

Short Communication

Increased incidence of allergic sensitisation and respiratory diseases due to mould exposure: Results of the Leipzig Allergy Risk children Study (LARS)*

Andrea Müller^a, Irina Lehmann^a, Annett Seiffart^b, Ulrike Diez^a, Heide Wetzig^b, Michael Borte^b, Olf Herbarth^{ac}

^a Department of Human Exposure Research and Epidemiology, UFZ-Centre for Environmental Research Leipzig-Halle Ltd., Leipzig, Germany

^b Department of Pediatrics, University of Leipzig, Leipzig, Germany

^c Department of Environmental Hygiene, University of Leipzig, Leipzig, Germany

Abstract

To investigate mould effects on health, the concentration of mould spores in air and dust was determined during a prospective cohort study. Clinical outcome was estimated by questionnaires and determination of specific IgE antibodies and intracellular cytokine production of T cells.

A significant association was observed between the incidence of respiratory tract infections and exposure to *Penicillium* spores. Moreover, *Aspergillus* exposure was found to be associated with allergic rhinitis or related symptoms. In addition, T cells of children exposed to *Aspergillus* showed a significantly lower content of TH1 cytokines (IFN- γ , TNF- α , IL-2) producing cells. Our data suggest that mould exposure is associated with several effects on health, depending on the species involved.

Key words: mould – indoor – allergy – respiratory diseases – allergy – cytokine

Introduction

In recent years, the health risk due to dampness and mould problems in homes has been the subject of considerable attention. Emissions from mould growth in homes are perceived as a potential risk factor for asthma and allergy, as well as for a range of health effects, such as chronic respiratory infections,

dry cough, eye irritations, skin symptoms and non-specific symptoms (Brunnekreef et al. 1989, Husman 1996, Peat et al. 1998). Since not all fungi have the same hazard potential, risk assessment of the health effects of moulds entails determining the exact fungal species (Miller 1992). To obtain more information about the underlying mechanism of mould effects on health, more specific methods are

* Following a presentation given as part of the International Congress on Environmental Health and the 4th Annual Meeting of the International Society of Environmental Medicine (ISEM), 1–4 October, 2000, Hannover, Germany.
Corresponding author: Andrea Müller, Department of Human Exposure Research and Epidemiology, UFZ- Centre for Environmental Research Leipzig-Halle Ltd., Permoserstr. 15, PF 02, D-04301 Leipzig, Germany, Phone: +49 341 235 2497, Fax: +49 341 235 2288, E-mail: amueller@expo.ufz.de

needed in order to quantify the specific fungal exposure which is relevant to health (Dillon et al. 1999). This paper presents data from a study in which the detailed determination of indoor exposure to airborne fungi was combined with an assessment of clinical outcomes and the measurement of both specific IgE antibodies and intracellular cytokine production of T cells in children.

Materials and methods

475 premature and atopic risk newborns (increased cord blood IgE, double positive atopic family history; birth weight 1500–2500 g) in Leipzig were recruited within a prospective cohort study ("LARS" – Leipzig Allergy Risk Children Study). Two hundred children at the age of 36 months participated in the here presented study. The concentration of mould spores in the air and dust in children's rooms was determined using an one stage air sampler MAS 100 (Merck, Darmstadt, Germany) ($n = 180$). 100 l of air was aspirated on Dichloran-Glycerol agar plates (DG18, Oxoid, Basingstoke, Hampshire, UK). To determine the concentrations of mould spores in dust, house dust samples were taken by a standardised method (Koch et al. 1995). 30 mg of fine dust was homogenised with 10 ml A. dest. and incubated on DG18 agar plates. After incubation at room temperature for 5–7 days the number of colony forming units (CFU) was counted. Microscopic (morphological) identification at genera and species-level was performed. The clinical outcome was estimated by questionnaires completed by parents. During medical examinations blood samples were taken to measure IgE antibodies and T cell cytokines (INF- γ , TNF- α , IL-2, IL-4). Total and specific IgE was analysed using the Pharmacia CAP system. Cytokine production was measured using intracellular cytokine staining. Whole blood samples were stimulated with 10 ng/ml phorbol ester, 1 μ M ionomycin and 2.5 μ M monensin for 5 hours, after which they were fixed in 4% paraformaldehyde and permeabilized with 0.1% saponin. Cytokines and cell surface markers were stained with fluorescence-labelled monoclonal antibodies (obtained from Beckman-Coulter) and flow cytometry ($n = 40$). Cells were analyzed on a flow cytometer (FACScan, Becton Dickinson). Data analysis was performed using STATISTIKA 5.1. Logistic regression was used to calculate the odds ratios (OR) for mould exposure and clinical outcomes.

