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MOULD SPECIFIC IgG ANTIBODIES CONNECTED WITH SINUSITIS IN TEACHERS OF MOULD DAMAGED SCHOOL: A TWO-YEAR FOLLOW-UP STUDY

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Abstract
Objectives: The aim of this study was to describe the relationship between mould exposure induced by moisture damage and mould specific immunoglobulin G antibodies to 20 common mould species and their association with respiratory diseases. **Materials and Methods:** Mould specific immunoglobulin G (IgG) antibodies were monitored in teachers in a follow-up after an extensive mould remediation process in school buildings. IgG antibodies to 20 different microbes were determined from the sera of 26 teachers (19 exposed and 7 references) by enzyme-linked immunosorbent assay (ELISA). The serum samples were drawn twice, firstly at the completion of the remediation in the spring of 1997 and secondly, two years later in the spring of 1999. Health data was collected with self-administered questionnaires. **Results:** No statistical differences were found in the overall concentrations of 20 mould-specific IgG-antibodies between the study and control groups at the beginning of the study. An association between sinusitis and elevated mould-specific IgG-levels for *Aspergillus fumigatus, Aspergillus versicolor, Aureobasidium pullulans, Chaetomium globosum, Cladosporium cladosporioides, Phialophora bubakii, Rhodotorula glutinis, Sporobolomyces salmonicolor, Stachybotrys atra,* and *Tritirachium roseum* was found in the study group. **Conclusions:** In a two-year follow-up the total concentration of the IgG antibodies for *Tr. toseum* was lower at the end than at the beginning of the follow-up and this remained significant for the group of teachers with sinusitis. The decrease in mould specific IgG to *Cl. cladosporioides, Geotrichum candidum, Ph. bubakii* and *Rhizopus nigricans* was associated with bronchitis. According to our knowledge, this is the first study in which the association between elevated mould specific IgG antibodies and sinusitis was found in the school environment.

Key words:
IgG-antibodies, Adults, Indoor air, Moisture damage, Sinusitis, Bronchitis

INTRODUCTION

Exposure in moisture and mould damaged buildings has recently received public attention because of the increased risk for respiratory, irritative and allergic symptoms reported among both adults [1–13] and children [14–18]. Excess moisture-related problems are common in the modern building stock and a conservative estimate on the prevalence of such observations is approximately 23% in Finnish residences [8], slightly varying prevalence

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estimates being presented in different studies [2,7]. Moisture problems have also been observed in schools and other public buildings. The association between moisture damage exposure and health is well documented, but the causal links between the exposing agents and adverse health effects are still poorly understood. Also the pathophysiological mechanisms are to be revealed. The health findings are mostly unspecific by their nature and therefore, diagnostic tools for detecting mould-associated health problems are urgently needed. Specific identification of the link between health findings and the building-related pollution sources would improve the diagnostics of such illnesses and help their prevention and remediation. In search of relevant approaches to characterize the exposure, the mould specific IgG antibodies would provide a direct link between environmental exposure to microbes and the human body, which is the target of this exposure. Serum mould specific immunoglobulin G antibodies (IgG) have been used as a marker of mould exposure in occupational environments with massive microbial exposures (10^5–10^6cfu/m^3 of viable microbes) such as agriculture [19] and sawmills [20]. In indoor environments the microbial levels are much lower, usually in the range of 10^1–10^3 cfu/m^3 of viable fungi in homes and offices [21]. In a school environment, the levels of airborne fungi may be even lower [15]. However, measurements carried out especially in moisture-damaged school buildings revealed slightly elevated fungal concentrations and different fungal genera compared to "non-damaged" school buildings, including a large variety of fungi in the indoor air [15,22–24). It has not been previously reported whether mould-specific IgG antibodies in teachers could reflect a possible relationship with school building-related exposure to moulds.

The aim of this study was to describe the relationship between mould exposure induced by moisture damage and mould specific immunoglobulin G antibodies to 20 common mould species and their association with respiratory diseases. The study is a part of an intervention study carried out in a school building that underwent a thorough remediation of moisture and mould damage. The details of the overall approach of the intervention and associated studies have been reported by Haverinen et al. [16]. The health status of the teachers was surveyed repeatedly during the remediation process of the school building. The follow-up included repeated measurements of mould specific IgG antibodies right after the completion of the repairs and two years thereafter.

