

Basidiomycete allergy: Measurement of spore-specific IgE antibodies

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RAST with 12 basidiospore extracts demonstrated that a significant percent of 42 individuals with symptoms of perennial rhinitis and/or asthma and skin reactivity to at least one spore extract had spore-specific IgE antibodies. These antibodies were not demonstrable in 14 atopic, symptomatic, skin test negative control subjects nor in five nonatopic, asymptomatic control subjects. There was a statistically significant association between RAST and skin test results for all but three of the spore extracts. A statistically significant association was also observed between RAST results obtained with most of the different spore extracts. A similar association was present for skin test results with different spore extracts. These results provide evidence that basidiospore extracts are suitable allergens for use in diagnostic studies of respiratory disease associated with exposure to basidiomycetes. (J ALLERGY CLIN IMMUNOL 1987;80:803-9.)

Basidiomycetes, the most morphologically complex of the fungi, consist of approximately 25,000 species and include mushrooms, puffballs, rusts, smuts, and bracket fungi.¹ High atmospheric concentrations of basidiospores have been reported in many parts of the world.²⁻⁴ Earlier studies from our laboratories demonstrated a correlation between elevated basidiospore levels in the environment, related to meteorologic changes, and increased seasonal incidence of hospital admissions for acute asthma.⁴ Subsequently, the presence of specific IgE antibodies to mycelial and metabolic fractions from in vitro cultured basidiomycetes was demonstrated by skin test and RAST.^{5, 6}

Abbreviation used

PBS: Phosphate-buffered saline

Basidiomycetes grown in vitro do not sporulate. Thus, reactions to in vitro growth extracts may not be relevant to respiratory allergic disease, since the fungal elements to which individuals are exposed are primarily spores. Our skin prick test results on subjects in New Orleans, La., and Seattle, Wash., confirmed that extracts of basidiospores are more appropriate allergens for clinical studies than mycelial or metabolic material. There was a good correlation between skin test results and questionnaire-derived history of respiratory symptoms,^{6, 7} although, in both populations, differences in the prevalence of skin reactions to different basidiospore preparations were observed. These findings support the hypothesis that basidiospores are major fungal aeroallergens in the New Orleans⁷ and Seattle⁸ environments.

The purpose of this study was to confirm and extend our evaluation of basidiospore-induced respiratory disease. We describe in this article the measurement of spore-specific IgE antibodies with RAST and analysis of relationships between and among skin prick test and RAST with these basidiospore extracts.

MATERIAL AND METHODS

Study population

The study population consisted of 42 adults with symptoms of respiratory allergy (perennial rhinitis and/or asthma)

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In conducting the research described in this article, investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

TABLE I. Skin test and RAST results with basidiospore extracts

Spore extract	% SKT +	% RAST + (highest % B)	% SKT and RAST +	Fisher's exact probability
<i>G. saccatum</i> *	55.6	31.0 (10.2)	44.4	ND
<i>C. quadrifidus</i>	54.8	31.0 (13.5)	31.0	$p = 0.001$
<i>Scleroderma</i> sp.	47.6	16.7 (8.9)	16.7	NS
<i>P. cubensis</i>	42.9	35.7 (23.9)	33.3	$p = 0.0003$
<i>P. ostreatus</i>	42.9	21.4 (8.7)	21.4	$p = 0.003$
<i>A. tabescens</i>	40.5	21.4 (10.5)	14.3	$p = 0.0145$
<i>G. lucidum</i>	38.1	21.4 (12.3)	16.7	$p = 0.009$
<i>C. cyathiformis</i>	33.3	16.7 (8.1)	16.7	$p = 0.0032$
<i>Boletus</i> sp.	33.3	4.8 (3.7)	2.7	NS
<i>A. amara</i>	33.3	26.2 (14.5)	23.8	$p = 0.019$
<i>A. muscaria</i>	31.0	16.7 (8.0)	7.1	NS
<i>C. molybdites</i>	31.0	19.0 (7.7)	14.3	$p = 0.006$
<i>P. tinctorius</i>	28.6	9.5 (5.7)	2.4	NS

SKT = skin test; B = binding; NS = not significant; ND = not determined.

*Skin test performed on nine individuals, and RAST performed on 42 individuals.

