

ORIGINAL PAPER

Mould-specific immunoglobulin antibodies quantified by flow cytometry reflect mould exposure in Norwegian children

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Summary

Background Studies from many countries have shown an association between dampness in buildings and airway symptoms. Little is known about the role of mould-specific IgG antibodies in this context.

Objective To examine the IgG antibody response to mould applying a new flow cytometric assay, compare the results with the standardized ImmunoCap® method, and evaluate the association of IgG to IgE antibodies, dampness in buildings, and airway symptoms like wheeze and asthma.

Methods A population of 3713 children 9–11 years of age living in Northern Norway was investigated for airway symptoms and dampness at homes by a parental questionnaire, using protocols of the International study of Asthma and Allergy in Childhood (ISAAC). Among these, a case-control study of 100 wheezers and 100 non-wheezers was established that included home inspection, a parental structured interview, and serum samples analysed for mould-specific IgG and IgE antibodies, total IgE, and specific IgE to an allergen panel (Phadiatop®).

Results Self-reported visible signs of mould or moisture at home during the child's first year of life were a significant risk factor for both wheeze and asthma. The levels of mould-specific IgG antibodies were associated with mould and moisture findings, but only when IgG antibodies were measured by flow cytometry.

Conclusions The results support that dampness at home can increase the risk of airway symptoms. IgG antibodies determined by flow cytometry reflect mould exposure better than antibodies measured by the conventional method. IgG antibodies measured by flow cytometry may be used as an indicator of mould exposure.

Keywords antibody, asthma, flow cytometry, mould, wheeze

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Introduction

Several epidemiologic studies have shown an association between dampness in buildings and respiratory disorders (reviewed in [1, 2]). Further, it has been estimated that 5% of the population are predicted to have, at some time, allergic symptoms as a consequence of exposure to fungal allergens [3]. The cause-and-effect relationship between mould exposure and symptoms is unclear. However, recent evidence has demonstrated a close association between fungal exposure and asthma severity [4]. The relationship between IgG and IgE antibody production in relation to allergic disease is poorly known, and the mechanism

behind allergen-induced antibody class switching has been suggested to differ between allergens [5].

Mould-specific IgG antibodies have been used as a marker of exposure to moulds that are associated with airway diseases like asthma and pneumonitis. Mould-specific IgG antibody production is clearly dependent on the extent and pattern of mould exposure [6], but the concentration of mould-specific IgG antibodies has never been established as a standard method for determining personal mould exposure. The ubiquitous occurrence of many fungi and the lack of specificity of fungal antigen extracts limit the usefulness of these types of tests. The most used methods for measuring concentration of

mould-specific IgG antibodies are based on antigen extractions, like the standardized ImmunoCap[®] methodology. As reviewed by Trout *et al.* [7], a poor association has often been found between mould exposure and IgG antibodies measured by extract-based immunoassays.

Recently, based on intact mould spores as capture antigens, we have developed a flow cytometric method for measuring the concentration of mould-specific IgG antibodies in serum [8]. This method utilizes the native antigens on the mould spore surface, which have been shown to contain the most important mould antigens for both IgG and IgE antibodies [9].

The aim of this study was to assess the association between mould exposure, antibody production, and airway symptoms among primary schoolchildren living in the subarctic environments of Northern Norway. Further, we wanted to investigate these parameters in relation to IgG antibody measurements by both ImmunoCap[®] and flow cytometry.

Methods

Study design

This study is part of Phase II of the International Study of Asthma and Allergies in Childhood in Europe (ISAAC) [10, 11]. Two of the northernmost counties of Norway, Troms and Finnmark, are localized north of the polar circle, and thereby represent contrasting exposure and living conditions to more southern regions. During the period from October 1999 to May 2000, all children in the two counties aged 9–11 years ($n = 4646$) were invited to participate in this cross-sectional study. The participation rate was 80% ($n = 3713$). A questionnaire, including questions related to asthma and other atopic diseases, family anamnesis, and home characteristics (present and retrospectively) was filled out by the parents.

