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Letters

Occupational allergy to rice involving α-amylase inhibitor as the relevant allergen

Rice (*Oryza sativa*), along with other cultivated cereals, belongs to the Poaceae family and is one of the most widely produced and consumed cereals in the world. Occupational allergic diseases with cereals are frequently related to asthma after inhalation of cereal flour, particularly wheat flour.[1–3] We report 2 cases of occupational rhinitis and urticaria in individuals after handling rice and adverse reactions after its ingestion.

A 30-year-old, female pizzeria worker (patient 1) experienced sneezing and rhinorrhea after handling rice powder for the last 2 years and presented with diarrhea and dysphagia after rice ingestion during the last year. One week before evaluation she presented with eyelid angioedema, chest tightness, and abdominal cramping after doing exercise right after eating rice. A 40-year-old, male professional cook (patient 2) presented with generalized urticaria within minutes after rice ingestion during the last month. He tolerated the inhalation of vapors during rice boiling but reported itchy skin and erythema after rice handling for the past several years.

Skin prick tests to commercial common aeroallergens, cereals (including rice, maize, wheat, barley, rye, oat, soy, and gliadin), and enzymes (including α-amylase, cellulase, papain, enolase, and bromelain) were performed, with positive results (wheal $\geq$3 mm) to only the rice and maize extracts for both patients. The results of skin prick-by-prick tests with rice powder were positive for both patients. Commercial serum specific IgE determinations (Pharmacia CAP System, Uppsala, Sweden) against rice revealed values for rice of 1.48 and 0.8 kU/L for patients 1 and 2, respectively (of total IgE levels of 23.7 and 32.8 IU/mL, respectively). Rice, rice powder, and maize extracts were prepared by homogenization in phosphate-buffered saline, dialyzation, and lyophilization. All these extracts were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli. SDS-PAGE IgE immunoblotting assays in nonreducing conditions were performed and revealed IgE reactivity with a 14-kDa protein in rice, rice powder, and maize extracts in patients 1 and 2 and with a protein level of 19 kDa in rice and rice powder extracts in patient 1 (Fig 1A). SDS-PAGE immunoblotting inhibition assays were later performed in both patients. When using maize as an inhibitor and rice powder in solid phase no IgE-binding inhibition was detected, suggesting rice as the primary sensitizer. Inhibition was complete when using rice powder as an inhibitor and maize in solid phase (Fig 1B). Total inhibition was also detected when electroluted, 14-kDa IgE-binding rice protein was used as an inhibitor, revealing the existence of IgE cross-reactivity between the 14-kDa bands from rice and maize. To identify this IgE-binding protein, the 14-kDa band from the rice powder extract was manually excised from the gel, digested with trypsin, and analyzed by matrix-assisted laser desorption/ionization–time of flight and liquid chromatography electrospray ionization tandem mass spectrometry. Protein identification was performed by searching a nonredundant protein sequence database (National Center for Biotechnology Information) using the Mascot program (http://www.matrixscience.com). To

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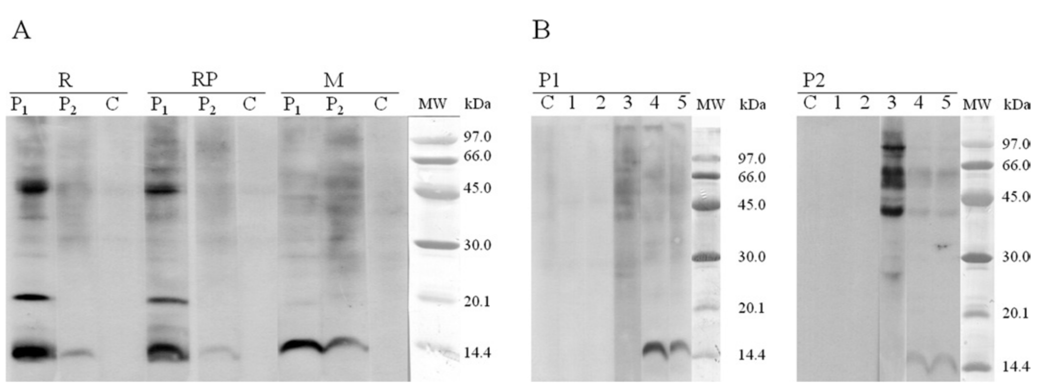

Fig. 1. **(A)** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting. R indicates rice extract; RP, rice powder extract; and M, maize extract. Lane P_1 is serum from patient 1; lane P_2, serum from patient 2; and lane C, control serum (pool of sera from nonatopic individuals). *(B)* SDS-PAGE immunoblotting inhibition assay. M in solid phase. P1 indicates patient 1; P2, patient 2; and MW, molecular weight. Lane C is control serum; lane 1, patient's serum preincubated with M (0.8 mg/mL) (homologous inhibition; positive control of inhibition); lane 2, patient's serum preincubated with electroluted, 14-kDa IgE-binding R protein (100 μg/mL) (electroluted protein heterologous inhibition); lane 3, patient's serum preincubated with R extract (0.8 mg/mL) (extract heterologous inhibition); lane 4, patient's serum preincubated with lamb extract (0.8 mg/mL) (extract negative control of inhibition); lane 5, patient's serum preincubated with bovine serum albumin (100 μg/mL) (purified protein negative control of inhibition).

