

Prevalence of basidiospore allergy in the Pacific Northwest

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Mold spore-induced respiratory allergy has been incompletely studied, and only a limited number of Fungi Imperfecti are well established as aeroallergens. Basidiomycetes, a complex and common group of fungi, which include mushrooms, rusts, smuts, brackets, and puffballs, have not been well studied. Although basidiospores can be present in high atmospheric concentrations, little is known of their aeroallergen potential. To examine this question, we performed skin prick and RASTs in 33 adult residents of Washington State using a panel of 15 common inhalant allergens that included four Fungi Imperfecti and 15 basidiospore extracts. Thirty-one of 33 (94%) subjects had positive immediate reactions to two or more common inhalants. Nine of 33 (27%) subjects responded to at least one Fungi Imperfecti; reactions were most common to Aspergillus sp. (21%), and least common to Penicillium sp., which were positive in 6%. Positive responses to basidiospore extracts were observed in 10 of 33 (30%) subjects. The prevalence of basidiospore reactivity was similar to that of Fungi Imperfecti, ranging from 18% for Scleroderma sp. to 6% for four different spore extracts. These results demonstrate that a significant number of subjects with respiratory allergies have skin test reactivity to basidiospore extracts, suggesting that these spores could be important aeroallergens in the Pacific Northwest. (J ALLERGY CLIN IMMUNOL 1988;82:1076-80.)

The significance of pollens as aeroallergens is well established, but the role of fungal spores is less clear. Of the four major classes of fungi, Deuteromycetes or Fungi Imperfecti are the best established as inhalant allergens.¹ Basidiomycetes, the most morphologically complex of the fungi, are distinct from Fungi Imperfecti and are a common group of fungi that includes mushrooms, rusts, smuts, brackets, and puffballs.² Although spores from Basidiomycetes can be present in high atmospheric concentrations,³⁻⁵ little is known

Abbreviation used

PBS: Phosphate-buffered saline

of their aeroallergenic potential.^{6,7} Recent studies performed on individuals living in New Orleans, La., demonstrated specific IgE antibodies to mycelial and culture filtrate (metabolic) fractions of Basidiomycetes, grown in vitro, and to basidiospore extracts, with skin prick and/or RASTs.⁸⁻¹¹

To determine whether Basidiomycetes play a role in respiratory allergies in a geographically and climatically distinct region, we evaluated 33 adult residents living in Washington State by skin testing and RASTs with basidiospore extracts. Our results demonstrate that the prevalence of reactivity to Basidiomycete spores is comparable to that of Fungi Imperfecti and is similar in the Gulf of Mexico and Pacific Northwest regions of the United States.

MATERIAL AND METHODS

Study population and testing

The study population consisted of 33 adults attending allergy clinics at the University of Washington affiliated

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TABLE I. Results of prick skin tests to common inhalants including Fungi Imperfecti in 33 adults with respiratory allergies

Allergen	No. reactors	% Reactors
House dust	25	76
<i>Dermatophagoides pteronyssinus</i>	23	70
Cat dander	23	70
Timothy grass	21	64
Velvet grass	21	64
Orchard grass	21	64
Alder	19	58
Spring birch	17	52
English plaitain	13	39
Dog dander	9	27
Feather	2	6
<i>Aspergillus</i> mix	7	21
<i>Cladosporium</i>	6	18
<i>Helminthosporium</i>	5	14
<i>Penicillium</i> mix	2	6

hospitals. There were twenty-two male and 11 female subjects; the mean age of the group was 32 years (22 to 64 years). Fifty-one percent ($N = 17$) had histories consistent with allergic rhinitis, 39% ($N = 15$) had histories of both allergic rhinitis and asthma, and 3% ($N = 1$) had a history of asthma only. Individuals gave informed consent in accordance with the guidelines of the University of Washington Human Subjects Review Committee. Blood samples were obtained from all individuals for RAST, and skin prick tests were performed with extracts of 15 common inhalant antigens, which included four Fungi Imperfecti (all extracts were obtained from Hollister-Stier Laboratories, Spokane, Wash.) and with 15 basidiospore extracts. Histamine di-phosphate (1 mg/ml) was the positive control, and glycerol PBS (1:1) was the negative control. A positive reaction was defined as a wheal of ≥ 5 mm.

