

Indoor airborne fungi as risk factors in IgE-mediated respiratory allergy

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Abstract

The purpose of this epidemiological study was to assess respiratory allergy in relation to the presence of indoor airborne fungi. The relationship between IgE-mediated respiratory allergy (skin test positivity) and the presence of fungi (CFU/m³) in the indoor environments of 104 subjects was assessed in a cross-sectional study by controlling for extraneous variables (age, gender, predisposition, asthma, rhinitis, skin positivity to ragweed and mite, and smoking). The qualitative and quantitative measurements of airborne seasonal fungi (*Alternaria* spp. and *Cladosporium* spp.) and non-seasonal airborne fungi (*Penicillium* spp. and *Aspergillus* spp.) were taken in the subjects' indoor environments twice in a 2-year period by volumetric methods (Burkard Personal Sampler). There was a significant association between skin test positivity to seasonal fungi and to ragweed (Adj. OR = 3.42, CI = 1.76–6.66). There was no association between skin test positivity to seasonal fungi and asthma (Adj. OR = 0.52, CI = 0.28–0.98), but a significant association was found between skin test positivity to seasonal fungi and rhinitis (Adj. OR = 5, CI = 2.03–12.32). In a logistic regression analysis (maximum likelihood estimates — model A), no statistical association was found indoors between skin prick test positivity to seasonal fungi (*Alternaria* and/or *Cladosporium*) and airborne *Alternaria* and/or *Cladosporium* concentrations (Adj. OR = 1.18, CI = 0.66–2.07). There was a significant association between skin prick test positivity to seasonal fungi and to non-seasonal fungi (Adj. OR = 12.81, CI = 1.67–98.34). There was no association between asthma and airborne *Penicillium* concentrations (Adj. OR = 1.86, CI = 0.47–7.33) nor between rhinitis and airborne *Penicillium* concentrations (Adj. OR = 0.18, CI = 0.03–1.19). In another logistic regression analysis (maximum likelihood estimates — model B) using non-seasonal fungi (*Aspergillus* and *Penicillium*), no statistical association was found indoors between skin prick test positivity to non-seasonal fungi and airborne *Penicillium* concentrations (Adj. OR = 0.33, CI = 0.07–1.69). These findings suggest an association between rhinitis and seasonal fungi. In the rhinitis stratum, subjects who had skin test positivity to ragweed had a higher risk of being sensitive to seasonal airborne fungal allergens. Subjects with non-seasonal fungal allergy had a high relative risk if they were also allergic to seasonal fungi. There was no association between asthma and airborne fungi, as the epidemiological study (cross-sectional design), by definition, does not allow an etiological evaluation of chronic disease. This would require a longitudinal study, i.e. the measurement of repeated exposure as an independent variable (allergen) and repeated measurement as a function of the disease as outcome in humans as a dependent variable.

Keywords: Colony forming units (CFU); IgE-mediated (skin tests positivity); Asthma; Rhinitis; OR (odds ratio: relative risk)

1. Introduction

Many investigators have proven that the inhalation of fungi induces the formation of IgE antibodies demonstrable both in vivo and in vitro, for example, by the

bronchoprovocation of fungal-induced asthma, clinical trials in relation to individual patterns of immediate skin reactivity to fungal extracts and fungal allergen characterization. Despite all the above, the estimation of respiratory allergy in relation to fungal type is the least understood facet of allergen-induced respiratory allergy.

Unlike for pollen and mite, the question of immune

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response and antigenic determinant still remains unresolved for fungi. Long-standing clinical observations indicate that some respiratory allergies are worse in the autumn. However, Bousquet (1990), Bruce and Norman (1977) and Ohman (1989) found that ragweed and the background effect of bronchitis contribute to the magnitude of autumn respiratory allergy in addition to seasonal fungi.

Since the outcome of IgE-mediated respiratory allergy is multifactorial, patients are seldom allergic to a single allergen. The determination of the magnitude of seasonal and non-seasonal fungal respiratory allergy can be assessed through a multivariate analysis in order to control for confounding variables (Miettenin, 1974; Kleinbaum et al., 1982; Hosmer and Lemeshow, 1989).

2. Materials and methods

In this study, the relationship between IgE-mediated respiratory allergy and the seasonal and non-seasonal airborne fungal colony forming units (CFU) presence was assessed by controlling for extraneous variables (age, sex, predisposition, skin test positivity to ragweed, mite, rhinitis, asthma and smoking).

In 1987, 122 mostly atopic human subjects were selected in a cross-sectional study by allergists who were affiliated with the allergy clinics of Hôpital Sainte-Justine, Hôpital Notre-Dame, Royal Victoria Hospital in Montréal, Hôpital Pierre Boucher in Longueuil, Clinique Concorde in Laval, and three other allergy clinics located in the east of Montréal, Westmount and Beloeil.

All the subjects were evaluated by allergists according to the following criteria: case history, signs, symptoms, pattern of symptoms, presence or absence of wheeze, shortness of breath, coughing, recurrent bronchitis, family history, profile of typical attacks, onset of the disease, seasonal or perennial respiratory allergy, precipitating or aggravating factors, as well as for the presence or absence of skin sensitivity to fungi along with skin sensitivity to pollen, mite and animal dander.

