

Serum IgE specific to indoor moulds, measured by basophil histamine release, is associated with building-related symptoms in damp buildings

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Received 8 May 2000; returned for revision 8 August 2000; accepted by E. Neugebauer 7 December 2000

Abstract. *Objective:* To study the relationship between basophil histamine release (HRT) to indoor moulds, indicating specific IgE, and building-related symptoms (BRS), asthma, and hay fever in individuals working in damp and mouldy buildings.

Methods: A cross-sectional study was performed among 86 school staff members, who on average had worked 143 months (range: 3–396) in moist buildings with mould growth in the constructions. A questionnaire concerning mucous membrane symptoms, facial skin symptoms, central nervous system symptoms, hay fever, and asthma was fulfilled by the participants, and blood samples were taken. Eight mould species growing on building constructions were identified and cultivated to obtain allergenic materials for testing. The presence in serum of IgE specific to moulds was verified by histamine release test (HRT) based on passive sensitization of basophil leukocytes. The validity of the method was confirmed by parallel testing of patients allergic to grass- and birch pollen and by the shift from positive to negative response after removal of serum IgE and by using sham sensitization.

Results: The prevalence of most BRS was between 32% and 62%. Positive HRT, showing serum IgE specific to one or more of the moulds, was observed in 37% of the individuals. The highest frequency of positive HRT was found to *Penicillium chrysogenum* and then to *Aspergillus* species, *Cladosporium sphaerospermum* and *Stachybotrys chartarum*. A significant association was found between most BRS and positive HRT, whereas no association was observed between positive HRT to moulds and self reported hay fever or asthma. *Conclusion:* Positive HRT to indoor moulds, showing the presence in serum of IgE specific to the fungi, was found to be related to BRS in individuals working in damp and mouldy buildings. Whether the association is of causal char-

acter is a question for further studies. The test may be useful in the evaluation and study of possible mould induced BRS.

Key words: Building-related symptoms – Sick building syndrome – Moulds – Histamine release – Specific IgE

Introduction

Moisture, condensation and water damages from leaks in the roof and pipes leading to mould growth in building constructions are quite common problems in the developed countries [1, 2]. Reports of adverse health effects such as building-related symptoms (BRS) and respiratory problems have recently been reported among persons occupying damp buildings [1, 3–5]. A higher risk of BRS seems to be associated with working or living in damp and mouldy buildings, and exposure to *Stachybotrys* species may be associated with BRS [6, 7]. Cases of asthma and allergic alveolitis due to low level indoor mould exposure have also been reported [8, 9]. An association between asthma symptoms and indoor concentrations of moulds has been demonstrated [10] and immediate type allergy to moulds could explain some of the respiratory effects observed [4]. Two preliminary studies indicate a possible association between mould-specific IgE indicated by histamine release test (HRT) and BRS in individuals not suffering from hay fever or asthma [11, 12]. In these individuals with BRS, working in damp buildings with growth of *Penicillium chrysogenum*, it was not possible to demonstrate specific IgE to this mould by the commercial standard extract used in skin prick test or RAST (Magic Lite) [12]. The relevant strain of *P. chrysogenum* from the building was therefore used. This is possible by using HRT. In addition, a degradation of epitopes in the mould caused by the high content of proteolytic enzymes was avoided. The aim of

the present study was to expand the examination of a possible relationship between basophil histamine release (positive HRT) to indoor moulds, indicating the presence in serum of mould-specific IgE, and the risk of building-related symptoms (BRS), asthma, and hay-fever in individuals working in damp and mouldy buildings. Therefore HRT was performed to all species of moulds found in the buildings. The methods were identical with those used before [11, 12]. Of ethical reasons it was not possible to perform skin prick test with the preparation of mould suspensions.

Subjects, buildings, and methods

Subjects and buildings

The study was a pooled cross-sectional study comprising 86 staff members from two municipal schools. The employees had a high frequency of complains over bad indoor air quality. Fifty staff members from school 1 and 36 staff members from school 2 were included in the study. Thirtytwo were male and 54 were female, with a mean age of 44 (range 25–62) years. The number of current smokers were 21 and the average duration of employment in the schools was 143 (range 3–396) months.

