

Mold-specific immunoglobulin G antibodies in a child population

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The determination of immunoglobulin G (IgG) antibodies to molds has been used as an objective evidence of significant mold exposure. Until present, no data have been published on antibody responses to molds in healthy children living in normal housing conditions. The microbe-specific IgG antibody concentrations of 21 molds and 3 actinobacteria were determined by enzyme-linked immunosorbent assay (ELISA) in 103 1- to 6-year-old children (12.4% of the population of that age), and in 111 7- to 14-year-old school children (12.1%). The international standard sera were available, and the IgG concentrations of the test sera could be expressed in mg/l. On average, IgG concentrations increased in relation to age until the age of 6–7 years. At school age the increase still continued but more slowly. Actinobacteria were the only exceptions; all three tested strains *Sreptomyces albus*, *S. griseus* and *S. halstedii* resulted in rather high concentrations until 3 years of age. If the children lived in a farm, mold-specific IgG concentrations increased at an earlier age than in other children. The results between farmers' children and other children differed significantly before school age for 20 of the 24 microbes tested, the four exceptions being the 3 actinobacteria and the mold *Aspergillus versicolor*. The reference values must be age related, and separate references are needed for farmers' children before school age.

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Water damage in a building leads to fungal growth in the structures, to emission of fungal products into the indoor air, and finally, may lead to health effects of the inhabitants (1–5). The connection between moisture in the houses and symptoms of the inhabitants, often mimicking allergic symptoms, has been documented in many epidemiological studies (6–10). Our recent studies have focused on the indoor air quality and health effect documentation in day care centres (11, 12) and primary schools (13, 14). An allergic sensitization to molds is rare (4, 13, 15–18), though an extensive exposure in agricultural and other working conditions may lead to mold-specific IgG production and, e.g. farmer's lung disease (19, 20). Therefore, the determination of IgG antibodies to molds has been used, at both an individual and a group level, as an objective evidence of significant mold exposure (19–23).

Molds are a natural part of the outdoor environment, and the same microbes are scattered all over the world (24). Indoor air fungal flora and concentrations reflect, depending on the climatic factors and the ventilation systems of the buildings, outdoor fungal flora and concentrations (25). Therefore, the fungi, yeasts and bacteria most commonly occurring in moistened building materials are similar around the world. Until present, no data have been published on the exposure and antibody responses of non-selected, healthy children to molds. So, the influence of, e.g. age and exposure in normal housing conditions to mold-specific IgG is still unresolved.

The aim of the present study was to evaluate the occurrence and concentrations of IgG antibodies to 21 molds and 3 bacteria signalling indoor moisture problems, in healthy children

aged 1 to 14 years living in normal conditions. Special attention was paid to the possible effects of age and agricultural environment. The final goal was to introduce age-related microbe-specific reference values for further epidemiological and clinical studies.

Materials and methods

Kiuruvesi is a small town with a total population of 10,600 people in Eastern Finland. The number of children under the age of 15 years was 1907 at the end of 1996. About 31% of the adult population work in agriculture or forestry, 51% in services and education, and only 15% in industry. As published recently, there were three school buildings with water damages and mold problems, leading to increased morbidity in both the teachers and school children (13, 26). Therefore, all municipal school buildings and day care centres were checked including technical investigation of the building and microbiological examination of indoor air and surface samples. The principles of the technical and microbiological evaluations of the buildings have been published elsewhere in more detail (27–31). Except for the three moisture and mold problem schools, other school buildings, as well as the day care centres were found to be 'healthy buildings'.

The data were collected and serum samples were obtained in 44 children aged 7–14 years from a healthy school in 1994 (26, 31), in 67 children of the same age from healthy schools in 1996, and in 103 preschool children aged 1–6 years visiting well-baby centres in 1998. We aimed to get data and serum samples from 15 to 20 children in each 1-year age group. The parents fulfilled a questionnaire including questions on home environment, such as observed moisture, observed odour or visible mold growth at home, questions on the social situation of the family including day care of the children, and questions on the occupations of the parents with special attention paid to the farming occupations. The questionnaire was the same as used in the previous studies of our study group (12).

