

Immunoblot Analysis of IgE-Binding Antigens in Paprika and Tomato Pollen

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Key Words

Allergy, occupational · Horticulture

Abstract

Background: The high incidence of occupational allergy in horticulture has only recently been recognized. We determined IgE against pollen and fruit from paprika and tomato plants in sera from 3 greenhouse workers and in 3 sera from food-allergic patients. **Methods:** Proteins in extracts of paprika and tomato pollen were incubated with patients' sera after covalent coupling of these proteins to agarose beads, or in immunoblots. **Results:** IgE against paprika pollen, but no IgE against tomato pollen, was found in serum from 2 greenhouse workers who worked with paprika plants only. IgE binding of these 2 sera to agarose-bound paprika pollen extract could be inhibited by paprika pollen but not by tomato pollen extract. A greenhouse worker, who cultivated tomato plants, had IgE against both tomato and paprika pollen. IgE binding of this serum to agarose-bound paprika pollen extract could be inhibited by both paprika pollen and tomato pollen extract. Three food-allergic patients also had IgE against tomato and paprika pollen. IgE from 2 food-allergic patients recognized IgE-binding structures in paprika or tomato pollen that were also present in fruit from the corresponding plant. In contrast, no substantial cross-reactivity was observed between paprika pollen and fruit towards IgE from 3 greenhouse workers. In 4 of

5 sera that were positive in the paprika pollen immunoblot major IgE binding to allergens of about 30 and 64 kD occurred. **Conclusion:** The presence of IgE against paprika or tomato pollen is not restricted to workers in horticulture; IgE against these pollen can also be present in food-allergic patients who have serum IgE against paprika and/or tomato fruit.

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Introduction

Allergic sensitization to workplace antigens can result in occupational asthma, rhinitis and dermatoses. Proteins, derived from natural sources encountered in the workplace, may induce the production of specific IgE antibodies. Subsequent exposure to these proteins can lead to clinical manifestations of allergy through release of mediators from mast cells and basophils and from inflammatory cells, attracted to the site of exposure [1].

We previously described a case of occupational allergy to paprika plants in a greenhouse worker and demonstrated specific IgE against the flower, leaf and stem from paprika plants in his serum [2]. Since that time IgE against paprika pollen could be demonstrated in 2 other greenhouse workers who experienced symptoms of allergy upon contact with paprika plants. Furthermore, IgE against tomato pollen was detected in serum from a patient who worked in a greenhouse where tomatoes were

Table 1. Measurement of IgE against paprika and tomato pollen and fruit using Sepharose-coupled allergen extracts

Patient	Total IgE kU/l	Specific IgE, kUa/l			
		paprika pollen	tomato pollen	paprika (fruit)	tomato (fruit)
1/F	2,898	3.8	1.3	ND	ND
2/F	780	25.4	28.3	35.5	32.5
3/G	328	10.5	<0.35	7.1	0.4
4/F	3,754	11.7	8.0	12.1	13.1
5/G	220	12.1	0.41	<0.35	<0.35
6/G	1,200	18.0	35.8	1.6	9.9
7c	350	<0.35	<0.35	ND	ND
8c	1,998	<0.35	<0.35	<0.35	<0.35
9c	2,800	<0.35	<0.35	ND	ND
10c	4,753	2.6	1.7	ND	ND
11c	771	ND	ND	<0.35	<0.35

F = Food allergy; G = greenhouse worker; c = control serum; ND = not done.

grown. With these 3 sera, we analyzed the pattern of IgE binding to paprika and tomato pollen by immunoblot analysis. By cross-inhibition experiments, we explored the presence of common IgE-binding structures in pollen from these two botanically related plants and in pollen and fruit from the same plant. Both plants belong to the Solanaceae family and are cultivated in large amounts in greenhouses.

Little is known about the properties of IgE-binding structures in paprika and tomato pollen. Allergens in paprika and tomato fruit were recently investigated by Jensen-Jarolim et al. [3, 4] and Petersen et al. [5], respectively. Jensen-Jarolim et al. [3, 4] characterized profilin as a major IgE-binding structure in fresh paprika fruits. They further identified a 23-kD pathogenesis-related protein as another IgE-binding structure in paprika fruit. Petersen et al. [5] demonstrated IgE binding to a profilin in SDS-PAGE separated extract of tomato fruit. They also demonstrated IgE binding to carbohydrate epitopes on several glycoproteins in the 18- to 70-kD range.

