

Indoor Air-Related Effects and Airborne (1→3)-β-D-Glucan

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In studies on the relation between indoor mold exposure and symptoms/disease, the exposure should be described in terms of biomass and not viability. This paper reviews field studies in which (1→3)-β-D-glucan was measured as a marker of biomass and was related to the extent of symptoms and measures of inflammation among exposed subjects. Increased levels of (1→3)-β-D-glucan were related to an increased extent of symptoms and markers of inflammation. The data suggest that (1→3)-β-D-glucan can be used as a risk marker in indoor environments. *Key words:* airways inflammation, (1→3)-β-D-glucan, indoor air, molds. — *Environ Health Perspect* 107(suppl 3):501–503 (1999).

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Background

Effects related to indoor air have been reported in a large number of studies from different countries (1,2). The major symptoms are irritation in the eyes, nose and throat; dry cough; fatigue/headache; and skin problems, all of which can be caused by nonspecific airway inflammation (3).

Many studies show a relationship between symptoms and a history of dampness or flooding of buildings (4). Humidity in building structures favors the growth of fungi and certain bacteria. These microorganisms contain several agents with important biologic properties, such as endotoxin, mycotoxins, (1→3)-β-D-glucan, and different allergens that can cause a variety of toxicologic and immunologic effects (5). Although the development of an infection requires that the organism be viable, these effects do not require viability. Risk estimations based on viability are thus not relevant, and indicators of cell biomass or measures of the specific toxic agent should be used.

Several agents in microbes have been used to determine biomass. Endotoxin is widely used for the estimation of Gram-negative bacteria (6). Ergosterol has been used to determine fungal biomass (7). (1→3)-β-D-glucan, a polyglucose compound present in the cell wall of fungi, certain bacteria and vegetable materials, has also been used (8).

A number of studies have used measurements of airborne (1→3)-β-D-glucan to estimate mold exposure and to evaluate the relation with effects present in the exposed populations. A review of these studies is presented in the following sections, preceded by a description of the analytical methods for determination of (1→3)-β-D-glucan.

Measurement of (1→3)-β-D-Glucan

(1→3)-β-D-Glucan is a polyglucose compound in which the biologic activity is caused by the unique (1→3)-β-bindings between the polyglucose rings. From an analytical point of view, a chemical analysis is thus very difficult, and the techniques available rely on biologic reactions.

One commonly used method was developed in Japan for the purpose of following the clinical progress of deep mycosis (9). The method is based on the reactivity of the amoeba present in the *Limulus* horseshoe crab to react specifically with the (1→3)-β-D-glucan molecule. The method is extremely sensitive and quantities can be detected as small as a few picograms.

In our laboratory, samples of air are taken by drawing air through Isopore filters (ATTP 0.8 μm, Millipore, Cambridge, MA) at a flow rate of 5 L/min for 30 min. The filters are shaken for 10 min in 10 mL of pyrogen-free water. Thereafter 0.3 M NaOH is added, and the filters are shaken on ice for 10 min to unwind the triple helix structure of the glucan and make it water soluble. Filter extract samples of 50 μL are placed in a microwell plate, and 50 mL specific glucan lysate (Fungitic G Test, Seikagaku Co., Tokyo, Japan) is added. The plate is incubated in a spectrophotometer (Wellreader, Scinics Corp., Tokyo, Japan), and the kinetics of the ensuing color reaction are read photometrically, transformed into absorbance units, and compared to a standard curve. The results are expressed as nanograms per milliliter of liquid. Using the value for airflow through the filter, this value is transformed to nanograms per cubic meter. The detection limit for the technique is 20 pg/mL. As the

method is very sensitive, even the slightest contamination with molds of the equipment or the laboratory space will introduce errors in the results, and control filter and solution always need to be analyzed in parallel.

