

Short communication

Food allergy to pumpkinseed – characterization of allergens

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In recent years, pumpkinseed has become increasingly popular as a foodstuff. Here we report the occurrence of allergic reactions (itching and swelling of oral mucosa, and asthma) to this member of the Cucurbitaceae family. We investigated three patients suffering from symptoms after ingestion of roasted pumpkinseed. All the patients fished for sport and used pressed pumpkinseed flour as bait. Sera were tested by the immunoblot technique for IgE reactivity with proteins of pumpkinseed extract. The immunoblot revealed pumpkinseed allergens of 13, 14, 36, 48, 77, and 87 kDa. Inhibition experiments with recombinant birch profilin were performed: IgE binding to the 14-kDa allergen was completely blocked by preincubation of the sera with recombinant birch profilin. In conclusion, type I allergy to pumpkinseed is rare, and the patients' histories suggest inhalation of pumpkinseed flour during fishing to be the relevant route of sensitization, leading to food allergy to pumpkinseed.

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In the search for nutritional variety, pumpkinseed has found a way into today's cuisine. Roasted pumpkinseed is a popular snack and is often added to bread as a condiment or to salad.

Besides its use as food, pumpkinseed flour is used by fishermen to lure fish. Food allergy to pumpkinseed (*Cucurbita pepo*) is rare and has not been previously described in the literature, although allergies to other members of the Cucurbitaceae family such as cucumber or melon have been reported (1–3). Enberg et al. (2) described the cross-reactivity between watermelon and ragweed allergens, and García Ortiz et al. (3) found shared allergens between melon and *Plantago*, a genus of weeds, and grasses. In the present study, we characterized the allergens of pumpkinseed by immunoblotting. A 14-kDa band was further characterized by inhibition experiments with recombinant birch profilin.

Material and methods

Characterization of allergic patients

Three male patients, aged 29–49 years, were studied (Table 1). All patients experienced allergic

symptoms immediately after ingestion of food containing pumpkinseed. The clinical symptoms ranged from oropharyngeal itching and swelling to bronchial asthma. A pool of sera from nonallergic subjects (negative case history, negative skin prick test [SPT], and negative RAST) was used as negative control.

SPT were performed according to the guidelines of the European Academy of Allergology and Clinical Immunology, the reactions being compared to a positive (histamine) control and a negative (NaCl) control (4).

Protein extracts

Pumpkinseed was obtained from a local grocery store. The seeds were ground and the proteins were extracted as previously described (5). The final concentration of pumpkinseed extract was 1.6 mg/ml. The extract was lyophilized and stored at –20°C.

Serologic determinations

RAST. The radioallergosorbent test (RAST) was performed according to the instructions of the

Table 1. Characterization of patients allergic to pumpkinseed

Patient no.	Age (years)	Sex	Clinical symptoms		Positive SPT	Specific IgE (RAST)	Total IgE
			Seasonal	Pumpkin			
1	49	M	rh, co	or, ang, as	Pumpkinseed, ragweed, goosefoot, wheat, corn, rye, mugwort, lobster	Pumpkinseed - 2 Grass - 2	139
2	29	M	rh, co, as	or, ang	Pumpkinseed, horse, cat, hamster, cow, dog, rye, grass, cock's foot, ragweed, mugwort, corn, wheat, rye flour, soybean, poison ivy, ribwort, alder, birch, hazel, ash tree, chrysanthemum, wood	Pumpkinseed - 2 Grass - 3 Birch - 3	115
3	46	M	rh, co, as	or, ang, as	Rye, grass, cock's foot, alder, birch, hazel, mugwort, bee and wasp venom, barley flour, celery, pea, anise, dog, salmon, almond, house-dust mite	Pumpkinseed - 2 Grass - 5 Mugwort - 3 Birch - 3 Bee, wasp venom - 2	258

as: bronchial asthma; rh: rhinitis; co: conjunctivitis; ang: angioedema; or: inflammation of oral mucosa.

manufacturer (Pharmacia Diagnostics AB, Uppsala, Sweden).

SDS-PAGE and immunoblotting. SDS-PAGE (12% homogeneous gels, 140×115×1 mm, 5% stacking gels) and protein transfer were performed as described previously (6). An amount of 60 µg extract per cm was loaded. Bound IgE was detected by rabbit antihuman IgE (Pharmacia).

