

***Boletus edulis*: a digestion-resistant allergen may be relevant for food allergy**

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Summary

Background Fungal components can cause allergic symptoms either through inhalation, ingestion or contact. Whereas respiratory allergy is thought to be induced by spores, allergic reactions following ingestion are attributed to other parts of the mushroom. Reports of food-related allergic reactions due to the edible mushroom *Boletus edulis* have occasionally been reported.

Objective The aim of the study was to investigate whether separate allergens may be detected in alimentary allergy to *Boletus edulis*.

Methods Sera of two subjects, one with recurrent anaphylaxis and the other with a predominantly oral allergy syndrome following ingestion of *Boletus edulis*, have been analysed by a time-course digestion assay using simulated gastric fluid and by SDS-PAGE immunoblotting. Sera of four *Boletus edulis* skin prick test-negative subjects and all without clinical symptoms to ingested *Boletus edulis* served as controls.

Results In lyophilized *Boletus edulis* extract, at least four water-soluble proteins were detected, the most reactive at 55 kDa and at 80 kDa. Following the time-course digestion assay, IgE binding was found to a 75-kDa protein, but only if the sera of the subject with recurrent anaphylaxis was used.

Conclusion The data indicate that *Boletus edulis* can cause an IgE-mediated food allergy due to a digestion-stable protein at 75 kDa. No IgE immune response to this protein was detected in the serum of a subject with respiratory allergy and oral allergy syndrome to *Boletus edulis* nor in control sera.

Keywords *Basidiomycetes*, *Boletus edulis*, fungal allergy, food allergy, food allergen, allergen stability
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Introduction

Boletus edulis (*BE*) (king bolete, porcini or cèpe) is an edible mushroom that belongs to the class of Basidiomycota and to the order of Boletales [1]. *BE* grows in temperate climates, mainly in late summer and autumn, and is considered as one of the gastronomically most delicious mushrooms. As shown in several studies, different components of the mushrooms can induce allergic, IgE-mediated symptoms either through inhalation, ingestion or contact [2–11]. Whereas spores are believed to cause respiratory allergies [2–4], edible parts of the mushroom seem to elicit allergic symptoms principally after ingestion [5–7]. In a recent report, three non-occupationally exposed individuals and one cook experienced symptoms like oral allergy syndrome or even life-threatening anaphylaxis following ingestion of *BE* [5]. Roncarolo et al. suggested a cross-reactive protein, most likely profilin, as a possible *BE* allergen responsible for abdominal pain, general itching, and diarrhea in an atopic women who suffered from these symptoms after eating raw or dried cooked *BE* [6].

Since fruiting bodies contain allergens, the recent reports of food induced allergic reactions from eating mushrooms are not surprising, and suggest that edible parts of the mushroom contain clinically relevant food allergens.

The aim of this study was to investigate, whether distinct allergens may be detected in alimentary *BE* allergy.

Patients and methods

Source material and preparation of extracts

Wild grown, fresh *BE* were obtained from the Institute of Botany, Ann Arbor, MI, USA University of Bern. Extracts were prepared as previously described [3,11]. Briefly, caps were peeled and the spore containing tissue was cut into cubes and frozen at -80°C . One gramme of spore-containing tissue was ground in liquid N_2 and prepared in 40 mL of 125 mmol/L ammonium bicarbonate buffer (pH 8.1) for 1 h with stirring at 4°C . The homogenate was centrifuged at 80 000 *g* for 30 min at 4°C and the supernatant was lyophilized. Extracts for skin prick test (SPT) were prepared as previously described [3]. For skin testing, lyophilized material was suspended in a solution of 50% glycerol, 85 mmol/L NaCl and 30 mmol/L sodium bicarbonate, and sterile filtrated with Acrodisc PF 0.8/0.2 μm (Gelman Science, Ann Arbor, MI, USA). Protein content of each extract was determined by the Bradford-based protein

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assay (Bio-Rad, Reinach, Switzerland) and adjusted to 10 mg/mL with diluent. All solutions were sterile filtrated and stored at -80°C . For immunoblot analysis a fraction of the lyophilized material was heated during 30 min at 96°C to denaturate proteins (lyophilized boiled).