From the original cohort, 100 current wheezers and 100 non-wheezers living in and near Tromsø city were included in a case-control study. The study design adhered to the ISAAC phase II protocol [10], and included home inspection, a structural interview of the parents, and blood sampling. Trained inspectors visited all homes, performing inspections for signs of mould or other moisture-related damages in the living area of the homes (bathroom, bedrooms, living room, and kitchen). In addition, the inspectors conducted a structured interview of the parents that included questions related to home characteristics like water damages and ventilation. Home visits were performed during the period from March 2001 to May 2002 (except June–August). Not all data were available for all children, and missing data were excluded from each single analysis. In total, the present case-control study is based on 200 home visits, 190 blood samples, and 165 questionnaires. The study was approved

by the regional committee for medical research ethics, Northern Norway.

Definitions

Wheeze and asthma incidences were defined as positive answers to the questions about symptoms in the questionnaire:

- Current wheeze: 'Has your child had wheezing or whistling in the chest in the last 12 months?'
- Ever wheeze: 'Has your child ever had wheezing or whistling in the chest at any time in the past?'
- Ever asthma: 'Has your child ever had asthma?'

A number of potential risk factors were examined: Gender, ever breastfeeding (yes/no), elder siblings (yes/no), parental history of asthma, hayfever and/or eczema (at least one symptom in the mother or father), socioeconomic status defined as university studies of minimum one parent (yes/no), and maternal smoking during pregnancy (yes/no). In addition, the following information regarding the child's home was obtained:

- Questionnaire: 'Has your child's home had damp spots on the walls or ceiling?' and 'Does or did your child's home have visible moulds or fungus on the walls or ceiling?' addressing the time of completion of the questionnaire and/or the first year of the child's life.
- Interview: 'Does your child's home presently have moisture marks, precipitation of salt on concrete walls, mould stains or mouldy smell?'

Findings reported by the inspectors were grouped into different definitions of exposure: 'any findings' included moisture spots, visible mould, mouldy smell, discoloured window frame, or salt precipitation on walls; 'mould and/or moisture' included visible mould, mouldy smell, or moisture spots; 'moisture' included moisture spots, and 'mould' included visible mould or a mouldy smell.

Antibody measurements

Blood samples were collected during the period from June 2001 to May 2002, at the Department of Clinical Research at University Hospital of Northern Norway. Clotted blood samples were centrifuged and sera were frozen and stored at -20°C until analysed. Total IgE and Phadiatop[®] were analysed by the ImmunoCap[®] system (Phadia AB, Uppsala, Sweden), in which the Phadiatop[®] score is based on specific IgE antibodies to common aeroallergens. The concentrations of IgG and IgE antibodies specific to *Aspergillus versicolor*, *Penicillium chrysogenum*, and *Cladosporium herbarum* were determined by ImmunoCap[®] (Cap ID for IgG: RGM25, m1 and Gm2; Cap ID for IgE: RGM25, m1 and m2, respectively, Phadia AB).

IgG antibody measurements were performed with serum diluted 1:100, while IgE analyses were performed on undiluted serum. At least 82 different mould species have been identified indoors, in which 36 were characterized as widely distributed [12]. However, we selected the three mould species *A. versicolor*, *P. chrysogenum*, and *C. herbarum*, because these are the most common mould species found indoors in Scandinavia [13, 14].

IgG antibodies to the same three moulds were also determined by flow cytometry as described previously [8]. In short, serum was incubated with 10^6 mould spores diluted in spore buffer (phosphate-buffered saline, pH 7.4, with 0.5% bovine serum albumin and 0.02% NaN_3), allowing the mould-specific antibodies to bind to the spore surface. Then, the spores were washed before incubation with an FITC-conjugated rabbit anti-human IgG γ -chain (Daco Cytomation, Glostrup, Denmark) diluted 1:10 in spore buffer. Finally, the spores were washed, before being analysed in an EPICS XL flow cytometer (Coulter, FL, USA) connected to a PC using EXPO32™ software (Applied Cytometry Systems, Sheffield, UK). A minimum of 10 000 spores per sample were analysed. The relative amount of mould-specific IgG antibodies in serum was determined by measuring the median FITC intensity of spores in each sample. For each experiment, the background fluorescence of the negative control (without serum) was adjusted to a median FITC intensity of 0.3.

