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Clinica Chimica Acta 305 (2001) 89–98

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Serum IgG and IgE antibodies against mold-derived antigens in patients with symptoms of hypersensitivity

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Received 12 June 2000; received in revised form 15 November 2000; accepted 22 November 2000

Abstract

Background: Exposure to mold in water-damaged buildings has been suggested to be responsible for various health problems such as hypersensitivity and upper respiratory tract diseases. However, only little information is available on possible diagnostic tools for examining mold-associated health problems. *Methods:* We used recently developed immunofluorometric IgG and IgE assays (UniCAP™) to examine serum IgG and IgE antibodies against mold-derived allergens from 70 mold-exposed individuals with ($n=55$) or without ($n=15$) symptoms of sensitization. Controls were healthy individuals ($n=31$) without any history of such exposure. *Results:* The IgG titers exceeded the upper normal limits of control individuals (mean±2 S.D.) in 35% of symptomatic men and in 25% of women. The IgG titers were usually higher in women than in men ($P<0.05$) showing no significant association with the severity of symptoms. During follow-up of eight mold-exposed subjects for 9–12 months the IgG titers remained relatively constant. Elevated anti-mold IgEs were found in six (11%) of the exposed subjects who were all symptomatic. *Conclusions:* Measurements of anti-mold IgGs may help to confirm exposure in patients with hypersensitivity symptoms and evidence of mold growth in living or working environment. Some exposed symptomatic patients present IgE-mediated responses. Combined measurements of IgGs and IgEs may prove to be of value in the comprehensive assessment and treatment of such patients.

Keywords: Mold; Water damage; Allergy; Immunoglobulins

1. Introduction

Adverse effects of indoor dampness have recently received increasing attention in countries which suffer from cold climate and frequent water damage in houses and office buildings. As a result of moisture condensation or leaks, excessive growth of mold may take place which, in turn, may lead to an increase in the incidence of respiratory, allergic and irritative symptoms among both children and adults living in such conditions [1–7]. Similarly, occupational exposure to mold-derived indoor allergens has been shown to cause symptoms to sensitized individuals [8,9].

The appearance of respiratory symptoms in sensitized individuals may proceed via a variety of mechanisms, including allergic, inflammatory and irritative pathways [8]. Exposure to foreign materials is known to trigger immune responses against antigenic structures as part of the defense against

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0009-8981/01/$ – see front matter
PII: S0009-8981(00)00424-1

potentially harmful invasion and as part of the development of tolerance. The existence of humoral immune responses and antibody production against mold-derived antigens has been previously well established from sawmill workers and from patients with farmer's lung disease [10–12]. Allergic bronchopulmonary aspergillosis (ABPA) is also known to be characterized by an immunologic reaction to *Aspergillus fumigatus* colonizing the bronchial lumen of affected individuals (for review see Ref. [13]). Malling and coworkers [14] have also previously reported allergic responses to *Cladosporium* in asthmatic patients. As yet, little information is, however, available on the appearance of mold-specific antibodies in other types of mold-exposed populations.

In order to clarify the relationship between mold exposure and humoral immune responses against mold-derived antigens, we used recently developed IgG and IgE antibody assays to measure antibodies against several specific mold-derived antigens from symptomatic and non-symptomatic mold-exposed individuals. In order to explore the clinical value of such measurements, the results were compared with those obtained from healthy non-exposed individuals.

2. Materials and methods

2.1. Patients and control subjects

The study population included 70 patients (41 females and 29 males) with mold-exposure in their living ($n=50$) or working ($n=20$) environment, as confirmed by expert technical investigations. Such examinations included detailed visual inspections, examinations of individual samples obtained from the contaminated materials, measurements of moisture contents from the selected targets, use of trained dogs to indicate growth of mold, and microbiological investigations. In the study material, there were 55 mold-exposed patients, who suffered from frequent respiratory and eye symptoms of sensitization at the time of the study, whereas 15 exposed patients were essentially non-symptomatic. The population of exposed subjects included 20 individuals (13 women, seven men) with occupational exposure from an office building with water damages and mold growth. Among these, 12 (60%) reported frequent respiratory symptoms which they presumed to be due to the working conditions. There were ten subjects with eye irritation, four had eczema, and three subjects suffered from asthma, whereas eight (40%) individuals reported no significant respiratory or eye symptoms.

