

Case report

Occupational asthma caused by exposure to asparagus: detection of allergens by immunoblotting

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Background Vegetables of the Liliaceae family, such as garlic or onion, have been reported to cause occupational asthma. However, there are few data on adverse reactions to asparagus. We evaluated the role of asparagus as a cause of asthma in a patient with respiratory symptoms occurring at work (horticulture) and studied relevant allergens.

Methods A 28-year-old man complained of rhinoconjunctivitis and asthma when harvesting asparagus at work. Eating cooked asparagus did not provoke symptoms. A positive skin test reaction was observed with raw asparagus, *Alternaria alternata*, and grass-pollen extracts. The methacholine test demonstrated mild bronchial hyperresponsiveness. The patient had an immediate asthmatic response after challenge with raw asparagus extract. Bronchial provocation tests with boiled asparagus, *A. alternata*, and control extracts were negative. Two unexposed subjects with seasonal allergic asthma did not react to the raw asparagus extract.

Results The double-blind, placebo-controlled food challenge with raw asparagus was negative. Serum asparagus-specific IgE was 13.9 kU_A/l. By SDS–PAGE immunoblot, at least six IgE-binding components, ranging from 22 to 73 kDa, were detected only in raw asparagus.

Conclusions We report a case of occupational asthma caused by asparagus inhalation, confirmed by specific bronchoprovocation. Immunoblot analysis showed that asparagus allergens are very labile and quite sensitive to heat denaturation.

Key words: allergen; asparagus; food hypersensitivity; occupational asthma.

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Inhalative exposure to food allergens can elicit respiratory symptoms, particularly in an industrial setting (1). Besides the classical baker's asthma, resulting from occupational airborne exposure to cereal proteins or contaminants contained in flour (2, 3), various foods of vegetable origin may cause occupational asthma, including vegetable dusts such as those of green coffee and castor bean (4), soybean (5), buckwheat (6), carob bean (7), fennel seed (8), onion (9), garlic (10), and sarsaparilla root (11). Allergic reactions to foods through inhalation have also been reported in housewives exposed to the steam from cooking vegetables (12–15). Asparagus, *Asparagus officinalis* L., is a herbaceous,

monocotyledonous plant of the Liliaceae family which is cultivated for its tender shoots, or spears, which emerge in early spring. It originated near the Mediterranean Sea and was considered a delicacy by the ancient Greeks, highly prized for its delicate flavor. "Asparagus" is the Greek word for "stalk" or "shoot". Moreover, the crude saponins from the shoots of asparagus have been found to have antitumor activity (16). Asparagus is cultivated and processed in many parts of the world; however, there is little information on occupational reactions to it. Paterson et al. (17) reported a nonimmunologic adverse reaction (severe botulism) after ingestion of home-preserved asparagus. Garcia et al. (18)

showed the role of asparagus as a cause of occupational asthma induced by inhalation and ingestion. Recently, Eng. et al. (19) reported two patients with a clinical history of itching conjunctivitis, running nose, tightness of the throat, and coughing during preparation of fresh asparagus. We describe a patient with IgE-mediated rhinitis and asthma induced by the inhalation of green asparagus.

Case report

A 28-year-old man had worked at harvesting, handling, and grading asparagus for 13 years. Eight years after he began this work, he noticed runny nose, nasal blockage, and sneezing. These symptoms occurred within 10 min after he began cutting asparagus spears, and handling and making bundles of recently picked asparagus. After 2 years, he experienced additional symptoms of cough, chest tightness, and wheezing. Symptoms were limited to the period February–June when he harvested this crop. During this period, he usually worked more than 8 h a day, including weekends, when the weather conditions were adequate. He became asymptomatic when the asparagus harvesting season was over. He handled onions, garlic, and leek without any symptoms and tolerated the consumption of these vegetables as well as cooked asparagus. He did not smoke and had no other history of respiratory diseases. Physical examination was normal. Results of sinus examination and chest radiography were normal. The white blood cell count was 5700 cells per cubic millimeter, of which 3.7% were eosinophils. Initial spirometry revealed an FEV₁ of 4.56 l (predicted, 4.78 l), FVC of 5.74 l (predicted, 5.75 l), and FEV₁/FVC of 79%.

Material and methods

Preparation of asparagus extracts

Freshly harvested green asparagus was provided by the patient. Raw (RA) and boiled (BA) (at 100°C for 20 min) asparagus (100 g) was extracted in PBS (pH 7.4), and the mixture (1:5 w/v) was stirred overnight at 4°C. The suspensions were centrifuged (12000 g) for 20 min at 4°C. The supernatant was subsequently dialyzed (Spectra/Por, Spectrum Medical Industries, LA, USA) (cutoff 3.5 kDa) at 4°C for 24 h against 100 v/v of 10 mM of glycine, centrifuged at 30000 g, and sterilized by 0.22-µm filtration (Millipore Corp., Bedford, MA, USA). The solutions were aliquoted and stored at -20°C until use. The same procedure was used to prepare an extract with green beans.

