Eur Respir J
1991, 4, 1143–1147

CASE REPORT

Occupational asthma to spores of *Pleurotus cornucopiae*

A. Michils*, P. De Vuyst*, N. Nolard***, G. Servais**, J. Duchateau**, J.C. Yernault*

Occupational asthma to spores of Pleurotus cornucopiae. *A. Michils, P. De Vuyst, N. Nolard, G. Servais, J. Duchateau, J.C. Yernault.*
ABSTRACT: We report the case of a young man who developed severe asthma a few months after starting work in a factory producing a single type of mushroom: *Pleurotus cornucopiae* (a basidiomycete). Immunological investigations, performed with material recovered from the filtering devices of the mushroom's bed, led to demonstration of specific IgE and IgG against spore extracts and to isolation of one discriminant antigen (molecular weight: 10.5 kd). Current data concerning the underestimated role of the basidiomycetes in allergic asthma are reviewed.
Eur Respir J., 1991, 4, 1143–1147.

* Dept of Pneumology, Erasme Hospital, University of Brussels.
** Dept of Immunology, Saint-Pierre Hospital, University of Brussels.
*** Institute of Hygiene and Epidemiology, Brussels.

Correspondence: A. Michils, Department of Pneumology, Erasme Hospital, route de Lennik, 808, 1070 Brussels, Belgium.

Keywords: Asthma; basidiospores; IgE antibodies; immunoblotting.

Received: December 12, 1990; accepted after revision June 26, 1991.

Recent studies indicate that allergy to spores of basidiomycetes (superior fungi) is frequent among atopic asthmatics [1–3]. The misknowledge of this kind of allergy results in part from the extremely wide variety of basidiospores that can be found in the atmosphere. Subsequently, exposure to one type of spore occurs rarely. But, with the development of mushroom culturing, one might expect an increasing incidence of occupational asthma related to intense and pure exposure to some of these antigens. Study of occupational cases could be a source of information about basidiospores allergy, *e.g.* helping to characterize allergens from spores of mushrooms and to prepare specific fungal extracts for diagnostic and casually therapeutic uses.

We report the case of a young man who developed severe asthma a few months after starting work in a factory which produces, for commercial purposes, a single type of edible mushroom: *Pleurotus cornucopiae.*

Case report

A 26 yr old man was evaluated for respiratory difficulties in the work place. He was a nonsmoker and had no past-history of allergic disease, including asthma. In January, 1988, he began to work in a mushroom bed, producing *Pleurotus cornucopiae,* a variety of mushroom close to the oyster mushroom (*Pleurotus ostreatus*). About six months after entering this workplace, he reported cough, followed three months later by dyspnoea during work.

The symptoms disappeared during the week-ends and holidays and worsened on the days where large quantities of mushrooms were harvested (up to 20 kg per day).

His physical examination and chest X-ray were normal. Ventilation functional tests were normal but demonstrated bronchial hyperreactivity to histamine (PC_{20} 1 mg·ml^{-1}). Total IgE were elevated at 268 U·ml^{-1} (N <200 U·ml^{-1}) and RAST were positive for grass and weed pollen, consistent with an atopic status. Measurements of peak expiratory flows demonstrated progressive decreases during the working day and recovering during the night and week-end (fig. 1), suggesting the presence of the responsible agent at the workplace. The patient left the factory and after a few months of eviction, he became asymptomatic confirming the diagnosis of occupational asthma.

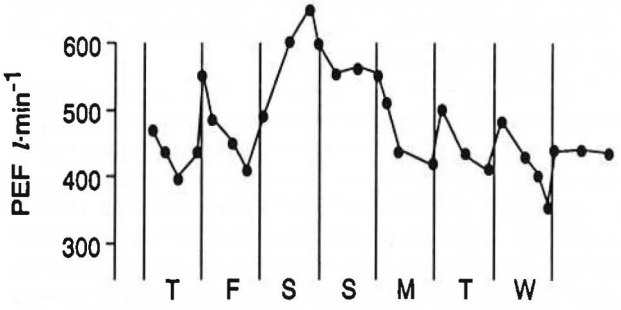

Fig. 1. – Peak expiratory flow (PEF) measurements showing a decline over the working day and recuperation during nights and week-ends.