In order to further clarify the specificity of the antibody assays we also examined three separate family samples. There were two four-membered families with mold exposure and respiratory symptoms at the time of the study. The daughter of the other family had not had recent symptoms after a tonsillectomy performed 8 months prior to sampling. For comparison, serum samples were also obtained from a non-symptomatic family with four members. The control population included 31 persons (18 females and 13 males) who were individuals without any evidence or history of exposure to mold-derived antigens either at home or at the work place. The control subjects also reported no history of current or frequent respiratory symptoms or any allergies. The age distribution of the control individuals (30 ± 15 years) was similar to that of the symptomatic patients (31 ± 16 years).

Serum samples were collected from all study participants. All subjects were thoroughly interviewed using standardized questionnaires on current and previous diseases and symptoms, medications, family predispositions, smoking, and living and working environments. Follow-ups were initiated from eight mold-exposed persons. All of them reported respiratory symptoms at the time of the first sampling. Four of them had been able to change their living or working environment during follow-up to avoid mold contamination with concurrent recovery in their clinical status, while the situation of the other four persons remained relatively stable.

All study subjects gave their informed consent prior to the study. The study protocol was approved by the Ethics Committee of the Central Hospital of Southern Ostrobothnia, Finland, and the study was carried out according to the principles of the Declaration of Helsinki.

2.2. Preparation of antigens and immunoglobulin measurements

For specific immunoglobulin measurements, we

used recently developed immunofluorometric UniCAP™ assays (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) for IgG and IgE antibodies against *Stachybotrys atra*, *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Trichoderma viride*, *Penicillium* spp., *Chaetomium globosum*, *Aspergillus niger*, and *Aspergillus fumigatus*, which are typical antigenic components of molds occurring on water-damaged house materials. For the preparation of these specific antigens molds are first cultivated as surface cultures. Harvested material consists of mycelium and spores. Culture media are not included. For comparison, IgG and IgE antibodies against *Micropolyspora faeni* (*Saccharopolyspora rectivirgula*), which has been reported to be associated with the development of farmer's lung disease [11,12], was also determined. UniCAP™100 analyzer, instrument version 1.0 (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) was used for the analyses.

2.3. Total immunoglobulin measurements

Serum total IgG concentrations were determined by the Behring Nephelometer II System (BNII), which measures immunonephelometrically the intensity of light, which is scattered by the antigen-antibody complexes formed in the sample.

2.3.1. Statistical methods

Values are expressed as mean±S.D. ANOVA was used to analyze the differences between groups, and the differences were considered statistically significant at $P<0.05$. Logarithmic transformation was used to yield non-skewed distributions, as appropriate. Linear regression analysis (r) or Spearman's rank-correlation test (r_S) was used to calculate correlations, as required. The reference ranges determined as the limits of normal values on the basis of the data obtained from healthy controls were calculated separately for women and men using logarithmically transformed values with non-skewed distributions (mean±2 S.D.). IgE values were considered elevated, when they were higher than 0.35 kUA/l (kilounits of allergen specific antibody/liter). Differences between prevalences were tested using χ^2-test.

