20 (TBST).[19] The nitrocellulose strips were incubated overnight with 80 μL of patients' sera and control serum from a nonallergic donor in 520 μL TBST containing 0.1% BSA. IgE antibody detection was performed with alkaline phosphatase-conjugated mouse antihuman IgE (1:1000, 4 hours; PharMingen, San Diego, Calif).

Immunoblot inhibitions were carried out on blot strips with carrot extract as solid phase. Patients' sera were diluted 1:7.5 and preincubated with one of the following inhibitors or with buffer as a control: 100 μg of protein from allergen extracts, 15 μg of recombinant allergens, or 15 μg of different glycopeptides. Bound IgE was detected according to the method discussed above.

N-glycans

N-linked glycopeptides with the glycan structure Manα1-6(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc (MUXF^3) and just 2 to 4 amino acid residues were prepared from extensively digested pineapple stem bromelain, as previously described.[20] The purity of the glycopeptides was checked by matrix-assisted laser-desorption ionization time-of-flight mass spectrometry,[21] amino acids and monosaccharides were analyzed,[22,23] and SDS-PAGE showed the absence of undigested protein.[21,24] A glycopeptide with the common pentasaccharide core Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc (MM), prepared from bovine fibrin, was used as a control.[25]

Data analysis

Sensitivity (SE) and specificity (SP) were calculated according to the method of Goldman[26]:

$$SE = TP/(TP + FN)$$
and
$$SP = TN/(TN + FP),$$

where *TP* is the number of true positives (patients with positive DBPCFC and positive SPT and/or CAP results), *FP* is the number of false positives (patients with negative DBPCFC and positive SPT and/or CAP results), *TN* is the number of true negatives (patients with negative DBPCFC and negative SPT and/or CAP results), and *FN* is the number of false negatives (patients with positive DBPCFC and negative SPT and/or CAP results).

RESULTS

Patients

A total of 26 patients (16 female and 10 male) entered the study. The mean age of the study population was 33.9 ± 12.7 years (range, 18 to 59 years). Case histories with respect to carrot allergy are summarized in Table I.

TABLE I. Specific IgE to birch pollen, rBet v 1, rBet v 2, and rBet v 6 as determined by CAP method and results of IgE reactivity to carrot proteins as detected on immunoblot in patients with positive and negative DBPCFC results

	Case history			CAP (kU/L)				Blot carrot extract†		
Patient no.*	Spring pollinosis	Carrot allergy	DBPCFC symptoms	Birch pollen	rBet v 1	rBet v 2	rBet v 6	12 kDa	17 kDa	34-70 kDa
DBPCFC-positive										
1	+	OAS, AE	OAS	41.8	36.3	neg	neg	–	+	–
2	+	AE, U, D, V	OAS	11.3	13.6	0.71	neg	–	(+)	+
3	+	OAS	OAS	>100	98.9	neg	neg	–	+	+
4	+	OAS, Th	OAS	63.1	70.6	neg	0.52‡	–	+	–
5	–	OAS, AE	OAS	>100	>100	0.84	0.73	–	+	+
6	+	Th, Dph	OAS, Dph	77.6	88.0	neg	neg	–	+	–
7	+	OAS	OAS	75.3	22.5	86.5	2.91	+	+	+
8	+	OAS	OAS	29.1	41.8	neg	neg	–	(+)	–
9	+	OAS	OAS	4.96	3.4	4.63	0.36‡	(+)	–	+
10	+	Dph, Ho	OAS	87.0	44.1	1.53	neg	–	+	–
11	–	OAS, Th	OAS	37.3	31.4	neg	neg	–	+	–
12	+	OAS, AE, Co	OAS	4.07	3.9	neg	neg	–	–	–
13	+	OAS, AE	F, C, D	26.1	26.8	neg	neg	–	+	+
14	+	OAS, R	OAS, R	>100	>100	37.6	neg	+	+	+
15	+	OAS	OAS	>100	>100	neg	0.46‡	–	+	+
16	+	OAS, AE	OAS	>100	>100	neg	neg	–	+	+
17	+	OAS, AE, R	OAS, P, C	10.8	11.8	0.79	neg	–	+	–
18	+	AE	OAS	34.6	37.8	1.77	neg	+	–	–
19	+	OAS, R	OAS	>100	>100	neg	neg	–	+	–
20	–	OAS	OAS	14.2	17.7	neg	neg	–	+	–
Total positive	85%			100%	100%	40%	10%	20%	85%	45%
DBPCFC-negative/OP-positive										
21	+	OAS	neg	>100	>100	41.1	neg	+	–	+
DBPCFC-negative/OP-positive										
22	+	OAS	neg	29.0	22.5	29.9	0.49‡	–	+	+
23	+	OAS, R	neg	6.21	5.72	neg	0.52‡	–	–	–
24	+	OAS	neg	55.3	78.8	neg	neg	–	+	–