Results

The mean viable fungal spore concentration was similar for all samples indoors and outdoors. However, analysis of genera and species revealed significantly higher concentrations of *Penicillium*- and *Aspergillus*-spores indoors than outdoors (for de-

Table 1. Fungal spore concentrations as determined indoors and outdoors. All counts are given in CFU/m³.

	indoors		outdoors	
	mean	range	mean	range
all samples	743	10–> 10,000	864	10–> 10,000
<i>Penicillium</i>	338	< 10–> 10,000	99	< 10–3,030
<i>Aspergillus</i>	80	< 10–2,400	27	< 10–> 10,000
<i>Cladosporium</i>	194	< 10–2,569	544	10–> 10,000
yeasts	65	< 10–4,000	125	< 10–6,300

tails see Table 1). Higher outdoor levels were found for *Cladosporium* and yeasts, with a significant seasonal maximum in summer. No association was observed between the total spore concentration and reported visible indoor mould or dampness. For a better assessment of an indoor source of mould we calculated the indoor concentration as the difference between the indoor and outdoor spore concentration for each genera. An indoor source was assumed when this difference exceeded a threshold of 100 CFU/m³ of each genera. So in 48.7% of children rooms an indoor source of mould was assumed. This indicated *Penicillium* and *Aspergillus* species, especially *P. chrysogenum*, *P. brevicompactum*, *P. expansum*, *P. commune*, *Aspergillus versicolor*, *Eurotium amstelodami* (anamorph, *Aspergillus amstelodami*), *Eurotium chevalieri* (anamorph, *Aspergillus chevalieri*) to be mainly indoor sources. *Aspergillus ochraceus*, *A. niger*, *A. flavus* and *A. fumigatus* occurred sporadically.

The determination of spores in house dust proved unsuitable for assessing an indoor source of mould.

Mould exposure was associated with several effects on health, depending on the species. A significant association was found between the incidence of respiratory tract infections and indoor exposure to *Penicillium* spores > 100 CFU/m³ (OR: 6.88, CI 95%: 1.21–38.9). *Aspergillus* exposure > 100 CFU/m³ was associated with an increased level of specific IgE against grass (OR: 5.28, CI 95%: 1.02–27.1). Results were adjusted to passive smoking and double positive atopic family history.

Investigations of T cell function showed the suppression of TH1 cytokine by mould exposure. In particular, exposure to *Aspergillus* spores, without exposure to *Penicillium*, was associated with a significant suppression of TH1 cytokine (INF- γ , TNF- α and IL-2) producing cells. The capacity of IL-4 production was not influenced. Exposure to other mould genera did not affect investigated cytokine production of T cells (Fig. 1).

