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Aerobiologia **16:** 393–396, 2000.
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Comparison of airborne spore concentrations and fungal allergen content

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Received 4 January 1999; accepted in final form 16 May 2000

Key words: airborne fungal allergens, *Alternaria*, *Cladosporium*, indoor air

Abstract

The exposure to spores causing health effects is usually assessed by determining the concentration of viable spores per cubic meter of air (CFU/m^3). Since allergens might also be present in dead spores or smaller particles, the objective of this study was to investigate the correlation between the viable spores of *Alternaria* and *Cladosporium* at different indoor and outdoor sites and the corresponding allergen concentration detected with a specially developed ELISA (Enzyme Linked Immunosorbent Assay). In outdoor air, the results show a strong correlation between the different sampling techniques applied for viable spores (Slit-Sampler and Multistage Liquid Impinger) and between the viable spores and the allergen concentrations detected in the liquid samples of the impingers. Indoors, the number of viable spores and the allergen concentration do not correlate and the allergen load is underestimated if colony counting methods are used.

Introduction

Mould spores are found in large numbers in indoor and outdoor environments and they have been recognised as important inhalant allergens. Clinical studies are being performed to correlate symptoms of allergic patients to the concentrations of allergenic fungal spores (colony forming units CFU/m^3 or the total spore counts) [DeKoster and Thorne, 1995; Cosentino and Palmas, 1996; Dotterud et al., 1996]. However, even in the absence of viable spores the air might carry allergens [ECA, 1993]. Allergenic proteins may be present in dead spores or in smaller fractions, similar to the findings that have been reported for various pollen allergens [Agarwal et al., 1984; Schäppi et al., 1996]. The objective of this study was to investigate if the concentrations of viable *Alternaria* and *Cladosporium* spores are a reliable indicator for the exposure to the respirable allergens of these moulds.

Materials and methods

Repeated air sampling was performed outdoors and in five mechanically and ten naturally ventilated buildings. A Slit-Sampler (FH2, Loreco Reckert GmbH) with petri dishes containing malt extract agar (MEA; 30 g malt extract, 5 g peptone, 15 g agar, 1 l distilled water) was operated for the collection of viable spores in the air. In addition, sampling was carried out with Multistage Liquid Impingers [May, 1966] filled with phosphate buffered saline (containing 0,05% Tween 20) for size-selective sampling (the approximate cut-off points for the three stages are 6, 3.5 and 0.8 μm). The viable spore concentrations were determined by plating out three 1 ml samples from each stage of the impingers onto MEA. Together with the agar plates from the Slit-Sampler they were incubated for five days, colonies were counted and identified microscopically to genus level.

For the quantification of the airborne fungal allergens collected in the liquid impingers a direct competitive ELISA for *Cladosporium herbarum* and *Alternaria alternata* was developed. Microtiter plates made

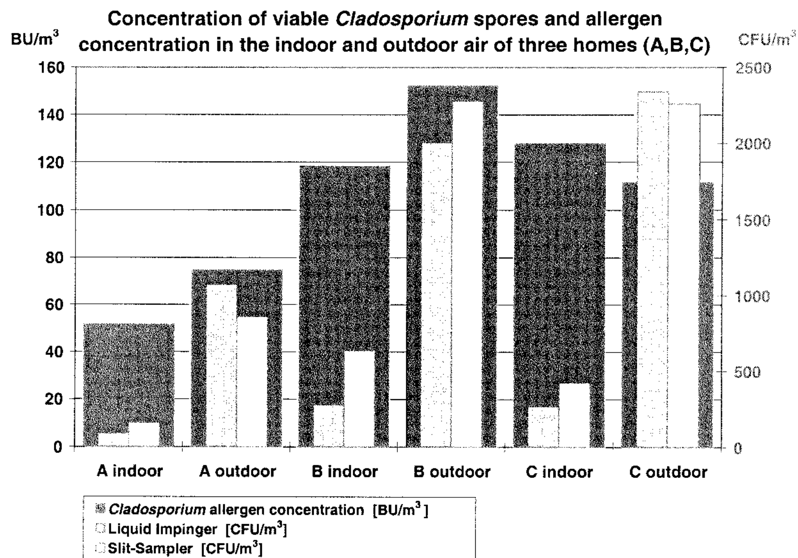

Figure 1. Concentration of viable *Cladosporium* spores and allergen concentration in the indoor and outdoor air of three naturally ventilated homes. The concentrations are averages of two samplings (May and July 1998).