IgE-inhibition experiments. All sera were diluted 1:10 in test buffer and preincubated overnight at 4°C under continuous agitation with 30 µg of purified recombinant birch profilin (Biomay Biotechnik Produktions- und Handelsgesellschaft mbH, Linz, Austria). Thereafter, preincubated sera were tested with nitrocellulose-blotted pumpkinseed allergens.

Results

IgE immunoblots

All sera showed IgE binding to a protein with molecular mass of 13 kDa and a protein of 14 kDa. Further IgE binding in the higher range (36, 48, 69, 77, and 87 kDa) could be detected (Fig. 1).

Immunoblot inhibition

Preincubation of patient sera with recombinant birch profilin led in all cases to the extinction of the 14-kDa band. IgE binding to other allergens was not affected by preincubation with Bet v 2 (Fig. 2).

Discussion

Fishing is a popular sport and several techniques have been devised to increase the catch. One trick

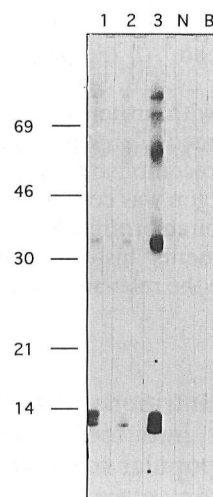


Fig. 1. IgE-binding proteins of pumpkinseed: lanes 1–3) pumpkinseed-allergic patients; N) serum pool from nonallergic subjects; B) buffer control.

consists of throwing bait into the water to lure fish to the area where the fisherman has positioned his rod. This bait is of powdery consistence and is usually made of a mixture of corn bran, wheat bran, and pumpkinseed flour. At the fishing place, water is added to the powder, and the resulting mass is molded into balls, which are then thrown into the water. All our patients were fishermen who lured their catch in this way. Two patients displayed slightly increased total serum IgE, and all of them showed additional sensitizations to common allergens, thus indicating an atopic disposition (Table 1). None of the patients had reacted to pumpkinseed before taking up fishing. Seasonal allergic symptoms prompted them to consult a physician, to whom they reported the occurrence of respiratory and oral problems after the ingestion of

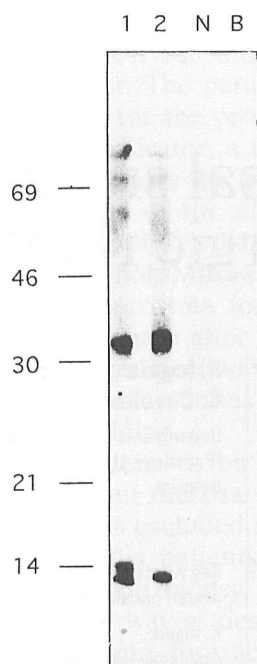


Fig. 2. Inhibition of IgE binding to pumpkinseed allergens (lane 1) by preincubation with recombinant birch profilin (lane 2). N) Serum pool from nonallergic subjects, B) buffer control.

pumpkinseed. Reactions to pumpkin pulp were not observed. After performance of SPT, prick to prick, and RAST, hypersensitivity to pumpkinseed was revealed. We assume a sensitization through the respiratory system, resulting from inhalation of the pumpkinseed flour mixture in its powdered form during preparation of the bait. Cross-reactions between inhaled and food allergies have been described earlier (2, 3, 5, 7–13). Axelsson et al. (10) reported four cases of allergic reactions to sunflower seeds in patients who kept birds in their home, and fed them on sunflower seeds, obviously sensitizing themselves through aerosolized particles.

In this study, we prepared a protein extract from whole pumpkinseed, and by immunoblotting we detected IgE binding to several proteins with molecular masses of 13, 14, 36, 48, 77, and 87 kDa. The 14-kDa band represents a homologue of the panallergen profilin (14), as it is completely inhibited by preincubation of the sera with recombinant birch profilin. Moreover, the 14-kDa protein was detected by sera from food-allergic patients containing profilin-specific IgE (not shown). Our findings agree with data published by Jordan-Wagner et al. (1), who described an IgE-binding 15-kDa band in cucumber and melon (both members of the Cucurbitaceae family), which presumably

represents profilin. Profilins constitute a ubiquitous family of conserved proteins, first described in birch pollen, that cause pollen–food cross-reactivity (15).

In conclusion, pumpkinseed is a potential cause of allergy, particularly in atopic patients. The route of sensitization in this food allergy seems to be inhalation of airborne pumpkinseed allergens. Profilin is one of its major allergens. Therefore, pumpkinseed should be considered potentially harmful to profilin-hypersensitive patients.

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