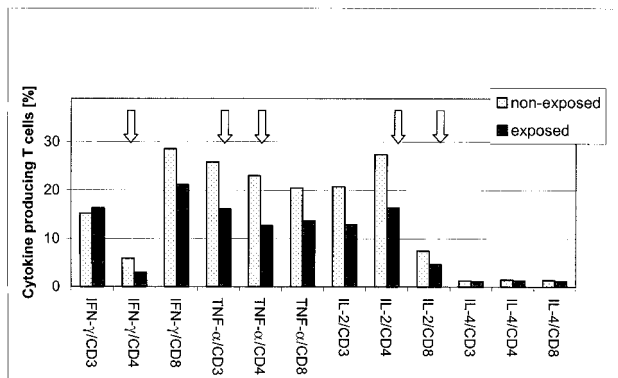


Fig. 1. Influence of *Aspergillus* exposure on cytokine producing T cells. Significant differences (Mann-Whitney-test, p value < 0.05) are marked with an arrow. Results show a significant suppression of TH1 cytokine (INF- γ , TNF- α , IL-2) producing cells, TH2 cytokine (IL-4) producing cells were not influenced.

Discussion

Our results show that mould affects health in several ways, depending on the genera and species involved. However, no association was observed between the total count of viable spores and the symptoms described. We found an association between *Penicillium* and respiratory diseases, as well as between *Aspergillus* and atopy. General toxic and immunosuppressant properties of some fungal metabolites, such as mycotoxins and MVOC could produce non-specific effects on the immune system (Garrett et al. 1998, Flannigan et al. 1991, Müller et al. 1999, Sorenson, 1999). Such an effect might explain the significant increase of atopy with exposure to *Aspergillus* spores since several *Aspergillus* species are known to produce mycotoxins. In our study the observed suppression of TH1 reactivity by *Aspergillus* exposure is significant, hence the reduction of TH1 cells promotes a TH2-dominated immune response, the basis for IgE mediated allergy (Lehmann et al. 1999). The great variation in the production of secondary metabolites by moulds may explain the different effects on health by several genera and species.

Acknowledgements. We would like to express our gratitude to the participating families for their valuable contribution to the study. We also thank Ms Jacqueline Kobelt for her excellent technical assistance in the identification of fungi. This study was supported by grants from the SMWK (Ministry of Science and Art of Saxony), Project No. 4-7531.50-03-UFZ/701.

References

- Brunnekreef, B., Dockery, D. W., Speizer, F. E., Ware, J. H.: Home dampness and respiratory morbidity in children. *Am. Rev. Respir. Dis.* 140, 1363–1367 (1989).
- Dillon, H. K., Miller, J. D., Sorenson, W. G., Douwes, J., Jacobs, R. R.: Review of methods applicable to the assessment of mould exposure to children. *Environ. Health. Persp.* 107, Suppl 3, 473–480 (1999).
- Flannigan, B., McCabe, E. M., McGarry, F.: Allergenic and toxigenic micro-organisms in houses. *J. Appl. Bact. Symp. Suppl.* 70, 61S–73S (1991).
- Garrett, M. H., Raymant, P. R., Hooper, M. A., Abramson, M. J., Hooper, B. M.: Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. *Clin. Exp. Allergy* 28, 459–467 (1998).
- Husman, T.: Health effects of indoor-air microorganisms. *Scand. J. Work Environ. Health* 22, 5–13 (1996).
- Koch, A., Heilemann, K.-J., Bischof, W., Burge, H., Heinrich, J., Wichmann, H. E.: Analyse von Schimmelpilzsporen im Hausstaub. *Allergologie*, 18, 501–505 (1995).
- Lehmann, I., Seiffart, A., Müller, A., Diez, U., Wetzig, H., Emmrich, F., Borte, M., Herbarth, O.: Suppression of the TH1 cell response by indoor mould exposure. *Immunobiology* 200, 515–516 (1999).
- Miller, J. D.: Fungi as contaminants in indoor air. *Atmos. Environ.* 26, 2163–2172 (1992).
- Müller, A., Herbarth, O.: Toxic effects of extracts from spores of moulds. *Umweltmed. Forsch. Prax* 44, 225 (1999).
- Peat, J. K., Dickerson, J. Li, J.: Effects of damp and mould in the home on respiratory health: a review on the literature. *Allergy* 53, 120–128 (1998).
- Sorenson, W. G.: Fungal spores: Hazardous to health? *Env. Health Persp.* 107 Suppl 3, 469–472 (1999).