The medical relevance of methods to sample indoor air microbial pollution

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ABSTRACT
The aim of the investigation was to evaluate different methods to sample microbial cell wall agents (MCWA) indoors and to relate the results to clinical markers of inflammation among persons ($n = 27$), living in houses with suspected mould problems. Airborne particles were sampled for 8 h or by agitating floor dust. Sedimented particles were collected from floor dust. Analysis was done for endotoxin and (1→3)-β-D-glucan. Endotoxin was found only in a few of the agitated floor dust samples. There was a relation between airborne endotoxin levels and the secretion of certain Th1-type cytokines from stimulated blood mononuclear cells and between (1→3)-β-D-glucan in floor dust and the total amount of IgE in serum. Boarder values were defined for endotoxin at 0.2 ng/m^3 and for (1→3)-β-D-glucan at 25 ng/mg floor dust.

INDEX TERMS
Microbial cell wall agents; Endotoxin; (1→3)-β-D-glucan; Inflammatory responses

INTRODUCTION

The microbial contamination indoors is often assayed by determining the number of airborne viable microbes. An important drawback is that agents in the microbial cell wall may retain their biological effect even after the death of the microbe and determinations of viability will thus not determine the actual dose of the causative agent. Previous investigations in home environments with microbial contamination have demonstrated that certain microbial cell wall agents (MCWA) may cause inflammatory responses in terms of an increased incidence of symptoms from the airways, decreased pulmonary function and changes in markers of inflammation in blood (Michel *et al*., 1996; Thorn and Rylander, 1998; Douwes *et al*., 2000; Beijer *et al*., 2002). A dose–response relationship has been shown between the degree of MCWA contamination and the ratio interferon gamma (IFNγ) and interleukin-4 (IL-4) secretion from stimulated blood mononuclear cells (PBMC) (Beijer *et al*., 2003).

There are no standardized methods at present to determine the indoor exposure to MCWA. A variety of sampling techniques has been used such as vacuumed floor dust, agitated floor dust sampled in the air or airborne dust over longer time periods. Dust from beds and from shelves has also been used.

The present study was undertaken to investigate different methods to sample MCWA indoor and to relate them to indicators of inflammation among persons living in dwellings with different levels of contamination.

MATERIAL AND METHODS

Study Group

Subjects ($n = 26$) were recruited by advertising in the local press for persons who knew or suspected that they had mould growth in their homes.

Exposure Determination

Airborne dust was sampled using two methods. The first employed a pump, drawing air at a rate of 5 l/min through two parallel filters (Isopore, ATTP 0.8 μm, Millipore Inc), placed 0.8 m above

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the floor. The pump was switched on by a person entering the room and automatically switched off after 30 min. It was switched on again if the person was still present in the room or when the person entered again. The sampling comprised at most four 30 min periods per day up to a total of 8 h sampling time (8 h airborne samples). The second method for airborne dust comprised agitation of the floor dust using an inverted vacuum cleaner and collecting airborne dust for 30 min as previously described (Thorn and Rylander, 1998) (agitated floor dust). Sedimented dust was collected by vacuuming 1 m^2 of the floor during 3 min. The dust was sieved through a 2 mm metal mesh into a Petri dish.

The amounts of endotoxin and (1→3)-β-D-glucan in the different dust samples were analysed with the Limulus test, using endotoxin- and glucan-specific lysates according to standardized methods described earlier (Tamura *et al*., 1994; Thorn and Rylander, 1998).

Medical Investigations

Atopic sensitization was determined using the prick test. Venous blood samples were drawn and the amount of eosinophilic cationic protein (ECP), C-reactive protein (CRP), monoperoxidase (MPO) and total amount of IgE were determined. Blood mononuclear cells (PBMC) were prepared from venous blood samples and the secretion of TNFα, IL-4 and IFNγ from cells stimulated with LPS or PHA was determined using standard techniques.

Statistical Analyses

The relations between exposure parameters as well as relations between exposure and medical parameters were examined by the Spearman rank correlation test. The data were analysed to reveal the presence of borderline exposure values above which significant differences in the clinical parameters were present, using Student's *t*-test.

RESULTS

In agitated airborne dust, endotoxin was only detected in two samples. (1→3)-β-D-glucan was detected in 21 of the 26 samples. In 8-h airborne dust, endotoxin was detected in 21 of the 24 samples and (1→3)-β-D-glucan in 20 of 24 samples. There was a relation between endotoxin and (1→3)-β-D-glucan in 8-h airborne dust ($r = 0.58$, $p = 0.003$). In contrast to this, there was no correlation in the sedimented dust.