Statistical analyses

Statistical analyses were performed with SPSS Version 14.0 (Chicago, IL, USA). Some of the groups had non-normal data, but all the distributions were symmetric, and we used parametric statistical methods. Categorical variables were compared by Pearson's χ^2 test. For continuous

variables, Student's *t*-test or one-way ANOVA tests were used for comparing two or multiple groups, respectively. When the ANOVA showed significant differences between all groups, pairwise comparisons were performed by the Sidak *post hoc* test for unequal group size. The level of significance was set to 5%. Logistic regression analyses were performed to establish possible predictors for wheeze and asthma, while linear regression analyses were performed to study the associations between exposure determinants and the concentration of mould-specific IgG antibodies. The independent variables entered into the regression analyses are shown in Table 1. Variables associated ($P < 0.2$) in bivariate analyses were included in the adjusted regression analyses. The logistic regression results were presented as odds ratios (OR) with 95% confidence intervals, while the linear regression analyses were presented as regression coefficients with their standard error and *P*-values. We have defined a minimum relevant change in the IgG concentration to be 20 U for the flow cytometric analyses (range 0–170 median FITC intensity) and 10 U for ImmunoCap® analyses (range 0–95 mgA/L). The associations between the minimum relevant change in IgG concentrations and current symptoms were analysed using the Multi Fractional Polynomials macro running under SAS Version 9.1.3 (Cary, NC, USA) to establish any non-linear association with the outcome [15]. These analyses were not adjusted for other determinants.

Results

In the cross-sectional questionnaire of 3713 children, 14.0% reported current wheezing, 32.6% reported ever wheezing, while 10.2% reported ever asthma (Table 1). In the same questionnaire, visible mould or damp spots on the walls or ceilings were reported currently in 3.4% of the

Table 1. Demography of 9–11-year old children with self-reported questionnaire in the cross-sectional ($n = 3713$) compared with the case-control study ($n = 165$)

	Cross-sectional		Case-control study	
	%	n/n_{tot}	%	n/n_{tot}
Male sex	50.1	1813/3622	56.2	91/162
Ever wheeze	32.6	1109/3397	64.4	105/163
Current wheeze	14.0	476/3411	54.6	89/163
Ever asthma	10.2	345/3368	19.4	31/160
Parental symptoms	53.6	1991/3713	58.8	97/165
Maternal smoking during pregnancy	34.5	1143/3312	33.3	52/156
Breast feeding	94.9	3434/3620	95.1	156/164
Elder siblings	61.6	2224/3613	55.8	91/163
Mould/moisture first year*	6.7	239/3576	5.5	9/164
Current mould/moisture*	3.4	120/3563	3.0	5/164

*Self-reported visible mould and/or moisture in the questionnaire.

There were no statistical differences between the cross-sectional and case-control study population in regard to any non-symptom characteristics (all $P > 0.1$).

Table 2. Logistic regression analyses for self-reported determinants associated with airway symptoms in the cross-sectional study ($n = 3713$)

	Current wheeze OR (95% CI)	Ever wheeze OR (95% CI)	Ever asthma OR (95% CI)
Male sex			
Crude	1.51 (1.24–1.84)	1.65 (1.43–1.91)	1.85 (1.47–2.33)
Adjusted	1.52 (1.23–1.87)	1.63 (1.39–1.90)	1.86 (1.46–2.36)
Parental symptoms			
Crude	1.75 (1.43–2.14)	1.53 (1.32–1.77)	2.25 (1.76–2.88)
Adjusted	1.77 (1.42–2.20)	1.55 (1.33–1.81)	2.28 (1.77–2.94)
Maternal smoking during pregnancy			
Crude	1.23 (0.99–1.51)	1.23 (1.05–1.44)	
Adjusted	1.18 (0.96–1.47)	1.20 (1.02–1.41)	
Elder siblings			
Crude			0.84 (0.67–1.06)
Adjusted			0.90 (0.71–1.14)
Mould/moisture 1st year*			
Crude	1.48 (1.04–2.10)	1.96 (1.49–2.58)	2.26 (1.59–3.22)
Adjusted	1.33 (0.92–1.95)	1.88 (1.41–2.52)	2.06 (1.43–2.97)
Current mould/moisture*			
Crude	1.45 (0.89–2.36)	NA	NA
Adjusted	1.45 (0.86–2.44)	NA	NA

Significant odds ratios are in boldface ($P \leq 0.05$). All variables from Table 1 were included in the analyses, but only variables that associated with airways symptoms with $P \leq 0.2$ are shown in the table. Each model is adjusted for all independent risk factors ($P \leq 0.2$ in crude analyses).

