

Original article

Immunoglobulin G antibodies to moulds in school-children from moisture problem schools

Background: The purpose of the present study was to evaluate mould-specific immunoglobulin G (IgG) antibodies in children exposed to moisture and mould problems in their school, and the association between IgG antibodies and mould allergy, active or passive smoking and respiratory symptoms.

Methods: IgG antibodies were studied to 24 moulds in 93 children from three moisture problem schools and in 33 children from a reference school. The antibodies were measured by enzyme-linked immunosorbent assay and compared to positive adult sera.

Results: There were no significant differences in mould-specific IgG concentrations between exposed and non-exposed school-children. Antibodies to moulds common in moisture damaged buildings were associated with allergic diseases, as well as with mould-specific immunoglobulin E (IgE) or skin prick test (SPT) findings. *Aspergillus fumigatus* and *A. versicolor* were the moulds with the most consistent findings. Active and passive smoking were associated with low levels of antibodies to many moulds. Though the association between asthma, wheezing or cough symptoms, and IgG to moulds was not significant, 7 (39%) of the 18 children with multiple (> 7) elevated IgG findings suffered from asthma or wheezing.

Conclusions: Allergy was, but asthma was not, associated with IgG antibodies to the moulds that can be found in moisture damaged buildings. However, no association was found between IgG antibodies to moulds and exposure to moisture and moulds in school.

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Moisture problems in a building can easily lead to fungal growth within its structures, exposing the inhabitants to spores and other mould products (1, 2). The association between moisture problems in the buildings, mould growth in the structures and respiratory symptoms of the inhabitants has been documented in several epidemiological studies (1, 3, 4). However, the mechanism by which mould exposure leads to clinical manifestations has thus far remained obscure (5–9). In recent clinical studies it has been found that allergy to moulds is rare in children, and that the symptoms associated with moisture and mould exposure are not caused by IgE-mediated allergy (1, 9–12). Instead, signs of nonspecific inflammatory responses have been reported (1, 8, 13–15).

The association between exposure to airborne fungi and the development of IgG antibodies has been shown in adults in occupational studies (16–18). In sero-epidemiologic studies, mould-specific IgG antibodies have been used as biomarkers of mould exposure (17, 19, 20). In adults, serum microbe-specific IgG antibodies have been reported in association with exposure levels above 10^4 cfu/m³ by methods assaying viable fungi and

above 10^5 spores/m³ by methods assaying non-viable fungi (19, 21). In public buildings like schools the microbial concentrations in indoor air rarely reach such levels (22, 23). Despite this, the indoor exposures associated with moisture and mould problems in schools and public buildings have been sufficient to induce serum IgG (20) and other immunological responses (8, 24, 25) in long-term users of these buildings. It is not known whether mould exposure in a moisture problem school can be assessed by IgG responses in the school-children. The aim of the present study was to evaluate IgG antibodies to moulds in children from moisture problem schools, with special emphasis on allergy, active or passive smoking and respiratory symptoms.

Material and methods

Study population

Originally, 133 school-children aged 7–13 years attended the clinical study in November 1994 (12). There were 99 children from three moisture problem (index) schools and 34 from a control school, forming 96% and 92% of all pupils attending these schools,

respectively. The study design, including enrolment of children and collection of clinical and exposure data, has been published earlier in more detail (10).

Moisture and mould growth documentation

A questionnaire study and technical investigations of the buildings, including microbiological studies, were carried out after the clinical study in September 1994 in all four study schools (10). All buildings were investigated by civil engineers from our study group, using a standardized protocol with special reference to water damage and visible signs of fungal growth (10). The personnel of each school were interviewed for the damage history (26). The technical investigations were the primary methods of classifying the schools into damaged or reference schools. Concentrations and flora of viable microbes were determined from air, surface and material samples of the buildings. The details of the microbial and damage characterization of the school buildings have been reported elsewhere (10, 26). In brief, the highest concentrations of viable airborne fungi were 140, 150 and 530 cfu/m³ in the air of the three index schools, compared to 160 cfu/m³ in the control school. The fungal flora found in the index schools and in the control school consisted of 31 and 20 different genera, respectively. They formed 68% and 47% of the genera with mould-specific IgG determinations available (see Table 1). There were three wooden buildings in the index schools and, based on inspection data, the moisture problems had been caused by either water leaking through the roofs, burst water pipes under the floor, missing or inadequate drainage or construction flaws in the insulation. Visible fungal growth was found on indoor surfaces in two index schools. The reference concrete school was similar in size to the others, and its construction was similar to the index schools. No moisture problems or fungal growth were observed during a detailed examination (10, 26).

