Occupational asthma caused by white mushroom

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Summary. The comercial growing of white mushroom (*Agaricus bisporus*) is a frequent activity in certain Spain regions as La Rioja.

We report two cases of white mushroom workers suffering from asthma caused by hypersensitivity to basidiocarp and spores of white mushroom.

Key words: Agaricus bisporus, white mushroom, basidiocarp, spores, occupational asthma.

Case report

Case nº 1

A 41 year-old-man who had been working as a white mushroom (WM) cultivator for eight years, began to present with symptoms of asthma whenever he was unloading and carrying sacks filled with WM seeds (bot barley and bot wheat grains covered with WM spores).

Case nº 2

A 40 year-old-white mushroom worker with a history of seasonal rhinitis, had experienced severe asthma attacks during the past 4 years only when he was at work. He never had attacks during the weekends and on holidays.

None of the 2 workers had reticulonodular shadows on chest radiography.

Skin prick test results and serum specific IgE determinations to common inhalants, including molds, were negative in both patients, except specific IgE against grass pollen with serum from patient n°2.

Skin prick tests were performed with extracts from basidiocarps of WM (*A. bisporus*), of oyster mushroom (*Pleurotus ostreatus*), of king bolete (*Boletus edulis*), from spores of oyster mushroom and WM, and from bot barley grain and bot wheat grain. They were strongly positive for WM basidiocarp in patient n°1 (wheal diameter 11 mm) and patient n° 2 (wheal diameter 9

mm). Prick test with WM spore was positive only in patient $n^{\circ} 1$ (9 mm). Prick tests with extracts from other tested mushrooms and from barley and wheat grains were negative in both patients.

Total IgE was 1161 kU/L in serum from patient n°1 and 1230 kU/L in patient n°2 serum. Specific IgE (EAST: HYTEC, HYCOR Biomedical Ltd. UK.) to WM basidiocarp and WM spore in patient n°1 serum was 34.5 kU/L (class 4) and 72.5 kU/L (class 4), respectively. In serum from patient n° 2, specific IgE to WM spore extract was 0.8 kU/L (class 2), and no specific IgE against WM spore was detected (Table I).

Serum precipitins to *Micropolyspora faeni* and *Thermoactinomyces vulgaris* extracts were determined with negative results.

Spirometric parameters were normal. Monitoring of bronchial hyperresponsiveness was performed in both patients by the dosimeter methacholine challenge testing both at workplace and after three weeks out of work showed a clear relationship between symptoms and the workplace. Patient n°1 showed a PC-20 FEV₁ value of 4.1 mg/ml at work and a value of 12 mg/ml out of work.

Patient n°2 showed a PC-20 FEV₁ value of 4.2 mg/ ml at work and negative result out of work.

SDS-PAGE immunoblotting of WM basidiocarp extract incubated with serum from patient n°1 showed two intense IgE-binding bands of 15.8 kDa and 13.8/ 14,5 kDa, and several minor bands between 24 and 39 kDa, whereas an intense IgE-binding band of 15.8 kDa was revealed when WM spores extract was used. Besides, inmunoblotting inhibition assay performed with patient n° 1 serum showed a total IgE-binding

		Patient nº 1 serum		Patient nº 2 serum	
Nº	Protein extract	kU/l	Class	kU/L	Class
1	Agaricus bisporus spore	72.5	4	<0.35	0
2	Agaricus bisporus basidiocarp	34.5	4	0.8	2
4	Pleurotus ostreatus spore	< 0.35	0	< 0.35	0
5	Pleurotus ostreatus basidiocarp	< 0.35	0	< 0.35	0
6	Boletus edulis	< 0.35	0	< 0.35	0
7	Barley grain	< 0.35	0	< 0.35	0
8	Wheat grain	< 0.35	0	< 0.35	0
9	Alternaria alternata	< 0.35	0	< 0.35	0
10	Cladosporium herbarum	< 0.35	0	< 0.35	0
11	Aspergillus fumigatus	< 0.35	0	< 0.35	0
12	Olea europaea pollen	< 0.35	0	< 0.35	0
13	Lolium perenne pollen	< 0.35	0	2.3	2
14	Plantago lanceolata pollen	< 0.35	0	< 0.35	0
15	Chenopodium album pollen	< 0.35	0	< 0.35	0

Table 1. Results of IgE determinations

inhibition on WM spore-15.8 kDa-protein band when the serum was previouly incubated with WM basidiocarp extract.

