

BRIEF COMMUNICATION

Allergy to beer in LTP-sensitized patients: beers are not all the same

O. Quercia¹, G. Zoccatelli², G. F. Stefanini¹, G. Mistrello³, S. Amato³, M. Bolla², F. Emiliani¹ & R. Asero⁴

¹Allergy Unit, Faenza Hospital, Faenza; ²Department of Biotechnology, University of Verona, Verona; ³Lofarma SpA, R & D, Milano;

⁴Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Italy

To cite this article: Quercia O, Zoccatelli G, Stefanini GF, Mistrello G, Amato S, Bolla M, Emiliani F, Asero R. Allergy to beer in LTP-sensitized patients: beers are not all the same. *Allergy* 2012; DOI: 10.1111/j.1398-9995.2012.02872.x.

Keywords

beer; food allergy; lipid transfer protein; maize.

Correspondence

Dr Riccardo Asero
Ambulatorio di Allergologia
Clinica San Carlo
Via Ospedale 21
20037 Paderno Dugnano (MI)
Italy.
Tel.: +39 02 99038470
Fax: +39 02 99038223
E-mail r.asero@libero.it

Accepted for publication 04 June 2012

DOI:10.1111/j.1398-9995.2012.02872.x

Edited by: Marek Sanak

Abstract

Background: Most cases of beer allergy reported so far have been associated with hypersensitivity to the non-specific lipid transfer protein (LTP). In view of the marked differences in brewing processes we assessed IgE reactivity as well as tolerance to many different beers in an allergic patient.

Methods: A 45 year-old man hypersensitive to grass pollen, cat dander and *Alternaria tenuis* with a history of urticaria and dyspnoea after drinking beer and a weak skin reactivity to commercial corn extract was studied. The patient underwent SPT with 36 different brands of beer and an open challenge with those scoring negative was performed. An immunoblot analysis was carried out using 2 SPT-positive beers, 2 SPT-negative beers, and barley, wheat, and maize extracts using both patient's serum and a maize LTP-specific in-house developed polyclonal antibody from rabbit. Further, the immune reactive LTP of one beer was separated by HPLC and the chromatogram was compared to that of purified maize LTP.

Results: Beer SPT scored positive in 30/36 cases. The immunoblot analysis showed IgE reactivity at about 10 kDa against the two SPT-positive beers and against maize with both patient's serum and the polyclonal anti-LTP rabbit serum, whereas the two SPT-negative beers, and barley extract scored negative. The immunodetected protein co-migrated with maize LTP.

Conclusion: In beer-allergic patients the diagnostic workup may point to the detection of some tolerated products that can be consumed risk-free.

Beer is one of the most popular alcoholic beverages all over the world. It is generally made with barley malt, hops, brewer's yeast, and water, but for the production of some special beers, other cereals (i.e., wheat, rice, or corn) and enzymes may be employed. Malt is obtained from germinated and heated cereals. Hops (*Humulus lupulus*) provide the characteristic bitterness and flavor of beer and prevent bacterial contamination. For the fermentation process, the yeasts *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* are mostly used.

Although not very common, cases of IgE-mediated urticaria and angioedema after drinking beer are reported in the medical literature (1). In most cases, allergy was associated with hypersensitivity to the nonspecific lipid transfer protein (LTP), a heat- and pepsin-resistant plant pan-allergen (2–5), although allergy to specific proteins in cereals (6, 7) and positive SPT in yeast-sensitized subjects (8) have been described

as well. However, different products are made using different ingredients, fermentation processes, and temperatures; as a consequence, the concentration of relevant allergens may change from one beer to another. In the present study we demonstrate that beer-allergic patients may show much variability in their tolerance/intolerance to different beers.

A 45-year-old man presented at the allergy unit of the hospital of Faenza reporting repeated episodes of diffuse urticaria, angioedema of the face, swelling of the oral mucosa, and dyspnoea, always shortly after drinking beer. The man had also a history of seasonal spring rhinoconjunctivitis and of rhinoconjunctivitis and asthma following exposure to cats.