Asthma subjects met the American Thoracic Society's definition of reversible airway obstruction. All the cases presented mild asthma, which was defined using many factors, including episodes of wheezing, response to and frequency of the administration of pharmacotherapy (beta agonist, corticosteroid, and/or Cromolyn and school or work attendance).

Predisposition to respiratory allergy was defined as allergists having asked whether the subjects's first degree relatives (parents and siblings) had ever had atopic disease (atopic dermatitis, asthma, allergic rhinitis, IgE-dependent food allergy).

Respiratory allergy to mite and ragweed was defined by means of skin prick test positivity to these allergens

and by allergists evaluating the symptoms in relation to seasonal and perennial exposure. None of the subjects were smokers. Very few cases had skin prick test positivity to grasses, trees or cat and dog dander. A very small number of the subjects presented rhinitis and asthma simultaneously. Given the low numbers, these categories could not be controlled for analysis.

All the patients had Miles Canada allergy extracts for skin prick testing with the same lot number for regional pollens (1:20) (trees, grasses, weed, and ragweed), fungi (1:10) (*Alternaria alternata* (*tenuis*), *Cladosporium cladosporioides*, *Aspergillus fumigatus*, *Cephalosporium acremonium*, *Helminthosporium maydis*, *Penicillium bifforme*), other inhalant allergens (mite (1:50), house dust (1:10), as well as cat and dog dander (1:10)). The skin prick test was performed on the volar aspect of the forearm. The skin reactions of all the subjects were measured for diameters of local redness and swelling for all the above allergens. A positive reaction was defined for fungal allergens as a wheal diameter > 3 mm over the size of the control. All the cases of fungal respiratory allergy demonstrated a skin prick test positivity to at least one fungus.

In the Montréal area and proximity, most seasonal fungi appear during the summer, with a peak in late summer along with the ragweed season, and they disappear with the first frost. Non-seasonal fungi can be present outdoors and indoors throughout the year.

The qualitative and quantitative seasonal and non-seasonal airborne fungal measurements were taken in 122 subjects' homes by volumetric methods (Burkard Volumetric Portable Air Sampler) by trained aerobiologists who did not know the subjects' histories. The authorization for aeroallergens measurements in the subjects' homes was obtained through formal and/or oral consent. The measurements were taken once between May and November 1987, in the peak airborne fungal season and once again between December 1987 and May 1988, in the following low fungal season.

The Burkard Volumetric Portable Air Sampler pulls air either through a rectangular orifice (1 × 14 mm) carefully designed to collect airborne particle matter directly onto the center of 75 × 25 mm glass slides coated with glycerine-gelatine for total spore collection or through an 8-cm diameter (Ø) plate with 35 ml of Dextrose Agar onto 9-cm Ø Petri dishes containing Sabouraud medium for colony forming unit collection. The impaction orifice of the Burkard Portable Sampler is mounted in the vertical plane and the instrument operates at a nominal air throughput of 10 l/min. It allows even quite small fungal spores like *Aspergillus* and *Penicillium* to be pulled into an orifice designed for impaction. The sampling time was 45 min (i.e. a total sample of 450 l of air). Total spores and colony forming units sampling were done simultaneously about 30 cm apart. The samplers were located 60–90 cm above the

floor, generally on a bedside or living room table. The subject's bedroom and the other room in the house with the highest level of humidity (which could have been the source of indoor fungi) were measured once in the peak fungal season (1987) and once in the low fungal season (1987/1988). The same rooms were sampled in both years. Temperature and relative humidity were also measured. Other housing characteristics in relation to fungal sources were evaluated by means of a questionnaire designed by the Laboratoire d'aérobiologie, Université de Montréal. At each site, outdoor fungal allergens from outside the subject's home were also measured as a control by using the same sampler, located on top of a car parked in front of the house. Particulate and culture were both assessed in the laboratory. Spore identification was carried out through microscopic examination ($\times 400$ or $\times 1000$) directly from the glass slide (covered with a cover slip together with gelatin and basic red fuschin) using the reference collection of the Palynology Laboratory, University of Montréal and Botton et al. (1990), but mainly Smith (1990) and Basset et al. (1978). Petri dish samples were incubated at 22–23°C for 5–7 days under controlled humidity and sub-cultured on a Malt agar medium. To accelerate sporulation of the fungi, they were exposed to ultra-violet light according to a 12-h cycle. Colonies that did not sporulate after these procedures were reported as *Mycelia sterila*. A small ($< 1 \text{ mm}^2$) conidial sample of sporulating pure colonies was observed under the microscope ($\times 400$ or $\times 1000$). They were put on a dish with a drop of lactophenol between a glass slide and a $22 \times 22 \text{ mm}$ cover slip. Identification was done using Botton et al. (1990) and Smith (1990). Colony forming units or spores per cubic meter of air were calculated using a simple rule of three, combining flux and sampling time. Only general or higher taxonomic levels are reported here. The indoor fungal allergens that were identified in the subjects' indoor environment were: *Acremonium*, *Agrocybe*, *Alternaria*, *Arthrospores*, *Ascospores*, *Aspergillus*, *Aureobasidium*, *Basidiospores*, *Botrytis*, *Candida*, *Chaetomium*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Cylindrocarpon*, *Drechslera*, *Epicoccum*, *Fusarium*, *Fusicladium*, *Ganoderma*, *Helicospores*, *Humicola*, *Lepiosphaeria*, *Mildew*, *Mucor*, *Myxomycetes*, *Nigrospora*, *Penicillium*, *Phoma*, *Pithomyces*, *Scopulariopsis*, *Smut*, *Stachybotrys*, *Stemphylium*, *Trichoderma* and Yeast.

