MICROBIAL EXPOSURE AND MOLD SPECIFIC SERUM IGG LEVELS OF SYMTOMATIC SCHOOLCHILDREN

A Hyvärinen*, T Husman¹, S Laitinen², T Meklin¹, T Taskinen³, M Korppi⁴ and A Nevalainen¹

¹National Public Health Institute, Kuopio, Finland
²Kuopio Regional Institute of Occupational Health, Kuopio, Finland
³Kiuruvesi Health Care Center, Kiuruvesi, Finland
⁴Kuopio University Hospital, Kuopio, Finland

ABSTRACT
The association between serum mold-specific IgG levels of 181 primary schoolchildren with asthma or wheezing or cough symptoms and the microbial exposure were determined. The study was conducted in a school with mold damage and in another without such damage. Microbial exposure was characterized with environmental sampling. Serum IgG antibody concentrations to 20 microbial strains were determined with ELISA. There was an association between elevated serum IgG levels to Penicillium notatum and moisture damage in the school. In addition, moisture damage when present both in school and at home was associated with elevated IgG levels to Penicillium notatum and Eurotium amstelodami. These observations were in concordance with microbial findings in the index school. No other positive associations between IgG antibodies and microbial findings of the school buildings were observed; in fact, the microbe-specific IgG levels of children were often higher in the reference school.

INDEX TERMS
Microbial exposure, Moisture damage, IgG, Schoolchildren, Respiratory symptoms

INTRODUCTION
Technical or self-reported observations of dampness and mold in buildings have often been used as criteria for indoor microbial exposure in epidemiological studies (Peat et al. 1998). In short-time air sampling of viable microbes differences in concentrations and composition of microbes have been associated with moisture problems in a building (Hyvärinen et al. 1993, Hyvärinen et al. 1999). However, a linear association between symptoms and concentrations of viable fungi or bacteria is seldom found (Verhoeff and Burge 1997).

An indirect monitoring of the exposure with serum microbe-specific immunoglobulin G (IgG) antibodies represents an attractive possibility. Mold-specific IgG levels have been shown to be a competent tool for exposure assessment in occupational environments with high microbial concentrations, such as in sawmills and agriculture (Eduard et al. 1992, Erkinjuntti-Pekkanen 1996). However, there is a lack of knowledge whether serum mold-specific IgG actually reflects exposure in sites with much lower bioaerosol concentrations e.g. in schools and private homes. The aim of this study was to evaluate whether there is an association between serum mold-specific IgG levels of schoolchildren and the microbial exposure in their school environment.

* Contact author email: anne.hyvarinen@ktl.fi
MATERIAL AND METHODS

Buildings studied
The study was conducted in two primary schools, which were categorized as a moisture damaged (index) school and a non-damaged (reference) school based on observations of moisture and mold in a thorough technical investigation by civil engineers. The standardized protocol has been described in detail by Nevalainen et al. (1998). The index school had frequent signs of moisture in its structures, mostly due to leaks in roofs and plumbing, missing or inadequate drainage and construction defects in the insulation. Visible mold was also observed in some parts of the school. The reference school had a few minor signs of moisture, assessed to be consequences of normal aging of the building.

Study population
A total of 212 children with respiratory symptoms, 170 from the index school (41 %) and 42 from the reference school (20%) were selected (Taskinen et al. 1999). From these, 181 (85%) children gave a blood sample for immunoglobulin G analyses and thus formed the cohort of this study; 148 (82%) came from the index school and 33 (18%) from the reference school.

Microbial characterization
Concentrations of airborne mesophilic fungi were determined with six-stage impactors (Andersen 10-800) on 2% malt extract agar (MEA) (Biokar, Beuvais, France) with streptomycin and on dichloran glycerol agar (DG18) (Oxoid, Basingstoke, England) with chloramphenicol. Tryptone yeast glucose (TYG) medium (Difco, Le Ponte Claix, France) with cycloheximide was used for mesophilic bacteria. Samples (10 min) were taken in the middle of the room at a height of 1-1.5 m in 20 different rooms in the index school and in 17 rooms in the reference school. Sampling was performed during the school day with normal activity and during wintertime when the outdoor air microbial levels are low due to snow cover (Reponen et al. 1992). Samples for fungi were incubated at 25 ºC for 7 d. Fungal colonies were identified morphologically at genus using an optical microscope. In addition, Aspergillus versicolor, Aspergillus fumigatus, Aspergillus niger and Aspergillus glaucus were identified at the species level. Bacterial samples were incubated in the dark at 20-23 ºC for 5 d and the total number of colonies was counted. The number of dryish actinobacteria–type colonies was counted after 14 days. Concentrations (colony forming units per cubic meter, cfu/m³) were calculated using flow rate, sampling time and the correction table for multiple impactions on individual sites (Andersen 1958).