*Self-reported visible mould and/or moisture in the questionnaire.

OR, odds ratio; CI, confidence interval; NA, not analysed.

homes, and in 6.7% of the homes during the child's first year of life (Table 1). Logistic regression analyses for determinants associated with airway symptoms showed that self-reported visible mould or moisture spots at home during the child's first year of life were significantly associated with all symptom categories in crude analyses ($P < 0.05$), and significantly associated with ever wheeze and ever asthma in the adjusted analyses (Table 2). Current visible mould or moisture spots at home had a non-significant association with current wheeze. As current exposure cannot influence previous symptoms, current exposure was not analysed for associations with ever symptoms. Reported spots of mould and moisture tended to be associated separately with symptoms, but the associations were stronger when mould and moisture were analysed together. Other factors associated with symptoms were male gender, parental symptoms, and maternal smoking during pregnancy (Table 2). Breastfeeding and elder sibling were of no significant importance.

In the case-control study, sera from 190 children were analysed for several different antibody specificities. Phadiatop[®] was found to be positive in 49% of the children, and the frequency of positive Phadiatop[®] was higher among wheezing (62%) than non-wheezing children (29%). Detectable levels of mould-specific IgE antibodies to *C. herbarum* were found in seven children (3.7%), to *P. chrysogenum* in five children (2.6%), and to *A. versicolor* only in one child (0.5%). All children with

elevated concentrations of mould-specific IgE antibodies were also Phadiatop[®] positive. Mould-specific IgG antibodies to *A. versicolor*, *P. chrysogenum*, and *C. herbarum* were analysed by both ImmunoCap[®] and flow cytometry. The IgG antibody levels showed correlation coefficients from 0.5 to 0.6 ($P < 0.001$) between the two methods. Children who tested positive to mould-specific IgE antibodies tended to have higher levels of mould-specific IgG antibodies for all three moulds compared with the mould-specific IgE-negative children. However, the number of mould-specific IgE-positive children was small, and the difference was statistically significant only for IgG to *C. herbarum* ($P = 0.008$ and 0.002 , measured by flow cytometry and ImmunoCap[®], respectively).

By combining data from the cross-sectional questionnaire and the case-control part of the study, associations were analysed between antibody measurements from the case-control study and current wheezing reported in the questionnaire (Table 3). Current wheeze was positively associated with IgE levels, both as total IgE and Phadiatop[®] positivity. In contrast, current wheeze was negatively associated with *A. versicolor*-specific IgG antibodies when measured by both flow cytometry and ImmunoCap[®] in crude analyses, but the associations were not significant in adjusted analyses. More relevant ORs were also calculated for changed IgG concentrations of 20 U in flow cytometry and 10 U in ImmunoCap[®] (Table 3). Current wheeze showed no associations with *C. herbarum*- or *P. chrysogenum*-specific IgG antibodies.

Table 3. Logistic regression analyses for self-reported and measured determinants associated with airways symptoms in the case-control study ($n = 165$)

	Current wheeze* OR (95% CI)
Parental symptoms*	
Crude	1.68 (0.89–3.16)
Adjusted	2.49 (0.83–7.46)
Maternal smoking during pregnancy*	
Crude	1.51 (0.81–2.82)
Adjusted	1.33 (0.61–2.92)
Elder siblings*	
Crude	1.86 (1.00–3.46)
Adjusted	2.06 (0.71–5.95)
Total IgE [†]	
Crude	1.001 (1.000–1.003)
Adjusted	1.000 (0.998–1.002)
Phadiatop [®] positive [†]	
Crude	3.98 (1.95–8.10)
Adjusted	5.46 (1.55–19.21)
<i>Aspergillus versicolor</i> IgG by flow cytometry [†]	
Crude	0.99 (0.98–1.00)
Adjusted	0.99 (0.94–1.00)
20 units	0.72 (0.56–0.92)
<i>A. versicolor</i> IgG by ImmunoCap ^{®†}	
Crude	0.97 (0.95–1.00)
Adjusted	0.98 (0.97–1.00)
10 units	0.68 (0.51–0.91)

Significant odds ratios are in boldface ($P \leq 0.05$). All variables from Table 1 were included in the analyses, but only variables that associated with airways symptoms with $P \leq 0.2$ are shown in the table. Each model is adjusted for all independent risk factors ($P \leq 0.2$ in crude analyses).