Study subjects

A 50-year-old-housewife (patient A) was first seen in 1995 for allergy evaluation of recurrent anaphylaxis after ingestion of cooked *BE*. Four times she experienced generalized urticaria, angioedema, emesis and diarrhea after meals containing *BE*; in two events a fall of blood pressure has been documented. Another patient, a 41-year-old lawyer (patient B), experienced itching and swelling of the oral mucosa, and sometimes rhinoconjunctivitis during or after eating of cooked *BE*. As a collector of mushrooms, he noticed acute rhinoconjunctivitis and sneezing following the inhalation of dried *BE* dust for several years. Symptoms due to ingestion of *BE* began about two years later. Four adult subjects (one of them had a relevant pollen allergy to grass) with a negative SPT to *BE* served as negative controls; all without any clinical symptoms to ingested *BE*. The characteristics of the study participants are outlined in Table 1.

Skin prick tests

All individuals were skin prick tested with nine common inhalant allergens (two species of house dust mites, grass, birch and mugwort pollen, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*), a mould-mix (*Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium chrysogenum*, *Pullularia pullulans*, *Rhizopus nigricans* and *Merulius lacrymans* (Allergopharma Hamburg, Germany)) and three mushroom extracts (*BE*, *Coprinus comatus* and *Pleurotus pulmonalis*) prepared in our laboratory [3,11]. SPTs were performed on the volar surface of the forearm according to the recommendations of the European Academy of Allergy and Clinical Immunology [12,13]. All skin reactions were recorded after 15 min and a mean weal diameter of 3 mm or greater than the 0.9% NaCl control was considered as positive. Blood samples were drawn and sera were stored at -20°C until used.

SDS-PAGE and immunoblotting

SDS-PAGE has been performed as described [3]. Fifteen μg per lane of the total protein extract were resolved on 12% SDS-

polyacrylamid gels and were transferred onto 0.2 μm pore size nitrocellulose (Bio-Rad). After blocking, the membrane was incubated overnight with 1:4 diluted patients and control sera at 4°C . Bound serum IgE was detected with goat anti-human IgE-POX (Nordic, Karlskopa, Sweden) diluted 1:2000 in PBS containing skim milk powder 1%, bovine serum albumin 0.2%, antiforam 0.01% and Tween 20 0.3%. Detection was enhanced by SuperSignal Chemiluminescent Substrate (Pierce, Rockford, IL, USA) and documented on X-ray films exposed for approx. five seconds.

Simulated gastric fluid (SGF) and time-course digestion assay (TCDA)

SGF was constituted with 0.3% pepsin from hog stomach (Fluka, Buchs, Switzerland), 30 mM NaCl and 63 mM HCl resulting in pH 1.2 [14,15]. 1.6 μL of the lyophilized extract, equivalent to 15 μg total protein, was diluted in 7.8 μL distilled water. After adding 5.6 μL of SGF, a digestion time-course was performed at 37°C . Reactions were stopped with 15 μL ice cold 160 mM Na_2CO_3 at the following times: 0.5, 1, 2, 4, 8, 16 and 30 min. Quenched reactions were immediately snap-frozen until used. Ten μL of loading buffer containing 4% β -mercapto-ethanol was added to the frozen, quenched samples and heated to 96°C for 8 min. Probes were immediately used for immunoblot analysis.

Results

Both *BE* food-sensitive patients showed a typical Type I immediate weal-and-flare reaction to the *BE* extract. However, they did not react to any of the common inhalant aeroallergens nor to the other basidiomycete extracts tested. Total IgE was 29 kU/L and 60 kU/L for patients A and B, respectively.