SPT with commercial extracts of pollens (including grasses, trees, and weeds), mites, animal dander, molds, and a large series of foods (all by Lofarma Allergeni SpA, Milan, Italy) were carried out. SPT were performed on the volar side of the forearm with sterile, disposable 1-mm-tip lancets (ALK-

Abello), pricking through the drop of the extract. SPT with normal saline and histamine 10 mg/ml were used as negative and positive controls, respectively. Readings were taken after 15 min. Reactions were expressed as the mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing by 2). A mean wheal diameter of 3 mm or more was considered positive (9). SPT showed hypersensitivity to grass pollen, cat dander, and *Alternaria tenuis*, and a weak skin reactivity to house dust mites. Among foods, a weak reactivity to corn was found, whereas SPT with wheat, barley, rice, and hops extracts scored negative. SPT with a commercial peach extract (Abello, Spain) was moderately positive. IgE specific for a series of airborne and food allergens measured by Immuno-CAP (Phadia, Uppsala, Sweden) showed significant levels of IgE to grass (9.1 kU/l), *Cladosporium herbarum* (20.7 kU/l), *Alternaria tenuis* (9.5 kU/l), Cat dander (>100 kU/l), barley (3.8 kU/l), and corn (5.0 kU/l), whereas no reactivity to the yeast *Saccharomyces cerevisiae*

was observed. IgE to peach was not measured. Finally, the patient underwent SPT with 36 different brands of beer by the prick-prick technique. Such SPT scored positive in 30/36 cases (Table 1). No skin reactivity to three different beers was observed in 10 nonatopic beer-drinking subjects and in 10 pollen-allergic patients used as controls.

The patient did not react to 100 mg of sodium metabisulfite challenged in a double-blind placebo-controlled fashion. Finally, after an informed written consent had been obtained, the man underwent an open challenge with the six brands of beer that scored negative on SPT (Table 1). Challenges were performed on six different days; 50 ml of beer were given 30 min apart up to a final dose of 500 ml. The patient tolerated all six beers and was allowed to drink them at home. He did not report further episodes of beer allergy since then.

Two beers scoring negative (Hoegaarden and Abbaye de Espérnee) on SPT and two scoring positive (Heineken and Forst) were selected for IgE immunoblotting analysis. Extracts from barley, wheat, and maize were also included. Cereal seeds were finely ground and extracted in PBS (1 : 5). Beer proteins were isolated by ammonium sulfate precipitation. Samples were diluted in SDS-PAGE sample buffer and analyzed by SDS-PAGE. Gels were electroblotted onto polyvinylidene fluoride

Table 1 List of beers used for SPT and oral challenges

Brand of beer	SPT	Oral challenge
1. Biere du demon	++++	ND
2. Peroni gran riserva doppio malto	++	ND
3. Leffe vieille cuvee	++	ND
4. Adelscott wisky malt	–	Tolerated
5. Carlsberg elephant	–	Tolerated
6. Peroni nastro azzurro	+++	ND
7. Aynger	+++	ND
8. Budweiser	++	ND
9. Lutece	–	Tolerated
10. Stella artois	++	ND
11. Bud	++	ND
12. Leffe radieuse	+++	ND
13. Loburg	++	ND
14. Brigand	+++	ND
15. Fisher	++	ND
16. Leffe blonde	++	ND
17. Leffe brune	++	ND
18. Carlsberg	++	ND
19. Chimay	++	ND
20. Duvel	++++	ND
21. Hoegaarden	–	Tolerated
22. Heineken	+++	ND
23. St bernardus 12	++	ND
24. Mort subite framboise	+++	ND
25. Mort subite krik	+++	ND
26. Urbach	++	ND
27. Biere du bucanier	++	ND
28. Forst premium	+++	ND
29. Mc ewans scotch ale	++	ND
30. Abbaye de espernee	–	Tolerated
31. Bjorne beer	++	ND
32. Franziskaner weissbier	++	ND
33. Chimay red	++	ND
34. Judas	++	ND
35. Leffe	++	ND
36. Desperados	–	Tolerated

