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Beer-induced anaphylaxis: identification of allergens

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Key words: barley; beer; cereals; food allergy; immunoblotting; malt.

Background: We report on a 21-year-old atopic woman who developed urticaria, angioedema of the face, and wheezy dyspnea shortly after drinking beer and after eating a corn-made snack.

Methods: Skin prick tests and specific IgE determinations to beer ingredients and cereal extracts were performed. Immunoblotting inhibition assays were carried out to investigate possible common allergens shared by barley and malt with corn.

Results: Skin prick tests and specific IgE measurements with beer, barley, malt, wheat, corn, rye, rice, and oat flour were positive. Ten pollen-allergic patients showed negative skin tests to beer. Double-blind, placebo-controlled, oral challenge tests with sodium metabisulfite and wheat flour were negative. Immunoblotting demonstrated several IgE-binding bands at 31–56 kDa in malt and barley extracts, and a major band at 38 kDa in the beer extract. Immunoblot inhibition assays showed that malt extract was able to inhibit most of the IgE-binding bands in wheat and corn extracts, whereas corn did not produce significant inhibition to barley and malt extracts.

Conclusions: This patient developed type I hypersensitivity to barley/malt and corn. Although she also showed IgE reactivity to wheat and other cereals, no symptoms were elicited upon ingestion of these cereals, probably indicating latent sensitization to them.

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Beer is one of the most popular alcoholic beverages all over the world. Beer mainly contains malt, hops, brewer's yeast and water. For the production of special beers, other cereals (e.g., wheat, rice, or corn) and enzymes may be added. Malt is obtained from germinated and heated or roasted barley. Hops

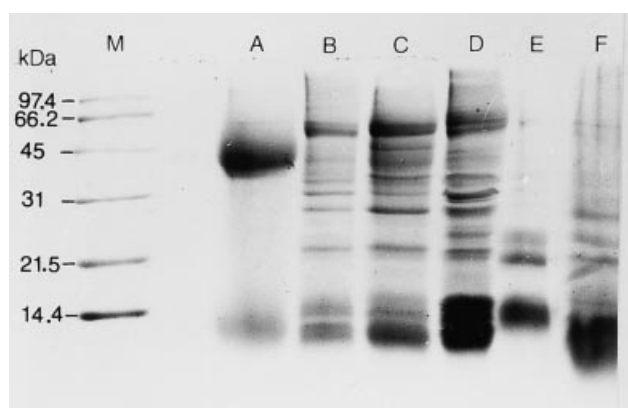


Figure 1. SDS-PAGE of precipitated beer proteins (lane A), barley (B), malt (C), wheat (D), rice (E), and corn (F). Molecular weight markers (M).

(*Humulus lupulus*) provides the characteristic bitterness and aroma of beer and prevents bacterial contamination. For the fermentation process, the yeast species *Saccharomyces cerevisiae* and *S. carlsbergensis* are used.

Beer has rarely been reported as a cause of type I allergic reactions (1–6). We describe the clinical and immunologic studies carried out in a patient in whom anaphylaxis developed after drinking beer.

Case report

The patient was a 21-year-old woman, with a history of seasonal rhinoconjunctivitis, allergy to mustard, oral allergy syndrome with Rosaceae fruits, and anaphylaxis to metamizol. She was referred to our clinic after she had developed two episodes of anaphylaxis in the last 6 months. In the first one, the patient experienced generalized itching and urticaria, angioedema of the lips and face, and wheezy dyspnea within 45 min of drinking beer. Worsening of symptoms required emergency treatment with systemic corticosteroids, epinephrine, oxygen, and antihistamines. Three months later, she experienced a similar reaction 1 h after eating a corn-made snack. She had not had other foodstuffs, beverages, or drugs coinciding with these episodes. The ingestion of bread, baking products, and rice elicited no symptoms.

Complete blood cell count, serum chemistries, chest radiographs, lung-function tests, determinations of complement factors (C₃, C₄, CH₅₀, and C₁INH), and serology for *Echinococcus granulosus* were normal. Total serum IgE was 447 kU/l.