*Self-reported in the cross-sectional questionnaire.

[†]Measured antibody levels in blood samples taken during the case-control study, 1–2 years after the cross-sectional study.

OR, odds ratio; CI, confidence interval.

Mould and moisture signs in the children's homes were divided into five different categories. In the interview, about 21% of the parents reported current moisture marks, precipitation of salt on concrete walls, mould stains, or a mouldy smell in any part of the home. The inspectors reported findings between 5% and 16% of the homes, depending on the severity of the finding (Table 4). Most definitions of exposure were associated with elevated levels of mould-specific IgG antibodies (Table 4). *P. chrysogenum*-specific IgG antibodies were significantly associated with inspector-reported findings of moisture, while *C. herbarum*-specific IgG antibodies were significantly associated with several categories of exposure, but more associated with findings of mould. IgG antibodies to *A. versicolor* showed no significant association with any exposure category. IgG antibody levels measured by flow cytometry were more often associated with exposure than IgG antibodies measured by ImmunoCap[®] (Table 4). The

level of mould-specific IgG antibodies that associated significantly with exposure was studied further, showing a trend of increased IgG concentrations towards more strictly defined exposure to visible mould, in particular for *C. herbarum*-specific IgG (Fig. 1). This was more clearly seen for IgG antibodies measured by flow cytometry than by ImmunoCap[®].

There was poor agreement between self-reported mould and moisture exposure in the questionnaire and the interview. Of the five families reporting current visible mould or moisture spots in the questionnaire, one family reported current signs of mould or moisture in the interview, and none of them had visible mould or moisture reported by the inspector. On the other hand, the agreement between inspector-administered interview and inspector-reported findings was good. Of those reporting current signs of mould or moisture in the interview, 88% were confirmed by inspector-reported findings.

Discussion

The prevalence of ever asthma in the present study was approximately the same as reported previously by the ISAAC questionnaire in Sweden (10–11%) [16], but higher than what was found by ISAAC in Finland (4–8%) [17]. In contrast, the prevalence of ever wheeze in this study (32.6%) was equal to the prevalence reported in Finland (28–33%), but somewhat higher than that in Sweden (23%). In a study of 10-year-old children in Oslo (situated in the south of Norway), the prevalence of ever asthma was 20.2% [18], twice as much as that in our study. Different asthma criteria may explain some of the difference. Thus, the prevalence of wheeze and asthma in our subarctic study is overall in accordance with the ISAAC-reported prevalence in other Scandinavian countries, situated in climate zones like Oslo. This suggests that the different climates in the subarctic Northern Norway and the more temperate southern part of Scandinavia do not have any major impact on the prevalence of wheeze and asthma.

Our results show that visible signs of mould or moisture at home during the child's first 12 months, as reported in the questionnaire, were significantly associated with wheeze and asthma, both ever and at the age of 9–11 years. Current mould exposure was associated with current wheeze, although not significantly. These associations are in accordance with previously reviewed studies showing a homogeneous positive association between mould exposure and airway symptoms, independent of the frequency of reported mould exposure [1, 19].