MATERIALS AND METHODS

Structural engineering study of the school buildings

The school center under study is a complex of three school buildings used by primary, secondary and high schools, located in a small town in central Finland. In the spring of 1996, the three buildings of the school center were first inspected by performing visual investigation, recording the signs of mould growth, environmental measurements (including temperature and moisture measurements from specific locations) and structural openings. Exposure assessment was continued with microbial sampling of indoor air, surfaces and damaged building materials. Based on those investigations, two of the school buildings, i.e., the secondary and high school buildings were considered as moisture-damaged (index) buildings and the primary school building as a non-damaged (reference) building.

The aim of the remediation process was to eliminate the causes of moisture damage and to replace the materials contaminated by mould. No major repairs were made in the reference building.

Microbial sampling

Indoor air microbes were collected with a six-stage impactor (Andersen 10–800) [25] from classrooms, halls and personnel rooms during school days when the buildings were occupied. Samples for fungi were taken on 2% malt extract agar (MEA) and dichloran 18% glycerol agar (DG18), and samples for bacteria on tryptone glucose yeast (TGY) agar. Sampling campaigns were carried out before and after the intervention, and during a three-year follow-up. Indoor/outdoor (I/O) ratio of fungal concentrations was used to evaluate differences between the three campaigns. Sampling time was 10 min and the detection limit (DL) 4 cfu/m^3. Fungi were incubated for 7 days at 25°C and then identified morphologically mainly to genus.

Bacteria were incubated for up to 14 days at about 20°C. The number of actinomycetes was counted after 14 days of incubation and that of other bacteria after 5 days of incubation. Detection of actinomycete colonies was based on their dry, actinomycete-like appearance.

Symptom questionnaire study

The health data of the teachers (n = 44) working in the three school buildings were collected with a self-administered questionnaire. The survey was repeated three times, in the spring of 1996 before the remediation, in the spring of 1997 after remediation and two years later in the spring of 1999. The questionnaire based on the Örebro questionnaire (MM40) [26] and the Tuohilampi set of questionnaires [27] included 70 questions on general and irritation symptoms (15 questions), respiratory infections (7 questions), allergy (3 questions) and medical treatment (2 questions). Of the 44 teachers, 26 were eligible for the study. The group consisted of 19 females and 7 males, including 3 smokers (mean age of 45 years). Mean duration of employment at school was 15 years. Five teachers reported moisture problems at home, and five teachers had diagnosed asthma.

Determination of serum immunoglobulin G antibodies

Serum samples for the mould-specific immunoglobulin G (IgG) antibody measurements were available for 26 (60%) teachers, of whom 19 worked in the index and 7 in the reference schools. The serum samples were drawn twice, firstly at the completion of the remediation in the spring of 1997 and secondly, two years later in the spring of 1999. The serum samples were stored at a freezer (-20° C) until all the serum samples were collected.

Serum IgG antibodies to 20 different microbes were determined by enzyme-linked immunosorbent assay (ELISA). The microbes in question were *Aspergillus (A.) fumigatus, A. umbrosus, A. versicolor, Aureobasidium pullulans, Cephalosporium curtipes, Chaetomium globosum, Cladosporium cladosporioides, Fusarium avenaceum, Geotrichum candidum, Paecilomyces variotii, Penicillium brevicompactum, Phialophora bubakii, Phoma macrostoma, Rhizopus nigricans, Rhodotorula glutinis, Sporobolomyces salmonicolor, Stachobotrys atra, Streptomyces albus, Trichoderma viride* and *Tritirachium roseum.* The microbes were selected to represent internationally recognized fungi indicative of moisture-damaged buildings [24] and other microfungi common in Finnish buildings [21,28,29]. Before manufacturing fungal antigen extracts, the identification of fungal species was confirmed by a reference laboratory.

