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SHIITAKE MUSHROOM (*LENTINUS EDODES*): A POORLY KNOWN ALLERGEN IN WESTERN COUNTRIES RESPONSIBLE FOR SEVERE WORK-RELATED ASTHMA

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Abstract

Objectives: The aim of this study was to investigate the IgE-mediated pathogenesis of severe asthma presented by a patient only after handling shiitake (*Lentinus edodes*) mushrooms (SM). **Material and Methods:** Skin tests were performed using in-house extracts from mushrooms that the patient usually handled, i.e., shiitake, porcini, oyster and black fungus mushroom varieties. Specific IgE to champignons and various molds were determined. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting was performed to detect IgE-binding components. Four negative controls were included in the study. **Results:** Skin prick tests performed with in-house mushroom extracts from varieties other than shiitake were completely negative, in contrast to the positive test obtained for shiitake mushrooms. Serum specific IgE levels for common molds and champignons were all negative. SDS-PAGE revealed many protein bands in the four mushroom extracts. Immunoblotting using the patient's serum showed allergenic bands at about 15 and 24 kDa exclusively for SM that were not shared with negative controls. Another faint band was detectable at approximately 37 kDa for SM and porcini varieties. **Conclusions:** Here, we present the first European case of SM-induced occupational asthma, a disease more frequently occurring in Asia. Asthma attacks stopped when the patient avoided contact with shiitake mushrooms. No skin reactions and no IgE-binding proteins by immunoblotting were detectable with the other mushrooms tested. The positive skin test with shiitake mushrooms and IgE-binding components in the shiitake extract confirmed the IgE-mediated etiology of the reaction.

Key words:
Asthma, IgE-immunoblotting, Mushroom allergy, Occupational disease, Shiitake

INTRODUCTION

A 41-year-old woman was referred to our Outpatient Department. She was suffering from a severe obstructive airway disease, breathlessness, wheezing and coughing. She did not report any inhalant or food allergy. Less than 1 year before, she had been working as an occasional employee for 2 months in a food industry. She was involved in the packaging of various dried mushrooms, changing the type of mushroom to package every 2–3 days. After about 3 weeks she reported strong rhinitis, coughing and dyspnea; specifically, these symptoms appeared the day after having handled shiitake mushrooms (SM), but not the other mushroom varieties. She quitted her occasional job and the pulmonary symptoms were treated

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as bronchitis. Two months before referring to our department, she started again to work in the same food industry, always packaging dried mushrooms. During the 1st week of working, she started again to suffer from rhinitis, coughing and dyspnea. She was treated once more with antibiotics and house rest. When she returned to work, the symptoms relapsed just when she was packaging shiitake mushrooms, and worsened during the subsequent days.

MATERIAL AND METHODS

The patient tolerated well the ingestion of cooked SM. She underwent the usual diagnostic allergy tests, such as clinical evaluation, spirometry, laboratory blood tests, and skin prick tests. Moreover, she provided a written consent to perform *in vivo* and *in vitro* tests to specifically evaluate the skin reaction to SM and the presence of IgE-binding proteins in the SM extract by means of IgE-immunoblotting.