For several years the buildings had suffered from repeated water damages due to leaks in the flat horizontal roof and drains leading to moisture and condensation in building constructions. The buildings had widespread visual mould growth in several places. Moulds growing on the building constructions were sampled with contact agar plates (V-8) and were cultivated for identification [13]. The identified moulds were: *Aspergillus sydowii*, *Aspergillus versicolor*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Stachybotrys chartarum* (Synonym: *Stachybotrys atra*) and *Ulocladium botrytis*.

Questionnaire and indices

The questionnaire was mainly based on questionnaires from earlier studies [14, 15]. Study participants were queried about mucous membrane/facial skin (MMS) symptoms (nasal congestion, irritation of eyes, nose, throat, and facial skin) and central nervous system (CNS) symptoms (fatigue, headache and lack of concentration). Positive MMS-index was defined as at least 2 out of 5 mucous membrane/facial skin symptoms present frequently, and positive BRS-index is obtained by positive MMS-index in connection with at least one of the three CNS symptoms present frequently. These BRS were defined as frequent when occurring in the building at least several days a week or daily. Asthma and hay fever questions were: "Have you in the past, or are you presently suffering from ...". These two questions were mainly included in the questionnaire for confounder control.

Serum and fungal test material

Venous blood was drawn from all participants and samples of sera were stored at -80°C for testing of specific IgE by HRT.

To obtain fungal test material, the identified indoor moulds were cultivated on V-8-agar [13], and harvested by scraping off the cultures in dry state and the material was freeze-dried and kept at -80°C until preparation of the mould suspensions. The material consisted of intact spores and a few and small hyphal fragments. Immediately before testing it was suspended in Tris-AMC medium. The aim was to avoid proteolytic degradation of fungal epitopes which may occur during the conventional procedure of extraction, caused by the high fungal content of proteolytic enzymes. The laboratory staff was not aware of the symptom status of the participants.

Basophil histamine release

Serum IgE specific to moulds was verified by HRT, based on passive sensitization of basophil leukocytes with serum from the participants, using the method described by Larsen et al. [16]. In short, lymphocytes with 2% basophil leukocytes were obtained by gradient centrifugation of umbilical cord blood. The cells were suspended in Tris-AMC medium. Transfer of serum IgE to the basophil cell surface was performed by passive sensitization, i.e. an equal volume of cell suspension and serum was incubated for 1 h at 37°C . To 50 μl samples of the sensitized leukocytes were added 5 μl of suspensions of moulds in different concentrations or 5 μl of Tris-AMC (spontaneous histamine release). The samples were incubated for 40 min at 37°C , and basophil histamine release was measured spectrofluorometrically after the reaction of histamine with o-phthaldialdehyde and expressed as a percentage of the total histamine content of the sample. Positive HRT was defined by a release exceeding the spontaneous release with 10% [16].

To show the relevance of the definition of positive HRT, sham sensitization was performed in parallel (Fig. 1), i.e. patient serum was replaced by Tris-AMC, individual control sera from healthy persons from healthy buildings or the patients' serum deprived of IgE. IgE was removed by anti-IgE sepharose, eliminating more than 95% of total IgE, but only 2% of total IgG in serum [16]. Additionally, the assay was controlled by the histamine release response to grass pollen or birch pollen extract (Soluprick-SQ, 10 HEP, ALK-Abello, Denmark) after passive sensitization with sera from six pollen allergic patients showing a high RAST response (Magic Lite technique, ALK-Abello, Denmark) [16]. These patients from healthy buildings had no BRS.

Figure 1 shows serum IgE specific to moulds verified by HRT. Four individuals with BRS showed positive HRT to either *Penicillium chrysogenum*, *Aspergillus sydowii*, *Aspergillus versicolor* or *Stachybotrys chartarum* (upper curves). No dose-response was obtained with sham sensitization using Tris-AMC, control serum or serum, from the four individuals mentioned above, deprived of IgE. These curves are identical with those obtained by the spontaneous histamine release (not included), showing the relevance of the definition of positive HRT. The figure also shows positive HRT obtained in pollen allergic patients tested with grass and birch pollen extracts, and the negative response obtained by the sham sensitization.

