INHALATION OF (1→3)-β-D-GLUCAN IN HUMANS

L Beijer, J Thorn and R Rylander
Department of Environmental Medicine
Göteborg University, Sweden

Abstract

Twelve subjects living in houses with levels of airborne glucan of at least 4 ng/m^3 (G+) and six subject living in houses with levels of airborne glucan of less than 1 ng/m^3 (G0), were exposed to grifolan suspended in saline as well as to saline alone. Spirometry, methacholine challenge and blood sampling were performed before, immediately after and 24 h after exposure. There was no difference between the groups comparing spirometry or airway responsiveness, neither before the two exposures or thereafter. Comparing the groups before exposure showed that they differed regarding the number of cytotoxic T cells in the blood ($CD8+S6F1_{strong}$). The G0 group had a higher ratio of cytotoxic/noncytotoxic T cells compared to G+, which might be interpreted as an increased trapping of cytotoxic cells in the lung tissue in subjects living in homes with glucan exposure. Blood mononuclear cells from G+ also showed an increased production of TNFα in *in vitro* non stimulated cells compared to cells from G0. After exposure to saline, the non stimulated TNFα production was increased in the G0 group while exposure to (1→3)-β-D-glucan resulted in an increase in both groups. An increase in the number of blood neutrophils and serum levels of myeloperoxidase was shown in both groups after both types of exposure.

Introduction

Numerous reports describe an association between molds or dampness in buildings and the presence of symptoms from the airways as well as general fatigue and headache. The character of the symptoms suggests that they are caused by a non-specific inflammation rather than by an allergic reaction [Rylander 1992]. Molds are a mixture of different bacteria and fungi, which contain (1→3)-β-D-glucan in the cell wall. It consists of a polyglucose with β-1→3 linkage and appears in a variety of forms with single or triple helix molecular structures, unbranched or branched to different degrees [Yadomae 1996]. (1→3)-β-D-glucans have a variety of effects on immune competent cells [Di Luzio 1985, Williams 1997]. Inhalation studies of glucan in animals have revealed an alteration of the inflammatory response to endotoxin with following consequences for the accumulation and activation of cells in the lung tissue and airways [Fogelmark et al 1992, 1994, Rylander and Holt 1998, Fogelmark et al 1997].

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:251-254 (1998)
National Cotton Council, Memphis TN

Previous studies have demonstrated a relation between the level of (1→3)-β-D-glucan indoors and the extent of different kinds of symptoms [Rylander et al 1996, 1997]. Investigations in an area of rowhouses demonstrated a relation between the blood level of myeloperoxidase and the level of (1→3)-β-D-glucan in the homes. A decrease in forced vital capacity during one second (FEV_1) and an increased prevalence of atopy among those living in houses with higher levels of (1→3)-β-D-glucan was also found [Thorn and Rylander 1998].

These relations could imply that (1→3)-β-D-glucan is the causative agent for the observed effects or that glucan serves as an indicator of molds, which contain other agents that cause these effects.

In order to further elucidate the role of (1→3)-β-D-glucan in the development of the inflammatory response seen among persons in moldy buildings, inhalation challenges with the pure substance were undertaken. The study comprises persons living in houses with higher levels of (1→3)-β-D-glucan and those living in houses with small or non-detectable levels. The two groups were compared regarding baseline values and the reactions seen after inhalation of pure (1→3)-β-D-glucan. The following is a preliminary report from the ongoing study.

Methods

Subjects

The subjects were recruited from the rowhouse study mentioned above [Thorn and Rylander 1998]. Based on the measurements of (1→3)-β-D-glucan in the homes, two groups were identified – one with an average level lower than 1ng/m^3 (G0) and one with an average level equal to or above 4 ng/m^3 (G+). Persons between 19 and 65 years old, without asthma, cardiovascular diseases or other disease that could put the person at risk during the experiment were invited to participate. Of the invited persons, 33% in low glucan homes and 60% in high glucan homes volunteered. Table 1 shows the number of persons and the personal characteristics.

