

Occupational Allergy in Horticulture: Demonstration of Immediate-Type Allergic Reactivity to Freesia and Paprika Plants

A.W. van Toorenenbergen^a, P.H. Dieges^b

^a Laboratory of Allergology, Department of Clinical Chemistry, and

^b Department of Allergology, University Hospital Rotterdam, The Netherlands

Abstract. Patients A and M developed allergic symptoms when working in a greenhouse with paprika plants and freesia plants, respectively. The possible involvement of an IgE-mediated mechanism was investigated with the skin prick test, radioallergosorbent test (RAST) and the histamine release test (HRT). Paprika flower, leaf and stem extract released 58, 47 and 43% of the total amount of histamine from washed leukocytes of patient A. In serum A IgE antibodies against paprika leaves and flowers could be demonstrated by RAST (11% binding of ¹²⁵I-anti-IgE added). Freesia flower and stem extract released 46 and 43% histamine, respectively, from washed leukocytes of patient M. In the RAST, specific IgE antibodies against freesia flowers and stems were found in serum M (37% binding of ¹²⁵I-anti-IgE added).

Introduction

Certain plant materials, to which occupational exposure is not unusual are very potent allergens. IgE-mediated allergies to dust of wheat flour, castor bean, green coffee bean and acacia gum are well-known causes of occupational asthma [1]. In this paper, we describe two patients who developed allergic symptoms when working in a greenhouse with paprika plants (*Capricum annum*) or freesia plants (*Freesia hybride*). An IgE-mediated allergy to freesia and paprika plants could be demonstrated with the skin prick test (SPT), radioallergosorbent test (RAST) and histamine release test (HRT).

Patients

Patient M, female, 24 years old, had worked since 1978 in a firm where various flowers (including freesia) were cultivated in green houses. Since early life she had eczema and rhinoconjunctivitis and since 1968 intermittent bronchial asthma; since autumn 1981, her asthma has been chronic. In serum from patient M, IgE against house dust mite and grass pollen was found by RAST (17% and 32% binding of ¹²⁵I-anti-IgE) but no IgE against cat dander. Her mother had eczema, rhinitis and bronchial asthma, her sister was allergic to cats and dogs.

Patient A, male, 20 years old, had worked since 1977 in a firm where paprika plants were cultivated in green houses. From April 1982 he has suffered from rhinoconjunctivitis and bronchial asthma when working with paprika plants. In serum from patient A, IgE against house dust mite and cat dander was found by RAST (23% and 10% binding of ¹²⁵I-anti-IgE, respectively) but no IgE against grass pollen. His mother had eczema, rhinitis and bronchial asthma; his sister was allergic to cats and dogs.

Materials and Methods

Allergens

Fresh freesia and paprika plants were supplied by patients M and A themselves. Flowers, stems, leaves and fruit of paprika and flowers and stems of freesia were subjected separately to acetone extraction: 2-10 g of plant material were homogenized with 100 ml acetone for 1 min at room temperature. The acetone was filtered off by suction and the residual plant parts were shaken for 1 h with 100 ml acetone at room temperature. After filtering off the acetone, the 1-hour acetone extraction was repeated twice. The remaining material was air-dried overnight at room temperature. The yield (expressed as percentage of the starting material; w/w) was 6, 10, 7 and 3% for paprika flower, leaf, stem and fruit, respectively, and 7 and 11% for freesia flower and stem, respectively. House dust mite culture (*Dermatophagoides pteronyssinus*) and *Dactylus glomerata* pollen were obtained from Laboratorium Diephuis (Groningen, The Netherlands) and used as controls. (Sheep antihuman IgE antiserum (SH 25-02 - PO8) was obtained from The Red Cross Laboratory, Amsterdam, The Netherlands.)

Table I. RAST for freesia-specific IgE antibodies in serum from patient M

Serum	Freesia		<i>D. glomerata</i>	Total IgE IU/ml
	flower	stem		
-	1	1		
Patient M	36	32	380	
Controls	1	2	36	300
	2	1	6	300
	3	1	2	500
	4	2	2	500

The results are expressed in percent binding of radioactivity. The specificity of the freesia-RAST was investigated with sera from controls without allergy to freesia.

Estimation of Total IgE and Allergen-Specific IgE

Total IgE was determined by a competitive binding assay (Radioassay IgE Test) according to the manufacturer's (Pharmacia) instructions.

Allergen-specific IgE was determined by RAST using Agarose beads as allergen support [2].

Allergens were coupled to CNBr-activated Sepharose 4B according to the manufacturer's (Pharmacia) instructions.