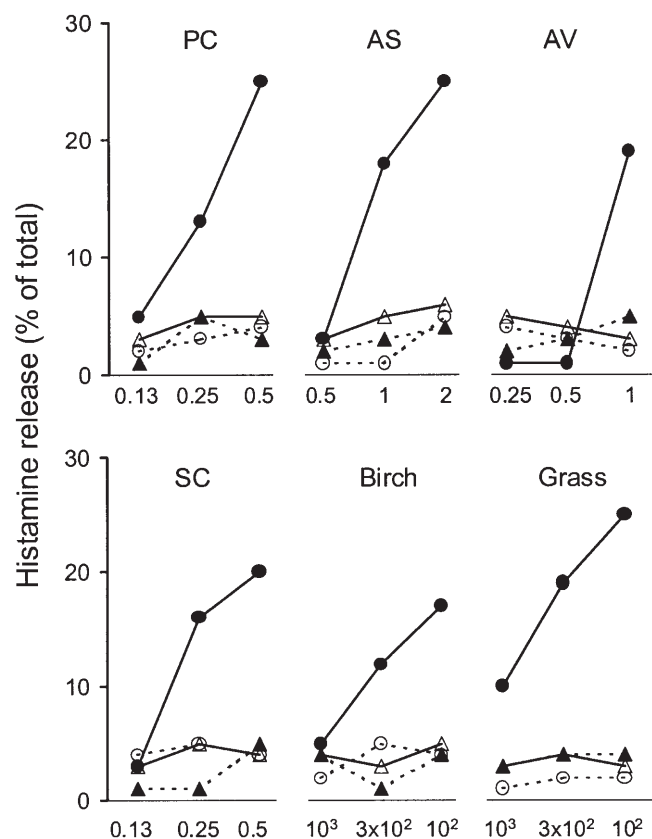


Fig. 1. Verification of serum IgE specific to indoor moulds in four individuals with building-related symptoms (BRS). Histamine release test showed specific IgE to *Penicillium chrysogenum* (PC), *Aspergillus sydowii* (AS), *Aspergillus versicolor* (AV) and *Stachybotrys chartarum* (SC) (upper curves). To compare, two patients allergic to grass or birch pollen, from healthy buildings and showing no BRS, were included. Serum, intact (●) and deprived of IgE (○) and controls, i.e. sham sensitization using medium (△) or control serum (▲). Testing with mould suspensions, grass and birch pollen extracts. X-axis: mould concentrations mg/ml and dilution of allergen extracts (ALK-Abello) 1:100, 1:300 and 1:1000.

Statistical analysis

The statistical analysis included χ^2 -test and logistic regression using the odds ratio (OR) as risk estimate adjusted for the following confounders: sex, smoking habits, years of employment, and hay fever. 5% was chosen as level of significance. The analyses were performed in the statistical package SPSS 6.0 for Windows.

Ethics

The study was in accordance with the second Helsinki declaration and approved by the local ethics committee.

Results

The prevalences of BRS in the 86 employees from the two schools are shown in table 1. Except for the facial skin irritation the prevalences of mucous membrane symptoms, central

Table 1. The prevalence of building-related symptoms (BRS), asthma, and hay fever among 86 staff members employed in two damp and mouldy schools.

Symptoms	Number	(%)
Eye irritation	48	(56)
Nasal irritation	36	(42)
Nasal congestion	39	(45)
Pharyngeal irritation	36	(42)
Facial skin irritation	12	(14)
Fatigue	53	(62)
Headache	39	(45)
Lack of concentration	28	(32)
Pos. MMS-indeks	49	(57)
Pos. BRS-indeks	40	(47)
Asthma	10	(9)
Hay fever	23	(27)

nervous system (CNS) symptoms as well as positive MMS- and BRS-indices were between 32% and 62%. Ten individuals were in the past or presently suffering from asthma, 23 from hay fever.

