HOME EXPOSURE TO MOLDS ALTERS BLOOD CELL CYTOKINE PRODUCTION

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Abstract

Persons with high (G-high) and low (G-low) airborne levels of (1→3)-β-D-glucan in their homes, as an indicator of mold exposure, were examined regarding baseline values for cytokine secretion from blood mononuclear cells (BMNC). All persons were then exposed during three hours to airborne, pure (1→3)-β-D-glucan (grifolan) and cytokines were again determined 24 hours after exposure. Exposure to saline was used as a reference.

The atopics in the G-high group had a larger secretion of TNFα in both unstimulated and *in vitro* LPS-stimulated BMNC, compared to the G-low groups. Non-atopic persons in the G-high group had a significantly higher baseline ratio IFNγ/IL-4, compared to the G-low group.

After exposure to (1→3)-β-D-glucan, the LPS induced TNFα secretion from BMNC was significantly lower among the atopics in the G-high group compared to the other groups. The exposure to (1→3)-β-D-glucan resulted in a further increased ratio IFNγ/IL-4 among the non-atopic in the G-high group but also the atopics in the G-high group showed a higher ratio compared to before exposure. The data suggest that mold exposure at home induces an inflammatory response and an altered reaction to (1→3)-β-D-glucan.

Introduction

Epidemiological and experimental data during the last decades suggest that mold exposure is an important agent for the development of a variety of symptoms related to indoor air. Almost 40 epidemiological investigations have demonstrated a relation between the presence of dampness and/or mold growth in buildings and respiratory as well as general symptoms. In the occupational environment, mold is a well-known risk factor for granulomatous pneumonitis (hypersensitivity pneumonitis).

Molds contain a variety of antigens and their capacity to induce an immunological IgE related response is well recognized [1]. It is increasingly realized, however, that the majority of symptoms reported in connection with mold exposure are not of an allergic origin but rather a non-specific airways inflammation [2]. In view of this, attention has in recent years focused on the inflammagenic properties of molds and particular substances therein. The most widely researched of these is (1→3)-β-D-glucan.

To elucidate the role of molds and especially (1→3)-β-D-glucan in the development of the inflammatory response, we studied persons living in houses with high and low levels of (1→3)-β-D-glucan. They were subdivided into atopic and non-atopic persons. The groups were compared regarding baseline values and regarding the reactions seen after inhalation of pure (1→3)-β-D-glucan. At last years Beltwide meeting, a report was presented showing a decreased number of cytotoxic T-cells in the blood of persons living in houses with increased levels of (1→3)-β-D-glucan, which could be indicative of an inflammation in the airways [3]. This year we will present data from determinations of cytokines from blood mononuclear cells.

Methods

Persons

The persons were recruited from a field study on moldy houses [4] and by advertising. Based on the measurements of (1→3)-β-D-glucan in their homes, two groups were identified – 20 persons with an average level of 1.2 ng/m^3 (G-low) and 15 persons with an average level of 9.6 ng/m^3 (G-high). Persons between 19 and 65 years of age, without cardiovascular diseases, asthma or other chronic inflammatory disease were invited to participate. Table I shows the number of persons and the characteristics of the two groups.

Exposure

The exposure to (1→3)-β-D-glucan was performed in a chamber, 2x3.5x2.45 m, furnished as a sitting room. One to three persons were exposed on the same occasion. Each subject was exposed to (1→3)-β-D-glucan in saline and to saline alone on two randomized occasions. For the (1→3)-β-D-glucan exposures, grifolan was used. This is a water-nonsoluble (1→3)-β-D-glucan extracted from *Grifolanum commune* (generous gift from prof N Ohno, Tokyo, Japan). The grifolan was suspended in 0.3 N NaOH to render it soluble and diluted in saline. The solution was aerosolized and led into the exposure chamber through the ventilation system. Sampling of the air in the room was done with Isopore filters. The exposure time was three hours.