For the preparation of *D. glomerata*, freesia- and paprika-Sepharose, 100 mg *D. glomerata* pollen or 100 mg acetone-treated plant material were extracted with 1–2 ml coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl, pH 8.5) for 1 h at room temperature. After centrifugation protein in the supernatant was coupled to 100 mg CNBr-activated Sepharose 4B. After 4 cycles of acid/base washings [2] the allergen-Sepharose preparations were washed once with RAST buffer (0.82% NaCl; 10 mM phosphate; 0.1% NaN₃; 0.2% Tween 20; 0.3% bovine serum albumin, Sigma, pH 7.4) and finally resuspended in RAST buffer. Per test 0.5 mg of *D. glomerata*-Sepharose, 2.5 mg of the various paprika-Sepharose preparations and 1.25 mg of the freesia-Sepharose preparations were used. An extract of house dust mite (*D. pteronyssinus*) was coupled to Sepharose 4B as described before [3]. 2 mg of mite Sepharose were used per test. Cat dander-Sepharose was prepared exactly in the same way as described above for *D. glomerata*-Sepharose. 2 mg cat dander-Sepharose were used per test. 50 µl patient serum were incubated for at least 4 h at room temperature with the indicated amounts of allergen-immunosorbent-Sepharose with horizontal shaking (300 cpm). After 3 washes with physiological saline, 0.2% Tween 20 and one wash with RAST buffer, radioiodinated rabbit antihuman IgE antibodies (Pharmacia) were added.

After an overnight incubation the Agarose beads were washed four times with physiological saline, 0.2% Tween 20. The percentage of bound radioactivity was counted in a LKB-Wallac gamma counter.

Histamine Release from Washed Leukocytes

The isolation of human leukocytes and the incubation of leukocytes with allergen were performed as described before [3]. In some experiments, the leukocytes were incubated in buffer that contained 50% deuterium oxide, to potentiate the histamine-release

Table II. RAST for paprika-specific IgE antibodies in patient A

Serum	Paprika				<i>D. glomerata</i>	Total IgE IU/ml
	flower	leaf	stem	fruit		
-	3	3	2	1		
Patient A	14	16	11	5	2	2,400
Controls	1	5	3	2		3,375
	2	5	4	2		2,400
	3	4	4	2		3,300

The results are expressed in percent binding of radioactivity. The specificity of the paprika-RAST was investigated with sera from 3 controls without allergy to paprika.

reaction [4]. The leukocytes were challenged with the same allergen preparations that were used in the RAST. For the use in the histamine release test, 10–20 mg house dust mite, pollen or acetone-treated plant material were incubated for 1 h at room temperature with 1 ml TACM at continuous rotation (TACM: 25 mM Tris, 120 mM NaCl, 5 mM KCl, 0.6 mM CaCl₂, 1 mM MgCl₂, 0.03% human serum albumin, pH 7.6). After centrifugation, the supernatant was used in the histamine release experiments. Histamine in samples was determined by an automated fluorometric method [5]. The amount of histamine released was expressed as the percentage of the total amount of histamine present in the cell suspension.

Skin Prick Test

1–2 mg of dry acetone-treated plant material and two drops of phosphate-buffered saline (PBS) were placed on the internal surface of the forearm. After mixing the plant material with the PBS, the skin was punctured through the fluid with a lancet. After 20 min the reactions were read. The reactions were graded in standardized plus signs [6].

Results

Allergen-Specific IgE Antibodies

In serum from patient M high levels of specific IgE antibodies were found that were directed against freesia plant material (table I); the IgE-binding component was found both in flower and stem parts of the plant. Table I also shows that there is no cross-reactivity between *D. glomerata* and freesia allergens. In serum from patient A specific IgE antibodies could be demonstrated that reacted primarily with flower and leaf components from paprika plants; IgE-binding was also seen with material extracted from paprika-stem and fruit (table II).

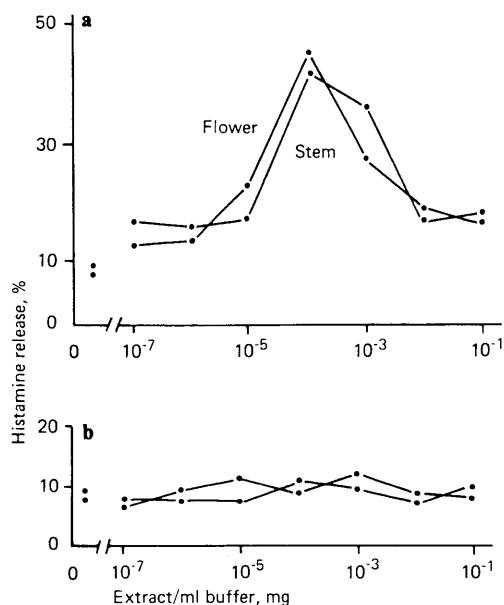


Fig. 1. Washed leukocytes from patient M (a) and from a non-allergic control (b) were incubated in TACM, 50% D₂O with extracts of freesia plant parts.

