

Respiratory allergy to mushroom spores: not well recognized, but relevant

Arthur Helbling, MD; Federica Gayer, MD; and Karl A Brander, PhD

Background: Although basidiospores are a major component of the air spora in many parts of the world, their clinical significance as triggers of respiratory allergy has rarely been demonstrated. Therefore, the class of basidiomycetes as an aeroallergen is not well known.

Objective: To demonstrate a cause and effect relationship between respiratory allergy and basidiospores, we illustrate this case report of a 38-year-old housewife.

Methods: Skin prick test, immunoblot, and active anterior rhinomanometry were used as diagnostic tools to verify specific reactivity of a *Pleurotus pulmonalis* spore extract. Two atopic subjects served as controls.

Results: The skin prick test positive study subject reacted with subjective and objective signs including a significant drop of the FEV₁ by nasal challenge at a concentration of 0.1 mg/mL of the *Pleurotus* spore extract while both controls were negative even at a higher test concentration. IgE-immunoblot revealed several distinct bands in the serum of the *Pleurotus*-sensitized subject.

Conclusion: Spores of *Pleurotus pulmonalis*, a common mushroom of the fungal class of basidiomycetes, can cause specific, IgE-mediated acute rhinoconjunctivitis and asthma in sensitized individuals.

Ann Allergy Asthma Immunol 1999;83:17–19.

INTRODUCTION

Basidiomycetes are the largest and morphologically most complex fungi and include mushrooms, puffballs, toadstools, and bracket fungi.¹ Though basidiospores represent up to 30% of the total spore count in some parts of the world^{1,2} and a recent study demonstrated that basidiomycetes are important sensitizers in Western Europe,³ the clinical significance of basidiomycetes as triggers of respiratory allergies is not completely accepted. In our outpatient allergy clinic, we routinely test subjects with respiratory allergies with basidiomycete extracts of *Boletus edulis*, *Coprinus comatus*, and *Pleurotus pulmonalis*. Selection of these basidiomycetes resulted from the widespread occurrence of these species in our area. To illustrate that basidiomycetes produce clinically significant allergens, we report the onset of acute rhinoconjunc-

tivitis and obstruction of the lower airways following nasal application of *P. pulmonalis* spore extract in a subject with asthma not sufficiently explained by pollen allergy.

CASE REPORT

A 38-year-old housewife was first seen in 1996 for allergy evaluation of her intermittent rhinitis and asthma in our Outpatient Clinic. Since 1993, rhinitic and asthmatic symptoms occurred mainly during nighttime and after physical exercise but noticeable less during the summer and in humid areas. With treatment of budesonide (800 µg per day) and terbutaline as needed, her asthma was well controlled without having nighttime symptoms or limitation of physical activity. By history, at the age of 6 to 16 years, she experienced asthma exclusively in the woods and occasionally during sports. From that time until 1993 at the age of 33 years, she realized slight rhinoconjunctival symptoms in the summer, but she was never bothered from asthma. Atopic dermatitis developed in the course of the

first 3 of 4 pregnancies (1981, 1983, 1985, and 1992) and promptly disappeared each time after birth. Family history was positive for hay fever (mother and one daughter). Physical examination and spirometry [FEV₁ 2.80 L (104% of predicted value)] were normal.

Methods

Skin prick tests were performed with a panel of 12 common inhalant allergens [Allergopharma Hamburg, Germany] as well as with 3 different in-house basidiomycete extracts.⁴ Results were recorded 15 minutes after testing. A positive response was defined as a mean wheal diameter of 3 mm or greater at the site of the test antigen. Spores of *P. pulmonalis* were collected in a sterile growth chamber of a commercial *Pleurotus* grower [P. Lussi, Oberdorf, Switzerland (Fig 1)]. Yields of spore collection and purity were determined by light microscopy. For extraction, 1 g of spores was prepared in 40 mL of 125 mM ammonium bicarbonate buffer (pH 8.1) for 1 hour with stirring at 4°C. The homogenate was centrifuged (80,000 g) for 30 minutes at 4°C and the supernatant lyophilized. For skin testing, lyophilized material was suspended in a solution of 50% glycerol, 85 mM NaCl, and 30 mM sodium bicarbonate and sterile filtrated with Acrodisc PF 0.8/0.2 µm (Gelman Science). Protein content of each extract was adjusted to 10 mg/mL with diluent, as determined by the Bradford-based protein assay (Bio Rad).