In Fig. 1 immunoblot with pooled serum of both *BE*-allergic subjects depict IgE-binding to lyophilized, boiled lyophilized, and digested lyophilized *BE* extracts. IgE-binding bands could be detected in the lyophilized and the lyophilized digested extract. The most reactive bands were at 55 kDa and at about 80 kDa. In the SGF-digested lyophilized extract, a strong IgE binding band was detected at about 75 kDa. Using the serum of patient B solely (Fig. 2), the binding pattern of the lyophilized

Table 1. Characteristics of the study subjects

Patients	Age/sex	Onset of Boletus allergy	Atopy ¹	Symptoms following ingestion of <i>Boletus edulis</i>	Reactivity to <i>Boletus edulis</i> extract ²
A	50/f	age 44	No	Angioedema, urticaria; emesis, diarrhea; drop of blood pressure	4 mm
B	41/m	age 27	No	OA ³ , rhinoconjunctivitis	10 mm
Controls					
1	30/f	—	Pollinosis	None	—
2	27/f	—	No	None	—
3	25/m	—	No	None	—
4	35/m	—	No	None	—

¹Atopy ≥ 1 positive SPT to common aeroallergens (weal diameter > 3 mm), symptoms of allergic asthma, rhinitis or conjunctivitis and family history.

²Weal size (compared to histamine with 3 mm). ³OA = oral allergy syndrome.

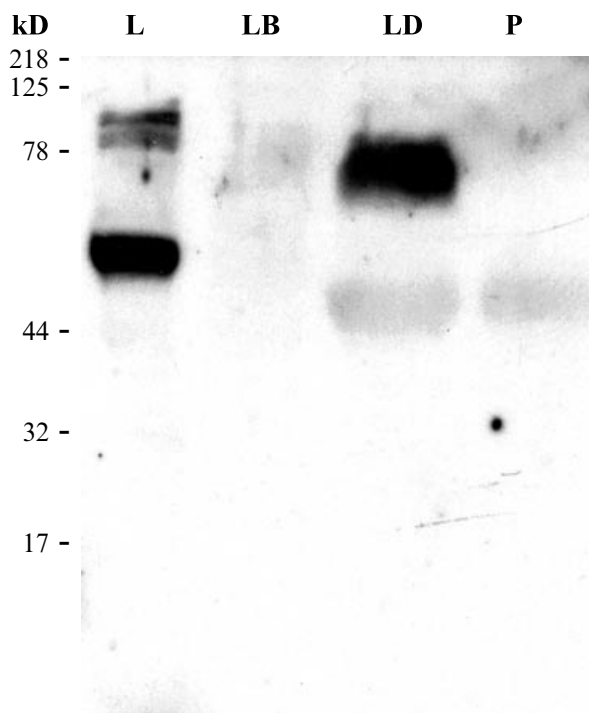


Fig. 1. IgE reactivity to lyophilized (L), lyophilized boiled (LB), pepsin digested lyophilized (LD) *BE* extracts, and the pepsin control (P). Fifteen μ g of *BE* protein per lane were applied and incubated with 1:4 diluted serum of both patients A + B.

BE extract was similar as described in Fig. 1, however, reactivity was completely abolished after the digestion process with SGF after 1 min.

Pooled serum of the control subjects showed two weak bands at approximately 44 kDa and a single IgE binding band at 75 kDa in the lyophilized *BE* extract (Fig. 3). After heating or after SGF-digestion no IgE binding was detectable.

Coomassie Brilliant Blue staining of the TCDA from 30 s to 30 min incubation in SGF is shown in Fig. 4(a). During the TCDA bands between 75 and 80 kDa lost intensity during the digestion process within half an hour. The uniform band at 45 kDa represents pepsin of the digestion solution. The same sample as in Fig. 4a was used for immunoblot analysis. The blot was incubated with serum from patient A and IgE-binding at 45 kDa, 55 kDa, and at 80 kDa can be observed (Fig. 4b). During TCDA an IgE binding to a 75-kDa protein persisted during the whole digestion process of 30 min.

