INHALATION OF (1→3)-β-D-GLUCAN (GRIFOLAN) CAUSES AIRWAY EOSINOPHILIA

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Abstract

Guinea pigs were exposed to an aerosol of grifolan (300 µg/m^3) 4 hours daily for five weeks. A lung lavage was performed and the number of inflammatory cells counted. Histological sections were prepared from the lung and the trachea. There was a higher number of eosinophils in the lung lavage from exposed animals as compared to controls (1.6(0.8) million cells/lung vs 0.8(0.6), $p<0.01$). In the airway epithelium, the number of eosinophils was also larger (68.6(13.6) vs 36.8(10.6), $p<0.001$). There was no increase in the number of neutrophils in the lavage. The results further support earlier findings that (1→3)-ß-D-glucan causes an inflammatory response in the airways which is different to the marked neutrophilia caused by endotoxin.

Introduction

The effects of (1→3)-ß-D-glucan on inflammatory and immune competent cells in general are well documented based on numerous *in vitro* and *in vivo* animal models [Di Luzio 1985; Williams 1997]. Water soluble as well as water nonsoluble (1→3)-ß-D-glucan is present in a variety of organic dusts such as dust from cotton, wood and compost. The origins of (1→3)-ß-D-glucan in these dusts are microbes and vegetable materials.

Human exposures in the above environments is by inhalation. In comparison to the abundance of data regarding the toxic effects of (1→3)-ß-D-glucan administered by injection or from *in vitro* models, the data regarding the effect of inhalation is limited. Previous reports from our laboratory have demonstrated that an acute inhalation of (1→3)-ß-D-glucan (curdlan) does not cause an invasion of neutrophils in the airways as is the case after exposure to endotoxin [Fogelmark *et al*. 1994]. After five weeks exposure, there was an increase in the number of neutrophils in the airways if endotoxin was administered together with (1→3)-ß-D-glucan, but not after exposure to (1→3)-ß-D-glucan only [Fogelmark *et al*. 1994]. In other chronic inhalation experiments, the adjuvant effect of endotoxin on the antibody formation of inhaled ovalbumin, was abolished by the simultaneous inhalation of (1→3)-ß-D-glucan [Rylander and Holt 1998].

The available data suggest that inhaled (1→3)-ß-D-glucan initiates an inflammatory response that is different from that induced by inhaled endotoxin. To further explore this phenomenon using other kinds of (1→3)-ß-D-glucan , experiments were undertaken to evaluate the inflammatory response in the lung after a prolonged exposure to grifolan.

Material and Methods

Animals

Male and female guinea pigs with an initial weight of about 700 g were used in the experiment. They were obtained from our own breeding colony. All animals were kept in cages supplied with filtered air at a slight overpressure. Food and water were supplied *ad lib*. The same proportion of females and males was kept in each exposure group. The animals were without signs of latent infections in the airways, as evaluated by a low number of neutrophil leukocytes in the lavage fluid of unexposed animals.

(1→3)-ß-D-Glucan Exposure

For the (1→3)-ß-D-glucan exposures, we used a water insoluble form (grifolan, kindly supplied by Dr. N. Ohno, Tokyo). It was suspended in 0.3 N NaOH and diluted with distilled water to a concentration of 100 μg/ml. An aerosol was generated using a Collison atomizer [Rylander 1968] and the animals were exposed in a small exposure chamber, in a continuous flow system. The animals were exposed for four hours a day, five days per week during five weeks.

The amount of (1→3)-ß-D-glucan in the chamber air was 300 μg/m^3, yielding an estimated dose of about 15 ng/animal/day, using previously published data on ventilation in guinea pigs [Schreider and Hutchens 1979].

Endotoxin Exposure

For the endotoxin exposure, we used a solution in water containing 25 μg/ml of lipopolysaccharide (LPS, *Escherichia coli* 026 B6, Difco lab*)* in a similar exposure equipment. The animals were exposed for 40 minutes a day prior to the (1→3)-ß-D-glucan exposure, five days/week for five weeks. The amount of LPS in the chambers was 75 μg/m^3, yielding a dose of about 4 ng/animal and day.

Lung Lavage Cells (LLC)

At 24 hours after the last exposure, the guinea pigs were deeply anesthetized with an i.p. injection of sodium pentobarbital (120 mg/ml). The thoracic cavity was opened and the lung vascular bed was perfused with chilled Dulbecco's PBS without Mg^{2+} and Ca^{2+} (DPBS, NordCell, Sweden). Perfusion was performed until the lungs were clear white, at which point the aorta was tied off.

The right lung was used for counting the number of inflammatory cells in the lavage fluid. The left lung was used for histological examinations and its main bronchus was tied off before the lavage procedure.

The right lung was subject to lung lavage *in situ*. A body weight of 700-800 g corresponded to 70 ml of saline and for each additional 100 g, the lavage volume was increased with

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10 ml. The saline was divided into 10 aliquots, which were slowly injected into the lung lobes via a cannula in the trachea. After each instillation, the fluid was withdrawn and collected in 50 ml centrifuge tubes placed on ice. The lavage fluid was centrifuged at 350 g for 10 minutes, the cell pellet was collected, resuspended and counted under a microscope. A cell differentiation was done using May-Grünewald-Giemsa stain. These cells are referred to as lung lavage cells.