OAS, Oral allergy syndrome; *AE,* angioedema; *neg,* negative; *U,* urticaria; *D,* dyspnea; *V,* vertigo; *Th,* tightness of throat or chest; *Dph,* dysphagia; *Ho,* hoarseness; *Co,* cough; *F,* flush; *R,* rhinitis; *C,* conjunctivitis; *P,* pruritus; *OP,* open provocation.

*Results of the 2 placebo responders have not been included.

†Values in parentheses indicate weak binding to immunoblots.

‡Value (<0.7 kU/L) has not been included in the number of positive results.

Twenty-two patients reported pollinosis symptoms during the flowering season of birch (Table I), and 7 patients reported pollinosis symptoms during the flowering season of mugwort.

Skin tests

All patients with positive DBPCFC results were positive for the prick-to-prick test with raw native carrot (SE = 100%) whereas the result of SPT with commercial carrot extract was positive in just 5 of 19 subjects tested (SE = 26%). In all patients with negative DBPCFC results, however, SPT with carrot extract was negative (SP = 100%) whereas prick-to-prick testing with raw carrot was positive (SP = 0%).

Of patients with a positive DBPCFC result, a positive SPT result was shown for birch, alder, and hazel pollen in 100% (n = 20), for ash pollen in 75% (n = 15), for mugwort pollen in 40% (n = 8), and for grass and rye pollen in 80% (n = 16) and 70% (n = 14), respectively.

In vitro diagnosis

Eighteen of 20 patients with positive DBPCFC results (all but patients 12 and 20) and 2 of 4 patients with negative DBPCFC results were CAP test–positive for carrot (≥0.7 kU/L). Thus, determination of specific IgE to carrot by the CAP method showed an SE of 90% and an SP of 50%.

Table I shows the CAP test results for rBet v 1, rBet v 2, and rBet v 6. In patients with positive DBPCFC results, the CAP test result was positive in 100% (n = 20) for birch pollen, in 60% (n = 12) for mugwort pollen, and in 85% (n = 17) for celery (data for mugwort and celery not shown).

