
Letters

EGG SENSITIZATION CAUSED BY IMMEDIATE HYPERSENSITIVITY REACTION TO DRUG-CONTAINING LYSOZYME

Lysozyme is an enzyme obtained from egg white. Its lytic activity on the cell wall of gram-positive microorganisms has led to its use in pharmaceutical products and food applications (cheese and wine industry).¹ However, hypersensitivity reactions to lysozyme as a result of drug intake have rarely been reported.^{2–6}

We describe a 20-month-old child with atopic dermatitis from the age of 2 months. For this reason, weaning was delayed and egg was not introduced into his diet. He presented with a severe adverse drug reaction to Narlisim (Baldacci Laboratories SpA, Pisa, Italy), a nasal decongestant that contains phenylpropylamine, thonzylamine, and lysozyme. He experienced rhinitis, bronchial asthma, and dysphonia within a few minutes after the nasal instillation of Narlisim.

He was referred to the Pediatric Unit of Fidenza for allergologic investigation supported by skin prick test (SPT) and ImmunoCAP assay (Phadia, Uppsala, Sweden). An SPT was performed with egg white, egg yolk (Lofarma, Milan, Italy), and Narlisim; a positive control (histamine, 10 mg/mL) and a negative control were also used. Serum specific IgE was also identified with the customized, commercially available allergen microarray (ISAC; VBC-Genomics, Vienna, Austria) in our research laboratories. The results of SPTs were positive with egg yolk (wheal mean diameter, 7 mm), egg white (wheal mean diameter, 12 mm), Narlisim (wheal mean diameter, 8 mm) as undiluted drug, and histamine (wheal mean diameter, 6 mm). Serum specific IgE was detected against egg yolk (1.20 kUa/L), egg white (6.02 kUa/L), ovalbumin (5.56 kUa/L), ovomucoid (3.38 kUa/L), and lysozyme (1.82 kUa/L). The allergen microarray revealed IgE antibodies against Gal d 4 (lysozyme) (1.04 kUa/L).

The parents did not give consent to perform a challenge test with Narlisim or a food challenge with egg. Therefore, currently the patient remains on an egg-free diet, and since diagnosis, the parents were advised to carefully examine the list of food and drug ingredients looking for lysozyme or E1105 (as lysozyme is occasionally identified).⁵

Clinical adverse reactions to egg may occur in infants or children who have never eaten egg. They may be sensitized or even react at first egg ingestion.⁷ Our observation suggests that an immediate hypersensitivity to drug-containing lysozyme may also represent the first clinical manifestation of egg sensitization. Indeed, a similar case has been recently reported.⁶ The allergen microarray detected specific IgE antibodies to Gal d 4 (lysozyme) but not to Gal d 1 (ovomucoid) or Gal d 2 (ovalbumin). On the contrary, SPT results were positive with egg yolk and egg white, and ImmunoCAP detected IgE antibodies not only against lysozyme but also against other egg proteins (ovomucoid and ovalbumin). This apparent discrepancy might be explained by the fact that the allergens coated on the microarrays are highly purified and do not contain contaminants compared with the natural extracts used for both SPTs and ImmunoCAP. This finding is in agreement with the study by Walsh et al,

who detected 4 distinct groups of hen egg allergens binding IgE in the sera of egg-allergic children using especially purified proteins.⁸

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ANAPHYLACTIC SHOCK CAUSED BY BUFFALO'S MOZZARELLA CHEESE

Buffalo's mozzarella cheese is an Italian delicacy made from milk of water buffalos (*Bubalus bubalis*) originally domesticated in Asia approximately 7,000 years ago and imported to Italy by the Huns. In Italy, buffalo's milk is used for mozzarella cheese production, whereas the milk of water buffalo accounts for more than 50% of drinking milk in certain developing countries, such as India, Pakistan, Egypt, and Nepal.¹

We report the case of a 70-year-old German patient who for the first time developed an episode of anaphylaxis during a stay in Italy. Fifteen minutes after dining, he developed pruritus on his scalp and oropharyngeal swelling. The symptoms regressed spontaneously. One year

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later he was referred to the emergency unit with generalized urticaria, severe angioedema, and dyspnea but no gastrointestinal symptoms approximately 15 minutes after ingestion of Italian mozzarella cheese, tomatoes, and chicken salad; 10 minutes later he lost consciousness. The patient had no other significant medical history, in particular no other history of atopy or food intolerance.

The results of skin prick tests with whey fractions of cow's and goat's milk (extracts from Lofarma, Milano, Italy) were negative. Skin prick tests with mozzarella and milk proteins of extracts from milk of 3 authentic water buffalos (extracts from the Nutrition and Food Research Center, Weihestephane, Germany) resulted in wheals of 20 × 20 mm, whereas prick-to-prick tests with cow's milk and mozzarella made from cow's milk and tomatoes and chicken meat were performed without reaction. The test results of buffalo's milk in control persons were negative.