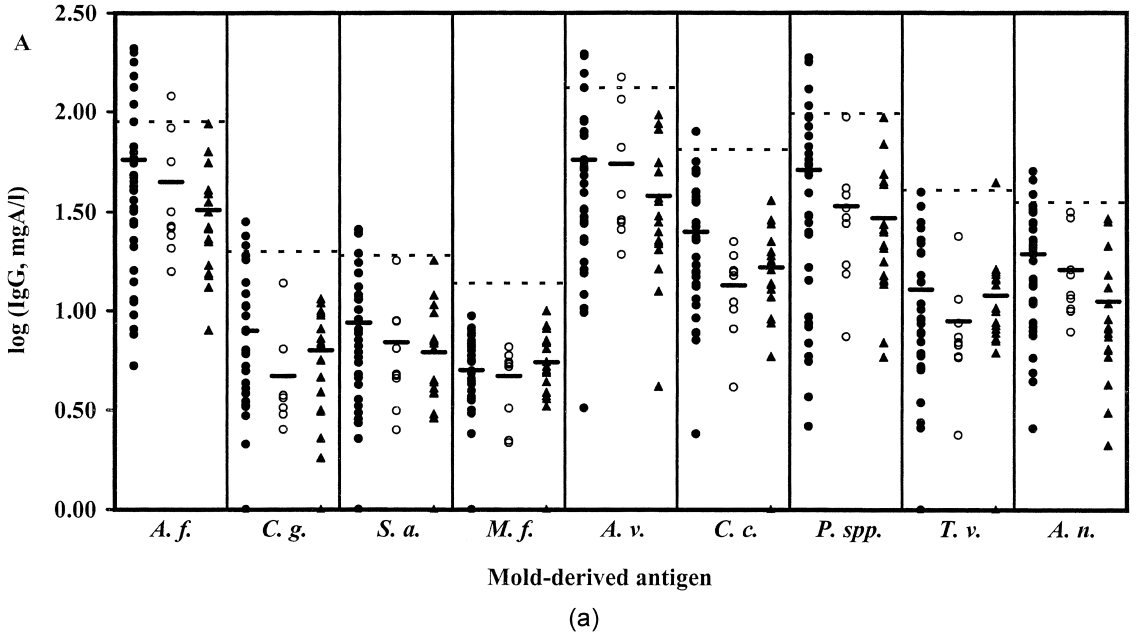
3. Results

The age and sex distributions and the prevalences of smoking were not significantly different between the study subgroups (Table 1). Among the mold-exposed symptomatic subjects, eight (15%) had a diagnosis of asthma, 22 (40%) had allergic rhinitis and 20 (36%) suffered from atopic eczema.

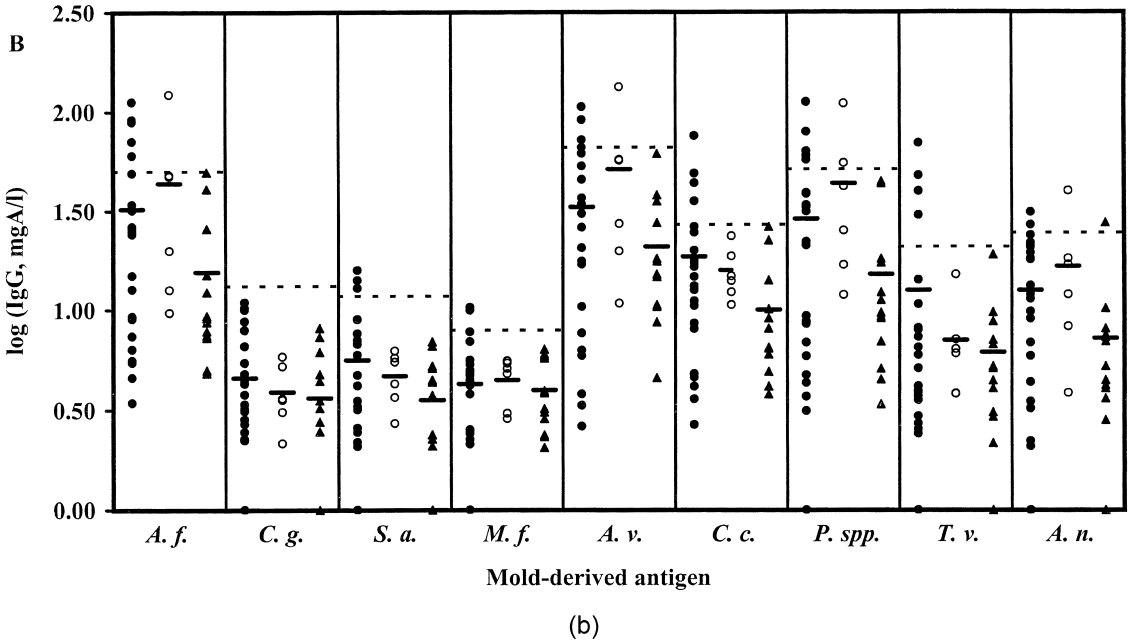
Specific IgG titers against the various mold-derived antigens are shown in Fig. 1. Among the symptomatic mold-exposed patients, the specific IgG titers were higher in women than in men for *A. fumigatus* ($P<0.05$), *A. versicolor* ($P<0.05$), *Penicillium* spp. ($P<0.05$), and *A. niger* ($P<0.05$) (Fig. 1A,B). In the healthy controls, women were also found to show significantly higher titers of mold-specific IgGs than men for several antigens, including *A. fumigatus* ($P<0.01$), *C. globosum* ($P<0.05$), *S. atra* ($P<0.05$), *A. versicolor* ($P<0.05$), *Penicillium* spp. ($P<0.05$), and *T. viride* ($P<0.05$) (Table 2, Fig. 1A,B). The IgG titers in the mold-exposed subjects were higher than in the control subjects but the differences in the mean values between the groups of mold-exposed subjects or healthy controls did not reach statistical significance, expect for *A. niger* ($P<0.05$). The prevalences of increased IgGs against the different individual an-