Positive HRT, showing the presence in serum of IgE specific to one or more moulds in the buildings, was observed in 32 individuals (37%). Positive test was obtained in 3% of the individuals to *Cladosporium herbarum*, 10% to *Stachybotrys chartarum*, 12% to *Aspergillus versicolor*, 18% to *Cladosporium sphaerospermum*, 20% to *Aspergillus sydowii* and the highest frequency of 27% was found to *Penicillium chrysogenum*. None of the individuals responded to *Aureobasidium pullulans* or *Ulocladium botrytis*.

Odds ratios (OR) of BRS, asthma and hay fever adjusted for possible confounders in relation to HRT are given in Table 2. A significant association was found between positive HRT to *Penicillium chrysogenum* and BRS such as nasal irritation, nasal congestion and fatigue, but not between HRT and eye irritation, pharyngeal irritation, facial skin irritation, headache and lack of concentration. Furthermore, a significant association was obtained to positive MMS-index and positive BRS-index with OR = 5.80, respectively 3.15. A significant association between HRT and BRS was also obtained when the results were calculated to include a positive HRT to one or more of the moulds found in the buildings (Table 2). No associations were found to self-reported asthma and hay fever.

Discussion

The significant associations observed between BRS and positive HRT to at least one of the 8 fungi growing in the buildings suggest that the HRT may be useful in the evaluation of possible mould induced BRS. Staff members who responded to one or more of the moulds had a 2–5 fold higher risk of BRS compared to the negative responders. The associations were even stronger for HRT to *Penicillium chrysogenum* probably due to a quantitative dominance of this species in damp buildings [2], a dominance also found in Swedish buildings [17, 18]. The risk estimates remained after adjustment for smoking, sex, years of employment in the schools and the presence of hay fever, factors that are known as

Table 2. Adjusted odds ratios (OR) of building related symptoms, asthma, and hay fever in relation to positive histamine release test (HRT) to one or more of the indoor moulds or *Penicillium chrysogenum*.

Symptoms	Positive HRT to one or more moulds N = 32			Positive HRT to <i>Penicillium chrysogenum</i> N = 23		
	n	OR	CI95%	n	OR	CI95%
Eye irritation	21	1.91	0.76– 4.80	16	2.21	0.78– 6.23
Nasal irritation	19	3.61	1.33– 9.77*	16	6.00	1.97–18.24*
Nasal congestion	19	3.00	1.14– 7.86*	17	6.17	2.00–19.08*
Pharyngeal irritation	17	2.62	1.00– 6.89*	13	2.69	0.95– 7.58
Facial skin irritation	8	2.93	0.81–10.62	7	3.53	0.97–12.88
Fatigue	22	1.81	0.70– 4.65	18	3.60	1.09–11.86*
Headache	15	1.41	0.55– 3.60	11	1.45	0.52– 4.03
Lack of concentration	11	1.28	0.49– 3.32	8	1.30	0.46– 3.68
Pos. MMS-index	24	4.67	1.63–13.42*	19	5.80	1.69–19.90*
Pos. BRS-index	19	2.70	1.04– 7.00*	15	3.15	1.11– 8.94*
Asthma	4	1.18	0.30– 4.72	3	1.24	0.28– 5.48
Hay-fever	6	0.57	0.19– 1.72	5	0.79	0.24– 2.59

OR: Logistic regression adjusted for sex, years of employment, smoking habits and hay fever.

OR analysis for hay fever and asthma: adjusted for sex, years of employment and smoking habits.

* Significant association between symptoms and positive HRT ($p < 0.05$).

important confounders in relation to severity and frequency of BRS [19]. Furthermore, possible exposure related confounders such as cleaning standard, detergents, room ventilation and temperature are eliminated in the present study, since the staff members showing positive and negative HRT were in general working in the same indoor environments.