Patients and Methods

Patients

3 sera (serum 3, 5 and 6 in table 1) were from patients who experienced symptoms of allergy when working with paprika or tomato plants in greenhouses. 3 other sera (serum 1, 2 and 4) were from

patients with symptoms of food allergy who were found positive in the paprika CAP-RAST or tomato CAP-RAST. Control sera were selected from sera, submitted for routine diagnostic IgE antibody measurements.

Patient 1, male, 6 years old, had eczema, food allergy and asthma. CAP-RASTs: house dust mite >100 kUa/l, *Dactylis glomerata* grass pollen 19.7 kUa/l, cat 10.2 kUa/l, egg white 9.3 kUa/l, milk 0.52 kUa/l, peanut 43.0 kUa/l, paprika >100 kUa/l, tomato 67.9 kUa/l.

Patient 2, female, 23 years old, had asthma, rhinitis and food allergy. There were positive skin tests for grass, birch and mugwort pollen. CAP-RASTs: peanut 43.1 kUa/l, hazelnut 21.3 kUa/l, tomato 39.7 kUa/l, apple 21.1 kUa/l, celery 39.3 kUa/l, tomato 39.7 kUa/l, paprika 38.9 kUa/l.

Patient 3, male, a 26-year-old greenhouse worker, rhinitis upon contact with paprika plants, no symptoms of food allergy. Negative skin tests for grass, birch and mugwort pollen.

Patient 4, female, 21 years old, rhinitis, eczema, food allergy. CAP RASTs: house dust mite 28.7 kUa/l, grass pollen >100 kUa/l, wheat 15.9 kUa/l, peanut 18.5 kUa/l, soy 9.5 kUa/l, hazelnut 5.7 kUa/l, tomato 20.1, apple 12.1, celery 16.7, peach 8.6 kUa/l, garlic 24.2 kUa/l, potato 24.0 kUa/l, paprika 18.1.

Patient 5, male, an 18-year-old greenhouse worker, rhinitis upon contact with paprika plants, no symptoms of food allergy. Positive skin tests for grass pollen and birch pollen.

Patient 6, female, a 24-year-old greenhouse worker, rhinitis and asthma upon contact with tomato plants. Positive skin tests for grass, birch and mugwort pollen. This patient has been described before [6]. There were no symptoms of food allergy.

Allergens

Pollen from paprika and tomato plants were collected in greenhouses; the complete stamen was removed from the flowers of both plants. Paprika and tomato fruit were obtained from a local grocery. House dust mite culture (*Dermatophagoides pteronyssinus*) was obtained from the Laboratorium Diephuis, Houten, The Netherlands.