TABLE II. Association between skin prick test results with different basidiospore extracts

	S.s.	G.s.	C.q.	A.m.	C.m.	B.s.	P.o.	P.t.	P.c.	A.a.	G.l.	C.c.	A.t.
<i>Scleroderma</i> sp.		ND	—	—	—	*	—	—	—	—	—	—	—
<i>G. saccatum</i>	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>C. quadrifidus</i>	—	ND		—	—	—	*	—	*	†	*	†	—
<i>A. muscaria</i>	—	ND	—		—	—	—	—	—	—	—	—	—
<i>C. molybdites</i>	—	ND	—		—	†	†	—	—	†	*	*	†
<i>Boletus</i> sp.	*	ND	—		†		*	—	*	†	—	†	*
<i>P. ostreatus</i>	—	ND	*	—	†	*		—	†	†	—	†	†
<i>P. tinctorius</i>	—	ND	—	—	—	—	—	—	—	—	—	—	—
<i>P. cubensis</i>	—	ND	*	—	—	*	†	—		†	†	†	*
<i>A. amara</i>	—	ND	†	—	†	†	†	—	†		*	†	†
<i>G. lucidum</i>	—	ND	*	—	*	—	—	—	†	*		*	†
<i>C. cyathiformis</i>	—	ND	†	—	*	†	†	—	†	†	*		—
<i>A. tabescens</i>	—	ND	—	—	†	*	†	—	*	†	†	—	—

ND = not done; — = not significant.

*0.0 $\times$ = p .†0.00 $\times$ = p .‡0.000 $\times$ = p .

attending the Charity Hospital of New Orleans, Tulane University Medical Center, or University of Washington Allergy Clinics, and who had positive skin prick reactions to one or more basidiospore extract (see next section for list). A control population of 14 atopic adults with symptoms of respiratory allergy but with negative skin prick tests to the basidiospore extracts and five nonatopic adults with no symptoms and negative skin prick test to the spore panel were also tested. Information on respiratory and allergic history was obtained on all study subjects, and blood samples were drawn. All individuals were skin prick tested with inhalant allergens common to the area. Individuals with two

or more positive skin prick tests to these panels were considered atopic.

Basidiomycete species used and collection of spores

Extracts of spores from basidiomycetes indigenous to the New Orleans region and reflecting a variety of species present in Louisiana, with different growing seasons, were prepared as described elsewhere.⁷ Basidiospore samples were collected from the puffballs *Scleroderma* sp., *Geastrum saccatum*, *Pisolithus tinctorius*, and *Calvatia cyathiformis*; a polypore, *Ganoderma lucidum*; and the gilled mushrooms,

TABLE III. Association between RAST with different basidiomycete spore extracts

	S.s.	G.s.	C.q.	A.m.	C.m.	B.s.	P.o.	P.t.	P.c.	A.a.	G.l.	C.c.	A.t.
<i>Scleroderma</i> sp.		**	*	**	***	—	**	*	**	**	*	***	*
<i>G. saccatum</i>	**		***	**	**	—	**	—	***	***	**	**	***
<i>C. quadrifidus</i>	*	***		**	**	—	*	—	***	***	**	*	***
<i>A. muscaria</i>	**	**	**		*	*	*	*	**	**	*	**	**
<i>C. molybdites</i>	***	**	**	*	*	*	**	**	**	**	*	***	**
<i>Boletus</i> sp.	—	—	—	*	*		*	*	—	—	*	*	*
<i>P. ostreatus</i>	**	**	*	*	**	*		*	**	**	*	**	*
<i>P. tinctorius</i>	*	—	—	*	**	*	*		*	*	*	*	*
<i>P. cubensis</i>	**	***	***	**	**	—	**	*		***	**	**	**
<i>A. amara</i>	**	***	***	**	**	—	**	*	***		*	**	**
<i>G. lucidum</i>	*	**	**	*	*	*	*	*	**	*		*	*
<i>C. cyathiformis</i>	***	**	*	**	***	*	**	*	**	**	*		*
<i>A. tabescens</i>	*	***	***	**	**	*	*	*	**	**	*	*	

— = Not significant.

*0.0 $\times$ or 0.00 $\times$ = *p*.**0.000 $\times$ or 0.0000 $\times$ = *p*.***0.00000 $\times$ = *p*.

Psilocybe cubensis, *Coprinus quadrifidus*, *Amanita muscaria*, *Chlorophyllum molybdites*, *Boletus* sp., *Pleurotus ostreatus*, *Agrocybe amara*, and *Armillariella tabescens*. Basidiomycetes were identified on the basis of morphology, growth patterns, and spore structure. *Boletus* sp., and *Scleroderma* sp., were mixtures of spores from different species of these genera.

Basidiospores were collected as described previously.^{7,9} Briefly, basidiomycete caps were placed, gills or pores down, on Whatman No. 1 filter paper or waxed paper and covered with aluminum foil or filter paper to obtain prints from which spores were scraped with a spatula. When fruiting bodies were puffballs, they were harvested at maturity, dried at room temperature, and cut in half, and the spore mass comprising the interior of the puffball was removed with a spatula.

