

Serological characterization of allergens in poppy seeds

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Summary

Background and objective Poppy seeds in food can induce immediate-type allergic reactions ranging from mild local symptoms to severe anaphylactic reactions. Previous publications showed that poppy seeds cross-react with other plant-derived allergens. The IgE-binding components have not been defined so far.

Methods We analysed sera from 11 patients with adverse reactions after ingestion of poppy seed-containing food by IgE-immunoblotting. Nine of 11 patients showed concomitant IgE binding to allergens of birch, mugwort or grass pollen in RAST-CAP, and suffered from characteristic seasonal symptoms.

Results Ten of 11 patients showed IgE binding to a 45-kDa protein, 4/11 to a 34-kDa, 5/11 to a 17-kDa, 5/11 to a 14-kDa, and 3/11 to a 5-kDa component. Furthermore, individual IgE binding to proteins of 20, 25, 30 and 40 kDa proteins could be observed. Periodate treatment of blots markedly reduced the IgE binding capacity of the 40- and 45-kDa compounds, indicating the existence of IgE epitopes of the carbohydrate type. Inhibition studies indicated the presence of homologues of pollen allergens in extracts from poppy seeds, i.e. Bet v 1 and Bet v 2.

Conclusion The serological analysis showed IgE binding to protein and sugar components of poppy seeds. The 40- and 45-kDa allergens are glycoproteins and contain IgE binding carbohydrate moieties. Moreover, cross-reacting homologues of pollen allergens including Bet v 1 and profilin were detected in poppy seed extract.

Keywords: Bet v 1, cross-reactivity, food allergy, IgE, Papaveraceae, pollen, poppy seeds, profilin

Clinical and Experimental Allergy, Vol. 29, pp. 1075–1079. Submitted 12 May 1998; re-revised 8 December 1998; accepted 22 December 1998.

Introduction

The poppy plant (*Papaver somniferum*) is a member of the botanical family Papaveraceae. Seeds of this plant are traditionally used in the cuisine, e.g. in the making of bread. However, during the recent years allergic reactions after the ingestion of poppy have been reported [1,2], with some incidences of anaphylactic systemic reactions [3,4]. Immunological cross-reactivity with allergens from nuts, cereals and sesame were recognized by case history, skin prick tests (SPT) and RAST inhibition experiments [5]. Vocks *et al.* reported common allergenic structures present

in hazelnut, ryegrain, sesame seeds, kiwi and poppy seeds [6]. The aim of this study was to characterize allergens from poppy seeds by immunoblotting. Many food allergies to plant-derived antigens have been described to be associated with pollinosis [7–10]. In this respect, profilin, an allergen first discovered in birch pollen, has been shown to be ubiquitously present in plants, and even in higher organisms [11]. The major allergen of birch pollen (Bet v 1) has been described in many reports as cross-reactive allergen in pollen-associated food allergies [9]. Recently, a group of allergens with higher molecular weight (40–70 kDa) has gained interest in pollen-associated food allergy [12–14].

In this study, we analysed IgE binding to poppy seed extract serologically and looked into putative associations between sensitization to pollen and food allergy to poppy.

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Table 1. Characteristics of patients with adverse reactions after the ingestion of poppy seeds

Patient no.	Symptoms			Pollen RASTs	Adverse reactions to other food	PRIST	Sex	Age (years)
	Local	Abdominal	Systemic					
1	OAS†		Rhinoconjunctivitis, pruritus	g = 2, w = 3*	Nuts, cinnamon	848	M	39
2	OAS		Urticaria, pruritus	g = 5, w = 2, t = 2	Nuts	413	W	16
3	OAS		Exacerbation of AD‡, cough	g = 0, t = 0	Nuts, sesame seeds	418	W	5
4	OAS		Urticaria	g = 0, w = 0	Nuts, kiwi, orange, lemon	119	W	12
5		Vomiting	Exanthema, wheezing	g = 3, w = 2, t = 3	Nuts, mustard	99	M	15
6	OAS		Exacerbation of AD, rhinoconjunctivitis	g = 6, t = 1	Nuts, soya, egg white	1035	M	8
7	OAS	Vomiting, abdominal pain, diarrhoea	Flush	g = 4	Pea, celeriac, sesame	106	M	28
8	OAS	Abdominal pain, nausea	Rhinitis, wheezing	g = 3.4, t = 2, w = 0	Nuts, apples	54	M	30
9			Asthma, exacerbation of AD	g = 2, w = 3	Nuts	1395	M	10
10			Anaphylaxis, eczema, exacerbation of AD	g = 3, t = 0	No	774	M	29
11	OAS	Nausea	Wheezing, pruritus	t = 3, w = 1	Nuts, strawberries, fish	170	F	40

