

Microbial Exposure and Mold-Specific Serum IgG Levels among Children with Respiratory Symptoms in 2 School Buildings

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ABSTRACT. In this study, the authors determined the association between serum mold-specific immunoglobulin G (IgG) levels in primary school children ($N = 181$) with asthma, wheezing, or cough symptoms and exposure to indoor mold in 2 schools, with and without mold damage. Microbial exposure was determined with environmental sampling. Serum IgG antibody concentrations against 24 microbial strains were determined with enzyme-linked immunosorbent assay. Children's microbe-specific IgG levels were often higher in the reference school. There was an association between elevated serum IgG antibody levels to *Penicillium notatum* and moisture damage in the school. In addition, moisture damage both in school and at home was associated with *Penicillium notatum* and *Eurotium amstelodami* IgG levels. These observations comported with microbial findings in the index school. No other positive associations between IgG antibodies and microbial findings for the school buildings were observed. IgG levels in sera of school children did not provide a specific means for assessing the microbial exposure associated with moisture and microbial problems in the school buildings. Thus, IgG analysis cannot be readily suggested as a routine method for the evaluation of these exposures.

<Key words: children, IgG antibodies, indoor air, mold, respiratory symptoms>

DAMPNESS AND MOLD in buildings are criteria for implementing epidemiologic studies of microbial exposure in indoor air.^{1,2} These exposures are often estimated with short-time air sampling of viable microbes. The types and concentrations of viable microbes may indi-

cate moisture and mold problems in a building,^{3,4} but a linear association between respiratory symptoms and concentrations of viable fungi or bacteria is seldom found.⁵ Cellular mechanisms of the effects of microbial exposure, or even the causative agents, are not suffi-

ciently known to permit a dose-response assessment. Hence, exposure assessment methods should still be aimed at identifying the most critical exposing agents.

Instead of estimating microbial exposure by environmental sampling, the biomonitoring of exposure with serum microbe-specific immunoglobulin G (IgG) antibodies represents an attractive possibility. Mold-specific IgG levels are a competent tool for exposure assessment in occupational environments with high microbial concentrations, such as sawmills and agriculture.^{6,7} There is a lack of knowledge about whether serum mold-specific IgG reflects exposures in sites with lower bioaerosol concentrations (e.g., schools and homes).

In this study, we evaluated whether there is an association between serum mold-specific IgG levels of school children and microbial exposure in their school environment. The underlying study hypothesis was that the indoor air of a mold-contaminated school is an interface where microbial exposure takes place, thus gradually inducing the production of IgG antibodies to the corresponding fungi. Crude extracts of the most important indoor fungi and actinobacteria were used as antigens to simulate the real exposure situation of the school environment with a diverse mycoflora.

Materials and Method

Buildings studied. The study was conducted in 2 primary schools located in a community in middle Finland. The schools were categorized as a moisture-damaged (index) school and a nondamaged (reference) school on the basis of observations of moisture and mold in a thorough technical investigation by civil engineers. The standardized protocol used has been previously described in detail.⁸ The index school had many signs of moisture in its structures. These signs resulted primarily from 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, but these signs were assessed as consequences of normal aging of the building. Thus, the index school was assessed as a building having moisture and mold problems, whereas the reference school was assessed as being in normal condition.

The index school—the main part of which was erected in 1952—was a brick building with a gable roof and natural ventilation. Its enlargement in 1968 included a flat roof; the total floor area was 5,520 m². The reference building, originally built in 1977 and enlarged in 1988, was made of wood and concrete. It has a flat roof, mechanical air supply/exhaust, and a floor area of 3,020 m².

Study population. A symptom questionnaire was originally distributed to all 815 students in the index and reference schools. Collectively, 622 children (index = 414,

reference = 208) returned a completed questionnaire (response rate = 76%).⁹ On the basis of the answers in the questionnaires, we invited a child to participate in the clinical study if he or she had doctor-diagnosed asthma or if he or she had experienced wheezing or cough symptoms during the previous 12 mo. A total of 212 children, 170 of the 414 from the index school (41%) and 42 of the 208 from the reference school (20%), were selected as potential participants. Of these, 181 children (85%) gave a blood sample for IgG analyses and thus formed the cohort for this study. Thus, the cohort comprised 148 children (82%) from the index school and 33 children (18%) from the reference school.