Since the unit of analysis is the human subject with skin test positivity to fungal allergens, only fungal allergens that were skin tested on the subjects' forearm at the baseline and also measured in the subjects' indoor environment were considered for epidemiological analysis, i.e. *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*. This was the only way of measuring exposure between the dependent and independent variables that respected epidemiological methodology.

2.1. Exposure assessment

Since fungi can appear to be everywhere, complete exposure assessment is impossible. However, indoor exposure, especially in the bedroom, is important, because it is where most people spend a lot of their time and where the indoor aeroallergen exposure is constant for at least 6 or 7 h every day. For this reason, in the planning of the study, we included the subjects who had skin test positivity to at least one fungal allergen and lived in the same home for a minimum of five consecutive years. For the dependent variable (skin test sensitivity to at least one fungal allergen), we had one stratum of skin test positivity to fungi $> 3 \text{ mm}$.

However, we planned to stratify fungal exposure as the independent variable, in order to assess for different levels of exposure, then assess the overall effect in different logistic models. Despite the fact that exposure is a continuous process, we had to dichotomize fungal exposure in terms of presence or absence. It is well known that subjects develop antibodies to a specific allergen through repeated exposure. However, depending on the nature of the exposure, it is difficult or sometimes impossible to measure and assess it as continuous exposure, with valid recognized epidemiological methodology, even if it is known that exact exposure assessment has to be continuous.

It is well documented that airborne fungal concentrations do not have a normal distribution. Fungi vary logarithmically, quantitatively and qualitatively in the same environment over time. Many natural factors can affect fungal concentration on a daily basis. For example, outdoor seasonal fungi can be relatively high in the morning. They decrease in the afternoon and can vary with rainy or sunny days. Furthermore, different patterns of variation exist for different fungi, e.g. Basidiospores are high in the early morning. For indoor fungi, the movements of human subjects and related factors, such as housekeeping activities, can also affect fungal dispersion, although the follow up of aerobiological pathways do not fall within the objectives of this paper.

We had many cases of skin test positivity without indoor exposure. If at the time of the aerobiological measurement, no fungal allergens were recovered, by the nature of the study design, the above subjects were categorized as non-exposed, given the aforementioned daily variation. In order to have an average indoor exposure for each season, the aerobiological measurement should have been a repeated measurement of the aeroallergen.

We stratified all the cases in relation to other covariates. Then we assessed these categories sequentially in relation to fungal exposure. Many categories of skin test reactivity in relation to the exposure to different fungal species divided the data too finely, resulting in imprecision in category specific estimates of exposure effect. In addition, subjects with skin test

positivity to other allergens, i.e. mites and ragweed, gave us some impractical subcategories, which dispersed the data, resulting in inefficient and imprecise analyses. In order to maintain precision while controlling important covariable of the baseline, we did a multivariate analysis to assess the overall effect of skin test positivity in relation to seasonal and non-seasonal indoor fungal exposure.

2.2. Statistical analyses

First a univariate analysis was done to evaluate the behavior of one variable at a time and to determine its distribution and frequency of distribution by SAS proc univariate. None of the fungal variables had a normal distribution. To keep it continuous we would have had to do a log transformation. Given the distribution, and the small number of cases, i.e. skin test positivity in relation to presence of the same fungal species in the subjects' indoor environment, we built two logistic regression models.

The variable of airborne fungi colony forming units per cubic meter (CFU/m³) was dichotomized according to the presence or absence of fungi in the subjects' homes. The results of the skin tests to the fungi were categorized as having skin test positivity or negativity to at least one fungal allergen. All cases presented skin test positivity to more than one fungal allergen. Asthma and rhinitis were also dichotomized according to presence or absence. Subjects were considered predisposed or non-predisposed, skin test positive or negative to mite, and skin test positive or negative to ragweed. Age was kept a continuous variable, given its normal distribution. Since all the subjects were non-smokers, this variable was omitted from further analysis. Bivariate analyses were then performed between the dependent and independent variables and between the main determinant and the potential confounder in order to decide which variable would be included in the models. In order to assess the relationship between fungal exposure and fungal respiratory allergy, while controlling for other

variables, two logistic regressions were performed. We used two models: A — skin test positivity to seasonal fungal allergens (*Alternaria*, *Cladosporium*) in relation to airborne colony forming units/m³ of *Alternaria* and/or *Cladosporium*, and B — skin test positivity to *Aspergillus* and/or *Penicillium* in relation to airborne *Penicillium* colony forming units/m³. It is to be noted that indoor airborne *Aspergillus* was not detected (low fungal season) in 1988. The potential confounders were included in the initial models. Potential statistical interaction (effect modification) among *Cladosporium* and/or *Alternaria* and other risk indicators were tested in the models. After all the interactions were tested, we kept those that were statistically significant in models A and B. To be considered significant in the model, α had to be smaller than 0.05. In order to decide whether or not to remove a variable which was not significant in a model, we compared β with and without the variable of interest. If β changed notably, the variable was kept in the model. If it did not change but the independent variable was important for validity, because of the literature and previous studies, we kept it in the model (e.g. predisposition). If β did not change and the variable was not felt to be necessary, it was discarded from the model because of missing variables. Only 104 of the original 122 subjects were considered for epidemiological analysis. In addition, in order to have a complete taxonomical level in the dependent/independent variable, only CFU/m³ data were used for model building.

