

Food allergy to *Boletus edulis*

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OD Optical density

Boletus edulis (boler) is a highly appreciated edible mushroom that is widely used in Italian cuisine. Adverse reactions after its ingestion were believed to be due to a deficiency of trehalase,¹ an enzyme required in the metabolism of trehalose, a glycidic component commonly present in mushrooms. Recently, a case of occupational allergy to boler was reported without any consideration about the possible allergens involved.² We describe a clinical case of food allergy to boler, including characterization of the patient's serum IgE reactivity.

Case report

A 38-year-old woman who had hay fever and asthma since the age of 12 years reported that in the last 5 years she had observed itching and swelling of the lips after eating fruits and vegetables (pear, apple, cherry, nuts, and kiwi). Recently, she had experienced abdominal pain, general itching, and diarrhea 1 hour after eating fresh raw or cooked dried boler. Skin prick test responses were positive to grasses, pellitory, birch, hazel, olive, and *Dermatophagoides* species. Prick-by-prick test results were positive to apple, pear, cherry, kiwi, nuts, and strongly positive to dried boler. Reactivity was lower with fresh mushrooms.

Methods

Boler samples were obtained by 10% wt/vol aqueous extraction of dried mushrooms. The protein content of the extract was 0.38 mg/ml, as determined by the BioRad method. Samples (molecular weight markers and boler extract) were electrophoresed according to the method of Laemmli³ at 40 mA in a 5% to 20% polyacrylamide gradient gel. The resolved proteins were transferred onto a nylon-cellulose membrane (Fluorotrans transfer membrane; Pall, Milan, Italy). The membrane was stained with 0.1% Coomassie Brilliant Blue or incubated with the patient's serum diluted 1:2 in Tris buffer. Bound specific IgE was detected by peroxidase-conjugated anti-human IgE serum (Sigma, Milan, Italy) by using an ECL western blotting kit (Amersham, Milan, Italy) as a substrate. Boler-specific IgE antibodies were detected and quantitated with a commercial version of the REAST method (Realtest; Lab. Farmaceutico Lofarma, Milan, Italy).⁴ Realtest was performed as indicated by the manufacturer. Briefly, 50 µl of standard IgE (0, 0.5, 1.0, 5.0, 10, and 25 kU/L) or patient's serum were added to wells coated with anti-IgE antibodies (Sigma, Milan, Italy). After washings, 100 µl of biotinylated anti-IgE (for the reference curve) or biotinylated boler or control extracts (for sample and control sera) were incubated for 1 hour. The use of peroxidase-conjugated streptavidin followed by addition of the enzyme chromogen and substrate give rise to a colorimetric reaction. Optical density (OD) of samples, read at 405 nm, was expressed as kilounits per liter by interpolation with the reference curve.

Biotinylated mushroom extract was prepared by adding 0.6 mg of activated biotin (Biotinyl-ε-aminocaproic acid N-hydroxysuccinimide ester) to 1 mg of extract. The highest concentration of biotinylated extract that caused an OD lower than the sensitivity limit of the assay (corresponding to the OD of the negative standard serum) when used with sera from subjects without boler sensitivity was used as the working dilution (0.5 µg/ml). We used biotinylated ambrosia and ovalbumin, prepared according to the same procedure as that used for boler, as negative control extracts.

