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Letters

Identification of a novel protein allergen in Mediterranean silverside fish species

Fish is one of the most common causes of food allergies. The main risk factors for fish allergy are atopy and exposure to fish. As new fish species are introduced into diets, allergic reactions begin to emerge. Parvalbumins are the major fish allergens, with molecular masses of 15 to 20 kDa.[1] However, other allergens that could be responsible for allergic responses to a single fish species have been identified.[2,3]

We describe 2 patients with symptoms after consuming silverside (*Atherina boyeri*). Patient 1 was a 49-year-old woman who, immediately after eating some frozen fried silversides, presented with epigastric pain, urticaria, vomiting, and dyspnea with wheezing. She has not eaten silverside since then and had previously tolerated other fresh fish. She did not present with any other allergic symptoms. Patient 2 was a 22-year-old man who, within minutes of eating some frozen fried silversides, presented with pruritus with pharyngeal foreign-body sensation and subsequent onset of widespread hives. Since then he has not eaten any fish. Some years ago he experienced hives and eyelid and lip angioedema 30 minutes after eating rice with shrimp. Since then he has eaten shrimp regularly without any symptoms.

A boyeri protein extract was prepared by homogenization in phosphate-buffered saline, dialyzation, and lyophilization. Skin prick tests to commercial extracts from fish (hake, cod, trout, tuna, salmon, sardine, and monkfish) (Bial Aristegui, Bilbao, Spain), *Anisakis simplex,* and *A boyeri* protein extract were performed. Both patients had positive skin prick test results (wheal $\leq$3 mm) for *A boyeri* protein extract only. The results of prick-by-prick tests with *A boyeri* were also positive in both patients. The result of a skin prick test with shrimp extract (Bial Aristegui) was negative in patient 2. Both patients refused administration of an oral challenge with silverside. *A boyeri* protein extract was analyzed by sodium dodecyl–polyacrylamide gel electrophoresis (SDS-PAGE). With the use of SDS-PAGE immunoblotting, the serum from patient 1 had IgE reactivity to only one 28.5-kDa protein and the serum from patient 2 reacted to 3 proteins (19, 28.5, and 38 kDa) (Fig 1). The 38- and 28.5-kDa bands from *A boyeri* extract were manually excised, digested with trypsin, and analyzed by mass spectrometry in tandem to identify the IgE-binding proteins.[4] Protein identification was performed by searching a nonredundant protein sequence database (National Center for Biotechnology Information). The analysis of the resulting peptides by mass spectrometry or mass spectrometry in tandem identified the 38-kDa band as glyceraldehyde-3-phosphate dehydrogenase (GADPH) from *Xiphophorus maculatus* (Southern platyfish) and the 28.5-kDa band as triosephosphate isomerase β from *Oreochromis niloticus* (Nile tilapia).

The Mediterranean silverside (*A boyeri*) is a teleost fish of the Atherinidae family and *Atherina* genus. It is a small, elongated fish that does not exceed 20 cm. Although a marine fish, silversides tolerate low-salinity water and can also be found in wetlands, coastal lagoons, and fresh water in the lower reaches of some rivers. It is found on the coast of the Mediterranean Sea, the Black Sea, and the Caspian Sea. In the Atlantic Ocean, it is found along the coast from Portugal to Mauritania.

GAPDH catalyses the conversion of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate. This is the sixth step in the glycolytic breakdown of glucose, an important energy and carbon molecule supply pathway that takes place in the cytosol of eukaryotic cells. It has been identified as an allergen in

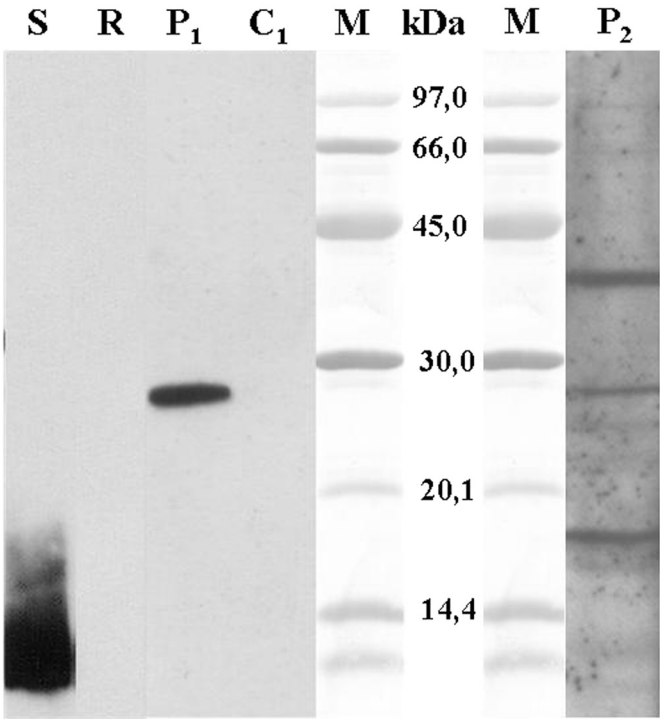

Figure 1. Sodium dodecyl–polyacrylamide gel electrophoresis immunoblotting with silverside crude extract. Lane P_1: patient 1 serum; lane P_2: patient 2 serum; lane R: control serum (pooled sera from nonatopic individuals); lane S: sardine antiparvalbumin rabbit serum; lane C_1: unimmunized rabbit control serum; and lane M: pattern of molecular masses.