3. Results

Table 1 shows selected characteristics of the study subjects. The mean age of the 104 subjects was 17.8 years, S.D. 15.7 years, and there were 45.5% females and 54.5% males.

The subjects' home environments were measured once in 1987 in the high fungal season (summer and autumn) and again in 1988 in the low fungal season (winter and spring). In 1987, *Cladosporium* had the highest propor-

Table 1
Subject characteristics. Results of skin tests to seasonal and non-seasonal fungi

Variable	Total (%) (n = 104)	Seasonal fungi		Non-seasonal fungi	
		Skin test + (%) (n = 47)	Skin test - (%) (n = 57)	Skin test + (%) (n = 64)	Skin test - (%) (n = 40)
Predisposition	47.1	38.8	61.2	59.2	40.8
Sex (M)	54.5	40	60	56.4	43.6
Sex (F)	45.5	50	50	67.4	32.6
Asthma	59.6	43.5	56.5	59.7	40.3
Rhinitis	47.8	52.4	47.6	64.4	35.4
Mite skin test +	20.2	61.9	38.1	71.4	28.6
Ragweed skin test +	38.5	60	40	77.5	22.5
Smoker	0	0	0	0	0

tion of indoor fungi and *Aspergillus* the lowest. The mean indoor airborne fungal concentrations (CFU/m³) for the 1987 high season were as follows: *Alternaria*: May 99.5, June 250.3, July 164.62, August 4.14, September 4.5, October 71.25; *Cladosporium*: May 3447, June 2112.1, July 439.75, August 65.6, September 47, October 376.75; *Aspergillus*: May 66.5, June 13.16, July 803.75, August 13.5, September 0, October 2679.75; and *Penicillium*: May 5, June 283.8, July 566, August 8.2, September 9.5, October 691.5. The mean indoor airborne fungal concentrations (CFU/m³) for the 1988 low season were as follows: *Alternaria*: February 1.4, March 2.9, April 6.4, May 3.3; *Cladosporium*: February 351, March 53.8, April 216, May 101.4; *Aspergillus*: February 38.8, March 47.2, April 32.7, May 48.2; and *Penicillium*: February 238.1, March 210.5, April 82.9, May 32.2. Figs. 1 and 2 show the mean indoor airborne fungal concentration in the high (1987) and low seasons (1988). Fig. 3 shows the mean outdoor airborne fungal concentration for Montréal as measured at the aerobiological station of the Université de Montréal in 1987 during the high season using the Burkard Seven-Day Spore Trap.

For the analysis of respiratory allergy in relation to seasonal fungi, *Alternaria* was chosen because the percentage of subjects with skin test positivity was the highest and 32% of the homes had indoor *Alternaria* CFUs. The second seasonal fungal allergen chosen for analysis was *Cladosporium* with the third highest percentage of skin test positivity. *Cladosporium* CFUs were present in 58% of the homes in the 1987 high season. *Helminthosporium* and *Cephalosporium* were tested, but it was not possible to consider them for analysis for the following reasons. In the aerobiological measurements, the presence of *Helminthosporium* in the subjects' indoor environment was not identified. It is also possible that this allergen corresponds to *Drechslera* and that the manufacturer is using an older taxonomical denomination. Only 11 cases had skin test sensitivity to *Helmin-*

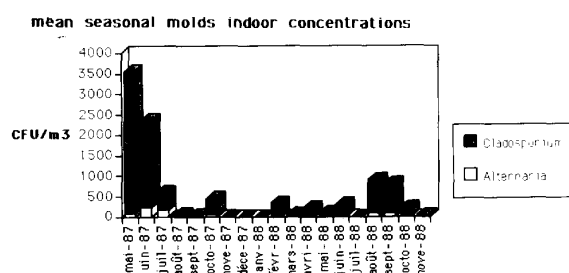


Fig. 1. Mean indoor fungal concentrations. The measurements of the indoor fungal colony forming units/m³ were taken in the human subjects' homes once in the peak season between May and November 1987 ($n = 93$) and once again in the low season between December 1987 and May 1988 ($n = 38$).