There was a rather poor agreement between exposure to mould or moisture reported in the parental questionnaire and the parental interview. All data were collected during the autumn, winter, and spring seasons; thus season did not have any major impact. The difference is more likely due to different times of data collection, as the inspections

Table 4. Linear regression analyses of mould exposure determinants related to the concentration of mould-specific IgG antibodies

		<i>Aspergillus versicolor</i>			<i>Penicillium chrysogenum</i>		<i>Cladosporium herbarum</i>	
	% positive*		Flow cytometry	ImmunoCap®	Flow cytometry	ImmunoCap®	Flow cytometry	ImmunoCap®
Self-reported†								
Any finding	20.9	<i>b</i>	3.93	3.80	10.17	0.67	7.58	1.74
		SE	7.34	4.37	4.24	1.04	3.53	2.25
		<i>P</i>	0.59	0.39	0.02	0.52	0.05	0.44
Inspector reported‡								
Any finding	15.9	<i>b</i>	5.03	− 1.94	6.47	1.28	10.57	2.85
		SE	8.08	4.87	4.85	1.13	4.68	1.34
		<i>P</i>	0.53	0.69	0.19	0.26	0.03	0.04
Mould and/or Moisture	14.4	<i>b</i>	3.21	− 3.92	7.49	1.37	12.63	1.94
		SE	8.41	4.83	4.73	1.12	4.83	1.39
		<i>P</i>	0.70	0.42	0.12	0.22	0.01	0.17
Moisture	10.1	<i>b</i>	7.19	− 0.80	13.49	2.87	8.48	3.02
		SE	10.49	6.06	5.79	1.38	6.15	1.75
		<i>P</i>	0.49	0.90	0.02	0.04	0.17	0.09
Mould	5.8	<i>b</i>	− 3.17	− 7.36	− 2.63	− 0.99	16.35	0.09
		SE	12.67	7.08	7.03	1.65	7.33	2.07
		<i>P</i>	0.80	0.30	0.71	0.55	0.03	0.97

Significant determinants are in boldface ($P \leq 0.05$). All parameters are adjusted for gender, parental symptoms, maternal smoking during pregnancy, breast feeding, elder siblings, total IgE and positive Phadiatop®.

*Percent children with findings among those with home inspection and blood sample.

†Reported in the interview and includes all parts of the house.

‡Includes only the living area of the house.

b, regression coefficient; SE, standard error.

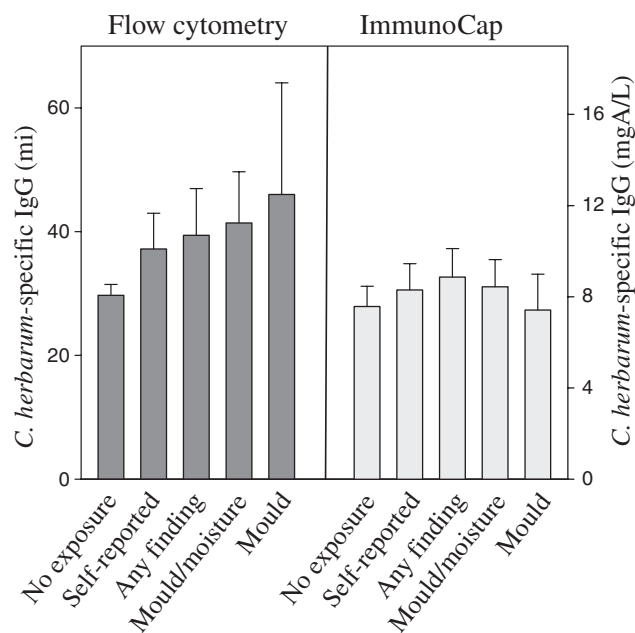


Fig. 1. Levels of *Cladosporium herbarum*-specific IgG antibodies with different definitions of mould and moisture findings at home; only significant associations are shown. Measurements by flow cytometry (dark grey) and ImmunoCap® (light grey) are shown as means (column) with their standard error. More strict definitions are placed to the right in each panel; the last three describe inspector-reported findings. mi, median FITC intensity.

and parental interview were performed 1–2 years after the questionnaire was administered. The living conditions could have changed during that period of time. The discrepancy may also have been caused by the families moving to a new home, uncertainty regarding the wording and definitions of questions in the questionnaires, and different interpretations of how to define signs of mould or moisture. However, the parental interview was in accordance with the inspector-reported findings, suggesting precise parental answers.

