

Case report

Hypersensitivity to carrot associated with specific IgE to grass and tree pollens

Gómez M, Curiel G, Mendez J, Rodríguez M, Moneo I. Hypersensitivity to carrot associated with specific IgE to grass and tree pollens. *Allergy* 1996; 51: 425–429. © Munksgaard 1996.

This study deals with a 34-year-old female cook with no previous history of atopy, who was studied because of allergic rhinoconjunctivitis and contact urticaria in both hands associated with severe itching when she handled raw carrot. The patient had had anaphylactic episodes after accidental ingestion of raw carrots, but she tolerated cooked carrots. Skin prick tests with carrot, celery, and olive, and birch, grass, and mugwort pollens were positive. Total IgE was 411 U/ml. Specific IgE to olive, grass, and weed pollens were 10.92, 6.17, and 2.4 AU/ml, respectively. The histamine release test was positive for carrot, celery, celeriac, and olive pollen up to a dilution of $1/10^6$. Immunoblot of raw carrot showed a single IgE-binding 18-kDa band. IgE reactivity for raw carrot immunoblot was completely inhibited by carrot and by celery, but not by olive or grass pollens. Specific IgE to olive pollen was not inhibited by carrot. The existence of monosensitization to an 18-kDa protein in carrot and specific IgE to olive pollen has not been reported in the celery-carrot-mugwort-spice syndrome.

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Key words: carrot, hypersensitivity

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Accepted for publication 22 February 1996

Patients with IgE-mediated hypersensitivity to pollen are frequently hypersensitive to allergens of fresh fruits or vegetables (1–9). This phenomenon has been characterized on the basis of clinical observations, skin tests, RAST, ELISA, and immunoblots. The common explanation of these observations is the presence of cross-reactive IgE. Recent progress in allergen research has resulted in the identification of several cross-reactive allergens (10–12). The most important allergen responsible for birch pollen–fruit cross-reactivity is the major birch pollen allergen *Bet v* I (10). Profilin is another allergen involved in pollen–vegetable cross-reactivity (13–15).

Carrot belongs to the Apiaceae family. Other members are celery, celeriac, and certain spices. Hypersensitivity to carrot is usually associated with allergy to celery and spices and sensitization to the pollen of the botanically unrelated species of mugwort and/or birch, a response known as celery-carrot-mugwort-spice syndrome (16–23).

In the present study, we describe a case of IgE-mediated occupational hypersensitivity to carrot.

Material and methods

The patient was a 34-year-old woman who had worked as a cook for the past 15 years. She presented rhinoconjunctivitis and contact urticaria on both hands associated with severe itching when handling raw carrots; this reaction had appeared 10 years before. She had no other history of previous atopic diseases and continues to work as a cook, avoiding contact with carrots.

She had presented rhinoconjunctivitis, swollen throat, wheezing, urticaria, and angioedema with laryngeal edema after accidental ingestion of raw carrot 5 years earlier. She tolerated boiled carrot, raw celery, raw celeriac, and spices of the Apiaceae family.

Allergen extracts

Extracts were prepared by mincing each vegetable, and adding phosphate-buffered saline (PBS). The mixtures were shaken at room temperature for 30 min and centrifuged for 10 min at 3500 g, and the supernatant was collected. The extracts were

dialyzed against PBS for 24 h and filtered (Millipore 0.22 µm).

Skin tests

Skin prick tests (SPT) were performed with a standard battery including pollens from grasses, trees mix, birch, weeds, mugwort, and olive, as well as mite, dog, and cat (Igoda-Merck, Barcelona, Spain). Raw carrot was tested by the prick-prick test (3). Histamine phosphate 10 mg/ml was used as positive control and saline as negative control. The tests were read after 15 min; SPT were considered positive when the wheal had a diameter > 3 mm (24).

With the antigens that gave positive results (grass, olive, birch, and mugwort), we carried out SPT at different dilutions in saline of up to 1/100. All these allergens were tested in a nonatopic subject.

Specific IgE detection

Specific IgE was determined for olive, grass, latex, weeds, and birch pollen by the commercial method of Kallestad Allarcoat EAST (Diagnostics Pasteur, Madrid, Spain).