* g: RAST-CAP coupled with timothy grass pollen extract; t: with birch pollen extract; w: with mugwort pollen extract.† OAS: oral allergy syndrome. ‡ AD: atopic dermatitis.

Materials and methods

Patients

Patients' characteristics are outlined in Table 1. All patients showed IgE reactivity to one or more pollen allergens in RAST, and all patients had characteristic seasonal symptoms. Ten of 11 patients also showed adverse reactions to foods other than poppy.

Skin prick tests (SPTs)

SPT was performed in all patients on the volar aspect of the forearm using Alutard SQ poppy extract (ALK, Hoersholm, Denmark) in a concentration of 10 HEP. As positive reference histamindihydrochlorid (10 mg/mL) was used. SPT was performed according to international guidelines.

RAST-CAP

RAST-CAP was performed according to the manufacturer's instructions, using RF224 (poppy seed extract), g₃ (timothy grass pollen), t₃ (white birch pollen) and w₆ (mugwort pollen) from Pharmacia (Uppsala, Sweden).

Protein extracts for immunoblots

Roasted poppy seeds were purchased in a local shop, homogenized in a food processor and grinded in liquid nitrogen before extraction. Pollen from white birch, timothy grass and mugwort were purchased from Allergon AB (Engelholm, Sweden). Proteins were extracted 10% (w/v) in 10 mmol/L potassium phosphate buffer (pH 7.0) containing 2% (w/v) suspended solid polyvinylpyrrolidone (PVPP), 2 mmol/L ethylene diamine tetraacetic acid (EDTA), 10 mmol/L sodiumdiethyldithiocarbamate (DIECA), and 3 mmol/L sodium azide, by overnight shaking at 4°C. After centrifugation at 40 000 *g* for 60 min at 4°C, supernatants were extensively dialysed. Dialysates were lyophilized and stored at -20°C. Protein concentrations were determined according to the method of Bradford [15].

SDS-PAGE and Immunoblotting

SDS-PAGE gels (12%) were run in vertical slab gel apparatus (Biometra, biomedizinische Analytik GmbH, Göttingen, Germany), samples were heated at 95°C, 5 min and 50 µg/cm gel loaded. The transfer of proteins was done using nitrocellulose with 0.4-µm pore size.

For IgE-immunoblotting, nitrocellulose strips were blocked in 50 mmol/L sodium phosphate buffer (pH 7.5), 0.5% (w/v) bovine serum albumin, 0.5% (v/v) Tween 20. Patients' sera were diluted 1:4 in blocking buffer and

incubated with blot strips overnight at 4°C. IgE was detected by ¹²⁵I-rabbit antihuman IgE (MALT Allergie System, IBL GmbH, Hamburg, Germany), 1:20 in blocking buffer. Blots were exposed to Kodak Biomax MS films at -70°C, for 2 or 5 days, respectively.

For inhibition experiments, sera were pre-absorbed with recombinant allergens rBet v 1, rBet v 2 (Biomay Biotechnik Produktions- und Handels GesmbH, Linz, Austria) (5 µg/mL diluted serum), or with pollen extracts (50 µg/mL), overnight at 4°C. For the detection of IgE-binding carbohydrate determinants on poppy proteins, serum 'pf 192' (kindly provided by Dr Ronald van Ree, Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands) containing anticarbohydrate IgE was used [16].