Clinical and questionnaire data

One of the authors (T.T.) interviewed the parents by using a standardized questionnaire and examined clinically all the children, as described earlier (10). Chronic, prolonged or repeated respiratory manifestations were present in 47 children and were classified into three groups: asthma ($n=9$), wheezing symptoms with no asthma ($n=17$) and cough symptoms with no asthma or wheezing ($n=21$) (10). Allergic diseases were present in 27 children and were classified into allergic rhinoconjunctivitis ($n=13$) and atopic dermatitis ($n=19$) (10, 27). In addition, a detailed questionnaire was used to obtain information from the parents on any moisture problems, mould odour or visible fungal growth in their homes. Details about active smoking were covered in the children's questionnaire, and details about passive smoking were covered in the parents' questionnaire.

As described previously (10), skin prick tests (SPT) were performed in all 133 cases with 11 common inhalation allergens and with 13 commercially available fungal allergens. Among them, *Aspergillus fumigatus*, *Fusarium roseum*, *Phoma herbarum* and *Rhodotorula rubra* were considered as moulds indicating moisture damage (28–30). SPT wheal responses to moulds were positive (3 mm) in 6 children and weak (1–2 mm) in 12 children (10, 31). Serum IgE determinations were performed in 54 children by enzyme immunoassay to 10 mould allergens, including children with positive, weak and negative SPTs to moulds (32). The moisture indicative moulds were *Aspergillus fumigatus*, *Fusarium moniliforme* and *Phoma betae* (28–30). The IgE concentration was elevated (>0.35 IU/ml) in seven children (32).

Subjects

In the present study, IgG antibodies were analysed to 24 microbes in 126 children from the original cohort of 133 children, consisting of 93 children from the moisture problem schools and 33 from the control school (10). The proportion of boys was 53%. The age distribution

was as follows: 29% were 7–8 years old, 33% were 9–10 years old, and 38% were 11–13 years old. Sixteen (13%) of these children reported moisture and/or mould problems at home and 83 (66%) lived on farms. Serum samples from seven children were not available; none of them had asthma, one child had wheezing symptoms and three had cough symptoms; one child had allergic dermatitis, but none had allergic rhinoconjunctivitis or positive SPT reactions to common allergens or to moulds.

Immunoglobulin G antibodies to moulds

IgG antibodies to 24 different microbes were determined from the sera of 126 children by ELISA (see Table 1). The moulds were selected based on the microbiology of Finnish, water-damaged buildings (2, 33, 34). Crude antigen extracts were prepared from cultures obtained originally from Finnish environmental samples, with the exception of *Streptomyces griseus*, which was originated from the UK. Before manufacturing the antigen extracts, the identification of fungal species and yeasts was assured by the international reference laboratory (Centraalbureau voor Schimmelcultures, Baarn, the Netherlands). *Streptomyces halstedii* was identified by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

The selected microbes were grown on agar plates at +25°C. The purity of the fungal isolates was verified before transferring them into peptone broth (2% malt extract, 1% mycological peptone, 4% glucose in sterile water). After incubation for 7 days, the cultures were autoclaved and separated from the fluid by filtration or by centrifugation. The cultures were washed with phosphate buffered saline (PBS), homogenised, and then treated by ultrasonification. The homogenates were centrifuged for 30 min at 23 300 g. After filtration through a filter of pore size 0.45 µm, the supernatants were stored at –70°C before being used as antigens. The working dilution of antigens was determined from the titration curves for each mould separately by using IgG positive sera diluted 1 : 100.