Immunological investigation

Soluble protein extracts were prepared from the spores allowing us to perform immunological investigations. The spores were collected on the air filtering devices connected with the room where the mushrooms were growing and were observed under scanning electron microscopy (fig. 2). One gram of this material was homogenized with a Braun homogenizer in 10 ml of ammonium bicarbonate buffer (0.4 % w/v) in order to extract soluble proteins. The homogenate was centrifugated at 15,000 g and the supernatant was concentrated ten fold through filtration on a Millipore® filter with a cut-off limit of 10 kdaltons. The final extract contained 50 $mg \cdot ml^{-1}$ of protein.

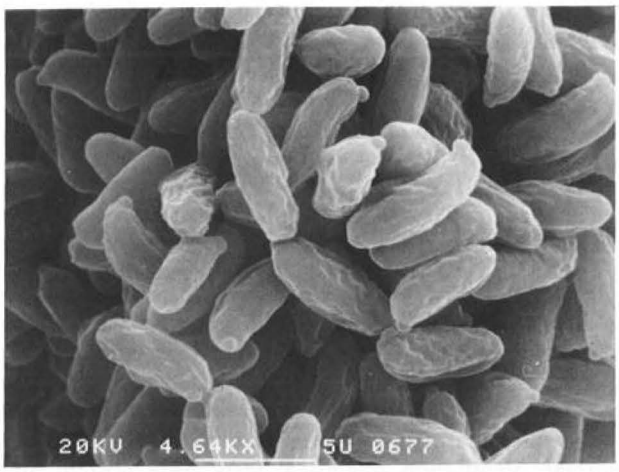

Fig. 2. – Scanning electron microscopic view of *Pleurotus cornucopiae* spores recovered from filtering devices (scale bar = 10 μm).

Sera from 9 subjects were obtained: 3 workers in contact with the mushroom's bed including our patient and, 6 control subjects without occupational exposure to basidiospores. The latter included 3 atopic subjects (serum IgE levels >200 $U \cdot ml^{-1}$) and 3 non atopic healthy blood donors.

Specific IgE and IgG antibodies against antigenic extracts from *Pleurotus* spores were measured in the serum of our subjects with two distinct procedures: the first consisting in a solid phase enzyme linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) and the second in an immunoblot.

ELISA tests were performed in 96 well polystyrene microplates (Cobind) passively coated with the antigen extract and saturated with human albumin before serum incubation. IgG fixation on the antigen was demonstrated by secondary retention of Peroxydase labelled protein A (Dako) using orthophenylenediamine as substrate. Nonspecific binding was measured in parallel with the same method but using microplates previously coated with human albumin only and no antigen extract. Results were expressed in net optical density corresponding to specific IgG binding.

Specific IgE antibodies were also detected with a solid phase assay using polystyrene tubes containing microbeads that were both passively coated with the same antigenic extract. Saturation of adsorptive capacities were achieved with horse serum before serum incubation. Nonspecific binding was estimated in an identical procedure using human albumin instead of the antigen extract. IgE binding was determined with I^{125} labelled anti-human IgE (Pharmacia) and results expressed as the ratio between radioactive counts obtained respectively for the test with antigen coated tubes and its albumin coated control. When superior to 2, this ratio is usually considered as indicating a significant fixation of the relevant IgE antibody (Pharmacia).

In an attempt to detect discriminant antigens, an immunoblot was performed as follows: the protein components of the antigen extract were first separated in a Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (15%/7% T/C) and then transferred on nitrocellulose sheets (0.45 um pore size-Amersham) under an electric field; finally specific antibodies against blotted proteins were measured in patient and control sera using peroxydase labelled anti-human IgE and IgG. To avoid false interpretation due to a non-specific binding of IgE antibodies to a lectin-like activity associated with any mushroom proteins, we tested in parallel 3 control sera selected for their high IgE levels. None of them displayed any IgE binding in our test.

Results

As shown in table 1, specific IgE antibodies were found in our patient and also in 2 atopic controls. High levels of specific IgG were found in the 3 workers and might be related to intense exposure.