Most of the children (> 80%) lived in either a single-family house or a detached house. Both moisture damage and a musty or moldy smell in the home were reported by 7% of the children attending the index school, whereas the corresponding percentages in the homes of the children attending the reference school were 3% and 9%, respectively. More than 50% of children from the index school and 30% of children from the reference school had pets. Passive smoking (13%) was equally common among children from both schools.

Microbial characterization. Concentrations of airborne mesophilic fungi were determined with 6-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. MEA allows the growth of the most common fungi in indoor environments, and DG18 favors the growth of xerophilic fungi (i.e., those preferring a drier growth medium). The use of these 2 media afforded detection of the most important fungi in indoor environments.¹⁰ Tryptone yeast glucose (TYG) medium (Difco [Le Pont de Claix, France]) with cycloheximide was used for mesophilic bacteria.¹¹

Samples were taken in the middle of the room at a height of 1 to 1.5 m in 20 different rooms in the index school, and in 17 rooms in the reference school. The sampling time was 10 min. Sampling was performed during the school day with normal activity, and during wintertime when the contribution of outdoor air to the microbial levels of indoor air is negligible as a result of snow cover.¹² Samples for mesophilic fungi were incubated in the dark at 25 °C for 7 days. The genera of the fungal colonies were identified morphologically with 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 °C for 5 days, after which the total number of colonies were 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.¹³

Surface samples were taken from both index ($n = 23$) and reference ($n = 14$) buildings, and samples of damaged building materials ($n = 23$) were taken during the dismantling of the damaged structures and renovation of the index building. Surface samples were taken with a sterile swab that was placed into sterile Tween 80 dilution buffer (distilled water with 42.5 mg/l potassium dihydrophosphate, 250 mg/l magnesium sulfate $\cdot 7 \text{ H}_2\text{O}$, 8 mg/l sodium hydroxide, and 0.02% Tween 80 detergent). The sampling area was 100 cm² for damaged surfaces and 200 cm² for undamaged surfaces. A series of dilutions was prepared and plated on MEA, DG18, and TYG, as was the case 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 min and in a shaker for 60 min. Dilution series were made and plated on MEA, DG18, and TYG media.

Health questionnaires. The questionnaire was based on the Örebro-questionnaire (MM-40),¹⁴ with additional questions on respiratory infections (bronchitis, common cold, otitis, pneumonia, sinusitis, tonsillitis),¹⁵ use of health care services, and background factors (e.g., housing conditions, parental professions, passive smoking, pets). The questions about asthmatic symptoms (dyspnea, wheezing, attacks with dyspnea and wheezing, prolonged dry cough apart from coughing with a cold or chest infection) and doctor-diagnosed asthma were similar to those used in the study by Timonen.¹⁶ The questionnaires were filled in by the parents.

Antibody determination. The serum samples were stored at -20°C until all 181 samples were collected. After the collection was completed, the antibody determinations were made simultaneously during a 2-wk period. In the laboratory, the samples from the index school and reference school were mixed and analyzed blinded, in random order. Serum IgG antibodies were determined to 21 fungal strains and 3 actinobacterial strains, covering the major microflora of the living environments.

Intracellular antigens for antibody determinations were prepared from microbial cultures as described by Laitinen et al.¹⁷ We determined the working dilutions of antigens from the titration curves for each microbe separately by using IgG positive sera diluted 1:100. Serum IgG antibody concentrations were determined by an enzyme-linked immunosorbent assay (ELISA).¹⁷ With ELISA, the absorbance values of a test serum were compared with those of a pooled control serum. The control serum was used on each microtiter plate. Pooled control sera had been collected from 321 adults positive to a few molds/microbes. Absorbances produced by a test serum are expressed as percentages from the absorbances of the corresponding microbes produced by the positive reference serum.

