

Reactivity of carrot-specific IgE antibodies with celery, apiaceous spices, and birch pollen

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Although carrot allergy is not well recognized in North America, the celery-carrot-mugwort-spice syndrome is well known in Europe. In the current study, cross reactivity of carrot, stalk celery, and spices, all members of the Apiaceae and birch pollen was assessed with serum from a patient reporting raw carrot-induced, raw stalk celery-induced, or spice-induced laryngeal edema and bronchospasm. By RAST inhibition, some cross-reactivity was demonstrated between raw carrot and stalk celery and other members of the Apiaceae. Immunoprint inhibition revealed common allergic epitopes on a 17-kD band shared by carrot, celery, and birch pollen. The results suggest that subjects sensitized to carrot may also have allergic reaction to other vegetables or spices of the Apiaceous family. Furthermore, carrot hypersensitivity can be associated with birch pollen allergy.

INTRODUCTION

Although all foods may have the potential to induce an allergic response, only some have been reported to cause hypersensitivity reactions.¹⁻⁴ Foods most commonly involved in immediate allergic reactions include milk, eggs, seafood, and legumes, particularly peanuts.^{1,3-5} These foods have been well documented to produce hypersensitivity reactions and their major allergens have been studied.^{1,4} In some European countries, carrot and celery are frequently implicated as causes of hypersensitivity reactions among adults.⁶⁻¹⁵ Although a number of reports in the European literature describe celery and carrot hypersensitivity, their antigenic/allergenic components have not been well studied.

Carrot and celery belong to the same botanical family, the Apiaceae (or Umbelliferae). Hypersensitivity

to carrot is usually associated with celery allergy and sensitization to the botanically unrelated species of mugwort (*Artemisia vulgaris*) and/or birch (*Betula* spp.).⁶⁻¹⁴ Studies of Apiaceous spices (such as anise, caraway, coriander, cumin, and parsley) indicate their involvement in the allergic responses of subjects sensitive to both celery and carrot, referred to as "celery-carrot-mugwort-spice syndrome."^{10,12,13} This syndrome is frequently described in the German literature. Based on skin tests, food-specific IgE antibodies and Prausnitz-Kustner serum transfer tests, carrot-induced, celery-induced, and spice-induced clinical symptoms are thought to be caused by IgE-mediated mechanisms.^{6,8-16}

There are no reports in the United States of allergic reactions following the ingestion of carrot. Recently, however, a patient with relevant pollinosis to mugwort and contact urticaria to carrot and celery was described.¹⁷ The current study explored the presence of allergens shared by carrot and other members of the Apiaceae and investigated the relationship of birch pollen allergens to carrot and celery allergens.

MATERIALS AND METHODS

Study Subject

A 48-year-old Caucasian woman with a history of seasonal rhinocon-

junctivitis in the spring and fall reported an itchy, runny nose, itchy eyes, and wheezing when shredding raw carrots and throat swelling, wheezing, and a squeaky voice upon ingestion of raw carrots. Symptoms were similar, although milder, after eating stalk celery, raw parsnip and fresh parsley, and cherries; occasionally, apples caused oral itching and throat swelling. Ingestion of cooked carrot, stalk celery, and parsnip and dried parsley did not produce symptoms.

Skin prick tests using commercial extracts (Hollister-Stier) were positive to grass, ragweed, birch, carrot, parsley, and pepper. Commercial RASTs (Pharmacia) were positive for celery (class 3), birch (class 3), ragweed (class 3), apple (class 2), anise (class 2), cumin (class 2), and coriander (class 2) but negative for carrot and grass. Sera of three atopic subjects with negative histories and skin tests to carrot were used as controls.