Discussion

The interesting aspect of this study is the finding that the two *BE* food-sensitive individuals with distinct clinical manifestation showed a separate pattern of reactivity in the IgE immunoblot and also disclosed a different digestion stability of the recognized proteins. Compared to the one subject with respiratory allergy to *BE* and the controls, an IgE immune response to a digestion-resistant protein was found only with serum of the patient with recurrent anaphylaxis. Both index subjects

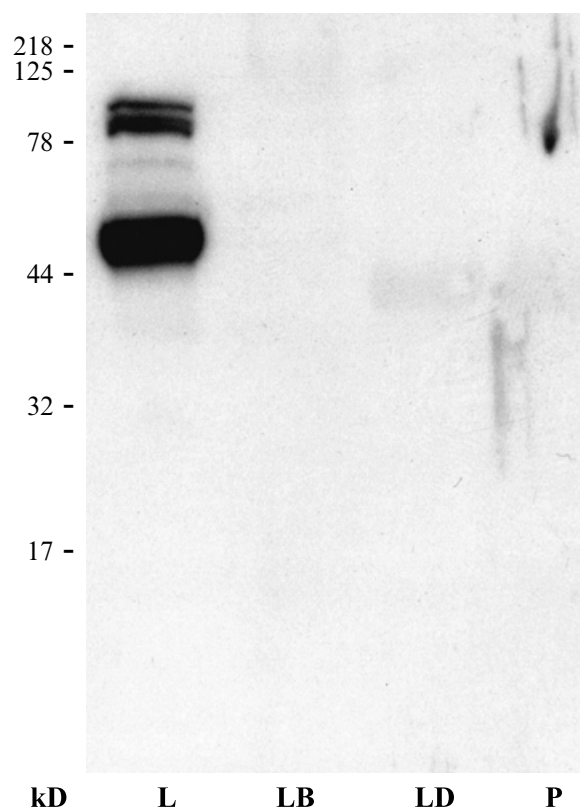


Fig. 2. Using the serum of patient B, IgE reactivity is detectable only with lyophilized (L) *BE* extract at 55 and at 80 kDa (double band).

had convincing histories of repeated allergic reactions following ingestion of cooked *BE*, which was obviously IgE-mediated as revealed by the clear-cut immediate weal-and-flare reaction to the *BE* extract and the results of the IgE binding on the SDS-PAGE analysis. Reliability of the extracts has been confirmed in a large population group [3]. Although certain reactive bands in the lyophilized *BE* extract were detectable also with control sera of non *BE*-allergic individuals the binding reactivity and number of reactive bands were less and different than with sera of the index patients. This phenomenon is a common finding [3], however, it still awaits an apparent explanation.

Stability to digestion and the ability to cross the mucosal membrane of the intestine is considered as a prerequisite of food allergens [15,16]. Though most of the detected *BE* proteins visually disappeared within 4 min during the simulated digestion model, a 75-kDa protein persisted for at least 30 min. Roncaloro et al. likewise described two thermostable IgE binding bands at 26 and 39 kDa using fresh *BE* extract [6]. These observations suggest that, depending on the porcini's preparation or growth condition, several potential *BE* allergens exist. The digestion-stable IgE-binding components at 75 kDa were only detected with serum of patient A who had anaphylaxis following ingestion of *BE*. In none of the other sera tested could this component be found. The hypothesis, whether this 75 kDa protein caused anaphylaxis, is speculative, the answer could eventually be given by a food challenge using this protein fraction. However, because of the dramatic course of recurrent

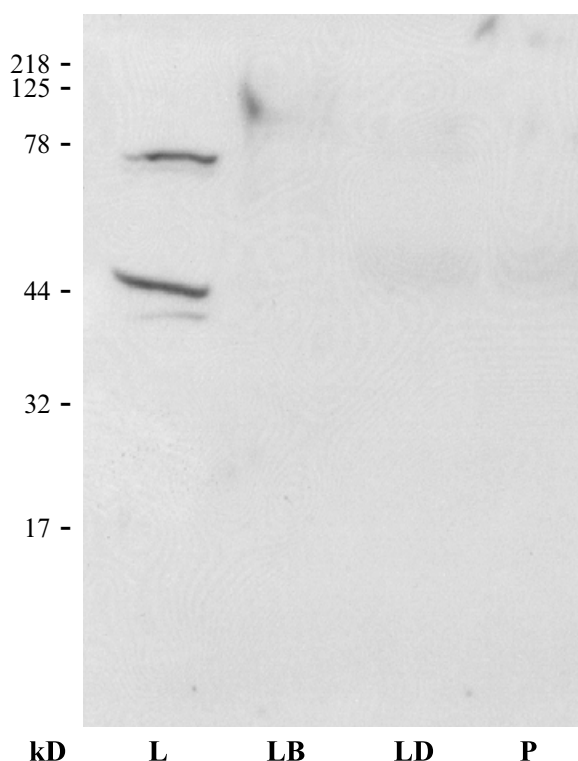


Fig. 3. Immunoblot analysis of lyophilized (L), lyophilized boiled (LB), and pepsin digested lyophilized (LD) *BE* extracts, including the pepsin control (P) using 1:4 diluted serum of the *BE* SPT-negative controls.