Identification of spore preparations, based on color, size, and shape, was confirmed by light microscopy. Purity was >98% for spores from gilled or pored mushrooms and at least 80% to 90% for spores from puffballs, with the major contaminant being sterile hyphae.

Preparation of basidiospore extracts

To prepare extracts, 1 gm of spores was homogenized in a Braun homogenizer in 20 ml of ammonium bicarbonate buffer (0.125 mol/L, pH 8.1), the homogenate was centrifuged at 48,000 $\times$ g, the supernatant was lyophilized, and the extract was stored at room temperature under desiccation.⁷ All extracts were tested for toxicity in female Swiss-Webster mice before use for skin prick testing and were demonstrated to be nontoxic.

Skin prick test reactions

Lyophilized spore extracts for skin testing were suspended in a sterile solution of 50% glycerol and PBS and

sterile filtered. Dry weight of each spore extract was adjusted to 5 mg/ml with PBS. Glycerol PBS (1:1) was used as the negative control, and 1 mg/ml of histamine diphosphate was used as the positive control. The mean of the largest wheal diameter and the diameter perpendicular to it was determined. A mean wheal diameter 2 mm greater than the diameter of the negative control wheal was considered positive.

RAST for specific IgE antibodies to spore extracts

Patients' sera were tested by RAST to determine levels of specific IgE antibodies to basidiospores. For coupling to RAST disks, spore extracts were adjusted to 10 mg/ml in pH 8.0 borate buffer (because of insufficient quantity, *A. amara* extract was used at 8.84 mg/ml) and coupled to CNBr-activated filter paper disks.¹⁰

For the test¹¹, 100 μ l of patient serum was incubated overnight with a test disk. All sera were tested in duplicate. After three washes with 2.5 ml of physiologic saline to remove unreacted serum, disks were incubated overnight with 100 μ l of ¹²⁵I-labeled anti-IgE antiserum (25,000 cpm), washed three times with saline to remove unreacted material, and counted in a gamma counter to determine radioactivity in counts per minute bound to the disks. A RAST binding $\geq 3\%$ of total activity added was considered positive; this value was >3 SD from mean percent binding obtained with sera from atopic control subjects skin test negative to basidiospores.

Statistical analysis

Descriptive statistics were obtained with the StatPro computerized program (Wadsworth Electronic Publishing Co., Boston, Mass.). Fisher's exact test¹² was used to evaluate the association of positive RAST results compared with

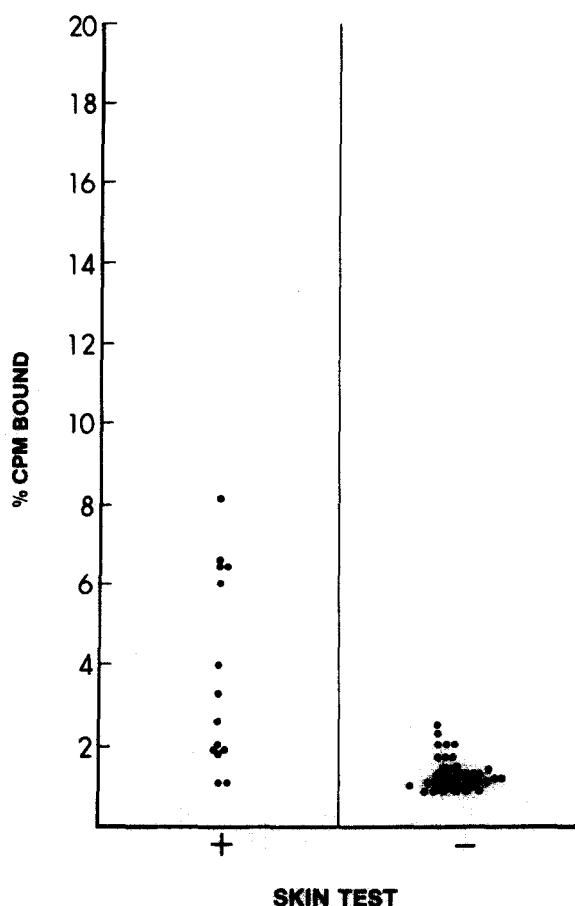


FIG. 1. Skin and RAST reactivity to spore extracts of *P. ostreatus*.

