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Allergy to kiwi, poppy seeds, and/or sesame seeds often occurs in patients with a simultaneous sensitization to nuts and flour. Previously cross reactions have been verified by RAST inhibition. In this study the nature of this cross-reactivity is further characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), followed by immunoblotting to nitrocellulose. The degree of cross-reactivity among kiwi, sesame seeds, poppy seeds, hazelnuts, and rye grain was found to be very high in the patients studied. The existence of both cross-reacting and unique components was observed; however, the cross-reacting and unique components could be different for different patients.

Key words: cross-allergenicity; plant food allergy; SDS–PAGE.

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Immunologic cross-reactivity between botanically closely related plants has been known for many years (12). Common antigens in different grass pollens were early demonstrated, and this knowledge led to a reduction in the number of allergens used for allergy testing and hyposensitization. Antigens shared between unrelated plants have also been demonstrated (1, 6, 7, 8, 11, 15, 21). There is a correlation between mugwort, celery, and carrot sensitivity and also between birch, nut, apple, and stone fruit sensitivity, commonly called the birch-pollen syndrome.

Allergy to kiwi, poppy seeds, and/or sesame seeds often occurs in patients being sensitized to hazelnuts and flour (4, 14, 17, 18, 19), and an allergenic relationship has been suggested by case history, skin prick test, and RAST results. By statistical analysis of RAST results and RAST inhibition experiments, the suspected allergenic relationship of this heterogenic group of botanically unrelated plants could be verified (13).

The aim of the present study was a qualitative characterization of the cross-reacting components by electrophoretic separation in a polyacrylamide gel, followed by immunoblotting to nitrocellulose.

Material and methods

Patient data (Table 1)

All serum samples were chosen from patients of the Klinik für Dermatologie und Allergie, Davos.

Patient 1, C.W., born 1978, male, was suffering from atopic dermatitis. After ingestion of nuts, especially hazelnuts, or poppy seeds, he developed angioedema of the glottis and anaphylactic shock. Corresponding positive results were found in skin prick test (SPT) (or respective skin rub test (5)) and RAST for hazelnuts and poppy seeds; furthermore, sensitizations to rye grain, sesame seeds, various pollens, animal dander, and house-dust mite were detected by RAST.

Patient 2, F.W., born 1982, male, had atopic dermatitis. Gastrointestinal symptoms (vomiting, diarrhea) and angioedema of the face were caused by

Table 1. Patient data

Patient	Disease	Food allergy		
		Clinical symptoms	Skin test	RAST
1. (CW)	AD	H, P	H, P	H, P, R, S
2. (FW)	AD	H, P	H, P	H, P, R
3. (CS)	AD, Rh	H, P	H, P	H, P, R
4. (DZ)	AD, A	H, P	H, P, R	H, P, R
5. (SG)	AD, A, Rh	H, P	H, P	H, P, R
6. (KJ)	AD	H, K	H, K	H, K, R
7. (CD)	AD, Rh	K	H, K, R	H, K, R
8. (GG)	AD, CrD	H, S	H, S	H, S, R

AD=atopic dermatitis, A=allergic asthma, Rh=allergic rhinitis, CrD=Crohn's disease, H=hazelnuts, P=poppy seeds, R=rye grain, S=sesame seeds, K=kiwi.

hazelnuts and poppy seeds. These sensitizations could be verified by SPT (or skin rub test) and RAST, but for rye grain, only by RAST.

Patient 3, C.S., born 1960, female, was suffering from atopic dermatitis and allergic rhinitis; she had a severe food allergy to hazelnuts and poppy seeds with generalized urticaria. SPT and RAST were positive for these two allergens, and RAST for rye grain; for more than 50 allergens, no further sensitization could be detected by RAST.

Patient 4, D.Z., born 1974, male, had atopic dermatitis and severe asthma. Hazelnuts and poppy seeds caused angioedema of lips and palpebrae. SPT and RAST showed a broad spectrum of immediate-type sensitizations, including hazelnuts, poppy seeds, and rye grain.

Patient 5, S.G., born 1968, female, was suffering from severe asthma, chronic allergic rhinitis, and atopic dermatitis. She developed very strong reactions to hazelnuts, poppy seeds, and apples, and moderate reactions to stone fruits, producing asthma, angioedema of the glottis, and anaphylactic symptoms. SPT and RAST were positive for the indicated foods; RAST was also positive for rye grain, various pollens, molds, and animal dander.