For the ELISA, the microtiter plates (Nunc Immuno Plate, Roskilde, Denmark) were coated with 200 µl/well of the antigen extract in phosphate buffered saline (PBS; pH 7.4), incubated at +37°C for 6 h, and then washed three times with deionized water. Serum samples diluted 1 : 100 in 10% FBS (10% fetal bovine serum in PBS) were added in a volume of 200 µl/well and the plates were incubated at +37°C for 2 h. After washing the wells twice with 0.05% Tween-20 in PBS and once with deionized water, alkaline phosphatase conjugated antihuman IgG (Sigma, Glostrup, Denmark) in 10% FBS was added in a volume of 200 µl/well at a dilution of 1 : 400 and incubated at +37°C for 2 h. The wells were washed as previously described and incubated with the substrate solution, 1 mg/ml p-nitrophenylphosphate (Sigma, Glostrup, Denmark) in diethanolamine-MgCl₂ buffer (Orion, Kuopio, Finland) 200 µl/well at +37°C for 30 min. The enzyme reaction was stopped with 100 µl/well of 2M NaOH, and the absorbances were measured at a wavelength of 405 nm with a spectrophotometer (Labsystems Multiskan MCC 1340, Helsinki, Finland).

In the ELISA, the absorbance value of a test serum was compared to that of the pooled control serum, which was used in every plate. The control sera have been collected from 321 adults who have reported exposure to moisture and mould problems and who have been positive for only one or a few moulds. There are no international standards available for the species-specific positive sera, and therefore, to minimise the effects of exceptionally high or low IgG concentrations, the control sera were pooled. The absorbance given by the test serum is expressed as a percentage from the absorbance given by the control serum.

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS) statistical package. The χ^2 test with Yates'

Table 1 Immunoglobulin G antibody levels to 24 moulds in 126 school-children from three moisture problem schools and one reference school, measured enzyme immunologically and expressed as percentages of the absorbances of the pooled control sera

Moulds	Water requirement for growth ²	Moisture problem schools (n=93)	Reference school (n=33)	All (n=126)
<i>Eurotium amstelodami</i> ¹	L	57 ³	53 ³	56 (24,914) ³
<i>Aspergillus versicolor</i> ¹	L	98	102	99 (50,123)
<i>Penicillium notatum</i>	L	57	59	58 (22,99)
<i>Penicillium brevicompactum</i>	L	71	68	70 (40,109)
<i>Paecilomyces variotii</i>	L	56	53	56 (24,97)
<i>Aspergillus fumigatus</i> ¹	H	79	84	80 (32,127)
<i>Rhizopus nigricans</i>	H	39	42	39 (17,77)
<i>Cladosporium cladosporioides</i>	H	71	75	72 (26,109)
<i>Fusarium oxysporum</i> ¹	H	31	26	30 (9,69)
<i>Aureobasidium pullulans</i>	H	56	54	56 (15,104)
<i>Geotrichum candidum</i> ^A	H	28	23	28 (8,75)
<i>Mucor circinelloides</i> ¹	H	24	23	24 (12,50)
<i>Rhodotorula glutinis</i> ^{1A}	H	73	76	73 (36,114)
<i>Chaetomium globosum</i>	H	40	36	39 (17,91)
<i>Stachybotrys chartarum</i> ¹	H	82	66	78 (26,145)
<i>Acremonium atrogriseum</i>	H	78	78	78 (42,120)
<i>Acremonium kiliense</i>	H	51	49	51 (19,114)
<i>Phoma macrostoma</i> ¹	H	22	27	23 (6,67)
<i>Trichoderma citrinoviride</i> ¹	H	64	58	62 (24,121)
<i>Tritirachium roseum</i>	H	42	37	41 (18,99)
<i>Sporobolomyces salmonicolor</i> ^{1A}	H	66	67	67 (29,112)
<i>Streptomyces albus</i> ^{1B}	H	84	85	85 (36, 154)
<i>Streptomyces griseus</i> ^{1B}	H	35	29	33 (16,92)
<i>Streptomyces halstedii</i> ^{1B}	H	73	66	69 (34,122)

1 = Microbes indicating moisture in buildings according to Samson et al. and Flannigan et al. (27–29).

2 = Minimal water activity (a_w) for growth: L = microbes which commonly grow on materials with a low water activity ($a_w < 0.8$), H = microbes which commonly have a high water activity demand for growth ($a_w > 0.8$). In the present study, the minimal water activity demand for growth was roughly estimated according to Samson et al. (27, 29).

