

Urticaria from beer: an immediate hypersensitivity reaction due to a 10-kDa protein derived from barley

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Summary

Background Urticaria from beer has been reported in atopic patients. In these subjects, the skin-prick test positivity to and presence of specific serum immunoglobulin (Ig)E for barley malt, the basic ingredient used in brewing, suggested a type I hypersensitivity to barley component(s).

Objective To identify the beer allergen(s) and to investigate the presence of related proteins in barley.

Methods Three patients with urticaria from beer and other atopic people, some of them suffering from baker's asthma, were examined for both prick test sensitivity to and the occurrence of serum-specific IgE for partially purified proteins from beer. Allergen identification in beer, malt and barley was performed by immunoblotting.

Results Skin-prick tests and detection of specific IgE by both solid-phase (RAST) and liquid-phase (AlaSTAT) assays demonstrated that the 5–20-kDa beer protein fraction contained the allergen. Immunoblot analysis with sera of patients with urticaria from beer showed that IgE bound only the 10-kDa protein band in beer and malt, whereas a main 16-kDa protein was revealed in barley in addition to a very faint 10-kDa band. With the serum of a patient suffering from baker's asthma no IgE binding bands were observed in beer, whereas specific IgE binding to several proteins, including a major 16-kDa component, were detected for both malt and barley.

Conclusions Urticaria from beer is an IgE-mediated hypersensitivity reaction induced by a protein component of approximately 10 kDa deriving from barley. This allergen does not seem to be related to the major barley 16-kDa allergen responsible for baker's asthma. Because of the severity of the allergic manifestations to beer we recommend testing atopic patients positive to malt/barley and/or who exhibit urticarial reactions after drinking beer for their sensitivity to this beverage.

Keywords: atopy, barley, beer, malt, urticaria, wheat

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Introduction

Some cases of contact urticaria with angioedema after drinking beer have been reported [1–4]. In these patients, the skin-prick test positivity to and the presence of specific

serum IgE for malt, the basic ingredient used in brewing derived from germinated barley, indicated a type I hypersensitivity to malt component(s).

Beer contains a variety of proteins and polypeptides with molecular weights (MW) ranging from approximately 5 up to more than 100 kDa [5]. Most of them originate from the proteolytic and chemical modifications of barley proteins occurring during brewing [6]. Only two major proteins have

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been characterized in beer, one with a MW of 40 kDa and another with a MW of approximately 10 kDa, derived, almost unmodified, from barley albumins/globulins protein Z [7,8] and lipid transfer protein 1 (BLTP 1) [9], respectively. However, several other protein bands with intermediate and even lower MWs can be detected in the SDS-PAGE profile of beer [6] including some hordein-derived polypeptides [10].

In our recent study concerning three cases of urticaria from beer, we found that skin-prick tests with the beer total protein fraction were strongly positive in all patients, suggesting the involvement of beer protein component(s) in the hypersensitivity reaction [4]. The data reported here confirm this assumption and demonstrate that the beer allergen is a low MW polypeptide of 10 kDa derived from barley.

Materials and methods

Materials

Different beer samples were bought at a local store. Barley malt was kindly provided from brewery Peroni (Padova, Italy). All reagents for liquid-phase IgE enzyme immunoassay (AlaSTAT) were from Diagnostic Products Corporation (DPC, Los Angeles, CA, USA), and those for RAST system from Pharmacia (Uppsala, Sweden). Cereal kernels (bread wheat var. *Randa* and two-row barley var. *Arda*) were from collections of the Botanical Garden of the University of Naples (Portici) and Department of Biotechnology (University of Padua). Bicinchoninic acid (BCA) protein assay reagent and SuperSignal ULTRA i.e. Chemoluminescent substrate were from Pierce (Rockford IL, USA), Sephadex G-50 medium from Pharmacia, nitrocellulose membranes (Immobilon NC 0.45 µm) from Millipore (Bedford MA, USA) and antihuman IgE peroxidase-conjugate monoclonal antibody from Sigma (St Louis MO, USA). All other chemicals were of analytical grade. Sera were from patients attending the Service of Allergy of San Gallicano Institute, IRCCS, Rome.

