

# Mushroom (*Basidiomycete*) allergy: Diagnosis established by skin test and nasal challenge

A. Helbling, MD, F. Gayer, W. J. Pichler, MD, and K. A. Brander, PhD *Bern, Switzerland*

**Background:** Fungal spores are universal components in the air and established as important causes of respiratory allergies. Whereas *fungi imperfecti* are accepted sources of allergic asthma and rhinitis, the significance of *basidiomycetes* as respiratory allergens is not established.

**Objectives:** The aims of the present study were to investigate the rate of sensitization in subjects referred to an allergy clinic to 3 *basidiomycetes* commonly found in Europe. We demonstrate the clinical relevance of *basidiomycete* sensitization by active anterior rhinomanometry and identified *basidiomycete* allergens by immunoblotting.

**Methods and Results:** Consecutive outpatient clinic attendees ( $n = 1207$ , >12 years of age) were given the skin prick test with a panel of common inhalant allergens and extracts of *Boletus*, *Coprinus*, and *Pleurotus*. To evaluate a cause-and-effect relation between *basidiomycete* allergens and respiratory allergies, 12 *Pleurotus pulmonalis* spore-sensitized subjects with respiratory symptoms and 6 control subjects were challenged by anterior rhinomanometry. SDS-PAGE immunoblots were used to identify *basidiomycete*-specific IgE antibodies in sera from subjects with positive skin prick test results. Of 1207 subjects tested, 48 (4%) reacted to at least 1 of the *basidiomycete* extracts. Whereas all of the 12 subjects with *Pleurotus pulmonalis*-positive skin test results showed a significant decrease in nasal air flow (mean 73%), none of the control subjects reacted. Immunoblots revealed several different IgE-binding proteins in *Pleurotus* and *Coprinus* extracts.

**Conclusions:** Our results demonstrate that sensitization to *basidiomycetes* in subjects with respiratory allergies is frequent. Furthermore, *Pleurotus pulmonalis* spore extracts can definitely induce respiratory allergy in sensitized subjects. Immunoblot assays disclosed different IgE-reactive bands indicating the existence of *basidiomycete* allergens. (*J Allergy Clin Immunol* 1998;102:853-8.)

**Key words:** Basidiomycetes, *Pleurotus pulmonalis*, *Coprinus comatus*, respiratory allergy, allergens, sensitization, diagnosis, nasal provocation test, immunoblot

Fungal spores are universal outdoor and indoor components and are generally recognized as possible sources of respiratory allergies.<sup>1-3</sup> Whereas *fungi imperfecti* such

as *Alternaria* spp or *Cladosporium* spp are well known causes of respiratory allergy,<sup>2-6</sup> the significance of *basidiomycetes* as respiratory allergens is not established. Basidiospores, however, are abundantly found in the outdoor air in many parts of the world,<sup>1-3</sup> and positive skin tests,<sup>7-11</sup> specific IgEs,<sup>12,13</sup> and bronchial reactivity<sup>14</sup> have been demonstrated in sensitive subjects. *Basidiomycetes* are the most morphologically complex of all the fungal classes and include mushrooms, puffballs, rusts, smuts, and bracket fungi. Recently, it has been shown that the sensitization to *basidiomycetes* in Europe and in the United States is comparable to that of the well-known *fungi imperfecti*.<sup>10</sup> Moreover, and in contrast to pollen, high basidiospore concentrations were significantly associated with worsening asthma.<sup>15</sup> In the current study we sought to examine the rate of sensitization to 3 *basidiomycetes* commonly found in Europe and to demonstrate the clinical relevance of *basidiomycete* skin test reactivity by active anterior rhinomanometry in an allergy clinic population. In addition, we identified allergens of 2 *basidiomycetes* by immunoblotting.