Under double-blinded, placebo-controlled labial provocation with buffalo's mozzarella, the patient developed a generalized reaction with an itchy throat, lip swelling, and rhinoconjunctivitis, rapidly followed by tachycardia (heart rate, 100/min) and a decrease in systolic blood pressure (100/60 mm Hg). The symptoms subsided after intravenous administration of 250 mg of methylprednisolone and 1 ampoule (4 mL) of dimethindene maleate. Oral provocation with cow's milk mozzarella was tolerated.

The total IgE level was elevated at 304 kU/L, whereas no specific IgE (CAP-FEIA, Pharmacia, Uppsala, Sweden) against the whey proteins α -lactalbumin and β -lactoglobulin from cow's milk was detected. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting revealed specific IgE reactivity against 2 bands (Fig 1a). However, in immobilized pH gradient isoelectric focusing immunoblot analysis, specific IgE reactivity could be reproduced against nonglycosylated and glycosylated buffalo κ -caseins, corresponding only to the 17-kDa bands, presuming that the 50-kDa band might be an oligomeric artifact in sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting. In the immunoblot with cow's milk, no specific IgE-antigen complex was observed compared with the positive control with serum samples from cow's milk allergic patients that form IgE-antigen complexes with cow's milk proteins (Fig 1b).

Here, an anaphylactic reaction to buffalo's milk in an adult without any reactivity to cow's milk is presented for the first time to our knowledge. Allergy to milk of goat and sheep, 2 phylogenetically closely related mammals, without allergy to cow's milk has been reported previously.³ Despite the close phylogenetic relationship between buffalo and cow, no cross-reactions between buffalo's and cow's milk could be found in our patient.^{4,5} Most patients allergic to cow's milk have specific IgE antibodies to more than 1 of