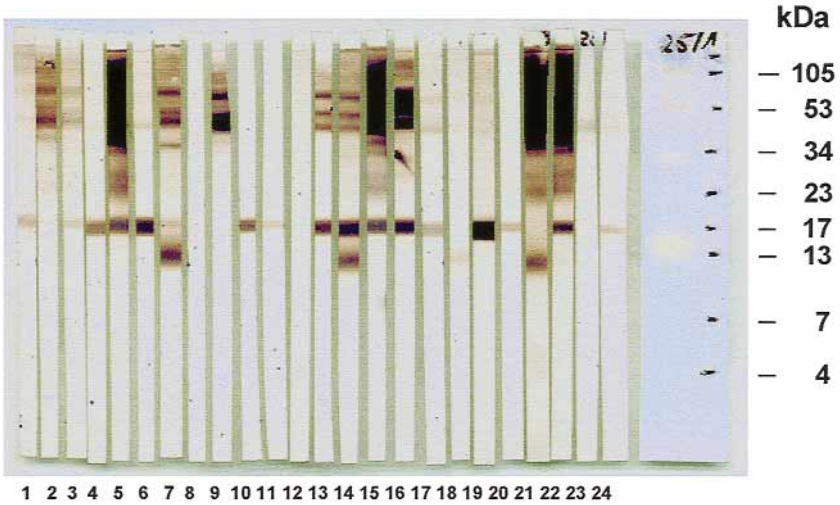

FIG 1. Immunoblot of carrot extract tested with patients' sera (*1-20,* DBPCFC-positive; *21,* nonresponder with a positive open challenge; *22-24,* nonresponders with negative open challenge).

DBPCFC with carrot

Twenty patients who reacted to the carrot-containing drink but not to the placebo drink were regarded as responders. Sixteen patients complained about symptoms strictly localized to the oral cavity (oral allergy syndrome; OAS) at a mean provocation dose of 1.9 ± 1.0 g of carrot. OAS appeared in 14 patients during the local mucosal challenge ("spit" phase) and in 2 patients after the swallowing of 13 mL of the active drink.

In 4 patients, symptoms were not restricted to the oral cavity (Table I) and occurred at a mean provocation dose of 1.7 ± 0.9 g of carrot—during the "spit" phase in 3 patients and after the swallowing of 13 mL of the active drink in 1 patient. Four patients responded to neither the carrot-containing drink nor the placebo drink (nonresponders). Two patients reported symptoms of OAS after the single-blinded challenge with the placebo drink and were excluded from the study as placebo responders.

Open provocation with carrot

Three of the 4 nonresponders did not complain about any symptoms when undergoing open provocation. The fourth patient reported symptoms of an OAS after swallowing 5 g of raw carrot.

Immunoblot analysis

IgE immunoblot analysis of carrot extract was performed with sera of all patients undergoing DBPCFC. IgE binding bands were detected at 12 kDa, at 17 kDa, and between 34 and 70 kDa. Results are presented in Fig 1 and summarized in Table I.

Immunoblot inhibition assays

Blot inhibition experiments were performed to confirm the SP of the detection and to identify the involved allergens. For this purpose, blots with carrot extract were incubated with the serum of patient 14 (recognizing allergens with molecular weights of 12 kDa, 17 kDa, and higher), with the serum of patient 15 (recognizing allergens with molecular weights of 17 kDa and higher), and with different inhibitors.

As shown in Fig 2, for both sera, complete inhibition was obtained with carrot extract (lanes 1), whereas milk extract as the negative control did not inhibit any IgE antibody reactivities to carrot (lanes 2). IgE binding to the 17-kDa allergen was strongly inhibited by preincubation with rBet v 1 in the serum of patient 15 and was not inhibited at all in the serum of patient 14 (lanes 5). Similarly, natural birch pollen extract inhibited IgE binding to the 17-kDa allergen in the serum of patient 15 but not in the serum of patient 14 (lanes 7). Pretreatment with rApi g 1, however, resulted in complete inhibition of IgE binding to the 17-kDa allergen in both sera (lanes 8). To confirm this surprising finding of incomplete inhibition of IgE binding to the 17-kDa allergen by Bet v 1 and natural birch pollen extract, we performed blot inhibition experiments in all sera of the 15 DBPCFC-positive patients that exhibited a strong IgE reactivity against the 17-kDa allergen. Preincubation with rBet v 1 and birch pollen extract resulted in complete inhibition in 9 sera and partial inhibition in 1 serum, whereas in 4 sera binding intensity was not influenced at all (Table II). Pretreatment with rApi g 1 resulted in complete inhibition in 11 sera and strong reduction of IgE binding in 4 sera (Table II). In the serum of patient 7, the band at 17 kDa was stained too weakly to assess the degree of inhibition. In patient 14, an increase of the Bet v 1 amount to 30 μg did not influence the inhibition (results not shown).