Periodate treatment of blotted poppy extract

Deglycosylation of nitrocellulose-blotted poppy seed extract was performed with 0.01 mol/L NaIO₄ in acetate buffer (pH 5.0) at room temperature in the dark. The reaction was stopped by extensive washes with dd H₂O.

Results and discussion

Eleven patients with positive poppy RAST-CAP and clinical symptoms (OAS, gastrointestinal complaints, or systemic reactions; Table 1) after ingestion of poppy-containing food were investigated. Testing their sera we could demonstrate IgE reactivity with separated blotted poppy seed proteins (Fig. 1a). Patients 1, 7 and 8 displayed positive RAST and IgE in immunoblot, but the skin tests with commercially available extracts were negative. However, the usefulness of skin testing is a controversial subject for diagnosis of food allergy [17]. Taking SPT (8/11 positive; Fig. 1a), RAST-CAP (all patients showed clearly detectable IgE in RAST-CAP) and immunoblot together, type I allergy to poppy could be diagnosed in all patients.

Nine of the 11 patients had symptoms induced by pollen during spring to late summer season already before development of the food hypersensitivity to poppy, and showed positive IgE to at least one pollen species in RAST-CAP. To investigate a putative cross-reactivity with pollen allergens we performed IgE inhibition experiments using recombinant allergens: rBet v 1a and rBet v 2 (profilin) (data not shown). These experiments showed that homologues of Bet v 1 and profilin, already known as broadly cross-reactive allergenic proteins [9,11], are also present in seeds of the poppy plant of the order Papaverales. One IgE binding protein in poppy had a molecular mass of 45 kDa and was recognized by 10/11 patients, this protein can therefore be considered as a major allergen. Moreover, IgE binding to this 45-kDa allergen was inhibited by