Ethics. The University Hospital of Kuopio Ethics Committee approved the study protocol. Information about

the study was delivered in open meetings with school personnel and the children's parents. Parents provided written consent for drawing the blood samples from the children.

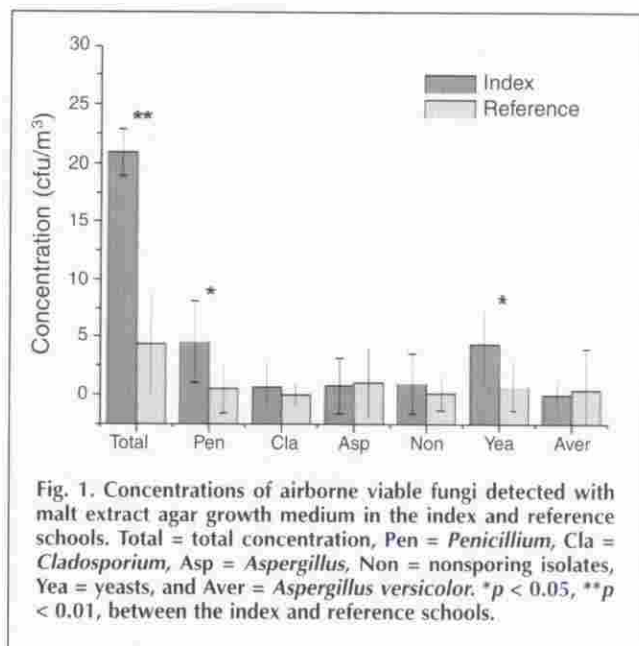
Statistics. Concentrations of fungi and bacteria did not exhibit normal distribution. Nonparametric statistical tests were used, and the data were described with geometric means (GMs) and geometric standard deviations (GSDs). Differences in fungal and bacterial concentrations between the index and reference schools were analyzed with Wilcoxon's test. Correlations between concentrations of total airborne viable fungi and fungal genera obtained from MEA and DG18 growth media were analyzed with Spearman's correlation analysis.

The overall differences in mold-specific IgG levels between the index and reference schools were analyzed with t tests for those with normal distributions (*Penicillium glabrum*, *Acremonium atrogriseum*, and *Trichoderma citrinoviride*) and with the Wilcoxon test for those that did not show normal distribution. The differences in crude rates of dichotomous variables, such as the occurrence of low or 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 75th percentile of IgG concentrations for the corresponding microbe in the entire present study population.

The differences in the occurrence of dichotomous symptoms and infection variables between the index and reference groups were tested with chi-square and Fisher's exact tests. SAS statistical software was used for all analyses.¹⁸

Results

Microbial results. Total concentrations of airborne viable fungi varied between less than 4 and 130 cfu/m³ ($GM_{MEA} = 21 \text{ cfu/m}^3$, $GM_{DG18} = 24 \text{ cfu/m}^3$) and less than 4 and 250 cfu/m³ ($GM_{MEA} = 4 \text{ cfu/m}^3$, $GM_{DG18} = 7 \text{ cfu/m}^3$) in the index school and reference school, respectively. The fungal concentrations on MEA are shown in Figure 1. 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. The results obtained with MEA and DG18 correlated well for total concentrations of viable fungi ($r = .74$) and for *Aspergillus versicolor* ($r = .72$), and moderately well for concentrations of *Penicillium* ($r = .53$) and yeasts ($r = .59$). The concentrations of airborne viable bacteria varied between 71 and 7,600 cfu/m³ in the index school ($GM = 924 \text{ cfu/m}^3$) and less than 4 and 2,100 cfu/m³ in the reference school ($GM = 250 \text{ cfu/m}^3$ [not significant [NS]]). Concentrations of actinobacteria were less than 11 cfu/m³ in both schools ($GM_{index} = 0.7 \text{ cfu/m}^3$, $GM_{reference} = 0.1 \text{ cfu/m}^3$ [NS]).



