

Letter to the Editor

Anaphylaxis provoked by ingestion of marshmallows containing fish gelatin

To the Editor:

The amount of gelatin used in the food industry is increasing worldwide. Most gelatins are of bovine and porcine origin. Because the use of bovine gelatin has caused concern in the context of the bovine spongiform encephalopathy crisis, gelatin from pig skin has become the most widely used gelatin. A series of articles concerning anaphylactic reactions provoked by gelatin-based additives in vaccines was published in this journal some years ago.^{1,2} Some of the patients also experienced allergic reactions to food-containing mammalian gelatin. A case of anaphylaxis after consumption of gummi bears without vaccine-related allergies has been reported in a young boy.³ For the vegetarian market, fish gelatin is, to some extent, an alternative to mammalian gelatins. Fish gelatin is a product also complying with some religious dietary principles. Fish is a food allergen for 0.4% of the population in the United States.⁴ Allergic cross-reactions to several fish species are explained by the dominant sensitization to the panallergen β -parvalbumin, which is present in considerable amounts in fish muscle. Commercial fish gelatin is extracted from the skin and bones of several fishes, such as cod, pollock, salmon, or tuna.⁴ Recent studies addressing the possible allergenicity of fish gelatins have been published.^{5,6} IgE antibodies to fish gelatin were identified in patients with fish allergy. However, no allergic reaction was provoked by means of oral challenge with 5 g of tuna gelatin in 3 sensitized patients⁷ and with a cumulative dose of 3.6 g of codfish gelatin in 3 patients with positive skin prick test responses to fish gelatin, except 1 mild subjective reaction at a cumulative dose of 7.61 g of fish gelatin.⁸ *In vitro* studies did not show IgE cross-reactivity between bovine and fish gelatins.⁷

The clinical case of a patient who experienced a severe anaphylactic reaction on ingestion of marshmallows containing fish gelatin prompted us to analyze in detail the IgE profile of this patient. The 12-year-old boy had a severe anaphylactic reaction, with urticaria, angioedema, and asthma starting 20 minutes after eating beef and several kosher marshmallows from a barbecue grill (no fish was prepared or consumed). He recovered after in-hospital emergency treatment. He was known to experience allergic reactions on consuming different fish species and to experience contact urticaria when touching fish. The young patient had a history of hay fever and occasional asthma attacks, mainly during viral respiratory tract infections. Skin prick tests showed a strong sensitization to tuna, cod, salmon, grass pollen, and house dust mites. *In vitro* IgE test results were positive for tuna (65 kU/L), salmon (32 kU/L), and cod (16 kU/L) but negative for beef (Phadia ImmunoCAP system, Uppsala, Sweden). Specific IgE for *Dermatophagoides pteronyssinus* was 63 kU/L, and that for meadow grass pollen was 64 kU/L. The total IgE level was 700 kU/L.

Parvalbumin isoform mixtures from tuna, cod, and salmon were prepared by means of 2-step chromatographic analysis. The patient's serum IgE antibodies recognized purified 10-kD parvalbumins from all 3 species in an immunoblot. Fish collagen was extracted from tuna skin by using acetic acid extraction. Two protein bands of 110 and 210 kD corresponded to the size of the

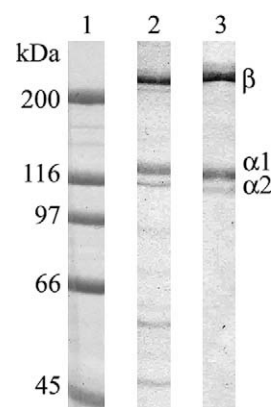


FIG 1. SDS-PAGE (7.5%) of type I collagen isolated from tuna skin. Lane 1, Marker. Lane 2, Coomassie staining visualizes tuna collagen chains $\alpha 1$ and $\alpha 2$ at 110 kD. The 210-kD band corresponds to the collagen β -chain (dimer of the α -chain). Lane 3, The patient's IgE strongly react with the $\alpha 1$, $\alpha 2$, and β tuna collagen bands in immunoblot.