The immunoblot revealed specific IgE against a protein band (molecular weight: ~ 10.5 kd) which seems to be one discriminant antigen and which is in agreement with prior results concerning other basidomycetes [4], including other species of *Pleurotus* [5]. Indeed, gel filtration data indicated a molecular weight of the *Pleurotus* allergenic components ranging between 10.5 and 28 kd [5]. This protein band could not be demonstrated in the 2 positive atopic controls, suggesting that they were most probably sensitized to antigens from other species of basidiospores cross reacting with proteins present in our extract, or alternatively that our present assay was lacking appropriate sensitivity. It must be remembered that immunoblotting was restricted to antigens with a molecular weight lower than 95 kd, at variance with the solid phase assays where the crude antigen extract was used allowing detection of antibodies against antigenic components with higher molecular weights. During this procedure, specific IgG could not be revealed, suggesting at least different antigen specificities for IgE and IgG or potential methodological limitations (fig. 3).

Table 1. – Immunological results

Subjects	RAST RATIO: CPM antigen / CPM human albumin	Immunoblot IgE	IgG Optical density × 10^4	Immunoblot IgG
1	3.45	+ (a)	1503	-
2	1.74		1116	-
3	1.54	-	1030	-
4	5.71	-	546	-
5	2.89	-	471	-
6	1.48	-	746	-
7	1.80	-	671	-
8	1.93	-	383	-
9	0.92	-	342	-

(a): protein band with molecular weight of ~ 10.5 kd; 1: patient; 2, 3: workers of the factory; 4, 5, 6: atopic controls; 7, 8, 9: non atopic controls: CPM: counts per minute.

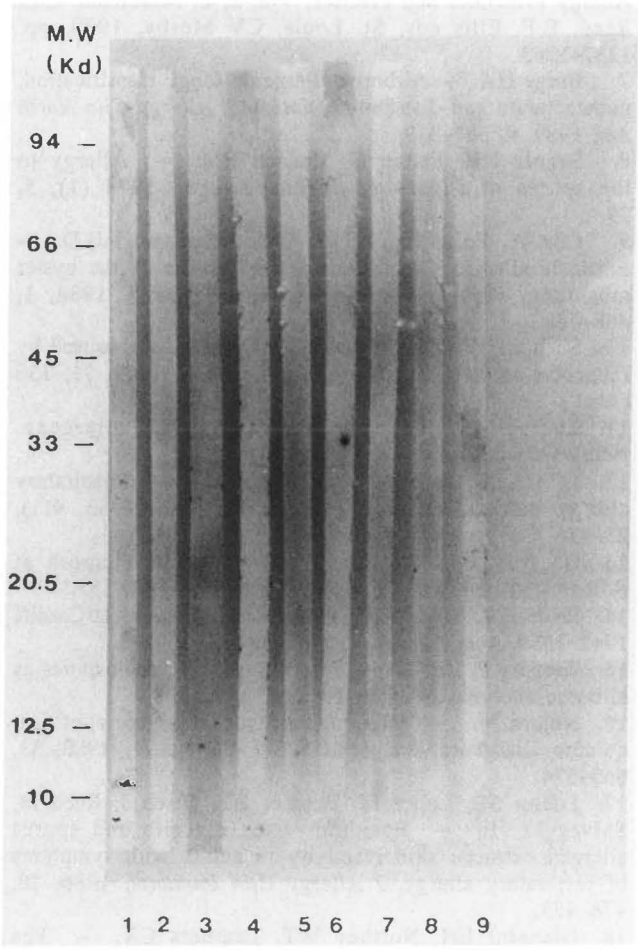

Fig 3. – Immunoblotting of the antigen extract showing in the serum of our patient (1) specific IgE antibodies against a protein band of ~ 10.5 kd molecular weight which appears to be one discriminant antigen. Specific IgE antibodies could not be detected neither in the sera of the 2 workers of the mushroom bed (2, 3) nor in the sera of the atopic (4, 5, 6) and non-atopic (7, 8, 9) controls. MW: molecular weight.

Discussion

Workers in mushroom plants are known to experience extrinsic allergic alveolitis due to bacteria (actinomycetes) coming from the compost on which the mushrooms are cultivated or to fungal spores themselves [6]. Depending in part on the antigen carrying particle, the pattern of exposure and the antigen itself, fungal spores can also induce asthma [7]. This is, to the best of our knowledge, the first case of asthma related to the exposure to spores of *Pleurotus cornucopiae* confirming the well-known allergenic power of *Pleurotus* species [8–10]. A European study, concerning occupational exposure, found that up to 30–40% of workers in contact with these basidiospores may develop allergic symptoms [11]. The reason why the *Pleurotus* species seem to be more allergenic than other cultivated mushrooms rests at least in part on differences on fruit body formation: they are constantly releasing spores to the atmosphere of the growing room ("spore fog"), giving rise to exposure to high concentrations of spores and a subsequent deposition on filtering devices.