Solutions for nasal challenge were prepared by dissolving lyophilized total protein extract in PBS to achieve a concentration of 10 mg protein/mL and subsequent 10-fold decreasing dilution (1 mg/mL, 0.1 mg/mL). All solutions were sterile filtrated and stored at –20°C.

Nasal airway resistance was measured by active anterior rhinomanom-

Institute of Immunology and Allergology, University Hospital, Bern, Switzerland.

Received for publication October 23, 1998.

Accepted for publication March 6, 1999.

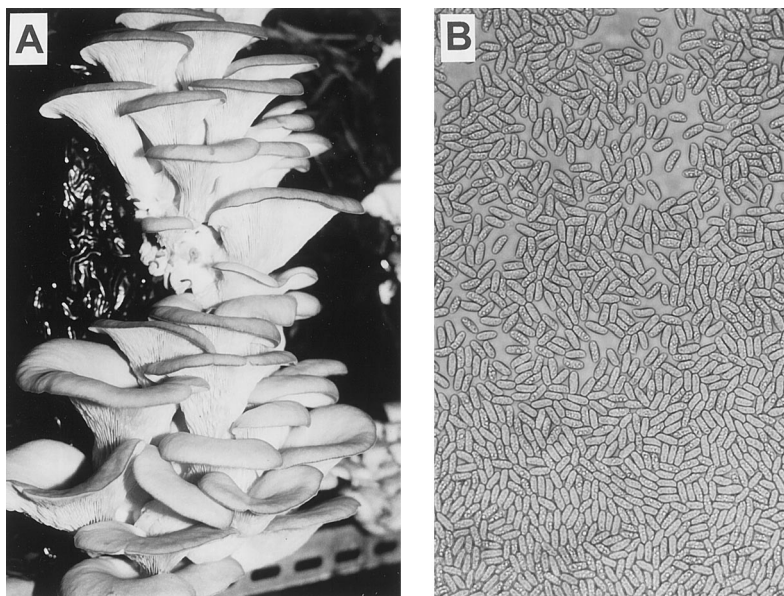


Figure 1. (A) Tree of *P. pulmonalis* grown in a sterile growth chamber. (B) Spores of *P. pulmonalis* (length $\times$ width: $\sim 5 \times 10 \mu\text{m}$ [light microscope with phase contrast $\times 400$]).

etry (RhinoTest MP 441, Germany) and recorded by a pneumotachograph (PM 8043/01 Philips, Japan) using a tightly fitted nose and mouth mask. All measurements were made at a reference pressure of 150 Pa according to the guidelines of the European Committee for the standardization of rhinomanometry.⁵ Resistance has been calculated by analysis of the pressure/flow curve. Following the baseline measurement with physiologic saline, 40 to 50 μL of the test solution was sprayed into one nostril in 15-minute intervals beginning at the lowest concentration (0.1 mg/mL). Subjects were instructed to breathe quietly through the nose. A 40% reduction in nasal airflow was considered as a positive reaction. In addition, objective signs such as sneezing or runny nose and subjective symptoms were recorded by asking whether they experienced nasal itch, blockage, or burning sensation. All protocols were reviewed and approved by the local ethical committee and subjects were informed about the design and the aim of the study and consented to participate. The study subject continued the antiasthmatic therapy but abstained, as well as two control individuals, from taking any other drugs 2

weeks before the challenge. Vital capacity and FEV_1 were measured using a ZAN 100 spirometer (Siemens, Germany) before and after nasal challenge.