In the index school, 4 of the 23 surface samples indicated microbial growth (concentration $> 1,000$ cfu/cm²), whereas in all 14 samples from the reference school, the levels were < 200 cfu/cm². Bacterial concentrations in both damaged and undamaged surface samples varied between less than 1 and 12,400 cfu/cm² in the index school. They varied between less than 1 and 450,000 cfu/cm² in the damaged samples and between less than 1 and 5,100 cfu/cm² in the undamaged samples from the reference school. Concentrations of actinobacteria in the index school varied from less than 1 to 820 cfu/cm² on damaged surfaces, and less than 1 to 180 cfu/cm² on undamaged surfaces, compared with less than 1 to 5 cfu/cm² and less than 1 to 59 cfu/cm², respectively, in the reference school.

Fungal concentrations in the 23 damaged building materials taken from the index school during dismantling varied from less than 45 to 9.7×10^5 cfu/gm ($GM_{MEA} = 2,400$ cfu/gm, $GM_{DG18} = 1,800$ cfu/gm). The ranges for total concentrations of viable bacteria and actinobacteria were less than 45 to 11.3×10^6 cfu/gm ($GM = 12,000$ cfu/gm) and less than 45 to 26,000 cfu/gm ($GM = 4$ cfu/gm), respectively. From the reference school, no such samples were available inasmuch as dismantling was unnecessary.

The fungal genera detected in indoor air, surface, and building material samples are presented in Table 1. The main genera detected in air samples of both index and reference schools were *Penicillium* (prevalence in the index and reference schools = 76% and 24%, respectively), *Cladosporium* (39%, 12%), *Aspergillus* (42%, 41%), and yeasts (68%, 26%), which contributed on average 70% of the total concentrations of airborne viable fungi. The difference between the schools was significant in the prevalence of *Penicillium* and yeasts ($p < 0.001$)

and *Cladosporium* ($p < 0.01$). Actinobacteria were found in 26% and 6% of the samples taken from the index and reference schools, respectively (NS). All of the fungal types from the reference school were also found in the index school, although several fungi were detected only in the index school: *Aspergillus niger*, *Chrysosporium*, *Exophiala*, *Hyalodendron*, *Monocillium*, *Mucor*, *Paezilomyces*, *Rhizopus*, and *Scopulariopsis*.

Penicillium was the most frequently found genus on damaged surfaces ($n = 13$) in the index school (MEA and DG18 = 54% and 38%, respectively), together with yeasts (31% and 15%), *Acremonium* (31% and 15%), and *Aspergillus versicolor* (38% and 38%). In the reference school, *Eurotium* was found in all 4 samples (DG18) (Table 2). In all, 25 different fungal types were found in the air or on surfaces of the index school, while 14 fungal types were found in the corresponding samples from the reference school. The main genera observed in building material samples were *Penicillium* (57%, 57%), yeasts (35%, 30%), *Acremonium* (43%, 22%), and *Aspergillus versicolor* (30%, 39%).

Symptoms and respiratory diseases. The 212 children selected as potential study cohort participants had either doctor-diagnosed asthma, wheezing symptoms, or prolonged cough during the previous 12 mo. The prevalence of these health outcomes in the original population was 41% in the index school and 20% in the reference school ($p < 0.001$).⁹ The prevalence of asthma (index = 10%, reference = 27%; $p < 0.01$), allergic rhinitis (index = 28%, reference = 48%; $p < 0.05$), and conjunctivitis (index = 16%, reference = 33%; $p < 0.05$), doctor calls due to asthma (index = 10%, reference = 24%; $p < 0.01$), as well as parent-reported wheezing attacks (index = 12%, reference = 36%; $p < 0.01$), were higher in the reference school than in the index school. Reported allergic diseases were prevalent, especially in the upper grades (4–6) of the reference school. The prevalence of night cough was significantly higher among the children attending the index school, compared with those attending the reference school (81% and 67%, respectively, $p < 0.05$).