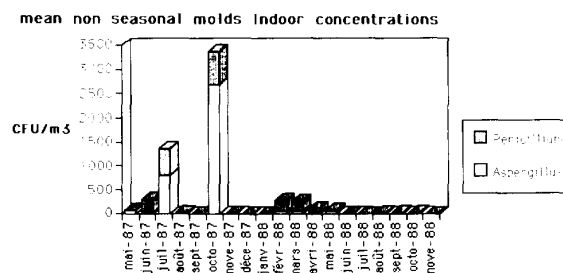


Fig. 2. Mean indoor fungal concentrations. The measurements of the indoor fungal colony forming units/m³ were taken in the human subjects' homes once in the peak season between May and November 1987 ($n = 93$) and once again in the low season between December 1987 and May 1988 ($n = 38$). The measurements from June to November 1988 were not considered in the epidemiological analyses, since we did not have the indoor environmental measurements for these houses in 1987 during the high season.

thosporium (*Drechslera*). Of these 11 cases, only 5% of the houses had *Drechslera* CFUs. Since other variables were missing, analysis was impossible. *Cephalosporium* was also excluded from analysis for the same reasons: first, it was not measured or recovered in the subjects' indoor environment, but could correspond to

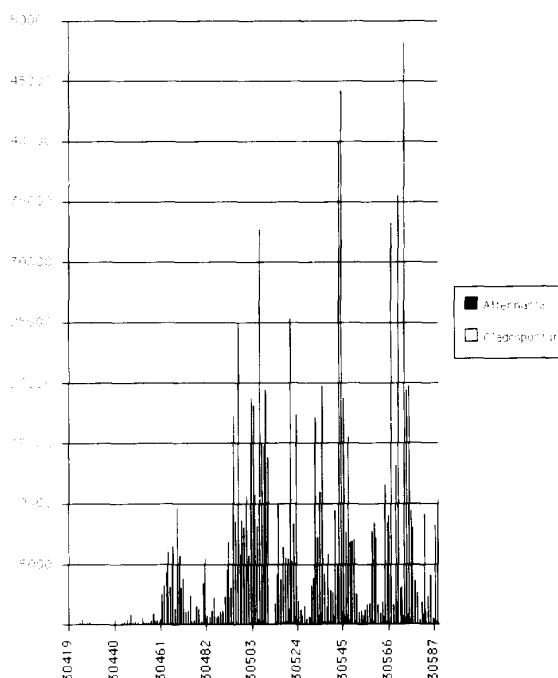


Fig. 3. Mean outdoor fungal concentrations for Montréal in 1987. Outdoor spore concentrations for Montréal as measured at the Aerobiological Station of the Université de Montréal in 1987 during the high season using the Burkard Seven-Day Spore Trap.

Acremonium CFUs, and second, only seven subjects had skin tests positivity to it.

For respiratory allergy in relation to non-seasonal fungal allergens, the subjects were skin tested for *Aspergillus* and *Penicillium*. The indoor airborne *Aspergillus* concentration was near zero in all the homes in 1987. This made it impossible to assess the relationship of *Aspergillus* skin test positivity to indoor airborne *Aspergillus* (CFUs). By multivariate analysis, we assessed skin test positivities to non-seasonal fungi in relation to the presence of indoor *Penicillium*.

It is common for a variable in an epidemiologic analysis to have only two values (binary dichotomous), corresponding to the presence or absence of disease and presence or absence of exposure. We stratified categories according to the presence or absence of skin test positivity to specific fungal species in relation to the presence or absence of the same indoor fungal allergen.

For the multivariate analysis, only the colony forming units in relation to skin test positivity were considered. Indeed, Platts-Mills (1989) stated that it is particularly difficult to relate spore counts to the measurement of allergen exposure, because the fungal spores do not release their protein content rapidly and may even require germination. It is also known that it is difficult to differentiate *Aspergillus* and *Penicillium* spores on spore trap slides reliably. Therefore, CFU/m³ seemed more precise as a measurement of airborne allergen exposure in relation to skin test sensitivity.

3.1. Multivariate analysis of seasonal fungi (logistic regression — maximum likelihood estimates)

Cladosporium was recovered in very few of the homes of subjects with skin test positivity to *Cladosporium* and *Alternaria* in very few of the homes of subjects with skin test positivity to *Alternaria*. The above distribution made it impossible to assess exposure in relation to each fungal species. We combined all the cases of skin test positivity to *Alternaria* with skin test positivity to

Cladosporium, as respiratory allergy in relation to seasonal fungal allergen exposure (*Alternaria* and/or *Cladosporium* CFUs). This made the analysis possible. The seasonal aeroallergens *Alternaria* and/or *Cladosporium* (AC2C) were present in 66% of the subjects who had skin tests positive to *Alternaria* and/or *Cladosporium* and absent in 34%.

3.2. Multivariate analysis for non-seasonal fungi (logistic regression — maximum likelihood estimates)

For non-seasonal fungi, skin test positivity to *Aspergillus fumigatus* was not statistically related to *Aspergillus* exposure. For example, 40 subjects had skin test positivity with zero degree of *Aspergillus* exposure, only one subject was exposed to 2 CFU/m³ and another to 28 CFU/m³. With such a distribution, it was impossible to do further analyses with these variables. Hence, *Aspergillus* CFUs were not considered in model B for respiratory allergy in relation to non-seasonal fungal allergens. Skin test positivity to *Aspergillus* and/or *Penicillium* in relation to *Penicillium* CFUs had to be combined in order to do further analyses.