SDS-PAGE and immunoblotting have been performed as described.⁴ Resolved proteins, 20 μg per lane, were transferred electrophoretically onto 0.2- μm pore size nitrocellulose (Bio Rad). After blocking, the membrane was incubated overnight with 1:15 diluted patient and control sera at 4°C. Bound IgE was detected with mouse anti-human IgE antibody TN 142 (R. Cramer, SIAF Davos) followed by goat anti-mouse IgG labeled with horseradish peroxidase (Nordic). Detection was enhanced by chemiluminescence (Pierce, UK) and documented on x-ray films exposed for 12 seconds.

RESULTS

The study subject was found to be atopic on the basis of the personal and family history and positive skin test responses to grass and birch pollen. She also had a positive skin test response to *P. pulmonalis* with a 5-mm wheal exceeding both the grass and birch pollen reaction. Total serum IgE was 259 kU/L. Results of commercial ImmunoCAP FEIA system (Pharmacia

Diagnostics AB, Uppsala, Sweden) to birch and grass pollen were negative. Two control subjects, both allergic to grass pollen, showed no skin reactivity to Pleurotus. While both controls had a completely negative nasal challenge (total dose of 555 μg of Pleurotus protein), slight symptoms (nasal itch and sneezing) were experienced by the study subject following application of the lowest concentration of the Pleurotus spore extract (0.1 mg/mL) without significant changes of the nasal air flow. After application of 1 mg/mL (accumulated dose of 55 μg of Pleurotus protein), oropharyngeal itch, rhinorrhea, conjunctivitis, and tearing occurred within 5 minutes and rhinomanometry showed a significant decrease in nasal air flow of 75%. In addition, the subject experienced audible breathing difficulties (wheezing). FEV_1 dropped 30% from the base line of 2.55 L (95% of the predicted value) to 1.78 L. Following inhalation of 2 puffs with terbutaline, full recovery was achieved within 20 minutes. No late phase reaction was observed.

IgE Immunoblot revealed several distinct bands, ranging from 30 to 150 kD; the most intense bands were at 37, 45, and 60 kD (Fig 2). In the control sera, no Pleurotus-specific IgE could be detected.

DISCUSSION

In this article, we demonstrate an acute onset of rhinoconjunctivitis and airway obstruction following nasal challenge with an extract of Pleurotus spores in a nonoccupational exposed individual. Sensitization to birch and grass pollen obviously did not explain paroxysmal rhinitis and intermittent airway obstruction because deterioration of asthma essentially occurred outside the pollen season. In addition to these pollens, only an impressive positive skin test reaction to Pleurotus was detected. Although it is generally accepted that airborne spores can cause respiratory allergy, reports documenting the clinical significance of a given sensitization to fungal allergens, particularly basidiomycetes, are rare.^{4,6-8} Pleurotus spp. are ubiquitous and grow in the woodland throughout the year though most

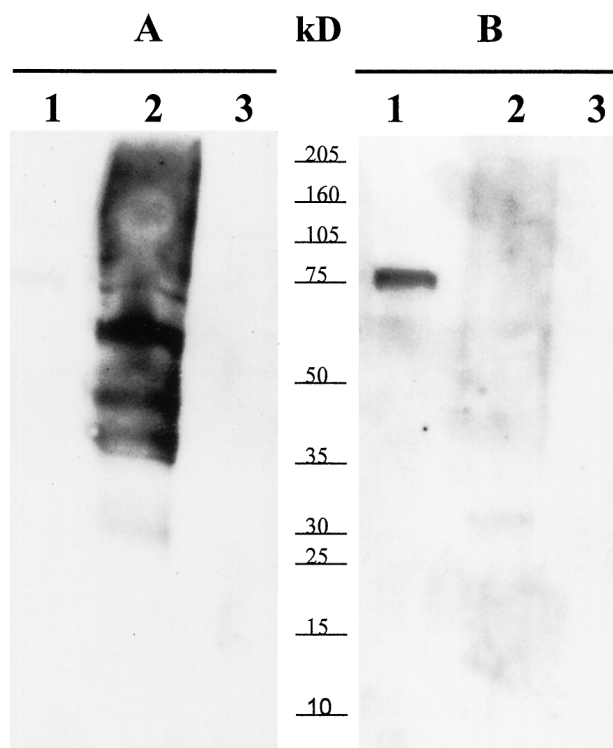


Figure 2. Immunoblot of *P. pulmonalis* spore extract separated by SDS-PAGE. (A) The results with study subjects serum. (B) Findings with the control subjects' sera (lane 1: molecular weight markers; lane 2: 20 µg *P. pulmonalis* spore extract; lane 3: blanc slot).