SDS-PAGE immunoblotting performed with WM basidiocarp extract and serum from patient n°2 revealed an intense IgE-binding band of 14.3 kDa which IgE-binding capacity was not inhibited by *Lolium perenne* pollen extract.

Discussion

The cultivation of common mushroom (*Agaricus bisporus*) involves handling of compost (organic material) for growing the mushrooms, culture and harvest (before spore sowing) of the mushrooms. Hipersensitivity pneumonitis (HP) that has been described as the main occupational disease in WM workers, is often produced by immunologic response to proteins from actinomycetes that live in the composting organic material using for the mushrooms cultivation [1,2].

However, it has also been reported that workers who raise other species of mushrooms, such as oyster mushrooms (*Pleorotus sp.*) or shiitake (*Lentinus edodes*), could develop HP and occupational asthma by mushroom spore proteins instead of proteins from actinomycetes [3,4].

The first reports on occupational bronchial asthma in champignon workers appeared in 1938 and 1951 [5,6]. In these reports, flies were considered to be the antigen source, but there was no further biologic identification of them and no *in vitro* specific IgE determination. Most recently, champignon flies from de Phoridae family and the Sciaridae family have been confirmed as a cause of asthma and rhinoconjunctivitis in these workers [7].

Symington et al. describe the development of asthma in a group of four factory workers who had occupational contact with different dried mushrooms (*Boletus edulis*, *Psalliata hortensis* and champignon (WM)) dust during the preparation of mushroom dried packeted soups. Bronchial challenge tests with dried mushroom (*P. hortensis*) were positive in all patients. The authors did not investigate if the other mushrooms (*Boletus edulis* and WM) were also implied [8].

In our report we add basidiocarp and spores from WM as two new sources of allergens that could elicit occupational asthma in WM workers.

We have found no cross reactivity with other mushroom species studied.



Figure 1. SDS-PAGE immunoblotting (A) WM spore extract (B) WM basidiocarp extract (C) *Lolium perenne* pollen extract. Lane P1: patient n° 1 serum; Lane C: control serum (pool of sera from non atopic subjects); Lane P2: patient n° 2 serum; Lane M: molecular mass marker. II) Immunoblotting inhibition using WM spore extract as solid phase. Lane 1: patient n° 1 serum (the apparent difference observed between this blott and part I, extract A, lane P1 blott is due to a different film developing time used in the blotting process); Lane 2: patient n° 1 serum previously incubated with WM spore extract as inhibitor (positive control); Lane 3: patient n° 1 serum previously incubated with WM basidiocarp extract as inhibitor; Lane 4: patient n° 1 serum previously incubated with lamb extract as inhibitor (negative control); Lane M: molecular mass marker. III) Immunoblotting inhibition using WM basidiocarp extract as solid phase. Lane 1: patient n° 2 serum; Lane 2: patient n° 2 previously incubated with WM basidiocarp extract as inhibitor (negative control); Lane M: molecular mass marker. III) Immunoblotting inhibition using WM basidiocarp extract as solid phase. Lane 1: patient n° 2 serum; Lane 2: patient n° 2 previously incubated with WM basidiocarp extract as inhibitor (positive control); Lane 3: patient n° 2 previously incubated with WM basidiocarp extract as inhibitor (positive control); Lane 3: patient n° 2 previously incubated with Lolium perenne pollen extract as inhibitor; Lane 4: lamb extract as inhibitor; Lane 4: not control); Lane M: molecular mass marker.

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