In summary, seasonal fungal skin test positivity to *Alternaria* and/or *Cladosporium* in relation to exposure to *Alternaria* and/or *Cladosporium* CFUs (AC2C) was kept for model A. Skin test positivity to *Penicillium* and/or *Aspergillus* was assessed in relation to exposure to *Penicillium* CFUs for multivariate analysis in model B.

3.3. Multivariate analysis of seasonal fungi (Model A)

The sample size for the analysis of seasonal fungi was 93. After considering the literature for important risk factors in IgE-mediated respiratory allergy, and the results of the univariate and bivariate analyses, the following variables were considered for logistic regression models (SAS Institution Program): dependent variable (skin test sensitivity to *Alternaria* and/or *Cladosporium*), independent variables (presence or absence of airborne

Table 2

Model A. Results of multiple logistic regression (maximum likelihood) skin prick tests to seasonal fungi and CFU seasonal fungi by controlling for selected risk factor ($n = 93$)

Variable	Estimated β	S.E.	P-value	Adj. OR	95% CI
CFU AC2C	0.16	0.29	0.58	1.18	0.66–2.07
Age	0.027	0.2	0.19	1.03	0.69–1.52
Predisposition	–0.6	0.33	0.7	0.55	0.29–1.05
Sex	0.21	0.28	0.46	1.24	0.71–2.14
Skin test + to ragweed	1.23	0.34	0.0003	3.42	1.76–6.66
Asthma	–0.65	0.32	0.046	0.52	0.28–0.98
Rhinitis	1.61	0.46	0.0005	5	2–12.32
CFU seasonal fungi* asthma	–0.6	0.31	0.059	0.55	0.3–1.01
CFU seasonal fungi* sex	–0.94	0.32	0.003	0.39	0.21–0.73

AC2C, *Alternaria* and *Cladosporium* CFU.

*Statistical interaction.

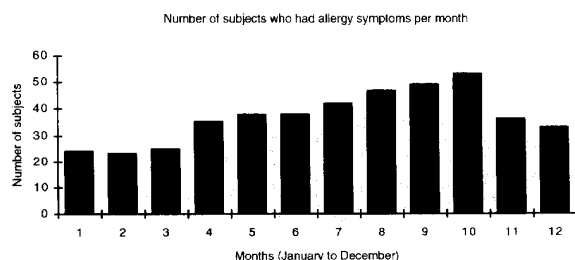


Fig. 4. Number of human subjects with respiratory allergy symptoms per month. Evaluation of respiratory symptoms in relation to rhinitis and asthma by allergists according to the month of the year. Most of the cases had rhinitis and asthma.

Alternaria and/or *Cladosporium* CFUs in patients' indoor environment). The following variables were also considered as secondary determinants of IgE-mediated respiratory allergy: age, predisposition, sex, skin test positive to ragweed, and presence or absence of rhinitis and asthma. Skin test positive to ragweed, as well as rhinitis and asthma were considered as potential confounders. Age, predisposition, sex, skin test positive to ragweed as well as rhinitis and asthma were considered as potential effect modifiers. If the probability of error of variable of interest was < 0.05 , and we knew a priori that the variable was important biologically, the variable was kept in the model, because it suggested that it was a determinant of respiratory allergy (Table 2).

3.4. Results: Model A

The results of the multivariate analysis agreed with the bivariate analysis for respiratory allergies in relation to seasonal fungi. In model A ($n = 93$; 43 cases and 50 controls), the relationship between airborne seasonal fungal CFUs and skin tests positive to *Alternaria* and/or *Cladosporium* was not statistically significant, (Adj. OR = 1.18 and CI = 0.66–2.07). The relationship between skin prick tests positive to seasonal fungi and skin prick tests positive to ragweed was statistically signifi-

cant (Adj. OR = 3.42 and CI = 1.76–6.66). Ragweed category subjects were 3.4 times as likely to be allergic to seasonal fungi. The relationship between skin prick test positivity to seasonal fungi and rhinitis was also statistically significant (Adj. OR = 5 and CI = 2.03–12.32). Rhinitis category subjects were five times as likely to be sensitive to seasonal fungi. The relationship between asthma and seasonal CFUs was also statistically significant (Adj. OR = 0.52, CI = 0.28–0.98). However, biologically, it does not make sense that fungal allergens would have a protective effect in asthma, since they are allergens, and, therefore, triggers of IgE-mediated respiratory allergy, like pollen and mite. The human subjects in this study had symptoms during 12 months. They were slightly more elevated in the high season (Fig. 4). The complexity of fungal exposure and fungal diagnostics will be addressed in the Discussion section.