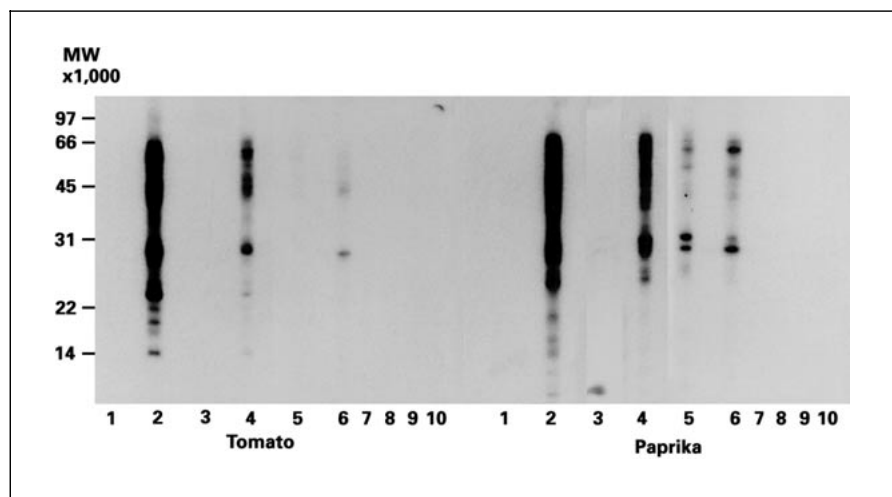
Methods

For immunoblotting, complete paprika or tomato stamens were extracted (10% w/v) in demineralized water for 1 h at 4 °C with shaking. After centrifugation, the supernatant was frozen in small aliquots at -20 °C. Protein in these supernatants was determined with the pyrogallol red method [7] and ranged between 4 and 5 mg protein/ml. Before electrophoresis, 1/10 dilutions (in sample buffer, including sodium dodecyl sulfate and dithiothreitol) of these supernatants were placed at 100 °C for 5 min.

SDS-PAGE and Immunoblotting

SDS-polyacrylamide gel electrophoresis and electroblotting were performed with the Mini Protean II system according to the manufacturer's instructions (BioRad Laboratories, Veenendaal, The Netherlands). Per slot 5 µg protein was applied for electrophoresis. The Western blots were incubated in the Mini Protean Multiscreen apparatus (Biorad) with patient sera, diluted 1:4 in phosphate-buffered saline, 0.2% Tween 20, 1% BSA, in a total volume of 600 µl per channel. For electrophoresis a 12% resolving gel and 4% stacking gel were used; for protein blotting a 0.45-µm pore size nitrocellulose (Biorad) was used. Bound IgE was, after incubation with radioiodinated anti-IgE antibodies (Pharmacia & Upjohn, Woerden, The Netherlands), visualized by autoradiography at -70 °C.

Fig. 1. IgE binding to SDS-PAGE separated extracts of tomato and paprika pollen after incubation with the same sera 1–10c as shown in table 1. In the paprika pollen blot, lanes 1, 2 and 4 were exposed to X-ray paper for 1 day and lanes 3 and 5–10 for 6 days. In the tomato pollen blot, lanes 1–4 were exposed for 1 day and lanes 5–10 for 6 days. Exposure of lanes 1–4 for 6 days also gave no bands for lanes 1 and 3.



IgE Antibody Measurements

Total IgE and specific IgE against common allergens were determined with the Pharmacia CAP system (Pharmacia & Upjohn) according to the manufacturer's instructions. This manufacturer has chosen paprika powder and frozen tomato juice as allergen source in the paprika and tomato CAP-RAST [8].

Specific IgE against paprika and tomato pollen was determined by RAST using agarose beads as allergen support [2]. 10 mg paprika or tomato stamen were extracted for 1 h in 2 ml coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl). After centrifugation, protein in the supernatant was covalently coupled to 100 mg CNBr-activated Sepharose 4B (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). After 3 cycles of acid-base washings, the pollen-Sepharose preparations were suspended in 25 ml RAST buffer (0.82% NaCl, 10 mM phosphate, 0.1% NaN₃, 0.2% Tween 20, 0.3% bovine serum albumin, pH 7.4). Per test, 2 mg paprika pollen-Sepharose or 1 mg tomato pollen-Sepharose were incubated for at least 4 h with 0.05 ml patient serum. After 3 washes with physiological saline, 0.2% Tween 20 and 1 wash with RAST buffer, the pollen Sepharose was incubated overnight with radioiodinated anti-IgE antibodies (Pharmacia & Upjohn). After 4 washes with physiological saline, 0.2% Tween 20, bound radioactivity was counted in a gamma counter (Packard, Groningen, The Netherlands). Bound radioactivity was converted into kUa/l by the CAP standard curve. Specific IgE against paprika and tomato fruit was also determined using agarose beads as allergen support [2]. Both fruits were extracted as described by Jensen-Jarolim et al. [3] for the extraction of bell peppers. After dialysis and lyophilization, extracts were stored at –20°C. 10 mg of lyophilized fruit extract, dissolved in 2 ml coupling buffer, was incubated with 100 mg CNBr-activated Sepharose as described above. Per test, 6 mg paprika fruit Sepharose or 6 mg tomato fruit Sepharose were incubated with 0.05 ml patient serum and processed further as described above for paprika and pollen Sepharose.