EAST inhibition

EAST olive pollen inhibition was used to measure the cross-reactivity between olive pollen and the raw carrot extract. Ten-microliter volumes of the antigens were used as inhibitors for 2 h at dilutions ranging from 1/5 to 1/625 (olive, carrot, grass) with 50 µl of the patient's serum. Thereafter, olive-pollen disks were added, and the procedure was performed according to the manufacturer's instructions.

Histamine release test

The following antigens were tested: raw carrot root, boiled carrot, celery, celeriac, chard, lettuce, birch, mugwort, grass, olive, mite, and cat in six serial dilutions, from 1/10 to 1/10⁶, obtained from the original extracts. An automated fluorometric method was used for these tests (25, 26).

Immunoblotting

After electrophoresis (27), gel equilibration was performed for 15 min in a transfer buffer (50 mM NaCl, 2 mM Tris HCl; pH 7.4), and the proteins were transferred to nitrocellulose for 24 h by diffusion with constant shaking, by a modified version of the method of Petit et al. (28). After four washes and blocking, the membranes were

incubated for 24 h with 10 ml of a 1/20 dilution of serum from the patient. The membranes were again washed four times, and 10 ml of 1/5000 dilution of monoclonal anti-IgE (labeled with biotin) (Ingenasa, Madrid, Spain) was added. The membranes were incubated for 3 h and, finally, after four washes, 10 ml of 1/2000 dilution of streptavidin alkaline phosphatase (Pierce Rockford IL, USA) was added for 1 h, after which the substrate was introduced (NBT-BCIP, Bio-Rad, Richmond, CA, USA).

Immunoblotting inhibition

Raw carrot extract was run on a 16% acrylamide gel and transferred overnight to nitrocellulose. Serial fivefold dilutions of the inhibitors (celery, grass, olive, and carrot) were prepared from the pure extracts, after which they were mixed with serum. The mixtures were placed on a Multiscreen Mini-Protean II (Bio-Rad), with overnight shaking. After several washings, the procedure continued as described above.

Results

Skin tests

SPT were positive for grass, olive, birch, mugwort, raw carrot root, celery, celeriac, parsley, and fennel. When grass, olive, birch, and mugwort were diluted with saline, the last two pollens produced negative results at a dilution of 1/100. In contrast, the response to grass and olive pollen antigens remained the same size. It was not possible to test further dilutions because the patient had no more space on the volar surface of either forearm.

In the prick-prick test, carrot produced a wheal measuring 20 × 5 mm and two satellite wheals measuring 8 × 5 and 6 × 5 mm, respectively.

Histamine release test

The test was highly positive for olive, celery, celeriac, and raw carrot root at dilutions up to 1/10⁶ (Fig. 1).

Immunoblotting

Specific IgE was not detected for parsley, fennel, grass, birch, or mugwort pollens (Fig. 2). The patient did recognize olive by this method, as well as an 18-kDa protein in carrot and celery.

Of the 17 patients selected with EAST >10 AU/ml to olive, none reacted to carrot (Fig. 3). Carrot immunoblotting was inhibited by both carrot and celery extracts, but not by olive or grass pollens (Fig. 4).

HISTAMINE RELEASE TEST

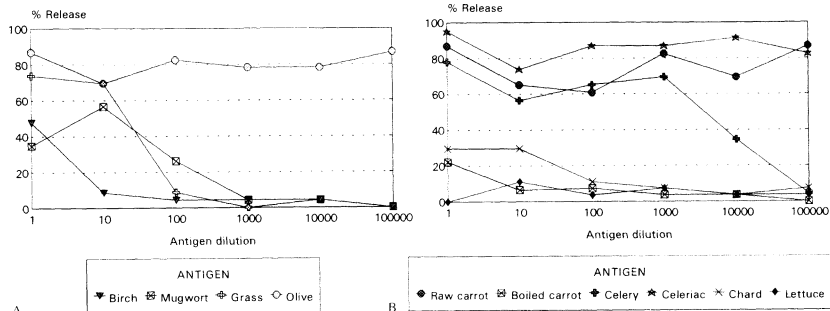


Fig. 1. Histamine release using serial 10-fold dilutions of different pollens (A) or vegetables (B).