Table 2 lists the prevalence of respiratory symptoms (e.g., dry cough, eye irritation, hoarseness, nasal congestion, night cough, nose bleeding, rhinitis, throat symptoms, upcoming phlegm) among subjects. In spring, dry cough and throat symptoms were more prevalent among children from the index school than among those from the reference school ($p < 0.05$). The odds ratios (ORs) for dry cough and throat symptoms in the index school, compared with the reference school, were 2.91 (95% confidence interval [CI] = 1.11, 7.90) and 2.99 (95% CI = 1.14, 8.11), respectively. The symptoms were more common during autumn in both schools. No significant differences were found in the occurrence of respiratory infections between the index group and the reference group (data not shown).

Table 1.—Occurrence of Fungal Genera or Groups and Actinobacteria in Air, Building Material, and Surface Samples in the Index and Reference Schools, in Relation to Number [n(%)] of Children with Elevated Immunoglobulin G (IgG) Levels

Microbe	Index					Reference†			
	Air	Material	Surface	IgG*		Air	Surface	IgG*	
				n	%			n	%
Actinobacteria	xx	x				x			
<i>Streptomyces albus</i>				35	24			11	33
<i>Streptomyces griseus</i>				34	23			12	36
<i>Streptomyces halstedii</i>				35	24			11	33
<i>Acremonium</i> spp.	x	x	x				x		
<i>Acremonium atrogriseum</i>				35	24			11	33
<i>Acremonium kiliense</i>				36	24			10	30
<i>Alternaria</i> spp.			x						
<i>Aspergillus</i> spp.	xx	x	x			xx	x		
<i>Aspergillus fumigatus</i>	x	x		37	25	x		9	27
<i>Aspergillus glaucus</i>	x					x			
<i>Aspergillus niger</i>	x								
<i>Aspergillus versicolor</i>	x	x	x	38	26	x		8	24
<i>Aureobasidium pullulans</i>	x	x	x	37	25	x		9	27
<i>Botrytis</i> spp.	x					x	x		
<i>Cladosporium</i> spp.	xx	x	x	34	23	x		12	36
<i>Chrysosporium</i> spp.	x								
<i>Eurotium</i> spp.	xx	x		42	28‡	x	x	4	12
<i>Exophiala</i> spp.	x	x							
<i>Fusarium</i> spp.		x		39	26			7	21
<i>Geotrichum</i> spp.				34	23			12	36
<i>Hyalodendron</i> spp.	x								
<i>Monocillium</i> spp.	x	x							
<i>Mucor</i> spp.	x	x		36	24			10	30
<i>Oidiodendron</i> spp.		x							
<i>Olpitrichum</i> spp.			x						
<i>Paecilomyces</i> spp.	x	x	x	37	25			9	27
<i>Penicillium</i> spp.	xx	x	x			xx	x		
<i>Penicillium glabrum</i>				40	27			6	18
<i>Penicillium notatum</i>				42	28‡			4	12
<i>Phialophora</i> spp.		x							
<i>Rhizopus</i> spp.	x			37	25			9	27
<i>Scopulariopsis</i> spp.	x	x							
<i>Sphaeropsidales</i> group		x	x				x		
<i>Phoma macrostoma</i>				36	24			10	30
<i>Sporobolomyces</i> spp.				34	23			12	36
<i>Stachybotrys</i> spp.		x		32	22			14	42§
<i>Trichoderma</i> spp.	x	x		37	25	x		9	27
<i>Tritirachium</i> spp.		x	35	24				11	33
<i>Wallemia</i> spp.	xx	x				x			
Yeasts (white and pink)	xx	x	x			xx	x		
<i>Rhodotorula glutinis</i>				32	22			14	42§

Notes: x = found, and xx = found in ≥ 5 air samples.

*A serum antibody level was defined as elevated if it exceeded the 75th percentile of IgG concentrations for the corresponding microbe in the whole study population.

†No material samples.

‡ $p = 0.05$ vs. reference school children.