3.5. Multivariate analysis of non-seasonal fungi (Model B)

For the following analysis, the sample size was 38. After considering the literature for important risk factors in IgE-mediated respiratory allergy and the results of the univariate and bivariate analyses, the following variables were considered for logistic regression models (SAS Institution Program): dependent variable (skin test sensitivity to *Aspergillus* and/or *Penicillium*), independent variables (presence or absence of airborne *Penicillium* in subjects' indoor environment). The following covariates were also considered as secondary determinants of IgE-mediated respiratory allergy: age, predisposition, sex, skin test positive to ragweed, mite and seasonal fungi, as well as the presence or absence of rhinitis and asthma. Skin test positivity to ragweed, mite and seasonal fungi were considered as potential confounders after Horst et al. (1990), Mailling and Dreborg (1986) and Prahl (1992). Age, predisposition, sex, skin test positivity to ragweed, mite and seasonal fungi, as well as rhinitis and asthma were considered as potential effect modifiers. If the probability of the variable of in-

Table 3
Model B. Results of multiple logistic regression (maximum likelihood) skin prick tests to non seasonal fungi and CFU *Penicillium* by controlling for selected risk factors ($n = 38$)

Variable	Estimated β	S.E.	P-value	Adj. OR	95% CI
CFU <i>Penicillium</i>	-1.1	0.83	0.18	0.33	0.07–1.69
Age	0.05	0.07	0.42	1.05	0.92–1.21
Predisposition	0.21	0.62	0.73	1.23	0.37–4.16
Sex	-0.05	0.67	0.45	0.61	0.16–2.24
Skin test + to ragweed	0.6	0.71	0.4	0.55	0.14–2.21
Skin test + mite	-0.92	1.07	0.39	0.4	0.05–3.25
Skin test + seasonal fungi	2.55	1.04	0.015	12.81	1.67–98.34
Asthma	0.62	0.7	0.37	1.86	0.47–7.33
Rhinitis	-1.7	0.96	0.074	0.18	0.03–1.19

terest was < 0.05 , the variable was kept in the model, because it suggested that it was a determinant of respiratory allergy to non-seasonal fungi (Table 3).

3.6. Results: Model B

In the multivariate logistic regression in relation to *Penicillium* CFU ($n = 38$; 21 cases and 17 controls), the variables were controlled for seasonal fungi, in addition to skin prick test positivity to mite and seasonal fungi. There was an agreement between the multivariate and bivariate results. There was no statistical significance between the *Penicillium* CFUs and skin prick test positivity to *Penicillium* (Adj. OR = 0.33, CI = 0.07–1.69). There was a statistically significant association between skin prick tests positive to seasonal and non-seasonal fungi (Adj. OR = 12.81, CI = 1.69–98.34). Subjects allergic to seasonal fungi were 12.8 times as likely to be allergic to non-seasonal fungi (*Aspergillus* and *Penicillium*). This issue is addressed in the Discussion section. There was no statistical association between rhinitis and *Penicillium* CFUs (Adj. OR = 0.18, CI = 0.03–1.19) nor between asthma and *Penicillium* CFUs (Adj. OR = 1.86, CI = 0.47–7.33).

4. Discussion

Both methods, bivariate and multivariate, failed to detect any statistical association between airborne fungal (CFU) exposure and skin test positivity; Al-Doory and Domson (1984), Burge (1992), Dreborg and Agrell (1986), Lehrer et al. (1982), Mailling and Dreborg (1986), Rosenwasser (1991), Salvaggio and Lopez (1976), Santilli et al. (1990), Vijay et al. (1979, 1991) have shown the presence of fungal antibodies, in vivo and in vitro, in IgE-mediated asthma. The presence of IgE indicate past or present exposure. The skin test positivity to fungi of the subjects in this study resulted from cumulative repeated exposure during their life time.

Many factors probably influenced the overall low recovery of airborne fungi in the indoor environment of skin test positive subjects at the time of the aerobiological measurement during the low season according to Chapman (1992) and Su et al. (1992). Low-season long-term repeated measurements are necessary to quantify overall exposure. This type of aerobiological design is feasible if only one or two units of analysis are involved (Su et al., 1992), i.e. office buildings, schools, or daycare centres, where a few measurements during the day over a few months are very easy as compared with 122 houses in two seasons in Greater Montreal. A very difficult task was setting up appointments for aerobiological measurements in the subjects' homes. Often the subjects were not at home, despite their appointment, or they were available only during the evening or early in the morning. Some changed their appointments so repeat-

edly during the high season that the two aerobiological measurements for these subjects were taken in the low season. These subjects were eliminated from the epidemiological analysis, because two measurements were necessary — high and low season exposure — in each subject's indoor environment. For this reason, we ended up with a smaller sample size, which in turn made it impossible to carry out the non-seasonal fungal effect measurements.

An attempt was made to assess the relationship between outdoor and indoor fungi. The question was whether any outdoor fungal allergens could influence the presence of indoor fungal allergens. In the 1987 high season, the geometric means of outdoor fungal spores/m³ were 3747 *Cladosporium*, 132 *Alternaria*, 115 *Aspergillus* and 89 *Penicillium*. In the 1988 low season, the geometric means of outdoor fungal spores/m³ were 86 *Cladosporium*, 16 *Alternaria*, 28 *Aspergillus* and 9 *Penicillium*. When we tried to determine whether there was a correlation between exterior and interior fungi, none was found, with the exception of *Alternaria* spores in 1987. In the 1987 high season, the Pearson correlation coefficient between indoor and outdoor colony forming units/m³ was $r = 0.031$ for *Cladosporium*, $r = 0.36$ *Aspergillus* and $r = 0.11$ *Penicillium*. *Alternaria* was not measured. The correlation between indoor and outdoor spores/m³ was $r = 0.11$ for *Cladosporium*, $r = 0.88$ *Alternaria*, $r = 0.11$ *Aspergillus*, and $r = 0.56$ for *Penicillium*.