In a previous study,⁹ 14 nonatopic adults without symptoms of respiratory allergy were skin prick tested with the same basidiospore allergens and gave uniformly negative results. Therefore, a similar control population was not included in the present study.

Basidiospore extract preparation

Extracts of basidiospore samples collected in the New Orleans area were prepared with previously described methods.⁹ Puffballs (*Pisolithus tinctorius*, *Scleroderma* sp., and *Calvatia cyathiformis*) were harvested at maturity and dried at room temperature, and the internal spore mass was removed. Spores from *Ganoderma lucidum*, a polypore, were collected in a plastic bag placed over the basidiocarp for 24 to 48 hours. All the other species were mushrooms. For these species, caps were collected and placed with gills

TABLE II. Number of subjects reacting to one or more basidiospore extracts

No. of basidiospore positive tests	No. of subjects	% Reactors of total basidiospore positive population
1	2	18
2	1	9
3	1	9
4	0	0
5	0	0
6	2	18
7	2	18
8	2	18
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	1	9
15	0	0

down on Whatman No. 1 filter paper (Whatman Inc., Clifton, N.J.) to obtain spore prints that were scraped from the filter paper. The purity of samples from mushrooms was $>98\%$, and for puffballs, 80% to 90%, with the major contaminant being sterile hyphae.

Extracts were prepared by homogenizing 1 gm of spores in a Braun (Infors AG, Basel, Switzerland) homogenizer in 20 ml of ammonium bicarbonate buffer (0.125 mol/L, pH 8.1). Homogenates were centrifuged at 70,000 g; the supernatant was lyophilized and stored at room temperature until use. All spore extracts were tested for toxicity in female Swiss Webster mice and demonstrated to be nontoxic. In conducting the research described in this article, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" that was prepared by the committee on care and use of laboratory animals of the Institute of Laboratory Animal Resources Commission on Life Sciences.

Before skin testing, lyophilized spore extracts were suspended in glycerol:PBS (1:1) and sterile filtered (0.45 μ m). Each extract was adjusted with diluent to a final concentration of 5 mg/ml. RAST was performed as described previously.¹² Antigen (10 mg/ml) in borate buffer (pH 8.0) was coupled with CNBr-activated filter-paper disks.¹¹ Serum (100 μ l) was incubated with the disks overnight and then were washed. ¹²⁵I-labeled antihuman IgE (approximately 25,000 cpm) (Pharmacia Diagnostics, Piscataway, N.J.) was added. The disks were washed and counted. Mean counts per minute was calculated, and data were expressed as percent binding of total radioactivity added. Assays were considered positive if percent binding was $>3\%$ of total; this value was >3 SD from the mean percent binding obtained with sera from atopic control subjects skin test negative to basidiospores.

TABLE III. Results of skin tests to basidiospore extracts

Spore	No. reactors	% Reactors of all subjects	% Reactors of total basidiospore positive subjects
<i>Scleroderma geaster</i>	6	18	55
<i>Agrocybe amara</i>	5	15	45
<i>Coprinus quadrididus</i>	5	15	45
<i>Psilocybe cubensis</i>	5	15	45
<i>Armillaria tabescens</i>	4	12	36
<i>Ganoderma lucidum</i>	4	12	36
<i>Pleurotus ostreatus</i>	4	12	36
<i>Boletus</i> sp.	4	12	36
<i>Chlorophyllum molybdites</i>	3	9	27
<i>Pisolithus tinctorius</i>	3	9	27
<i>Calvatia cyathiformis</i>	3	9	27
<i>Amanita muscaria</i>	2	6	18
<i>Cantharellus cibarius</i>	2	6	18
<i>Boletinus merulioides</i>	2	6	18
<i>Inonotus ludovicianus</i>	2	6	18

TABLE IV. RAST results, expressed as a percent of ¹²⁵I-labeled anti-IgE added, from three subjects with positive responses

Basidiospore species	Study subject		
	R. B.	V. S.	J. M.
<i>Scleroderma</i> sp.	2.6	2.0	3.2*
<i>Coprinus quadrididus</i>	4.7*	3.8*	3.2*
<i>Amanita muscaria</i>	4.6*	1.8	1.5
<i>Chlorophyllum molybdites</i>	2.2	2.0	3.1*
<i>Boletus</i> sp.	1.0	1.6	1.0
<i>Pleurotus ostreatus</i>	1.8	3.8*	2.6
<i>Pisolithus tinctorius</i>	1.3	2.3	1.1
<i>Psilocybe cubensis</i>	6.2*	9.8*	4.7*
<i>Agrocybe amara</i>	4.1*	2.9	4.6*
<i>Ganoderma lucidum</i>	4.9*	3.5*	2.5
<i>Calvatia cyathiformis</i>	1.9	2.3	3.3*
<i>Armillaria tabescens</i>	2.9	2.7	2.3

*Positive response.