3 = Median; 4 = 10%, 90% percentiles.

A = Microbes belonging to yeasts, B = microbes belonging to actinobacteria.

Statistical significance: there were no significant differences in mould-specific IgG antibodies between the children from moisture problem and reference schools.

correction was used to test the significance of differences between groups. The absorbance data were non-normally distributed, also after logarithmic transformation. Therefore, the nonparametric Wilcoxon test and the one-way analysis of variance, the Kruskal–Wallis test and Spearman's correlation analysis were used. In the present paper, the absorbance values are presented as medians and 90% percentiles, indicating 0.5 and 0.1 probability, respectively, to have the same or greater concentration of mould antibodies. Since the material consists of 126 subjects, 13 cases form the group >90% percentile for each mould specific IgG. After univariate analyses, the main topics of the study (the association between mould-specific IgG and exposure in school, and the association between mould-specific IgG and asthma, wheezing or cough) were checked by multivariate analysis using logistic regression. These analyses were adjusted for age, atopy, moisture and/or mould exposure at home, and active and passive smoking.

Ethics

The Ethics Committee of Kuopio University Hospital approved the study protocol. Written consent was obtained from the parents of all children.

Results

Serum IgG antibodies were measured to the antigens of 24 microbial species in 126 school-children. In Table 1, the tested microbes are listed and grouped according to their moisture requirements. There were no significant

differences for any moulds between the 93 children from the moisture problem (index) schools and the 33 from the reference school. The result remained the same when data from the three index schools were analysed separately. Moisture and mould exposure was present only in school buildings for 79 children, only at home for 2, at both places for 14 children and in either place for 31. When IgG antibody levels were compared within these four exposure groups, or between the three exposure groups and the one non-exposed group, no significant differences were found (data not shown). Despite these negative results, the highest IgG antibody levels were to four moisture indicative microbes: *A. versicolor*, *Stachybotrys chartarum*, *Streptomyces albus* and *A. fumigatus* (Table 1). Serum IgG antibody levels to 24 microbes in the farmers' children did not differ significantly from the other children's IgG antibody levels (data not shown).

IgG antibodies to moulds showed some association with age but not with gender of the children. The antibody levels to *A. versicolor* were higher ($P < 0.05$) in children over 10 years of age than younger ones (data not shown). Eight (6%) children reported tobacco smoking and 24 (19%) had smoking parents. Active smokers, in comparison to nonsmokers, had lower IgG antibody levels against *S. griseus* ($P < 0.05$). In addi-

Table 2 Immunoglobulin G antibody levels to 24 moulds measured by enzyme immunoassay and expressed as percentages of the absorbances of the pooled control sera, in relation to respiratory disorders in 126 school-children

Moulds	Respiratory disorders			
	Present ²		Not present	
	(n=44)	(10%,90%)	(n=82)	(10%,90%)
<i>Eurotium amstelodami</i> ¹	54 ³	(27, 83)	59	(24, 92)
<i>Aspergillus versicolor</i> ¹	101	(39, 126)	98	(55, 121)
<i>Penicillium notatum</i>	58	(15, 94)	59	(24, 101)
<i>Penicillium brevicompactum</i>	70	(30, 111)	70	(46, 108)
<i>Paecilomyces variotii</i>	51	(21, 88)	56	(26, 100)
<i>Aspergillus fumigatus</i> ¹	76	(25, 131)	81	(39, 124)
<i>Rhizopus nigricans</i>	38	(14, 77)	39	(18, 77)
<i>Cladosporium cladosporioides</i>	72	(21, 110)	70	(30, 104)
<i>Fusarium oxysporum</i> ¹	25	(6, 69)	30	(12, 65)
<i>Aureobasidium pullulans</i>	53	(12, 93)	60	(20, 104)
<i>Geotrichum candidum</i> ^A	33	(8, 80)	27	(8, 70)
<i>Mucor circinelloides</i> ¹	19	(10, 44)	26	(12, 54)
<i>Rhodotorula glutinis</i> ^{1A}	72	(35, 115)	74	(41, 113)
<i>Chaetomium globosum</i>	37	(13, 98)	40	(18, 79)
<i>tachybotrys chartarum</i> ¹	63	(23, 145)	83	(28, 145)
<i>Acremonium atrogriseum</i>	76	(30, 121)	79	(45, 117)
<i>Acremonium kiliense</i>	53	(12, 99)	50	(21, 124)
<i>Phoma macrostoma</i> ¹	26	(6, 65)	22	(7, 67)
<i>Trichoderma citrinoviride</i> ¹	62	(15, 121)	64	(30, 112)
<i>Tritirachium roseum</i>	41	(15, 89)	41	(22, 106)
<i>Sporobolomyces salmonicolor</i> ^{1A}	64	(29, 115)	67	(26, 112)
<i>Streptomyces albus</i> ^{1B}	72	(29, 154)	87	(38, 153)
<i>Streptomyces griseus</i> ^{1B}	27	(14, 74)	34	(17, 92)
<i>Streptomyces halstedii</i> ^{1B}	62	(25, 105)	73	(36, 127)