anaphylaxis, no provocation with *BE* could be performed. Since this digestion-stable protein at 75 kDa has not been found in the serum of the *BE*-allergic patient B, this protein may represent a real food allergen which is actually distinct from the *BE*-associated food allergens described by Roncaloro et al. [6]. The data clearly support the finding that *BE* occasionally may cause an IgE-mediated food allergic reaction in sensitive subjects.

An interesting question is how sensitization to ingested *BE* does occur. In the adult population most food allergy is thought of as the result of cross-reactivity induced by the sensitization to an aeroallergen such as pollen [17,18]. This may be the case in the 41-year-old patient (patient B), a previously passionate mushroom collector, who developed *BE* food allergy years after a known respiratory sensitivity. In patient A with recurrent anaphylaxis however, an airborne sensitization is unlikely as she never experienced seasonal or persistent respiratory symptoms. Therefore, this patient may have developed a real food allergy to the ingested *BE* proteins, mainly the 75 kDa protein. However, the mechanism of sensitization is not clear. Because cross-reactivities due to common inhalants or foods in this non-atopic subject seems implausible, one might speculate that sensitization to this protein occurred in a susceptible period of the intestines such as during a viral infection or mucosal inflammation.

Our data suggest that ingested *BE* is capable of causing food allergy in two ways: first causing oral or mild systemic symptoms probably due to cross-reactivity to inhaled, atmospheric

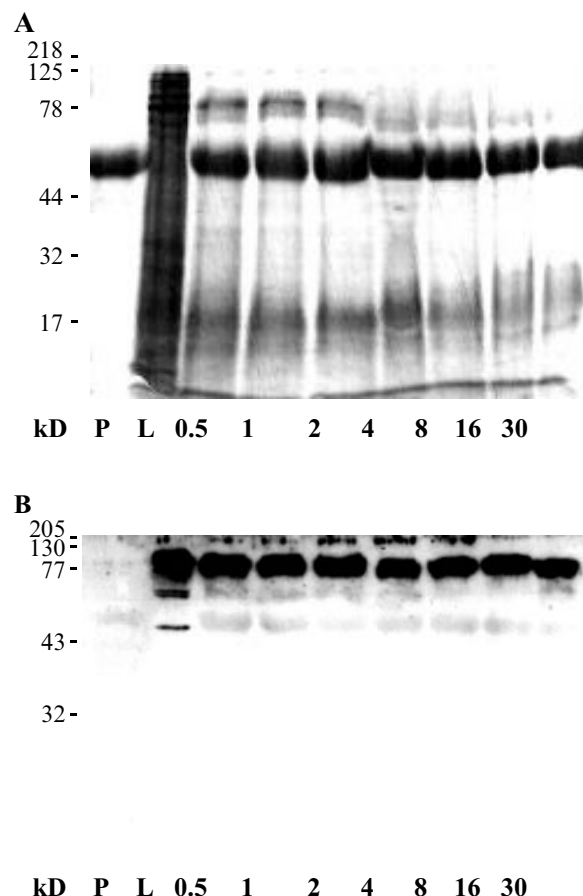


Fig. 4. Coomassie blue staining of a time-course digestion assay of the lyophilized *BE* extract in simulated gastric fluid over 30 s to 30 min. The most intense staining band at 45 kDa represents pepsin (a). Immunoblot of the corresponding assay tested with 1:4 diluted serum of patient A shows a persisting IgE binding band at 75 kDa (b).

BE allergens (patient B) and second to a digestion-stable 75 kDa protein (patient A).

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