## METHODS

### Source material and preparation of extracts

*Coprinus comatus* mycelia were obtained from a German mushroom supplier (Pilzbrut Dieskau, Dieskau-Zwintschöna, Germany). Under recommended conditions, fruiting bodies were produced in collaboration with the Institute of Botany, Bern (S. Amsler). After collection, gills were separated, peeled, and frozen at  $-80^{\circ}\text{C}$ . Species of *Boletus edulis* were obtained by the Institute of Botany (Dr B. Senn-Irlet) from professional mushroom collectors. Caps were peeled, and the spore containing tissue was cut into cubes and frozen at  $-80^{\circ}\text{C}$ . Spores of *Pleurotus pulmonalis* were collected in a sterile growth chamber of a commercial *Pleurotus* grower (P. Lussi, Lupi Speisepilze, Oberdorf, Switzerland). Yields of spore collection and purity were determined by light microscopy. For extraction, 1 g of spore-containing tissue was ground in liquid  $\text{N}_2$  and prepared in 40 mL of 125 mmol/L ammonium bicarbonate buffer (pH 8.1) for 1 hour with stirring at  $4^{\circ}\text{C}$ . The homogenate was centrifuged (80,000g) for 30 minutes at  $4^{\circ}\text{C}$  and the supernatant lyophilized.

For skin testing, lyophilized material was suspended in a solution of 50% glycerol, 85 mmol/L NaCl, and 30 mmol/L sodium bicarbonate and sterile filtrated with Acrodisc PF 0.8/0.2  $\mu\text{m}$  (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).

### Study population

Consecutive outpatient clinic attendees ( $n = 1207$ , >12 years of age) were given the skin prick test with a panel of common inhalant allergens (house-dust mites, grass, birch and mugwort pollen, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*

From the Institute of Immunology and Allergology, University Hospital. Supported by the Swiss National Science Foundation 32.40402.94, Bern, Switzerland

Received for publication Dec 10, 1997; revised May 26, 1998; accepted for publication July 13, 1998.

Reprint requests: A. Helbling, MD, Institute of Immunology and Allergology, University Hospital, 3010 Bern, Switzerland.

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0091-6749/98 \$5.00 + 0 1/1/93064

**TABLE I.** Skin test reactivity to groups of common inhalant allergens in *basidiomycete* skin prick test–positive and skin prick test–negative atopic individuals

| Group of allergens | <i>Basidiomycete</i><br>SPT-positive<br>subjects<br>n = 48* (%) | <i>Basidiomycete</i><br>SPT-negative<br>atopic subjects<br>n = 412 (%) | Subjects with<br>positive SPT<br>(% of all<br>1207 tested) |
|--------------------|-----------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------|
| Pollen             | 39 (81)                                                         | 248 (60)                                                               | 287 (23.8)                                                 |
| House dust mites   | 25 (52)                                                         | 137 (33)                                                               | 162 (13.4)                                                 |
| Animal dander      | 27 (56)                                                         | 105 (25)                                                               | 132 (10.9)                                                 |
| Mold               | 22 (46)                                                         | 18 (4.4)                                                               | 40 (3.3)                                                   |

SPT, Skin prick test.

\*Three subjects reacted to *basidiomycete* extracts only.

and a mold-mix (*Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans*, and *Merulius lacrymans* [Allergopharma Hamburg, Germany]) as well as with in house extracts of *Boletus*, *Coprinus*, and *Pleurotus*. Skin prick tests were performed on the volar surface of the forearm according to the recommendations of the European Academy of Allergy and Clinical Immunology.<sup>16</sup> All skin reactions were recorded after 15 minutes and a mean wheal diameter of 3 mm or more than the 0.9% NaCl control was considered as positive. Serum samples were collected from subjects with positive skin prick tests to *basidiomycete* extracts and all sera were stored at –20° C until used.

