

Short Communications

Skin test reactivity of allergic subjects to basidiomycetes' crude extracts in a tropical environment

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Fungal allergies can be detected by the skin prick test with extracts of the organisms, but not all fungi, including the basidiomycetes, are being examined. We determined the level of sensitization to basidiomycetes in allergic subjects and compared their reactivity to commercial extracts commonly used to detect allergies. Crude spore extracts of the basidiomycetes *Ganoderma applanatum*, *Chlorophyllum molybdites*, and *Pleurotus ostreatus*, which are known to release numerous spores, were examined along with commercial extracts on 33 subjects with asthma, allergic or non-allergic rhinitis. Overall, affected subjects showed the highest reactivity to mites (36%), followed by *Ganoderma applanatum* (30%), grass (27%) *Chlorophyllum molybdites* (12%) and *Pleurotus ostreatus* (12%). Allergic rhinitis patients were most reactive to mites (58%), grass (42%), *Ganoderma applanatum* (25%), *Penicillium* spp. (25%), and cat (17%). Those with asthma primarily responded to mites (44%), *Ganoderma applanatum* (44%), grass (33%), and *Pleurotus ostreatus* (22%). IgE levels correlated with positive basidiomycetes extracts. This finding, coupled with higher reactivity to basidiospores as compared to mitosporic, and the similar sensitivities of patients to *G. applanatum* and mites, suggest that basidiomycetes are important allergen sources in the tropics.

Keywords asthma, allergic rhinitis, basidiomycetes, extract, skin-prick test (SPT)

Introduction

Numerous studies have associated fungal allergens with asthma symptoms and morbidity [1–4]. It is estimated that 10% of the population is sensitive to some fungal allergens [4]. Even though fungi have been connected with fatal cases of asthma [4], much of the information on fungal allergens comes from studies with mitosporic fungi (fungi imperfecti or deuteromycetes), and which have been performed in countries with temperate climates [1]. In many tropical environments, such as in Puerto Rico [5],

basidiospores are the predominant fungal particulates in the atmosphere [6]. However, their contribution to allergic respiratory diseases have not been as extensively studied as mitosporic fungi because they are difficult to manipulate under laboratory conditions and numerous fruiting bodies are necessary to prepare extracts.

Considering that Puerto Rico has a high incidence of respiratory diseases [7], and that there are elevated concentrations of basidiospores in the atmosphere [8], we wanted to study the skin prick test (SPT) reactivity among subjects diagnosed with some kind of respiratory allergy to crude spore print extracts of basidiomycetes. Following protocols used in the USA and Europe [8], we tested basidiomycetes' species that are common in the area and produce numerous spores, and compared the results to those obtained with commercial extracts used to diagnose allergies. Our hypothesis is that, contrary to findings in countries with temperate climates

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[2,9], subjects with respiratory allergies in Puerto Rico have a higher rate of SPT reactivity to basidiospores extracts.

Materials and methods

While numerous fruiting bodies were collected in the field, extracts were prepared from only those of *Ganoderma applanatum*, *Chlorophyllum molybdites*, and *Pleurotus ostreatus* because they were the most abundant. These basidiomycetes, with the exception of *P. ostreatus* which is cultivated for commercial purposes, are common in Puerto Rico which made it feasible to collect sufficient quantities to prepare spore prints (spore deposit). The latter were made by cutting the stipes from the fruiting bodies of *P. ostreatus* and *C. molybdites* and laying the basidiocarps, gills facing downward, onto Petri dishes for 24 h. In the case of *G. applanatum*, spore prints were prepared in the field with spore traps consisting of Petri dishes over metallic nets placed under the basidiocarps. Precautions were taken to insure that the Petri dishes did not make contact with basidiocarp to avoid contamination of the spore deposit. The extraction method was previously described by Lehrer [10]. A spore print suspension for each basidiomycete was prepared and extracted at 50 mg of spore print/ml of ammonium bicarbonate. The homogenate was centrifuged at 800 rpm for 5 min at 4°C with a Sorvall RT6000B Refrigerated Centrifuge (Dupont, Wilmington, DE, USA). The resulting supernatant was centrifuged at high-speed (48,000 rpm) for 1 h at 4°C in an Optima L-100 XP Centrifuge (Beckman Coulter, Brea, CA, USA). The supernatant was filter-sterilized with a Nalgene 115 ml Filter Unit (Nalge Nunc International) and lyophilized with an Edwards Modulyo Freeze-Dryer (Edwards High Vacuum International, Crawley, UK). The resulting solid was re-suspended in 10 ml (volume used was dependent on the amount of solid, but in a ratio of 5 mg/ml) of a solution containing 50% glycerol (Fisher Scientific), 85 mmol sodium chloride (Fisher Scientific), and 30 mmol sodium bicarbonate (Fisher Scientific). This re-suspension buffer is similar to that previously used by Lehrer [8]. The protein concentration of the extracts was determined with a BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions. The protein concentrations were 150, 220, and 914 µg/ml for *G. applanatum*, *C. molybdites*, and *P. ostreatus*, respectively. Crude extracts were tested in their non-diluted states because, as stated by Horner *et al.*, we did not know the factors affecting the stability of each of the extracts [4].