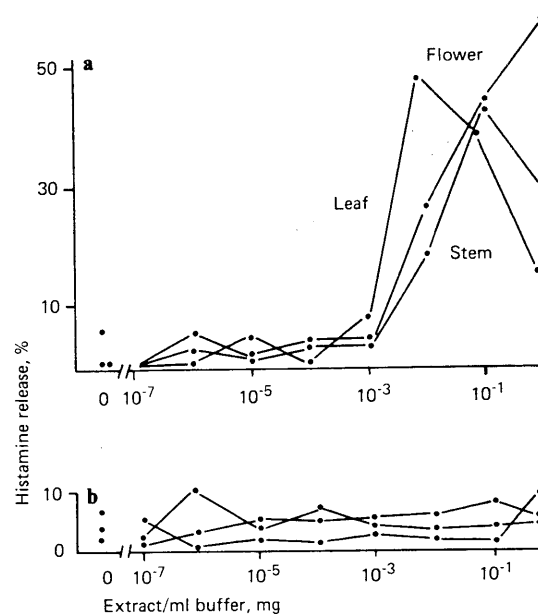


Fig. 2. Washed leukocytes from patient A (a) and from a non-allergic control (b) were incubated in TACM with extracts of paprika plant parts.

Table III. Skin prick test

	Flower	Stem	Leaf	Fruit	Buffer
Patient A	1.5+	2+	1.5+	1.5+	0
Paprika					
Patient M	2+	1.5+			0
Freesia					

Patients M and A were skin-tested with freesia and paprika plant parts, respectively.

Histamine Release from Washed Leukocytes

Figure 1 shows that components from both freesia flower and stem were able to release histamine from washed leukocytes of patient M. A maximal percentage of 25% histamine release from washed leukocytes of patient M was found after incubation with anti-IgE antiserum. After incubation with freesia stem and flower extract, leukocytes from a normal nonallergic donor did not release more histamine than leukocytes incubated in buffer alone. Figure 2 shows the release of histamine that was found after incubation of leukocytes from patient A with extracts of the various paprika plant parts. Leukocytes from a normal donor did not release histamine after incubation with these

extracts. A maximal percentage of 25 and 45% histamine release from washed leukocytes of patient A was found after incubation with anti-IgE antiserum and house dust mite extract, respectively.

Skin Prick Test

Table III shows that patients M and A scored positively in the SPT when tested with freesia or paprika plant parts. No response was seen when 3 controls were skin-tested with the freesia or paprika plant parts.

Discussion

The personal history of allergy of the two patients described above suggested that antigens from the working environment were the elicitors of the hypersensitivity reactions. The positive skin tests (table III) with paprika plant parts (patient A) and freesia plant parts (patient M) pointed to an IgE-mediated allergy to these materials.

The demonstration of specific IgE antibodies in serum from patient M (table I) and patient A (table II) corroborate the skin test results. In serum from patient M a high level of IgE antibodies against *D.*

glomerata pollen was also found. Table I shows, however, that the IgE-binding component from freesia does not cross-react to a noticeable extent with *D. glomerata* pollen: control serum No. 1 contained a large amount of IgE antibodies against *D. glomerata* pollen extract, whereas no IgE was found that was directed against freesia plant parts. Moreover, table I shows that the freesia-Sepharose used in the RAST had a negligible nonspecific IgE-binding at the serum total IgE levels tested. A high total IgE level was found in serum A; table II shows that the IgE binding from serum A to the four paprika-Sepharose preparations was distinctly higher than the IgE-binding of serum A with total IgE levels equal to or higher than the IgE level of serum A. This demonstrates that the IgE binding of serum A to the paprika-Sepharose preparations was not caused by nonspecific IgE binding. In serum A, no IgE antibodies against *D. glomerata* pollen were found; this suggests that the IgE-binding allergens from paprika plants do not cross-react with *D. glomerata* allergens.

The results of the histamine release test, in which washed leukocytes were incubated with extracts of the various plant parts (fig. 1, 2), further confirm the presence of an IgE-mediated allergy to freesia and paprika plant parts. Leukocytes from normal donors did not release histamine at concentrations of plant part extracts, where leukocytes from patient M and A released a substantial percentage of histamine; this demonstrates that the release of histamine from leukocytes of patients A and M was not caused by a cytotoxic effect of the various plant part extracts.

From tables I and II, and from figures 1 and 2, it can be concluded that IgE-binding antigens are present in several different parts of freesia and paprika plants but especially in stem and leaves. Busse et al. [7] have reported that other parts from ragweed plants than pollen grains also contain skin-test-active ragweed antigen. Rebhun et al. [8] studied the antigenic relationship of the pollen, seed and leaves of giant ragweed. From their [8] data, it can be calculated that leaves of giant ragweed contain about 100 times less skin-test-active material than giant ragweed pollen grains. In accordance with this, Shafiee et al. [9] showed that proteins extracted from mixed stem and leaves of short ragweed, contained 100 times less skin-test-active material than proteins extracted from pollen grains of the same plant.

In conclusion, the present study adds two new cases to the growing list of occupational allergies and

describes the technology for in vitro diagnosis of IgE-mediated allergies to freesia and paprika plants.

Both patients had a strong personal and familial history of atopy; this suggests that such patients are at particularly high risk to develop occupational allergy.

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Correspondence to: Dr. A.W. van Toorenenbergen,
Laboratory of Allergology,
University Hospital Dijkzigt,
Dr. Molewaterplein 40,
NL-3015 GD Rotterdam (The Netherlands)