Blood samples were drawn in the laboratory of the local health care centre. The obtained blood volume was 5–10 ml; serum was immediately separated and frozen, and within 1 week, sent as frozen to the study laboratory. There the samples were stored at -20°C until studied simultaneously and in a random order in 1999. The 103 1- to 6-year-old children represented 12.4%, and those 111 7- to 14-year-old children 12.1% of the child population of that age in the town. In total, 56 (54%) under school-aged children

attended day care outside the home, mainly in a day care centre. In 78 (36%) cases, one or both of the parents were farmers; the figure was 25 (24%) for under school-aged and 53 (48%) for school-aged children. In 63 families, the parents had daily contacts with animals, working in most cases on dairy farms.

Immunoglobulin G (IgG) antibody concentrations in serum samples were measured to antigens of in total 24 microbes including *Aspergillus fumigatus**, *Acremonium kiliense*, *Cladosporium cladosporioides*, *Geotrichum candidum*, *Penicillium glabrum*, *A. atrogriseum*, *Rhodotorula glutinis** (yeast), *Sporobolomyces salmonicolor* (yeast), *A. versicolor**, *Chaetomium globosum*, *Fusarium oxysporum**, *Phoma macrostoma**, *Stachybotrys chartarum**, *Streptomyces albus** (actinobacterium), *Trichoderma citrinoviride**, *Tritirachium roseum*, *Aureobasidium pullulans*, *Eurotium amstelodami**, *Mucor circinelloides**, *Paecilomyces variotii*, *P. notatum*, *Rhizopus nigricans*, *S. griseus** (actinobacterium) and *S. halstedii** (actinobacterium). The microbes were selected to cover as well as possible the strains found, based on the literature and on our own experience, in buildings with moisture or mold problems (24, 26, 32, 33). According to Samson et al. (33) 13 microbes (*) were classified as dampness indicative molds or bacteria. *Stachybotrys*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Streptomyces* and *Trichoderma* genera are the genera most often found in water-damaged buildings, including homes, day care centres or school buildings in our area (29, 33).

Microbe-specific IgG antibody concentrations of 19 fungi, 2 yeasts and 3 bacteria were determined by enzyme-linked immunosorbent assay (ELISA), according to Laitinen et al. (22). Crude antigen extracts were prepared from cultures obtained originally from Finnish environmental samples, with the exception of *S. griseus*, which was originated from the UK. Before manufacturing of the antigen extracts, the identification of fungal species and yeasts was assured by an international reference laboratory (Centraalbureau voor Schimmelcultures, Baarn, the Netherlands). The culture of microbes and preparation of antigen extracts, as well as the detailed performance of the ELISA test, have been published elsewhere (22, 26). In the ELISA, the absorbance values were compared with the values of pooled, positive adult sera specific for each microbe, and the control serum was used on each microtitre plate to avoid possible differences in the assays done at different times. The positive control sera had been collected from 321 adults who had reported exposure to moisture and mold

problems, and who had significant amounts of IgG antibodies only to one or a few molds. To minimize the effects of exceptionally high or low IgG concentrations, the positive control sera were pooled. The absorbance given by the test serum was expressed as a percentage of the absorbance given by the positive control serum. Thereafter, the pooled control sera were tested against the international standard serum (World Health Organization's International Reference Preparation for Human Serum IgG, IgA and IgM, number 67/86, National Institute for Biological Standards and Controls, London, UK), and the concentrations in mg/l for the test sera were calculated from the observed absorbance values. Finally, the mold- and bacterium-specific IgG concentrations of the test sera (mg/l) were presented separately for the four age groups (1–3, 4–6, 7–9 and 10–13 years).