1 = Microbes indicating moisture in buildings according to Samson et al. and Flannigan et al. (27–29).

2 = Children with asthma, repeated wheezing symptoms and prolonged cough symptoms combined.

3 = Median; 4 = 10%, 90% percentiles.

A = Microbes belonging to yeasts, B = microbes belonging to actinobacteria.

Statistical significance: there were no significant differences between the groups for any mould-specific IgG antibody level. Likewise, the differences between children with asthma, wheezing symptoms or cough symptoms, when analysed separately, and children with no respiratory symptoms were not significant.

tion, children exposed to passive smoking had low IgG antibody levels to many moulds; *Penicillium glabrum* ($P < 0.05$), *Chaetomium globosum* ($P < 0.05$), *Mucor circinelloides* ($P < 0.05$), *Penicillium notatum* ($P < 0.01$) and *Rhizopus nigricans* ($P < 0.01$) reached statistical significance when compared to non-exposed children.

There was no association between asthma, wheezing or cough, and serum IgG antibody levels to moulds (Table 2). The result remained the same when asthma, wheezing and cough groups were analysed separately. In contrast, allergy did seem to have an association. Children with allergic diseases had higher IgG antibody levels than nonallergic children to *Aspergillus* species, *A. versicolor* ($P < 0.05$) and *A. fumigatus* ($P = 0.06$). IgG antibody levels were low to *Eurotium amstelodami*, the anamorph which belongs to the genus *Aspergillus*, and no difference was found between allergic and nonallergic children. Children with positive SPT reactions to animal dander more often than SPT negative children carried specific IgG antibodies to *A. versicolor* ($P < 0.01$) and to *A. fumigatus* ($P < 0.05$), but again not to *E. amstelodami*. In contrast, no association was found between SPT reactions to house dust mites or pollens, and IgG antibodies to moulds (data not

shown). Children with allergic rhinoconjunctivitis had elevated IgG antibody levels to three additional moulds: *Acremonium kiliense* ($P < 0.05$), *Cladosporium cladosporioides* ($P < 0.05$) and *Trichoderma citrinoviride* ($P < 0.05$).

There were six children with positive (> 3 mm) SPT reactions to moulds in this cohort. Positive SPT reactions to moulds were associated with serum IgG antibodies to six agents: *A. kiliense* ($P < 0.05$), *C. globosum* ($P < 0.05$), *P. macrostoma* ($P < 0.05$), *T. citrinoviride* ($P < 0.01$), *Aureobasidium pullulans* ($P < 0.05$) and *Paecilomyces variotii* ($P < 0.05$) (data not shown). The IgG antibody levels to *C. globosum*, *P. macrostoma* and *T. citrinoviride* were elevated in three (50%) of these six SPT positive children. *P. herbarum* reactions in SPTs were significantly correlated with IgG antibodies of *P. macrostoma* in serum; all other correlations between IgG antibodies and positive SPT results to the same mould were not significant.