With the approval of the University of Puerto Rico, Medical Sciences Campus (UPR-MSC) Institutional Review Board (IRB), subjects living in the San Juan metropolitan area were recruited from participating allergists' ambulatory clinics, as well as staff and student volunteers from the UPR-MSC. Inclusion criteria for the study included a self-reported

history of suffering from asthma or nasal allergies, having a stable medical regime for the previous 30 days, being non-institutionalized, and > 15 years of age. Exclusion criteria were current oral steroid-dependent asthma or immune suppressor treatment, any type of cancer within the last six months, upper respiratory tract infection within the past two weeks, smoking, dementia or other conditions that would not allow self-reporting of data, self-reported poor compliance with any medical therapy, institutionalization, and pregnancy. Each individual was provided an explanation of the purpose of the study and presented with an informed consent form. A total of 33 participants consented to be part of the study. All were > 22 years of age with female volunteers being predominantly represented (30 females and three males). Ten milliliters of blood were obtained from each participant and the levels of serum immunoglobulin E (IgE) were determined at a reference laboratory with the chemoluminescence method ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY, USA).

Allergic rhinitis was diagnosed based on the history of current use of medications for the condition, nasal symptoms and/or physician diagnosed allergic rhinitis, in addition to having a history of a positive skin test to relevant antigens. Non-allergic rhinitis was determined based on the presence of symptoms or medications used and in the absence of a positive test or elevated total IgE. Negative controls were asymptomatic subjects who did not use any medications for rhinitis and who had negative or irrelevant skin test results. Asthma was determined on the basis of pulmonary symptoms of wheeze, cough, shortness of breath, medications used, and physician diagnosed asthma. Chronic obstructive pulmonary disease (COPD) was diagnosed based on pulmonary symptoms and smoking history.

A board-certified allergist performed the SPTs with various extracts on the volar surface of the forearm or on the back of the 33 subjects. The commercial allergen extracts in the panel from Greer Laboratories (Greer Laboratories, Lenoir, NC, USA) were those from mitosporic fungi (imperfect fungi such as *Penicillium* spp., *Aerobasidium* spp., *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Aspergillus* spp., *Acremonium* spp., and an ascomycetes (*Chaetomium* spp.), pollens (trees, Bermuda, Johnson, and Bahia), pets (dog and cat dander), cockroaches, mites (Der p and Der f), positive (histamine 15 mg/ml) and negative (glycerol-phosphate buffered saline) controls. The wheal and flare was recorded for each subject after 15 min by measuring the orthogonal diameters, i.e., a wheal of 3 mm diameter or more than that of the negative control was considered as a positive test.