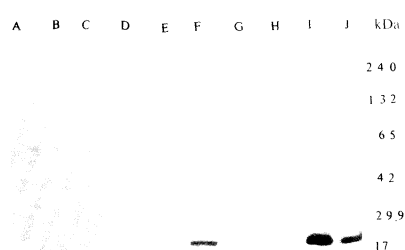


Fig. 2. Specific IgE immunoblot (12% acrylamide gels) to: lane A) grass pollen, lane B) mugwort pollen, lane C) birch pollen, lane D) fennel, lane E) parsley, lane F) olive pollen, lane G) raw celeriac, lane H) carrot leaf, lane I) raw carrot root, lane J) raw celery.

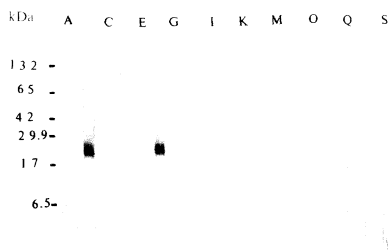


Fig. 3. Specific IgE immunoblot to carrot (16% acrylamide gels) using patient's serum (lanes B and F) and 17 olive-positive sera, randomly located, blank in lane T.

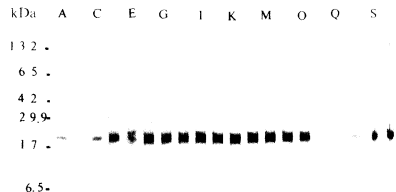


Fig. 4. Carrot immunoblotting (16% acrylamide gels) performed with patient's serum inhibited with serial fivefold dilutions: celery (lanes A-E), grass pollen (F-J), olive pollen (K-O), and carrot (P-T).

Specific IgE detection by EAST

The serum of the patient had positive EAST results in response to grass (6.17 AU/ml), olive (10.92 AU/ml), and weed mix (2.40 AU/ml); however, it was negative for latex, birch pollen, and cat epithelium (used as a control). There was no evidence of inhibition of the olive EAST by carrot (data not shown).

Discussion

IgE-mediated reactions after ingestion of fruits and/or vegetables are often associated with pollinosis (4, 7, 12, 15, 29, 30). Among the most frequently established associations are those of birch-apple, mugwort/birch-celery, and ragweed-banana-melon (2, 3, 8-11, 19-22, 29). The study of allergens has led to the identification of several structures implicated in cross-reactivity between

pollens and fruits and/or vegetables (10). A number of studies (5, 29) have demonstrated the existence of common epitopes in fruit, vegetable, and pollen antigens.

In their study of patients who were allergic to celery, Wüthrich et al. (21) found clinical evidence of mugwort-induced pollinosis in 87%, sensitivity to carrot in 52%, and sensitivities to different spices ranging between 26% for caraway and 3% for aniseed.

Pauli et al. (18) demonstrated the existence of common epitopes in celery and pollens from mugwort and birch by RAST inhibition and immunoblotting. Recently, Vieths et al. (20) described a 16-kDa celery allergen that cross-reacts with *Bet v* I and a 15-16-kDa double band recognized by mugwort-allergic patients.

We have found no mention of occupational sensitivity to carrot in the literature. In the case reported here, the study revealed that the clinical reaction shown by the patient upon handling carrots was IgE-mediated, and, although she showed no such reaction to other members of the Apiaceae family, we did detect IgE to celery and to celeriac, but not to spices. Although our patient, like others previously described, also presented positive skin tests to birch, grass, and mugwort, the dilution study disclosed that only grass and olive pollens remained positive at a dilution of 1:100. This type of dilution study appears to be highly useful in the characterization of the response to pollens in these patients, although it has not been carried out by other authors. Sensitization to olive pollen has not been previously reported. In contrast to other studies, we have been unable to demonstrate a cross-reaction between carrot and olive pollen, a fact that suggests the existence of cosensitization.

Our immunoblotting study showed that the patient recognized a single band of approximately 18 kDa and that the recognition was destroyed by cooking the vegetable, supporting previous studies on the thermostability of this antigen (17, 21). A protein with a molecular mass of 18 kDa, recently described by Zhou et al. (31), exhibits carotenoid-binding activity. If the antigen described by us coincides with this protein, then the allergen belongs to the large family of a carotenoid-protein complex found in fish, algae, plants, and bacteria (23).

Acknowledgments

This study was supported by a grant from the Fundación de la Sociedad Española de Alergología e Inmunología Clínica.

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