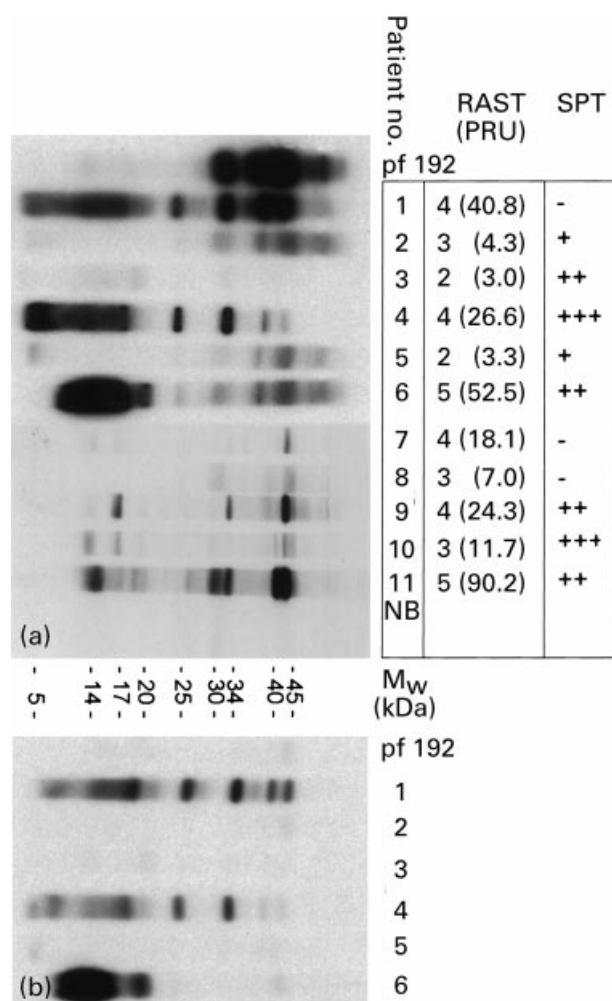


Fig. 1(a) Comparison of results of RAST-CAP, IgE immunoblots and SPT with poppy seed extract of 11 patients with food allergy to poppy seeds (lanes 1–11) in comparison with serum of a patient with high levels of anticarbohydrate IgE (lanes 'pf 192'). Patient numbers correspond with numbers in Table 1. A SPT reaction was recorded as +++ when the weal was equal to the histamine control, ++ were given for a weal half the positive control, + for smaller than half. Immunoblot: diluted patients' sera were applied to blots and bound IgE detected with ^{125}I -labelled antihuman IgE. Lane N: serum of a nonatopic volunteer, lane B: buffer control. The autoradiogram is shown. Molecular masses (kDa) of poppy allergens calculated from comparisons with graduated markers. (b) Poppy extract was treated by periodate (deglycosylation) before IgE-immunoblotting. Patient numbers correspond with numbers in (a).

pre-absorption with extracts of birch, mugwort and timothy grass pollen (Fig. 2), indicating additional cross-reactive structures in pollen and poppy extracts. In contrast, IgE binding to the 34-kDa poppy allergen was not affected (Fig. 2). This allergen might therefore represent a poppy-specific allergen.

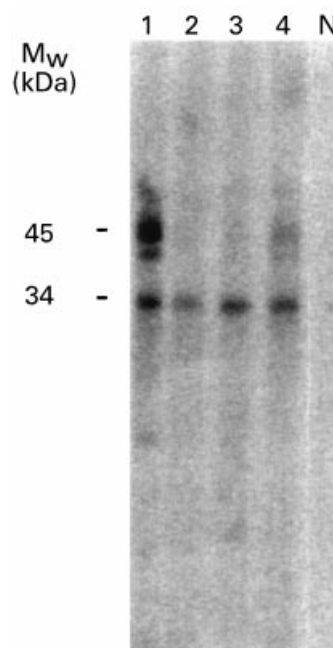


Fig. 2. Immunoblot testing serum of patient number 8 for IgE binding to poppy extract. IgE binding is shown before (lane 1) and after pre-incubation with extracts from birch pollen (lane 2), mugwort pollen (lane 3) or timothy grass pollen (lane 4). Lane N: serum of a nonatopic volunteer.

Carbohydrate determinants on plant-derived glycoproteins are possible candidates for broadly cross-reacting IgE epitopes [16,18–21]. To further characterize the nature of these higher molecular weight allergens in poppy, we used serum 'pf 192' with high levels of anticarbohydrate IgE for immunoblotting [16]. We observed intense IgE reactivity with the 30-, 40- and 45-kDa poppy allergens, which was completely abolished after periodate treatment of blotted poppy extract (Fig. 1b). The same phenomenon was observed when testing sera of poppy-allergic patients, indicating that the band structure between 40 and 50 kDa consists of glycoproteins which harbour carbohydrate epitopes for IgE. Therefore, regarding this allergen, carbohydrate epitopes alone, or in concert with protein epitopes, might be responsible for IgE binding and serological IgE cross-reactivity with pollen allergens. In sera of patients number 2, 5, 7 and 8 (Fig. 1a), however, the higher molecular weight allergens were recognized exclusively. Therefore, we suggest that in poppy seed allergy also anticarbohydrate IgEs might be of clinical importance, even when described to be of low biological activity [16].

In species belonging to the family Apiaceae (anise, fennel, coriander and cumin) a similar situation could be observed [13]. Interestingly, sera of poppy-allergic patients also displayed IgE binding to extracts from anise, fennel,

coriander and cumin (data not shown), again indicating the presence of homologous cross-reactive structures in these plant-derived foods. In conclusion, the reactivity pattern is highly reminiscent of the IgE binding proteins responsible for the 'celery–mugwort–spice syndrome' [7,12,21], where the cross-reactive allergens could also be identified as profilin, Bet v 1 and molecules with higher molecular mass.

In synopsis with published data on cross-reactivity between poppy and plant food (e.g. kiwi, nuts or flour [4–6]) we suggest a common mechanism related to sensitization with pollen allergens [22].

Acknowledgements

This work was supported by S06704-MED from the Fonds zur Förderung Wissenschaftlicher Forschung (FWF), Vienna, Austria. We thank Ms Magdolna Vermes for excellent technical assistance.

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