

ORGANIC DUST INDUCED PULMONARY DISEASE – THE ROLE OF MOULD DERIVED B-GLUCAN

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Rylander R: Organic dust induced pulmonary disease – the role of mould derived β -glucan. *Ann Agric Environ Med* 2010, **17**, 9–13.

Abstract: The objective of the study was to evaluate the role of the mould cell wall agent β -glucan in environmentally related pulmonary disease. All published articles where β -glucan was administered by the airways, either as intratracheal injection or as inhalation were utilised as data sources. Data reporting consisted of analysis of data reports concerning the effects of β -glucan on the immune system on the cellular level, particularly on the aggregation of inflammatory cells or production of inflammatory cytokines. High doses of soluble and particulate β -glucan cause an inflammatory response characterized by cytokine activation and neutrophil invasion in the lung tissue. At lower doses, closer to environmental exposure levels, the predominant effect is an influence on the response to antigens, the reactivity of eosinophils and other Th2 driven immune responses. It is concluded that, β -glucan can induce Th1 as well as Th2 driven immune responses. The pathology of atopy/allergy, hypersensitivity pneumonitis, and toxic pneumonitis might be induced by exposure to β -glucan. Measurements of β -glucan in different environments are useful for risk control and prevention.

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Key words: β -glucan, endotoxin, mould, inhalation, inflammation.

INTRODUCTION

Organic dust is dust of vegetable, animal, and microbial origin [18]. It is found in a large number of occupational and general environments, such as in industries handling organic material, the farming environment, garbage handling, and indoors, particularly in dusty or mouldy buildings. Exposure to organic dust causes an increased risk of a variety of pulmonary disease such as airways inflammation, toxic pneumonitis, hypersensitivity pneumonitis and asthma.

Agents of a microbial origin play an important role for this disease risk. During the 1980s and 1990s it became apparent that endotoxin from Gram-negative bacteria could cause an inflammatory response in the airways, mediated through CD14 and Toll Like Receptor 4 (TLR-4) [19, 20, 31]. Apart from endotoxin, organic dust contains a variety of other microbial cell wall agents (MCWA) originating from Gram-positive bacteria and moulds. Mould exposure

has been related to respiratory disease and atopy/allergy in a number of studies, and there is a well known relation between exposure to mouldy hay and hypersensitivity pneumonitis [2, 17, 26, 28, 42].

The cell wall of moulds contains a skeleton of a particular polyglucose compound – glucan. A general overview of the toxicology of glucan has been presented previously [34]. The purpose of this review is to evaluate the role of glucan in organic dust induced pulmonary disease. Although it is clear that most agents in the environment work in conjunction, it is justified to focus on one single agent and its characteristics, as this knowledge will facilitate the understanding of both the role of glucan as such and that of mould cells.

WHAT IS GLUCAN?

Structure and cellular activity. Glucan is a group of glucose polymers that includes cellulose. They are present

in a variety of forms, characterized by the length of the polymer, the degree of branching and water solubility [16]. Their biological activity is strongly related to the binding characteristics of the polyglucose chains in the molecule. β -1,4-glucan (cellulose) is relatively innocuous, whereas β -1,3-glucan has important immunological properties [10]. Examples of β -1,3-glucan that have been extensively used in experimental work are grifolan from *Grifola frondosa*, curdlan from *Alcaligenes fecalis*, and laminan from *Laminaria digitata*. Zymosan from *Saccharomyces cerevisiae* is not a pure β -1,3-glucan but also contains mannan and chitin, which have immunomodulating properties in themselves. In spite of this, the effect of zymosan has been included in this review.

β -1,3-glucan is attached to cells through the Dectin-1 receptor which is present in myeloid cells such as macrophages, dendritic cells, and neutrophils [32]. The complement receptor 3 may also play a role. Dectin-1 induces activation of NF κ B which initiates the production of several inflammatory cytokines.