Group 1: patients suffering urticaria from beer

Patient 1. A 28-year-old florist had developed generalized urticaria and laryngeal oedema with dyspnea on two occasions immediately after drinking beer. He was treated with corticosteroids and antihistamines and improved within hours. He also had a previous history of seasonal rhinoconjunctivitis. Prick tests and specific IgE for pollens of *Compositae* mix and grass mix were positive. Total serum IgE was 347 kU/L.

Patient 2. A 17-year-old student reported several episodes of generalized urticaria with swelling of oral mucosa and pruritus shortly after drinking beer. The most recent episode included generalized urticaria and laryngeal oedema with dyspnea, requiring emergency treatment with systemic corticosteroids. He had a previous history of seasonal rhinoconjunctivitis with prick tests positivity and specific IgE for pollens of *Compositae* mix, grass mix and for *Dermatophagoides farinae*. Total serum IgE was 308 kU/L.

Patient 3. A 32-year-old worker had experienced episodes of mild facial angioedema and itching of the mouth after drinking beer. Recently, he also suffered generalized urticaria, facial angioedema and general malaise immediately after drinking beer. He was treated at the emergency room with corticosteroids and antihistamines. His condition improved shortly afterwards and he was asymptomatic within 24 h. Skin-prick tests and specific IgE for *Compositae* mix were positive. Total serum IgE was 428 kU/L.

Group 2: patients suffering from baker's asthma

Four patients (one baker, two pizza-makers and one pastry cook) suffered from rhinitis (or rhinoconjunctivitis) and asthma caused by inhalation of wheat flour. Skin tests and specific IgE for wheat flour proteins were positive in all patients. Total serum IgE ranged between 300 and 2020 kU/L.

Group 3: atopic patients suffering allergic conjunctivitis

Nine patients with personal and family history of atopy were chosen as the control group. They suffered rhinitis (or rhinoconjunctivitis) and asthma. Prick tests and RAST were positive for different inhalants, but not for cereals. Total serum IgE ranged between 250 and 4000 kU/L.

All the patients were informed on the nature and purpose of the tests. Informed consent was obtained in all cases.

Protein extraction

Kernels from the various cereals were milled into flours, that were extracted with 0.5 M NaCl in a solid to solvent ratio of 1:10 (w/v) for 1 h at room temperature. The obtained suspensions were centrifuged (10000 g, 20 min) and the supernatants stored frozen for subsequent use (protein extracts). The same procedure was also used for the extraction of salt-soluble proteins from barley malt.

Partial purification of beer proteins

Proteins were precipitated from 660 mL of beer by ammonium sulphate addition to a final concentration of 80%, as

previously described [6]. Salted-out material was re-dissolved in distilled water (100 mL) and dialysed exhaustively (cut-off 3.5 kDa) against water. After centrifugation of the dialysed solution, the supernatant was put in a dialysis tube (cut-off 3.5 kDa) and concentrated to approximately 1/4 of the original volume against solid polyvinylpyrrolidone 40-T to obtain the beer total protein fraction (BPF). BPF was added of 0.1 volume of 0.2 M TRIS-HCl, pH 7.4, containing 8% (w/v) NaCl, 0.2% (w/v) KCl (concentrated TBS) and, finally, the solution was made 5% (v/v) 2-mercaptoethanol. After standing overnight at room temperature, 6 mL of the reduced solution was loaded onto a Sephadex G-50 column (90 × 2.6 cm) equilibrated with concentrated TBS diluted 1:10 with water. The column was eluted with diluted TBS at a flow rate of 0.5 mL/min and 1 mL fractions were collected. The absorbance at 280 nm of the collected fractions was measured, and fractions pooled accordingly. Protein content of the pooled peaks was measured by the BCA method [11]. The pooled fractions of each peak were dialysed against water (cut-off 3.5 kDa), lyophilised and dissolved at appropriate concentrations for the experiments described below.