IgE Inhibition Experiments

For IgE inhibition experiments, 10 mg house dust mite culture, paprika stamen or tomato stamen were incubated for 1 h with 1 ml RAST buffer under rotation. 10 ml lyophilized paprika fruit extract

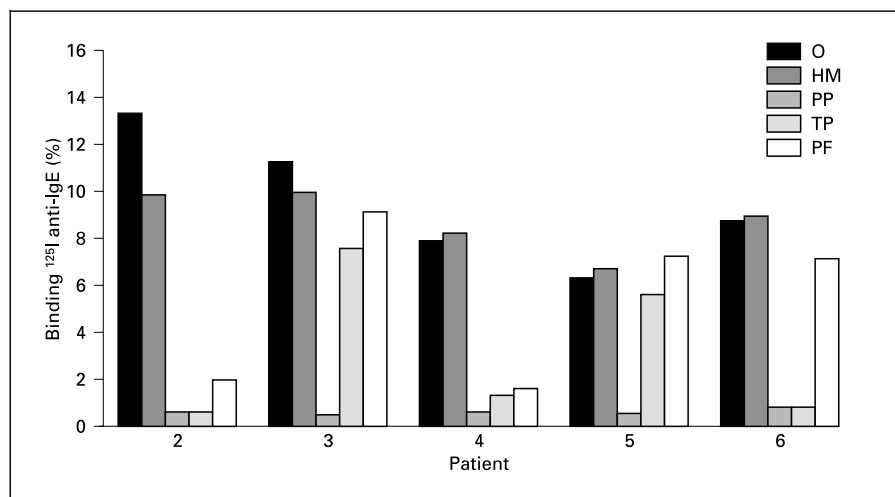
or tomato fruit extract were mixed for 1 min with 1 ml RAST buffer. After centrifugation, 0.05 ml supernatant was incubated overnight at room temperature with 0.05 ml serum diluted in RAST buffer (sera 2 and 6 were diluted 1:3, and sera 3, 4 and 5, 1:2 in RAST buffer, see table 1). After centrifugation, 0.05 ml supernatant was incubated with paprika pollen and tomato pollen Sepharose as described above.

Results

Specific IgE against paprika pollen could be demonstrated in all 3 patients with occupational allergy to paprika or tomato plants, but also in 3 patients with food allergy selected because they were found positive in the paprika and tomato CAP-RAST (table 1). Sera 3 and 5 were negative and borderline positive, respectively, in the tomato pollen IgE assay; these patients only worked with paprika plants. Patient 6 had worked for 4 years in a greenhouse where tomato plants were grown; in her serum more IgE against tomato pollen was found than against paprika pollen.

Only sera with elevated specific IgE levels (8 kUa/l or higher) were positive in immunoblotting experiments. Serum from patient 3 only showed IgE binding to a low (<14 kD) band in paprika pollen, which bound no substantial IgE after incubation with sera 5 and 6; IgE from these 2 sera was preferentially bound to bands of about 30 and 64 kD. Sera 2 and 4 gave the strongest IgE binding in these experiments. The difference in the extent of IgE binding between sera 2 and 4, and the other sera is even larger than figure 1 suggests, because lanes 2 and 4 had been exposed to X-ray paper for 1 day and the other lanes for 6 days.

Fig. 2. IgE binding to paprika pollen Seph-
rose after preincubation of patient serum
with RAST buffer (O) and extracts in RAST
buffer of house dust mite culture (HM), pa-
prika pollen (PP), tomato pollen (TP) and
paprika fruit (PF).



In IgE inhibition experiments, IgE binding of sera 2 and 4 (both from 2 patients with food allergy) to paprika pollen could be strongly inhibited by preincubation of these 2 sera with extracts of both paprika and tomato pollen, as well by an extract of paprika fruit (fig. 2). In contrast, with sera 3 and 5 (both patients worked with paprika plants in greenhouses) IgE binding to paprika pollen could be inhibited by preincubation with an extract of paprika pollen only. With serum 6, from a patient who cultivated tomato plants, IgE binding to paprika pollen could be inhibited by preincubation with both paprika and tomato pollen extract; no inhibition was observed after preincubation of this serum with paprika fruit extract. IgE binding of sera 2 and 4 (both from patients with food allergy) to tomato pollen could be inhibited by preincubation of these 2 sera with extracts of both tomato and paprika pollen as well by an extract of tomato fruit (fig. 3). With serum 6, from the patient who cultivated tomato plants, IgE binding to tomato pollen could be completely inhibited by preincubation of this serum with tomato pollen extract. Preincubation of serum 6 with paprika pollen and tomato fruit extract caused partial inhibition of IgE binding to tomato pollen.

