

Anaphylaxis to wheat isolates: Immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge

To the Editor:

The increase in food allergy prevalence highlights studies on the allergic risk caused by hidden allergens in human food intake¹ and studies on allergen modification by means of technologic processes.²⁻⁴ This is the case of proteins from various foodstuffs used as ingredients in the food industry. We report a case of food allergy to a wheat isolate used in the meat industry, without there being any allergic reaction to native wheat flour.

A 24-year-old woman had 2 episodes of angioedema with generalized urticaria less than 30 minutes after the ingestion of a sausage and pork pie. She regularly eats bread and pork-based products without any reaction. Skin prick tests performed with 25 common food allergens showed no reaction except to wheat flour and gluten (wheat responses of 4 and 5 mm, respectively). Skin prick tests performed with purified wheat flour proteins (S. Denery-Papini, Laboratoire d'Etude des Céréales, Nantes, France) showed positive responses to γ -gliadin (2.5 mm) and ω -gliadin (6 mm). Skin prick test responses to α - and β -gliadin and to high- and low-molecular-weight glutenins were negative.

Realistic skin prick test responses to the fresh meat pie components (crust, crumb, meat, and sausage) were positive (ie, wheal responses of 5 to 10 mm). At our request, the manufacturer informed us that the pork pie contained a wheat isolate. Native skin prick test reactions to this wheat isolate (isolate A) were positive, with 19-mm and 8-mm wheal responses (raw and cooked, respectively).

Low levels of specific IgE were detected by means of CAP testing (Pharmacia): 1.6 kU/L to wheat flour and 3.1 kU/L to gluten. Leukocyte histamine release results were negative to wheat flour, and results were positive (10%) to wheat isolate.

An open food challenge showed negative results to 30 g of wheat flour in association with 3 g of gluten followed by exercise. A double-blind, placebo-controlled food challenge (DBPCFC) was performed for 2 days with wheat isolate A. Clinical symptoms (ie, nasal itching, sneezing, and pharyngeal discomfort, followed by cough and laryngeal itching) appeared after the ingestion of 2 g of wheat isolate. There were no changes in the arterial pulse, blood pressure, or peak expiratory flow. The control test result with bread not containing wheat isolate was negative.

Three wheat flour isolates were studied: wheat isolates A and B from 2 different producers were obtained by means of acid treatment and heat, and wheat isolate C was obtained by means of enzymatic treatment. Wheat isolates were extracted for 2 hours at a 1:20 w/v ratio in Coca buffer (0.55% NaHCO₃, 1% NaCl).

Two fractions of native wheat flour were prepared.

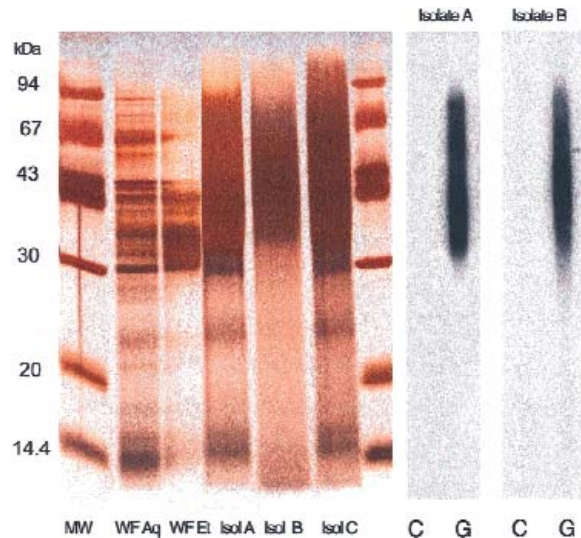


FIG 1. SDS-PAGE of native wheat flour aqueous-soluble fraction (WF Aq), native wheat flour ethanol-soluble fraction (WF Et) and wheat isolate A (Isol A), wheat isolate B (Isol B), and wheat isolate C (Isol C) reduced with 0.5% dithiothreitol and stained with silver (left). Immunoblots were incubated with serum G (diluted 1:20) or blocking buffer (C). MW, Molecular weight.

The aqueous soluble fraction was extracted as described above. After the centrifugation step, the pellet was extracted for 2 hours with a 70% ethanol solution to obtain gliadin fractions.

Extracts were centrifuged at 4°C and filtered onto a 0.22- μ m membrane. Specificity of IgE was assessed by means of RAST, ELISA, and SDS-PAGE, followed by immunoblotting, as previously described.⁵ Briefly, IgE ELISA was performed by using Immulon-2 microtitration plates and alkaline phosphatase-labeled goat anti-human IgE (KPL).

Serum G was studied in comparison with the sera of 2 patients (sera 1 and 2) with food allergy to native wheat flour and a control serum (serum 3) from a patient with food allergy to cow's milk protein and peanut. Rabbit anti-wheat gliadin antibodies (Sigma) were also tested by means of ELISA and detected by using alkaline phosphatase-labeled mouse monoclonal anti-rabbit IgG.