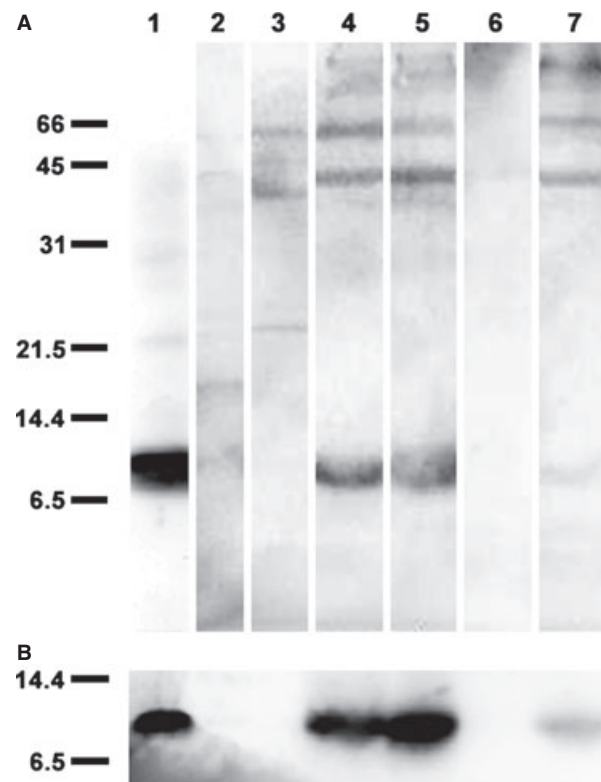


Figure 1 (A) Immunoblotting analysis of cereal and beer proteins developed with patient's serum (Panel A) and with anti-maize LTP (Panel B, detail of the low molecular weight range). Lane 1: maize PBS extract; lane 2: barley PBS extract; lane 3: wheat PBS extract; lane 4: Heineken; lane 5: Forst; lane 6: Abbaye de Espernee; lane 7: Hoegaarden.

(PVDF) membrane (Millipore, Milano, Italy). IgE immunoblotting was carried out with patient's serum diluted 1 : 5 in blocking solution (3% defatted dry milk in PBS, 0.05 Tween 20) overnight. IgE was detected by HRP-conjugated monoclonal anti-human IgE (Southern Biotechnology, Birmingham, AL, USA) diluted 1 : 1000 followed by chemiluminescence detection (LiteAbiot Extend, Euroclone, Milano, Italy). Maize LTP detection was achieved by a specific in-house-developed polyclonal antibody from rabbit (1 : 1000 dilution) followed by incubation with a HRP-conjugated anti-rabbit IgG (Sigma, Milano, Italy) diluted 1 : 5000.

The immunoblotting analysis showed IgE reactivity at about 10 kDa (this molecular weight corresponds to that of LTP) against the two beers that scored positive on SPT and for maize extract, whereas no such reactivity against the two beers which did not react on skin tests or against barley extract was found (Fig. 1A). Two further bands around 40–55 kDa were immunodetected using patient's IgE, with the lower molecular weight one highly reminiscent of the barley protein Z previously reported as an allergen. As these two bands were detected also in Hoegaarden beer that scored negative on SPT, we suppose that they are probably not involved in the allergenicity of the beers analyzed here. The results suggested that the 10-kDa band immunodetected in the beers was the maize LTP. To confirm this hypothesis, a second immunoblotting was carried out using the polyclonal antibody specific for maize LTP (Fig. 1B). This showed a clear reactivity against the two beers positive on SPT, a very faint band against Hoegaarden, and no reactivity to Abbaye de Espernee, in accordance with IgE-immunoblotting results.

We tried then to separate the immune reactive LTP from a protein preparation from Heineken beer by HPLC and to compare the chromatogram with that of purified maize LTP.

A volume of 2.5 ml of Heineken beer was dialyzed against water by PD10 column (GE Healthcare, Milano, Italy). The dialyzed sample was lyophilized, resuspended in 0.1% trifluoroacetic acid, and injected into a reverse phase c18 column (Gemini 250 × 4.60 mm, Phenomenex, Bologna, Italy) connected to a System Gold HPLC chromatographer (Beckman Coulter, Milano, Italy). The elution was carried out by 15–35% acetonitrile gradient at 1 ml/min flow rate. The eluted fractions were collected, lyophilized, and analyzed by SDS-PAGE and IgE-immunoblotting with the anti-maize LTP polyclonal antibody. The results showed the presence of an immunodetected protein eluted in different fractions and comigrating with maize LTP (Fig. 2), thus confirming its identity. Noteworthy, the immunoreactive protein eluted at a longer retention time than the maize LTP. This could be explained taking into account the strong modifications that proteins nor-