Exploratory data analysis revealed that the distributions of the data obtained in this study were not normal, and the distributions remained as non-normal even after logarithmic transformations. Therefore, the results are expressed as medians, and in addition, 95% percentiles are presented. Non-parametric tests were used to evaluate the significances of the differences between the groups. Wilcoxon's rank sum test was used to analyze the significance of the differences in species-specific IgG concentrations between farmers' children and other children.

The study protocol was approved by the Ethics Committee of the National Public Health Institute, Finland. After receiving thorough information, a written consent was obtained from the parents and, in addition, also from school-aged children.

Results

Serum IgG concentrations were measured to the antigens of in total 24 microbial species. On average, IgG concentrations increased in relation to age until the age of 6–7 years. At school age the increase still continued but more slowly. Actinobacteria were the only exceptions; all three tested strains *S. albus*, *S. griseus* and *S. halstedii* resulted in rather high IgG concentrations until 3 years of age, showing thereafter first a decreasing and then a slowly increasing tendency. There was a rather wide variation of specific IgG for all microbes within all age groups.

In earlier studies, the mold-specific IgG values have been expressed as ratios or percentages of the absorbances of the study sera from the in-house control sera of each laboratory. When tested against the international standard, the

concentrations of the control sera of our laboratory varied from 30.5 (*Stachybotrys atra*) to 67.4 mg/l (*F. oxysporum*). The concentrations were within a 20-mg/l interval, between 40.0 and 60.0 mg/l for 16 (67%) of the total 24 microbes (data not shown).

About a third (24% of those under and 48% of those over 7 years) of the children lived in a farm house and their parents were farmers. In them, mold-specific IgG concentrations increased in an earlier age than in other children. The results between farmers' children and other children differed significantly before school age for 20 of the 24 microbes tested. The cumulative figure was three (13%) at 1–2 years, six (23%) at 3–4 years and 20 (83%) at 5–6 years of age. Thereafter, no differences were found between these two child groups. The four microbes not having produced higher amounts of antibodies in farmers' children were the mold *A. versicolor*, and the actinobacteria *S. albus*, *S. griseus* and *S. halstedii*.

Serum IgG concentrations of molds were not dependent on the gender of the children. About half (54%) of the under school-aged children attended day care outside the home, and no association was found between day care attendance and microbial IgG antibodies (data not shown). Thirteen per cent of the parents reported moisture problems, 6% odour problems and 4% visible fungal growth at home. At least one of these observations was reported in 16% of the current homes of the families. These parent-reported observations, when analyzed either alone or combined, did not have any significant effects on microbial antibody results (data not shown).

Tables 1–3 present IgG antibody concentrations of 24 microbes in four age groups, being expressed as medians and 95% percentiles. Before school age, the results are presented separately for farmers' children and other children. In school-aged children, the concentrations were rather similar in these two groups, and therefore, the results at school age are presented as combined.

In under school-aged children, the medians of species-specific IgG antibody concentrations varied from 2.9 (*G. candidum*) to 37.4 mg/l (*A. versicolor*) (Table 1). The median IgG levels to *A. kiliense*, *G. candidum*, *M. circinelloides* and *P. macrostoma* were <10 mg/l in both age groups. The median concentrations were >25 mg/l for *A. versicolor* and *S. halstedii* in both age groups, and *A. atrogriseum* in the group of 4- to 6-year-old children.

In farmers' children, the medians of species-specific IgG antibody concentrations varied from

Table 1. Serum IgG antibody concentrations of 24 microbes in 78 preschool-aged children (farmers' children excluded) expressed as medians and 95% percentiles

