

Immunoglobulin E antibodies against coriander and other spices

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Patient M, working with spices in the food industry, developed asthma on inhalation of dust from spices. Skin prick test results with curry, coriander, and mace were strongly positive. With RAST, specific IgE antibodies against coriander, curry, mace, ginger, and paprika powder could be demonstrated in serum from patient M. It could be demonstrated by RAST inhibition that the IgE-binding components from coriander did not cross-react with the IgE-binding components from ginger and paprika. Leukocytes from a normal donor, after passive sensitization with serum from patient M, released a substantial ($\geq 50\%$) amount of histamine on challenge with extracts of coriander, mace, and curry powder. This study demonstrates that the inhalation of dust from spices can result in an IgE-mediated allergy toward these materials. (J ALLERGY CLIN IMMUNOL 76:477-81, 1985.)

Symptoms of occupational asthma may be the result of an IgE-mediated mechanism; they can also be caused by irritant, nonimmunologic effects of inhaled industrial fumes, dusts, or gases or by direct toxic release of histamine from airway mast cells.¹ Organic dusts of wheat flour, castor bean, green coffee bean, and acacia gum are well-known elicitors of occupational asthma.¹ Recently, Falleroni et al.² described a patient who developed asthma on inhalation of garlic dust. In this article we report a new case of occupational asthma induced by exposure to the powdered spices coriander, curry, and paprika. With the use of skin prick test, RAST, and histamine-release test, the involvement of IgE antibodies in this case of occupational asthma could be demonstrated.

Case report

Patient M is a 26-year-old Portuguese man who came to Holland 15 years ago. At age 20 years he started working in a spice factory. It was his job to operate a machine that mechanically packages a constant amount of powdered spices in small plastic containers. Various spices were processed by this machine; among them were curry, coriander, mace, paprika, and ginger. The spices were fed to the ma-

Abbreviation used

PBS: Phosphate-buffered saline

chine through a large funnel. The machine stood in a large room and visibly produced dust when it was operating.

During the first 2 years of his work in the spice factory, patient M started to experience symptoms of rhinitis and shortness of breath that aggravated from Monday to Friday; symptoms were absent during weekends and at holidays. In the third year serious attacks of dyspnea with wheezing started to occur, especially at night. At this time he consulted his general practitioner who prescribed for him theophylline and who referred him to this hospital (Dijkzigt University) for further evaluation of his complaints of asthma and rhinopathy. Patient M did not smoke and had never experienced symptoms of atopic disease before his work in the spice factory. Patient M lived in a dry house; the presence of a cat at home did not evoke symptoms of allergy. There was no evidence of parasitic infection, and the history of atopic diseases in his family was negative. Physical examination and chest x-ray film were normal.

Intracutaneous skin tests were negative with extracts of grass, mugwort, and birch pollen and cat and dog dander. Only the highest concentration of house dust mite that was tested elicited a small immediate wheal-and-flare reaction. The control solution, PBS, elicited no reaction; the response to a 0.01 mg/ml histamine solution was in the normal range. The levels of specific IgE antibodies against house dust mite, grass pollen, and cat dander, when these were tested by RAST, were undetectable to low (4%, 5%, and 3% binding of ¹²⁵I-anti-IgE added).

In 1983 patient M was assigned another job that did not expose him to spices. Since that time all symptoms of atopic disease have disappeared.

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TABLE I. IgE RAST

		% Binding of ¹²⁵ I-anti-IgE		
	Patient M	Control		Blanc
		1	2	
Coriander	45	8	3	3
Curry	44	5	3	2
Mace	26	3	5	1
Paprika	30	2	2	1
Ginger	27	3	3	1
White pepper	13	1	1	1
Mugwort	5	4	3	2
Total IgE (IU/ml)	4000	4000	5250	—

Blanc = no patient serum added. Measurement of specific IgE against various spices in serum from patient M and in two control sera with high total IgE levels.

MATERIAL AND METHODS

Allergens

Powdered spices were obtained from a local grocery. The spices used were coriander (*Coriandrum sativum*), mace (*Myristica fragrans*), white pepper (*Piper nigrum*), paprika (*Capsicum tetragonum*), ginger (*Zingiber officinale*), and curry. Curry is a mixture of various spices. Important constituents of curry are coriander, *Capsicum* peppers, and black pepper.^{3,4} Mugwort pollen (*Artemisia vulgaris*) was obtained from Laboratorium Diephuis, Groningen, The Netherlands. The same allergen preparations were used in RAST, skin prick test, and histamine-release test.