Skin testing

Skin tests were performed by the prick technique, with a dilution 1:10 w/v in PBS-glycerol of the RA and BA extracts. A battery of commercially available common inhalants and food allergens (ALK-Abelló, Madrid, Spain) were also prick tested. In addition, RA, BA, garlic, onion, chive, and leek skin testing was carried out by the prick-prick method. Skin testing with RA and BA extracts was carried out in five nonallergic control subjects. Isotonic glycerol saline was used as a negative control, and 10 mg/ml histamine hydrochloride as a positive control. A wheal at least 3 mm larger than that of the negative control was considered a positive reaction.

Specific IgE determinations

Serum specific IgE levels were determined by the fluoroenzymatic method CAP-FEIA (Pharmacia Diagnostic, Sweden). Results were expressed by kU/l, according to the manufacturer's instructions. CAP-FEIA values equal to or greater than 0.35 kU/l were considered positive.

Bronchial provocation tests (BPT)

Bronchial provocation testing was performed according to the report prepared by the EAACI Subcommittee on provocation tests with allergens (20). These tests were performed during an asymptomatic period, after at least 7 days without exposure to asparagus. The substance was delivered by a De Vilbiss 646 nebulizer (De Vilbiss Co., Somerset, PA, USA) with an output of 0.28 ml/min, and intermittent aerosol generation. Each dose of aerosol consists of five breaths, taken slowly from residual volume to the total lung capacity with a 2–5-s breath-hold. The different allergen concentrations were administered at 20-min intervals. The test was considered positive when a FEV₁ decrease of at least 20% was obtained. BPT with allergenic extracts were performed as for the methacholine tests, with a previous end-point titration beginning with a dilution of 1:1 000 000 w/v of the extract and progressively increased by 10-fold, to select a safe initial allergen dose for inhalation. For both methacholine and specific bronchial challenges, the delivery system was identical and pulmonary function was measured by assessment of FEV₁ (Sensor Medics, Anaheim, USA). Spirometric controls were performed at 10-min intervals until a positive response was obtained and, from this moment, at 10-min intervals up to hour 1. Afterward, FEV₁ values were followed hourly for the next 12 h. The next morning, a new spirometric

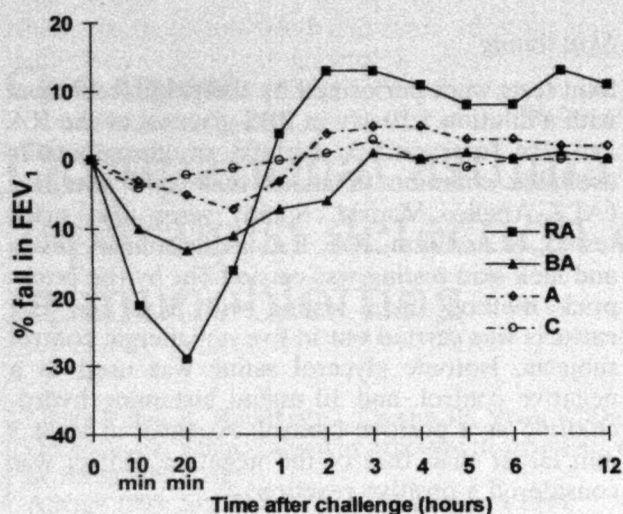


Fig. 1. Bronchial provocation tests with raw (RA) and boiled (BA) asparagus, *Alternaria alternata* (A), and control (C) (green bean) extracts.

procedure was performed. Two unexposed patients with seasonal allergic asthma were also challenged with a 1:10 w/v dilution of RA extract after informed consent was obtained.

Double-blind, placebo-controlled food challenge (DBPCFC)

DBPCFC was carried out with freeze-dried RA. Increasing doses from 100 mg to 17.5 g of the freeze-dried material were administered, at 30-min intervals, in a mixture of pineapple and tomato juice as a vehicle, as described elsewhere (21). The juice, without the active material, was used as placebo. The test was performed over 2 days, with serial FEV₁ measurements every 30 min up to the last dose, and hourly until hour 5. From this moment on, the patient was given a peak-flow meter, with hourly measurements, until bedtime.