Preparation of Extracts

Extracts were prepared from fresh, raw carrot, boiled carrot (250 g in 1 L of deionized water for ten minutes at 100 °C), water remaining after boiling the carrots, raw stalk celery, cumin, anise, and coriander; all were purchased from a local supermarket. Foods were homogenized and extracted overnight in phos-

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phate buffered saline (PBS) (0.1 mol/L; pH 7.2) at 4 °C under constant stirring. Extracts were centrifuged for 30 minutes at 70,000 g, supernatants concentrated by ultrafiltration with Amicon YM1 (Danvers, MA) (M.W.C.O 1 kD), dialyzed (M.W.C.O 3.5 kD) against deionized water and stored at -20 °C. Birch extract (birch mix: paper, river and white birch, Hollister Stier, USA) was dialyzed (M.W.C.O 3.5 kD) against deionized water, and concentrated by dialysis against Ficoll 400 (Pharmacia LKB, Sweden) at 4 °C. Dry weight was determined and protein concentrations were measured by Coomassie Plus Assay (Pierce, Rockford, IL) following manufacturer's instructions. Protein concentrations were 13.7 mg/mL for raw carrot, 1.14 mg/mL for boiled carrot, 0.76 mg/mL for carrot cooking water, 7.6 mg/mL for stalk celery, 20.0 mg/mL for birch pollen, 16.0 mg/mL for anise, 18.0 mg/mL for coriander, and 17.0 mg/mL for cumin.

RAST, RAST Inhibition, and Determination of Total IgE

Raw carrot, boiled carrot, carrot cooking water, and stalk celery RAST were performed by the method of Ceska and Lundkvist.¹⁸ Paper disks were coated with 1 mg dry weight of extract per disk. Duplicate antigen-coated disks were incubated overnight with 100 µL undiluted serum. After washing with saline (0.9%), 100 µL of ¹²⁵I-labelled anti-IgE (15,000 cpm/disk; Kallestad, Chaska, MN) was added and the disks incubated overnight. Disks were washed and bound ¹²⁵I (cpm) measured in a gamma counter (Gamma 5500, Beckman, Irvine, CA). Results were expressed as mean percent cpm binding of total radioactivity added.

RAST inhibition was performed using the method of Gleich.¹⁹ Fifty microliters of patient serum plus 50 µL of increasing concentrations (0.01, 0.1, 1.0, and 10.0 mg/mL) of inhibitor extract were tested for inhibition of raw carrot RAST. Ex-

tract of cabbage, a member of the Brassica family, was used as a negative control. Duplicate inhibitions were performed and mean values determined.

Total serum IgE levels were measured by Kallestad QuantiCLONE kit (Chaska, MN) using ¹²⁵I-labelled anti-IgE.

SDS-PAGE and Immunoprint

SDS-PAGE was performed by the method of Laemmli,²⁰ using 15% T/2.7%C separation gels (16 × 18 × 0.15 cm) and 4% T/2.7%C stacking gels in an SE 600 electrophoresis chamber (Hoefer Scientific Instruments, San Francisco, CA). Samples were heated at 100 °C for five minutes in sample buffer containing 125 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol. Electrophoresis was conducted at 60 mA of constant current per gel until bromophenol tracking dye was 1 cm from the bottom of the gel. For staining, proteins were fixed for one hour in 3.5% sulfosalicylic acid in 11.5% trichloroacetic acid and stained with 0.1% Coomassie blue R 250. For immunoblotting, proteins were transferred electrophoretically to a cyanogen bromide-activated, 0.2-µm pore size nitrocellulose membrane.²¹ Unreacted membrane sites were blocked with phosphate buffer (40 mM; pH 7.5) containing 0.05% Tween 20 and 1% bovine serum albumin. Nitrocellulose membranes were washed in PBS with 0.05% Tween 20 and incubated overnight with subject (1:8 dilution) or control sera (1:2) diluted with phosphate buffer. To adjust the mean total IgE to comparable levels, serum dilution from the study subject was increased 4 times. After washing with PBS/Tween, radiolabeled anti-IgE (diluted to 50,000 cpm/strip with buffer) was added and the membranes incubated at room temperature overnight. Unbound anti-IgE was removed by washing and the membrane was allowed to dry. Autoradiography was performed on x-ray film (Eastman Kodak

Company, Rochester, NY) with an intensifier screen (Dupont Cronex Lightning plus) for one to three days at -70 °C.

Immunoblot Inhibition

Following separation by SDS-PAGE of 15 µg per 0.5-cm gel of raw carrot, stalk celery or birch pollen, proteins were blotted onto nitrocellulose membranes which were then cut in 0.5-cm strips. IgE serum reactivity was inhibited by adding increasing concentrations of raw carrot, celery, or birch pollen protein extracts (0.01, 0.1, 1.0, and 10.0 mg/mL) to subjects' (1:8) or pooled control serum (1:2) diluted with PBS. Standard immunoprint assay was conducted as previously described.²²

Statistical Analysis

The inhibition results of raw carrot were log transformed and analyzed by least-squares-multiple linear regression. Slopes and intercepts were determined.