Discussion

The present study extends and confirms previous reports [2, 6] on IgE against paprika pollen in paprika growers. Our results indicate that IgE against proteins from paprika and tomato pollen extracts is not only demonstrable in sera from greenhouse workers, but also in sera from

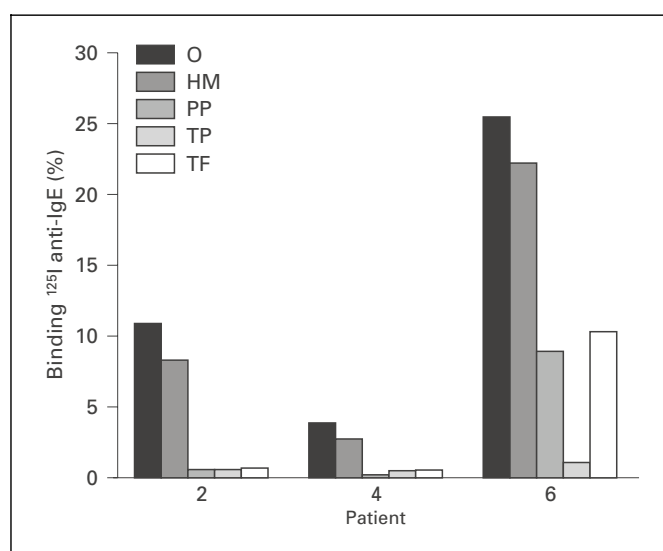


Fig. 3. IgE binding to tomato pollen Sepharose after preincubation of patient serum with RAST buffer (O) and extracts in RAST buffer of house dust mite culture (HM), paprika pollen (PP), tomato pollen (TP) and tomato fruit (TF).

food-allergic patients with IgE antibodies against paprika and tomato fruit.

In a study on spice allergy in patients with food allergy, many positive skin tests to paprika powder were observed by Niinimäki and Hannuksela [9]. Respiratory allergy to paprika powder has been described as well [10, 11]. This probably led to the choice of paprika powder as allergen source for the preparation of commercially available

(CAP)-RAST reagents [8]. Jensen-Jarolim et al. [3] suggested that allergens in paprika fruit can be destroyed during processing to paprika powder. Therefore, we extracted fresh paprika and tomato fruit according to Bjorksten et al. [12] and Jensen-Jarolim et al. [3]. IgE binding to such extracts, covalently coupled to Sepharose (table 1), was rather slightly lower than to the commercially available CAP-RAST reagents (see Patients). In the paprika (powder) CAP-RAST serum 5 scored higher than serum 3; the reverse was seen when using the paprika (fruit) Sepharose preparation (table 1). Besides a difference in processing, horticultural strain differences may be the cause of this discrepancy [3].

Jensen-Jarolim et al. [3] found predominant IgE binding to a 14-kD profilin in the paprika fruit. Patients 2 and 4 with food allergy showed some IgE binding to a 14-kD

band in our experiments (fig. 1), but more IgE binding to other bands with a higher molecular weight was observed.

None of the 3 patients with occupational allergy to paprika or tomato pollen reported symptoms of allergy when consuming paprika or tomato fruit. However, in a previous report [6] we described a patient with rhinoconjunctivitis upon contact with paprika plants. This paprika grower experienced facial angioedema the next day after having eaten paprika fruit.

In summary, this study demonstrates the presence of IgE against tomato and paprika pollen in both 3 greenhouse workers and 3 food-allergic patients. The IgE inhibition experiments indicate a complex pattern of cross-reactivity between paprika and tomato pollen and between pollen and fruit from the same plant.

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