Patient 6, K.J., born 1967, female, had a severe atopic dermatitis. Angioedema of lips and tongue occurred immediately after eating hazelnuts, kiwi, banana, or tomatoes. SPT and RAST were positive for hazelnuts and kiwi; RAST with broad spectrum showed, in addition, sensitizations to rye grain and various other allergens, especially inhalants.

Patient 7, C.D., born 1965, female, was suffering from atopic dermatitis and allergic rhinitis. She complained of reactions to kiwi and fish with oral tingling. SPT and RAST with kiwi, hazelnuts, and rye grain (flour) were positive.

Patient 8, G.G., born 1948, male, had severe atopic dermatitis with alopecia totalis and Crohn's disease. He reported a severe food allergy to sesame seeds, with angioedema of the tongue and glottis, and similar reactions to hazelnuts. SPT and RAST were positive for sesame seeds and hazelnuts; RAST showed additional sensitizations to rye grain and various other allergens.

Sera from healthy nonatopic volunteers without (N) or with (S) myeloma IgE added (3000 kU/l) were used as negative controls.

Patient sera

The patient sera used in this study were chosen from an earlier study in which cross-reactivity between hazelnuts, rye flour, sesame seeds, poppy seeds, and kiwi was demonstrated by RAST inhibition (13); sera were chosen on the basis of case history and SPT and RAST results. The serum samples used in

this study were, however, not the same as those used in the first study, but another set of samples from the same patients taken shortly after the first.

Sera were diluted 1:2 [SG (patient 5), CD (patient 7)], 1:3 [FW (patient 2), GG (patient 8)], 1:4 [CW (patient 1), CS (patient 3), KJ (patient 6)] and 1:6 [DZ (patient 4)] for immunoblotting and immunoblotting inhibitions, depending on RAST scores and volumes available.

Allergen source materials

Hazelnut, rye grain, kiwi, sesame seeds, and poppy seed extracts were prepared in a 0.1 phosphate buffer pH 7.4 for 2 h at 8°C, followed by centrifugation for 20 min at 20 000 rpm. The supernatants were vacuum filtered by an OOH Munktell filter paper, and desalted on PD 10 columns (Pharmacia Diagnostics AB, Uppsala, Sweden) if used for SDS-PAGE and immunoblotting (IB) (9). The following extraction concentrations were used:

hazelnuts: 1/15
rye: 1/15
kiwi: 1/5
sesame seeds: 1/15
poppy seeds: 1/15.

RAST assays

Phadebas RAST® disks f5, f10, f17, f84, and Rf224 (Pharmacia Diagnostics AB, Uppsala, Sweden) were used. Assays (22) were run in duplicate, according to the manufacturer's instructions.

Electrophoretic separation

The SDS-PAGE gels were molded in a BIORAD Protean Dual Slab Cell device (BIORAD Laboratories, Richmond, CA, USA) according to operating instructions. The electrophoresis was carried out essentially as described by Neville (10), with a polyacrylamide stacking gel (6%) on top of a gradient separation gel (7.5–20%). The stacking gel buffer was Tris-SO₄, pH 6.1; the upper reservoir buffer was Tris-Borate, pH 8.6, with 0.1% SDS. The lower reservoir and separation gel buffer was Tris-HCl, pH 9.18. The sample buffer contained 3% 2-mercaptoethanol, 2% SDS, 7% glycerol, and 0.001% Bromphenol Blue as tracking dye. The electrophoresis was run at 6 mA/gel for 18–20 h, and approximately 10 mg of sample was added to each gel.

Electrophoretic blotting

The separated proteins were transferred from the separation gel to a nitrocellulose membrane

(Hyperfilm-MP 0.45 μ m, Amersham, UK) by electrophoretic transfer, as previously described (2, 16, 20). The gel and nitrocellulose membrane (NC) were equilibrated for 45 min in the blotting buffer, which was composed of 25 mmol Tris, 192 mmol glycine, and 20% methanol, pH 8.3. Blotting was performed in a BIORAD Trans-blot Cell filled with precooled transfer buffer and blotted for 4 h at approximately 0.45 A, according to the operating instructions. After transfer, unoccupied protein-binding sites were blocked by incubation with a phosphate buffer, pH 7.4, containing 0.5% Tween 20 for 1 h at 37°C. The blotted gel and an unblotted strip of the gel were stained with Coomassie brilliant blue R250 (in 30% methanol and 7% acetic acid) to evaluate the effectiveness of transfer.

Immunologic detection

The NC membrane with blotted proteins was kept in blocking solution at 8°C until used, and was then cut into strips and incubated overnight on a shaker at room temperature with patient serum, diluted 1:4, in a phosphate buffer pH 7.4, containing 0.5% Tween 20.