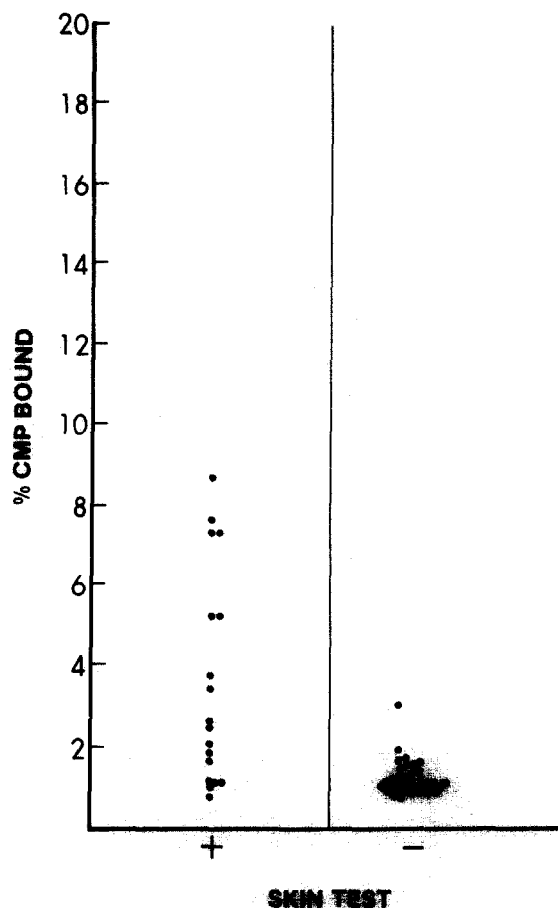


FIG. 2. Skin and RAST reactivity to spore extracts of *C. cyathiformis*.

positive skin prick tests obtained for each basidiospore extract and to test for associations among the individual spore extracts.

RESULTS

Skin prick testing with basidiospores

Prevalence of basidiospore-specific positive skin prick tests for each spore extract, in this selected population, is presented in Table I. Prevalence varied from a low of 28.6% for *P. tinctorius* to a high of 55.6% for *G. saccatum* (5/9) and 54.8 for *C. quadrifidus*. Mean wheal diameter of skin prick tests varied from 2 to 7 mm, with the largest reactions observed with *P. ostreatus*.

Statistical analysis for determination of association between spore extract skin test results

There was a strong association between skin test results to many of the different basidiospores (Table II). The strongest associations were observed between *P. cubensis* and *C. cyathiformis*. No associa-

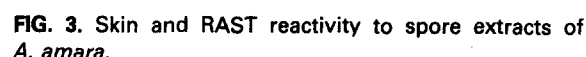
tions were obtained with *A. muscaria* and *P. tinctorius* extracts. *Scleroderma* sp. demonstrated an association with *Boletus* sp. only.

RAST with basidiospores

The percent binding of individual sera varied markedly depending on the basidiospore extract used. *P. cubensis* demonstrated the highest RAST values with maximal percent binding of 23.9%. Other basidiospore extracts also demonstrated significant percent binding (Table I). *Boletus* sp. demonstrated the lowest RAST values with maximum percent binding of only 3.7%. No positive results were obtained with sera from skin test negative atopic or nonatopic asymptomatic individuals.

Statistical analysis for determination of association between spore extract RAST results

With the exception of *Boletus* sp. and *P. tinctorius*, there was a strong statistical association between RAST with the different basidiospore extracts (Ta-



Comparison of RAST results with skin prick test

A scatter plot showing the relationship between SKIN TEST results and % CPM BOUND. The y-axis is labeled '% CPM BOUND' and ranges from 0 to 24 in increments of 2. The x-axis is labeled 'SKIN TEST' and has two categories: '+' and '-'. A vertical line separates the two categories. For the '+' category, there are 20 data points showing a wide distribution of values, with a notable outlier at 24. For the '-' category, there are 10 data points that are tightly clustered at low values, mostly between 1 and 2.5.

SKIN TEST	% CPM BOUND (approximate values)
+	1.0, 1.2, 2.2, 3.4, 4.1, 4.5, 4.6, 6.1, 6.2, 7.7, 11.0, 12.7, 19.0, 23.8
-	1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 2.4

FIG. 4. Skin and RAST reactivity to spore extracts of *P. cubensis*.

onstrated the greatest percentages of RAST positive responses; *P. cubensis* demonstrated up to 35.7% RAST positive, and both *C. quadrididus* and *G. saccatum* had up to 31.0% positive RAST results. RAST with other basidiospore extracts, such as *P. tinctorius* and *Boletus* sp., which demonstrated the lowest percent (<10%) of positive RAST results, did not correlate with skin test reactivity. These results demonstrate that IgE antibodies to basidiospore allergens are, in general, present in sera of skin test positive individuals.