Questionnaire

The subjects were interviewed using a slightly modified standard questionnaire for the assessment of organic dust-induced effects which has been used in several previous investigations [Rylander *et al.*, 1990]. It contained a series of items about the domicile concerning water damage, signs of dampness, odors of molds and the presence of pets. Personal questions about occupation and existing diseases were followed by a series of questions on different symptoms. The symptoms were cough (dry or with phlegm), chest tightness, shortness of breath, irritation in the eyes, nose or throat, and nose congestion and itchy nose. Questions were also posed on headache, unusual tiredness, wheezy chest and skin problems. Special questions related to subjective airway reactivity, chronic bronchitis and asthma. Finally, the questionnaire contained items on physician-verified allergy and smoking habits. Chronic bronchitis was defined as cough

with sputum for at least three months a year for a period of at least two years.

Exposure

One to three persons were exposed at the same time and each subject was exposed on two randomized occasions to (1→3)-β-D-glucan in saline and to saline alone, as a control. For the glucan exposures we used grifolan, a water-nonsoluble (1→3)-β-D-glucan extracted from *Grifolanum commune* (generous gift from prof N Ohno, Tokyo, Japan). Grifolan was dissolved in 0.3 N NaOH over night and then diluted with saline to a concentration of 6 µg/ml. The concentration of NaOH in the final exposure solution was 0.003 N. The exposures were performed in a chamber with 2x3.5 meters surface, furnished as a sitting room. The solution was aerosolized using a Collison spray [Rylander 1968] and led into the exposure chamber through the ventilation system. The exposure time was three hours. Sampling of the air in the room was done with Isopore filters.

The amounts of airborne (1→3)-β-D-glucan on the filters were determined using a specific Limulus lysates (Fungitec G Test®, Seikagaku Co, Tokyo, Japan) [Tamura *et al.*,1994]. Filters from the saline exposure were also analyzed for endotoxin using specific endotoxin lysate (Endospecy®). No endotoxin was detected on these filters.

The average concentration of (1→3)-β-D-glucan in the chamber was about 30 ng/m^3 (range 15.3 – 43.2 ng/m^3) which was about 3-10 times higher than the glucan concentration measured in the subject's homes.

Spirometry and Airway Responsiveness

Spirometry was performed using standard techniques using a Vitalograph model S with a PFT printer. The subjects performed at least three technically acceptable trials, and the largest value for the forced expiratory volume in one second (FEV_1) was registered and compared with predicted values [Knudson *et al.*, 1983].

Airway responsiveness was assessed using the methacholine challenge test according to Yan *et al.* [1983], with some modifications. The subjects initially inhaled one dose of saline. The spirometric values obtained one minute after this inhalation were used as the baseline values for the methacholine test. The methacholine was administered in increasing doses at three-minute intervals up to a total amount of 1.2 mg. The results were expressed as the decrease in FEV_1 after the highest dose of methacholine given.

Total and Differential Blood Cell Count and Lymphocyte Subsets

Five ml of blood was collected in EDTA tubes using the vacutainer technique. A sample was used for determination of the total cell count using a Bürker chamber. Differential cell counts was analysed before, immediately and 24 hours after exposure. Slides were stained with May-Grünwald-Giemsa and counted in light microscope. The results were expressed as 10^9 cells/l blood.

Lymphocyte subsets were quantified before exposure only, using flow cytometry. Helper/inducer (CD4+), cytotoxic/suppressor (CD8+) T cell populations were identified. CD8+ cells were analyzed together with a monoclonal antibody binding S6F1, an epitope of the α-chain in the LFA-1 complex (CD11a). CD8+ T cells expressing S6F1 have been classified as cytotoxic cells, while CD8+ T cells with a weak staining for S6F1 are either naive cells or suppressor effector cells [Morimota *et al* 1987].