of polystyrene with high protein affinity were coated with extracts of *Alternaria* and *Cladosporium* (Allergopharma Joachim Ganzer KG) diluted to 10 μg protein/ml. After blocking of the plates with buffer containing 1% bovine serum albumin, the samples from the liquid impingers or dilutions of the extracts for the standard curve were mixed with diluted serum from a patient allergic to *Cladosporium* (RAST class 3) or to *Alternaria* (RAST class 4), added to the plates and incubated for two hours at 37 °C. The remaining IgE-antibodies to *Cladosporium* or *Alternaria*, which had not reacted with the air samples and were therefore free to be captured by the coated antigens, were detected with enzyme labelled (alkaline phosphatase) mouse anti-human antibodies. The results are given in Biological Units (BU; defined by the Nordic Guidelines, 1989, and used to quantify the commercial extract used in this study: 5000 BU/vial) per m^3 of air. The sera used were tested for a large number of allergens and showed also reactivity against other allergens than the allergens of *Cladosporium* or *Alternaria* used in this study (e.g. grass pollen, animals). However, the design of the IgE-Inhibition ELISA suggests that possible cross-reactivity of the sera with unknown allergens in our samples will not show up. Cross-reactivity to other fungi known to produce similar allergens can not be eliminated, but the identification of the viable spores provides some information on the presence of such other fungi.

Results

The total viable spore concentrations obtained with the Slit-Sampler and the Multistage Liquid Impingers at all the investigated locations correlate strongly (Spearman $r_s = 0.85$; $p < 0.001$). This is also the case for viable *Cladosporium* spores sampled with both methods ($r_s = 0.90$). During peak season slightly higher concentrations of total viable spores (mainly *Cladosporium*) were sampled with the liquid impinger than with the Slit-Sampler. With the plated out liquid samples from the impingers less overgrowing occurred than on the Slit-Sampler agar plates. Since the sampling took place in summer an average of 80% (range 62–98%, no difference between sampling techniques) of the spores outdoors were *Cladosporium*. *Alternaria* made up only about 1% of all the viable spores collected with the Slit-Sampler and the liquid impinger.

Figure 1 represents the concentrations of viable spores obtained with the Slit-Sampler and the Multistage Liquid Impinger as well as the allergen concentrations sampled in the impingers and detected with the ELISA. The concentrations of viable *Cladosporium* spores and the *Cladosporium* allergen concentration in the indoor and outdoor air of three investigated homes with natural ventilation are shown.

The allergen concentrations collected in the liquid impingers and detected with the ELISA did not always