Table 1
Characteristics of healthy controls and mold-exposed subjects with or without eye/respiratory symptoms

	Healthy controls ($n=31$)	Mold exposed subjects ($n=70$)	
		Subjects without eye and respiratory symptoms ($n=15$)	Subjects with eye and respiratory symptoms ($n=55$)
Age (years, mean±S.D.)	29.6±14.9	25.2±11.8	31.0±16.1
Sex (F/M)	18/13	9/6	32/23
Smoking (prevalence, %)	19	13	18



(a)



(b)

Fig. 1. Specific IgG antibody titers against mold-derived antigens: *A. fumigatus* (A. f.), *C. globosum* (C. g.), *S. atra* (S. a.), *M. faeni* (M. f.), *A. versicolor* (A. v.), *C. cladosporioides* (C. c.), *Penicillium* spp. (P. spp.), *T. viride* (T. v.), and *A. niger* (A. n.) in A: mold-exposed women with eye/respiratory symptoms (●), mold-exposed non-symptomatic women (○) and healthy control women (▲); and in B: mold-exposed men with eye/respiratory symptoms (●), mold-exposed non-symptomatic men (○) and healthy control men (▲). The bars (–) indicate the means of the values. Abbreviation: mgA/l, milligrams of allergen specific antibody/liter. The dashed line (---) indicates the tentative limits of the normal values, as obtained by calculating the mean±2 S.D. of the results from healthy female (n=18) and male (n=13) controls, respectively.

Table 2
The prevalences of increased mold-specific IgG and IgE titers in mold-exposed women ($n=32$) and men ($n=23$) with respiratory/eye symptoms[a]

Mold-specificity	IgG				IgE	
	Prevalence		Limit of normal value (mgA/l)		Prevalence	
	F, %	M, %	F	M	F, %	M, %
A. fumigatus	19	22	89.74	50.07	6	13
A. niger	9	9	35.17	24.67	0	0
A. versicolor	13	13	130.55	66.39	0	0
C. globosum	9	0	19.97	13.13	0	0
C. cladosporioides	3	17	64.53	26.74	6	9
M. faeni	0	9	13.72	7.95	0	0
Penicillium spp.	13	22	96.90	51.24	0	4
S. atra	9	13	19.06	11.84	0	0
T. viride	0	17	41.14	20.71	0	0

[a] The upper normal limits for mold-specific IgG are also shown. F, women; M, men; mgA/l, milligrams of allergen specific antibody/liter The upper normal limits of IgG results are based on the results obtained from healthy control individuals (mean±2 S.D.). The IgE values were considered elevated when they were higher than 0.35 kUA/l.

tigens, as determined in comparisons with the tentative reference values (mean±2 S.D. of the values from healthy controls), among the symptomatic patients are shown in Table 2. Overall, increased IgG titers against one or several antigens occurred in 35% of men and in 25% of women. The antibody concentrations against these antigens in farmer's lung disease patients, as determined for comparison purposes, were typically 10–70 times higher than those in the present population of mold-exposed subjects (data not shown).