Material and methods

Preparation of extracts

Hops, brewer's yeast, malt, barley, wheat, corn, oats, rice, and rye were purchased from local stores and prepared at a concentration of 20% w/v: 2 g of source material was extracted in 10 ml phosphate-buffered saline (PBS). After magnetic stirring for 60 min at room temperature, the soluble components were separated from the insoluble ones by centrifugation at 5000 rpm for 20 min. The supernatant was collected, dialyzed in distilled water, and sterilized by filtration through a membrane of 0.22- μ m pore diameter (Millipore, Bedford, MA, USA). Bromelain and papain (Sigma Chemical Co., St Louis, MO, USA) extracts were made at a concentration of 10 mg/ml in saline solution 0.9%.

Beer proteins were precipitated with ammonium sulfate, as previously described (7). The precipitation was carried out by adding the salt to the extract solution up to 95% saturation, and the solution was then left overnight at 4°C. The precipitated fraction was separated by centrifugation at 11 000 rpm for 30 min at 4°C, and then dissolved in PBS, dialyzed, and freeze-dried.

The protein concentration of the extracts, as determined by Bradford's method (8), was as follows: 1.5 mg/ml in barley, malt, rye and rice, and beer (precipitated); 0.2 mg/ml in oats and corn; and 0.05 mg/ml in hops and brewer's yeast extracts. For *in vitro* experiments, the protein concentration of all the allergenic extracts was adjusted to 1 mg/ml.

Skin prick tests

Prick tests were carried out with commercially available extracts of pollens (grasses, trees, and weeds), mites (*Dermatophagoides pteronyssinus* and *D. farinae*), animal dander (cat and dog), molds (*Alternaria*, *Aspergillus*, and *Penicillium*), α -amylase, and foods (fruits, nuts, legumes, and cereal grains) (Abelló Lab., Madrid, Spain). Prick tests were also carried out with beer and home-made extracts of hops, brewer's yeast, malt, barley, wheat, corn, rice, oats, rye, bromelain, and papain.

Histamine phosphate at 10 mg/ml and normal saline were used as positive and negative controls, respectively. A wheal with a mean diameter 3 mm or greater with erythema, occurring 15 min after testing, was defined as a positive reaction, the skin prick test with the saline being negative at the same time.

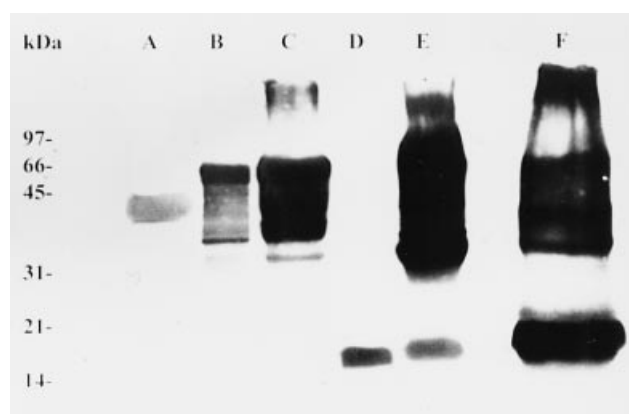


Figure 2. Immunoblot analysis of binding of IgE antibodies from patient's serum to proteins in extracts of precipitated beer (A), barley (B), malt (C), rice (D), wheat (E), and corn (F).

Specific IgE antibodies

Specific IgE antibody levels against the above-mentioned allergens were determined by the CAP technique according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). A CAP score greater than 0.35 kU/l was considered positive.

Oral provocation tests

Double-blind, placebo-controlled, oral challenge tests were performed with sodium metabisulfite (5, 10, 20, 50, 100, and 200 mg) by the method previously described (9) and with wheat flour in opaque capsules (125, 250, 500, and 1000 mg). Moreover, an open oral challenge test with blended whisky was carried out from 10 up to 50 ml.

SDS-PAGE and immunoblotting

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out by the method of Laemmli (10). Polyacrylamide concentrations of 15% and 5% were used for separating and stacking gels, respectively. The samples were mixed with Tris-ClH 0.055 M, adjusted to pH 6.8, containing 2% (w/v) SDS, 10% (v/v) glycerol, and 0.005% (w/v) bromophenol blue. An amount of 30 µg protein was applied per lane. Six different samples were separated by SDS–PAGE: ammonium sulfate-precipitated beer, malt, barley, rice, wheat, and corn extracts.