RESULTS

RAST and RAST Inhibition

The patient's serum had positive RAST reactivity to raw carrot (41.9%), boiled carrot (31.4%), carrot cooking water (35.3%) and stalk celery (42.8%). Negative control sera did not show significant RAST binding (<3%) to any of the extracts tested. Raw carrot RAST was maximally inhibited by stalk celery (46%), boiled carrot (46%), anise (45%), cumin (44%), and coriander (42%) (Fig 1). Cabbage, a negative control, did not inhibit raw carrot RAST.

SDS-PAGE and Immunoprints

At least 13 raw carrot proteins, ranging from 17 to 61 kD, were demonstrated by SDS-PAGE; the most intense Coomassie blue staining bands were at approximately 17, 21, 32, 45, and 61 kD. Seven Coomassie blue staining bands each were detected in celery (17 to 66 kD) and in birch pollen (17 to 37 kD), 12 bands in anise (17 to 66 kD), 5 in

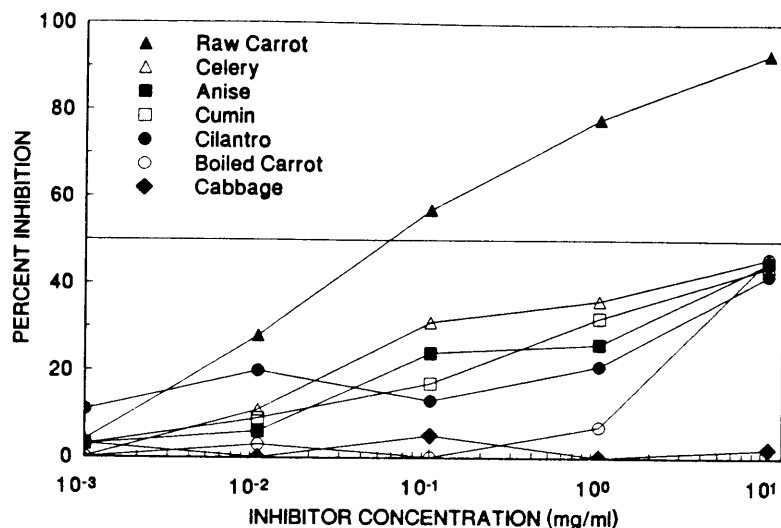


Figure 1. Inhibition of raw carrot RAST with carrot, stalk celery, and spices of the Apiaceae.

coriander (17 to 64 kD), and 10 in cumin (17 to 41 kD). The ten protein bands in both boiled carrot and carrot cooking water were identical to each other (17 to 43 kD) and similar but more intense (except 17 kD band) than those bands in raw carrot.

Immunoprints of raw carrot showed seven IgE binding bands at 17, 25, 32, 36, 41, 45, and 61 kD; the 17 kD being the most intense (Fig 2). All IgE binding bands corresponded to Coomassie blue staining protein bands. In boiled carrot and carrot cooking water, only a single band at 17 kD was detected (Fig 2). Birch pollen had five IgE binding bands at 17, 21, 32, 45, and 50 kD; celery, nine bands at 17, 21, 23, 31, 34, 40, 43, 45, and 66 kD; anise, three bands at 17, 21, and 23 kD (Fig 3); and coriander and cumin, one single band each at 17 kD (data not presented). The 17-kD allergen was the most intense IgE binding band in all extracts. No IgE binding components were detected by negative control sera (data not presented).