When the data from the sample of 20 employees working in an office building, in which growth of *Chaetomium*, *A. versicolor*, *Cladosporium* and *Penicillium* had been found by the technical investigations, no significant differences were noted between the different study groups for any of the antigens (data not shown). There were also no significant correlations between the duration of employment and the antibody titers ($r_S=-0.3$–0.01).

Interestingly, the family samples showed very similar patterns of IgG responses towards the different antigens (Fig. 2). The specific IgG titers obtained in the symptomatic families (Fig. 2A,B) were also significantly higher than those in the non-symptomatic family for *A. fumigatus* ($P<0.01$), *A. versiclor* ($P<0.01$), *C. cladosporioides* ($P<0.05$), *Penicillium* spp. ($P<0.05$), and *A. niger* ($P<0.01$).

In the total study material ($n=101$) no significant correlations emerged between age and any of the specific IgGs ($r=-0.002-0.2$). There were also no significant correlations between serum total IgG concentration and the specific IgG titers ($r=0.08-0.4$, $n=20$). Significant correlations were, however, found between the different individual mold-derived antigens (Table 3). During follow-up of 9–12 months, IgG titers remained relatively constant in all patients (Fig. 3).

Elevated IgEs against mold-derived antigens were found in six (11%) of the symptomatic patients (Table 2), four of whom showed increased IgE titers for more than one antigen. All patients with increased specific IgE titers reported blocked or runny nose and rhinitis and associated the symptoms to the moldy environment. Two of the patients were multiallergic. The data on the distribution of clinical symptoms of the mold-exposed patients classified according to both the specific IgG and IgE levels are summarized in Table 4. The patterns of symptoms were found to vary between the groups. Interestingly, among the exposed individuals there was a tendency towards a lower degree of hypersensitivity-related clinical symptoms in individuals with the highest titers of IgGs, whereas all the patients with elevated IgEs showed symptoms of hypersensitivity. The differences did not, however, usually reach statistical