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rambutan fruit,[5] wheat flour in baker's asthma,[6] and also as a potential allergen in fish processing workers.[7] Triosephosphate isomerase plays an important role in glycolysis and is essential for efficient energy production. Triosephosphate isomerase has been found in nearly every organism, including mammals, insects, fungi, plants, and bacteria. It has been identified as an allergen in plant-derived food allergies (wheat, lychee, and watermelon),[4] in shrimp,[8] and as a fish allergen in sole[9] and amago salmon.[10]

Because neither of the patients consented to an oral challenge with silverside, the reaction could not be reproduced, but the positivity of the skin test results and the identification of IgE reactivity in SDS-PAGE immunoblotting with silverside extract in both patients suggest an IgE-mediated hypersensitivity to this fish. For this reason and because no other family members who ate the same fish had similar symptoms, the possibility of scombroid poisoning was ruled out.

We report 2 cases of allergy to silverside (*A boyeri*) without allergy to other fish. To our knowledge, this is the first description of allergy to this fish. Two proteins were identified as the allergens involved: triosephosphate isomerase (patients 1 and 2) and GADPH (patient 2). Triosephosphate isomerase was recently described as a new fish allergen, and therefore the present work is the third description of the involvement of this allergen in cases of fish allergy without sensitization to fish parvalbumin. To our knowledge, this is the first time that GADPH has been identified as a fish allergen.

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Omalizumab effectively prevents recurrent refractory anaphylaxis in a patient with monoclonal mast cell activation syndrome

Monoclonal mast cell activation syndrome (MMAS) is a clonal mast cell disorder, first described in 2007,[1,2] that is characterized by episodic symptoms owing to mast cell degranulation in patients who do not meet the diagnostic criteria for systemic mastocytosis. Clinically, patients may present with recurrent unprovoked episodes of flushing, lightheadedness, abdominal symptoms, loss of consciousness, and life-threatening hypotension owing to mast cell mediator release.[3] According to diagnostic criteria established at a 2007 consensus conference, patients with MMAS meet only 1 or 2 of the minor criteria for mastocytosis and do not exhibit the clusters of bone marrow mast cells ($\geq$15 mast cells) that define mastocytosis.[3] The natural history of MMAS is not known, but in 1 prospective study,[4] one third of patients initially diagnosed with a clonal mast cell activation disorder eventually fulfilled the 3 minor criteria for systemic mastocytosis after developing sustained elevations in serum baseline tryptase. Treatment involves antihistamines, antileukotrienes, and mast cell stabilizers, with the goal of relieving and preventing symptoms of mast cell degranulation, but control can be difficult to achieve.[3] There are few reports of the use of omalizumab in mast cell activation disorders. The authors present a case of MMAS with recurrent unprovoked life-threatening anaphylaxis responding to treatment with omalizumab.

A 31-year-old woman presented with 6 multisystem reactions over the course of the previous 8 months. Her typical symptoms peaked within 5 to 10 minutes of onset and included cutaneous (urticaria, swelling), respiratory (cough, shortness of breath, chest tightness, dysphagia, dysphonia), gastrointestinal (vomiting, cramping abdominal pain), and cardiovascular involvement. Blood pressure was recorded at 80/40 mmHg by paramedics during 1 reaction and loss of conscious occurred on 1 occasion. All 6 episodes had been treated in the emergency department and required 2 to 3 doses of epinephrine. The patient was not taking angiotensin-converting enzyme inhibitor, β blocker, nonsteroidal anti-inflammatory, or opioid medication. Typical triggers, including foods, drugs, latex, insect stings, exercise, menstruation, and alcohol, were ruled out and skin testing was noncontributory.

Complete blood cell count with differential was normal. Baseline serum tryptase was minimally elevated on 2 separate occasions at 13.7 and 12.2 ng/mL (reference range $<$11.4 ng/mL). Serum tryptase was elevated acutely after 2 separate reactions at 73.6 and 37.8 ng/mL. Bone marrow biopsy showed mildly increased mast cells and eosinophils, with 1 compact cluster of mast cells (not meeting the major criterion for mastocytosis). Occasional mast cells

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