Immunoprint Inhibition

IgE reactivity of raw carrot immunoprint was completely inhibited by homologous extract and stalk celery extract at the concentration of 50 μ g; a partial dose-dependent inhibi-

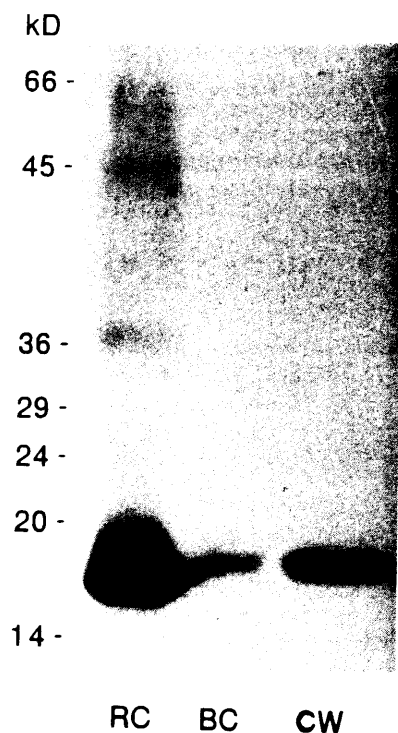


Figure 2. IgE reactivity to raw carrot (RC), boiled carrot (BC), and carrot cooking water (CW). Seventy-five micrograms of protein per lane were applied and incubated with 1:8 diluted patient's serum.

tion was achieved with birch pollen extract. Stalk celery immunoprints were also completely inhibited by 50 μ g homologous and raw carrot extract; birch pollen; inhibition was also partially dose-dependent. Birch

pollen immunoprints were completely inhibited by as little as 50 μ g of homologous extract and were partially inhibited in a dose-dependent fashion by raw carrot and stalk celery extracts (Fig 4).

DISCUSSION

Vegetable, nut, and/or fruit hypersensitivity is not uncommon among atopic adults, particularly individuals allergic to birch and/or mugwort pollens.⁶⁻¹⁶ Although a relationship between carrot or celery with birch/mugwort hypersensitivity has been reported in the European literature,⁶⁻¹⁴ its existence has not been well recognized in North America. In this investigation, cross-reactivity between carrot and other members of the Apiaceae was assessed and their allergenic relationship with birch pollen determined using a serum from a patient who had laryngeal edema and bronchospasm after ingestion of raw carrot, raw stalk celery, raw parsnip or fresh parsley.

Although the subject studied was skin test positive to carrot extract, skin testing with commercial food extracts is not always reliable. The limitation of commercial carrot and celery extracts is illustrated by a recent case report in which suspected contact urticaria from these vegetables could only be confirmed by skin testing with fresh food extracts.¹⁷ Unfortunately, skin testing using freshly prepared food extracts is not always easily performed and, therefore, is not a general practice.^{1,3}

Commercial carrot RAST failed to detect carrot-specific IgE antibodies in our patient's serum suggesting, inadequacy of antigen-coated commercial carrot disks. High levels of carrot-specific IgE antibodies, however, were detected with our laboratory-prepared disks. Nonspecific IgE binding is unlikely since negative control sera did not bind significantly to the disks. The lack of reactivity of commercial carrot disks agrees with other studies revealing that commercial carrot RASTs detect only half of the

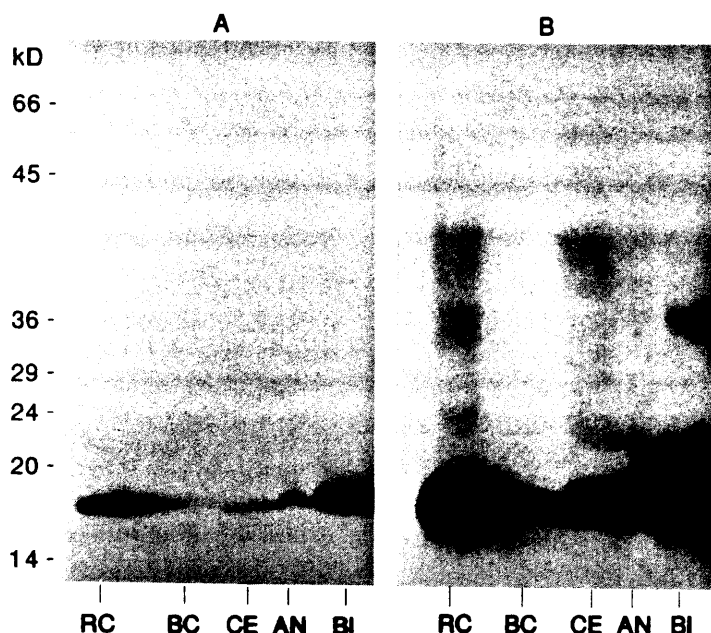


Figure 3. IgE reactivity to raw carrot (RC), boiled carrot (BC), stalk celery (CE), anise (AN), and birch pollen (BI) extracts. Sixty micrograms of protein per lane of each extract were loaded into separate wells. After blotting, nitrocellulose membrane was incubated with 1:8 diluted patient serum. Autoradiography was developed after 6 (A) and 72 hours (B).