The immunoblotting inhibition experiments were performed by incubating 0.5 ml of the allergen extract with 0.5 ml of patient serum and 1 ml of phosphate buffer for 2 h before incubating with the nitrocellulose strip. The NC strips were washed three

times 10 min with saline containing 0.5% Tween 20 and incubated with isotope-labeled anti-IgE (Phadebas RAST) diluted 1:4 on a shaker for 4 h at room temperature.

NC strips incubated with isotope-labeled antibodies were washed three times 10 min with saline containing 0.5 Tween 20 and two times with distilled water before being taped onto glass plates, placed on radiographic film (Hyperfilm-MP, Amersham, UK), and exposed in a Kodak (Eastman Kodak, Rochester, NY, USA) X-omatic cassette, equipped with a Du Pont Cronex intensifying screen, for 60 h at 70°C.

Results

RAST

All patients were positive in RAST to the expected allergens, and RAST-values obtained for the second set of samples corresponded well with the data obtained from the first set of samples (13) (results not shown).

SDS-PAGE followed by immunoblotting

Patients positive in RAST were also positive in immunoblotting to the expected allergens. A broad spectrum of IgE specificities was detected for all the allergens studied, and a visual resemblance between the different patterns obtained was noticed (Fig. 1).

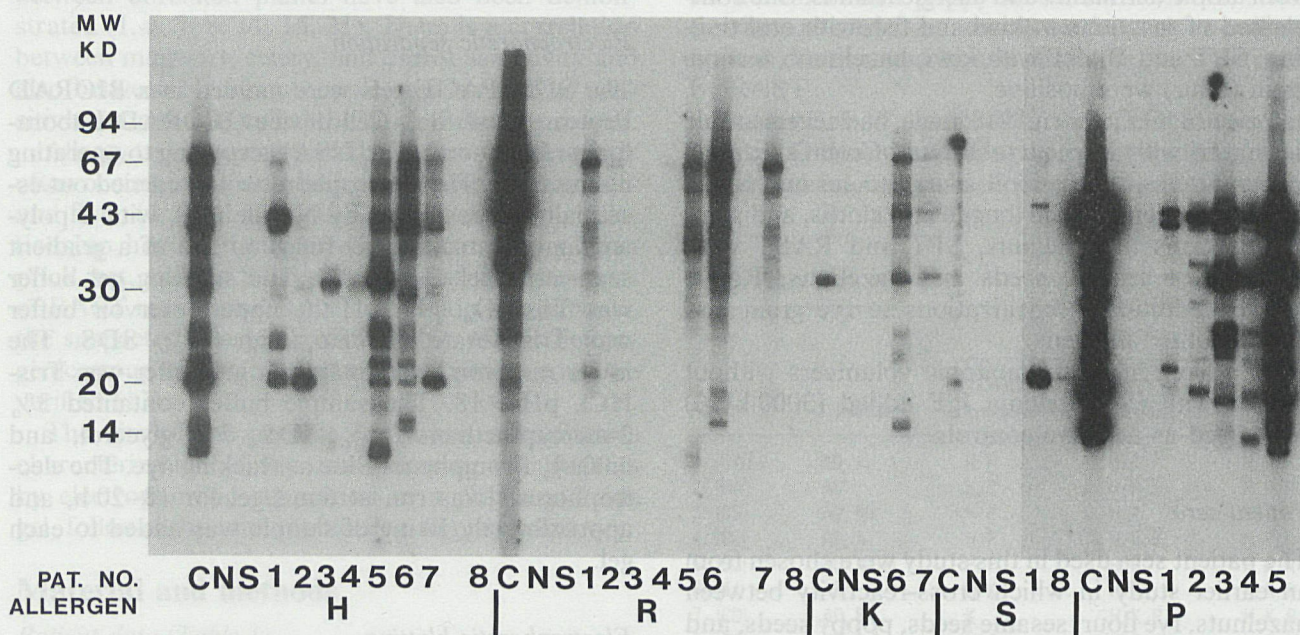


Fig. 1. Immunoblotting from SDS-PAGE gel. Allergen blotted onto strip: H – hazelnut, R – rye, K – kiwi, S – sesame, P – poppy. Patient sera: C – positive control, N – negative control, S – negative spiked control, 1–8 – positive patient sera. IgE was detected with isotope-labeled antibody.