The amount of airborne (1→3)-β-D-glucan on the filters was determined using a specific Limulus lysate (Fungitec G Test®, Seikagaku Co, Tokyo, Japan) [5]. The filters were also analyzed for endotoxin using specific endotoxin lysate (Endospecy®). No endotoxin was detected on the filters. The average concentration of (1→3)-β-D-glucan in the chamber was 30 ng/m^3 (range 15.3 – 43.2 ng/m^3). The total dose of (1→3)-β-D-glucan inhaled during the three hours´ exposure was approximately 20 ng.

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Atopy

Atopy was determined by measuring 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).

Cytokine Production

Venus blood was collected before and 24 hours after exposure for cytokine analysis. Mononuclear cells (BMNC), $1x10^6$/ml, were isolated and suspended in AIM-V medium (Gibco BRL) supplemented with 2-mercaptorthanol, $4x10^{-5}$M, and incubated with or without phytohaemagglutinin (PHA, Murex Diagnostics Ltd, UK), final concentration 500 µg/ml or endotoxin 500 pg/ml (LPS lipopolysaccharide *Escherichia coli* 026:B6 Difco). PHA was used to study mainly lymphocyte-secreted cytokines (IFNγ and IL-4) and LPS to study mainly monocyte-secreted cytokines (TNFα and IL-10). After 48 hours incubation at 37°C in 5% CO_2, the supernatants were collected and stored at -25°C till cytokine analysis.

TNFα from unstimulated BMNC was analyzed using an ELISA kit with a sensitivity of 0.1 pg/ml (Quantikine high sensitive, R&D Systems). IFNγ and IL-4 from PHA stimulated BMNC and TNFα and IL-10 from LPS-stimulated BMNC were analyzed using PeliKine-compact™ human cytokine ELISA (CLB, The Netherlands).

Cytokine data before the (1→3)-ß-D-glucan exposure (baseline) were obtained from two blood samples taken one to two weeks apart.

Results

Baseline Characteristics

Table II shows the TNFα secretion in BMNC measured before the experimental exposure. In the unstimulated cells, the secretion was higher among the atopics in the G-high group but the difference to the other groups was not statistically significant.

In cells stimulated with LPS, the non-atopic G-high group had a lower secretion of TNFα as compared to the G-low non-atopic group (p=0.017). On the contrary, the atopic G-high group had a significantly higher TNFα secretion compared to the atopics in the G-low group (p=0.042).

The secretion of IL-4, IL-10 and IFNγ in non-stimulated BMNC was below the detection limit in most cases. In stimulated BMNC, there was a tendency to a higher IFNγ production among atopics as well as non-atopics in the G-high group compared to the G-low group. The variation was, however, rather large and the differences was not statistically significant. There were only small differences between the groups in IL-4 and IL-10 secretion.

Table III shows the ratio IFNγ/IL-4 in PHA stimulated cells before exposure. Among the non-atopics, the ratio was significantly higher in the G-high group than in the G-low group (p=0.009). There was no difference between the atopics in the G-low and G-high groups.

Exposure Effects

Inhalation of saline or (1→3)-β-D-glucan did not affect the baseline secretion of any of the cytokines in unstimulated BMNC.

The LPS induced secretion of TNFα is shown in Table IV. There were only small differences between the TNFα secretion before and after exposure to saline with a tendency to an increased secretion among the G-high groups. After exposure to (1→3)-ß-D-glucan, there was also a small increase in all groups except the G-high atopic group, which showed a marked reduction in the TNFα secretion. This difference is, however, based on an unusually high value before the exposure. The results are also shown as the mean change in LPS induced secretion of TNFα after the exposures in Table V. Inhalation of saline caused a slightly increased secretion of TNFα in three of the groups, suggesting a slight inflammatory effect. After inhalation of (1→3)-β-D-glucan, the LPS induced secretion of TNFα was significantly reduced among atopics in the G-high group compared to the G-low group (p=0.017).

Table VI shows the ratio IFNγ/IL-4 after inhalation of saline or (1→3)-β-D-glucan. Similar to the finding before exposure, the ratio was higher among the non-atopics in the G-high group as compared to the G-low group after both inhalations. In the G-high group, also the atopics had a slightly higher ratio after exposure to (1→3)-β-D-glucan as compared to saline exposure.