The differences in exposure reported by parental questionnaire and parental interview indicated that the time delay between sampling may have had a major impact on the results. Combining data from the cross-sectional and the case-control study, associations between current wheeze and antibody levels were analysed even though the blood sample was taken 1–2 years after the questionnaire was administered. Symptoms of current wheeze were associated with increased levels of total IgE and Phadiatop® positive results, whereas there was a negative association between current wheeze and *A. versicolor*-specific IgG antibodies. This is in accordance with the general understanding that IgG antibodies, in contrast to IgE, are not directly related to symptoms. IgG can be both protective [20] or aggravate allergic symptoms [21]. With regard to the association between levels of IgE and IgG antibodies, those testing positive for any mould-specific IgE in the

case-control study had higher IgG concentrations than non-sensitized individuals. This suggests that those responding with mould-specific IgE production also produce mould-specific IgG antibodies. In addition, all individuals having mould-specific IgE antibodies were also Phadiatop[®] positive, suggesting that sensitized individuals are more likely to be sensitized to more than one allergen.

Mould exposure reported both by interview and inspection was overall more often associated with the level of mould-specific IgG antibodies when measured by flow cytometry compared with the ImmunoCap[®] methodology. This was further supported by increasing levels of IgG antibodies, when measured by flow cytometry, using more strict definitions of exposure, and thus more reliable and certain exposure. The difference due to methodology could indicate that the flow cytometric assay utilizes different and more relevant antigens than does the ImmunoCap[®]. ImmunoCap[®] is based on mould extracts, where both internal and external antigens are basically present [9]. However, all immunological-relevant antigens are not necessarily dissolved and presented in the mould extract, and therefore available for antibody binding in the ImmunoCap[®] system. As antibodies in general are produced against the foreign surface of invading organisms, in addition to secreted antigens, surface antigens will usually be the most important in the host response. Transmission electron microscopy has shown that IgG and IgE bind more densely to cell walls than to cytoplasmic compounds of *A. fumigatus* spores and hyphae [9]. By using intact mould spores in the flow cytometric assay, the surface antigens are naturally present and no extraction procedure is needed. The low correlation coefficients (0.5–0.6) between IgG measurements by the two methods support this explanation.

The most pronounced associations between IgG antibodies and exposure to mould/moisture were found with antibodies specific to *P. chrysogenum* and *C. herbarum*, and no association was found with *A. versicolor*-specific IgG antibodies. Air sample measurements from the present study showed that *Penicillium* and *Cladosporium* were the dominating mould genera indoors, while *Aspergillus* was rarely found (Strømsnes et al., unpublished). Thus, the mould genera dominating indoors were in accordance with the genera specificity of IgG antibodies determined by flow cytometry. The association between mould-specific IgG antibodies and symptoms of wheezing was, on the other hand, significant only for antibodies specific to *A. versicolor*. However, these analyses were performed on data combined from both the cross-sectional and the case-control study, and could have been strongly influenced by the time difference between the two parts of the study.

Self-reported information about symptoms and mould exposure was obtained from the same questionnaire. If

symptomatic individuals over-reported and/or symptom-free individuals under-reported the occurrence of mould in their homes, this might have resulted in a recall bias. Particularly, this could influence the retrospective report of mould or moisture during the child's first year of life. However, our OR, ranging from 1.8 to 2.2, was similar to what has been shown previously in many studies with different study designs [22]. IgG antibody measurement is an objective parameter that can be used to confirm exposure without any recall bias. We have no information regarding the children's exposure to moulds elsewhere than at home, e.g. at school. Anyway, the level of serum IgG antibodies will reflect the total mould exposure, which will also be the most important parameter related to health effects.

In summary, our results support the notion that dampness can increase the risk of airway symptoms. Owing to limited methodology for mould-specific IgG antibody measurements, it has previously been difficult to relate IgG antibodies to either exposure or symptoms. IgG antibodies measured by the novel spore-based flow cytometry assay seem to reflect the levels of exposure better than the extraction-based ImmunoCap[®] methodology. Flow cytometric measurements of mould-specific IgG antibodies can be a helpful tool to improve understanding of the relation between mould exposure, immune responses and airway symptoms.