In the 1988 low season, no correlation was found between indoor and outdoor fungi because the measurable airborne fungi were extremely low. The correlation between indoor and outdoor colony forming units/m³ was $r = 0.45$ for *Cladosporium* and $r = 0.58$ for *Penicillium*. No correlation for indoor and outdoor *Aspergillus* colony forming units/m³ was found, since no indoor *Aspergillus* was found.

The lack of an indoor and outdoor correlation of airborne fungi in the high season in our study partially resulted from averaging the measurements that took place from May through to the end of October 1987. Following the clinical allergy evaluation of the subjects, their indoor environment was measured once during the high season and once during the low season. Within the high season, there is a monthly variation. However, if one takes the peak of the high season, a correlation between the indoor and outdoor airborne fungi should exist, when the measurements are taken repeatedly. Nevertheless, Verhoeff et al. (1992) did not find any correlation between CFU/m³ in living rooms and outdoors ($r = 0.13$) or between CFU/m³ in bedrooms and outdoors ($r = 0.19$), despite repeated measurement over 6 months. In conclusion, it is probable that the factors influencing the presence of airborne fungal allergens outdoors are different from those influencing airborne fungal allergens indoors. Indoor airborne fungal disper-

sion and related factors in time and space are not part of this epidemiological study, since in any clinical epidemiology study, the unit of analysis is the human being, and therefore, the measure of specific IgE.

Another factor in our study was that the selected cases came mostly from referrals in the spring and summer of 1987. If we had waited for all the selected cases, then we could have done the aerobiological measurements within 2 weeks and we would have gone into late November and December. The high season would have been over and, in addition, very few patients had expressed an interest in indoor aeroallergen measurements during December, which would have resulted in our losing more cases.

There is a lack of association in the asthma category, because asthma is a chronic multifactorial disease according to Metzger and Donnelly (1983), Packe et al. (1985) and Tseng et al. (1992), with different causal mechanisms, in relation to specific (allergens) and non-specific exposure (cold, exercise, pollutants). Tseng et al. (1992) and Tuchinda et al. (1987) documented cases of the hospitalization of subjects (especially children under 14 years in Asia and in developed countries) presenting asthma in relation to sulphur dioxide (SO₂), ozone (O₃), total suspended particles (TSP), respiratory suspended particles (RSP), nitrogen dioxide (NO₂), and nitrogen oxides (NO_x). It is possible that, in our study, asthma cases had skin test positivity to fungal allergens and that the latter initiated the inflammation. Then ozone, allergens or pollutants could have a synergistic effect in provoking bronchial hyperreactivity. However, our study was not designed to answer such questions. Asthma is a complex disease, there are many factors and there is always the possibility of epidemiological interaction (Gayers, 1986; Horst et al., 1990; Prahl, 1992).

Fungi produce different proteins under different growth conditions, and a variety of strains and relevant epitopes have to be present in the standardized diagnostic fungal-extract cocktail for a sensitive and reproducible diagnosis that can be assessed in relation to airborne fungal exposure according to Bousquet (1990) and Bruce and Norman (1977). Hypothetically, even if we had well-defined and standardized allergens for diagnosis, there would be other major issues one would have to consider when assessing asthma in relation to specific and non-specific environmental exposure or primarily in relation to airborne fungi. Documented respiratory allergy solely in relation to fungi, to which all signs and symptoms can be attributed, is infrequent. In this population study, there was not one case of skin test positivity to fungi without skin test positivity to other inhalants. Since there are a lot of categories in respiratory allergy, the number of subjects for the study is important for effect measurement.

Documentation on respiratory allergy and gender is infrequent and comes mostly from observational stud-

ies. It is not restricted to the allergen category, i.e. food or inhalant allergy. Our data consistently suggested that there was potential effect modification between fungi and gender and that females were more sensitive to fungi than were males. In the model A, there was statistical interaction between seasonal fungal exposure and gender (adj. OR = 0.387, $P = 0.003$). We could not separate the male and female in the multivariate analysis, while considering other variables (see model A). However, in the bivariate analysis of potential effect modification in relation to seasonal fungi and gender, gender F was more sensitive to fungi than gender M.

Gender M: Unadj. OR = 0.57, $n = 53$

Gender F: Unadj. OR = 2.24, $n = 44$

In model B, for non-seasonal fungi, given the very small sample size ($n = 38$; 21 cases, 17 controls), we could not do any interaction (effect modification) testing for gender. However, in the stratified analysis of potential effect modification, gender F was more sensitive to *Penicillium* than was gender M.

Gender F: Unadj. OR = 2.91 $n = 20$

Gender M: Unadj. OR = 0.53 $n = 21$

Since asthma is a chronic multi-factorial disease, the epidemiological interaction (additive model) of the gene (predisposition, family history) and the environment (pollutant, ozone and allergen) is important. However, the baseline of each category has to be well measured and clearly defined. Better environmental and pharmacological intervention will only be possible if further research can be done in aerobiology, epidemiology and immunology in relation to respiratory diseases.

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