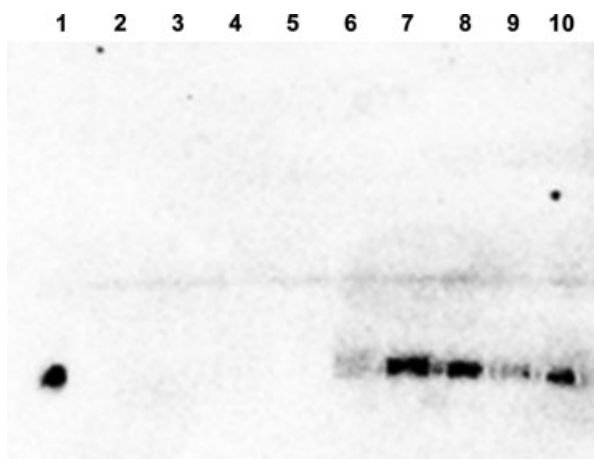


Figure 2 Analysis of HPLC eluted fractions by SDS-PAGE and immunoblotting with anti-maize LTP antibody. 1: maize LTP; 2–10: Heineken protein fractions eluted by HPLC.

mally undergo during malting and along brewing steps, especially when the wort is boiled together with hops. The severity of these processes can denature the proteins and in some cases modify the aminoacid sequence by glycation as a result of Maillard reaction (10). All these factors contribute to alter the hydrophobicity of the proteins, thus helping explain the different retention time in the reverse phase column.

Although our patient was sensitized to LTP, surprisingly enough he reported only beer allergy and tolerated peach and other *Rosaceae* (11). Skin tests with 36 different beers showed variable degrees of IgE reactivity, suggesting differences in allergen concentration as a result of the use of different ingredients (rice, barley, maize, and wheat malt), fermentation processes and temperatures, pasteurization, filtration, storage, etc. Depending on the specific country regulations, maize grits are often added during brewing to give special flavor characteristics to beer (12). In the present case the addition of this cereal may represent the allergen source. Surprisingly enough, the patient contacted again after the results of the immunoblotting, confirmed his good tolerance to both polenta and pop corn. It is tempting to speculate that the presence of alcohol in beer enhanced the rate of intestinal absorption of the allergen, thus inducing the allergic reactions.

Altogether, this study suggests that in beer-allergic patients the diagnostic workup may point to the detection of some tolerated products that can be consumed risk free. This may certainly improve the quality of life of the allergic patient.

References

1. Bonadonna P, Crivellaro M, Dama A, Senna GE, Mistrello G, Passalacqua G. Beer-induced anaphylaxis due to barley sensitization: two case reports. *J Invest Allergol Clin Immunol* 1999; **9**:268–270.
2. Curioni A, Santucci B, Cristaudo A, Canistraci C, Pietravalle M, Simonato B et al. Urticaria from Beer: an immediate hypersensitivity reaction to a 10 kDa protein derived from barley. *Clin Exp Allergy* 1999; **29**:407–413.
3. Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier M, Ciurana CL et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000; **122**:20–32.

4. Asero R, Mistrello G, Roncarolo D, Amato S, van Ree R. A case of allergy to beer showing cross-reactivity between lipid transfer proteins. *Ann Allergy Asthma Immunol* 2001;**87**:65–67.
5. Garcia Casado G, Crespo JF, Rodriguez J, Salcedo G. Isolation and characterization of barley lipid transfer protein and protein Z as beer allergens. *J Allergy Clin Immunol* 2001;**108**:647–649.
6. Figueredo E, Querce S, del Amo A, Cuesta J, Arrieta I, Lobo C et al. Beer-induced anaphylaxis: identification of allergens. *Allergy* 1999;**54**:630–634.
7. Vidal C, Gonzalez-Quintela A. Food-induced and occupational asthma due to barley flour. *Ann Allergy Asthma Immunol* 1995;**75**:121.
8. Kortekangas-Savolainen O, Savolainen J, Lantto R, Kalimo K. Immediate hypersensitivity to bakery, brewery and wine products in yeast-sensitive atopic dermatitis patients. *Clin Exp Allergy* 1994;**24**:836.
9. Dreborg S, Frew A. Allergen standardization and skin tests. EAACI position paper. *Allergy* 1993;**48**:49–75.
10. Jégou S, Douliet JP, Mollé D, Boivin P, Marion D. Purification and structural characterization of LTP1 polypeptides from beer. *J Agric Food Chem* 2000;**48**:5023–5029.
11. Asero R, Mistrello G, Roncarolo D, Amato S, Caldironi G, Barocci F et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy* 2002;**57**:900–906.
12. Briggs DE. *Malts and Malting*. London, UK: Blackie Academic & Professional, 1998.