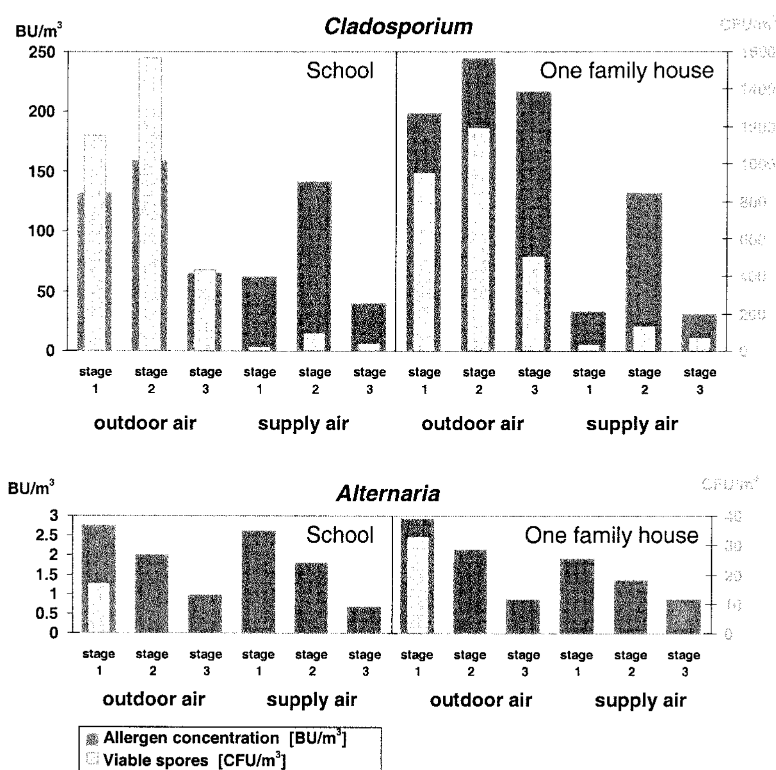

Figure 2. Viable spore and allergen concentration for *Cladosporium* (top) and *Alternaria* (bottom) in the outdoor air and in the supply air of two mechanically ventilated buildings investigated in August 1998. The concentration for each of the stages (1–3) of the liquid impinger is given. The large *Alternaria* spores are all captured in stage 1 while smaller particles carrying *Alternaria* allergens are also collected in stages 2 and 3. *Cladosporium* allergen particles were collected on all three stages with most of the single spores in stage 2, clumps of spores in stage 1 and additional ill defined spore fractions such as allergen coated dust particles in stage 3.

correlate well with the viable spore concentrations in the samples. Comparing all the outdoor and the indoor samples of the investigated buildings a weak but significant correlation between the *Cladosporium* allergen concentration and the viable *Cladosporium* spores collected in the liquid impinger or the Slit-Sampler can be observed (both $r_S > 0.35$; $p < 0.05$). The allergen concentrations of *Alternaria* show only a correlation with the viable *Alternaria* spores on the Slit-Sampler agar plates ($p < 0.05$) and do not correlate with the viable spores in the liquid impinger.

The separate analysis of indoor and outdoor measurements shows, that the correlation between viable spores and allergen concentration is always much stronger outdoors than indoors and that the relation of BU/CFU is much higher indoors than outdoors. For *Cladosporium*, the relation BU/CFU in the air of the naturally ventilated homes and the outdoor air was significantly different ($p < 0.01$; indoor: Median 1.01, Max. 5.45; outdoor: 0.16, 2.47). For *Alternaria* in the homes and for *Alternaria* and *Cladosporium* in the supply air of the mechanically ventilated buildings the concentrations of the viable spores were extremely low and no correlation between the viable spore concentration and the allergen content could be observed. This finding is demonstrated in Figure 2 showing that in the supply air of the ventilation systems, no viable spores of *Alternaria* and *Cladosporium* or extremely low amounts were found while allergens could still be detected.

Discussion

Sampling with a liquid impinger followed by an ELISA analysis seems to be an appropriate way for

detecting fungal allergens in the air. A good correlation between allergen concentration and CFU/m^3 can be established for outdoor samples. It is interesting that a difference in the ratio of allergen concentration and viable spores between indoor and outdoor samples can be observed. The reasons for this finding in both naturally and mechanically ventilated buildings need to be investigated in more detail. The identification of the viable spores did not show higher indoor concentrations of other fungi producing similar allergens which might cross-react in the ELISA. The relatively high indoor concentrations of allergens compared to the low CFU/m^3 indicate that indoors the spores of *Cladosporium* or *Alternaria* may have lost their viability while the allergens are still present and may contribute to an allergic reaction. The size fractionation indicates that outdoors as well as indoors a considerable amount of allergen might be present in dead spores, hyphae, small spore fragments or allergen coated dust particles.

Acknowledgements

The authors would like to thank Allergopharma Joachim Ganzer KG, Reinbek, Germany for providing the allergen extracts and all the building owners for their cooperation.

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