Basophil HRT has been used for the detection of specific IgE to moulds where commercial extracts were not available for skin prick test or RAST-methods. An advantage of the HRT is the possibility of using the suspected moulds in the buildings rather than standard moulds from other sources, since the relevant strain may be important for the testing [20]. Furthermore, in the HRT, the risk of degradation of fungal epitopes is minimized using intact fungal spores harvested in dry state and kept freeze-dried. Multiple studies have demonstrated the clinical significance of the HRT to specific antigens including pollen, animal dander, mite and moulds in allergy testing. When using the patients' leukocytes a 80 to 90% concordance was found between the results of HRT and RAST or skin prick test in children and adults [21–24]. Additionally, IgE-sensitization to fungi was verified in patients with atopic dermatitis [25]. The clinical significance of HRT performed on basophils, sensitized with serum from the patients, was demonstrated in previous studies [26–28], and a high coincidence rate was found to RAST [26]. In previous studies HRT showed positive response to indoor moulds in patients with BRS, indicating the presence of basophil-bound IgE and serum IgE, both directed against the moulds [11, 12]. That IgE was responsible for the positive response was demonstrated by removal of cell-bound IgE leading to reduced or abolished histamine release [11]. In analogy, when IgE was removed from the patients serum before the passive sensitization, the results of HRT was changed from positive to negative [11]. The present study confirm these results which were observed in individuals with BRS as well as in pollen allergic patients, confirming the validity of the method to measure specific IgE.

The prevalence of self-reported asthma and hay fever did not differ between individuals with and without histamine release response to the moulds. This is in agreement with earlier findings [11, 12]. Accordingly, an association was found between mould specific histamine release response and BRS without a history of a manifest type I allergic disease.

Whether BRS could represent a subgroup or subclinical state of type I allergic diseases is unknown. Further investigation may include a double blinded provocation test to examine an association between positive HRT and provoked BRS. Monitoring the inhaled dosis of the present test material is essential to differentiate between responsible and irrelevant fungi (unspecific stimuli) in hyperreactive individuals.

In conclusion, positive HRT to indoor moulds, showing the presence in serum of IgE specific to the fungi, was found to be related to symptoms in damp and mouldy buildings. Whether the association is of causal character is a question for further studies. The test may be useful in the evaluation and study of possible mould induced BRS.

Acknowledgements. This study was supported by the National Health Fund for Research and Development (Ministry of Health), Research Centre for Environmental Health's Fund, National Housing and Building Agency, the Allergy fund of 1981, and the Danish Union of Teachers.

References

- [1] Flannigan B, Miller JD. Health implications of fungi in indoor environment-an overview. In: Samson RA, Flannigan B, editors. Health implications of fungi in indoor environments. Air Quality Monographs. vol 2. Amsterdam: Elsevier, 1994: 3–28.
- [2] Gravesen S, Frisvad JC, Samson RA, editors. Microfungi. Copenhagen: Munksgaard, 1994: 1–168.
- [3] Husman T. Health effects of indoor-air microorganisms. Scand J Work Environ Health 1996; 22: 5–13.
- [4] Norbäck D, Björnsson E, Janson C, Palmgren U, Boman G. Current asthma and biochemical signs of inflammation in relation to