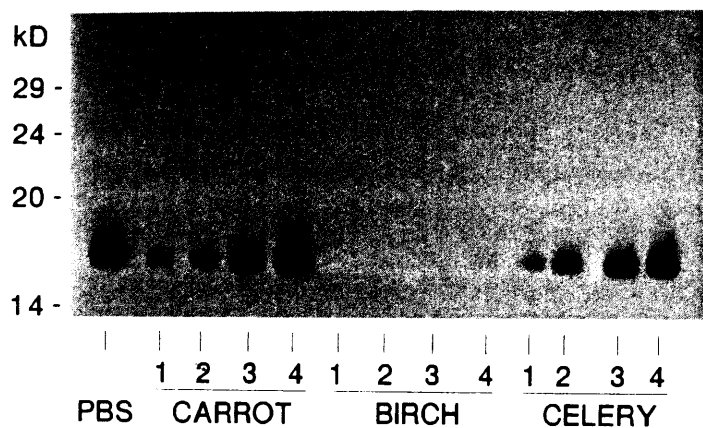


Figure 4. Inhibition of birch pollen immunoprint. Raw carrot, stalk celery and birch pollen were tested for inhibition at a final concentration of 5000 μ g (1), 500 μ g (2), 50 μ g (3), and 5 μ g (4). Phosphate buffered saline (PBS) was used as positive control.

carrot-sensitive subjects.^{10,25} RAST with boiled carrot showed decreased IgE binding, possibly from destruction or extraction of carrot allergens by the cooking process. Some carrot allergens are extracted by the cooking process as shown by the positive RAST to carrot cooking water.

Immunoprints demonstrated less IgE reactivity of the 17-kD protein

in cooked carrot and carrot cooking water than in raw carrot. This finding of reduced allergenicity in cooked carrot and carrot cooking water may be due to allergen destruction as suggested clinically. The patient reportedly can eat cooked carrot without any difficulty. This is supported by the common observation that most individuals who are

allergic to fresh vegetables or fruits can tolerate the offending foods if cooked.^{10-12,25}

Could the association of pollen with food hypersensitivity be due to common epitopes or allergens? Such relationships have been investigated in other studies, especially between mugwort or birch pollens and celery.^{11,13,15,16,26,27} Among both pollens and celery, similar allergens have been demonstrated by different methods. Immunoprint inhibition of the 17-kD allergen illustrated that birch pollen allergen shares common epitopes with carrot and celery allergens. Two other studies using immunoprint inhibitions also suggest common epitope structures in birch pollen, vegetable and fruit allergens in the molecular weight range of 14 to 20 kD.^{26,27} Our RAST inhibition studies demonstrated some cross reactivity of raw carrot with celery and Apiaceous spices. Celery was a potent inhibitor of raw carrot RAST, which is supplemented by clinical observation that many subjects who are allergic to carrot are also sensitized to celery.¹⁰⁻¹² Mugwort was not tested in this study because it was not a common local aeroallergen.

Recently, a 15-kD allergen has been identified in celery tuber that is immunochemically similar to allergens present in other plant species.²⁸ This 15-kD allergen was identified as profilin with a rabbit polyclonal antibody specific to recombinant birch pollen profilin. Profilins have been proposed as a novel family of plant pan-allergens.²⁹ Although the size and presence in carrot, stalk celery, and birch pollen of our 17-kD band suggest it could be related to profilin, further studies are needed to confirm this.

This investigation demonstrates that carrot can produce IgE antibodies, even when cooked. Carrot, a member of the Apiaceae, cross-reacts with stalk celery and certain spices (also members of the Apiaceae family). Carrot and celery

share epitopes with birch pollen, although they are botanically unrelated. Since birch is a common North American tree, the association of birch pollen allergy with carrot and celery hypersensitivity may be more prevalent in the United States and Canada than clinically recognized.

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