In vivo skin-prick tests

These were performed in accordance with the recommendations of the European Academy of Allergology and Clinical Immunology [12] using the different cereal extracts at a protein concentration of 0.5 mg/mL. Commercial beer samples containing approximately 0.5 mg/mL of protein were tested undiluted.

Detection of specific IgE

Detection of specific IgE to inhalants and to cereals (wheat and barley) was performed by using the commercial RAST kits (Pharmacia) according to the manufacturer's instructions. Detection of specific serum IgE to beer protein fractions eluted from Sephadex G-50 column (F1 and F2, see Results) was performed with both the AlaSTAT and RAST systems. In the case of the AlaSTAT assay, approximately 50 mg of protein was coupled to the soluble matrix and processed by the scientific section of the DPC, as previously reported [13,14]. Specific IgE were then determined according to the AlaSTAT protocol (DPC) [13]. For the RAST assay, F1 and F2 were coupled to paper discs using the method of Ceska *et al.* [15]. Proteins were dissolved in Ceska buffer at 4 mg/mL and coupled to CnBr-activated paper discs in a ratio of 0.2 mL/paper disc. The detection of specific IgE was then performed with Phadezym RAST tracer reagents following the method of Wide *et al.* [16].

Competitive inhibition of IgE binding to the beer low MW protein fraction (F2) by protein extracts from different

cereals (barley, bread wheat, einkorn wheat, rye and rice) was carried out by incubating 50 µL of each extract (protein concentration 2.5 mg/mL) and 50 µL of undiluted serum of either patient 1 or patient 2 overnight at 4°C. The reaction mixture was then tested for specific IgE using the AlaSTAT assay.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to Laemmli [17] utilizing a mini-protein II cell Bio-Rad, Richmond CA, USA. Total acrylamide (T) and bis-acrylamide (C) concentrations were 18 and 2.5%, respectively. Samples were prepared by a 0.2-fold dilution with 0.5 M Tris-HCl, pH 7.4, containing 30% (w/v) glycerol, 5% (w/v) SDS and 10% (v/v) 2-mercaptoethanol. Electrophoresis was run at 50 mA constant current until the tracking dye bromophenol blue reached the bottom of the gel. Gels were stained according to Koenig *et al.* [18] or used for blotting.

Immunoblot analysis

Protein bands separated by SDS-PAGE were transferred by semidry blotting onto nitrocellulose sheets as previously described [19]. Blotted bands were visualized by soaking the membranes for a few minutes in Ponceau S (0.1% in 3% trichloroacetic acid) and marked with a pencil before destaining with water. Membranes were blocked with TBS containing 0.05% Tween 20 (TBS-T) and 5% skim milk powder (M-TBS-T) for 2 h, and incubated overnight with single patient sera diluted in TBS-T as reported in Fig. 3. After washing five times with M-TBS-T, blots were incubated for 1 h with monoclonal antihuman IgE peroxidase-conjugate antibody (Sigma) diluted 1:5000 in M-TBS-T. After four washes in M-TBS-T and one with TBS, bound IgE were visualized by chemiluminescence using the Super-Signal detection kit (Pierce), following the instructions given by the manufacturer. The whole procedure was performed at room temperature.

Results

Clinical data of selected patients

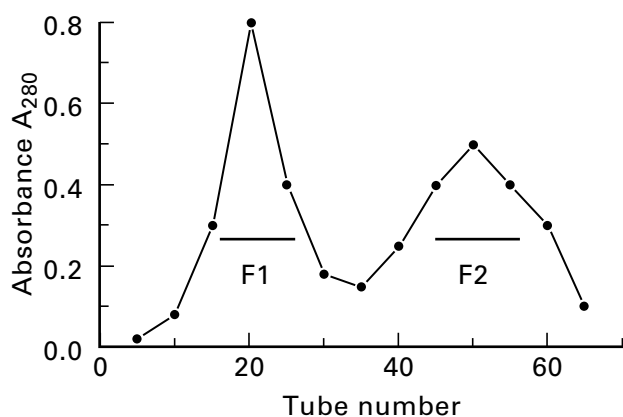
We have examined numerous atopic patients showing different allergic manifestations (Table 1). On the basis of the symptoms and the presence of specific IgE to inhalants, and to wheat and barley proteins, these patients could be divided in three groups. Group 1 (cases 1–3) included three patients with urticaria from beer: one of them (patient 2) was that reported previously as case 3 [4] whereas the other two were new patients. All of them were unreactive when