The microbial strains were grown on agar plates at +25°C for seven days to obtain spores and hyphal fragments for transferring into peptone broth (2% malt extract, 1% mycological peptone and 4% glucose in sterile water). The purity of each inoculated isolate was verified before the transfer. After incubation for seven days at +25°C in cell culture bottles (Roux), microbial mass was separeted from culture fluid by filtering and subsequent washes with phosphate bufferd saline (PBS). The microbial yield in PBS (1:1, v/v) was then autoclaved, homogenized and ultrasonicated. The homogenates were centrifuged for 30 min at 23 300 g with a cooled centrifuge. After filtration through a 0.45 μm filter, the supernatants were stored at -70°C until use. Each microbial extract was serially diluted and used as capture antigen in ELISA using IgG positive sera diluted 1:100 and alkaline phosphatase conjugated goat anti-human IgG as detecting antibody in order to get optimal dilution for each antigen for further use in ELISA. The antibody determination of the samples was made as a one set. For the laboratory analyses, the order of the samples from the index and reference schools was mixed and analyzed blinded in random order.

The 96-well microtiter plates (Nunc MaxiSorp™, Roskilde, Denmark) were coated with 200 μl/well of the antigen extract in PBS (pH 7.4), incubated at +20°C over night, and then washed three times with deionized water. Serum samples diluted 1:100 in 10% fetal bovine serum (FBS) 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 goat antihuman IgG (Daco) was added in a volume of 200 μl/well at washed, as described earlier, and incubated with 200 μl/well of substrate solution, 1 mg/ml p-nitrophenylphosphate (Sigma, Glostrup, Denmark) in diethanolamine-

$MgCl_2$ buffer (Reagena Ltd, Kuopio, Finland) at 37°C for 30 min. The 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 Multiscan MS200, Helsinki, Finland).

The pooled positive control serum diluted 1:100 was incorporated on each plate as an interplate calibrator. The levels of microbe specific IgG are expressed as absorbances at a wavelength of 405 nm.

Ethics

All the studied individuals were volunteers. The study protocol was approved by the Ethical Committee of the Kuopio University Hospital.

Statistical analysis

Mann-Whitney U test was used to test the differences between indoor-outdoor (I/O) ratio of the microbial concentrations. The absorbance values of the mould specific IgG antibodies were not normally distributed and therefore non-parametric tests were used and data were described with medians. Wilcoxon`s test and Mann-Whitney U test were used to compare absorbance values of mould specific IgG values between the teachers of index and reference schools and in the follow-up to analyze the change in absorbance values. The differences in the occurrence of the dichotomous symptoms and infection variables between index and reference groups were tested with chi-square test and Fisher's exact test. The SPSS statistical package was used for all analyses [30].

RESULTS

The summary of microbes detected from the buildings during the three different sampling campaigns is shown in Table 1. The airborne concentrations of fungi varied from 4 to 357 cfu/m^3. Concentrations of fungi in samples of damaged materials varied between below the limit of detection (<45 cfu/g) and 22 000 000 cfu/g. The number of various fungal types in the index school was initially 27, and it decreased to 17 and 12 after the remediation and follow-up. The corresponding numbers for the control school were 18, 10 and 5.

The I/O ratio of concentrations of viable fungi are shown in Table 2. It was significantly higher ($p = 0.005$) in the index schools (1.46) than in the control school (0.33), but after remediation the values reverted to almost the same level (0.70–0.86), and I/O ratio of the two most common fungi, *Penicillium* and *Cladosporium,* decreased.

To sum up the health findings, five teachers (26%) in the index group had diagnosed asthma; seven (37%) reported one or more episodes of sinusitis; five (26%) reported one or more episodes of bronchitis; fifteen (79%) reported allergic rhinitis; and three (19%) clinical atopy during the twelve months before the remediation. After remediation and in the three-year follow-up no new cases of asthma were recorded. The number of teachers with sinusitis decreased to two (11%) after remediation and to one (5%) in the follow-up, with bronchitis to zero and to one (5%), and with symptoms of allergic rhinitis to nine (47%) in both periods. All the exposed participants complained about hoarseness. Of the seven reference teachers, none reported asthma and the numbers of individuals with other symptoms were: three with sinusitis, one with bronchitis, four with symptoms of allergic rhinitis, one with atopic eczema and five with hoarseness.

IgG-antibody concentrations at the beginning of the study (1997) in the index and reference groups are shown in Table 3. Also in Table 3 there are shown the IgG concentrations of the index group with and without infection episodes and symptoms of allergic diseases during the previous twelve months. At the beginning of this study, no statistical differences were found in the overall concentrations of mould-specific IgG antibodies between the index and reference groups.