Another method is based on a highly specific inhibition enzyme immunoassay (EIA), developed for the quantitation of (1→3)-β-D-glucan in dust samples from the occupational and residential environment (10). Immunospecific rabbit antibodies are produced by immunization with bovine serum albumin-conjugated laminarin [a (1→3)-β-D-glucan] and affinity chromatography. The laminarin-based calibration curve in the inhibition EIA ranges from approximately 40–3,000 ng/mL (15–85% inhibition). Another (1→3)-β-D-glucan, curdlan, shows a similar inhibition curve but is 3 to 5 times less reactive on a weight basis. Other polysaccharides such as β(1→6)-glucan and mannan do not react in the inhibition EIA. The assay can detect heat-extractable (1→3)-β-D-glucan in dust samples collected in a variety of occupational and environmental settings such as compost facilities, swine confinement buildings, and homes. Both fungal and plant (1→3)-β-D-glucans are detectable in the assay. Based on duplicate analyses of dust samples, the coefficient of variation is approximately 25%.

In the studies described in this paper, the air samples were taken using aggressive sampling. We used a device stirring up the air from the floor using an air stream, which is passed over the surface of the room in which the sampling is performed. Without this procedure, the dust (and mold) particles will rapidly set on the floor surface and air sampling would give a false

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negative value in environments where there is no or little movement of persons. Alternatives are to sample over a prolonged period, e.g., 8 hr during periods of normal movement in the room or to use a personal sampler.

Studies Performed

The first published study reported an investigation of a number of flats in a small town in Sweden (11). The presence of symptoms was evaluated using questionnaires, and measurements of (1→3)-β-D-glucan were made in the houses. The exposure was divided into three classes—high, medium, and low glucan. The proportion of persons reporting symptoms indicative of airways inflammation was related to the amount of glucan measured indoors. A similar relationship was found for fatigue.

The second study involved a number of buildings where complaints had been made about indoor air-related symptoms—a day-care center, a post office, and two primary schools (12). An office building where no symptoms had been reported served as a control. All persons working full time in the buildings received a questionnaire originally developed to assess the effects of organic dusts and modified for indoor conditions. Questions were asked about symptoms in the eyes and airways as well as fatigue and headache. Of the 442 subjects selected for the study, 437 participated. A total of 39 persons in the buildings with symptoms and 405 persons in the control building was investigated. Measurements were made of the amount of airborne endotoxin and (1→3)-β-D-glucan. The results are summarized in Table 1. It is seen that the extent of different symptoms was related to levels of airborne (1→3)-β-D-glucan.

A case study in Switzerland comprised a clinical investigation of two boys living in a house with indoor mold growth and

airborne (1→3)-β-D-glucan levels ranging between 5 and 106 ng/m³ (mean 41.9) (13). The boys developed severe airway inflammation with cough, wheezing, and fatigue after about 6 months of living in the house; one boy became sensitized to house dust mites.

The symptoms disappeared when the boys moved out of the house. About a year later, the parents developed airway inflammation and they were also forced to move from the house. A similar case history was reported in Sweden (14). In this study, three children reported intense symptoms of airway inflammation and fatigue/headache. Two of the children were on medication with bronchodilators. Levels of (1→3)-β-D-glucan ranged from 21.8 to 115.2 ng/m³ in different rooms of the house. The children finally moved to their grandmother's house, and the symptoms cleared within a few months. These case studies demonstrate that children living in home environments with molds may develop severe airway symptoms as well as fatigue and headache. Except for the sensitization to mites in one child, no evidence of allergic reactions was present. The rapid improvement in both cases when the children moved out of their homes supports a hypothesis of causality.

One study was performed in a day-care center with a history of dampness (15). For several years, symptoms of nasal swelling, throat irritation, headache, and fatigue had been reported by the staff. The 15 staff members were investigated by questionnaire, and a methacholine test was performed to determine airway responsiveness. A few months later, the center was closed for renovation and the personnel worked elsewhere. After the renovation, they returned to the center and were examined 1 and 2 years later.