RESULTS

Thirty-one of the 33 (94%) individuals had positive prick skin tests to two or more common inhalant allergens, and these subjects were classified as atopic. Prevalence of reactivity ranged from highs of 76% to house dust and 70% to *Dermatophagoides pteronyssinus* and cat dander, to a low of 6% to feathers. The prevalence of individuals reacting to the Fungi Imperfecti ranged from a high of 21% to *Aspergillus* mix to a low of 6% to *Penicillium* mix (Table I).

Eleven of 33 (33%) individuals demonstrated at

least one, and as many as 14, positive skin test reactions to basidiospore extracts (Table II). Four of the 11 (36%) individuals had a history of allergic rhinitis, six (55%) had both allergic rhinitis and asthma, and one (9%) individual had asthma alone. Results of prick skin tests to individual basidiospore extracts are summarized in Table III. The prevalence of positive skin test reactions ranged from a high of 18% to *Scleroderma* sp. to a low of 6% to four different species: *Amanita muscaria*, *Cantharellus cibarius*, *Boletinus merulioides*, and *Inonotus ludovicianus*. Preva-

lence of skin test reactivity to basidiospores was similar to that of the Fungi Imperfecti.

Sixty-three percent of individuals reacting to common inhalant extracts failed to react to basidiospore extracts. There was no significant correlation between reactivity to common inhalant allergens and basidiospore extracts in those individuals reactive to both. However, there was a significant correlation between the number of positive reactions to Fungi Imperfecti and basidiospores in subjects reactive to both classes of fungi ($r = 0.91$; $p < 0.001$; Fig. 1).

Three of the 11 basidiospore skin test positive subjects had positive RASTs (binding $>3\%$) to one or more basidiospore extract (Table IV). The most potent RAST reactivity was to *Psilocybe cubensis* (percent binding: 4.7, 6.2, and 9.8). No positive RAST reactions were detected to *Boletus* sp., *Pisolithus tinctorius*, and *Armillaria tabescens*.

DISCUSSION

Basidiomycetes are a major class of fungi that includes mushrooms, puffballs, smuts, rusts, and bracket fungi and number about 25,000 species. Concentrations of basidiospores in the atmosphere can be significant,²⁻⁵ and recent studies have documented the importance of Basidiomycetes in inducing respiratory allergies in patients living in the New Orleans and Gulf of Mexico regions.^{1, 3, 14, 15} To determine whether members of this fungal class may be important aeroallergens in the Pacific Northwest, skin testing and RAST was performed in 33 individuals with allergic rhinitis and/or asthma to common aeroallergens, Fungi Imperfecti, and to a selection of 15 basidiospores. Our results indicate that basidiospore reactivity is present in atopic patients living in the Pacific Northwest and that these fungi may be important aeroallergens in this region. The prevalence of reactivity to the basidiospore extracts was similar to that for Fungi Imperfecti used in this study.

In general, our data are similar to data reported previously in a group of atopic patients from New Orleans who were tested with the same basidiospore antigens.⁹ The two populations were generally comparable with respect to reported respiratory symptoms; however, New Orleans subjects had a higher percentage of individuals 26/150 (17%) reporting symptoms of asthma only. Prevalence of reactivity to common inhalant allergens, particularly the Fungi Imperfecti, as well as basidiospore reactivity, was also similar in both studies. The New Orleans study population demonstrated a prevalence of basidiospore skin test reactivity that ranged from 5% for *Cantharellus cibarius* to 17% for *Scleroderma* sp. In