### Anterior rhinomanometry

Nasal airway resistance was measured by active anterior rhinomanometry (Rhinotest MP 441, Germany). A tube connected to a pressure sensor was placed into one nostril and fixed by tape. Air flow through the other nostril was recorded by a pneumotachograph (PM 8043/01 Philips, Japan) in 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.<sup>17</sup> Resistance has been calculated by analysis of the pressure/flow curve.<sup>17</sup> Following the baseline measurement with physiologic saline, 40 to 50 µL of the test solution was sprayed into the nostril in 15-minute intervals beginning at the lowest concentration. For nasal challenge, 3 different concentrations of *Pleurotus pulmonalis* extract (0.1, 1.0, and 10.0 mg protein/mL PBS) were used. The subjects were instructed to breathe quietly through the nose. A 40% reduction in nasal air flow 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 all patients were informed about the design and the aim of the study and consented to participate.

### Pulmonary function test

Vital capacity, FEV<sub>1</sub>, and FEV<sub>25-75</sub> were measured with a ZAN 100 (Siemens, Germany) before and 30 minutes after the last nasal challenge dosage.

### SDS-PAGE and immunoblotting

SDS-PAGE has been performed as described.<sup>13,18</sup> Resolved proteins, 20 µg per lane, were transferred electrophoretically onto 0.2 µm pore size nitrocellulose (Schleicher & Schuell) by tank blotting for 3 hours at 100 µV constant current in Towbin transfer buffer with 20% methanol and 0.03% SDS. After transfer, unreacted sites were blocked, initially with 0.15% casein in PBS at 42° C for 90 minutes, followed by blocking buffer B (PBS containing 0.2% BSA,

**TABLE II.** Frequency of skin test positivity to *basidiomycete* and mold extracts in 48 *basidiomycete* skin test–positive individuals

| Fungal species               | Positive skin reaction<br>n (%) |
|------------------------------|---------------------------------|
| <i>Pleurotus pulmonalis</i>  | 38 (79)                         |
| <i>Coprinus comatus</i>      | 28 (58)                         |
| <i>Boletus edulis</i>        | 19 (40)                         |
| <i>Alternaria alternata</i>  | 14 (29)                         |
| <i>Cladosporium herbarum</i> | 12 (25)                         |
| Indoor mold mix*             | 10 (21)                         |

\*Extract containing *Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans*, and *Merulius lacrymans* [Allergopharma Hamburg, Germany].

1.0% skim milk powder, 0.02% sodium acid, and 0.01% antifoam A) at room temperature for 90 minutes. The membrane was incubated overnight with 1:19 diluted sera in blocking buffer B containing 0.3% Tween 20 at 4° C. Membranes were washed 3 times with PBS containing 0.1% Tween 20. Bound IgE was detected with the use of polyclonal goat anti-human IgE antibody labeled with horseradish peroxidase (Nordic) diluted 1:2500 in the same buffer. Detection was enhanced by chemiluminescence (Amersham, UK) and documented on x-ray films exposed for approximately 1 minute.

### Statistics

Chi-square analysis was used to evaluate sensitization to mold in individuals with positive and negative reactions to *basidiomycete* in the skin test. A value of  $P = .05$  was considered to be significant.

## RESULTS

Four hundred fifty-seven (37.8%) of 1207 subjects were atopic on the basis of history and 2 or more positive skin prick tests to common aeroallergens, and 289 (23.9%) had a history of asthma. Skin test reactivity to groups of common inhalant allergens in *basidiomycete* skin test–positive and skin test–negative atopic individuals are presented in Table I. *Basidiomycete* skin test–positive subjects were statistically significant ( $P < .001$ ) more sensitized to molds than *basidiomycete* skin test–negative, atopic persons. Forty-eight (4.0%) of 1207 tested subjects reacted to at least 1 of the *basidiomycete* extracts. The mean age of the 48 *basidiomycete* skin test–positive population was 28 years (range 13 to 67 years), and 44% were female. Forty-seven (98%) of 48 subjects complained of respiratory symptoms, 28 (58%) of asthma, and 19 (39%) of rhinitis; signs of atopic dermatitis were detected in 11 (23%) patients. Of the *basidiomycete* skin test–positive individuals, only 3 (6%) did not react to common inhalant allergens. Two of the 3 individuals reacted to *basidiomycete* extracts solely, including the 1 subject without respiratory symptoms. They developed adverse reactions after meals containing edible *boletus*. The third subject had a respiratory allergy to *Ficus benjamina* (weeping fig).