Estimation of total IgE and allergen-specific IgE

Total IgE was determined by a noncompetitive binding assay (Tandem IgE kit, Hybritech, Inc., La Jolla, Calif.). Allergen-specific IgE was determined by RAST by use of agarose beads as allergen support.⁵ One hundred milligrams of spices were extracted with 1 to 2 ml coupling buffer (0.1 mol/L NaHCO₃, and 0.5 mol/L NaCl, pH 8.5) for 1 hour at room temperature. Protein in the supernatant was coupled to 100 mg of CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Two milligrams per test of the various spice-Sepharose preparations, suspended in 0.5 ml of RAST buffer (0.82% NaCl, 10 mmol/L of phosphate, 0.1% NaN₃, 0.2% Tween 20, 0.3% bovine serum albumin [Sigma Chemical Co., St. Louis, Mo.], pH 7.4), were incubated for at least 4 hours with 0.05 ml of patient serum. After four washes, radioiodinated rabbit anti-human IgE antibodies (Pharmacia) were added. After an overnight incubation and four washes, the percentage of bound radioactivity was measured.

RAST inhibition

Twenty milligrams of the various spices or mugwort pollen was mixed with 0.2 ml of RAST buffer for 1 hour at room temperature. After centrifugation, 0.1 ml of supernatant or 0.1 ml of tenfold dilutions in RAST buffer of the supernatant was incubated for at least 4 hours with 0.1 ml of a 1/100 dilution of serum from patient M in RAST buffer. After addition of 1 mg of coriander-Sepharose, the mixture was incubated overnight at room temperature. The procedure was identical thereafter to the last steps of the RAST.

Histamine release from washed leukocytes

The isolation of human leukocytes and the incubation of leukocytes with allergen were performed according to the method of Siraganian and Hook.⁶ Leukocytes from a non-allergic donor were passively sensitized in vitro according to the method of Levy and Osler.⁷ Passively sensitized leukocytes were incubated with allergen in buffer that contained 50% deuterium oxide to potentiate the histamine release reaction.⁸ Histamine in samples was determined by an automated fluorometric method.⁶

Skin prick test

One to two milligrams of powdered spices and two drops of PBS were placed on the internal surface of the forearm. After mixing the spices with the PBS, the skin was punctured through the fluid with a lancet. After 20 minutes the reactions were read. The combined wheal and erythema reactions were graded according to the skin test grading system of Norman.⁹

RESULTS

Skin prick test

Skin prick tests with powdered coriander, curry, and mace were strongly positive with patient M. Mace and curry elicited a 2½+ reaction (average erythema diameter ≈ 25 mm; average wheal diameter ≈ 10 mm), and coriander elicited a 4+ reaction (average erythema diameter ≈ 40 mm; average wheal diameter ≈ 15 mm). PBS alone produced no reaction. Four patients, nonallergic to spices, were negative in the skin prick test with mace, curry, and coriander.

Specific IgE antibodies against spices

Table I illustrates that the inhalation of dust from spices had resulted in a high level of specific IgE antibodies against various spices. The total IgE level of serum from patient M was high (4000 IU/ml); sera with comparable total IgE levels elicited much less IgE binding to the various spice-Sepharose preparations than serum from patient M (Table I). Thiel and Fuchs⁴ have suggested that common constituents of certain spices (essential oils) are responsible for the allergenic activity of these spices. To test this 1 gram