The teachers of the index school with reported episodes of sinusitis during the previous 12 months had significantly higher mould-specific IgG levels to 10 moulds than those without sinusitis. These 10 moulds were *A. fumigatus, A. versicolor, Au. pullulans, Ch. globosum, Cl. cladosporioides, Ph. bubakii, Rh. glutinis, S. salmonicolor, St. atra,* and *Tr. roseum.*

The teachers of the index school with symptoms of allergic rhinitis had higher IgG-levels to *Rh. glutinis* and *S. salmonicolor* than the teachers without these symptoms.

Table 1. The presence of microbes in air (x) and material (o) samples collected from the studied schools

Mould	Index school			Reference school		
	1996 Before remediation (n_a = 9, n_m = 9)	1997 After remediation (n_a = 11)	2000 After follow-up (n_a = 10)	1996 Before remediation (n_a = 6, n_m = 1)	1997 After remediation (n_a = 6)	2000 After follow-up (n_a = 3)
Acremonium	x o	x		x	x	
Aspergillus	x o	x	x	x	x	
A. fumigatus	x o			x		
A. niger	x					
A. penicillioides			x			
A. versicolor	x o	x	x	x		
Aureobasidium	x	x		x	x	
Botrytis	x			x		
Chaetomium	o					
Chrysosporium			x			x
Cladosporium	x o	x	x	x	x	x
Eurotium	x o		x	x		
Geomyces	x o	x		x		
Geotrichum	x			x		
Gonatorrhodiella				x		x
Oidiodendron	o					
Olpitrichum	x o			o		
Paecilomyces	x o	x	x			x
Penicillium	x o	x	x	x	x	
Phialophora	o					
Rhinocladiella		x				
Rhizopus	o	x				
Sphaeropsidales	x o					x
Non-sporing	x o	x	x	x	x	
Trichoderma	x o	x				
Tritirachium	o					
Ulocladium	x	x				
Wallemia	x	x		x	x	
Yeasts	x o	x	x	x	x	
Actinomycetes	x o	x	x	x	x	
Other bacteria	xo	x	x	xo	x	

n_a –number of air samples.
n_m – number of material samples.

Table 2. Indoor-outdoor (I/O) ratio and geometric mean concentration of the airborne fungi, cfu/m^3

	1996 Index building (n = 9)	1997 Index building (n = 11)	1996 Reference building (n = 6)	1997 Reference building (n = 8)
Total	1.46 (123)	0.70 (74)	0.33 (245)	0.86 (21)
Penicillium	1.95 (13)	0.54 (28)	0.95 (33)	– (3)
Cladosporium	1.71 (15)	0.40 (5)	0.27 (65)	– (0.6)
Yeasts	1.28 (14)	1.38 (21)	1.62 (8)	– (3)
A. versicolor	– (2)	– (0)	– (0.7)	– (0)
A. fumigatus	– (1)	– (0)	0.48 (0.8)	– (0)
Trichoderma	– (0.6)	– (0.3)	– (0)	– (0)

Not detected from outdoor air (–).

Table 3. The IgG antibody concentrations in the index and reference schools and in the index school teachers with or without infection episodes or symptoms of allergic disease