Surface samples were taken from both index (n=23) and reference (n=14) buildings and samples of damaged building materials (n=23) during the dismantling and renovation of the index building. Samples from damaged surfaces were taken with a sterile swab into sterile Tween 80 dilution buffer (distilled water with 42.5 mg/L KH₂PO₄, 250 mg/L MgSO₄ x 7H₂O, 8 mg/L NaOH and 0.02% Tween 80 detergent). Reference samples were taken from corresponding, undamaged, clean surfaces. The sampling area was 100 cm² for damaged surfaces and 200 cm² for undamaged surfaces. A series of dilutions were prepared and plated on MEA, DG18 and TYG as in the air sampling. Building material samples were weighed, homogenized and extracted with sterile Tween 80 dilution buffer. Suspensions were held in an ultrasonic bath for 30 minutes and in a shaker for 60 minutes. Dilution series were made and plated on MEA, DG18 and TYG media as described above.

Antibody determination
The serum samples were stored at –20 ºC until all the serum samples were collected. The samples were analyzed blinded in a random order. Serum IgG antibodies were determined to
19 fungal strains, and three 1 actinobacterial strain, including the most common molds found in moisture damage buildings. Intracellular antigens for antibody determination were prepared from microbial cultures as described by Laitinen et al. (1999). The working dilutions of antigens were determined from the titration curves for each microbe separately by using IgG positive sera diluted 1:100. Serum immunoglobulin G (IgG) antibody concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) (Laitinen et al. 1999). In the ELISA, the absorbance values of a test serum were compared to those of a pooled control serum collected from 321 adults positive to a few molds/microbes. The control serum was used on each microtiter plate. Absorbances produced by a test serum are expressed as percentages from the absorbances of the corresponding microbes produced by the positive reference serum.

Statistics
Concentrations of fungi and bacteria were not normally distributed and the data were analyzed with non-parametric tests. Differences in fungal and bacterial concentrations between the index and reference schools were analyzed using Wilcoxon’s test. The differences in the occurrence of low/elevated mold specific IgG-levels were first tested with chi-square and Fisher’s exact tests and the effects of confounding factors were adjusted with a logistic regression analysis. A serum antibody level was defined as elevated if it exceeded the 75 percentile of IgG concentrations for the corresponding microbe in the whole present study population. The SAS statistical was used for all analyses (SAS 1990).

RESULTS
Microbial results
Total concentrations of airborne viable fungi varied between <4 – 130 (GMMEA=21 cfu/m³, GMDG18=24 cfu/m³) and <4 - 250 cfu/m³ (GMMEA=4 cfu/m³, GMDG18=7 cfu/m³) in the index school and reference school, respectively. Total concentrations of viable fungi, and the concentrations of *Penicillium* and yeasts grown on both media were significantly higher in the index school than in the reference school. Concentrations of actinobacteria were <11 cfu/m³ in both schools (GM_index=0.7 cfu/m³, GM_reference=0.1 cfu/m³).

The occurrence of fungal genera, to which IgG antibodies were determined, in indoor air, surface and building material samples are presented in Table 1. All of the fungal types of the reference school were also found in the index school. In addition to the fungi in the Table 1, there were fungi that were only detected in the index school: *Alternaria, Aspergillus niger, Chrysosporium, Exophiala, Hyalodendron, Monocillium, Mucor, Oidiodendron, Olpitrichum, Phialophora* and *Scopulariopsis*.

Microbe-specific immunoglobulin G antibodies
More children with elevated antibody levels came from the reference school than from the index school (Table 1); the difference was significant for *Stachybotrys chartarum* and *Rhodotorula glutinis*. In contrast, 28 children in the index school had elevated IgG levels to *Penicillium notatum* and *Eurotium amstelodami* (p=0.052 vs reference school).