Frequency of skin test reactivity to *basidiomycete* and mold extracts in the 48 *basidiomycete* skin test–positive individuals is given in Table II.

Twenty-three (48%) of the 48 subjects sensitized to *basidiomycetes* reacted to only 1 species; 13 (27%) of 48

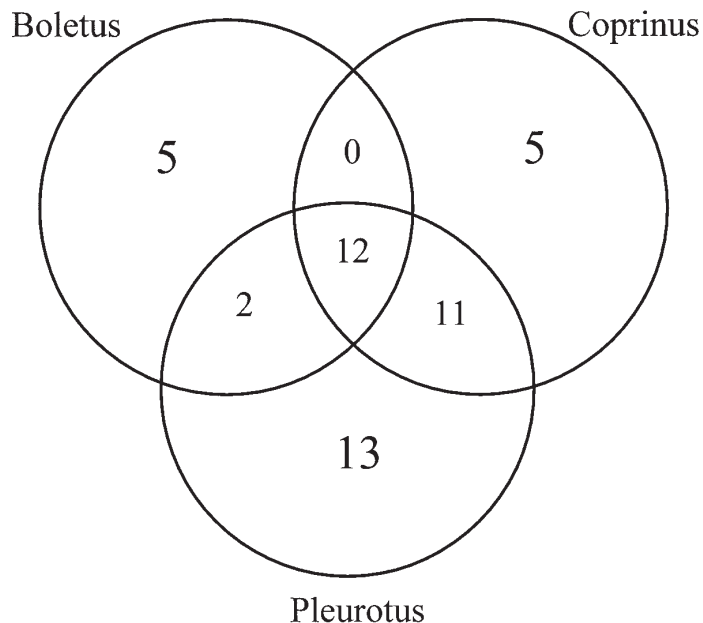


**FIG 1.** Overlap of skin prick test reactivity to 3 *basidiomycete* extracts in 48 *basidiomycete*-sensitized individuals. Numerals indicate number of positive tests.

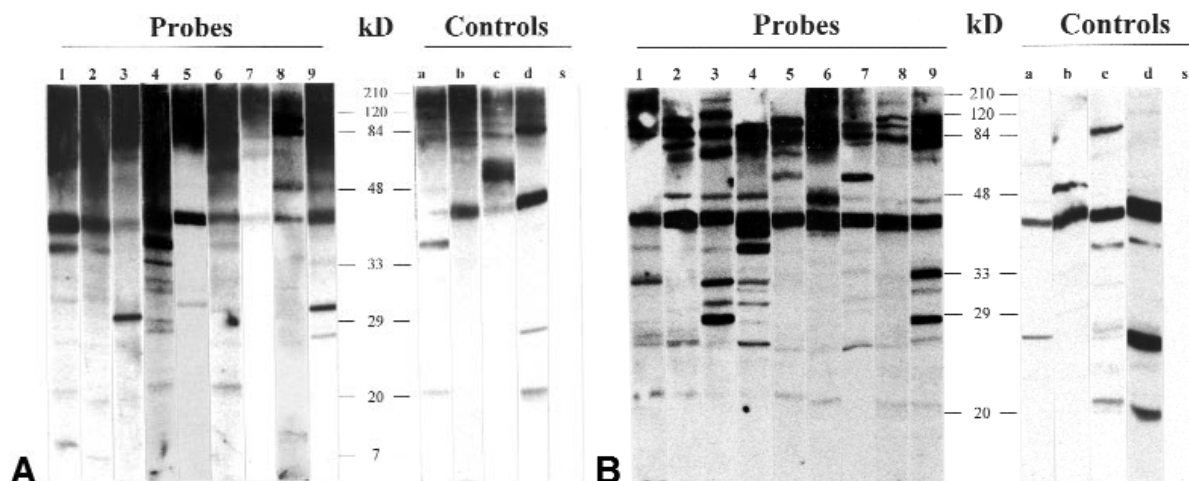


**FIG 2.** IgE reactivity to crude proteins (20 µg/lane) of *Pleurotus pulmonalis* (A) and *Coprinus comatus* (B) separated by SDS-PAGE. Sera applied were from 9 skin prick test-positive (lanes 1 through 9) and 4 skin prick test-negative subjects. Nonatopic control sera (lanes a through d) and control without serum (lane s). Exposure time was approximately 45 seconds.