DISCUSSION

These results extend our previous studies of basidiomycete-specific IgE antibodies in patients with

symptoms of respiratory allergy.⁵ The findings confirm earlier studies that basidiospore extracts are useful reagents for skin prick testing to detect basidiospore hypersensitivity.^{7, 8, 13, 14, 15} Our in vitro testing for specific IgE antibodies also demonstrated that basidiospore allergens can be coupled to CNBr-activated disks for use in the RAST assay. RAST results demonstrated that a high proportion of skin test positive individuals had basidiospore-specific serum IgE antibodies. We also demonstrated that, for most spore extracts tested, RAST and skin test results had a strong statistical association. There was a highly significant association between positive skin prick test and positive RASTs with *C. quadricaudatus*, *C. molybdites*, *P. ostreatus*, *P. cubensis*, *A. amara*, *G. lucidum*, *A. tabescens*, and *C. cyathiformis* extracts. The other basidiospores tested did not demonstrate a good correlation with skin test reactivity or demonstrated little or no RAST reactivity. Of particular note were RAST results with *P. tinctorius* that demonstrated little or no IgE antibody binding.

The reasons for disparity in skin reactivity and magnitude of RAST reactivity for some basidiospores are not known. It is certainly possible that some basidiospores are present in the environment in greater concentration or for longer time periods than other basidiospores and therefore have the potential to stimulate higher titers of specific IgE antibodies in susceptible individuals. Unfortunately, because of difficulties in identifying and quantifying atmospheric concentrations of specific basidiospores, currently, the best estimates can only be obtained by relating data on the number of basidiocarps growing in the wild and the number of spores collectible from them. Based on this crude measurement, it appears that some basidiocarps, such as *P. tinctorius* and *C. molybdites*, which probably release tremendous numbers of spores, do not induce significant specific IgE responses, as measured by skin test or RAST reactivity. This is in contrast to other basidiocarps, such as *A. amara*, which, in our experience, produce less fruiting bodies for a shorter time period (and, presumably, less spores), and yet are significant inducers of specific IgE antibodies. A possible explanation for these observations is that some basidiospores may be more potent allergens than others.

Another perplexing observation is the discrepancy between skin test and RAST results for several basidiospore extracts. The RAST reaction essentially binds protein antigens to CNBr-activated disks. It is possible that some basidiospore allergens have fewer free amino groups to bind to the disk or may be composed of nonproteinaceous materials; thus, other methods must be used to insolubilize these allergens for mea-

surement of specific IgE antibodies. It is also possible that extraction of allergen is not optimal for some of these basidiospores.

An important observation was the high prevalence of multiple skin test reactivity to different basidiospore preparations among individuals with histories of perennial rhinitis and/or asthma. This was true for all but three of the basidiospores tested (*A. muscaria*, *P. tinctorius*, and *Scleroderma* sp.), the latter two being puffballs. These results suggest the possibility of common allergenic epitopes. Preliminary RAST-inhibition testing of basidiospore extracts suggests that there may be shared antigenic structures among different basidiospore preparations¹⁵; however, further analysis of spore extracts will be necessary to determine if this is indeed the case.

Since the collection of basidiospores is a laborious, time-consuming procedure, the possibility that common allergenic epitopes exist has important economic, clinical, and diagnostic implications. Future studies could use a limited panel of more easily obtained basidiospores with common allergenic epitopes to determine IgE-mediated hypersensitivity rather than the use of extracts from a wide range of basidiospore species. Similarly, if immunotherapy proves feasible for treatment of basidiospore sensitivity, a limited panel of basidiospores with common allergenic epitopes could be used rather than attempting to desensitize patients with the large numbers of basidiospore species to which they are exposed.

Our results demonstrate that basidiospore extracts are suitable diagnostic reagents for both in vivo and in vitro testing of patients allergic to basidiomycetes. Since these studies were performed in selected individuals, they do not provide information regarding prevalence of reactivity to basidiospores in the general population; however, as we now have adequate quantities of extracts to test individuals, this can be determined. It should also now be possible to begin to apply laboratory immunologic methods, such as those described by Swanson et al.¹⁶ to measurement of atmospheric concentrations of specific basidiospores. Since it is usually extremely difficult to identify basidiospores morphologically, use of such immunologic assays to measure allergenic basidiospore concentrations would be an important development. In addition, since commercially available basidiospore extracts are being increasingly used for testing patients, our ability to identify elevated levels of specific IgE antibodies in serum and to develop RAST to detect these antibodies suggest that it will be possible to develop techniques that can be used for standardization of commercial basidiospore allergen preparations for clinical and diagnostic use.

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