Discussion

The study is so far based on relatively few subjects, particularly atopics in the G-high group. There is thus a need to address certain shortcomings, which may affect the results.

The subjects chosen for the study were selected with regard to amount of (1→3)-β-D-glucan in their homes. The lysate used is highly specific for (1→3)-β-D-glucan and there is very little cross-reactivity for other glucans and no cross-reactivity for endotoxin [5]. Measurements of (1→3)-β-D-glucan represent an estimation of mold biomass (living and dead cells) [6]. (1→3)-β-D-glucan is also present in certain bacteria, pollen and vegetable materials such as cotton fibers [7]. There is no reason to believe that (1→3)- β-D-glucan from these other sources has a different biological activity. As

this study shows an effect after inhalation of pure (1→3)-ß-D-glucan, the amount of (1→3)-β-D-glucan found can thus be used as an indicator of risk, although (1→3)-ß-D-glucan may not be the only agent in the microbes causing the observed effects.

The measurements were made on one occasion and in two rooms in the house; it is thus not certain that they are representative for an exposure over a longer time period. Also there is little knowledge about when and where the relevant exposures in home environments take place; when sitting down or in bed or when moving around indoors. In spite of these possible shortcomings, previous studies have shown dose-response relationships between indoor levels of (1→3)-β-D-glucan and the extent of symptoms as well as lung function and inflammatory mediators [8].

The data show that an increased secretion of TNFα was present among persons living in homes with higher levels of (1→3)-β-D-glucan. The increased secretion was, however, only found among atopics. A tendency for an increased level of IFNγ was also found in the G-high group, in this case both for atopics and non-atopics. TNFα is secreted from macrophages and IFNγ from lymphocytes, suggesting that (1→3)-ß-D-glucan stimulates macrophages and lymphocytes through different pathways. A conclusion regarding an ongoing low-grade inflammation in the G-high group agrees with earlier findings where the lower number of cytotoxic CD8+ T-cells in blood could be interpreted as an enhanced trapping of these cells in the lung tissue or airway epithelium [3].

In BMNC from subjects who had inhaled saline, the *in vitro* LPS stimulated a slight increase in TNFα secretion. However, in BMNC from subjects who had inhaled glucan+saline, this cytokine secretion was not present among atopics in the G-high group. Even if this observation is based on a few individuals, an ability of (1→3)-ß-D-glucan to block TNFα secretion is in accordance with some previous data. In an *in vitro* test, pretreatment of BMNC with (1→3)-β-D-glucan markedly reduced the LPS induced production of TNFα [9,10]. In guinea pigs, exposure to endotoxin induced a migration of neutrophils into the airways, while preexposure to (1→3)-β-D-glucan decreased this neutrophil influx [11]. In another experiment, the adjuvant effect of endotoxin on the formation of albumin antibodies was abolished by (1→3)-β-D-glucan [12]. In the study presented here, inhalation of (1→3)-β-D-glucan thus induced an alteration of the normal inflammatory reaction to saline but only among those who were atopic.

Inhalation of (1→3)-β-D-glucan also caused an increase in the secretion of IFNγ among those exposed to molds in their homes, irrespectively of their atopic status. The capacity of (1→3)-β-D-glucan to block the LPS induced secretion of TNFα was confined to atopics. As the macrophage is the main target cell for (1→3)-β-D-glucan, it could imply that the regulation of macrophage TNFα secretion is influenced by the atopic status of the individual.

The clinical consequences of these alterations of cytokine secretion are unknown. Conceivably an increase baseline as well as increased *in vitro* induced secretion of TNFα reflects an ongoing inflammation, in this case probably in the airways. This status and the susceptibility to inhalation of (1→3)-β-D-glucan was, in this study confined to atopics. A previous study has shown that atopic children in a school with high levels of (1→3)-β-D-glucan had a higher extent of upper respiratory symptoms, compared to non-atopic children in the same school or atopic children in a school with low levels [13].