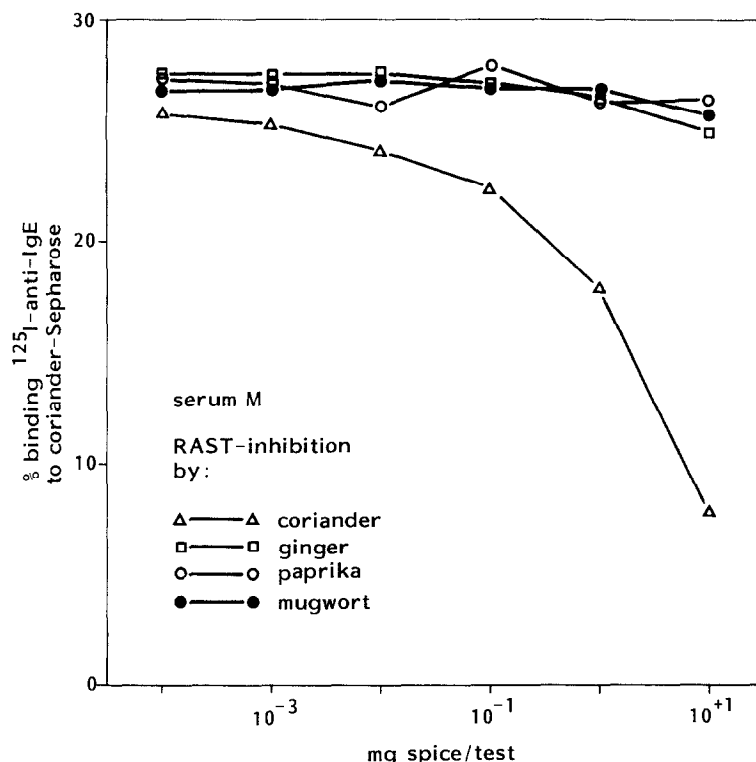


FIG. 1. Estimation of the cross-reactivity between coriander, ginger, paprika, and mugwort extract by RAST inhibition.

of powdered coriander was extracted twice with 10 ml of diethyl ether and finally dried at room temperature. An extract of this ether-treated coriander was coupled to Sepharose 4B as described above for untreated coriander. The ether extraction did not affect the binding of IgE from serum of patient M to coriander-Sepharose. Incubation of serum from patient M with Sepharose-coupled, ether-treated coriander resulted in 45% binding of ^{125}I -anti-IgE. A control serum with a total IgE level of 3500 IU/ml elicited 3% binding of ^{125}I -anti-IgE.

The cross-reactivity between various spices

With the use of RAST inhibition, the cross-reactivity between various spices was investigated. In preliminary experiments it was found that incubation of 0.1 ml of a 1/100 dilution of serum from patient M with coriander-Sepharose resulted in half the binding of ^{125}I -anti-IgE that was found with 0.05 ml of serum from patient M. Therefore, the RAST inhibition was performed with 0.1 ml 1/100 diluted serum from patient M. Fig. 1 illustrates the dose-related inhibition by coriander extract of the coriander IgE RAST. Extracts of paprika powder, ginger powder, and mugwort

pollen did not inhibit the coriander IgE RAST (Fig. 1). This demonstrates that IgE from serum of patient M did bind to different antigenic determinants on the allergen molecules that were extracted from coriander, paprika, and ginger, respectively.

Histamine release by extract from spices

After incubation of washed leukocytes from patient M with curry, coriander, and mace extract, the maximal percentage of histamine release was 86%, 75%, and 61%, respectively. However, when the leukocytes were incubated in buffer alone, a high spontaneous release of histamine was observed ($37\% \pm 5$ [SD]; $n = 6$). Therefore, leukocytes from a nonallergic donor were passively sensitized with serum from patient M. Sensitized leukocytes released a substantial percentage of histamine after incubation with coriander, curry, and mace extract (Fig. 2), whereas unsensitized cells from the same donor, simultaneously tested with the three spices, did not release histamine (Fig. 2). In a second experiment with leukocytes from another nonallergic donor, no histamine was released on incubation with coriander and curry extract.

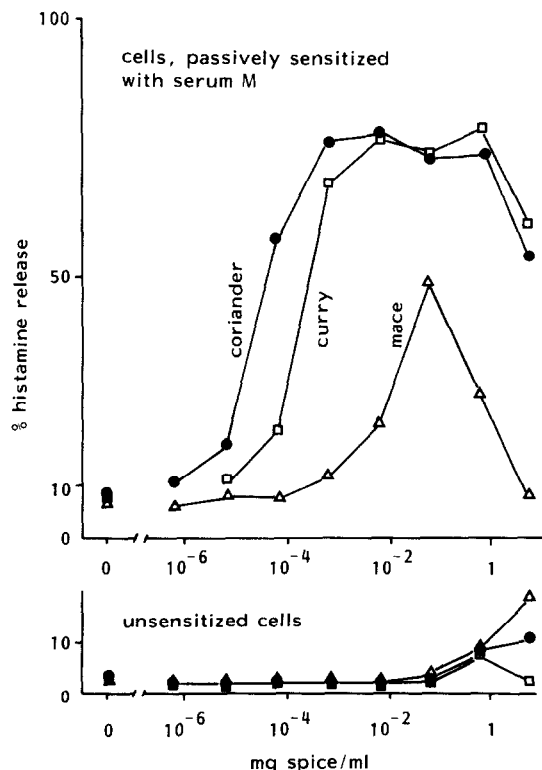


FIG. 2. Passive sensitization of leukocytes from a normal donor with serum from a patient allergic to various spices.