Native wheat flour fractions and wheat isolates (isolates A, B, and C) were separated by means of SDS-PAGE (Fig 1). The aqueous soluble fraction extracted from wheat flour showed a wide diversity of proteins between 67 and 10 kd, corresponding to albumins and globulins. The ethanol-soluble fraction extracted from wheat gluten showed proteins located between 43 and 30 kd, corresponding to gliadins. Wheat isolates showed a common nonresolved pattern between 67 and 37 kd. Wheat isolate B showed a slight difference by the absence of proteins at 22 and 15 to 16 kd. Immunoblotting showed strong IgE binding of serum G to the nonresolved proteins located between 67 and 35 kd in wheat isolates A and B. Wheat

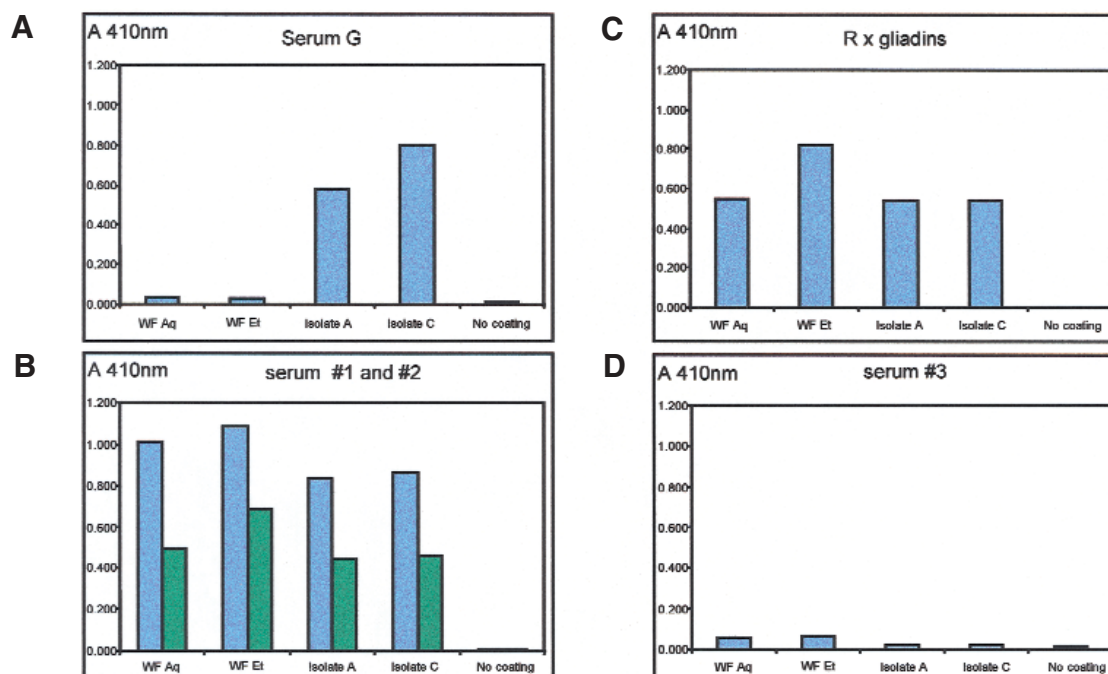


FIG 2. Detection by means of ELISA of specific IgE to wheat flour fractions (aqueous extract [WF Aq], ethanol extract [WF Et]) and wheat isolates A and C. Nonspecific binding was controlled (no coating). Antigens were coated at 20 μ g/mL, and human sera was diluted 1:20. IgE was detected by means of alkaline phosphatase-labeled goat anti-human IgE. **A**, Serum G, patient with allergy to wheat isolate. **B**, Sera 1 and 2, patients with food allergy to wheat flour. **C**, Rabbit anti-wheat gliadin antibodies (Sigma) diluted 1:4000. **D**, Serum 3, patient with allergy to peanut and cow's milk.

isolate allergens were found to be acid after isoelectrofocusing and immunoblotting (data not shown).

RASTs were performed with wheat isolate A–coupled cyanogen bromide disks⁶ calibrated with monoclonal anti-IgE disks by using World Health Organization international IgE standards. Wheat isolate–specific IgE levels were found to be greater than 100 kU/L. Specific IgE from the 4 sera was also assessed by means of ELISA (Fig 2). The control serum (serum 3) showed no IgE binding to the whole panel of extracts (wheat flour, gluten, and wheat isolates A and C). Two sera from patients with wheat flour allergy (patients 1 and 2) showed strong IgE binding to gliadin fractions, to aqueous soluble wheat flour fraction, and to the 2 wheat isolates. Serum G showed strong IgE binding to the wheat isolates (isolates A and C), whereas no IgE binding was found against native wheat flour fractions (both aqueous-soluble and ethanol-soluble fractions). Positive detection of all extracts by means of rabbit anti-wheat gliadin antibodies confirmed that wheat isolates are wheat gluten–derived products.

IgE inhibition assays by means of ELISA showed that the 3 isolates were able to inhibit serum G IgE binding to adsorbed wheat isolate A, whereas no inhibition was observed with the ethanol-soluble fraction extracted from native wheat flour (data not shown).