	Index school 1997 (n = 19) Median	Reference school 1997 (n = 7) Median	P	Sinusitis (n = 7) Median	No sinusitis (n = 12) Median	P	Bronchitis (n = 5) Median	No bronchitis (n = 14) Median	P	Allergic rhinitis (n = 15) Median	No allergic rhinitis (n = 4) Median	P	Atopic eczema (n = 3) Median	No atopic eczema (n = 16) Median	P
A. fumigatus	1.359	0.736	0.473	1.51	1.02	**0.000**	1.38	1.27	0.689	1.38	0.612	0.124	1.43	1.20	0.363
A. umbrosus	0.944	1.000	0.825	1.19	0.904	0.083	1.28	0.933	0.127	0.988	0.621	0.260	1.03	0.933	0.307
A. versicolor	1.106	1.240	0.530	1.32	0.765	**0.016**	1.28	1.07	0.561	1.25	0.595	0.079	1.28	0.933	0.256
Au. pullulans	0.516	0.439	0.954	0.862	0.424	**0.000**	0.539	0.475	0.293	0.539	0.300	0.060	0.477	0.528	1.000
C. curtipes	1.031	1.024	0.356	0.864	1.22	0.863	0.846	1.51	0.694	1.03	0.954	0.575	1.57	0.846	0.913
Ch. globosum	0.300	0.315	0.856	0.473	0.223	**0.044**	0.294	0.307	1.000	0.310	0.184	0.126	0.168	0.313	0.173
Cl. cladosporioides	0.387	0.359	0.931	0.675	0.215	**0.003**	0.430	0.333	0.385	0.432	0.214	0.235	0.344	0.430	0.655
F. avenaceum	1.454	1.048	0.113	1.02	1.49	0.536	1.42	1.56	0.628	1.45	1.03	0.796	1.57	1.42	0.822
G. candidum	0.567	0.733	0.741	0.920	0.463	0.070	0.582	0.556	0.628	0.569	0.424	0.646	0.833	0.463	0.105
P. variotii	0.715	0.463	0.697	0.917	0.651	0.070	0.715	0.690	0.386	0.715	0.552	0.470	0.736	0.663	0.491
Pe. brevicompactum	0.734	1.131	0.576	1.125	0.582	0.123	0.795	0.583	0.252	0.795	0.407	0.152	1.23	0.661	0.257
Ph. bubakii	0.757	0.349	0.205	0.836	0.661	**0.021**	0.774	0.739	0.770	0.780	0.391	0.280	0.851	0.739	0.432
Pho. macrostoma	0.280	0.210	0.269	0.307	0.244	0.585	0.338	0.255	0.687	0.307	0.224	0.426	0.141	0.307	0.127
R. nigricans	0.563	0.484	0.737	0.573	0.548	0.429	0.702	0.549	0.755	0.573	0.305	0.079	0.420	0.569	0.714
Rh. glutinis	0.881	0.885	0.576	1.46	0.711	**0.005**	0.742	0.828	0.443	1.24	0.507	**0.036**	0.844	0.786	1.000
S. salmonicolor	0.398	0.568	0.418	0.730	0.293	**0.035**	0.390	0.573	0.694	0.573	0.198	**0.018**	0.293	0.573	0.362
St. atra	0.341	0.199	0.356	0.766	0.309	**0.035**	0.609	0.309	0.498	0.506	0.215	0.126	0.371	0.311	1.000
Str. albus	0.345	0.252	0.931	0.384	0.275	0.480	0.190	0.363	0.335	0.370	0.301	0.958	0.190	0.378	0.136
T. viride	0.595	0.649	0.912	0.595	0.572	0.533	0.539	0.600	0.892	0.550	0.806	0.745	0.435	0.600	0.793
Tr. roseum	0.151	0.113	0.386	0.322	0.135	**0.003**	0.179	0.136	0.335	0.179	0.121	0.102	0.157	0.145	0.913

A. – *Aspergillus.*
Au. – *Aureobasidium.*
C. – *Cephalosporium.*
Ch. – *Chaetomium.*
Cl. – *Cladosporium.*
F. – *Fusarium.*
G. – *Geotrichum.*
P. – *Paecilomyces.*
Pe. – *Penicillium.*
Pho – *Phoma.*
Ph – *Phialophora.*
R. – *Rhizopus.*
Rh. – *Rhodotorula.*
S. – *Sporobolomyces.*
St. – *Stachybotrys.*
Str. – *Streptomyces.*
T. – *Trichoderma.*
Tr. – *Tritirachium.*

Table 4. The IgG antibody concentrations in the index school at the end of the follow up (1999) and the change in the IgG concentrations during the follow-up period (1997–1999) with and without infections and symptoms of allergic disease