Table 1. Skin-prick tests and specific IgE to various cereal allergens and to the partially purified beer protein fractions in selected patients.

Patient	Prick						AlaSTAT ^a		RAST ^a			
	F1	F2	W	B	M	Be	F2	F1	F2	W	B	
Group 1												
1	0	4+	0	3+	3+	4+	2	0	3	0	2	
2	0	4+	0	4+	4+	4+	2	0	3	0	2	
3	0	4+	0	2+	2+	2+	1	0	2	0	2	
Group 2												
4	0	0	4+	NT	NT	0	0	0	0	4	2	
5	0	0	3+	3+	3+	0	0	0	0	4	2	
6	0	0	3+	3+	3+	0	0	0	0	4	3	
7	0	0	3+	3+	3+	0	0	0	0	4	3	

^a AlaSTAT or RAST class. Group 1: patients suffering urticaria from beer. Group 2: patients suffering 'baker's asthma'. W, bread wheat (kernel); B, barley (kernel); M, malt; Be, beer; F1, high molecular weight beer protein fraction; F2, low molecular weight beer protein fraction. NT, not tested.

prick-tested with extracts of hops and brewer's yeast (at a concentration of 1 mg/mL of protein, not shown), which are the additional ingredients used in beer production. These patients were IgE positive to barley, but not to wheat. They reacted strongly in prick tests with beer and extracts of barley malt and barley, but were unreactive to wheat. Group 2 (cases 4–7) included four bakers suffering from rhinitis and asthma caused by wheat flour (baker's asthma). These patients reacted to wheat and barley in both RAST and prick. Group 3 (cases 8–16) comprised patients suffering from rhinoconjunctivitis to inhalants. They were IgE negative to wheat and barley.

**Fig. 1.** Gel filtration on Sephadex G-50 medium of the salted-out beer proteins. The two collected fractions (F1 and F2) are indicated by horizontal bars.

Partial purification of the beer proteins

In order to positively identify the proteins responsible for the allergic reaction, BPF was fractionated by gel filtration chromatography. This procedure allowed us to obtain two major peaks (Fig. 1). The first peak (F1), by SDS-PAGE analysis, was shown to contain the major 40-kDa protein in

