

Letters

NEW ALLERGEN INVOLVED IN A CASE OF ALLERGY TO *SOLEA SOLEA*, COMMON SOLE

In the last few years, food allergy has increased among the inhabitants of developed countries. Fish and fish-derived products play an important role in human nutrition because they are a valuable source of polyunsaturated fatty acids, vitamins, and high-assimilation proteins. In Spain, where the consumption of fish is relatively high (in 2000, the average Spaniard consumed 31 kg of fish per year, according to the Spanish Ministry of Health), fish constitute one of the most important groups of allergens in the induction of immediate (type 1) food hypersensitivity, along with eggs, cow's milk, and peanuts.¹ Fish allergy is more frequent in children (30%) than in adult food allergic patients (12%).² Common sole is one of the most widely consumed types of fish in Spain. Cod parvalbumin was the first fish allergen to be described and reported as a major allergen in fish.³ Most cases of fish allergy are due to sensitization to parvalbumin. However, little has been studied on allergic reactions to common sole or the main allergens implicated in these reactions.

We report the case of a 2-year-old Spanish girl with a history of food allergy to fish and other foods (cow's milk, eggs, kiwi, and banana). At 1 year of age she developed urticaria and vomiting after eating haddock. Skin prick testing to commercially available fish extracts yielded positive results for haddock (5 mm), common sole (5 mm), sardine (5 mm), and anchovy (8 mm). Skin testing with the prick-by-prick method elicited positive responses to cooked sole (16 mm) and haddock (10 mm).

Specific IgE determinations (Pharmacia CAP System; Pharmacia, Uppsala, Sweden) were positive for salmon (0.43 kU/L), haddock (0.91 kU/L), and cod (0.92 kU/L) and were negative for tuna (<0.35 kU/L). An open oral challenge test was performed, eliciting a urticarial reaction 15 minutes after eating 10 g of cooked common sole. The results of an oral challenge test with tuna were negative, and no adverse reactions occurred. The fish extract was prepared from sole muscle as previously described.⁴ Briefly, 30 g of cooked sole filets (100°C for 30 minutes) were extracted in 10 volumes (wt/vol) of phosphate-buffered saline, pH 7.2, with a protease inhibitor cocktail at 4°C with constant stirring for 24 hours. After centrifugation, the supernatant was delipidated with diethyl ether and dialyzed against 0.1M ammonium bicarbonate. Protein extracts were lyophilized and stored at 4°C. Proteins from common sole extracts were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Figure 1), and specific IgE-binding bands were evaluated by immunoblotting.⁵ Serum samples from individuals not sensitized to fish were used as negative controls. Incubation with patient serum showed a main IgE-binding band with an apparent molecular weight of approximately 28 kDa.

To confirm whether parvalbumin (main fish allergen) was involved in reactivity, an IgE enzyme-linked immunosorbent assay experiment with purified parvalbumin from sole was performed. The

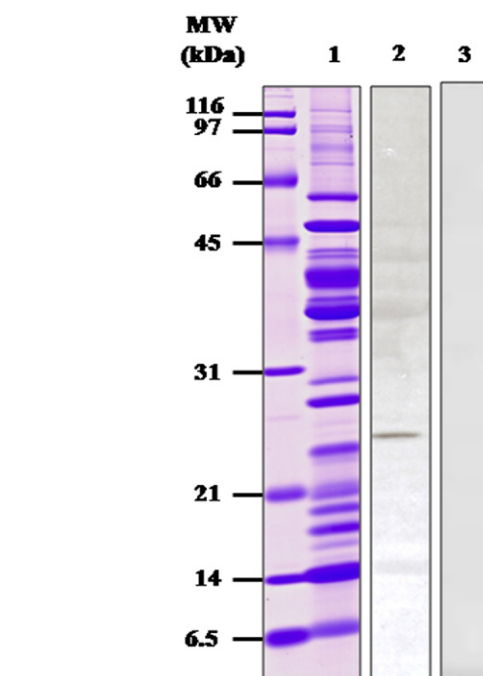


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot of sole extract. Coomassie blue–stained SDS-PAGE (lane 1). Western blot assay of sole extract using patient serum (lane 2) and serum from a nonatopic patient used as negative control (lane 3). Molecular weight markers are indicated in kilodaltons.

purified parvalbumin was obtained specifically. Following extraction of sole proteins, parvalbumin was enriched from supernatant by ammonium sulfate precipitation. The parvalbumin-enriched fraction was dialyzed in Tris (10mM, pH 7.5) and applied to a MonoQ column (GE Healthcare, Uppsala, Sweden). Fractions containing purified parvalbumin isoforms were eluted with a linear salt gradient from 0 to 1M sodium chloride.

The 28-kDa band was extracted from the gel and digested with trypsin, and the proteins were identified by mass spectrometry using liquid chromatography–tandem mass spectrometry, as previously described.⁶ The sequence of several internal peptides was obtained with the following sequences: KFFVGGNWK and VVLAYEPVWAIGTGK. Research conducted with protein databases identified the 28-kDa protein as triosephosphate isomerase β (GI:15149252) from *Xiphophorus maculatus* (southern platyfish). Triosephosphate isomerase β was confirmed to be the IgE-binding band in sole extract (Figure 1).