β -1,3-glucan in the environment. A number of studies have measured the amount of β -1,3-glucan in occupational and general environments. The analysis methods used have been either a highly specific biological method – the *Limulus* assay with a glucan specific lysate – or an immune method using the ELISA technique. These methods measure different endpoints – for the *Limulus* assay the capacity of freely exposed β -1,3-glucan on the mould cell surface or on cell fragments, whereas the ELISA assay detects antibodies against specific mould species that have been used for the manufacture of the immune reagent. The *Limulus* assay is much more sensitive although it has been demonstrated that it does not “recognize” all available β -glucan, only the portion that is directly exposed to the exterior, akin to the findings on the biological effect of endotoxin and the *Limulus* test efficiency [21, 35]. No extensive comparisons between the methods have been published. Airborne β -1,3-glucan has been measured in a variety of environments and examples of values found are given in Table 1.

The table does not present an inventory of the large number of measurements that have been published, but rather

Table 1. Examples of levels of airborne β -1,3-glucan in different environments.

Site	Range
<i>Limulus</i> test	
Homes	5.9–57.9 ng/m ³
Waste collection	10.8–36.4 ng/m ³
School with mould	9.2–27.4 ng/m ³
Immunological test	
Composting	0.5–3.0 μ g/m ³
Waste handling	2.0–16.0 μ g/m ³
Swine confinement	0–38.5 μ g/m ³

examples to illustrate the difference in values obtained between the *Limulus* and the immunological methods and the variability in levels between different sites. There is also a large variation between different research groups (ng vs μ g) reflecting methodological difficulties. No extensive inter-laboratory comparisons have been made. Apart from variations caused by methodological differences, airborne levels are determined by a variety of factors such as activity during sampling, degree of contamination and efficiency of the sampling equipment.

Regarding a human exposure dose, precise calculations are difficult. Theoretically an airborne value of one microgram/m³, presuming a 50% deposition in the lungs, and an eight hour work day, would yield a total dose of 1.5 μ g per person or 20 ng/kg body weight (bw). It is likely, however, that much higher doses could be present in particularly dusty environments or environments with a high amount of moulds, such as when handling mouldy hay or cleaning mouldy buildings.

There are some 6,000 publications on the biological properties and effects of β -1,3-glucan. A large number of the studies relate to the injection of high doses in the research to evaluate the anti-infectious and anti-cancer effects of the compound. This review will only deal with studies where the effects of β -glucan were studied after administration via the airways. This has been done either by intra-tracheal injections or after inhalation. Data are available both from animal and human experiments. Data from epidemiological studies are less relevant in this context as the exposure in such studies is always mixed, comprising several MCWA such as endotoxin, chitin, proteases, lipoteichoic acid, peptidoglycans, mannan, and enzymes. Precise conclusions regarding the effects of β -1,3-glucan thus cannot be drawn from such studies.

ANIMAL STUDIES

Intra-tracheal injections. An experimental model where rats were exposed by intra-tracheal instillations of zymosan was first published in 2001 [36]. The dose levels were up to 5 mg/kg bw. There were dose-related increases in a variety of indicators of pulmonary inflammation such as number of polymorphonuclear leukocytes, amounts of albumin and lactic dehydrogenase (LDH) in the bronchi, and nitric oxide production of alveolar macrophages. The interaction between β -1,3-glucan and endotoxin was studied in an experiment where endotoxin was administered before or after β -glucan (2.5 mg/kg bw) [37]. If endotoxin was given one day after the β -1,3-glucan exposure, the production of LDH and TNF α was significantly lower. Endotoxin given simultaneously with β -1,3-glucan or before did not influence any of the inflammatory effects. In a subsequent study, it was demonstrated that particulate β -1,3-glucan induced a more severe pulmonary inflammation than a water soluble form at a dose level of around 2 mg/kg bw [38]. The effects on the immune response were evaluated

at day 1 up to day 4 after exposure to zymosan (2.5 mg/kg bw) [39]. The increase in neutrophils had disappeared at day 4, whereas lymphocytes recovered from lymph nodes reached a maximum on day 6. There was also an increase in the number of inflammatory cytokines on day 1 (TNF α , interleukin (IL)6, IL-10, and IL-12).