The association between IgG and IgE antibodies to moulds was evaluated in the 54 cases with both determinations available. Seven children had elevated (> 0.35 IU/ml) mould-specific IgE. Four of them (57%)

Table 3 Demographic characteristics, atopic status, respiratory status and mould-specific skin test data in 18 subjects who carried elevated IgG antibody levels to seven or more moulds

Child	Age/Sex (years,M/F)	Allergystatus (n=26)	Respiratory status ^A (n=44)	SPTs to moulds ^B (n=18)	Mould specific IgG elevated ^C (n=24)
1	7 F	AR-/AD-	Healthy	Negative	7
2	7 F	AR-/AD-	Healthy	Negative	10
3	7 M	AR+/AD-	Healthy	Negative	10
4	8 F	AR-/AD-	Healthy	Weak	9
5	9 M	AR-/AD-	Healthy	Negative	12
6	10 M	AR+/AD+	Wheezing	Positive	8
7	10 M	AR-/AD+	Asthma	Weak	7
8	10 M	AR+/AD+	Wheezing	Negative	7
10	11 M	AR+/AD-	Wheezing	Negative	8
11	11 M	AR-/AD+	Healthy	Weak	8
12	11 M	AR+/AD-	Asthma	Positive	15
13	12 M	AR-/AD-	Healthy	Negative	10
14	12 F	AR+/AD+	Cough	Negative	8
15	13 M	AR-/AD+	Healthy	Negative	8
16	13 F	AR-/AD-	Healthy	Negative	8
17	13 M	AR+/AD-	Asthma	Positive	8
18	13 F	AR-/AD-	Healthy	Negative	15

A=Asthma, wheezing or cough symptoms; for definitions, see the text.

B=Mould-specific skin prick test reactions to moulds(3 mm (positive), 1–2 mm (weak) or not present (negative).

C=Cases with mould-specific IgG >90% percentiles were defined as elevated. There were 75 children with at least one elevated result. The figures in the table indicate the numbers of elevated values in each child.

AR=Allergic rhinoconjunctivitis, AD=atopic dermatitis.

had elevated IgG antibodies to *C. globosum* ($P<0.05$) and three to *Fusarium oxysporum* ($P<0.05$), *P. macrostoma* ($P<0.05$) and *T. citrinoviride* ($P<0.05$). However, there were no cases with elevated IgE and IgG antibodies to the same mould.

Since age, allergy, and exposure to tobacco smoke influenced the serum IgG antibody concentrations, these factors, as well as exposure to moisture and mould problems at home, were included as covariates in the logistic regression analysis. The main results remained the same as in univariate analysis. There were no significant differences in serum IgG antibodies between index and control schools, or between children with and without respiratory manifestations.

We focused in more detail on those 18 children who had elevated (>90% percentile) levels in IgG antibodies to seven or more (>25%) of the 24 moulds (Table 3). Eleven (61%) of them were boys (NS), and 13 (72%) were aged 10 years or more ($P<0.05$ vs. younger children). In addition, 10 (56%) children were allergic ($P<0.001$ vs. nonallergic children) and six (33%) either had asthma or had suffered from wheezing (NS).

Discussion

Allergic diseases, rhinoconjunctivitis in particular, were associated with elevated IgG antibody levels to two *Aspergillus* species, *A. fumigatus* and *A. versicolor*. Moreover, positive SPT and IgE responses to moulds correlated with IgG findings to certain microbes, e.g., *C. globosum*, *F. oxysporum*, *P. macrostoma* and *T. citrinoviride*. The presence of all these microbes

suggests a moisture problem in buildings, at least in communities outside the agricultural environment (19, 22, 30, 33, 35). Though allergy is common, specific allergy to moulds is rare, being seen in less than 5% of children in our area (10, 11). Therefore the numbers of mould-specific findings remain small in population-based studies, and there is a risk for false-negative results. Despite the rarity of mould allergy, an association has been suggested for allergic symptoms and home dampness (8, 36–38). Thus, by combining exposure, clinical and laboratory data, it may be possible to identify mould species or genera that may make a significant contribution to indoor air quality and health effects. Our results combining data on allergy, including SPT and IgE findings, with IgG data, suggest such a role for the *Aspergillus* genus, mainly for *A. fumigatus* and *A. versicolor*.