In 1 patient (patient 7), we identified IgE binding to a 35-kDa protein that was completely abolished by preincubation with rBet v 6 (result not shown).

IgE binding to the 12-kDa protein was completely inhibited in the serum of patient 14 (Fig 2, *A*) by birch

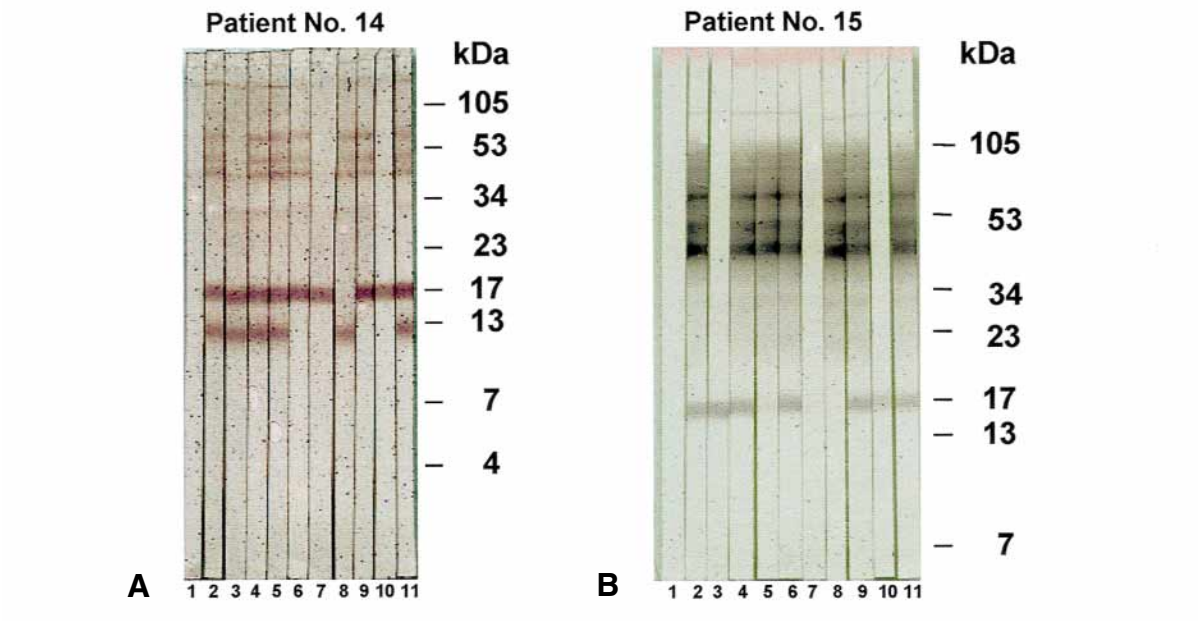

FIG 2. Immunoblot inhibition with carrot extract tested with sera of patients 14 (**A**) and 15 (**B**). *Lanes: 1,* carrot extract; *2,* milk protein; *3,* bromelain glycopeptide MUXF^3; *4,* fibrin glycopeptide MM; *5,* rBet v 1; *6,* rBet v 2; *7,* birch pollen extract; *8,* rApi g 1; *9,* rApi g 4; *10,* mugwort pollen extract; *11,* no inhibitor. Inhibitor concentrations (in micrograms of protein): extracts, 100; allergens and glycans, 15.

and celery profilin (lanes 6 and 9) and by birch and mugwort pollen extract (lanes 7 and 10). These findings and the fact that all 4 sera that exhibited IgE reactivity against the 12-kDa protein were CAP-positive to Bet v 2 suggest that the 12-kDa protein is carrot profilin.

IgE binding to proteins of high molecular weight was suspected to be caused by cross-reactive carbohydrate determinants (CCDs) on glycoproteins. Therefore, we performed inhibition experiments with highly pure N-linked glycopeptides MUXF^3 and MM (Fig 2, lanes 3 and lanes 4, respectively). Preincubation with the bromelain glycopeptide MUXF^3 abolished IgE binding to components of high molecular weight completely in the sera of patients 14 and 15; the same result was obtained with birch and mugwort pollen extract. The fibrin glycopeptide MM did not influence IgE binding.