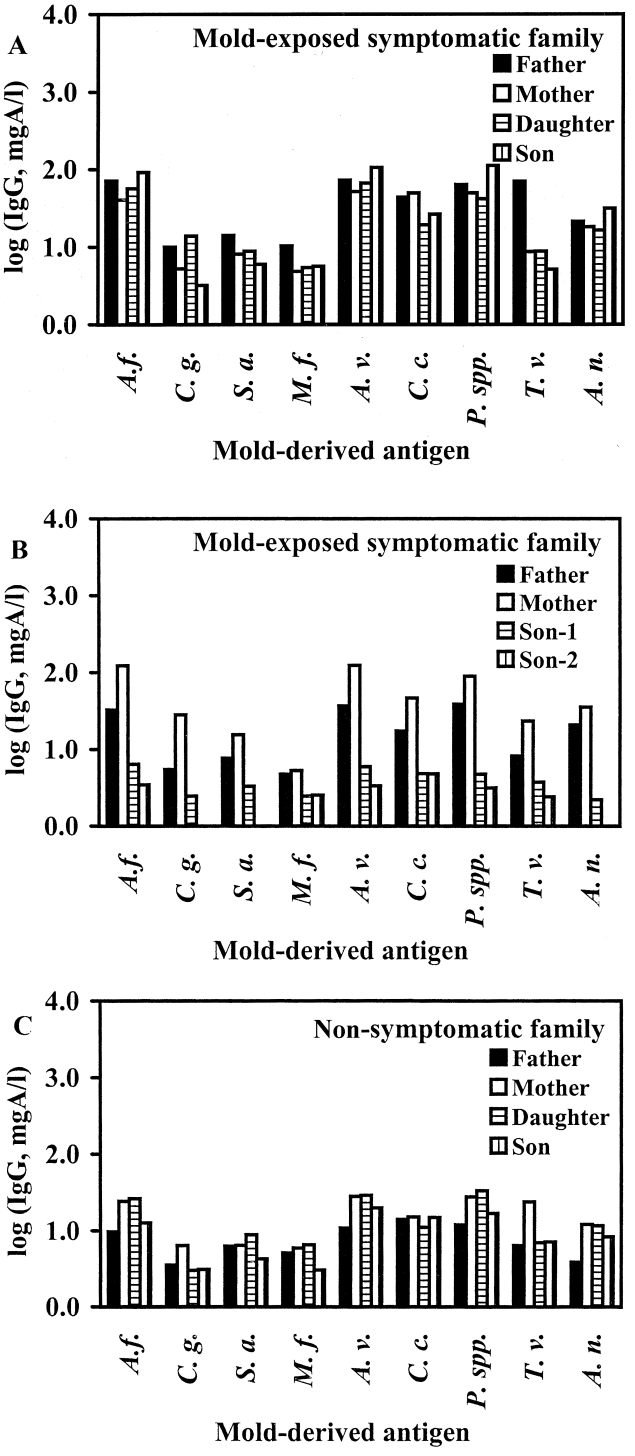


Fig. 2. Mold-specific serum IgG titers from members of two mold-exposed families with respiratory symptoms (A and B) and from those of a non-symptomatic family (C).

Table 3
Correlations (*r*) between mold-specific IgG titers[a]

IgG-specificity	IgG-specificity							
	A.f., *r*	C.g., *r*	S.a., *r*	M.f., *r*	A.v., *r*	C.c., *r*	T.v., *r*	A.n.
C.g.	0.415***							
S.a.	0.473***	0.749***						
M.f.	0.493***	0.306**	0.472***					
A.v.	0.980***	0.315**	0.368***	0.494***				
C.c.	0.669***	0.593***	0.662***	0.592***	0.627***			
T.v.	0.276*	0.489***	0.532***	0.500***	0.232*	0.491***		
A.n.	0.918***	0.380***	0.371***	0.523***	0.942***	0.646***	0.303**	
P.spp.	0.849***	0.281*	0.293**	0.499***	0.869***	0.588***	0.277*	0.905***

[a] A.f., *A. fumigatus*; A.n., *A. niger*; A.v., *A. versicolor*; C.g., *C. globosum*; C.c., *Cladosporium cladosporioides*; M.f., *Micropolyspora faeni*; P.spp., *Penicillium* spp.; S.a., *S. atra*; T.v., *T. viride*.

* $P<0.05$.

** $P<0.01$.

*** $P<0.001$.

significance. A separate analysis of individuals with atopy and multi-allergies ($n=8$), although no exposure to mold, revealed no elevated values towards any of the mold-derived antigens. The mean IgG values were as follows: 14.96±17.20 for *A. fumigatus*, 2.48±1.37 for *C. globosum*, 2.84±1.75 for *S. atra*, 6.31±6.85 for *M. faeni*, 17.62±18.00 for *A. versicolor*, 7.88±4.85 for *C. cladosporioides*, 12.28±14.89 for *Penicillium* spp., 5.01±3.62 for *T. viride* and 7.79±9.41 for *A. niger*. The IgE titers against mold-derived antigens remained below the limit of detection in all of these subjects (data not shown).

4. Discussion

The present study demonstrates that both IgG and IgE antibodies against mold-derived antigens can be found from the sera of mold-exposed individuals using the quantitative UniCAP™ assay system. These assays are designed to measure antibodies against several antigenic components, which are known to occur on mold-contaminated indoor materials. Although several recent studies have suggested that symptoms of hypersensitivity may be caused by exposure to mold [1–9], laboratory tests for detecting the severity of exposure have not been widely available for such purposes. Use of such tests may also have been hampered by the fact that there are no gold standards for the extent of mold exposure and therefore, reference intervals for such assays have not been established.