The study of such cases of occupational asthma might contribute to a better understanding of basidiospore allergy. Indeed, while fungal spores are present in high concentrations in the air in different parts of the world, largely in excess of pollen grains [12], they have been, until recent past, relatively ignored as potential aeroallergens except for fungi imperfecti. Although spores of basidiomycetes (superior fungi), may constitute an important part of all fungal spores present in the atmosphere, more than 20% in various parts of the world [13–15] (up to 10 percent in a Belgian study over a 10 yrs period [16]) they have not been considered as potential allergens until 1952 [15].

The complexity with basidiospores arises from the number of species of basidiomycetes (about 25,000)

and from the sporulation period which is more variable in time than pollination. Yet, more recent reports revealed that basidiospores can be implicated in exacerbation of asthma during the summer [1] and the fall season [2].

Other data [3, 17, 18] report that, among patients suffering from atopic asthma, 30–60% show positive skin reactivity to one or more basidiospore extracts used as reagents for skin prick tests which is also suggested from our own immunological findings *i.e.* detection of specific IgE antibodies in 2 of the 3 atopic controls' sera. SPRENGER *et al.* [3] found a linear relationship between the number of positive skin tests to basidiospores and to moulds, indicating a similar importance in allergy. Bronchoprovocation studies were performed by LOPEZ *et al.* [19] in atopic asthmatics showing positive skin tests to basidiospores extracts compared to atopic asthmatic subjects without skin reactivity to the same extracts. They observed a decline of the forced expiratory volume in one second over 20%, after aerosol inhalation of basidiospores extracts, in most positive patients whereas none of the negative controls responded. This suggests a potential role of basidiospores in provoking respiratory symptoms.

Because natural exposure occurs mainly to airborne spores, spore extracts are generally used as reagents for diagnostic tests (skin prick tests and RAST assays). They seem to be more relevant than cap and mycelia extracts, even though there is evidence for shared epitopes carried by somatic and spore extracts and even though they are more difficult and time-consuming to prepare [20, 21].

Interestingly, there is a high prevalence of skin test reactivity to multiple basidospores extracts in the same patients, suggesting the possibility of common epitopes [17, 22]. RAST inhibition and Ouchterlony technics have confirmed the presence of shared allergenic determinants (which is also highly suggested from our present immunological findings), with some exceptions [23]. This finding has economic and clinical implications by allowing us to prepare some representative panels of spore extracts for diagnostic and eventually therapeutic uses. In this regard, the study of occupational asthma to basidiomycetes could not only allow us to study the relationship between the development of respiratory symptoms and pure exposure, but also to collect enough samples of different types of spores (difficult in natural conditions) in order to better characterize allergens and cross reactions and to prepare such panels. Being aware that there is a potential medical risk, the *Pleurotus* growers are using various protective masks, but these appear to be insufficient help, and prevention should now be orientated to the culture of growing sterile species.

Acknowledgements: We thank C. Chasseur and L. Devos for their help during the study and M. Onrubia for preparing the manuscript.