The results from calculations of the correlation between MCWA and markers of inflammation demonstrated that endotoxin related to cytokines secreted from PBMC whereas (1→3)-β-D-glucan related to serum levels of total IgE (Table 1). Boarder values were found for floor dust (1→3)-β-D-glucan regarding total IgE in serum. Table 2 shows the boarder values for the whole group and non-atopics separately. For both groups, a significantly higher level of IgE was found if the exposure exceeded 25 ng/mg floor dust for (1→3)-β-D-glucan. No border value was found for (1→3)-β-D-glucan in airborne 8 h or agitated floor dust.

Table 1 Relation between endotoxin and (1→3)-β-D-glucan in different dusts and markers of inflammation

Dust	Marker	r_{xy}	p
Endotoxin			
8 h airborne	IFNγ/IL-4	0.43	0.04
Agitated airborne			No relation
Floor			No relation
Glucan			
8 h airborne			No relation
Agitated airborne			No relation
Floor	IgE, serum	0.45	0.02

Table 2 Floor dust (1→3)-β-D-glucan and border values for total IgE; median and range (within parenthesis)

Glucan (ng/mg)	<25	≥25	*p*
n	15	10	
IgE	9.0 (2.0–46.7)	57.7 (10.7–115.0)	0.002
Nonatopics			
n	13	4	
IgE	8.60 (2.0–46.7)	48.5 (24.7–80.6)	0.023

Border values were also found for airborne 8 h airborne endotoxin regarding L-selectin, ECP, IFNγ and TNFα/IL-4 (Table 3). L-Selectin and ECP were lower at higher exposures to endotoxin and IFNγ and TNFα/IL-4 were higher (boarder value about 0.2 ng/m^3). For (1→3)-β-D-glucan, L-selectin was also lower at higher exposure values. No other border values were found in the material.

Table 3 Airborne 8 h endotoxin border values for L-selectin, IFNγ, ECP and TNFα/IL-4; median and range (within parenthesis)

Endotoxin (ng/m^3)	<0.2	≥0.2	*p*
n	19	4	
L-selectin	810 (499–1491)	653 (384–717)	0.015
IFNγ	1830 (288–13880)	4380 (3024–11839)	0.029
	<0.17	≥0.17	
n	20	5	
ECP	3.5 (1.5–28.1)	2.9 (2.1–4.2)	0.032
TNFα/IL-4	305 (57–877)	552 (485–1330)	0.016

COMMENTS

The results are based on relatively few persons and the number of comparisons was large, increasing the risk for random significance. On the other hand, the results showed some consistent trends and similarities with previous data, supporting their relevance.

The medical relevance of sampling airborne endotoxin has been demonstrated in previous studies in occupational environments where relations to subjective symptoms and lung function have been shown. In the samples of agitated airborne dust, sampled during 30 min, endotoxin levels were below the threshold of detection in most samples. Contrary to this, detectable levels were found in most 8-h airborne samples, emphasizing the importance of sample size when investigating home environments where the exposure levels may be low.

The relations between endotoxin in different fractions of the dust samples and the Th1-type cytokines and inflammatory markers support previous knowledge on the inflammagenic properties of endotoxin (reviewed in Rylander, 2002). Determinations of endotoxin in dwellings where symptoms of airways inflammation are present are thus justified from risk assessment and remedial purposes.

The relation between the total amount of serum IgE and (1→3)-β-D-glucan in floor dust can be compared with data from a previous study where a relationship was found between airborne viable spores and total IgE levels (Su *et al*. 2003). This suggests that the exposure to moulds and the MCWA (1→3)-β-D-glucan influences the immune system in a Th2-positive way. This hypothesis is supported by previous data on humans and animals where (1→3)-β-D-glucan was found to have a blunting effect on the endotoxin induced inflammation and antibody adjuvance effect of

endotoxin as well as a promoting effect on the secretion of IL-10 (Rylander and Holt, 1997; Rylander and Lin, 2000; Rylander 2002) This justifies that investigations in dwellings where MCWA contamination is suspected, also include determinations of (1→3)-β-D-glucan. In view of the results presented here, sampling of sedimented floor dust seems to be a suitable method for this MCWA.

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