Electrophoresis was performed by applying 100 V for 3 h, using as buffer of electrodes Tris 300 mM, pH 8.3; glycine 1.92 M; and SDS 34.7 mM. The gels were stained with Coomassie brilliant blue, or used for immunoblotting as

described below. Separated protein bands were electrophoretically transferred to nitrocellulose membrane (Bio-Rad, Richmond, CA, USA) by the method of Towbin et al. (11), in transfer buffer (0.025 mM Tris, pH 8.3; 0.192 mM glycine, 0.005% SDS; and 20% methanol) at 70 mA for 120 min. After transfer, the membrane was saturated for 60 min with 10 mM Tris-buffered saline (TBS) containing 3% bovine serum albumin (BSA). The membranes were then incubated with the patient's serum diluted 1:5 in 10 mM TBS containing 3% BSA and 1% polyoxyethylenesorbitan monolaurate (Tween 20) for 12–14 h. After four washes with 0.1% Tween 20 (v/v) in PBS, the membranes were incubated with antihuman IgE conjugated with peroxidase (The Binding Site, Birmingham, UK) for 3 h. The protein bands were developed by chemiluminescence with ECL (enhanced chemiluminescence) (Amersham International, Buckinghamshire, UK).

Immunoblotting inhibition assays were carried out to investigate possible common allergens shared by barley and malt with corn. Briefly, the patient's serum diluted 1:5 in TBS containing 3% BSA and 1% Tween 20 was incubated for 2 h at 4°C with either malt or corn. BSA was used as a negative inhibitor control. After incubation, the serum solution was applied to nitrocellulose strips containing the separated cereal proteins, and immunoblotting was conducted as described above. In all the immunoblot inhibition assays, an amount of 50 µg of protein inhibitor allergen was used.

Results

Skin prick test

Immediate positive responses were observed with grass pollen, Rosaceae fruits, nuts (almond, peanut, and hazelnut), and mustard. Positive skin tests were also found to beer (12×6 mm), malt (11×8 mm), barley (10×10 mm), corn (7×6 mm), wheat (4×4 mm), rye (8×6 mm), rice (7×6 mm), and oats (5×5 mm) flour extracts. Prick test with molds, mites, animal dander, hops, brewer's yeast, and enzymes (papain, bromelain, and α-amylase) were negative. Ten beer-drinking controls and 10 pollen-allergic patients showed negative skin tests to beer and malt.

Specific IgE antibodies

Specific IgE antibodies were detected in the patient's serum against malt (2.88 kU/l), barley (3.86 kU/l), corn (7.18 kU/l),

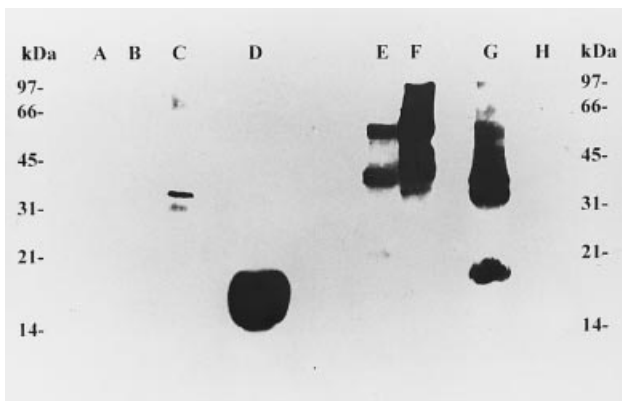


Figure 3. IgE immunoblots with barley (A, E), malt (B, F), wheat (C, G), and corn (D, H) after preincubation of patient's serum with malt (lanes A–D) and corn (E–H).

wheat (3.05 kU/l), rye (3.17 kU/l), rice (1.5 kU/l), and oats (12.2 kU/l).

Oral provocation tests

The double-blind, placebo-controlled, oral challenge test with sodium metabisulfite and wheat flour elicited no reaction. The patient tolerated whisky without ill effect.

