Brief communication

Isolation and characterization of barley lipid transfer protein and protein Z as beer allergens

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Beer has recently been implicated as the causative agent of contact urticaria and severe IgE-mediated anaphylaxis. However, no allergen from beer has as yet been isolated and characterized. Two major components of 45 kd and 9 kd were detected in crude protein preparations from beer. Both components were purified; they were identified as barley protein Z_4 (45 kd) and lipid transfer protein 1 (LTP1; 9 kd). Protein Z_4 was recognized by the 4 individual sera tested but provoked weak positive responses to skin testing in 2 of 4 beer-allergic patients. Purified LTP1 showed reactivity with 3 of 4 individual sera and induced strong positive skin prick responses in all 4 patients tested. Barley LTP1 and protein Z_4 have been identified as the main beer allergens. (J Allergy Clin Immunol 2001;108:647-9.)

Key words: *Beer; barley; anaphylaxis; lipid transfer protein; protein Z_4; allergen; double-blinded, placebo-controlled food challenge*

Beer is a drink widely consumed all over the world. Malt derived from germinated barley is the basic ingredient used in brewing. Therefore, the beer polypeptides originate mainly in barley proteins, either relatively unmodified or as products of the proteolytic and other chemical modifications involved in seed barley processing.[1]

Several cases of contact urticaria induced by beer have been described since 1980.[2,3] Moreover, ingestion of beer has recently been implicated as the causative agent in cases of severe IgE-mediated anaphylaxis.[4,5] Despite the increasing number of clinical cases of beer allergy, no allergen from beer has been isolated and identified. The purpose of this study was to isolate and characterize IgE-binding components responsible for beer hypersensitivity and to assess in vivo and in vitro allergenicity of the purified allergens.

Abbreviation used
LTP: Lipid transfer protein

METHODS

Each of 4 patients (22 to 38 years of age) with positive immediate skin prick test (SPT) responses to beer had either a clinical history of immediate allergic reactions after isolated intake of beer on 1 or more occasions (patients 1, 2, and 4) or a positive response to double-blinded, placebo-controlled food challenge with beer (patient 3). Details of the challenge procedure and interpretation were described previously.[4] All 4 patients showed specific IgE levels to malt and barley above 6.9 kU/L and 6.0 kU/L, respectively. Skin prick tests were performed with the crude protein preparation from beer (10 mg/mL) and purified allergens (200 μg/mL).

Freeze-dried beer was extracted with PBS buffer. The crude protein preparation was separated by reverse-phase HPLC on a semipreparative Nucleosil 300-C4 (Sugelabor, Madrid, Spain); elution was with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (10% to 100% in 150 minutes; 1 mL/min). Peaks containing the corresponding allergen were identified by SDS-PAGE and immunodetection. Fractions containing the LTP were further repurified by cation-exchange chromatography on an Acell Plus CM Waters Sep-Pak cartridge (Waters Corp, Milford, Mass). Elution was with 20 mmol/L formic acid, pH 4.0, and the retained protein was then eluted with 0.5 mol/L NaCl in the same buffer (1 mL/min). SDS-PAGE was carried out on 4% to 20% precast polyacrylamide gels (Bio-Rad, Hercules, Calif), according to the method of Laemmli.[6] N-terminal amino acid sequences were determined by standard methods through use of an Applied Biosystems 477A gas-phase sequenator (Foster City, Calif).

Immunodetection after SDS-PAGE separation with the serum pool from beer-allergic patients or with control sera (1:2 dilutions) was performed as described previously.[7] The 4 individual sera from the selected beer-allergic patients (1:2 dilutions), as well as control sera, were also used to determine the IgE-binding capacity of the purified allergens (3 μg) by dot-blotting.

RESULTS

A crude protein preparation from freeze-dried beer (11% protein content) was obtained by PBS buffer extraction and $(NH_4)_2SO_4$ precipitation. SDS-PAGE fractionation of this crude preparation showed 2 major bands with apparent molecular sizes of approximately 9 kd and 45 kd (Fig 1, *A*). Only the 45-kd band was recognized in specific IgE immunodetection assays using a serum pool from the patients with beer allergy (Fig 1, *B*). The 2 main beer components (9 and 45 kd) were isolated

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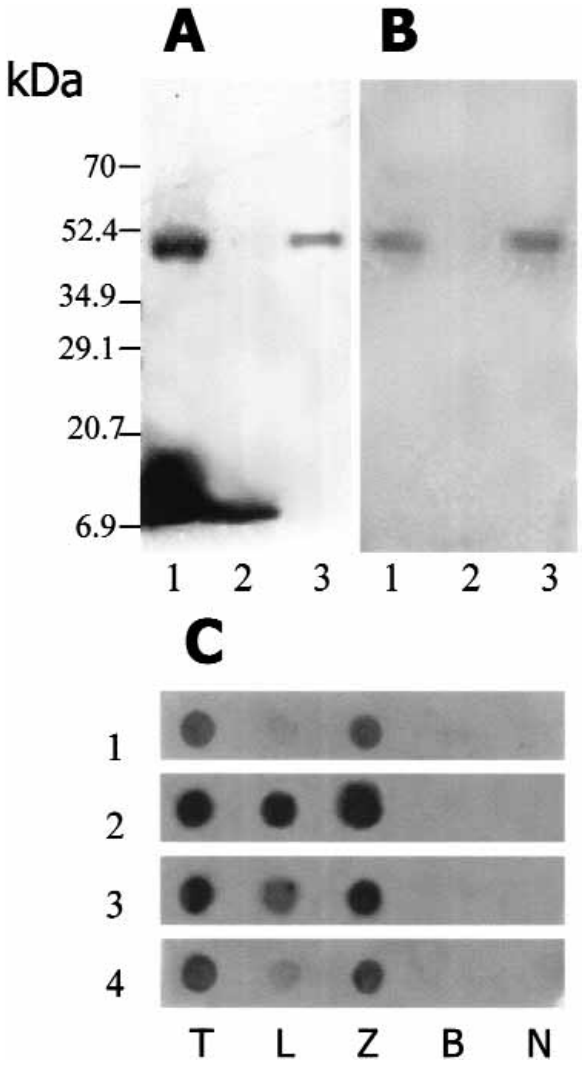