	1997 Index school (n = 19) Median	1999 Index school (n = 19) Median	P	Sinusitis (n = 7) Median	No sinusitis (n = 12) Median	P	Bronchitis (n = 5) Median	No bronchitis (n = 14) Median	P	Allergic rhinitis (n = 15) Median	No allergic rhinitis (n = 4) Median	P	Atopic eczema (n = 3) Median	No atopic eczema (n = 16) Median	P
A. fumigatus	1.359	1.313	0.502	0.030	0.001	0.594	-0.083	0.033	0.089	-0.037	0.080	0.262	0.263	0.001	0.428
A. umbrosus	0.944	1.030	0.857	0.059	-0.019	0.650	0.062	-0.019	0.690	-0.010	-0.023	0.590	0.111	-0.033	0.136
A. versicolor	1.106	1.153	0.366	0.093	-0.003	0.212	0.001	0.001	0.830	0.003	-0.006	0.700	0.001	-0.003	0.477
Au. pullulans	0.516	0.595	0.888	-0.061	-0.023	0.221	-0.021	-0.030	0.894	-0.034	-0.021	0.725	-0.020	-0.030	0.719
C. curtipes	1.031	0.960	0.732	-0.100	0.006	0.126	-0.100	0.017	0.395	-0.033	0.032	0.187	0.041	0.002	0.424
Ch. globosum	0.300	0.273	0.400	-0.023	-0.009	0.538	-0.028	-0.009	0.848	-0.025	0.002	0.644	-0.041	-0.009	0.359
Cl. cladosporioides	0.387	0.422	0.369	-0.040	0.000	0.126	-0.039	0.001	**0.021**	-0.036	0.001	0.187	-0.035	0.000	0.304
F. avenaceum	1.454	1.383	0.705	-0.021	0.032	0.370	-0.061	0.032	0.248	-0.021	0.051	0.507	-0.021	-0.005	0.312
G. candidum	0.567	0.546	0.764	0.020	-0.040	1.000	-0.062	0.024	**0.048**	-0.043	0.067	0.507	-0.137	0.020	**0.003**
P. variotii	0.715	0.850	0.096	0.121	0.055	0.633	0.121	0.055	0.617	0.121	-0.025	0.463	0.165	0.055	0.625
Pe. brevicompactum	0.734	0.760	0.793	0.114	-0.038	0.537	0.114	-0.038	0.754	0.024	-0.056	0.262	0.187	-0.038	0.797
Ph. bubakii	0.757	0.718	1.000	0.002	-0.014	0.480	-0.076	0.004	**0.037**	-0.022	0.044	0.100	0.072	-0.014	0.359
Pho. macrostoma	0.280	0.336	0.924	0.002	-0.021	0.203	-0.031	0.002	0.054	-0.006	-0.008	0.948	-0.021	-0.005	0.312
R. nigricans	0.563	0.597	0.476	-0.109	-0.044	0.484	-0.208	-0.018	**0.008**	-0.034	-0.063	0.960	-0.175	-0.032	**0.048**
Rh. glutinis	0.881	0.856	0.992	0.013	-0.006	0.438	-0.013	0.006	0.576	0.013	-0.063	0.520	0.013	-0.004	0.972
S. salmonicolor	0.398	0.372	0.151	0.008	0.014	1.000	0.014	0.012	0.636	0.017	0.008	0.718	0.029	0.012	1.000
St. atra	0.341	0.406	0.795	-0.055	0.008	0.104	-0.030	-0.013	1.000	-0.022	-0.008	0.948	-0.033	-0.013	0.655
Str. albus	0.345	0.333	0.061	-0.031	-0.028	0.287	-0.011	-0.037	0.070	-0.024	-0.033	0.693	-0.012	-0.030	0.934
T. viride	0.595	0.501	0.953	-0.017	0.010	0.484	0.056	-0.015	0.344	-0.013	0.002	0.590	0.056	-0.006	1.000
Tr. roseum	0.151	0.127	**0.020**	-0.086	-0.018	**0.009**	-0.037	-0.033	0.571	-0.040	-0.015	0.100	-0.038	-0.023	0.576

Abbreviations are explained in Table 3.

There were no such differences in IgG concentrations in respondents with bronchitis and atopic eczema.

The initial total concentrations of IgG antibodies and IgG changes in the index group at the end of the follow-up are shown in Table 4. In the two-year follow-up, the IgG-antibody concentration for *Tr. roseum* was significantly lower than that at the beginning of the study. In the reference group there were no significant changes (data not shown).

The change in the antibodies to *Tr. roseum* remained statistically significant among the teachers of the index schools with one or more episodes of sinusitis; those with self-reported bronchitis during the previous 12 months in the initial phase of the study showed significant decrease in IgG antibodies to *Cl. cladosporioides, G. candidum, Ph. bubakii* and *R. nigricans* and those with atopic eczema to *R.nigricans* and *G. candidum.*

As for teachers with allergic rhinitis in the follow-up, there were no changes in either group.

Gender or having asthma did not make any difference in the IgG levels in teachers of both groups at the beginning of the study or in the follow-up (data not shown). At the beginning of the study there were no significant differences between the groups in the age, gender distributions, prevalence of smoking, years at school or self-reported moisture or mould problems at home.