Microbes	1–3 years		4–6 years	
	Median	95%	Median	95%
<i>Aspergillus fumigatus</i>	10.3*	26.6	19.0	42.3
<i>Acremonium kiliense</i>	7.4	26.9	8.2	29.3
<i>Cladosporium cladosporioides</i>	10.5	33.0	17.2	36.8
<i>Geotrichum candidum</i>	2.9	9.2	9.2	20.9
<i>Penicillium glabrum</i>	12.1	30.7	21.4	47.7
<i>Acremonium atrogriseum</i>	14.1	38.6	25.1	47.1
<i>Rhodotorula glutinis</i>	8.1	25.9	15.1	39.9
<i>Sporobolomyces salmonicolor</i>	6.3	32.3	15.7	36.9
<i>Aspergillus versicolor</i>	25.1	56.3	37.4	56.4
<i>Chaetomium globosum</i>	7.8	23.0	13.0	28.2
<i>Fusarium oxysporum</i>	8.8	44.5	12.8	31.7
<i>Phoma macrostoma</i>	3.9	21.2	7.4	32.5
<i>Stachybotrys chartarum</i>	9.2	27.1	14.3	25.9
<i>Streptomyces albus</i>	18.7	35.5	19.1	38.3
<i>Trichoderma citrinoviride</i>	10.5	35.0	19.1	35.5
<i>Tritirachium roseum</i>	9.1	21.5	11.9	37.9
<i>Aureobasidium pullulans</i>	13.9	29.2	18.6	35.7
<i>Eurotium amstelodami</i>	9.7	26.2	18.9	37.1
<i>Mucor circinelloides</i>	6.8	23.4	9.2	30.8
<i>Paecilomyces variotii</i>	7.6	28.1	15.7	32.4
<i>Penicillium notatum</i>	15.7	33.0	21.6	40.6
<i>Rhizopus nigricans</i>	11.0	33.7	20.8	45.3
<i>Streptomyces griseus</i>	18.1	47.0	11.9	32.3
<i>Streptomyces halstedii</i>	28.6	63.7	25.1	54.3

There were 48 1- to 3-year-old and 30 4- to 6-year-old children.

*mg/L.

2.9 (*G. candidum*) and 4.8 mg/l (*Phoma macrostoma*) to 40.2 (*A. versicolor*) and 43.8 mg/l (*A. atrogriseum*) (Table 2). For all molds, the median IgG concentrations were > 10 mg/l at the latest at 4 years of age. The 25-mg/l IgG concentration was exceeded for *P. glabratum*, *A. versicolor*, *E. amstelodami*, *S. albus*, *S. griseus* and *S. halstedii* before the age of 3 years. In farmers' children, on average, the 10- to 13-year level (see Table 3) was achieved before school age for 18 (75%) of the 24 microbes.

In school-aged children, the medians were > 25 mg/l for 15 (63%) of the 24 microbes (Table 3). The lowest concentration (10.6–12.6 mg/l) was for *P. macrostoma*. The highest concentration (55.8–54.7 mg/l) was for *A. versicolor*.

Discussion

There are two main results in the present study on serum IgG antibodies to molds in healthy, Finnish children. First, IgG antibody concentrations increased rather constantly in relation to age for the first 6–7 years of life. At school age, the concentrations remained rather stable or the increase continued but more slowly than before

Table 2. Serum IgG antibody concentrations of 24 microbes in 25 preschool-aged, farmers' children expressed as medians and 95% percentiles