In the present work, increased IgG titers against one or several mold-derived antigens were found in 35% of men and in 25% of women exposed to mold using the mean±2 S.D. from the values of the control population as the cut-off level. It should be noted, however, that the number of control samples here may be too small to establish a definitive reference range at this time. Lack of apparent correlation between the clinical symptoms and the IgG antibodies suggests that anti-mold IgGs are rather associated with mold exposure than the severity of clinical symptoms. Several types of mechanisms may account for clinical symptoms at an individual level, such as the direct toxic effects of mycotoxins. According to previous findings by Hogan et al. [15], allergic airway disease can occur via pathways associated with interleukin (IL)-5 and eosinophilic inflammation independently of IL-4 and allergen-specific immunoglobulins. The patterns of IgG responses observed here in the members of families exposed to similar environmental conditions indicates, however, that the antibody formation is clearly dependent on the extent and pattern of mold exposure.

Apparently, individual sensitivity for different types of antigenic stimuli may play a role in the appearance of symptoms, antibody responses and generation of tolerance. Moderately or highly elevated IgG titers here were often associated with a

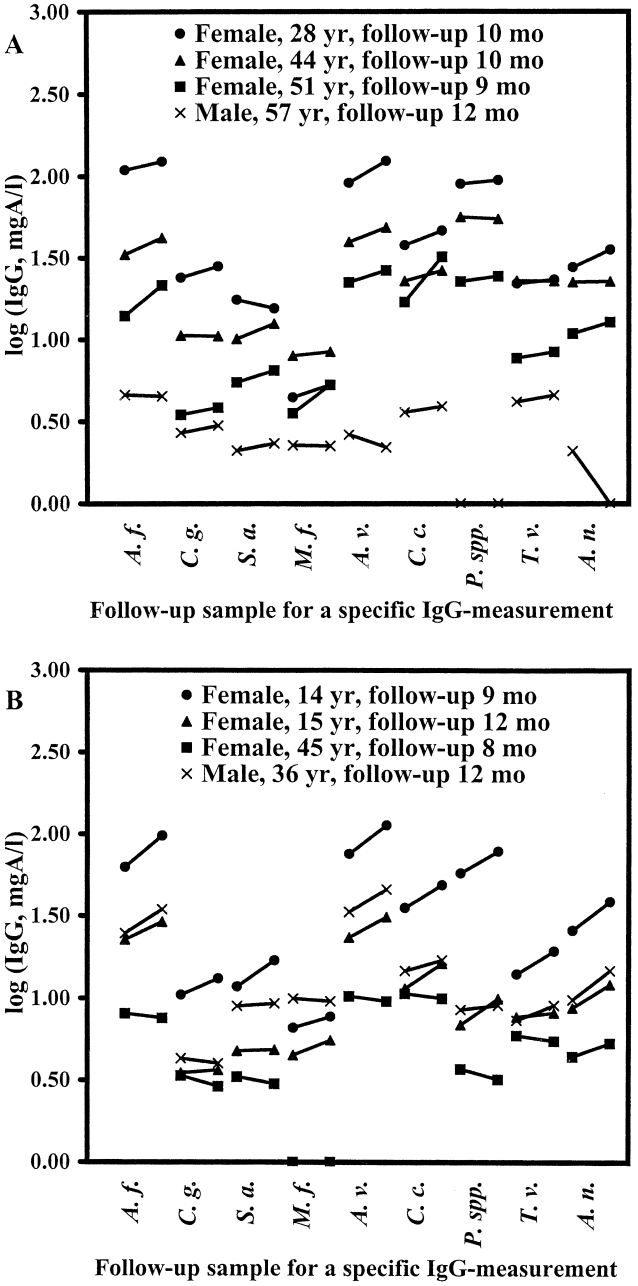


Fig. 3. Follow-up of specific IgG titers (9–12 months) in mold-exposed symptomatic patients, who had had no apparent alterations in their environmental conditions (A) or who had changed their living or work place and reported concomitant improvement in their clinical status (B).

relatively low degree of clinical symptoms (Table 4), which is in accordance with previous findings in hypersensitive beekeepers indicating that IgG antibodies could actually mark the degree of protection [16,17]. As expected, the IgG titers for all mold-derived antigens in the present type of study popula-

Table 4
Prevalences of clinical symptoms in mold exposed patients classified according to the titers of mold-specific antibodies[a]