The amount of airborne (1→3)-β-D-glucan before the renovation was 11.4 ng/m³ (*n* = 24, SD = 2.3) and 1.3 ng/m³ (*n* = 13, SD = 0.9, *p* < 0.0001) after the renovation. The results of measurements of airway responsiveness (Table 2) showed that there was a tendency for the average

decrease in forced expiratory volume (FEV₁) after methacholine challenge to be lower at 2 and 3 years after the renovation compared to before. The number of persons with decreases in FEV₁ larger than 4% was lower at 2 and 3 years (*p* = 0.03 and 0.08, χ^2 , Yate's correction). Two of the staff reported that they had developed an allergy (to cats) at the reexamination at 2 years.

A recent study was performed in an area with rowhouses, some of which had problems with dampness and mold growth (16). The study comprised 75 houses with indoor air (1→3)-β-D-glucan levels ranging from 0 to 19 ng/m³. The tenants were invited to a clinical examination and 127 persons participated. The amount of myeloperoxidase in blood was higher among those living in houses with levels of (1→3)-β-D-glucan > 1 ng/m³ (*p* = 0.03). This group also showed a significant inverse relationship between the baseline FEV₁ and the number of years the person lived in the house, when controlling for age, gender, smoking, asthma, atopy, and house pets. The proportion of atopic persons was higher among those living in houses where levels of (1→3)-β-D-glucan exceeded 4 ng/m³ (24.6 vs 13.2%), although the difference was not statistically significant.

A follow-up study was made with volunteers from the rowhouse study (17). A blood sample was taken and blood monocytes were isolated for determination of cytokine production before and after *in vitro* cell stimulation. After comparing persons with an increased level of (1→3)-β-D-glucan in their homes with those in homes with low or undetectable levels, an increased baseline production of tumor necrosis factor α was found.

Several studies have investigated the children's environments. In an investigation in Sweden, two schools—one with mold problems and another without problems—were studied (18). The extent of respiratory symptoms was significantly higher in the school with higher levels of (1→3)-β-D-glucan (15.3 ng/m³) than in the school with low levels (2.9 ng/m³). Among atopic children, the extent of symptoms of dry cough, cough with phlegm, and hoarseness was similar to those of the nonatopics in the low (1→3)-β-D-glucan school but significantly more common in the high (1→3)-β-D-glucan school.

Comments

A general conclusion from the studies reviewed here is that there was a relation between exposure to (1→3)-β-D-glucan as

Table 1. Airborne levels of (1→3)-β-D-glucan in relation to extent of symptoms.^a

	Control	Day-care/ post office	School 1/2
<i>n</i>	405	19	20
Glucan (ng/m ³) ^b	<0.1	1.3	5.2
% with symptoms			
Nasal irritation	16	37	50
Throat irritation	11	37	45
Dry cough	6	5	35
Headache	2	5	30
Fatigue	21	58	75

^aSummarized by Rylander et al. (12). ^bAdjusted according to later modification of the Limulus assay.

Table 2. Airway responsiveness before and after building renovation. Change of FEV₁ after inhalation of 1.2 mg methacholine.^{a,b}

	Before	2 years	3 years
<i>n</i>	11	11	11
FEV ₁ (%)	-7.8 (6.2)	-4.0 (6.6)	-5.9 (8.8)
<i>n</i> > 4%	8	2	3

FEV₁, forced expiratory volume. ^aFigures in parentheses are standard deviations and numbers of persons with a decrease larger than 4%. ^bSummarized from Rylander et al. (12).

an indicator of mold biomass and the extent of symptoms of airway inflammation, fatigue, and headache. Some data show that objective measurements of inflammation were also related to the exposure. In some of the studies, dose-response relationships could be demonstrated. For field measurements, (1→3)-β-D-glucan may thus be a suitable indicator of risk.

Even if there is a relation between indoor air effects and the amount of (1→3)-β-D-glucan, this cannot be taken as evidence for a causal relationship. (1→3)-β-D-glucan is an indicator of fungal biomass, but fungi contain a variety of agents with biologic effects. Support for causality would require exposures to isolated (1→3)-β-D-glucan, preferably using atopic and asthmatic subjects. Although a few such studies have been reported and have confirmed an effect on the airways (19), there is still a great need for further research in that area.

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