- building dampness in dwellings. *Int J Tuberc Lung Dis* 1999; 3: 368–76.
- [5] Taskinen T, Meklin T, Nousiainen M, Husman T, Nevalainen A, Korppi M. Moisture and mould problems in schools and respiratory manifestations in schoolchildren: clinical and skin test findings. *Acta Paediatr* 1997; 86: 1181–87.
- [6] Johanning E, Biagini R, Hull D, Morey P, Jarvis B, Landsbergis P. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *Int Arch Occup Environm Hlth* 1996; 68: 207–18.
- [7] Platt SD, Martin CJ, Hunt SM, Lewis CW. Damp housing, mould growth, and symptomatic health state. *BMJ* 1989; 298: 1673–78.
- [8] Burr ML, Mullins J, Merrett TG, Stott NCH. Indoor moulds and asthma. *JRSH* 1988; 108: 99–101.
- [9] Thörn Å, Lewné M, Belin L. Allergic alveolitis in school environment. *Scand J Work Environ Health* 1996; 22: 311–14.
- [10] Björnsson E, Norbäck D, Janson C, Widström J, Palmgren U, Ström G et al. Asthmatic symptoms and indoor levels of micro-organisms and house dust mites. *Clin Exp Allergy* 1995; 25: 423–31.
- [11] Larsen FO, Meyer HW, Ebbenhøj N, Gyntelberg F, Sherson D, Netterstrøm B, Gravesen S, Norn S. Are fungi-specific IgE found in staff suffering from non-allergic sick building syndrome? *Inflamm Res* 1997; 46 Suppl 1: S79–S80.
- [12] Meyer HW, Larsen FO, Jacoby HH, Poulsen LK, Clementsen P, Gravesen S et al. Sick building syndrome: Association of symptoms with serum IgE specific to fungi. *Inflamm Res* 1998; 47 Suppl 1: S9–S10.
- [13] Gravesen S. Identification and quantitation of indoor airborne micro-fungi during 12 month from 44 Danish homes. *Acta allergologica* 1972; 27: 337–54.
- [14] Skov P, Valbjørn O, and DISG. The “sick” building syndrome in the office environment: The Danish Town Hall Study. *Environm Int* 1987; 13: 339–49.
- [15] Andersson K. Epidemiological approach to indoor air problems. *Indoor Air* 1998; 4: 32–9.
- [16] Larsen FO, Norn S, Mordhorst CH, Stahl Skov P, Milman N, Clementsen P. *Chlamydia pneumoniae* and possible relationship to asthma, serum immunoglobulins and histamine release in patients and controls. *APMIS* 1998; 106: 928–34.
- [17] Dahlqvist M, Alexandersson R. Acute pulmonary function impairment in school staff working in a sick building: a pilot study. *Indoor Environ* 1993; 2: 179–85.
- [18] Norbäck D, Wålinder R, Wieslander G, Smedje G. Indoor air pollutants in schools: nasal patency and biomarkers in nasal lavage. *Allergy* 2000; 55: 163–70.
- [19] Menzies D, Bourbeau J. Building-related illnesses. *N Eng J Med* 1997; 337: 1524–31.
- [20] Savolainen J, Viander M, Einarsson R, Koivikko A. Allergenic variability of different strains of *Candida albicans*. *Int Arch Allergy Appl Immunol* 1989; 90: 61–6.
- [21] Faraj BA, Camp VM, Lollies P. Histamine-dependent allergy blood test. *Allergy* 1986; 41: 526–31.
- [22] Nolte H, Schiøtz O, Stahl Skov P. A new glass microfibre-based histamine analysis for allergy testing in children, results compared with conventional leukocyte histamine release assay, skin prick test, bronchial provocation test and RAST. *Allergy* 1987; 42:366–73.
- [23] Nolte H, Storm K, Schiøtz PO. Diagnostic value of a glass fibre-based histamine analysis for allergy testing in children. *Allergy* 1990; 45: 213–23.
- [24] Siraganian RP. Automated histamine analysis for in vitro allergy testing, correlation of skin test results with in vitro whole blood histamine release in 82 patients. *J Allergy Clin Immunol* 1977; 59: 214–22.
- [25] Nissen D, Petersen LJ, Esch R, Svejgaard E, Stahl Skov P, Poulsen LK et al. IgE-sensitization to cellular and culture filtrates of fungal extracts in patients with atopic dermatitis. *Ann Allergy Asthma Immunol* 1998; 81:247–55.
- [26] Nolte H, Stafanger G, Skov PS, Schiøtz PO. Passive sensitization of basophil leukocytes from a non-atopic adult by plasma from allergic children. *Allergy* 1988; 43: 32–8.
- [27] Stahl Skov P, Jensen C, Nolte H, Mosbech H, Norn S. Regulatory role of cell-bound IgE on histamine release. In: Schou JS, Geisler A, Norn S, editors. *Drug receptors and dynamic processes in cells. Alfred Benzon Symposium 22*. Copenhagen: Munksgaard, 1986: 245–58.
- [28] Skov PS, Pelck I, Ebbesen F, Poulsen LK. Hypersensitivity to the diphtheria component in the Di-Te-Pol vaccine. A type I allergic reaction demonstrated by basophil histamine release. *Pediatr Allergy Immunol* 1997; 8: 156–8.



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