Regarding cytokine secretion patterns, much attention has been paid to the concept of Th1/Th2 type cells [14]. This offers an interesting model to assess the consequences of altered cytokine secretion patterns. In the present study, the ratio IFNγ/IL-4 was higher among non-atopics in the G-high group as compared to the G-low group. The G-high atopics had a ratio close to the G-low atopics as well as the G-low non-atopics. The lower ratio among the atopics, compared to non-atopics, in homes with high levels of (1→3)-β-D-glucan suggests a Th2 dominating lymphocyte activity in this group, which results in depressed IFNγ production.

Conclusion

The results suggest a relation between living in homes with higher levels of glucan (indicator for molds) and effects on the inflammatory and immunological system. Indications of an ongoing inflammation were assessed through an increased TNFα secretion from blood cells in the atopics and an increased ratio IFNγ/IL-4 in the non-atopics.

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Table I. The results from determinations of (1→3)-β-D-glucan at home (ng/m^3, average from 4 filters) and basic characteristics of the subjects. G-low = in homes with low and G-high = homes with high levels of glucan.

	G-high	**G-low**
n	20	15
Glucan in the home		
- mean	1.2	9.6
- range	0.2 – 2.7	4.2 – 51.8
Females	10	5
Atopic	8	5
Age		
- mean	45	42
- range	23 – 66	19 - 59

Table II. TNFα production in blood mononuclear cells. Mean from two determinations.

	N	**mean**	**SD**	**p**
Unstimulated				
G-low nonatop	12	7.44	3.43	
G-high nonatop	8	8.75	3.49	
G-low atop	8	7.99	4.30	
G-high atop	4	15.38	8.95	NS
LPS stimulated				
G-low nonatop	12	142	85.9	
G-high nonatop	8	64.6*	32.8	p=0.017
G-low atop	8	95.4	90.1	
G-high atop	4	258**	158	p =0.042

* Significantly different from G-low non-atop
****** Significantly different from G-low non-atop

Table III. The ratio IFNγ / IL-4 in PHA stimulated cells before exposure. Mean from two determinations.

	n	**IFNγ/IL-4**	**SD**	**p**
G-low nonatop	12	158	156	
G-high nonatop	8	512*	520	0.009
G-low atop	8	270	185	
G-high atop	4	271	209	

* Significantly different from G-low non-atop

Table IV. TNFα production in LPS-stimulated cells. after exposure.

	n	**TNFα**	**SD**
Before NACl exposure			
G-low atop	8	135	151
G-high atop	4	140	66
G-low nonatop	12	156	96.1
G-high nonatop	8	72.0	39.2
After NaCl			
G-low atop	8	118	119
G-high atop	4	173	106
G-low nonatop	12	185	114
G-high nonatop	8	115	116
Before glucan exposure			
G-low atop	8	55.3	38.3
G-high atop	4	377	278
G-low nonatop	12	128	85.4
G-high nonatop	8	57.0	33.2
After glucan exposure			
G-low atop	8	80.6	47.8
G-high atop	4	160	69
G-low nonatop	12	155	134
G-high nonatop	8	61.6	62.7

Table V. Change in TNFα production (pg/ml medium) in LPS-stimulated cells after exposure.

	N	Δ NaCl	Δ glucan
G-low nonatop	12	28.5 (63.4)	27.7 (102)
G-high nonatop	8	43.3 (87.9)	4.6 (47.6)
G-low atop	8	-17.9 (88.5)	25.3 (26.0)
G-high atop (p=0.017)	4	32.9 (77.3)	- 217 (268)*

* Significantly different from G-low non-atop

Table VI. The ratio IFNγ / IL-4 in PHA stimulated cells after exposure.

	n	IFNγ/IL-4	SD	p
After NaCl				
G-low nonatop	12	165	230	
G-high nonatop	8	505	768	
G-low atop	8	227	154	
G-high atop	4	155	164	
After Glucan				
G-low nonatop	12	145	192	
G-high nonatop	8	638*	845	0.01
G-low atop	8	205	170	
G-high atop	4	334	294	

* Significantly different from G-low non-atop