§ $p < 0.05$ vs. index school children.

Microbe-specific IgG antibodies. In general, mean IgG antibody levels were higher among students from the reference school. The differences, however, were significant only for *Cladosporium cladosporioides*, *Rhodotorula glutinis*, and *Phoma macrostoma* ($p < 0.05$).

The antibody level for each microbe was defined as elevated if it exceeded the 75th percentile, yielding a total of 46 out of 181 school children with elevated IgG. 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* ($p < 0.05$). In contrast, 42 children from the index school had elevated IgG levels to *Penicillium notatum* and *Eurotium amstelodami* ($p = 0.052$ vs. reference school).

To estimate the effect of exposure time, children were divided into 2 groups according to their grade in school: (1) grades 1–3 (7–9 yr of age) and (2) grades 4–6

Table 2.—Prevalence (%) of Respiratory and Eye Irritation Symptoms among the Studied Children in the Index and Reference Schools

Symptom	Autumn				Spring			
	Index school		Reference school		Index school		Reference school	
	n	%	n	%	n	%	n	%
Nasal congestion	118	80*	25	76†	83	56	16	48
Rhinitis	116	78‡	24	73†	91	61	15	45
Throat symptoms	93	63‡	15	45†	66	45§	7	21
Hoarseness	54	36†	8	24	36	24	3	9
Nose bleeding	31	21	11	33	19	13	5	15
Dry cough	82	55†	20	61‡	65	44§	7	21
Upcoming phlegm	46	31	12	36	42	28	7	21
Eye irritation	43	29	7	21	36	24	7	21

* $p < 0.001$ (autumn vs. spring).

† $p < 0.05$ (autumn vs. spring).

‡ $p < 0.01$ (autumn vs. spring).

§ $p < 0.05$ (index school vs. reference school).

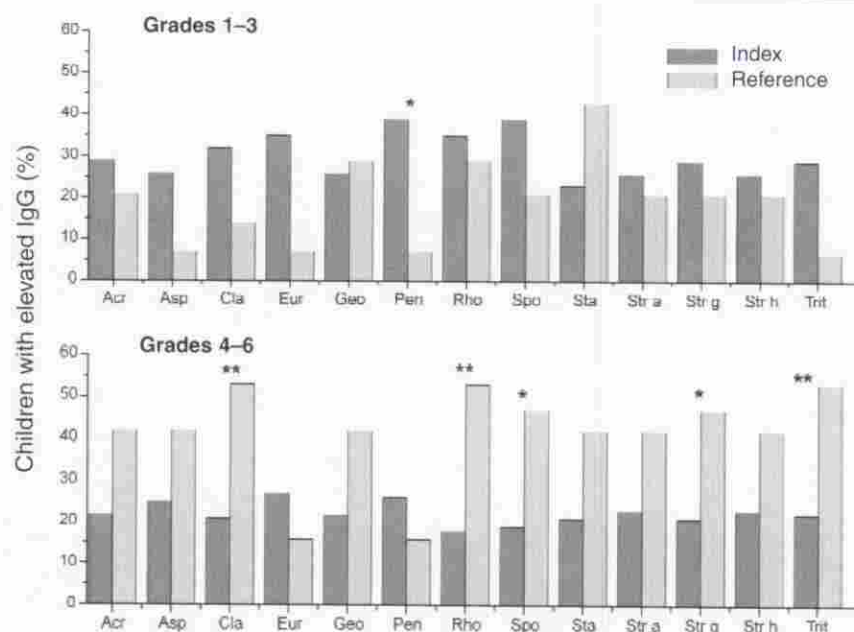


Fig. 2. Percentages of children with elevated immunoglobulin G (IgG) levels for 13 microbes having the largest differences between the schools. Acr = *Acremonium kiliense*, Asp = *Aspergillus fumigatus*, Cla = *Cladosporium cladosporioides*, Eur = *Eurotium amstelodami*, Geo = *Geotrichum candidum*, Pen = *Penicillium notatum*, Rho = *Rhodotorula glutinis*, Spo = *Sporobolomyces salmonicolor*, Sta = *Stachybotrys chartarum*, Str a = *Streptomyces albus*, Str g = *Streptomyces griseus*, Str h = *Streptomyces halstedii*, Trit = *Tritirachium roseum*. * $p < 0.05$, ** $p < 0.01$, between the index and reference schools.