reacted to 2 and 12 (25%) of 48 to all 3 species (Fig 1). The most frequent *basidiomycete*-positive skin test was to *Pleurotus* (79%), followed by *Coprinus* (58%) and *Boletus* (40%). With regard to all 457 atopic subjects, *Pleurotus* elicited a positive skin reaction in 8.3%. *Copri-*

*nus* ranked second (6.2%), and *Boletus* demonstrated the least reactivity (5.4%).

Twelve *Pleurotus pulmonalis* skin test-positive subjects with respiratory symptoms (asthma [9], rhinitis [3]) and 6 *Pleurotus pulmonalis* skin test-negative control

**TABLE III.** Characteristics of study subjects and results of nasal challenge tests with *Pleurotus pulmonalis* spore extracts in skin test–positive subjects and skin test–negative control subjects

|                  | Age<br>(y) | Sex | Clinical<br>symptoms | Concentration<br>eliciting positive<br>response (mg/mL) | Decrease of<br>nasal flow<br>(%) | Symptoms<br>at positive<br>challenge dose |
|------------------|------------|-----|----------------------|---------------------------------------------------------|----------------------------------|-------------------------------------------|
| Patients         |            |     |                      |                                                         |                                  |                                           |
| 1                | 21         | M   | A                    | 0.1                                                     | 100                              | P, R                                      |
| 2                | 25         | F   | A                    | 0.1                                                     | 70                               | B, R                                      |
| 3                | 26         | F   | A, R, D              | 0.1                                                     | 60                               | B, R                                      |
| 4                | 32         | F   | A, R, D              | 0.1                                                     | 75                               | B, R                                      |
| 5                | 50         | M   | R, D                 | 0.1                                                     | 44                               | R                                         |
| 6                | 20         | M   | A, R                 | 1                                                       | 100                              | B, P, R                                   |
| 7                | 23         | M   | A, R                 | 1                                                       | 86                               | B, R                                      |
| 8                | 24         | F   | R, D                 | 1                                                       | 66                               | B, P, R                                   |
| 9                | 34         | F   | A                    | 1                                                       | 55                               | P, R                                      |
| 10               | 33         | M   | R                    | 1                                                       | 77                               | B, R                                      |
| 11               | 38         | F   | A                    | 1                                                       | 75                               | B, R, P, A                                |
| 12               | 34         | M   | A, R                 | 10                                                      | 77                               | B, R                                      |
| Control subjects |            |     |                      |                                                         |                                  |                                           |
| 13               | 30         | F   | A, R                 | >10                                                     | 5                                | —                                         |
| 14               | 33         | M   | R                    | >10                                                     | 23                               | —                                         |
| 15               | 35         | M   | —                    | >10                                                     | 0                                | —                                         |
| 16               | 35         | F   | —                    | >10                                                     | 14                               | S                                         |
| 17               | 37         | M   | —                    | >10                                                     | 31                               | —                                         |
| 18               | 39         | F   | —                    | >10                                                     | 6                                | S                                         |

A, Asthma; B, nasal blockage; D, atopic dermatitis; P, pruritus; R, rhinitis; S, burning sensation.