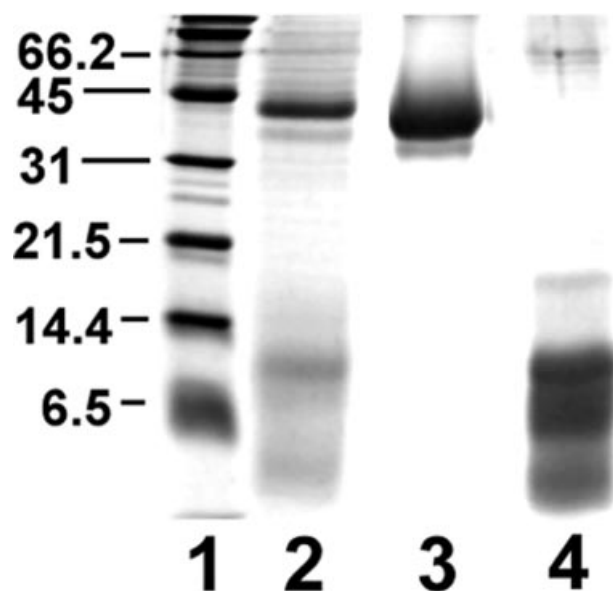
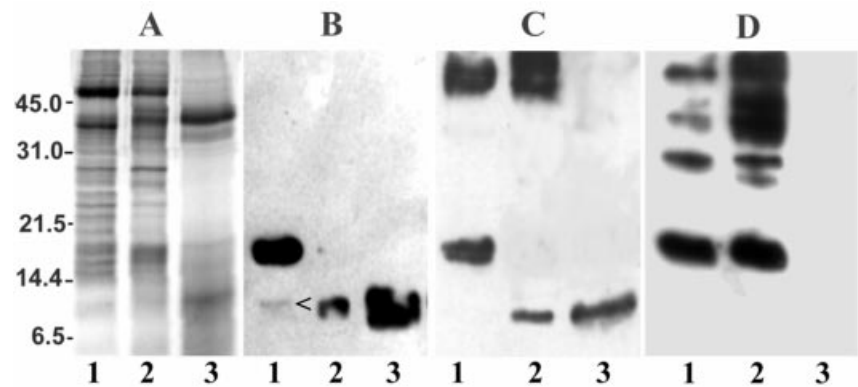
**Fig. 2.** SDS-PAGE analysis of total salted-out beer proteins (lane 2), and fractions collected from Sephadex G-50: F1 (lane 3) and F2 (lane 4). Molecular weight standard proteins are in lane 1.

Fig. 3. SDS-PAGE analysis of the salt-soluble proteins from barley (lanes 1) and malt (lanes 2) and of the salted-out beer proteins (lanes 3). Proteins were stained with Coomassie (A) or IgE immunodetected with the sera of two patients suffering from urticaria from beer (B, patient 1; C, patient 2) and of a patient suffering from baker's asthma (D, patient 5). Sera of patients 1 and 2 were used undiluted and the serum of patient 5 was diluted 1:2. Molecular weights are indicated to the left.



a nearly pure form (Fig. 2, lane 3), whereas the second one (F2) comprised the bulk of the low MW polypeptides (Fig. 2, lane 4), with apparent MWs ranging from approximately 20 to 5 kDa, which is the lowest MW detectable in a 18% polyacrylamide SDS gel. The gel filtration was run under nonreducing conditions (TBS buffer) which, although giving a poor resolution, allowed recovery of the proteins of the two separated fractions in their native state and their safe subsequent use on patients because of the absence of potentially toxic denaturing compounds.

Prick tests and detection of specific IgE with partially purified beer protein fractions

No reaction was found in the patients of group 1 when pricked with F1, whereas a strong positive response was evident with F2, indicating that this complex mixture of polypeptides contained the allergen(s). Patients of group 2 did not react when pricked with both F1 and F2 (Table 1). Patients of group 3 and 10 healthy subjects were also unreactive to both preparations (data not shown).

Specific IgE antibody levels against any component(s) of F2 were determined by both AlaSTAT and RAST methods. Only the sera of group 1 patients showed to contain specific IgE against this fraction (Table 1). No specific IgE against F1 was present in the sera of both groups, confirming the results of the skin-prick tests (Table 1).

Competitive inhibition of IgE binding to F2 (AlaSTAT assay) by extracts from different cereals (bread wheat, einkorn wheat, rye and rice) using the serum of either the patient 1 or patient 2 showed that barley and malt were completely inhibitory (100%) at a protein concentration of 2.5 mg/mL, whereas the other cereals were ineffective at all even at a protein concentration 10 times higher (data not shown). These results indicated that no protein cross-reacting with F2 from barley occurred in the other cereals tested.