Histology

The trachea was cut off below the larynx and together with the left lung removed and fixed by slowly injecting 4% buffered formaldehyde into the trachea. After dehydration and embedding, the trachea was sectioned longitudinally, including the first bifurcation and stained with Weighert haematoxylin - eosin.

The degree of eosinophil infiltration in the epithelial layer was calculated from three different levels in the trachea. One level selected right below the bifurcation, another near the fifth gristle ring and third level near the seventh gristle ring.

Statistics

The mean numbers of each cell type in the LLC preparation were calculated for each group of animals and the statistical significance of differences between groups was evaluated using two sided Student's t-test.

Results

The numbers of lung lavage cells is shown in Table 1.

It is seen that in animals exposed to LPS, the number of all cells was higher as compared to controls.

In animals exposed to grifolan, the number of eosinophils was slightly higher than among controls ($p<0.02$).

In animals exposed both to LPS and grifolan the number of lymphocytes was higher than among animals exposed to LPS or grifolan only ($p<0.001$). The number of neutrophils was slightly lower than after exposure to LPS only (NS). The number of eosinophils was higher than in animals exposed either to LPS or grifolan only.

The numbers of eosinophils in the airway epithelium are shown in Table 2.

The table shows that the number of eosinophils among animals exposed to grifolan was higher than among control animals ($p<0.001$). In animals exposed to LPS, the number was slightly lower (NS) and in animals exposed to grifolan and LPS, the number was slightly lower than for grifolan only (NS).

Comments

Regarding dose levels, the animals in this study exposed by inhalation received doses far inferior to those used in *in vitro* experiments. A calculation of the amount of (1→3)-ß-D-glucan deposited in the lung and with reference to the number of macrophages in the lung [Snella 1986] demonstrates that the dose corresponds to about 1.5 ng/10^6 macrophages in *in vitro* conditions. Against that background, the differences previously reported between the reactions occurring after *in vitro* and inhalation experiments may be a matter of dose. It cannot be excluded, however, that the differences reflect different reactions in different compartments of the lung.

The exposure to grifolan caused no increase in any of the inflammatory cells in lung lavage. This is consistent with previously reported studies using curdlan [Fogelmark *et al* 1994; Sjöstrand and Rylander 1997; Rylander and Holt 1998]. This shows that the mechanism through which (1→3)-ß-D-glucan exerts its actions on pulmonary cells is different to LPS, which causes a marked infiltration of all kinds of inflammatory cells as seen here and also reported previously [Snella and Rylander 1982; Fogelmark et al 1994; Rylander and Holt 1998].

The results from the study show that a 5 weeks exposure to (1→3)-ß-D-glucan induced an increase in the number of eosinophils in the airway epithelium. This is generally regarded as a phenomenon related to allergic asthma. There is increasing evidence, however, that the inflammatory reactions induced by antigens are of a non-specific nature and that they are mimicked by exposure to agents without an IgE induction. Experience from field studies on persons exposed to elevated levels of (1→3)-ß-D-glucan in their homes, suggests that there is an increase in inflammatory markers related to the exposure to (1→3)-ß-D-glucan and that there is an increased risk for atopy among persons exposed to high levels of (1→3)-ß-D-glucan in their homes [Thorn and Rylander 1998].

The reason why LPS decreased the epithelial eosinophil response to (1→3)-ß-D-glucan is not known. The effect is, however, in principle consistent with a previous study, where an ovalbumin induced eosinophilia in lung lavage was decreased by a simultaneous exposure to LPS [Rylander and Holt 1998].

A possible mechanisms behind the accumulation of eosinophils is an effect of (1→3)-ß-D-glucan on macrophages. It has previously been shown that (1→3)-ß-D-glucan decreases the neutrophil response induced by an acute exposure to LPS [Fogelmark *et al* 1992; Fogelmark *et al* 1997). This could be due to a decreased secretion of TNFα - major neutrophil chemotactic agent secreted by macrophages over T cells, this could lead to an increased secretion of IL-5 from T cells, stimulating migration of eosinophils in the ling tissue [Thepen *et al* 1992].

In conclusion, the results from the present study support previous data that (1→3)-ß-D-glucan induces a different response among lung inflammatory cells as compared to LPS. The data further suggest that (1→3)-ß-D-glucan could cause an eosinophil dominated inflammatory response in the airway epithelium, probably through a defect in macrophage control of T cell function. Finally, the results illustrate the need to evaluate different compartments of the lung . airway spaces, epithelium and lung parenchyma - in order to understand the complicated cell dynamics which constitute the response to inhaled agents.

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Table 1. Lung lavage cells (10^6 cells/g lung tissue) in guinea pigs exposed to grifolan, LPS and grifolan + LPS . Macrophages=M, Lymphocytes=L, Neutrophils=N, Eosinophils=E.

Exposure	n	M	L	N	E
LPS	10	7.9(4.5)	0.2(0.1)	1.0(0.5)	1.2(0.6)
Grifolan	10	1.8(0.6)	0.1(0.1)	0.02(0.02)	1.6(0.8)
Grifolan+LPS	10	6.4(2.3)	0.8(0.9)	0.7(0.3)	2.6(1.0)

Table 2. Eosinophils in airways epithelium below bifurcation. Cells/field, 40x.

Exposure	n	Eosinophils
Control	10	36.8(10.6)
LPS	10	22.7(12.7)
Grifolan	9	68.6(13.6)
Grifolan+LPS	10	32.2(20.5)