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References

- 1 Bornehag CG, Sundell J, Bonini S *et al.* Dampness in buildings as a risk factor for health effects, EUROEXPO: a multidisciplinary review of the literature (1998–2000) on dampness and mite exposure in buildings and health effects. *Indoor Air* 2004; 14:243–57.
- 2 Bush RK, Portnoy JM, Saxon A, Terr AI, Wood RA. The medical effects of mold exposure. *J Allergy Clin Immunol* 2006; 117:326–33.
- 3 ACOEM council on Scientific Affairs. *American College of Environmental and Occupational Medicine position statement. Adverse health effects associated with molds in the indoor environment.* Elk Grove Village, IL: ACOEM, 2002.

- 4 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006; 27:615–26.
- 5 Stern DA, Riedler J, Nowak D *et al*. Exposure to a farming environment has allergen-specific protective effects on T(H)2-dependent isotype switching in response to common inhalants. *J Allergy Clin Immunol* 2007; 119:351–8.
- 6 Makkonen K, Viitala KI, Parkkila S, Niemela O. Serum IgG and IgE antibodies against mold-derived antigens in patients with symptoms of hypersensitivity. *Clin Chim Acta* 2001; 305: 89–98.
- 7 Trout DB, Seltzer JM, Page EH *et al*. Clinical use of immunoassays in assessing exposure to fungi and potential health effects related to fungal exposure. *Ann Allergy Asthma Immunol* 2004; 92:483–91.
- 8 Rydjord B, Namork E, Nygaard UC, Wiker HG, Hetland G. Quantification and characterisation of IgG binding to mould spores by flow cytometry and scanning electron microscopy. *J Immunol Methods* 2007; 323:123–31.
- 9 Reijula KE, Kurup VP, Fink JN. Ultrastructural demonstration of specific IgG and IgE antibodies binding to *Aspergillus fumigatus* from patients with aspergillosis. *J Allergy Clin Immunol* 1991; 87:683–8.
- 10 Weiland SK, Bjorksten B, Brunekreef B, Cookson WO, von Mutius E, Strachan DP. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur Respir J* 2004; 24:406–12.
- 11 ISAAC Steering Committee. Phase II Modules of The International Study of Asthma and Allergies in Childhood (ISAAC). Available from <http://isaac.auckland.ac.nz/Phasetwo/Phs2Frame.html>. Cited March 2007.
- 12 Vesper S, McKinstry C, Haugland R *et al*. Development of an environmental relative moldiness index for US homes. *J Occup Environ Med* 2007; 49:829–33.
- 13 Meklin T, Husman T, Vepsäläinen A *et al*. Indoor air microbes and respiratory symptoms of children in moisture damaged and reference schools. *Indoor Air* 2002; 12:175–83.
- 14 Gravesen S, Nielsen PA, Iversen R, Nielsen KF. Microfungal contamination of damp buildings – examples of risk constructions and risk materials. *Environ Health Perspect* 1999; 107 (Suppl. 3):505–8.
- 15 Royston P, Altman DG. Regression using fractional polynomials continuous covariates: parsimonious parametric modelling. *Appl Stat* 1994; 43:429–67.
- 16 Annus T, Bjorksten B, Mai XM *et al*. Wheezing in relation to atopy and environmental factors in Estonian and Swedish schoolchildren. *Clin Exp Allergy* 2001; 31:1846–53.
- 17 Pekkanen J, Remes ST, Husman T *et al*. Prevalence of asthma symptoms in video and written questionnaires among children in four regions of Finland. *Eur Respir J* 1997; 10:1787–94.
- 18 Lodrup Carlsen KC, Haland G, Devulapalli CS *et al*. Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. *Allergy* 2006; 61:454–60.
- 19 Zock JP, Jarvis D, Luczynska C, Sunyer J, Burney P. Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 2002; 110:285–92.
- 20 Niederberger V, Horak F, Vrtala S *et al*. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004; 101 (Suppl. 2):14677–82.
- 21 Janeway CA, Travers P, Walport MJ, Shlomchik M. *Immunobiology – the immune system in health and disease*, 5 Edn. London: Garland Publishing, 2001.
- 22 Bornehag CG, Blomquist G, Gyntelberg F *et al*. Dampness in buildings and health. Nordic interdisciplinary review of the scientific evidence on associations between exposure to “dampness” in buildings and health effects (NORDDAMP). *Indoor Air* 2001; 11:72–86.