The pulmonary clearance of *Listeria monocytogenes* was studied after acute and repeated exposures to zymosan (0.6–2.5 mg/kg bw). After an acute exposure there was an increase in the clearance [40], but after repeated exposures (4 doses over a 4 week period) the clearance of *Listeria* was decreased [41]. There was a down-regulation of the Th1 driven response with an increase of Th2 characteristics, based on measurements of different categories of CD cells.

Mice were used in another animal model with intra-tracheal administration. The instillation of a soluble β -1,3-glucan from *Candida albicans* (25–100 μ g/animal) induced a neutrophil and eosinophil inflammation, with increased local expression of a variety of inflammatory cytokines (IL-1 β , IL-6, macrophage proteins and RANTES) [11]. Similar responses were seen with the same model using soluble β -1,3-glucan from *Aspergillus* (12.5–100 μ g/animal) [12]. In an experiment with repeated exposures to ovalbumin (OVA), the addition of a soluble *Candida albicans* β -1,3-glucan (12.5–25 μ g/animal) enhanced the OVA induced eosinophilia and the expression of Th2 cytokines and IL-17A [13].

Inhalation. A number of experiments have been reported where guinea-pigs were exposed to an aerosol of different β -1,3-glucans in a continuous air flow exposure chamber. An acute exposure to non-soluble curdlan, schizophyllan, and zymosan (300 μ g/m³ for 40 minutes) had no effect on the number of neutrophils in the airways, but a tendency to a decreased number of macrophages and lymphocytes [8]. When curdlan was solubilised using NaOH, there was a large increase in the number of neutrophils. In another experiment, water non-soluble curdlan, schizophyllan, and pullulan were used and none produced an inflammatory response [23].

The effect of repeated exposures has been evaluated in several studies. The effect of β -1,3-glucan on the immune response induced by OVA was explored in a 5 week exposure study (300 μ g/m³) [24]. When OVA was administered together with endotoxin, there was a significant increase in the levels of OVA IgG antibodies. This response was completely abolished in animals also exposed to β -glucan. In an experiment with repeated inhalations of particulate curdlan (30 μ g/m³) and endotoxin over a 5 week period, there were only small changes in the number of free lung cells after exposure to endotoxin (reflecting the adaptation to this agent) or β -1,3-glucan [7]. When the agents were given together, there was a significant increase in the number of macrophages, lymphocytes and neutrophils, compared to when the agents were given alone. In a study using soluble grifolan (30 μ g/m³), guinea-pigs were exposed for 5 weeks

daily (dose 1.5 ng/animal) resulting in an increase of the number of eosinophils and lymphocytes in lung lavage and lung tissue [9].

A number of endpoints were used in experiments where mice were exposed to an aerosol of soluble grifolan (10 μ g/m³) [33]. The exposure potentiated an allergen induced infiltration of eosinophils and suppressed the down-regulation of an antigen induced IgE response. There was also an up-regulation of IL-10 and a down-regulation of IL-12 mRNA. Mice sensitized to fungal extracts by ip injection were exposed to an aerosol of water soluble grifolan (120 μ g/m³) 7 times during a 21 day period [15]. Occasional slight irritation was observed but there were no effects on the histology of the nasal cavity or IgE levels.