FIG 1. SDS-PAGE fractionation of a crude protein preparation (*lane 1*; 15 μg of protein) and of purified 9-kd (*lane 2*) and 45-kd (*lane 3*; 3 μg) proteins from beer, stained with Coomassie blue (**A**) or electrotransferred to nitrocellulose membrane and immunoblotted with a serum pool from beer-allergic patients (**B**). **C,** IgE immunodetection after dot-blotting (3 μg of protein) a crude protein preparation from beer *(T)*, and purified barley LTP1 *(L)* and protein Z_4 *(Z)*. Bovine serum albumin *(B)* and PBS buffer *(N)* were used as negative controls. Four individual sera (1-4; numbers correspond to those in Table I) from selected beer-allergic patients were used.

TABLE I. Results of skin prick testing with a crude protein preparation from beer (T), purified barley LTP1, and protein Z_4 used as testing agents

Patient no.	Skin prick test result (mm)		
	T	LTP1	Protein Z_4
1	13.2	7.5	–
2	7.5	7.0	–
3	12.2	6.0	3.2
4	5.0	7.0	3.5

Samples were tested at 10 mg/mL *(T)* or 200 μg/mL (purified proteins). –, Negative response. Values are means (n = 2).

from the crude protein preparation (Fig 1, *A*). The purified 45-kd protein, but not the 9-kd polypeptide, reacted in IgE antibody immunodetection tests using the serum pool from beer-allergic patients (Fig 1, *B*).

The N-terminal amino acid sequence of the 9-kd polypeptide (LNXGQVDS) was identical to the first 8 N-terminal residues of the LTP1 (molecular mass, 9.7 kd) from barley seed.[8] The N-terminal amino acid sequence of the purified 45-kd beer protein (VDLVDFANH) fully fitted that of an internal region (residues 363 to 372) of barley protein Z_4.[9] Treatment of the isolated protein Z_4 with trifluoroacetic acid, a reagent used in the initial step of N-terminal amino acid sequence determination, produced a modification of the electrophoretic mobility that corresponded to a decrease in apparent molecular size of approximately 4 kd, suggesting a cleavage of an internal peptide bond (not shown).

The in vitro allergenicity and the in vivo allergenicity of the purified proteins are shown in Fig 1, *C* and Table I, respectively. Dot-blotted purified protein Z_4, under nonreducing and nondenaturating conditions, induced positive signals when incubated with individual sera from the 4 patients allergic to beer, but only 2 patients had positive skin test results. The purified LTP1 induced positive skin reactions by prick tests in all of the allergic patients but reacted with sera from 3 patients in dot-blot experiments.

DISCUSSION

The 2 beer allergens characterized in this study, with apparent molecular weights of 9 kd (LTP1) and 45 kd (protein Z_4), are the main barley proteins that could survive the malting and brewing processes and thus become the major components of the beer protein fraction.[10] In fact, these 2 proteins were previously characterized as the major components of the proteinaceous material present in beer.[1]

The purified 9-kd component was identified as barley LTP1 on the basis of its determined partial amino acid sequence and molecular size. Members of the LTP family have been recently identified as major allergens in several plant foods, especially fruits.[7,11] Purified LTP1 from beer exhibited different in vitro and in vivo allergenic capacity. It did not react with the sera from allergic patients after SDS-PAGE fractionation but was recognized by 3 of 4 individual sera after dot-blotting when denaturating and reducting agents were omitted. However, the reaction with each serum was weaker than that of protein Z_4 under the same conditions. On the contrary, LTP1 induced positive in vivo responses in all 4 patients tested. These data suggest that LTP1 is a relevant beer allergen, and they indicate that in its molecular form, purified from beer, it loses its IgE-binding capacity after SDS-PAGE fractionation.

The N-terminal amino acid sequence of the isolated 45-kd beer component matches to an internal region of barley protein Z_4.[9] Native protein Z_4 (399 residues and a molecular mass of 43.1 kd) is N-terminally blocked, but

the presence of sensitive bonds to cleavage at acidic conditions around residues 357 to 359 has been described.[9] Protein Z_4 was the single beer polypeptide that reacted with the sera from beer-allergic patients in IgE immunodetection assays after SDS-PAGE separation. In this way, only an uncharacterized component of approximately 40 kd has been previously detected in beer extracts through use of sera from patients with beer-induced anaphylaxis.[4,5] Purified protein Z_4 was also recognized by all 4 individual sera from the selected patients. In contrast, the protein showed a poor in vivo allergenicity, inducing weak positive responses in 2 of 4 patients studied (Table I).

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