Inflammatory Markers and Atopy

Eosinophilic cationic protein (ECP) was assayed in serum by a fluorescent enzyme immunoassay technique (CAP ECP FEIA, Pharmacia Diagnostics AB, Uppsala, Sweden) and expressed as µg/l. Myeloperoxidase (MPO) was assayed by a radio immunoassay technique (CAP MPO RIA, Pharmacia Diagnostics AB, Uppsala, Sweden) and expressed as µg/l.

Atopy was determined by the concentration of specific serum IgE antibodies against ten airborne allergens using a fluorescent enzyme immunoassay technique (CAP Phadiatop FEIA, Pharmacia Diagnostics AB, Uppsala, Sweden). The results were expressed as positive (atopic) or negative (non-atopic). The test is well characterized and is clinically used for the diagnosis of atopy in several countries in Europe. The specific antibodies measured and standard allergens used are known only to the manufacturer. Atopy is defined as a reaction above a certain limit (decided by the manufacturer) against any of the ten inhalant allergens. The method sensitivity and specificity are 0.91 and 0.93 based on 21 studies.

Cytokine Production

Venus blood was collected before and 24 hours after exposure for cytokine analysis. About 16 ml of blood was drawn into cell preparation tubes (CPT, Becton Dickinson). After centrifugation the mononuclear cells (MNC) was collected, washed twice in Hank's salt buffer supplied with 10% homologues serum and then suspended in AIM V medium (Gibco BRL) supplemented with 2-mercaptorthanol, $4x10^{-5}$M, to a concentration of $2x10^6$/ml. The MNC were incubated in tissue culture plates with an area of 2 cm^2, diluted in an equal volume of either medium alone or medium containing phytohaemagglutinin (PHA, Murex Diagnostics Limited), final concentration 500 µg/ml. After 48 hours incubation at 37 °C in 5% CO_2 , the supernatants were collected and stored at -25°C till cytokine analysis. TNFα was analyzed using an ELISA kit with a sensitivity of 1 pg/ml and intra/inter-assay reproducibility of less than 10% (PeliKine-compact™ human cytokine ELISA, CLB, The Nederlands).

Results

Regarding spirometry and airway responsiveness, there were no differences between the groups in their baseline values and the exposure to saline or glucan had no effect on those parameters.

Table 2 shows the number of blood neutrophils and MPO before and immediately after the three hours exposure. It is seen that the baseline value for the number of neutrophils was slightly lower (not significant) in the G+ group. Both saline and glucan exposure gave an increase in neutrophils. MPO, which is an indicator of activated neutrophils, was elevated in G0 as well as G+ after both exposures (significantly for G+). At 24 hours after exposure, the values returned to baseline levels. The baseline numbers of monocytes, lymphocytes and eosinophils were similar in the two groups and very small effects were seen in these cells after exposure.

Table 3 shows the lymphocyte subpopulations measured before exposure. The ratio CD4+/CD8+ was higher for G+ compared to G0. The number of CD8+S6F1$_{weak}$ cells was higher in the G+ group and the ratio of the S6F1$_{strong}$/S6F1$_{weak}$ was significantly lower in this group (p=0.035).

Table 4 shows the values of TNFα production in non-stimulated cells. Due to technical errors, some analyses had to be discarded and the number of subjects in the G+ group was seven for this analysis. This G+ group had a significantly higher baseline production of TNFα in non stimulated mononuclear cells compared to the G0 (p=0.04). Exposure to saline caused an increase in non stimulated TNFα production in G0 while exposure to (1→3)-β-D-glucan caused an increase in both G0 and G+.

Regarding TNFα production in PHA stimulated cells, we could observe a trend that cells from G0 produced less TNFα after exposure to saline or (1→3)-β-D-glucan, compared to before exposure, while cells from G+ produced more TNFα after those exposures.