No association was found between IgG antibodies to moulds and exposure to moisture or moulds in the school when it was assessed objectively. In addition, no relationship was found with living in an agricultural environment, where early exposure to moulds would be anticipated (16, 19, 35, 39). The study material allowed evaluation of the effect of agricultural environment, since over half of the children (66%) lived on farms. On the other hand, the indoor air concentrations of viable fungi in the schools were rather low, much lower than the concentrations of 10^4 cfu/m³ proposed as the threshold in occupational health studies. Although no objective data were available from homes, the above mentioned limit is rarely exceeded in Finnish houses (34, 40). In addition, IgG assays were available for only two-thirds of the moulds cultured from the buildings.

Though the production of IgG antibodies to foreign material, like moulds or their products, is a normal response (21, 41), some profiles of mould-specific antibodies may be the result of excessive exposure or exposure caused by certain harmful moulds. Therefore, IgG antibodies to selected moulds have been used as biomarkers of exposure to moulds, particularly at the group level (17, 19, 20).

None of the individual IgG antibodies to moulds had any significant association with asthma or other long-term pulmonary problems. On the other hand asthma and wheezing cases were over-represented among children with multiple IgG findings. In earlier Finnish studies a clear association was shown between farmers with lung disease and elevated IgG antibodies to certain moulds (16, 17, 19). In many studies respiratory manifestations like asthma and wheezing, and especially respiratory infections, have been shown to be increased in children from moisture damaged day-care centres and schools (10, 11, 13, 42, 43). The mechanism is only rarely IgE-mediated (10–12, 32, 44) and the present results suggest that IgG antibodies play only a minor role, if any. Thus, it is essential to identify new approaches to examine the effects of exposure to moulds, such as measurements of pro-inflammatory cytokine mediators or exhaled nitric oxide (15, 25, 45).

On average, children have lower IgG levels to moulds than adults (39). A significant correlation between age and IgG levels was seen for only three of the 24 moulds. Two of these moulds, *A. versicolor* and *F. oxysporum*, are generally found in moisture problem buildings as well as in the agricultural environment (19, 22, 30, 35). However our study subjects were 7–13 years old, and age-dependence is more probable before school age (39). As in other studies there were no differences in IgG antibody levels to moulds between males and females (16, 17, 19). Smokers, compared to non-smokers, seem to have lower immunoglobulin levels to moulds as well as to other antigens (17, 46, 47). In our study this was seen in serum IgG antibodies to five moulds in children exposed to passive smoking. The effect was paralleled, though not significantly, in the small group of actively smoking children. Concomitant with decreased immune responses to environmental factors like moulds, smoking increases respiratory symptoms. Therefore, smoke exposure is an important confounding factor to be included in analyses of health studies, as was done in the present study.

There are three strengths of the present study. First, the school buildings were investigated both technically and microbiologically, and all children

were examined clinically. Second, the number of subjects was large, over 130 school-children, and the participation rate was high, over 90%; despite this, the distributions of IgG antibody levels were wide for all moulds, reflecting the large variation between different individuals. Third, IgG antibodies were measured to as many as 24 different moulds, including the most important indoor and moisture indicative moulds in our area (22, 23, 26, 33, 34). A large panel is essential, since the number of moulds associated with indoor air problems is high and the roles of individual moulds are poorly understood (20, 24, 25, 36). However, because each statistical test was performed 24 times it is possible when using the 0.05 level of significance that there will be one or two spuriously significant results. Therefore, the few positive associations must be interpreted very carefully. The main difficulties in interpretation of the present results are the lack of international reference sera, of standardised antigen extracts and of population-based reference values for different ages. The control sera used in the present study were from exposed adults positive for mainly one mould, thus neither representing any population nor references for normal or pathological values. The ELISA we used is appropriate for comparisons between groups such as exposed and non-exposed children, or atopic and nonatopic children, as in the present study.

In conclusion, IgG antibodies were measured to 24 moulds in 126 school-children, and no clear association was found with exposure categories. Atopy was associated with high levels of *A. fumigatus* and *A. versicolor* antibodies, and smoking was associated with low levels of antibodies to many moulds. In general, mould allergy was associated with IgG antibodies to moisture indicative moulds, but asthma, wheezing and cough symptoms were not associated with mould-specific IgG in school-children.

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