(10–12 yr of age). Figure 2 presents the largest differences between schools for the percentages of children with elevated IgG levels to 13 microbes. The number of children with elevated *Penicillium notatum* IgG levels was significantly higher in the index school than in the reference school among grades 1–3. In comparison, the numbers of children with elevated IgG to *Rhodotorula glutinis*, *Cladosporium cladosporioides*, *Sporobolomy-*

ces salmonicolor, *Tritirachium roseum*, and *Streptomyces griseus* were higher in the reference school among children in grades 4–6.

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* (Fig. 1) and the more frequent occurrence of *Eurotium* in the

index school (Table 1). In contrast, the higher IgG levels to *Stachybotrys chartarum*, *Rhodotorula glutinis* (Table 1), *Sporobolomyces salmonicolor*, *Tritirachium roseum*, and *Streptomyces griseus* (Fig. 2) in the reference school did not comport with microbial findings (Table 1).

Associations between individual mold-specific IgG levels and housing mode, ownership of pets, and passive smoking showed a few significant differences, with no apparent consistency in the results (data not shown). Likewise, associations between mold-specific IgG levels and doctor-diagnosed asthma, other reported atopic diseases, and skin test positivity to common aeroallergens were analyzed. The results showed inconsistent associations in different age groups and schools, and for different microbes. For example, doctor-diagnosed asthma was significantly associated with high IgG levels to several microbes: *Fusarium oxysporum* ($p < 0.01$), *Acremonium atrogriseum* and *Trichoderma citrinoviride* ($p < 0.05$), and *Sporobolomyces salmonicolor* ($p = 0.057$) among the older children (grades 4–6) in the reference school (data not shown).

The associations between moisture damage in school, at home, or at both places and mold-specific IgG levels were analyzed with logistic regression. Age, asthma, and atopy (allergic conjunctivitis or rhinitis, or atopic eczema), gender, having pets, housing mode, and passive smoking were included in the model. The associations were analyzed for individual microbes and for a combined microbe variable (elevated levels of IgG to any 7 or more of the microbial strains studied).

The analyses revealed a significant association between moisture damage in the school and elevated IgG levels to *Penicillium notatum* ($p < 0.05$). In addition, elevated IgG levels to *Penicillium notatum* ($p < 0.01$) and *Eurotium amstelodami* ($p < 0.05$) were associated significantly with moisture damage both in the school and at home. Significant negative associations, however, were observed between elevated levels of *Stachybotrys chartarum* or *Rhodotorula glutinis* and moisture damage in the school ($p < 0.05$). The negative associations between high IgG levels of *Sporobolomyces salmonicolor*, *Tritirachium roseum*, and *Streptomyces griseus* and microbial exposure seen in the crude analyses disappeared in the model. Passive smoking was associated positively with elevated levels of *Phoma macrostoma* ($p < 0.05$). Children with asthma often had elevated IgG levels to *Geotrichum candidum* (OR = 2.5, 95% CI = 0.9, 6.8) or to any of 7 combined microbial strains (OR = 2.3; 95% CI = 0.9, 6.3).