Comments

The results are so far based upon a small number of persons, particularly the group with low levels of (1→3)-β-D-glucan in their homes, and should thus be interpreted with caution. The data analyzed show that a major effect found related to living in a house with higher levels of (1→3)-β-D-glucan was an increase in the number of S6F1$_{weak}$ cells, a higher number of CD4+cells and an increased baseline production of TNFα. In a previous study where subjects were exposed to cotton dust, a relation was found between the number of S6F1$_{weak}$ cells and the decrease in FEV$_1$ after exposure [Beijer et al 1995]. The S6F1 is a marker of cytotoxic effector cells and it has been shown that S6F1 expression is necessary for the migration of CD8+ cytotoxic cells through the endothelium [Berman et al 1995]. Taken together, these data suggest that a larger proportion of S6F1$_{weak}$ cells in blood could be due to homing of S6F1$_{strong}$ cells to the lung tissue or airway epithelium, reflecting an ongoing inflammation, also reflected by the increased baseline production of TNFα.

The exposure to saline induced increased numbers of neutrophils and MPO in blood.

The saline exposure also caused an increase in baseline production of TNFα among those living in houses with low levels of (1→3)-β-D-glucan but not among those living in houses with high levels. Again the small number of subjects may be responsible for these differences but it is interesting that animal experiments have demonstrated a decreased reactivity to endotoxin after exposure to (1→3)-β-D-glucan [Fogelmark et al 1992]. The subjects in homes with higher levels of (1→3)-β-D-glucan might thus have been less sensitive to or react in a modified way to the slight inflammatory effect of saline. Further work is needed to assess this conclusion.

Summary

The preliminary results from this study in progress suggest that persons living in homes with increased levels of (1→3)-β-D-glucan have signs of an ongoing inflammation in the airways, reflected by a higher production of TNFα in non stimulated blood mononuclear cells and a higher number of CD8+S6F1$_{weak}$ lymphocytes in blood. An acute exposure to (1→3)-β-D-glucan caused an increased production of TNFα, both among those living in houses with higher levels and those living in houses with lower levels of (1→3)-β-D-glucan. This suggests that (1→3)-β-D-glucan could be a causative agent for effects seen among persons living in moldy homes.

Acknowledgment. The study was supported by funds from the Center for Indoor Air Research (contract 96-09) and Vårdalstiftelsen (grant A96 082).

References

Beijer L., R R Jacobs, B Boehlecke, B Andersson, R Rylander. 1995. Monocyte responsiveness and a T-cell subtype predict the effect induced by cotton dust exposure. Am J Respir crit Care 152:1215-20.

Berman J.S., K Mahoney, J J Saukkonen, J Masuyama 1995. Migration of distinct subsets of CD8+ blood t cells through endothelial cell monolayers in vitro. J. Leukoc. Biol. 58:317-324.

Di Luzio N.R. 1985. Update on the immunomodulating activities of glucans. Springer semin Immunopathol 8:387-400.

Fogelmark, B., H. Goto, K. Yuasa, R. Rylander. 1992. Acute pulmonary toxicity of inhaled (1Õ3)-ß-D-glucan and endotoxin. Agents Actions 35:50-55.

Fogelmark, B., R. Rylander. 1994. Pulmonary inflammation induced by repeated inhalations of (1Õ3)-ß-D-glucan and endotoxin. Int J Exp Pathol 74:85-90.

Fogelmark, B., M. Sjöstrand, D. Williams, R. Rylander. 1997. Inhalation toxicity of (1Õ3)-ß-D-glucan : recent advances. Mediators Inflamm 6:263-266.

Knudson RJ, M D Lebowitz, C J Holberg, B Burrows 1983. Changes in the normal maximal expiratory flow-volume curve with growth and aging. Am Rev Respir Dis 127:725-734.

Morimoto C, C E Rudd, N L Letvin, S F Schlossman 1987. A novel epitope of LFA-1 antigen which can distinguish killer effector and suppressor cells in human CD8 (T8) cells. Nature 330:479-482

Rylander, R. 1968. Pulmonary defense mechanisms to airborne bacteria. Acta physiol Scand suppl 306: 1-89.