Considering the limited sample size ($n = 33$), a Fisher's exact test was used to determine potential association between subjects' SPT reactivity results with the allergen sources (Fig. 1). A Pearson product moment correlation coefficient was calculated to find correlations between the

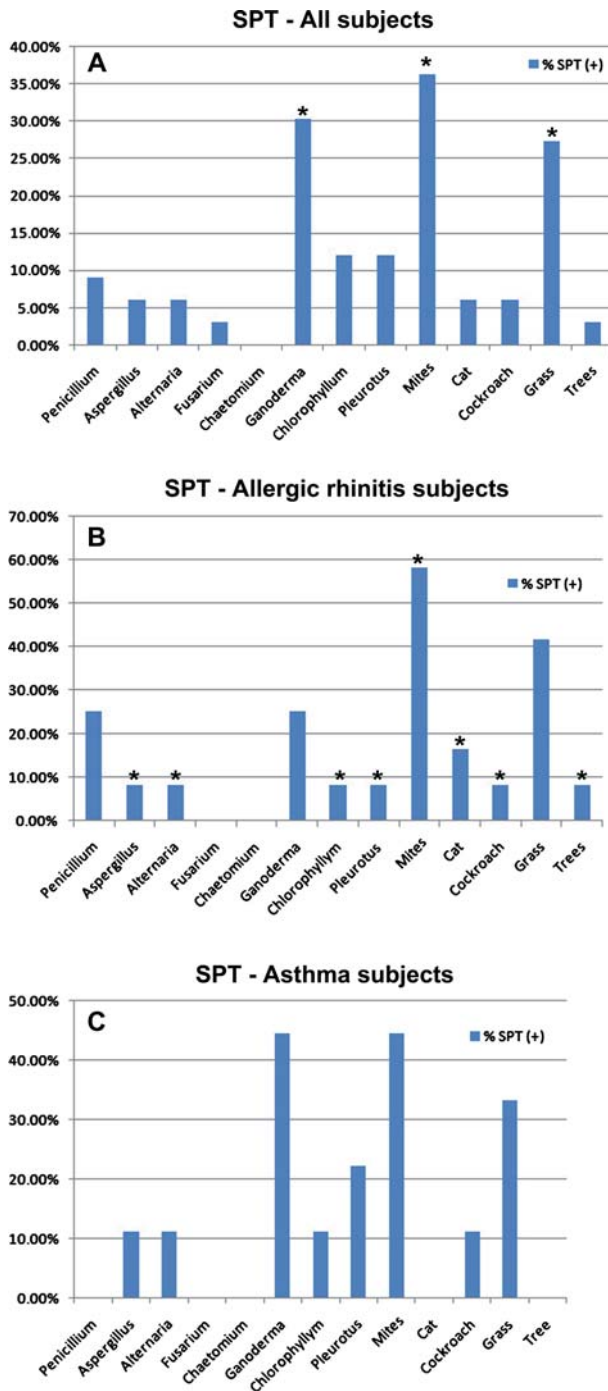


Fig. 1 Percentages of skin-prick test (SPT) positives when all (A), allergic rhinitis (B), or asthmatic patients (C) were evaluated. * $P < 0.05$ was considered significant.

SPT results (extracts with the highest % of reactivity) and for the total number of extracts testing positive per subject, with the predictors of IgE levels and respiratory condition (Table 1). For this purpose, each disease was given the Current Procedural Terminology code, in which 472, 477.8,

Table 1 Pearson correlation between predictors (IgE levels and disease) and extracts with highest reactivities and total extracts positive.

	IgE (pearson coefficient) (p-value)	Disease
Ganoderma	0.425	0.159
	0.022	0.394
Mite	0.209	0.207
	0.278	0.263
Grass	0.575	0.139
	0.001	0.457
Extracts positive	0.683	0.247
	0.000	0.181

and 493 corresponded to non-allergic rhinitis, allergic rhinitis, and asthma, respectively. A $P < 0.05$ value was considered significant in the statistical tests used.

Results

Allergic rhinitis and asthmatic subjects (20 of 21) were positive to at least one of the basidiospores extracts. Among the non-allergic rhinitis patients, one was positive to *G. applanatum*, *C. molybdites*, and grass, another to only *G. applanatum*, and a third to mite and all three basidiomycetes (clinically non-relevant sensitivity). The percentage of subjects positive to each allergen, as shown in Fig. 1, were; 36% to mite, 30% to *G. applanatum*, 27% to grass, 12% to *C. molybdites* and *P. ostreatus*, 9% to *Penicillium* spp., 6% to *Alternaria* spp., *Aspergillus* spp., cockroach and cat, and finally 3% to *Fusarium* spp. and trees. Sensitivity of mite, grass, and *G. applanatum* were significantly different from those to the other extracts.