DISCUSSION

Earlier studies have shown a clear association between high fungal exposure and elevated IgG antibodies to certain moulds [19,20,31]. Elevated mould-specific IgG antibodies have been associated with a disease, such as farmer's lung but also with no mould-related disease, thus being rather a marker of exposure [32].

A significant association between episodes of sinusitis and elevated mould specific IgG antibodies was found among teachers working in the moisture-damaged buildings. These subjects had higher levels of IgG-antibodies to 10 of 20 moulds tested. Eight of the ten microbes were also isolated from the building, either in material or indoor air samples. An increased occurrence of sinusitis has been frequently connected with exposure to moisture or mould [11], but the association with IgG antibodies has not been previously reported. Futhermore, the decrease in IgG antibodies for four moulds were significant in the two-year follow-up among the exposed teachers with episodes of bronchitis before the remediation. Increased episodes of bronchitis have also been connected with indoor moisture and mould problems [8]. It has been shown earlier that long-term exposure to indoor environments with moisture problems may increase the risk for hyperreactivity of the upper airways among teachers [33]. This acquired hyperreactivity may persist over years and its decrease is very slow even after taking successful remedial measures [34]. Inflammation and swelling of the mucosa during sinusitis or bronchitis increase its permeability to any external exposure and may promote the exposure. On the other hand, mould exposure and related immunological response may affect immunoresponses against common infective agents. The causative agents of sinusitis or bronchitis are assumed to be common respiratory pathogens, but usually they are found in the environment. Thus, sinusitis or bronchitis associated with exposure to indoor moulds may be a result of a still unknown chain of events between exposing agents and the immune defence system of the host.

Symptoms of allergic rhinitis were common in the exposed group with the prevalence of 79%. The IgG antibodies for *Rh. glutinis* and *S. salmonicolor* were significantly higher among the exposed subjects with allergic rhinitis than among those without it. The antibodies to those two moulds were also elevated among the exposed subjects with sinusitis. While sinusitis and allergic rhinitis are often found in the same patients, no association between these conditions and elevated IgG antibodies has previously been reported.

The association between atopic eczema and mould exposure has not been emphasized in previous studies reported in the literature. It is evident that all irritants, including mould spores may worsen the symptoms of acute eczema. Atopic skin is more permeable to exposing agents because of the altered lipid concentration structure of the skin, and especially in the acute phase of eczema the skin is less protected to airborne dust exposure, which may possibly lead to IgG activation.

An exceptionally large number of asthma cases were found in the index group; three of them had been diagnosed as an occupational disease induced by mould exposure, and all the asthmatic teachers were under regular medication. However, the levels of IgG antibodies to moulds did not differ between asthmatic and non-asthmatic teachers. In earlier studies, a preventive effect of smoking on IgG antibodies has been reported [35], but this finding was not confirmed in this study. Females have been suggested to have higher mould specific IgG antibodies than males [36], but the number of males in this study was too small to draw any conclusions.

Overall concentrations of IgG antibodies to 20 moulds in the teachers of the index and reference schools did not differ. Similar findings have recently been reported in school children [37–39]. These microbes can also be found in other environments, outdoors or in other buildings. Similarities in the presence of airborne indicator of fungi in index and reference schools before and shortly after remediation suggest certain resemblance of microbial exposure, which may obscure the differences in teachers' antibody findings in these particular schools.

During the two-year follow-up, IgG antibodies to *Tr. roseum* decreased significantly among the index group and even more specifically among those individuals who had reported previous episodes of sinusitis. *Tritirachium* is an unusual mould found occasionally in indoor air and its health effects are practically unknown except for two isolations in clinical case reports [40,41]. However, the genus of this mould often grows on moisture damaged materials, most frequently in paints and glues but also occasionally on wood, mineral insulation, gypsum boards or plastics [42]. In one of the index schools examined, *Tr. roseum* was found in one material sample before the remediation, thus being a possible source of this fungus.

This is a pilot study, where the association between elevated concentrations of mould specific IgG antibodies and respiratory infections has been found in mould-exposed employees in damaged school buildings. The study population was small but the differences were detectable and statistically significant. More understanding is needed on the pathophysiological mechanism and inflammatory processes occurring in common respiratory infections during mould exposure.

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