Some compatibility between elevated serum IgG levels of *Penicillium notatum* and *Eurotium amstelodami* (Table 1) and measured microbial exposure was observed in higher levels of *Penicillium* and the more frequent occurrence of *Eurotium* in the index school (Table 1). Instead, the higher IgG levels to *Stachybotrys chartarum, Rhodotorula glutinis* (Table 1), in the reference school were not in concordance with microbial findings (Table 1).
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<th>Microbe</th>
<th>Index</th>
<th>Reference</th>
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<td>Air</td>
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<td>Actinobacteria</td>
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<td>Aspergillus versicolor</td>
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<td>Aureobasidium pullulans</td>
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<td>Cladosporium</td>
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<td>Paecilomyces</td>
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<td>Rhizopus</td>
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<td>Sphaeropsidales group</td>
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<td>Sporobolomyces</td>
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<td>Tritirachium</td>
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<td>Yeasts (white and pink)</td>
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<sup>A</sup> = A serum antibody level was defined as elevated if it exceeded the 75 percentile of IgG concentrations for the corresponding microbe in the whole study population; <sup>B</sup> = no material samples; <sup>1</sup> = Streptomyces albus; <sup>2</sup> = Acremonium atrogriseum; <sup>3</sup> = Penicillium notatum; <sup>4</sup> = Phoma macrostoma (belongs to Sphaeropsidales group); <sup>5</sup> = Rhodotorula glutinis (belongs to yeasts)

<sup>*</sup> p<0.05 versus the index school children; <sup>0</sup> p=0.05 versus the reference school children
The associations between moisture damage in school, at home or at both places and mold-specific IgG levels were analyzed also with the logistic regression. Age, gender, housing mode, having pets, passive smoking, asthma and atopy (allergic rhinitis, allergic conjunctivitis or atopic eczema) were included in the model. The analyses confirmed the significant association between moisture damage in the school and elevated IgG levels to Penicillium notatum \((p<0.05)\). In addition, elevated IgG to Penicillium notatum \((p<0.01)\) and Eurotium amstelodami \((p<0.05)\) were significantly associated with moisture damage both in the school and at home. The negative associations between elevated IgG to Stachybotrys chartarum or Rhodotorula glutinis and moisture damage in the school remained significant.

**DISCUSSION**

The two school buildings were defined as a moisture damaged (index) school and as a reference school according to objective technical investigations with a structured protocol performed by a civil engineer (Nevalainen et al. 1998). According to the results of microbial measurements, concentrations of airborne viable fungi were higher in the index than in the reference school. In addition, the index school had higher concentrations of Penicillium and yeasts, and a larger diversity of fungal genera.

There were only two IgG findings that supported the hypothesis that indoor exposure increases mold-specific IgG levels: the higher proportion of children with elevated IgG levels in the index school to Penicillium notatum and Eurotium amstelodami. This is in line with preliminary findings among adults published by Makkonen et al. (2001). On the contrary, most of the IgG-levels in the reference schoolchildren with no school-related exposure in the sense of the microbial findings were similar to the index school, or even higher as was seen in IgG to Stachybotrys chartarum and Rhodotorula glutinis. Hence, it can be concluded that elevated IgG-levels of schoolchildren with respiratory symptoms were predominantly not associated with measured microbial exposure or observed technical findings of moisture damage in the school building. This finding is supported by similar studies concerning homes and schools (Hyvärinen et al. 1999, Taskinen et al. 2001), and in a study carried out in an office environment (Malkin et al. 1998). However, this disagrees with observations performed in adults working in environments such as in agriculture and sawmills (Eduard et al. 1992, Erkinjuntti-Pekkanen 1996, Bünger et al. 2000), and among workers of a water-damaged military hospital (Seuri et al. 2000), where exposure to mold has been related with serum IgG levels.

The study population was selected based on respiratory symptoms and therefore, the results cannot be extrapolated to the general child population. The IgG levels found in the serum reflect the total life-time exposure to microbes. The development of IgG antibodies is not sufficiently known. Young children spend only about 4-6 hours per weekday in the school, and so, other exposures, especially at home, outdoors and in previous life may play an important role in the development of mold-specific IgG antibodies.

In conclusion, an association between elevated serum IgG antibodies to Penicillium notatum and moisture damage in the school was found among schoolchildren with respiratory symptoms and allergic diseases. In addition, there were associations between elevated IgG levels to Penicillium notatum and Eurotium amstelodami and moisture damage when present both in school and at home. Serum IgG antibodies in the children to other microbes did not, however, correlate with the microbial findings of the school buildings. Measurement of IgG antibodies from schoolchildren cannot be recommended as a routine method for assessment of building moisture-related microbial exposure.
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REFERENCES