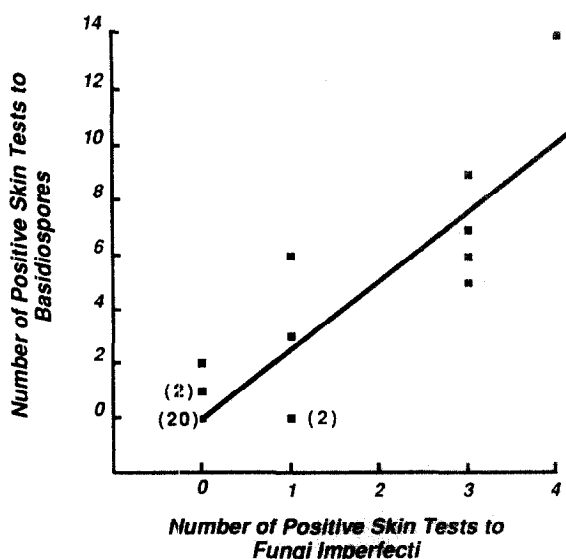


FIG. 1. Linear relationship between number of positive skin tests to basidiospore and number of positive skin tests to *Fungi imperfecti*; $r = 0.91$; $p < 0.001$.

addition, the prevalence of skin test reactivity to basidiospores in the New Orleans population was similar to that observed for Fungi Imperfecti, as was also found in the current study.

One noteworthy difference between results from the two studies was different reactivity to *I. ludovicianus*. In the Washington state population, only 2/33 (6%) subjects reacted to this extract, whereas 9/76 (12%) subjects in Louisiana reacted to *I. ludovicianus*. These discordant observations may result from the distribution of that species. *I. ludovicianus*, in contrast to most Basidiomycete species used in this study, is limited to the southeastern United States. Although related species are found in the Pacific Northwest,¹⁶ cross-reactivity among species in this genus has yet to be determined. Our findings suggest that there may be both shared and unique allergens.

Minor variations in observed reactivity to other Basidiomycete species are probably the result of several factors, including climatic conditions, such as wind, humidity, and temperature, and the relative numbers of spores present in a particular region. Accurate estimates of atmospheric concentrations of specific basidiospores are difficult to obtain with currently available aeroallergen collection techniques. Relative estimates, based on the number of basidiocarps growing in a particular area, along with the number of spores harvestable from basidiocarps, permit crude estimations of atmospheric spore concentrations. Different basidiospore species may vary in their ability to induce IgE responses caused by the relative aller-

genic potency of basidiospore proteins, the duration of time that they are suspended in the air, and antigenic cross-reactivity among various species.¹⁷ Indeed, in the current study population, we demonstrated a high prevalence of positive skin tests to two or more basidiospore extracts. This may be due in part to shared allergenic epitopes among basidiospore species, as has been suggested previously.¹⁸

RAST results between the Washington state and Louisiana study populations are less easily compared.¹¹ In both groups, *Coprinus quadrifidus* and *Psilocybe cubensis* had the greatest number of positive responses, whereas *Boletus* sp. and *Pisolithus tinctorius* had the least. Results from other extracts were generally discordant, with the most striking example being *Armillaria tabescens*. In the present study, no individuals were RAST positive to that allergen; however, 21.4% of subjects residing in the New Orleans and Gulf of Mexico regions had in vitro IgE antibodies. In addition, percent binding of total anti-IgE added appeared lower in the population from Washington state. The most likely reason for the discrepancies may be the limited sample size from Washington, although climatic variables listed above may also play a role. Both our present and previous¹¹ studies demonstrated a generally poor correlation between skin test and RAST results. The reason(s) is not clear, but it is possible that some basidiospore extracts have fewer free amino groups available to bind with the disk or that the allergen(s) is not proteinaceous.

A significant question that remains to be answered is whether skin test reactivity is an adequate predictor of clinical symptomatology. Previous studies⁸⁻¹⁴ have demonstrated a relationship between episodes of allergic asthma and increased concentrations of basidiospores in the atmosphere. Of our study population, all patients reacting to the basidiospores had histories of clinically significant perennial respiratory symptoms, including rhinitis, bronchospasm, or both. Recently, a relationship between bronchospasm and inhalation of basidiospores was demonstrated.¹⁹ Five of seven individuals with asthma and with positive skin tests to basidiospores demonstrated reductions in FEV₁ of >20% after aerosol inhalation challenge with extracts of *Pleurotus ostreatus*, *Coprinus quadrifidus*, *Ganoderma lucidum*, or *Psilocybe cubensis*. These data clearly indicate the importance of basidiospore allergy in provoking respiratory symptoms. Further studies with basidiospore extracts with subjects from additional geographic regions are necessary to confirm the emerging information about the prevalence of basidiospore allergy.

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