The results of this report are relevant for 2 facts: (1) parvalbumin, the primary fish allergen, was not involved in the allergy of this patient, and (2) triosephosphate isomerase β was identified as a novel sole allergen. Triosephosphate isomerase plays an important role in glycolysis and is essential for efficient energy production. Triosephosphate isomerase has been found in nearly every organism studied to search for the enzyme, including mammals and insects, as

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well as fungi, plants, and bacteria. It has been identified as an allergen in plant-derived food allergy, such as wheat, lychee, and watermelon,⁷ and recently described as an allergen in amago salmon (*Oncorhynchus masou ishikawae*).⁸ In the future it would be of interest to determine whether this report constitutes an exceptional case of fish allergy or whether it is an important fish allergen affecting patients who are allergic to sole.

MARINA PÉREZ-GORDO, BSc*
CARLOS PASTOR VARGAS, PhD*†
BÁRBARA CASES, BSc*
MANUEL DE LAS HERAS, MD*
AROA SANZ, BSc*
FERNANDO VIVANCO, PhD†‡
JAVIER CUESTA-HERRANZ, PhD, MD*

*Department of Allergy
Fundación Jiménez Díaz
Madrid, Spain

†Department of Biochemistry and Molecular Biology I
Universidad Complutense
Madrid, Spain

‡Department of Immunology
Fundación Jiménez Díaz
Madrid, Spain
cpastor@fjd.es

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THREE CASES OF RICE-INDUCED OCCUPATIONAL ASTHMA

In many Asian countries, rice is the major agricultural crop and staple food. There have been several case reports^{1–4} and one immunologic study⁵ of IgE-mediated rice allergy. However, occupational asthma and rhinitis induced by inhalation of rice powder are not common. We treated 3 patients with rice allergy induced by exposure to rice allergen in a work environment. Brief histories of each patient follow.

The first patient was a 21-year-old man who visited our allergy clinic with a history of recurrent asthma exacerbations. History

revealed that respiratory symptoms were aggravated when he stayed in his house, which was next to a rice mill. Patient 2 was a 30-year-old woman with cough, rhinorrhea, and nasal obstruction for a year, which got worse especially at her workplace. She had cultivated rice for 3 years, and respiratory symptoms appeared 3 months ago. The third patient was a 61-year-old woman who had been diagnosed as having bronchial asthma for several years. She had been working at a rice mill for more than 10 years, and her respiratory symptoms developed a few years ago, which were aggravated while making rice flour and relieved during holidays.

To investigate the pathogenic mechanism involved, we measured the level of specific IgE antibodies to rice extracts. Raw rice was ground into powder after soaking in water for 30 minutes using a grinder, which was extracted with slow overhead stirring at 4°C overnight in phosphate-buffered saline. Particles were removed by centrifugation at 10,000g for 30 minutes at 4°C, and the supernatant was dialyzed overnight against phosphate-buffered saline. The protein concentration of the extract determined using the Bradford method⁶ was 2.0 mg/mL.

The second and third patients had positive responses to common inhalant allergens, including house dust mites and pollens, as well as to rice extract on a skin prick test, whereas the first patient showed a positive response only to the rice extract on a skin prick test. As controls, 10 nonatopic, healthy individuals underwent skin prick tests with the same rice extract, and all showed negative results. All 3 showed positive responses to a methacholine bronchial challenge test, with methacholine provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20% ranging from 0.17 to 7.94 mg/mL. A bronchoprovocation test with rice extract (1.0 mg/mL) showed immediate and late asthmatic responses in all 3 patients.

All 3 patients had an elevated serum total IgE level (222 to 1,425 IU/mL) and a high level of specific IgE to rice extract, compared with levels in unexposed healthy controls (Figure 1A). The cutoff value for a positive serum specific IgE level was set at a value that was greater than 3 SDs from the mean absorbance value of healthy controls. To confirm the binding specificity, enzyme-linked immunosorbent assay IgE inhibition tests were performed with additions of rice extract and an unrelated allergen, house dust mite extract. Rice extract was inhibited at approximately 77% to 82% with serial addition of rice extract (1, 10, and 100 µg/mL), whereas inhibition by house dust mite extract was 12% to 33%. Moreover, 2 IgE binding components (12 and 15 kDa) were identified on IgE immunoblots (Figure 1B).

With increasing consumption, rice has become a food allergen capable of inducing various allergic reactions, including rhinitis, asthma, contact urticaria, and atopic dermatitis.^{1,2,4} In addition, the occupational exposure of rice millers and farmers may affect their respiratory health. A cross-sectional study in rice farmers reported the prevalence of respiratory symptoms, such as chronic bronchitis (6.3%), physician-diagnosed asthma (7.1%), and persistent wheezing (8.8%).⁷ The present study demonstrates that rice powder inhalation can induce occupational asthma and rhinitis through an IgE-mediated response, as evidenced by positive responses to rice extract on skin prick and bronchoprovocation tests and the presence of high serum specific IgE levels.

Several rice allergens have been described, including the major rice pollen allergen Ory s 1 (35 kDa), a 14- to 16-kDa member of the α-amylase–trypsin inhibitor family present in rice seed, a 33-kDa allergen with glyoxalase I activity, and a 9-kDa lipid transfer protein, which sensitized via the oral route.^{4,5} In this study, the 2 IgE binding components of 12 and 15 kDa detected in an immunoblot analysis may be comparable to the 14- to 16-kDa rice allergen previously reported in patients presenting with a rice-induced food allergy.^{5,8} However, further studies are needed to identify the aller-

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