SDS-PAGE immunoblotting

SDS-PAGE was performed with a 12% polyacrylamide separating gel and a stacking gel of 4%. A volume of 20 μ l at a final concentration of 1 mg/ml of each allergenic extract was applied to each well. Electrophoresis was performed for 1 h at 130 V per gel for the 8 cm by 7.5 cm gels (Mini-Protean II System, Bio-Rad Laboratories, Richmond, CA, USA). Proteins were electrophoretically transferred from the separating gel to a nitrocellulose membrane in a transfer buffer, as previously described (22). After transfer from the transblot apparatus, the nitrocellulose was placed in blocking solutions for 1 h. The nitrocellulose blot was then washed four times with TTBS and incubated with

the patient's serum (1:3 v/v) overnight with rocking. The nitrocellulose blot was again washed four times with TTBS, and alkaline phosphatase-conjugated goat antihuman IgE (1:2000 v/v) was added and incubated for 6 h. After an additional wash with TBS three times, the blot was developed with 250 μ l of 30 mg of nitro-blue tetrazolium in 70% dimethylformamide and 250 μ l of 15 mg of 5-bromo-4-chloro-3-indolyl-phosphate in 70% dimethylformamide solution in 25 ml of carbonate buffer (0.2 mol/l, pH 9.8) for 15 min. The immunoblots were scanned in the DeskTop Densitometer (PDI, Inc., New York, NY, USA) and analyzed by Diversity One software (PDI, Inc., New York, NY, USA).

Results

Positive skin prick tests were observed with RA, *Alternaria alternata*, and grass-pollen extracts. The results of skin testing with *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*, *Mucor mucedo*, *Dermatophagoides* spp., storage mites, cat dander, dog dander, and BA extracts were negative. Skin tests by the prick-prick method were positive to RA and negative to BA, garlic, onion, and leek. The five control subjects had no response to the tested allergens. Methacholine challenge demonstrated mild bronchial hyperresponsiveness (PD₂₀, 72 CBU). The patient had an immediate asthmatic response after bronchial challenge with the 1:10 w/v dilution of the RA extract. As depicted in Fig. 1, at 20 min, there was a 29% fall in FEV₁, which returned to the baseline approximately 1 h later. No late reaction was observed. BPT with BA, *A. alternata*, and control (green bean) extracts were all negative. The two unexposed subjects with seasonal allergic asthma did not react to the 1:10 w/v dilution of RA extract. Neither symptoms nor changes in the FEV₁ were detected after oral provocation with RA.

Serum asparagus-specific IgE to asparagus was 13.9 kU/l. The IgE reactivity profile of the patient's serum with nitrocellulose-blotted raw and boiled asparagus extracts is shown in Fig. 2. Components with molecular weights of approximately 73, 59, 52, 44, 31–36, and 22–26 kDa were detected in RA with the patient's serum. There was no IgE-specific binding to any BA protein.

Discussion

Our patient experienced respiratory symptoms after years of work at harvesting asparagus. At that time, the onset of nasal and bronchial symptoms within minutes of exposure to asparagus at work and the improvement when the asparagus

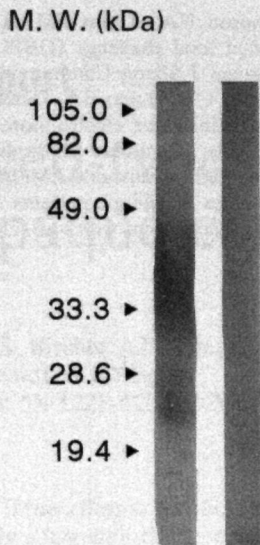


Fig. 2. IgE immunoblots of asparagus with patient's serum. Lane 1) raw asparagus extract; lane 2) boiled asparagus extract.

harvesting season was over suggested the occupational origin of the asthma. In spite of his positive methacholine challenge, the seasonal pattern of symptoms and the sensitization to grass pollens and *A. alternata* argue that the dusty environment at work simply aggravated seasonal allergic asthma. Nevertheless, the negative bronchial challenges to *A. alternata*, BA, and control extracts in the patient and the negative bronchial response to a similar concentration (1:10 w/v) of RA extract in two unexposed subjects with seasonal allergic asthma indicate a true specific immediate bronchospastic reaction to RA in the patient. These findings are also sustained by the results of skin testing and *in vitro* studies. The complete data indicate that RA was the antigen causing asthma in our patient. *Alternaria* spores are commonly recovered during asparagus harvesting throughout the year, and we should note the possibility that a contaminant accounts for the antigenicity of raw asparagus (23). However, the negative results of the *Alternaria*-specific bronchoprovocation test rule out this possibility. Our results also demonstrate that bronchial reactivity was limited to raw, not boiled, asparagus extract. This observation is not surprising because of the complete lack of IgE reactivity to nitrocellulose-blotted boiled asparagus extract, and it indicates that asparagus allergens are quite sensitive to heat denaturation. Interestingly, our patient tolerated the ingestion of asparagus either raw or cooked, showing the lability of its allergens to digestion and enzymatic processes. These findings agree with those of Eng et al. (19), who showed by CAP-RAST inhibition that asparagus-specific IgE antibodies could be inhibited by raw, but not by heated, asparagus. Although

immunologic cross-reactivity between asparagus and other plants belonging to the Liliaceae family has been shown by RAST inhibition (10), our patient was specifically sensitized to asparagus.

In summary, although the causative agents of asthma in a farming environment could be multiple and polysensitization is frequent, a seasonal pattern of symptoms could be related not only to spores or pollen grains, but also to vegetables, such as asparagus, of which the harvest season extends for several months in spring.

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