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identify the 14-kDa protein, we performed tandem mass spectrometry and obtained the sequence of an internal peptide with the sequences CQPGMGYPMYSLPR and QCVGHGAPGGAVDEQLR. Research conducted with protein databases identified the sequence as α-amylase inhibitor from *O sativa*.

α-Amylase is an enzyme that hydrolyses polysaccharides, yielding glucose and maltose. The α-amylase inhibitor (α-AI) family comprises salt-soluble proteins, which play a key role in plant defense against heterologous α-amylase from insects, mites, mammals, and/or bacteria but not against the endogenous α-amylase.[1] α-AI is found in the seeds of cereals. The α-AI subunits are 12- to 16-kDa polypeptides with 4 to 5 intrachain disulfide bridges that are essential for their inhibitory activity.[1] Amino acid sequence identity among members of the family ranges from approximately 30% to 95%. The α-AI family has been reported as the main culprit in baker's asthma[1–3] and has been isolated from wheat flour,[1] rye flour,[4] and barley flour.[5] Allergens from the α-AI family have been reported to sensitize not only by inhalation but also by ingestion.[6] α-AIs have also been described in rice seeds.[7,8] Cases of occupational rhinoconjunctivitis and asthma to rice involving a 12- to 15-kDa protein and a 19-kDa protein have been reported,[9,10] but the allergens involved were not identified.

In the present study we report an IgE-mediated occupational rice allergy in 2 patients who handled rice and rice powder, suggesting α-AI as the relevant allergen. Both patients began with occupational symptoms (rhinitis and urticaria) but later started with symptoms after ingestion. Patient 1 even presented with an anaphylactic episode after rice ingestion before exercise. Although rice allergy is uncommon, physicians should take into account that exposure to potential allergens might develop symptoms. Furthermore, coexistence of additional mast cell mediator–activating triggers, as exercise, may worsen previous symptoms.

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Allergy academy: a novel program for allergy nursing and clinic staff education

Nurses and staff working in an allergy-immunology clinic have an extensive and specialized skill set that includes allergy testing, immunotherapy, food challenges, and other procedures. Many of these skills are not typically taught as part of the standard nursing curriculum. In addition, the knowledge base in allergy-immunology is fairly specific and unique. Patient education is crucial in the care of children with atopic conditions, and nurses are the key personnel to provide this education to those seen in the clinic. There are many strategies to ensure that the staff working in an allergy-immunology office has the necessary expertise, but many learn on the job. This method may or may not suit the needs of all, does not ensure that all topics are discussed with all staff, and does not guarantee that all staff members have the needed proficiency. To make sure that our staff has the required abilities, we instituted a novel educational program for the staff at the allergy-immunology clinic at Emory University and Children's Healthcare of Atlanta.

Allergy Academy is an organized program that contains didactic lectures, workshops, and small group discussions aimed at educating the staff. A comprehensive curriculum was developed with input from medical professionals, current allergy staff, and nursing leadership. The course outline is given in Table 1. It is composed of 4 modules that address all the major topics in allergy and immunology. The program was taught by the allergy-immunology faculty (3 board-certified allergists-immunologists and 1 nurse practitioner specializing in allergy and immunology). The program was held during 4 days and took approximately 28 hours to complete. The program was designed so that the knowledge base gained during the didactic lectures was applied in practical workshops that followed.

Ten individuals (1 nurse practitioner, 5 registered nurses, 2 licensed practical nurses, and 2 medical assistants) from 2 allergy-immunology clinics (Emory Children's Center and Hughes Spalding Asthma Center) attended the 4-day Allergy Academy and completed the 4 modules. The primary outcome was learning as measured by pretests and posttests. We also assessed acquisition of practical skills by return demonstration, a mock anaphylaxis scenario, and the percentage of the class who received certification.

We used repeated-measures analysis of variance to evaluate changes in testing scores (before and after each learning module). There was significant improvement in the mean (SD) pretest and posttest scores for the entire program (56% [13%] vs 75% [30%]; $P = .02$; mean change, 19%), module 1 (54% [0.13%] vs 71% [13%]; $P = .004$; mean [SD] change, 19% [15%]), module 2 (50% [6%] vs 66% [15%]; $P = .03$; mean [SD] change, 19% [15%]), module 3 (72% [10%] vs 80% [11%]; $P = .04$; mean [SD] change, 8% [8%]), and module 4

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