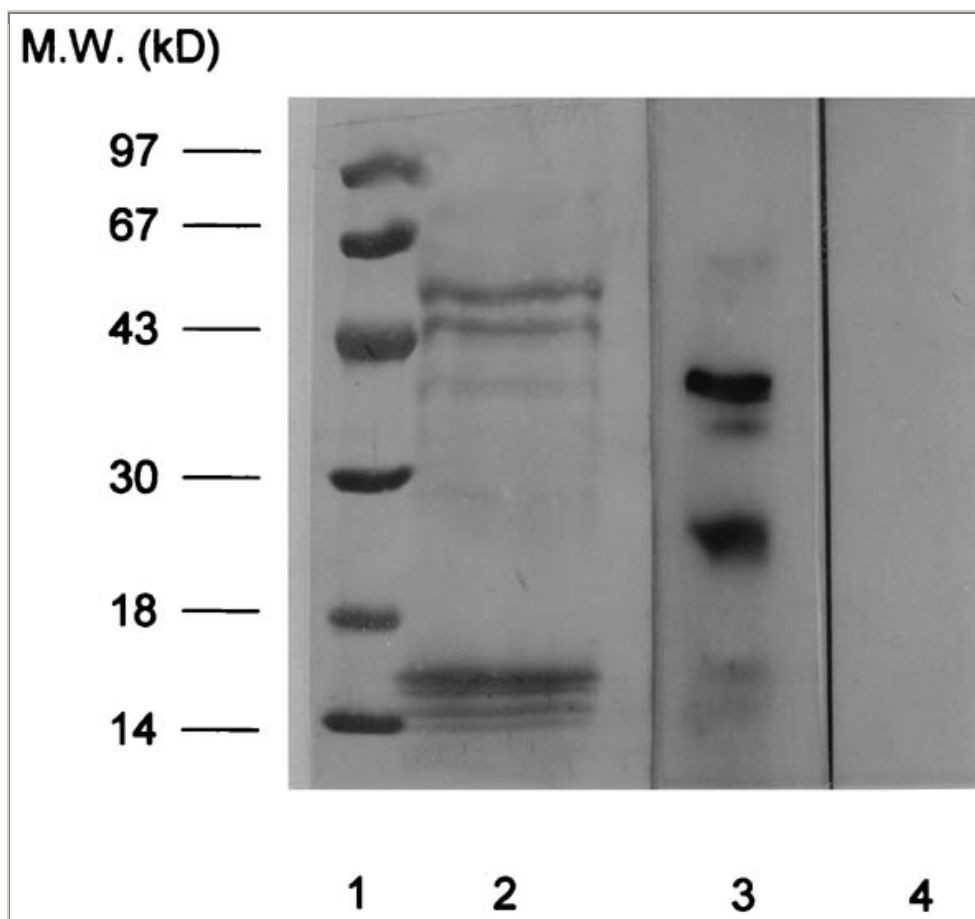
Results

Realtest findings showed that the patient's serum contained boler-specific IgE antibodies. OD was significant (0.445 AU) with biotinylated boler extract. Uncorrelated biotinylated allergen controls (ambrosia and ovalbumin) caused ODs very close to those found with the negative standard serum (approximately 0.085 AU). Results were also negative with sera from allergic subjects without boler sensitivity who were tested with biotinylated boler extract.

Interpolating the OD value of the patient's serum on the reference curve, we obtained 1.6 kU/L of boler-specific IgE, corresponding to a class-1 positive reaction.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis analysis of mushroom extract (Fig. 1, *lane 2*) showed several components from 14 to approximately 90 kd.

Fig. 1. Sodium dodecylsulfate–polyacrylamide gel electrophoresis and immunoblotting experiments. *Lanes 1 and 2* show molecular weight markers and mushroom extract profile after transfer onto nylon-cellulose membrane. *Lanes 3 and 4* show IgE reactivity of patient's and RAST-negative sera against mushroom extract. *M.W.*, Molecular weight.



Three bands of 16, 48, and 54 kd were the most intensely stained. In agreement with the Realtest data, an immunoblotting experiment (Fig. 1, lane 3) showed several components with IgE-binding properties in the boletus extract. Two of them, at approximately 26 and 39 kd, seemed to be the most important, although a weak signal also appeared at approximately 14 kd.

Discussion

Our findings are consistent with a diagnosis of pure food allergy to boletus without any respiratory sensitization. In this sense our report differs from that of Torricelli et al.,² who describe a cook with food allergy to boletus consequent to previous occupational allergic asthma, and that of Brander and Helbling,⁵ who describe cases of respiratory sensitization to common mushroom spores (e.g., *Coprinus comatus* and *Pleurotus ostreatus*). In our patient the fact that symptoms arose after ingestion of both raw and cooked boletus suggests that at least some *Boletus edulis* allergens are thermostable. Because this patient also had skin reactions to birch pollen extract and some fruits and vegetables, it could be interesting to check for allergen cross-reactivity between those substances and boletus extract. In view of the immunoblotting findings of a component at approximately 14 kd, we suggest that profilin might be the cross-reactive allergen.

References

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