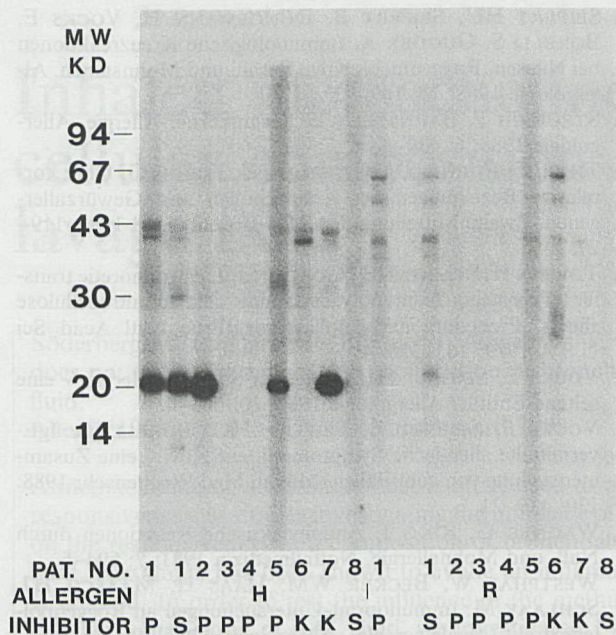


Fig. 2. Inhibition immunoblotting from SDS-PAGE gel. Allergen blotted onto strip and inhibiting extract: H – hazelnut, R – rye, K – kiwi, S – sesame, P – poppy. 1–8 – positive patient sera. IgE was detected with isotope-labeled antibody.

Inhibition immunoblotting

The weakening of hazelnut and rye bands by preincubation of patient sera with extracts of kiwi, sesame seed, or poppy seed allergen extracts indicated that cross-reacting components were present (Figs. 1 and 2).

The inhibition of hazelnut strips by kiwi, sesame seeds, and poppy seeds was high for most IgE-binding components. For CS (patient 3) and DZ (patient 4), the hazelnut bands were almost extinct, while for CW (patient 1), FW (patient 2), SG (patient 5), and CD (patient 7), one remaining band of approximately 20 kDa was observed. For those patients, this component seemed to be hazelnut specific; however, KJ (patient 6) seemed to recognize that this component cross-reacted with kiwi.

The bands detected on rye strips were considerably reduced by inhibition of kiwi, sesame seed, and poppy seed extracts with all the patient sera studied. This was particularly clear for SG (patient 5) and KJ (patient 6), whose results showed a large number of densely stained bands on the noninhibited strips.

Discussion

Laboratory results

The presented results indicate the existence of several components present in hazelnuts, rye grain, kiwi, sesame seeds, and poppy seeds which give rise to cross-reacting IgE antibodies. This corresponds well with earlier obtained RAST-inhibition results (13).

Because of the different levels of specific IgE in different sera, it is impossible to make a single exposure of the radiographic film that will satisfactorily show all bands in all patients. Figs. 1 and 2 show an intermediate exposure time selected as the most representative.

In five (patients 1, 2, 5, 6, 7) out of eight hazelnut-allergic patients' sera, a very strong band with the molecular weight of 20 kDa was seen. This band could not be inhibited by poppy seed or sesame seed extract with any of the sera used. This component was thus a unique hazelnut allergen for those patients; however, the 20-kDa component was inhibited by the kiwi extract, with no. 6 patient's serum, but was not inhibited by kiwi extract with another patient's serum (no. 7). Studying sera for rye grain sensitization, we found that most antirye IgE-bindings could be inhibited with sesame seed, poppy seed, or kiwi extracts, and no unique rye grain component could be detected.

Clinical implications

Our own clinical findings in patients with birch-pollen syndrome and selective intolerance of carrot and celery also suggest that the number of allergenic epitopes in plant food is smaller than anticipated. Possible relations between the birch-pollen syndrome and the mugwort-celery-syndrome were reported by Halmepuro & Löwenstein (6). Common protein/glycoprotein structures and enzymes may exist that are shared by many different plants.

Clinical experience shows that some foods are differently tolerated at different times (11) and may influence the course of the atopic disease. It is possible that the cumulative effects of weakly sensitizing allergens cross-reacting with the main allergen give rise to the increased clinical symptoms.

In contrast to hazelnut sensitization, sensitization to rye grain or flour is in most cases without symptoms. According to clinical experience, hazelnuts are strongly allergenic to most patients whether cooked or not, while rye flour is commonly allergenic only as unprocessed flour (e.g. baker's allergy). One explanation could be the presence of heat-stable, unique hazelnut components influencing the clinical expression.

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