DISCUSSION

The personal history of patient M and the results of the skin prick test strongly suggested the presence of an IgE-mediated allergy toward various spices. With RAST, specific IgE antibodies against components from various spices could be demonstrated in serum from patient M (Table I); sera with total IgE levels equal to or higher than this level in serum from patient M demonstrated much lower IgE binding to the various spice-Sepharose preparations than serum from patient M (Table I). This demonstrates that the IgE binding from serum of patient M to these spice-Sepharose preparations was specific and was not caused by nonspecific, nonimmune IgE binding. Coriander extract was able to inhibit the coriander IgE RAST with serum from patient M, thus providing additional proof of the specificity of the coriander IgE RAST. In contrast, extracts of ginger and paprika powder and mugwort pollen did not inhibit the coriander IgE RAST. These spices or pollen are derived from botanically unrelated species. *Coriandrum sativum* (coriander) belongs to the umbelliferae, *Zingiber officinale* (ginger) belongs to the zingiberaceae, *Capsicum tetragonum* (paprika) belongs to the solanaceae, and *Artemisia vulgaris* (mugwort) belongs

to the compositae. Table I and Fig. 1 demonstrate that, in each of these three spice extracts, IgE from serum of patient M recognized different IgE-binding components. In contrast, Thiel and Fuchs⁴ have suggested that coriander, ginger, and paprika possess common IgE-binding structures; they suggested that essential oils from these spices could be part of the IgE-binding structure(s); however, coriander, subjected to ether extraction, had fully retained its IgE-binding capacity. Essential oils do not appear to be important for the binding of IgE from serum of patient M to coriander-Sepharose. In serum from patient M, no IgE antibodies against mugwort were present (Table I). Furthermore, an extract of mugwort pollen did not inhibit the coriander IgE RAST (Fig. 1). In contrast, a close association has been reported before between a positive skin test toward aromatic plants and a positive skin test toward mugwort pollen.^{4, 10} All 14 patients that were found skin test positive to celery by Kauppinen et al.¹⁰ were also positive to mugwort. Thiel and Fuchs⁴ found only three patients that had a negative skin test to mugwort in a group of 49 patients that were skin test positive to various spices. It should be realized however that there is an important difference between patient M and the patients described by Kauppinen et al.¹⁰ and Thiel and Fuchs.⁴ In these groups of patients, spices had entered the body through the gastrointestinal route. In contrast, patient M was exposed to spices by inhalation of dust from spices. Antigen handling in the lungs may be different from antigen handling at the intestinal mucosal surface. The results of the histamine-release test further confirm the presence of an IgE-mediated hypersensitivity toward spices (Fig. 2). Leukocytes, not sensitized with serum from patient M, were unresponsive toward spice extracts (Fig. 2). This demonstrated that the release of histamine from sensitized cells was not caused by a direct toxic effect of the spice extracts on the leukocytes.

In summary, this article describes a case of occupational allergy to various spices. Furthermore, it describes the preparation of solid-phase coupled spice extracts that can be used for the estimation of specific IgE antibodies against spices.

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Skin testing with an aqueous extract of cotton bract

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Airway challenge with cotton bract extract (CBE) causes reversible bronchospasm in most volunteers never before exposed to CBE or the textile industry. Lung function abnormalities develop slowly after inhalation with a maximum effect reached within 2 hours and lung function slowly improving thereafter. When CBE was injected intradermally in three naive subjects with negligible airway response to CBE, we observed an initial wheal-and-flare reaction within minutes followed by erythema and subsequent induration that persisted for 24 to 48 hours. Microscopic examination of skin biopsy specimens obtained during the course of these reactions revealed edema in the early phase followed by perivascular infiltration of a mixed cellular nature in the subsequent stages of the reaction. Degranulation of mast cells was noted throughout the course of the reaction. These findings indirectly suggest that a nonspecific inflammatory reaction initiated by mast cell-derived mediators and sustained by the presence of infiltrating cells may be responsible for some of the airway effects observed in byssinosis. (J ALLERGY CLIN IMMUNOL 76:481-7, 1985.)

Byssinosis is an occupational lung disease associated with the inhalation of cotton and other textile dusts.¹⁻⁴ Early manifestations of this disease are characterized by reversible symptoms such as chest tightness and shortness of breath that accompany exposure to cotton dust, particularly early in the work week.

Abbreviations used

CBE: Cotton bract extract
MEF: Maximal expiratory flow
DEAE: Diethylaminoethyl

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These symptoms are usually accompanied by changes in lung function that slowly abate after exposure.⁵

The symptoms and physiologic changes of early byssinosis can be reproduced in healthy, naive subjects never before exposed to cotton dust by the inhalation of an aerosol of a water-soluble extract of cotton bracts.⁶ Although most healthy subjects will show some decrease in lung function after exposure