A case of food anaphylaxis to a wheat isolate has been proved by means of DBPCFC. The ingested amount of isolate could be estimated as 1 g for the sausage and 300 mg for the pork pie. The DBPCFC result was positive to

2 g, whereas 30 g of wheat flour associated to 3 g of gluten was tolerated.

Wheat isolates are produced from wheat gluten, which can be submitted to a chemical process by heating gluten at acidic pH.⁷ This treatment induces the solubilization of gliadins in aqueous buffers by means of deamidation of part of the asparagins and glutamins, resulting in aspartic and glutamic acids. Wheat isolates can also be produced by means of enzymatic treatment with transglutaminase for deamidation.^{8,9} The high protein content and solubility in water of wheat isolates give interesting technologic properties for their use in food industry. Wheat isolates can be used at a maximum 5% level as a food emulsifier, foam stabilizer, water-retention agent, thickening and gelling agent, film formation aid, and stretchability agent in meat products, sauces, and soups. Recently, wheat isolate has been used as a clarifying agent in red wines.¹⁰

We have compared wheat isolates with native wheat flour proteins, both aqueous-soluble fractions (albumins-globulins) and ethanol-soluble fractions (gliadins). Whereas native gliadins are well-resolved molecules between 30 and 43 kd after SDS-PAGE, wheat isolate proteins, fully soluble in aqueous buffer, showed a diffused zone between 35 and 67 kd with nondiscriminated bands.

Serum from patients tolerating wheat flour but allergic to wheat isolate showed strong levels of specific IgE to wheat isolate (class 6), but no specific IgE to wheat flour fractions were detectable by means of ELISA, and only a weak level was detectable by using the more sensitive

CAP-RAST technique. Patients with food allergy to wheat still had specific IgE to wheat isolates, showing the absence of allergen denaturation during the industrial process of gliadin solubilization.

The absence of ELISA inhibition of wheat isolates by means of gliadin fractions could argue for a neoallergenicity. Deamidation of the gluten fraction by means of technologic processes (chemical or enzymatic) could have induced the exposure of cryptic allergenic epitopes or the formation of new allergenic epitopes. Because digestion has been shown to reveal masked epitopes of proteins and the patient is not allergic to wheat flour, the first hypothesis is not retained. Thus we can conclude that the treatments used for gluten deamidation generate new allergenic epitopes. Furthermore, recent works have shown that deamidation of gliadins or gliadin peptides by tissue transglutaminase has a major role in celiac disease.^{11,12} A case of contact urticaria has been recently attributed to hydrolyzed wheat in cosmetics combined with a generalized urticaria induced with the ingestion of sausages with lentils and a French cassoulet.¹³ This patient could also eat cereal-based products without any problem.

Wheat isolates are currently labeled as wheat proteins, achieving patient protection affected with celiac disease. This label might not be precise enough for patients tolerating the native wheat food but specifically allergic to the deamidated forms. Because wheat isolates are new food ingredients, the prevalence of their allergenicity is not known, but wheat isolates should be tested when a food allergy to finished foods is suspected.

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The marble test for gastric outlet obstruction in chronic granulomatous disease

To the Editor:

Chronic granulomatous disease (CGD) is a genetically heterogeneous disorder of the nicotinamide dinucleotide phosphate oxidase complex that results in impaired generation of reactive oxygen species and defective phagocyte antimicrobial activity against catalase-positive organisms. Most patients with CGD have chronic inflammatory complications, including potentially obstructive granuloma of the genitourinary or gastrointestinal tracts.¹ We describe a child with CGD in whom gastric outlet obstruction was suspected without radiographic evidence of delayed gastric emptying, because of an unusual finding.

A 20-month-old boy with CGD was evaluated in the immunology clinic for 2 weeks of nausea and nonbilious, nonbloody vomiting. He was afebrile, and his daily activity, appetite, stools, and sleep patterns were unaffected. Before this clinic visit, his regular medications, trimethoprim-sulfamethoxazole and IFN- γ , had been stopped for 4 days, but the vomiting had persisted. His primary pediatrician had prescribed ranitidine for anti-reflux prophylaxis.

The patient was diagnosed with CGD at 17 months of age, when he had a culture-negative cervical abscess that required surgical drainage, despite appropriate oral and intravenous antibiotics. The diagnosis was confirmed by nicotinamide dinucleotide phosphate oxidase immunoblotting and DNA analysis. After diagnosis, he remained healthy, other than several episodes of folliculitis that responded well to oral cephalixin.

Physical examination revealed a thriving toddler in no distress. The abdomen was nondistended, had good bowel sounds, and was nontender, with no palpable masses and no hepatosplenomegaly. No enlarged lymph nodes were palpable. The remainder of the physical examination was normal.

The differential diagnosis of this persistent vomiting included gastritis, gastric outlet obstruction, chronic reflux, and medication-induced vomiting from IFN- γ . An upper gastrointestinal series revealed normal gastric emptying, but a small marble that the child had reportedly swallowed several weeks earlier was visible in the stomach (Fig 1).

The child was started on 0.1 mg/kg metoclopramide 3 times daily in addition to the ranitidine. Two weeks later, he returned to the clinic with continued vomiting and had