SDS-PAGE and immunoblotting

SDS-PAGE of barley and malt extracts showed similar patterns of protein bands, and several of these bands were also present in wheat extract. On the other hand, rice and corn showed a different pattern of separated proteins from barley and malt. In the precipitated protein beer extract, a dense protein band with apparent mass of 38 kDa, and two faint bands at 56 and 12 kDa, were observed (Fig. 1). IgE immunoblots showed many different allergens in the cereal flours in a wide range of MW (Fig. 2). Several IgE-binding bands were detected at 31–56 kDa in malt and barley extracts, and a major band at 38 kDa in the beer extract. Interestingly, immunoblotting showed greater IgE binding to malt than to barley proteins. On the other hand, a broad spectrum of IgE-binding bands were detected in wheat, in the range of 17–97 kDa.

Immunoblots of nitrocellulose strips incubated with the patient's serum in the presence of inhibitors is shown in Fig. 3. No IgE binding to beer and barley/malt proteins was observed with serum from a control nonatopic patient (not shown). Malt extract completely inhibited IgE binding to the malt and barley proteins, whereas the BSA control did not inhibit IgE binding to these cereals. Malt extract was also able to inhibit most of the IgE-binding bands in wheat and

corn extracts, with the exception of a strong band at 16–18 kDa in corn extract. Immunoblot inhibition assays using corn extract as inhibitor allergen showed complete inhibition of IgE binding to corn, whereas in the same experiment, it did not produce significant inhibition of IgE binding to barley and malt extracts (Fig. 3).

Discussion

We report a patient who experienced severe anaphylactic reactions after drinking beer and eating a corn-made snack. The results of *in vivo* and *in vitro* tests strongly suggest that these reactions were due to an IgE-mediated mechanism against barley/malt and corn allergens. Although specific IgE against other cereals were also found in the patient's serum, the only clinically relevant allergens seem to be those from malt and corn flour. In fact, oral challenge with wheat flour was negative and the ingestion of bread and baking products elicited no symptoms in this patient. Moreover, the negative results of challenge tests with sulfites and ethanol (whisky) rules out sensitivity to these compounds.

Beer allergy has seldom been reported (1–6). In the previous papers on beer allergy, urticaria was the commonest clinical manifestation; anaphylaxis has rarely been reported. van Ketel (1) described two patients with immediate-type allergy to beer; one of them developed an itchy swollen face after entering a bar and anaphylactic shock on drinking some beer, and the other patient had recurrent urticaria after drinking beer. The scratch test to malt was positive in both patients. Jover et al. (2) studied 12 patients with immediate hypersensitivity to beer. The 12 patients had positive scratch tests to beer, malt, brewer's yeast, and rice, and nine of them were positive to hops. Since oral challenge tests with raw rice and yeast powder yielded negative results in these patients, malt was considered the main causative antigen of beer allergy. Keller & Schwanitz (3) reported a patient with angioedema after drinking beer or eating fresh white bread or cake, the disorder being attributed to wheat and barley allergy. Gutgesell & Fuchs (5) described a patient with hives on her hands upon contact with beer at work; however, drinking beer elicited no symptoms in her. Santucci et al. (6) studied three patients in whom urticaria developed after drinking beer, suggesting that malt was the component causing urticaria. Recently, Vidal & González-Quintela (4) reported a patient who developed asthma after both occupational exposure to barley flour and ingestion of beer.

In all these reports, malt was implicated in the elicitation of allergic symptoms, and this indicates that anaphylactic reactions to beer are likely to be due to malt/barley sensitization. However, to the best of our knowledge, allergenic components of malt in patients with beer allergy have not been previously identified.

By using immunoblotting, we could identify several allergens in malt and barley. Interestingly, more and stronger IgE-binding bands were identified in malt than in barley itself. A possible explanation of this finding might be that during the germination process to produce malted barley several enzymes are produced that turn starches into brewing sugar. In fact, malt quality for brewing relies very much on the enzyme content. Thus, the malted grain may

contain new allergens or epitopes that appear during the germination and/or heating of barley grains, so enhancing the allergenicity of barley. Immunoblotting assays demonstrated that the main IgE-binding component in precipitated beer proteins was a 38-kDa allergen. With serum from this patient, IgE binding to barley/malt proteins could not be inhibited by corn extract, suggesting that most of the relevant IgE epitopes in barley/malt were not present in corn extract.

In conclusion, our patient developed type I hypersensitivity to barley/malt and corn. Although she also showed IgE reactivity to wheat and other cereals, no symptoms were elicited upon ingestion of these cereals, probably indicating latent sensitization to them.

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