Microbes	1–3 years		4–6 years	
	Median	95%	Median	95%
<i>Aspergillus fumigatus</i>	16.3*	39.8	29.3	58.0
<i>Acremonium kiliense</i>	11.7	21.8	24.2	38.2
<i>Cladosporium cladosporioides</i>	17.2	32.5	32.5	53.1
<i>Geotrichum candidum</i>	2.9	7.5	15.4	39.6
<i>Penicillium glabrum</i>	25.8	44.4	39.5	66.9
<i>Acremonium atrogriseum</i>	19.8	43.3	43.8	69.2
<i>Rhodotorula glutinis</i>	12.0	32.1	29.0	42.2
<i>Sporobolomyces salmonicolor</i>	15.3	44.4	33.0	55.0
<i>Aspergillus versicolor</i>	26.8	62.5	40.2	60.3
<i>Chaetomium globosum</i>	12.2	22.6	20.1	37.8
<i>Fusarium oxysporum</i>	11.5	56.6	22.2	56.6
<i>Phoma macrostoma</i>	4.8	16.0	16.9	31.6
<i>Stachybotrys chartarum</i>	13.4	24.4	29.9	50.6
<i>Streptomyces albus</i>	25.9	35.7	20.4	41.7
<i>Trichoderma citrinoviride</i>	21.8	30.0	36.9	51.4
<i>Tritirachium roseum</i>	14.6	28.3	20.1	67.6
<i>Aureobasidium pullulans</i>	19.0	34.3	33.8	58.9
<i>Eurotium amstelodami</i>	26.2	44.5	36.5	56.6
<i>Mucor circinelloides</i>	11.1	20.9	16.0	28.3
<i>Paecilomyces variotii</i>	13.2	21.0	29.1	44.8
<i>Penicillium notatum</i>	21.6	50.3	39.5	62.2
<i>Rhizopus nigricans</i>	16.6	28.2	25.7	51.4
<i>Streptomyces griseus</i>	25.7	44.2	12.9	25.7
<i>Streptomyces halstedii</i>	42.0	63.7	26.3	45.6

There were 11 1- to 3-year-old and 14 4- to 6-year-old children.

*mg/L.

school age. Second, the final concentrations were achieved earlier in the farmers' children than in other children, suggesting a more extensive exposure to microbes and earlier antibody responses in an agricultural environment. The results stressing the role of farming environment agree with earlier observations in adult farmers (19, 20, 24), but disagree with our recent observations in selected children exposed to moisture and molds in the school (31). Because as many as 36% of the children lived in a farm house, mainly on dairy farms, the present study was especially suitable for the evaluation of the influence of an agricultural environment. To our knowledge, the development of mold-specific antibodies in a child population has not been studied earlier. To minimize the confounding environmental effects, only children from 'healthy' day care centres and school buildings were included, and before school age, the farmers' children are presented separately.

The ELISA test was used to measure mold-specific IgG antibodies, and the absorbances were compared with pooled positive sera (22, 31, 35). These control sera of our laboratory have been obtained from moisture and molds exposed

Table 3. Serum IgG antibody concentrations of 24 microbes in 100 school-aged children (farmers' children included) expressed as medians and 95% percentiles

Microbes	7–9 years		10–13 years	
	Median	95%	Median	95%
<i>Aspergillus fumigatus</i>	45.0*	67.8	47.7	68.3
<i>Acremonium kiliense</i>	16.8	36.7	24.6	42.5
<i>Cladosporium cladosporioides</i>	29.6	64.5	34.9	55.4
<i>Geotrichum candidum</i>	13.3	38.8	16.3	36.3
<i>Penicillium glabrum</i>	35.6	60.8	42.2	70.1
<i>Acremonium atrogriseum</i>	38.2	66.4	43.3	57.5
<i>Rhodotorula glutinis</i>	25.5	46.1	31.3	51.1
<i>Sporobolomyces salmonicolor</i>	26.3	57.4	30.3	50.7
<i>Aspergillus versicolor</i>	55.8	67.5	54.7	61.4
<i>Chaetomium globosum</i>	15.6	47.3	19.5	36.0
<i>Fusarium oxysporum</i>	20.9	46.5	23.6	56.6
<i>Phoma macrostoma</i>	12.6	30.3	10.4	27.3
<i>Stachybotrys chartarum</i>	20.1	41.2	23.5	43.3
<i>Streptomyces albus</i>	29.3	62.1	37.0	75.2
<i>Trichoderma citrinoviride</i>	26.4	53.4	30.0	57.8
<i>Tritirachium roseum</i>	17.8	40.2	19.7	42.0
<i>Aureobasidium pullulans</i>	25.1	45.0	29.7	52.4
<i>Eurotium amstelodami</i>	33.5	61.5	42.6	60.9
<i>Mucor circinelloides</i>	16.0	38.2	16.0	37.6
<i>Paecilomyces variotii</i>	26.7	54.4	32.9	47.2
<i>Penicillium notatum</i>	31.9	61.1	41.1	63.8
<i>Rhizopus nigricans</i>	25.7	69.8	26.9	55.7
<i>Streptomyces griseus</i>	14.3	33.3	16.6	34.7
<i>Streptomyces halstedii</i>	38.5	59.0	43.8	61.9

There were 48 7- to 9-year-old and 63 10- to 14-year-old children, and 53 (48%) were farmers' children.