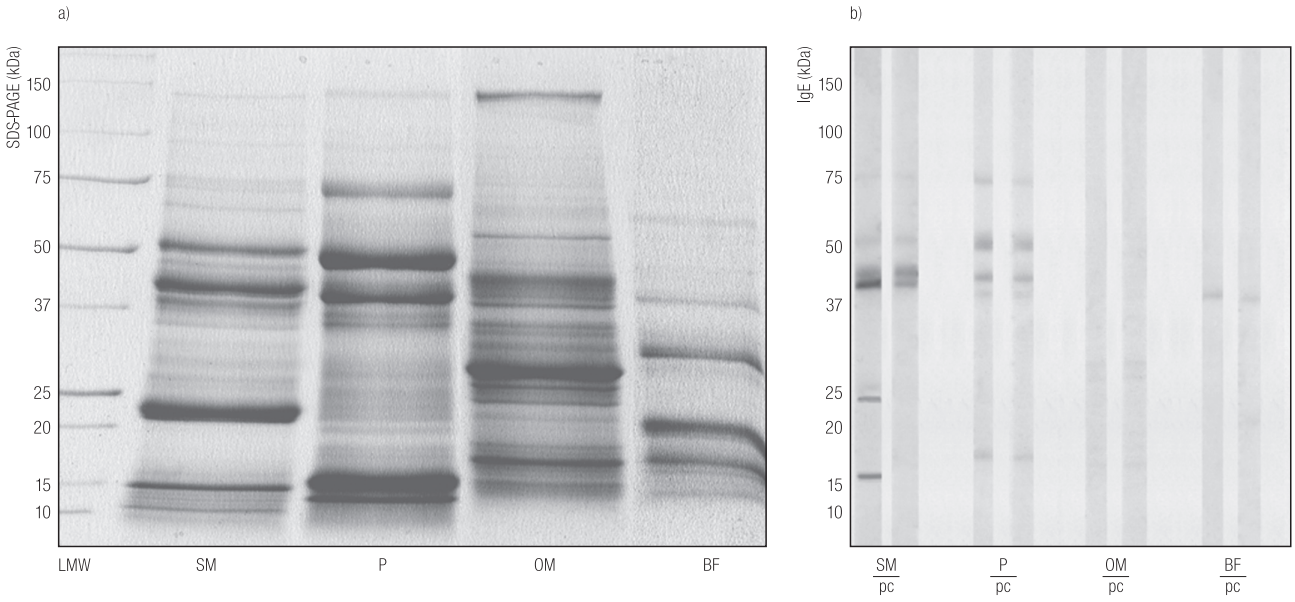
RESULTS

Skin prick tests [1] with commercial aeroallergens and food allergens were completely negative, confirming the negative allergic history. The initial spirometry test confirmed the airflow obstruction: forced expiratory volume in 1 s (FEV_1) = 1.33 l, and forced vital capacity (FVC) = 1.84 l (predicted values of 2.27 l and 2.67 l, respectively), and a bronchodilator test (salbutamol 400 μg) gave a positive result – FEV_1 = 1.67 l and FVC = 1.95 l, with a percentage increase in pulmonary function parameters of 25% and 6%, respectively.

Exhaled nitric oxide (FeNO), a marker of eosinophilic airways inflammation [2], resulted 61 ppb. The total IgE titre was normal (13 kUA/l), and serum specific IgE levels for common molds (*Aspergillus fumigatus*, *Alternaria alternata*, *Penicillum notatum*, *Cladosporium herbarum*) and champignons (*Agaricus bisporus*), determined using the ImmunoCAP System (Thermo Fisher Scientific, Milan, Italy), were all negative. *Aspergillus fumigatus* precipitins were negative; the erythrocyte sedimentation rate (ESR) and C-reactive protein were also negative, and peripheral blood eosinophil count was 190 cells/mm^3. CT was negative for lung bronchiectasis, interstitial lung disease and pneumonia foci. All the performed tests allowed us to reject the diagnosis of allergic bronchopulmonary aspergillosis.

Considering the mushrooms usually handled by the patient, namely oyster mushroom (OM, *Pleurotus ostreatus*), black fungus (BF, *Auricularia polytricha*) and porcini (P, *Boletus edulis*), in-house mushroom extracts were obtained by rehydrating the dried mushrooms provided by the patient in saline solution (1:10) overnight. After centrifugation, the protein content assessed by means of the Lowry method resulted to be 2.4 mg/ml for SM, 2.6 mg/ml for OM, 2.8 mg/ml for P, and 2 mg/ml for the BF extract. Skin prick tests, performed with glycerinated mushroom extracts titrated at 1 mg/ml, were completely negative, apart from the ones concerning SM, which were clearly positive (7 mm). Four healthy negative controls were also skin tested, and the results obtained for them proved completely negative.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and IgE-immunoblotting were performed as described previously [3], using a 6% stacking gel and a 7.5–20% gradient separation gel. The SDS-PAGE analysis (Photo 1a) revealed many protein bands for all of the mushroom extracts considered, whereas IgE-immunoblotting (Photo 1b) showed SM allergenic bands at approximately 15 kDa and 24 kDa that were not shared with the negative controls pooled serum and were not detected in the other mushroom extracts. An IgE-binding protein above 37 kDa, shared with the porcini extract, was detected using the patient's serum; this band was minimally detected using the pooled serum from the negative controls. No IgE-binding proteins were clearly discernible in the OM and BF extracts with either the patient's serum or the pooled negative-control sera.