DISCUSSION

Although clinical manifestations of carrot allergy are rather frequent, there have been few attempts thus far to study this food allergen in representative collectives of patients. In this study, we present the first clinical and in vitro data of 20 patients whose carrot allergy had been confirmed by DBPCFC.[5]

The history of allergic reactions to carrot was confirmed by means of DBPCFC in 77% of patients tested. History revealed systemic reactions in 14 patients and symptoms strictly localized to the oral cavity (OAS) on carrot consumption in 6 patients. During DBPCFC, however, only 4 of 20 responders developed reactions more severe than OAS. Thus our 2-step procedure prevented more severe systemic reactions in over 70% of patients. Amazingly,

TABLE II. Results of blot inhibition of IgE binding to Dau c 1 with rBet v 1, birch pollen extract, and rApi g 1 in carrot-allergic patients sensitized to the major carrot allergen

	Inhibitors		
Patient no.	**Birch pollen extract**	**rBet v 1**	**rApi g 1**
1	–	–	++
3	++	++	+
4	++	++	++
5	++	++	+
6	–	–	+
10	++	++	++
11	++	++	++
13	++	++	+
14	–	–	++
15	+	+	++
16	–	–	++
17	++	++	++
19	++	++	++
20	++	++	++

+, Partial inhibition.
++, Complete inhibition.
–, No inhibition.

mean provocation doses of carrot to elicit OAS and systemic reactions were comparable (1.9 ± 1.0 g and 1.7 ± 0.9 g, respectively). In celery-allergic patients, the mean provocation dose to elicit a systemic reaction to celery was more than 10 times higher than the dose provoking OAS (19.3 ± 11.8 g vs 1.3 ± 1.4 g).[4] One patient with a negative DBPCFC result complained about OAS in the open provocation, possibly because of dilution or degradation of carrot allergens in the challenge mixture. Hence, as in

DBPCFC with celery,[4] false negative DBPCFC results can occur during diagnosis of carrot allergy.

CAP determination of carrot-specific IgE antibodies showed an SE of 90% in our study population. The SE was 26% for SPT with a commercially available carrot extract in DBPCFC-positive patients and 100% for the prick-to-prick test with crude carrot.

SPTs with food extracts are often affected by false negative reactions because of instability of allergens, particularly of Bet v 1–related allergens, and/or as a result of a lack of standardization.[27-29] Therefore, skin testing is often performed with fresh food instead of commercial extracts.[30] This approach is often more sensitive, but it is not standardized at all. Because our study population was highly selected—ie, there was always a positive history of carrot allergy—and DBPCFC was used as criterion for true positives, the estimates of SE and SP of the diagnostic procedures cannot be transferred to a normal unselected population.

In adults, food allergy mainly develops secondarily as a consequence of sensitization to inhalant allergens on the basis of IgE cross-reactivity.[10] Carrot allergy is well known to be highly associated with a sensitization to birch or mugwort pollen.[2,3] Correspondingly, of our patients with positive DBPCFC results for carrot, 100% were sensitized to birch pollen and 60% to mugwort pollen.

In the present study, we identified 4 birch pollen–related carrot allergens. Sera of 85% (n = 17) of DBPCFC-positive patients recognized a 17-kDa allergen, recently described as Dau c 1; Dau c 1 exhibits a sequence similarity of 61% (38% identity) with Bet v 1 and 93% (81% identity) with the major celery allergen Api g 1.[11]