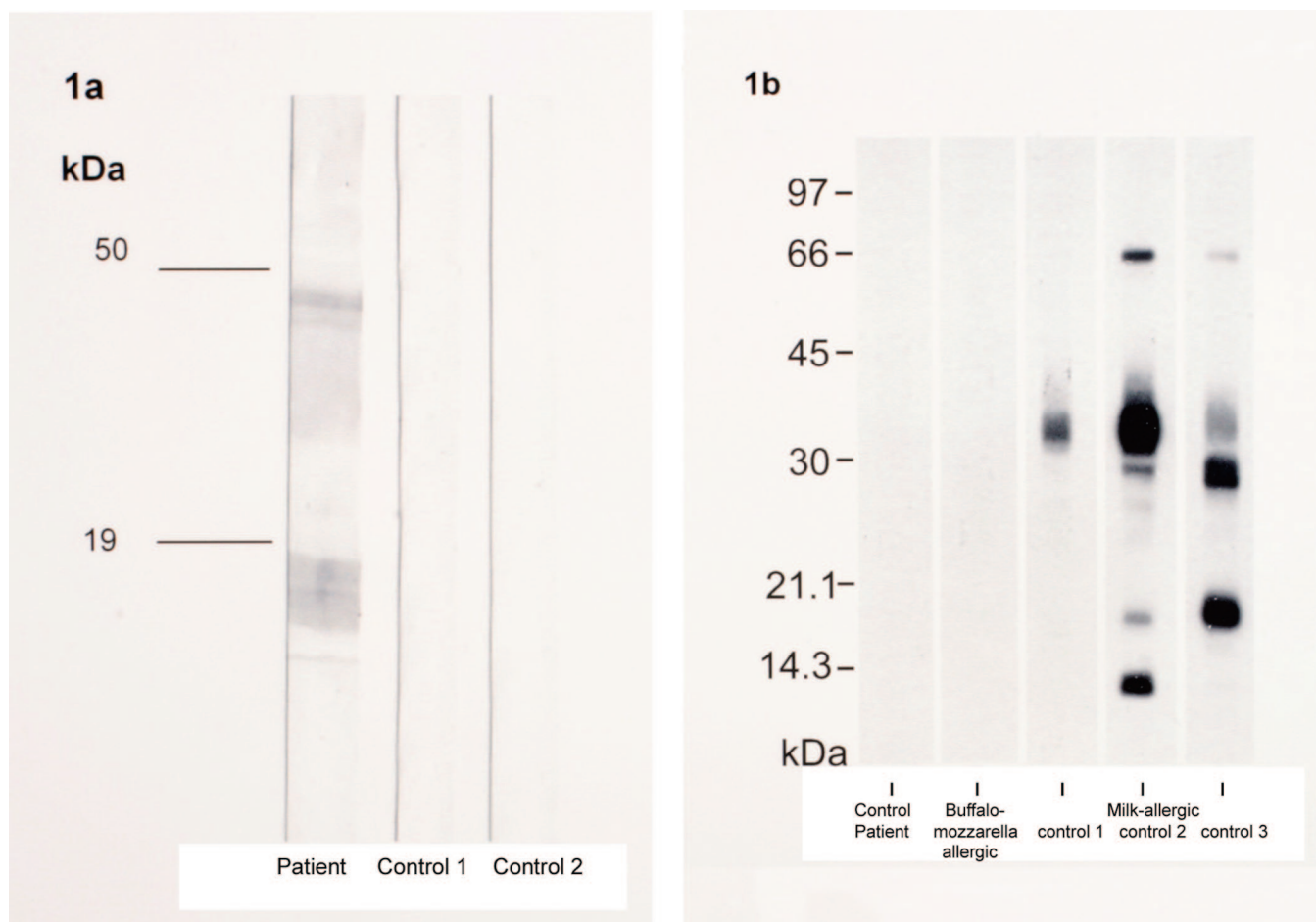


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting.

the milk proteins, and clinical practice indicates that patients allergic to 1 type of milk protein will not tolerate milk proteins of other species.² An anaphylactic reaction to goat's milk in a cow's milk allergic infant has been mentioned.⁶ Cross-reactivity between milk proteins from different animal species, namely, cow, goat, ewe, and buffalo, has been shown in vitro in cow's milk allergic patients.⁷ These were discovered to be due to homologies in amino acid composition and the phylogenetic relationship.^{7,8} Interestingly, our patient showed an isolated anaphylactic reaction to buffalo's milk.

Allergy to bovine milk belongs to the most common types of food allergy.^{4,6} Milk consists of many proteins divided into caseins that make up 80% of milk proteins and whey proteins. Caseins and β -lactoglobulin appear to be the major allergens in cow's milk.² Our patient did not show elevated specific IgE levels against bovine caseins and β -lactoglobulin, and also prick testing and labial provocation with cow's milk remained without any positive reaction. The patient was educated to strictly avoid bufylline milk products and to initiate therapy in case of an unintended ingestion.⁸

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RAW PARACENTROTUS LIVIDUS AND ALLERGY

Sea urchins are members of a large group of marine invertebrates in the phylum Echinodermata, subphylum Echinozoa, class Echinoidea. The sea urchin of gastronomical interest is the *Paracentrotus lividus*. Their shell is globular in shape and is armed with spines.¹ The spines, long and sharp, protect the urchin from predators and can inflict a painful wound. Skin reactions to sea urchin have been described in the medical literature.² The reproductive organs of sea urchins can be eaten cooked or raw. The use of these sea urchins as food is common in Korea, Japan, Spain, and Italy. In southern Italy, they are usually eaten raw. Reactions after eating sea urchin roe (boiled and served with sushi) have been previously described.^{1,3}

We describe a patient who developed allergy-related symptoms to raw sea urchin on 3 different occasions. The patient, a 28-year-old woman with a history of perennial allergic rhinitis, reported that the first episode consisted of urticaria and gastric pain after eating raw sea urchin, mussels, and shellfish. In the second and third episodes, the patient again had urticaria and gastric pain after ingestion of raw sea urchin only.

Raw sea urchin proteins were extracted by stirring for 2 hours at 4°C in 0.15-mol/L phosphate-buffered saline (pH 7.2). Insoluble particles were removed by means of centrifugation at 15,000g for 30 minutes at 4°C, and the supernatant was dialyzed against distilled water. The protein concentration of the extract was determined using Bradford analysis with bovine serum albumin as a standard.⁴ The resulting extract was stored at –20°C until required, dissolved in sample buffer, and heated at 100°C for 5 minutes. Twenty micrograms of sea urchin extract was applied on 4% to 12% polyacrylamide precast running gel (NuPage Bis Tris; Invitrogen, Milan, Italy)⁵ and stained with 0.1% Coomassie brilliant blue. The sodium dodecyl sulfate–polyacrylamide gel electrophoresis profile of raw sea urchin showed a band of 118 kDa (Fig 1). Proteins were electrophoretically transferred to nitrocellulose membrane (Schleicher & Schuell BioScience GmbH, Dassel, Germany).⁶ The nitrocellulose membrane was saturated with 0.1-mol/L tris-buffered saline and 0.5% fat-free milk powder and was incubated overnight at 4°C with patient serum. Bound specific IgE was detected by using peroxidase-conjugated anti-human IgE antibodies from goat (1:1,000 in saturation buffer) (Sigma-Aldrich, Milan) and by using the chemiluminescence method (ECL Western Blotting Kit; Amersham Biosciences, Buckinghamshire, England). Skin prick tests with anisakis simplex (ALK-Abellö, Milan), common inhalant allergens, food commercial extracts (including oysters, clams, mussels, and shrimp) (Stallergenes, Milan), and diluted extracts (1:1,000, 1:100, and 1:10) of raw sea urchin were performed using saline solution and histamine (10 mg/mL) as negative and positive controls, respectively.

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