LMW – low molecular weight; SM – shiitake mushroom; P – porcini; OM – oyster mushroom; BF – black fungus.

Photo. 1. a) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profile and b) IgE-immunoblotting performed using the patient's serum (p) and healthy negative controls' pooled sera (c) for the 4 mushroom extracts analyzed

The patient willingly agreed to avoid SM packaging during the entire therapy period. The treatment began with oral prednisone 50 mg/day for 3 days, halving the dosage every 5 days, and salmeterol/fluticasone propionate 50/500 µg twice a day. After 40 days, spirometry parameters improved (FEV_1 = 2.10 l; FVC = 2.64 l) as well as the results concerning fractional exhaled nitric oxide (FeNO = 35 ppb), so the therapy continued with salmeterol/fluticasone 50/250 µg administered twice a day for 30 days. A significant improvement in the spirometry test results was detected only after additional 2 months of treatment. Finally, the measured spirometry parameters and FeNO (20 ppb) were contained in the normal range and the patient continued the therapy only with an inhaled steroid.

CONCLUSIONS

Allergy to edible *Basidiomycetes* (i.e., gilled fungi) has rarely been described in Western countries and has mainly been reported for porcini mushrooms [4–7]. In contrast, in the Far East, where mushrooms like shiitake and oyster are traditionally and extensively consumed and cultivated, several authors have described cases in which individuals who worked with mushrooms, for a period from a few to several years, developed respiratory problems (asthma) and particularly hypersensitivity pneumonitis [8]. The authors of this review underlined the lack of diagnostic tests able to screen employers working in the mushroom industry for potential development of mushroom lung diseases. Furthermore, cutaneous (contact dermatitis, toxicodermia) symptoms were reported [9]. Very recently, a case report of hypersensitivity pneumonitis by SM spores in a French Caucasian worker has been published [10]. Due to the severe asthma symptoms, our patient refused to undergo the specific inhalative provocation test. However, the patient's history, the immediate positive skin reaction solely to the SM extract, the presence of specific SM IgE-binding proteins detected by immunoblotting and elevated FeNO supported the allergic, IgE-dependent, pathogenesis of this occupational asthma. In addition, other clinical investigations, including

laboratory findings and high-resolution computed tomography, excluded fungal pulmonary diseases. The administered therapy successfully resolved asthma attacks, together with the removal from the packaging workplace.

To our knowledge, this is the first European case of IgE-mediated SM occupational asthma, as documented by SDS-PAG and IgE-immunoblotting. Two specific IgE-binding bands were identified in the SM extract at approximately 15 kDa and 24 kDa and not detected in the other mushroom extracts either with the use of the patient's serum or the pooled sera from the control subjects. These 2 IgE-binding bands seemed to be responsible for the patient's inhalatory allergic symptoms. Another IgE-binding band at approximately 37 kDa was identified in the SM extract, also identified with lower radiostaining in the porcini extract with the use of the patient's or the control subjects' sera. Previously, some porcini-specific IgE-binding proteins were identified at medium-high molecular weights, not corresponding to our protein band [6,7] and probably representing a shared fungal allergenic protein, not clinically relevant herein since our patient tolerated well exposure to other mushrooms.

In this report, we want to underline how in medical practice careful attention has to be paid to clinical history and, in particular, to patient's occupation, in view of the possible exposure to potential workplace allergens. Furthermore, the knowledge of foreign diseases related to exotic/imported foods is fundamental, since globalization influences dietary attitudes, adding new foods and occupational allergens, which an allergist has to take into consideration.

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