hetero α -chains and β -chain of tuna collagen. Food-grade fish gelatins (Rousselot, Courbevoie, France) and fish gelatin-containing marshmallows (Glatech Productions, Lakewood, NJ; Shneider's, Pantin, France) were purchased from specialized trade. No contamination of collagen and gelatin preparations by parvalbumin could be shown by means of ELISA with a sensitive anti-parvalbumin mAb (detection limit, 9 ng per well). The patient's IgE antibodies reacted with the α - and β -chains of tuna collagen (Fig 1) and a complex pattern of proteins in hydrolyzed food-grade gelatin samples. Skin prick tests performed with cold-water fish gelatin (Sigma, St Louis, Mo) and 2 fish gelatin-containing marshmallow samples produced wheal-and-flare reactions down to gelatin concentrations of 4.5 μ g/mL sterile saline. Specific IgE titers were quantified for purified parvalbumins from tuna, salmon, cod, and different fish gelatins by means of ELISA (Table I). As controls, 10 sera from nonallergic patients, 90 sera from allergic patients, and 10 sera from patients with fish allergy (specific IgE, >10 kU/L) were used. From this control population, only 1 subject with fish allergy had IgE antibodies (titers, 3-5 kU/L) to fish gelatin. For this patient, no information is available concerning possible adverse reactions to products containing fish gelatin. Comparison of our patient's specific IgE titer to fish extracts determined by means of CAP and specific IgE level to fish parvalbumins quantified by means of ELISA showed comparable titers for cod. Higher CAP values were obtained for salmon and still higher values for tuna, suggesting that the patient reacted to an additional fish allergen different from parvalbumin that is present in tuna and salmon CAP coatings. When serum was tested for specific IgE against different fish gelatins by means of ELISA (Table I), IgE titers ranged from 5 to 22 kU/L, and the highest titer was obtained for 2 hydrolyzed food-grade gelatins at about 20 kU/L. IgE binding was lower for high-molecular-weight fish collagen and for highly degraded cold-water fish gelatin (Sigma). The IgE titer of 20 kU/L against fish gelatin could be an explanation for the difference of IgE obtained with tuna total extract in CAP (65 kU/L) and purified tuna parvalbumin in ELISA (30 kU/L). CAP inhibition was performed by using hydrolyzed fish gelatin at a final concentration

TABLE I. The patient's IgE antibody quantification by means of ELISA with different purified parvalbumin and fish gelatin preparations

Coating antigen	IgE (kU/L)
Parvalbumins	
Tuna	30
Salmon	18
Cod	20
Gelatin	
Tuna skin collagen	5
Cold-water fish (Sigma)	13
Marshmallow (United States)	15
Marshmallow (France)	16
FGH-N, food grade (Rousselot)	17
FGH, food grade (Rousselot)	22

FGH-N, Fish gelatin hydrolyzed at low molecular weight; FGH, fish gelatin hydrolyzed.

of 1 mg/mL. This resulted in a decrease of the specific IgE value to tuna extract from 65 down to 23 kU/L. The obvious clinical history, the specific IgE binding to fish gelatin (ELISA, immunoblot, and CAP inhibition), and the positive skin test responses with fish gelatin are strong evidence for an anaphylactic reaction provoked by the consumption of fish gelatin contained in kosher marshmallows. Indeed, it is important to note that no parvalbumin was detected in the cold-water fish and marshmallow fish gelatin preparation.

Even though a series of persons sensitized to fish gelatin did not experience allergic reactions on oral challenge,^{7,8} this case shows that fish gelatin can be a trigger for life-threatening anaphylaxis.

In the United States fish gelatin as an ingredient derived from an allergenic source must be declared.⁹ In the European Union there is an exemption for fish gelatin labeling in processed food. Taking into account that the amount of fish gelatin in gelatin products is growing, one can assume that the risk for allergic

reactions to fish gelatin-containing products will increase. Testing for sensitization to fish gelatin should be included in the diagnostic analysis of patients with fish allergy to give them proper advice.

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Supported by grant no. FNR/03/07/03, SECAL Project I and II of the Fonds National de la Recherche, Luxembourg.

Disclosure of potential conflict of interest: A. Kuehn and F. Hentges are employees of CRP-Santé and have received research funding from the Ministry of Research (Luxembourg). C. Hilger declares that she has no conflict of interest.

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Available online ■■■.
 doi:10.1016/j.jaci.2008.12.012