fruiting bodies are found from late fall until springtime (B. Senn-Irlet, PhD, University of Botany, Bern, personal communication). *P. pulmonalis* exists in the forest but is usually produced in chambers because fruiting bodies are essentially cultivated for gastronomic purposes. Even though the degree of exposure to *Pleurotus* spores is not known in our study subject, her living conditions allow the assumption of such a sensitization since she has been raised and still lives in a rural area surrounded with forests.

The mechanism of response following nasal challenge conceivably is IgE-mediated, as demonstrated by the positive skin prick test, immunoblot IgE reactivity and the acute full spectrum of rhinoocular and bronchial symptoms and signs. A non-specific reaction seems very unlikely since none of the control subjects showed a positive response neither in the skin nor in the provocation test. Nasal challenge test

was performed in August when her asthma was stable under the constant treatment with budesonide and the subject completely free of any symptoms.

Rhinomanometry was used to corroborate the skin test result and to demonstrate the clinical relevance, although the study subject predominantly suffered from asthma. Results of the nasal challenge definitely confirm that *Pleurotus* spores can induce respiratory allergy in sensitized individuals. In addition to the nasal symptoms, the patient developed a precipitous drop in FEV₁ probably due to concomitant mucosal hyperreactivity of the lower airways. These findings confirm the reliability of the skin test reactivity to *Pleurotus* indicating appropriate production and processing of the extracts.⁴ Because *Pleurotus* (eg, oyster mushroom) as well as other species of basidiomycetes are ubiquitous and also perennial producers of airborne allergens, these sources should

be considered as potential cause in subjects with respiratory symptoms which are not completely explained by other inhalant allergies. Since no suitable extracts are commercially available, the production of well-defined allergenic extracts and the characterization of specific allergens at the molecular level is needed.

ACKNOWLEDGMENTS

This study was supported by the Swiss National Science Foundation 32.46798.96, Bern Switzerland.

REFERENCES

1. Horner WE, Helbling A, Salvaggio JE, Lehrer SB. Fungal allergens. Clin Microbiol Rev 1995;8:161-79.
2. D'Amato G, Spiekma FTHM. Aerobiologic and clinical aspects of mould allergy in Europe. Position paper. Allergy 1995;50:870-7.
3. Lehrer SB, Hughes JM, Altman LC, et al. Prevalence of basidiomycete allergy in the USA and Europe and its relationship to allergic respiratory symptoms. Allergy 1994;49:460-5.
4. Helbling A, Gayer F, Pichler WJ, Brander KA. Mushroom (Basidiomycete) allergy: diagnosis established by skin test and nasal challenge. J Allergy Clin Immunol 1998;102:853-8.
5. Clement PAR. Committee report on standardization of rhinomanometry. Rhinology 1984;22:151-5.
6. Licorish K, Novey HS, Kozak P, et al. Role of Alternaria and Penicillium spores in the pathogenesis of asthma. J Allergy Clin Immunol 1985;76:819-25.
7. Malling HJ. Diagnosis and immunotherapy of mould allergy. III. Diagnosis of Cladosporium allergy by means of symptom score, bronchial provocation test, skin prick test, RAST, CRIE and histamine release. Allergy 1986; 41:57-67.
8. Lopez M, Voigtlander JR, Lehrer SB, Salvaggio JE. Bronchoprovocation studies in basidiospore-sensitive allergic subjects with asthma. J Allergy Clin Immunol 1989;84:242-246.

Request for reprints should be addressed to:
A Helbling, MD
Institute of Immunology & Allergology
University Hospital
3010 Bern
Switzerland