subjects were challenged by active anterior rhinomanometry (Table III). Of the control subjects neither the 2 atopic, pollen-allergic, nor the 4 nonatopic subjects reacted objectively with any of the *Pleurotus pulmonalis* concentrations tested. In contrast, all of the *Pleurotus pulmonalis* skin test–positive subjects had a significant decrease in nasal air flow (mean reduction of 74%, range 44% to 100%) and subjective symptoms. Five (42%) of the 12 *basidiomycete*-sensitive subjects reacted at the lowest concentration of 0.1 mg protein per mL, 6 (50%) of 12 at 1.0 mg/mL, and only 1 subject showed a positive response at 10 mg/mL, the highest dose tested. Spirometry performed before and after nasal challenges remained within normal limits in 18 (95%) of 19 subjects. In 1 patient (No. 11), while experiencing breathing difficulties, a significant fall of the FEV<sub>1</sub> from 2.6 L to 1.8 L (30%) was documented.

Immunoblots of *Pleurotus* and *Coprinus* revealed a distinct IgE-binding pattern (Fig 2, A and B). In *Pleurotus* extract, IgE-binding proteins were evident at 10, 29, 30 and 33 kd, as well as some IgE that bound to larger molecular structures (Fig 2, A). With *Coprinus*, bands from 22 to > 100 kd were detected, the most intense at 29, 33, and 55 kd (Fig 2, B). Some reactive bands were also detectable with control sera of nonatopic individuals. However, the binding reactivity and the number of reactive bands were less and different than with sera of skin test–positive subjects.

## DISCUSSION

For decades, airborne spores have been recognized as possible causes of respiratory allergies. Although relative

amounts of basidiospores are estimated to range between 5% and 30% of the total spore count<sup>2,3,7</sup> and despite a recent study demonstrating *basidiomycetes* as important sensitizers in Western Europe,<sup>10</sup> spores of *basidiomycetes* are not yet established causes for respiratory allergies. The prevalence of *basidiomycete* skin test reactivity observed in 1207 consecutive subjects tested in our outpatient allergy clinic was 4.0%. Compared with other studies demonstrating a *basidiomycete* reactivity ranging from 10% to 91%,<sup>7-11</sup> our data seem relatively low. However, we tested consecutively all clinic attendees and did not select subjects with respiratory allergies. Moreover, with regard to individuals with skin prick test–positive results to common aeroallergens, the sensitization rate to *basidiomycetes* rises to 9.8%, which is within the reported range.

Because 96% of the 1207 subjects tested did not reveal any skin test reactivity to these extracts, a nonspecific reaction seems very unlikely. Overall, the frequency of positive skin tests to *basidiomycetes* in our clinic is less than to pollen, house-dust mites, and cat dander but higher than to dog dander, molds, or latex (detailed data not shown). These results suggest that *basidiomycetes*, particularly their spores, are potential sources of aeroallergens.

The majority of *basidiomycete*-sensitive subjects is atopic because of their history, clinical symptoms, and skin test reactivity to common inhalant allergens. Only 2 (4%) subjects had a skin test positive exclusively to *basidiomycete* extracts. One patient tolerating other edible fungi had recurrent anaphylactic reactions with facial angioedema, generalized urticaria, and diarrhea after ingestion of *boletus*, and the second patient complained about intestinal

symptoms, malaise, and dizziness after a meal containing various mushrooms. Although sensitization to *basidiomycetes* are usually found in subjects with other inhalant allergies, isolated sensitization to *Boletus edulis* have been described.<sup>19</sup> This occurrence suggest that *basidiomycetes* contain clinically relevant and specific allergens.