Discussion

According to the findings of both technical and microbiological investigations, the index school was moisture- and mold-damaged. We found 2 IgG levels

supporting the hypothesis that indoor exposure increases IgG levels: elevated IgG levels in the index school, compared with the reference school, for *Penicillium notatum* and *Eurotium amstelodami*. These findings comport with preliminary findings among adults published by Makkonen et al.¹⁹ On the other hand, we found that most of the IgG levels in the reference school—with no excess exposure to the microbes—were similar to those for the index school. We found elevated serum IgG levels to *Stachybotrys chartarum* and *Rhodotorula glutinis* in children of all age groups, as well as to 4 other microbes in older children in the reference school. Hence, we concluded that elevated IgG levels for children with respiratory symptoms were not predominantly associated with measured microbial exposure or observed technical findings of moisture damage in the school building. This finding is supported by the results of similar studies sited in schools and homes,^{4,20} and in a study carried out in an office environment.²¹ Our findings contrast with those obtained from adults working in agriculture and sawmills, where microbial levels are high.^{6,7} Thus, the weak compatibility between the microbial findings and mold-specific IgG levels of the current study may result in part from low levels of airborne fungi in the schools (< 250 cfu/m³).

Cough and throat symptoms were associated with mold problems in the school, even though IgG levels were low among the children in the index school. The association between mold exposure and night cough is of special importance, since night cough is known to often precede the development of asthma.²² In general, we found irritation symptoms more prevalent in the index school than in the reference school. In addition, the differences between the index and reference schools were clearer in spring than in autumn, a finding that suggested that environmental exposures during the school year might have influenced our results. This trend was present despite the fact that, in this cohort, asthma and allergy were more prevalent in the reference school, a fact that may have biased our results with higher mold-specific IgG antibodies in the reference population. On the other hand, the results did not change when the analysis was performed in a multivariable model adjusting for asthma and atopy. The association of respiratory symptoms with indoor exposure to building-related moisture and mold is consistent with previous studies.^{23–25} Recently, B nger et al.²⁶ showed an association between airway and skin diseases and increased IgG antibody concentrations to molds.

The duration of exposure, measured as the number of years spent in the school, was not associated with serum IgG levels. For individual microbes, elevated IgG antibody levels to *Penicillium notatum* were associated with moisture damage in the school, and elevated IgG

levels to *Penicillium notatum* and *Eurotium amstelodami* were associated with the presence of moisture damage both in the school and at home. These microbes were more abundant in the index school; *Penicillium* was found at higher levels, and *Eurotium* was found more frequently. Moisture damage in the school was associated negatively with elevated IgG levels of *Stachybotrys chartarum* and *Rhodotorula glutinis*. Both of these microbes are moisture-indicative fungi,¹⁰ and *Stachybotrys* was present only in the index school. These observations were consistent, being the same in both univariate and multivariate analyses, after adjusting for potential confounding factors. We concluded that the mold-specific IgG levels among the children in our cohort did not reflect the moisture and mold-related exposures in their school environments with sufficient sensitivity.

Previous findings have demonstrated that active and passive smoking can suppress the immune response to inhaled antigens.^{20, 27} In this study, passive smoking was associated positively with elevated levels of *Phoma macrostoma*. No other associations were found.

The study population was selected according to respiratory symptoms and, therefore, the results cannot be extrapolated to the general child population. The selection process led to a high proportion of children with asthma in this cohort, especially among children in the reference school. The development of IgG antibodies may possibly occur during the early years of childhood. Children spend only about 4–6 hr each weekday in school, so their additional exposures, especially at home or outdoors, as well as previous exposures in daycare, may play a role in the development of mold-specific IgG antibodies. Although a large panel of 24 microbes was available in the IgG measurements, the panel included only 75% of the molds found in the school buildings. The large IgG panel is essential, given that many molds (32 in the present study) exist in damaged buildings. Their importance as exposing agents is poorly understood.

An association between elevated serum IgG antibody levels to *Penicillium notatum* and moisture damage in the school was found among school children with respiratory symptoms and allergic diseases. In addition, there were associations between elevated IgG levels to *Penicillium notatum* and *Eurotium amstelodami* and moisture damage both in school and at home. The children's levels of IgG antibodies against other microbes did not, however, correlate with the microbial findings for the school buildings. We conclude that the serum IgG levels of the school children in our cohort did not specifically assess the microbial exposure associated with moisture and microbial problems of the school buildings tested. IgG analysis, therefore, cannot be readily suggested as a routine method for the evaluation of such exposure.

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