*mg/L.

adults positive only for one or a few molds. Thus, the in-house control sera are highly specific for each individual mold minimizing the risk for cross-reactions, but due to this selection, they do not represent any population or defined group. The control sera were tested against the international standard serum, and for most microbes, the concentrations varied within a rather narrow interval of 20 mg/l. Low concentrations (< 40 mg/l) were to *S. atra*, *C. cladosporioides*, *R. glutinis* and *A. kiliense*. The concentrations were high (> 60 mg/l) for *E. amstelodami*, *M. circinelloides*, *R. nigricans* and *F. oxysporum*. Though a comprehensive panel of 24 microbes was applied, the selected strains constituted only two-thirds of molds or other microbes commonly found in water-damaged buildings (31).

The presence of IgG antibodies to selected molds has been used as biomarkers of exposure to molds, mainly at a group level in occupational environments such as agriculture (20, 23, 34). In the conditions with high-level exposure, these antibodies have been considered to reflect exposure also in cases with no association with mold-related diseases (21). Our two recent studies in

children from moisture and mold problem schools gave negative results, that is no association was found between exposure or microbial findings in the schools, and mold-specific IgG levels in the school children (31, 35). In all studies, the interpretation of mold-specific antibody results has been difficult, because no reference values have been available. The references used for adults in occupational environments are too high for children, being based on positive samples taken from subjects with high-level exposure (22). The figures of the present paper can be used as references for children, and the expression of the concentrations in mg/l allows the comparison of different research and clinical data.

The present data do not allow any estimation of which mold-specific IgG values should be considered as pathological. The concentrations were age-related being higher in farmers' children, they were non-normally distributed having wide variations, and no comparison was possible with health effects of the molds. Therefore, 95% percentiles are presented for four age groups, indicating a 0.05 probability to have an equally high concentration of mold antibodies at the corresponding age in the corresponding population. On the other hand, elevated antibodies to occasional molds may not be very informative, because exposure in the cases of water damage or excessive moisture in the building takes place concomitantly to numerous different molds. The profiles of species-specific antibodies may be the result of an excessive exposure or an exposure caused by certain harmful molds, and thus are more important than single elevated antibody concentrations.

The presence of IgG antibodies to microbes indicates an exposure but tells nothing about the time when the exposure took place, or how long or severe it was. Therefore, one must be careful when applying mold-specific IgG antibodies as markers of exposure at an individual level. The results may be more useful at the group level, and usually should be combined with microbial data of the environment (23). When IgG antibodies are positive for many identical microbes in the family members, in the day care group or in the group of school children, a common source of exposure is possible. Further, if the microbes that have induced antibody production are also detected in the environment of the child, the connection between symptoms and environmental exposure becomes still more probable. Therefore, reference values are needed not only for serum antibody concentrations, but also for

microbial concentrations/amounts in the air and other samples from the buildings.

In conclusion, serum IgG antibody concentrations of 21 molds and 3 actinobacteria in 214 healthy, 1- to 14-year-old Finnish children were measured. The goal of the study was to assess reference values for further clinical and epidemiological purposes. Before school age, concentrations were higher in farmers' children than in other children, and different reference values are presented for these two groups. At school age, the groups did not differ, and common reference values are presented. The study design does not allow the estimation of pathological limits, and 95% percentiles of microbe-specific IgG concentrations are presented as possible limits for further studies.

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