Sensitization to molds has been detected in less than half of the *basidiomycete* skin test–positive subjects. This observation further supports the finding that an allergenic relation between *basidiomycetes* and *fungi imperfecti* is minimal,<sup>2,20</sup> which emphasizes the need of testing with representative species from both classes of fungi. Cross-reactivity among *basidiomycetes* is thought to be extensive,<sup>21,22</sup> although some taxonomically distinct species, such as *Ganoderma* spp, appear to share fewer allergens.<sup>23</sup> In our study group, *Pleurotus pulmonalis* elicited the most and *Boletus edulis* the least positive skin test reaction. Because most of the *basidiomycete*-sensitive subjects responded to the *Pleurotus pulmonalis* extract (79%) and unique sensitization to *Coprinus* or *Boletus* (10.5% each) was much less frequent, it may be assumed that *Pleurotus pulmonalis* spores contain most of the allergenic properties common to the others. However, a proportion of 21% of *basidiomycete* skin test–positive subjects would not have been identified by using *Pleurotus* extract only. On the basis of our data with 3 species and the occurrence of low cross-reactivity between *basidiomycetes* and molds as well as *basidiomycetes* among themselves,<sup>2,20</sup> sensitization to *basidiomycetes* must be more prevalent than expected.

Molds, especially *Alternaria* and *Cladosporium* spp, are generally considered as important incitants of asthma.<sup>4,6</sup> In a few studies, patients' symptoms have been correlated with spore counts of both species.<sup>4,5</sup> In a recent investigation, however, only high *basidiospore* but not pollen or mold counts were correlated significantly with an increased risk of high medication use and awakening at night in asthmatic subjects.<sup>15</sup> The clinical course of our patients could not be correlated to seasonal spore counts because aerobiologic data are not yet available in our area. Therefore, we choose nasal challenge tests to demonstrate the clinical relevance of one of the *basidiomycete* species. By active anterior rhinomanometry, all tested *Pleurotus pulmonalis*–sensitized patients with respiratory allergies reacted to the spore extract of this mushroom with a significant reduction of nasal air flow and subjective symptoms compatible to an inhalant allergy. Because none of the control subjects, including 2 subjects allergic to pollen, did respond in this test, the results clearly indicate that basidiospores can induce an early allergic nasal response in susceptible subjects.

On the basis of our findings, skin prick and nasal provocation tests with basidiospore extracts are reliable methods to corroborate the role of *basidiomycetes* in respiratory allergy. Considering that most individuals sensitized to *basidiomycetes* have other multiple sensitivities, and the contribution of this class of aeroallergens cannot be determined by history, provocation tests are necessary to demonstrate the clinical relevance. In our hands, anterior

rhinomanometry has been established as a useful method.

In contrast to the clinical findings by the immunoblot technique, generally judged as a useful tool in detecting allergens,<sup>13,23,24</sup> *basidiomycete*-sensitized subjects could not be accurately identified because nonatopic individuals also showed some IgE-binding components to *basidiomycetes*. Nonspecific binding of IgE could not be suppressed completely despite the use of various anti-IgE antibodies in combination with methodic variations of this technique.<sup>24</sup> On the basis of our experience, we found that anti-IgE antibodies are cross-reacting or interacting with *basidiomycete*-specific IgG antibodies. For the demonstration of IgE-binding *basidiomycete* proteins, we selected a very sensitive secondary antibody displaying moderate background. The presumption that IgE antibodies can be directed to cross-reactive carbohydrate determinants as described in vegetable food, invertebrates, insect venom, and pollen<sup>25–27</sup> seems less likely. It has been postulated that this cross-reactive IgE has poor biologic activity, thus causing positive *in vitro* tests without clinical significance.<sup>27</sup> Nevertheless, the pattern of reactive bands demonstrates the existence of specific allergens that are only recognized by serum IgE from *basidiomycete*-sensitized individuals. However, to confirm the existence of *basidiomycete*-specific allergens, the production of recombinant allergens is warranted (KA Brander et al, manuscript in preparation).

In conclusion, sensitization to 3 selected and common *basidiomycetes* is frequently in our area in subjects with respiratory allergies. Diagnosis of *basidiomycete* allergy can be postulated by positive skin prick tests and confirmed by nasal provocation challenges with in-house extracts. Our data corroborate other studies attesting *basidiomycetes* being potential aeroallergens. Obviously, a sensitization to *basidiomycetes* is accompanied by a higher frequency of sensitizations to other aeroallergens. Results of immunoblot analysis support the hypothesis of *basidiomycetes* producing important allergens. However, further investigations are necessary.

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