Symptom	Prevalence, %			
	I	II	III	IV
Eye dryness, irritation or itching	52	71	33	60
Blocked or runny nose	76	93	78	100
Rhinitis	67	64	33	100
Dry or hoarse throat	43	71	67	40
Cough	76	79	67	60
Difficulty in breathing	48	21	33	40
Wheezing	33	14	33	20
Phlegm (mucus)	52	57	44	20
Fever	19	21	22	0
Repeated respiratory tract infections	67	71	33	40
Medication to eye or respiratory symptoms	80*	33*	38	60

[a] The patients were classified as follows. Specific IgE titers <0.35 kUA/l and IgG titers against one or several of the nine mold-derived antigens: I, $<$mean$\pm$1 S.D. (low); II, $\geq$mean$\pm$1 S.D. but $<$mean$\pm$2 S.D. (slightly elevated); III, $\geq$mean$\pm$2 S.D. (clearly elevated) as compared with the IgG titers from healthy controls; or IV, specific IgE titers against some of the nine mold-derived antigens ≥ 0.35 kUA/l.

* Significant difference ($P<0.05$) between values.

tion are also far lower than those in symptomatic farmer's lung disease patients, in which the generation of a humoral immune response is a well-established pathogenic factor [11,12]. The occurrence of moderate IgG titers in the healthy controls suggests that all people become exposed to mold-derived antigens to some extent. There were strong correlations between some antigens (e.g. *A. niger* against *A. fumigatus*, *A. versicolor* and *Penicillium* spp.) indicating the possibility of cross-reactivity between these species. This observation is also in line with previous findings by Shen and coworkers [18] indicating common antigenic determinants between *Penicillium* and *Aspergillus* species.

A significant sex difference in the immune responses against mold-specific antigens has not been previously reported. Based on the present data it appears that separate reference intervals may be necessary for men and women. While at this time we cannot rule out the possibility that the higher levels of the anti-mold IgG antibodies in women could be due to sensitization to possible cross-reacting fungal antigens, such as *Candida* [19] or the conserved regions of yeast enolases [20], it should be noted that none of the individuals included in the present reference material had suffered from *Candida* infection. Current data may also suggest a more active immunological responsiveness in women than in men. Women are known to be more prone to several types of autoimmune diseases, such as SLE or thyroid diseases. Previously, we have also found higher prevalences of autoantibodies against acetaldehyde-modified proteins in alcohol consuming women than in men [21]. Sex steroid hormones have been previously suggested to play a role in the regulation of immune responses, although the specific mechanisms have remained obscure [22]. For instance, it has been shown that estradiol inhibits the suppressive activity of a subset of T-lymphocytes bearing Fc-receptors for immunoglobulin G [23].

Current follow-up data indicates that the IgG titers remain constant for prolonged periods, despite the fact that the apparent antigen exposure discontinues, suggesting a long steady level of IgG following immune stimulus, which is in accordance with earlier vaccination studies [24,25].

IgE-associated responses were found here in ~11% of the mold-exposed patients, suggesting that this type of hypersensitivity may also occur in mold-exposed patients. Corey et al. [26] previously reported IgE-mediated responses in 44% of subjects with allergic rhinitis in a Midwestern allergy practice. Besides locational differences, which may influence the results, it is also possible that the selection criteria of the participants may have been different between the studies. However, in agreement with Corey and co-workers [26], particularly *Aspergillus*, *Cladosporium*, *Penicillium*, and *Trichoderma* antigens were also shown here to be the primary inducers of IgE responses. It should further be noted that the patients with IgE responses were also consistently symptomatic suggesting that IgE antibodies may be more directly related to disease which may be responsive to therapy.

In summary, despite considerable overlapping between symptomatic and non-symptomatic individuals, combined measurements of mold specific IgGs and IgEs may prove to be useful in confirming mold exposure in patients, who show clinical symptoms of hypersensitivity towards such antigens and have evidence of mold growth in their living or working

environments. IgG antibodies may suggest exposure to molds but no clear association appears to exist between the severity of clinical symptoms and the serum concentrations of IgG antibodies.

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