Rylander R, Y Peterson, K J Donham, 1990. Questionnaire evaluating organic dust exposure. Am J Ind Med 17:121-126.

Rylander R, K Persson, H Goto, K Yuasa, S Tanaka 1992. Airborne ß,1-3 glucan may be related to symptoms in sick buildings. Indoor Environment 1:263-267

Rylander R. 1996. Airway responsiveness after inhalation of endotoxin and (1→3)-β-D-glucan. Indoor + Built Environ 5:106-111.

Rylander R. 1997. Airborne (1→3)-β-D-glucan and airway effects in a day care center before and after renovation. Arch Environ Health 52:281-85

Rylander R., P G Holt 1998. Modulation of immune response to inhaled allergen by coexposure to the microbial cell wall components (1→3)-β-D-glucan and endotoxin. Mediators of Inflammation (in press)

Tamura H, Y Arimoto, S Tanaka, S Yoshida, T Obayashi, T Kawai 1994. Automated kinetic assay for endotoxin and (1→3)-ß-D-glucan in human blood. Clinica Chimica Acta 226:109-112.

Thorn J, Rylander R. 1998. Airways inflammation and glucan in a rowhouse area. Am J Respir Crit Care Med (in press).

Williams DL. 1997. Overview of (1Õ3)-ß-D-glucan immunobiology. Mediators of Inflammation 7; 247-250.

Yadomae T., N Ohno 1996. Structure-activity relationship of immunomodulating (1→3)-β-D-glucans. Reccent. Res. Devel. In Chem. and Pharm. Sciences 1:23-33.

Yan K, C Salome, A J Woolcock 1983. Rapid method for measurements of bronchial responsiveness. Thorax 38:760-765.19.

Table 1. Number of persons invited, participants and their basic characterisitcs

	low-glucan	high-glucan
	G0	**G+**
Persons Invited	18	20
Participants	6	12
% participants	33	60
Females	4	6
Males	2	6
Atopic	3	5
Smokers	0	1
Age		
- mean	55	41
- range	35-66	19-61

Table 2. Neutrophils in blood and MPO in serum among subjects living in homes with low (G0) and high (G+) levels of (1→3)-β-D-glucan. Figures are mean and SD in parenthesis.

	G0	**G+**
n	6	12
PMN in blood, 10^9/l		
- baseline	3.41 (1.06)	2.76 (1.15)
- after NaCl	3.99 (1,39)	3.98 (1.77)
- baseline	3.38 (1.32)	2.73 (1.17)
- after grifolan	4.11 (1.25)	3.44 (1.19)
Myeloperoxidase in serum, µg/l		
- baseline	186 (69)	209 (117)
- after saline	210 (62)	247 (98)
- baseline	183 (31)	184 (70)
- after grifolan	222 (62)	214 (84)

Table 3. Lymphocyte subpopulations in blood among subjects living in homes with low (G0) and high (G+) levels of (1→3)-β-D-glucan. Figures are mean and SD in parenthesis.

	G0	**G+**
n	6	12
CD4+	0.77 (0.31)	0.87 (0.29)
CD8+	0.50 (0.13)	0.47 (0.38)
CD4+/CD8+	1.44 (0.78)	2.55 (1.42)
$CD8+S6F1_{strong}$	0.46 (0.17)	0.31 (0.34)
$CD8+S6F1_{weak}$	0.14 (0.06)	0.25 (0.12)

Table 4. TNFα production in non stimulated monomuclear blood cells among subjects living in homes with low (G0) and high (G+) levels of (1→3)-β-D-glucan. Figures are mean and SD in parenthesis.

	G0	**G+**
n	6	12
- baseline	1.14 (1.39)	2.16 (1.44)
- after saline	3.01 (2.48)	2.19 (1.70)
- baseline	0.53 (0.62)	1.97 (1.40)