Results of SPT for allergic rhinitis subjects showed that 58% were reactive to mites, 42% to grass, 25% to *G. applanatum* and *Penicillium* spp., 17% to cat, 8% to *Aspergillus* spp., *Alternaria* spp., *C. molybdites*, and *P. ostreatus* (Fig. 1B). A total of 44% of asthmatic subjects (Fig. 1C) were reactive to mites, as well as *G. applanatum*, 33% to grass, 22% to *P. ostreatus*, 11% to *Aspergillus* spp., *Alternaria* spp., *C. molybdites* and cockroach. We found a significant Pearson product moment correlation between IgE levels and the percentages of positive SPTs to *G. applanatum* and grass extracts (Table 1). In addition, there was a significant correlation between the number of extracts to which the subjects were positive and the IgE levels. There was no correlation between respiratory disease and the percentages of SPT positive.

Discussion

The prevalence of fungal sensitization, including to basidiomycetes, is one of the least studied conditions and most difficult to diagnose. One of the reasons is the limited

availability of commercial extracts for many fungi, including basidiomycetes [11].

A considerable proportion of subjects (30%) were sensitive to *G. applanatum* with the reactivity not significantly different to that of mite extract. The latter is a common indoor allergen [12,13] despite some debate in the literature [14]. Possible explanations for this finding could be that *Ganoderma* is a perennial and widespread wood-rotting basidiomycete in tropical environments releasing trillions of spores daily [15] and is always present in the atmosphere of San Juan [5].

Results of this study demonstrate that sensitivity to basidiomycete was significantly higher than to mitosporic fungi. This data are similar to those reported in various studies performed in temperate regions, in which the reactivity was around 30% and sensitization to basidiomycete was as prevalent as other well-characterized allergenic molds [8,10,11]. These results have important implications for Puerto Rico because basidiospores are the most common aeroallergens in the island's atmosphere, representing close to 60% of the outdoor fungal spores [5]. To the best of our knowledge, this is the first report to demonstrate the significance of basidiomycete spores as potential allergens in the tropics, including the Caribbean. Nevertheless, further studies with larger sample sizes should be performed to further establish the prevalence of sensitization to basidiomycetes among subjects with respiratory allergies.

The significance of the correlation seen with the outdoor allergens (*G. applanatum* and grass pollen) was surprising because there are conflicting reports regarding the association between IgE levels and SPT results [16]. However, to definitely conclude the presence of IgE reactivity to these basidiomycetes' allergens, immunoblots, in which the sera of the patients are challenged with electrophoresis-separated patterns of different fungal extract, must be performed [2]. We were not able to conduct such studies as the sera from the subjects in our study were tested for reactivity to airborne fungal particulate using the halogen immunoassay [17].

The small sample size in this study must be considered as a limitation to the performance of the statistical analyses. As a result, the percentages of reactivity of basidiospore extracts over mitosporic fungi may have been favored although the extraction methods used for the basidiomycetes were similar to those previously used by Lehrer *et al.* [8,10]. Another limitation of the present investigation was that protein concentrations of basidiomycetes extracts were not normalized with serial dilutions prior to their use in skin testing as performed in previous studies [8,10]. This could have made the comparison between commercial and non-commercial extracts difficult. Because we wanted to test the extracts in their crude form, and to avoid any modification of possible allergenic proteins [4], we did not dilute the extracts.

In summary, this is the first report in a tropical environment in which the reactivity to basidiomycetes in subjects with asthma or rhinitis was studied. Although the percentage of basidiomycetes reactivity might suggest a role in exacerbation of allergic respiratory diseases, further clinical, immunological, and molecular biology studies are warranted to evaluate the role of basidiospores in allergy and asthma.

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