All of our DBPCFC-positive patients with Dau c 1–specific IgE antibodies were also sensitized to rBet v 1, which suggests a primary sensitization to birch pollen in this group of patients. In a subset of patients (n = 4), however, IgE binding to Dau c 1 was not inhibited by rBet v 1 (15 μg) or birch pollen extract (100 μg), and a 2-fold increase of the Bet v 1 amount showed the same results. This is clearly divergent from other pollen-related food allergens, such as Pru av 1 from cherry, Cor a 1.0401 from hazelnut, and Api g 1 from celery; in similar experiments, complete IgE inhibition to these allergens was obtained with 15 to 40 μg of rBet v 1.[8,9,31,32] Two of these 4 patients showed IgE reactivity exclusively against the 17-kDa allergen in immunoblot analysis. Our data do not allow us to differentiate whether in these patients primary sensitization to birch pollen was followed by a secondary sensitization to new epitopes on Dau c 1 or whether a clinically relevant primary sensitization to food allergens of the Bet v 1 family can occur in a subgroup of carrot-allergic patients independently of a primary sensitization to birch pollen allergens. In support of the second hypothesis, Moneo et al[33] recently described in 4 carrot-allergic patients a monosensitization to an 18-kDa protein in carrot extract but no concomitant sensitization to birch pollen. The characterization of the 18-kDa protein allowed to assign this allergen to the Bet v 1 family.

An allergen with a molecular mass of approximately 12 kDa was recognized by sera from 20% of DBPCFC-positive patients. It was identified as carrot profilin, inasmuch as IgE binding to the 12-kDa allergen was inhibited by rBet v 2 and celery profilin, rApi g 4. Furthermore, all patients who exhibited IgE binding to the 12-kDa allergen were sensitized to Bet v 2 (birch profilin). The sensitization rate in our group of carrot-allergic patients corresponds well with the profilin sensitization in 20% of patients with pollen allergy described by Valenta et al.[12]

Recently, a new minor allergen from birch pollen, Bet v 6, was identified and cloned.[15,34,35] Bet v 6 has a molecular weight of 35 kDa and exhibits a high degree of sequence identity to isoflavone reductase and isoflavone reductase homolog proteins from several plants. Approximately 12% of birch pollen–allergic patients are sensitized to this protein.[35] In accord with these results, 10% of sera from the patients in our carrot-allergic study population, all of whom were sensitized to birch pollen, contained specific IgE to rBet v 6 (≥0.7 kU/L), as determined through use of the CAP method. IgE binding to a 35-kDa protein in carrot extract was clearly visible in 1 patient (patient 7), and SP of binding was confirmed by blotting inhibition with rBet v 6 (result not shown). The clinical importance of Bet v 6 in initiation of cross-reactive food allergy has not been established thus far.

CCDs are present in taxonomically distant allergenic sources, such as pollen, food, and arthropods, and might induce specific IgE.[21,36] The role of specific IgE directed to these epitopes is still controversial. In 45% of DBPCFC-positive patients, multiple binding to carrot allergens of high molecular weight was detected by means of immunoblot analysis. Inhibition experiments with N-glycan containing α1,3 fucose and β1-2-xylose (MUXF^3) identified them as CCDs. Furthermore, cross-inhibition with birch and mugwort pollen extract, known to contain CCDs, confirmed the IgE reaction against these ubiquitous carbohydrate moieties and their presence in carrot extracts.

We could not find a correlation between sensitization pattern of DBPCFC-positive patients in terms of allergen recognition and type of clinical reaction mentioned in the patient history.

In conclusion, for the first time, we were able to confirm carrot allergy in 20 of 26 patients using a DBPCFC protocol with carrot. DBPCFC-positive patients exclusively produced IgE antibodies to birch pollen–related carrot allergens, Dau c 1 being the major allergen.

We thank Susan Marti, Irène Cuhat, and Marie-Claire Weber for their superb technical assistance; the nurses of the Allergy Unit for their cooperation; and Pascale Heuschmann for creating the recipe for the test meals. We also thank Dr S. Scheurer for supplying rApi g 4 and Dr J. Lidholm, Marina Moro, and Pirjo Lethonen, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden, for producing and providing Bet v 6-ImmunoCaps. We are grateful to Dr G. Reese of the Paul-Ehrlich-Institut for critically reviewing the manuscript.

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