

## Basidiomycete Allergy: What Is the Best Source of Antigen?

M.		Lopez
B.T.		Butcher
J.E.		Salvaggio
J.A.		Olson
M.A.		Reed
M.L.		McCants
S.B.		Lehrer

Clinical Immunology Section, Tulane University School of Medicine, New Orleans, La., USA

### Abstract

Skin prick test activity and antigenicity of extracts of in vitro growth of the Basidiomycete *Pleurotus ostreatus* (PO) were compared to extracts of spores from PO growing in the wild. Patients demonstrated significant differences in skin test reactivity to the PO extracts. Some reacted only to in vitro growth extracts, others only to the spore extracts and 1 patient to all extracts. Further studies analyzed antigens present in all extracts with rabbit antisera to PO. Common as well as unique antigens were present in the spore extracts as compared to those from in vitro preparations. The fact that spores contain unique antigens suggests that basidiospores may be the best source of relevant allergens for clinical studies.

Correspondence to: Dr. M. Lopez, Clinical Immunology Section, Tulane University School of Medicine, 1430 Tulane Av., Room 7209, New Orleans, LA 70112 (USA)

Basidiomycetes are the most advanced of all fungi classes and include about 25,000 species. This class of fungi comprises many diverse forms, including mushrooms, puffballs, rusts, smuts and mirror yeasts. In earlier studies we have shown that extracts from selected Basidiomycetes cultured in vitro, were aller-genic in man and antigenic in the rabbit [1, 2]. Since basidiospores are present in the air in large quantities they are of interest as a potentially important source of spore aeroallergens [2,3].

In order to determine the incidence of skin test reactivity to Basidiomycete allergens, six Basidiomycetes (*Cantharellus cibarius*, *Coprinus comatus*, *Dacry-myces deliquescens*, *Naematoloma sublateritium*, *Pleurotus ostreatus* and *Xylobolus frustulatus*) were chosen as representative of families that grow in the region of New Orleans. Cultures of each of these organisms obtained from the American Type Culture Collection were grown in dialyzable yeast malt broth. The culture filtrate was used as a source of the metabolic antigen and the remaining fungal growth, extracted by sonication in phosphate-buffered saline (pH 7.2) was the source of somatic antigen.

Sterile preparations of the metabolic and somatic antigens were prepared at 10mg/ml in 50% glycerol and used for skin prick testing of individuals presenting at the allergy clinic with a history of asthma and/or rhinitis. Skin test reactions of 2 mm or greater in mean wheal diameter were considered positive. Most of these patients were atopic as defined by positive prick tests to 2 or more extracts from 10 common inhalant allergens. The skin test results (table I) demonstrate that prevalence of positive skin test reactions to basidiomycete

extracts ranged from as low as 7.7% for *N. sublaterium* to as high of 10.9% for *C. comatus* antigen extracts. Of the 147 patients tested 23.1 % reacted to at least one of the extracts. Since the incidence of positive skin tests was lower than anticipated, it was postulated that this might be due to the lack of spore production in the basidiomy-

Table I. Skin reactivity of allergic individuals to basidiomycete extracts

Results are expressed as number of positive subjects/total subjects tested. 1 Mean wheal diameter  
^ 2 mm.

170

Lopez/Butcher/Salvaggio/Olson/Reed/McCants/Lehrer

In vitro growth extracts  
metabolic      somatic

Fig. 1. Reactivity of *P. ostreatus* antigen extracts with rabbit antisera. Extracts of PO basidiocarp (CAP), spore extract No. 1 and 2 (Spore), somatic fraction (Som) and metabolic fraction (Met) were tested for reactivity with rabbit antisera to metabolic fraction (Anti-Met), to somatic fraction (Anti-Som) or to spores (Anti-Spore) by double diffusion in agarose.

Table II. Skin test reactions to *P. ostreatus* extracts

tabolic) and spores extracts No. 1 and 2. Skin tests results from 11 patients summarized in table II, demonstrate that there is a significant difference in the skin test reactivity to the extracts. Some patients react only to the in vitro extracts, others only to the spore extracts and 1 patient to both. These results suggest that there may be unique allergens in the spore extracts. In subsequent studies, New Zealand white rabbits were hyperimmunized with somatic, metabolic and spore extracts prepared in complete Freund's adjuvant. Resulting antisera were tested for reactivity to various *Pleurotus* extracts. The gel double diffusion results in figure 1 demonstrates that common as well as unique antigens are present in the spore extracts as compared to the in vitro preparations. A comparison of extracts by isoelectrofocusing also showed that the in vitro and the spore extracts contain components with similar isoelectric points as well as distinct components with different isoelectric points.

These studies demonstrate that extracts prepared from *Pleurotus* spores have distinct antigens as demonstrated by gel double diffusion and isoelectric focusing and show a different pattern of skin reactivity in allergic patients when compared to extracts from the same Basidiomycete grown in vitro. The finding of unique antigens present in basidiospores suggests that extracts from spores may be better sources of relevant allergens for clinical studies.

Results are expressed as wheal diameter in mm.

cetes grown in vitro. To test this hypothesis we selected the Basidiomycete *P. ostreatus* since it is readily grown in synthetic media and spores can be collected from the fungi grown in the wild. Spores were extracted by a two-step procedure using 0.125 M ammonium carbonate buffer (pH 8.1). In the first step, spores were extracted in the buffer, overnight at 4°C, centrifuged, and supernatant lyophylized (spore No. 1). Spore pellet was resuspended in the buffer, homogenized and extracted overnight at 4°C, (second step). Following centrifugation, supernatant was lyophylized (spore No. 2). Sterile preparations of the extracts (10 mg/ml in 50% glycerol) were used for skin prick testing.

48 atopic patients were skin prick tested with extracts of *P. ostreatus* grown in vitro (somatic and me-

#### Acknowledgement

This project was supported by a grant from the NIH-NIA1D, AI 20331. The authors gratefully acknowledge the helpful assistance of Beverly Morgan in manuscript preparation.

#### References

Lopez, M.; Salvaggio, J.E.; Butcher, B.T.: Allergenicity and immunogenicity of basidiomycetes. *J. Allergy clin. Immunol.* 57: 480–488(1976).

Lehrer, S.B.; Aukrust, L.; Salvaggio, J.E.: Fungal induced respiratory allergy. *Clin. Chest Med.* 40: 23–41 (1983).

Gregory

P.; Hirst

J.: The summer airspore at Rothamsted. *J. gen. Microbiol.* 17: 135–152(1957).