References

1. Herxeimer H, Hyde HA, Williams DA. – Allergic asthma caused by basidiospores. *Lancet,* 1969, 19, 131–132.
2. Santilli J, Rockwell WJ, Colius R. – The signification of the spores of the basidiomycetes (mushrooms and their allies) in bronchial asthma and allergic rhinitis. *Allergy,* 1985, 55, 469–471.
3. Sprenger JO, Altman C, O'Neil LE, Ayars JH, Butcher BT, Lehrer SB. – Prevalence of basidiospore allergy in the Pacific Northwest. *J Allergy Clin Immunol,* 1986, 82, 1076–1080.
4. Ibanez MD, Horner WE, Liengswangswong V, Sastre J, Lehrer SB. – Identification and analysis of basidiospore allergens from puffballs. *J Allergy Clin Immunol,* 1988, 82, 787–795.
5. Elliot W, Ibanez MD, Liengswangswong V, Salvaggio JE, Lehrer SB. – Characterization of allergens from spores of the oyster mushroom, *Pleurotus ostreatus. J Allergy Clin Immunol,* 1988, 82, 978–986.
6. Fink JN. – Hypersensitivity pneumonitis. *In:* Allergy Principles and Practice, Vol. 2, E. Middleton, C.E. Reed, E.F. Ellis eds, St. Louis, CV Mosby, 1988, pp. 1237–1262.
7. Burge HA. – Airborne allergenic fungi: classification, nomenclature and distribution. *Immunol Allergy Clin North Am,* 1989, 9, 307–319.
8. Schulz KH, Felten G, Hausen BM. – Allergy to the spores of *Pleurotus florida. Lancet,* 1974 (1), 5, 29.
9. Cox A, Folgering HTM, Van Griensven LJLD. – Extrinsic allergic alveolitis caused by spores of the oyster mushroom *Pleurotus ostreatus. Eur Respir J,* 1988, 1, 466–468.
10. Shehtari YAH. – Mushroom workers lung caused by *Pleurotus sapidus. J Allergy Clin Immunol,* 1983, 71, 136 (Abst.).
11. Zadrazil F. – *Pleurotus* spores als allergene. *Natuurwissenchaften,* 1974, 61, 456–459.
12. Lehrer SB, Aukrust L, Salvaggio JE. – Respiratory allergy induced by fungi. *Clin Chest Med,* 1983, 4(1), 23–41.
13. Gregory P, Hirst J. – The summer airspora at Rothamsted in 1952. *J Gen Microbiol,* 1957, 17, 135.
14. Hyde HA, Adams KF. – Airborne allergens at Cardiff 1942–1959. *Acta Allergol,* 1960 (Suppl.), 159.
15. Gregory P, Hirst J. – Possible role of basidiospores as airborne allergens. *Nature,* 1952, 170, 414.
16. Nolard N. – Importance des champignons en tant qu'aéro allergènes. *Bul Acad R Sci Outre Mer,* 1988, 33, 569–574.
17. Lehrer SB, Lopez M, Butcher BT, Olson J, Reed M, Salvaggio JE. – Basidiomycetes mycelia and spores allergen extracts: skin reactivity in adults with symptoms of respiratory allergy. *J Allergy Clin Immunol,* 1986, 78, 478–485.
18. Giannini EH, Northey WT, Leathers CR. – The allergenic significance of certain fungi rarely reported as allergens. *Ann Allergy,* 1975, 35, 372–376.
19. Lopez M, Voigtlander JR, Lehrer SB, Salvaggio JE. – Bronchoprovocations studies in basidiospores-sensitive allergic subjects with asthma. *J Allergy Clin Immunol,* 1989, 84(2), 242–246.
20. Lopez M, Butcher BT, Salvaggio JE, Olson J, Wright

MA, MacCants ML, Lehrer SB. – Basidiomycetes allergy: what is the best source of antigen? *Int Archs Allergy Appl Immunol,* 1985, 77, 169–170.
21. Weissman DN, Halmepuro L, Salvaggio, Lehrer SB. – Antigenic/allergenic analysis of basidiomycetes cap, mycelia and spore extracts. *Int Archs Allergy Appl Immunol,* 1987, 84, 56–61.
22. Butcher BT, O'Neil CE, Reed MA, Altman L, Lopez M, Lehrer SB. – Basidiomycetes allergy: measurement of spore - specific IgE antibodies. *J Allergy Clin Immunol,* 1987, 80, 803–809.
23. O'Neil CE, Hughes JM, Butcher BT, Salvaggio JE, Lehrer SB. – Basidiospore extracts: evidence for common antigenic/allergenic determinants. *Int Arch Allergy Appl Immunol,* 1988, 85, 161–166.

Asthme professional aux spores de Pleurotus cornucopiae. A. Michils, P. De Vuyst, N. Nolard, G. Servais, J. Duchateau, J.C. Yernault.

RÉSUMÉ: Nous rapportons le cas d'un jeune homme qui a développé un asthme sévère quelques mois après son entrée en fonction dans une champignonnière produisant une seule espèce de champignon: la pleurote corne d'abondance (un basidiomycète). L'étude immunologique réalisée avec le matériel récolté au niveau des filtres d'aération de la champignonnière a permis de détecter dans le sérum du patient des anticorps IgE et IgG contre les spores du champignon ainsi que d'isoler un antigène majeur (poids moléculaire 10.5 kd). Les données actuelles concenant le rôle sous-estimé des basidiomycètes dans l'asthme allergique sont revues.

Eur Respir J, 1991, 4, 1143–1147.