HUMAN STUDIES

In comparison to animal studies, human exposure experiments using β -1,3-glucan have been limited to acute exposures using low doses, comparative to those in the environment. Subjects with a previous history of airway reactivity were compared to subjects without symptoms in an inhalation study using endotoxin (0.2 ng/m³) and particulate curdlan (210 ng/m³), and airway responsiveness evaluated with the methacholine test [25]. The responsiveness was unaffected by the β -1,3-glucan exposure but subjective throat irritation was slightly increased in the group with previous airway symptoms, compared to saline control exposure. In another study, subjects from homes with high or low levels of moulds inhaled soluble grifolan (28 ng/m³) during 3 hours, and the endotoxin induced secretion of cytokines from peripheral blood mononuclear cells (PBMC) was measured [1]. In the group with high mould levels at home, the secretion of TNF α was lower after inhalation of β -1,3-glucan and the number of blood lymphocytes was increased. One study comprised healthy volunteers who inhaled 125 ng of soluble grifolan [30]. The TNF α -secretion from PBMC was decreased after β -1,3-glucan inhalation, compared to after inhalation of saline. There was no effect on several markers of inflammation or pulmonary function. These results on a decreased secretion of TNF α are supported by a study where β -1,3-glucan was administered by injection [27]. The application of 5 or 50 ng of soluble grifolan into the eyes of persons with pollen reactivity did not produce any increase in the number of eosinophils, or on the concentration of eotaxin in nasal lavage [3].

SYNTHESIS

Cell reactions. The data reviewed suggest that β -1,3-glucan has the potential to induce a variety of important effects on the immune system. The effects described are related both to Th1 and Th2 driven responses. At low doses there was no inflammatory reaction in terms of a neutrophil invasion and inflammatory cytokine triggering, but eosinophilia and antigen related effects were present. At high

dose levels there was a pronounced inflammatory response with activation of several cytokines, suggesting a Th1 driven response.

The relation between environmental exposure levels as depicted in Table 1 and the dose in the experimental studies is difficult to ascertain. There are, however, some limitations in environmental measures using the *Limulus* assay and there is no knowledge on particle sizes which is important for penetration into and deposition in the lungs. Exposure to 300 $\mu\text{g}/\text{m}^3$ – the highest dose in the inhalation experiments, and presuming a 50% deposition – would yield a dose of about 5 μg in a guinea-pig. Taking the highest measured environmental value of around 1 $\mu\text{g}/\text{m}^3$, the human dose – again accounting for a 50% deposition – would be around 3 μg during an 8 hour exposure. This is in contrast to experiments with intratracheal installations where the dose levels were in the order of mg.

Based on the above assumptions, although very approximate, it can be suggested that Th2 driven immunomodulating effects, rather than the inflammatory effects, are the most important after low environmental exposures to β -1,3-glucan. Such doses would be found indoors in mouldy buildings or in workplaces with little or no agitation of the organic materials treated. A pronounced inflammatory effect would be present at high exposure levels, eg such as when organic material is agitated or when mouldy material is handled such as stacking of mouldy hay.

Diseases. The cell reactions demonstrated after exposure to β -1,3-glucan in small doses, suggest that it plays a role in the development of atopy and allergic disease. This hypothesis is supported by findings in epidemiological studies although such data cannot be used to confirm causality [17, 26, 28]. The β -glucan induced decreased secretion of inflammatory cytokines might also explain an increased frequency of respiratory infections often reported by persons living in homes with mould damage. A down-regulation of immune defence mechanisms may also lead to a late hypersensitivity reaction. This mechanism is involved in the mould induced disease hypersensitivity pneumonitis.

Regarding acute inflammatory reactions with a neutrophil invasion into the lung, release of inflammatory cytokines, and severe symptoms, the clinical case would be toxic pneumonitis (organic dust toxic syndrome) which can be found among subjects exposed to high levels of organic dust. The first account of this reaction in connection with mould exposure was published by Blackley [4]. He inhaled *Penicillium* spores and described his reaction as “The spores of the microscopic fungi, I have reason to believe, will ... generate symptoms not unlike those of hay fever in some respects but differing in others – being much more like those of ordinary influenza”. Toxic pneumonitis could thus be due to a very high dose of β -1,3-glucan, but also to bacterial endotoxin [20].

CONCLUSION

Knowledge about the negative effects of agents in the environment is never complete. For β -1,3-glucan there is, however, enough information to suggest that it is an important agent for the development of pulmonary diseases, both of an inflammatory and an allergic nature. The risk can be assessed by measuring β -1,3-glucan, although precise standards are not available at present, and decreased by lowering the exposure, either through improved work practices or by cleaning of moulds.

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