Identification of the allergens by immunoblotting

Immunoblotting experiments were carried out with the sera of patients 1 and 2 (group 1) and 5 (group 2). The serum of patient 3 (group 1) was not included in this experiment because it was no longer available. In addition, the serum of a healthy subject was included in the experiment as a negative control. The IgE binding pattern was tested on the SDS-PAGE patterns of the BPF and also on the salt-soluble proteins from the ungerminated barley kernels and from a commercial beer malt. As shown in Fig. 3, the IgE of the sera from patients 1 and 2 strongly recognized only one band with an apparent MW of 10 kDa among the beer proteins (Fig. 3B,C, lane 3). For these patients, a band with the same MW also was revealed in the malt extract (Fig. 3B,C, lane 2), although with a lower intensity. In barley, the strongest binding took place at approximately 16 kDa, whereas a weak reaction was observed with a band of 10 kDa for patient 1 (Fig. 3B, lane 1, arrowhead). In contrast, the serum of the patient of group II did not react with any band of the beer protein pattern (Fig. 3D, lane 3), but, as expected [20], a complex IgE binding pattern was seen with barley and malt (Fig. 3D, lanes 1 and 2, respectively). In both these latter extracts, one of the main IgE binding component was a band with an apparent MW of approximately 16 kDa, which corresponds to that of one of the major allergens involved in baker's asthma [20]. No IgE binding bands were observed for the healthy subject with beer, barley and malt (not shown).

Discussion

The data here reported show that the urticaria from beer observed in the patients examined in this study is an IgE-mediated hypersensitivity reaction induced by a protein component of approximately 10 kDa derived from the barley malt used in beer production. A protein with an almost identical MW (9.7 kDa) has been previously identified in the beer as the Lipid Transfer Protein 1 of the barley

kernel (BLTP 1) by amino acid sequence and immunoblotting. BLTP 1, which occurs in relatively low amounts in the barley kernel, survives the malting and brewing processes, being particularly resistant to both wort boiling and proteolysis, and thus can be found intact in the beer, where it represents one of the major protein components [9]. Therefore, if the beer allergen corresponds to the BLTP 1, the different extent of IgE binding observed in the three different preparations (i.e. barley, malt and beer) could reflect differences in the levels of BLTP 1 relative to the total protein present in each sample.

In barley, the main IgE-binding component for the sera of patients suffering from urticaria from beer showed a MW of approximately 16 kDa (Fig. 3B,C, lane 1). However, the reactivity to this protein disappeared in both the malt extract and in the BPF (Fig. 3B,C, lanes 2 and 3, respectively), indicating that the 16-kDa barley allergen was modified during malting. Therefore, the possibility that such a modification gives rise to the IgE-binding 10-kDa protein observed in the malt and in the beer must be taken into account.

Beer contains some hordein-derived polypeptides [10] and wheat prolamins have been recently reported to be involved in cereal hypersensitivity [22]. Thus, a possible relation of the 10-kDa allergen to hordein cannot be excluded, although no protein bands with a MW around 10 kDa were detected in immunoblotting experiments with antibodies against hordeins [10].

The serum of the patient presenting the classical symptoms of the occupational disease known as 'baker's asthma', examined by immunoblotting, did not react with any protein from beer (Fig. 3D, lane 3), confirming the results of prick tests, AlaSTAT and RAST, but had IgE that bound a 16-kDa protein present in the barley and malt extracts. A protein with a similar MW, belonging to the barley α -amylase inhibitor family, has been reported to be one of the major barley allergens involved in baker's asthma [20].

It is noteworthy that the sera of the patients of groups 1 and 2 examined by immunoblotting all contained IgE that reacted with a protein of approximately 16 kDa in the barley pattern. However, the occurrence of IgE-reactive 16 kDa band in barley but not in malt for the patients of group 1 and in both barley and malt for the patient of group 2 suggests that, although showing a similar MW, the barley 16 kDa components recognized by the sera of the patients of the two groups are different proteins. This is consistent with the well-known heterogeneity of the barley kernel proteins showing a MW of approximately 16 kDa [21].

The data from the literature [1–4] and reported here indicate that people suffering from urticaria from beer are atopic patients, as evidenced by their relatively high total IgE levels and allergic manifestations to inhalants. The allergic reactions to beer may vary from mild urticarial manifestations to life threatening events such as laryngeal

oedema with dyspnea and shock. Therefore, we recommend testing for 'beer allergy' in all patients allergic to malt/barley and/or exhibiting urticarial reactions after drinking beer.

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