Health risks by microbial cell wall agents indoors

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ABSTRACT
This presentation will review two major microbial cell wall agents (MCWA)—endotoxin and (1→3)-β-D-glucan—concerning their toxic effects after inhalation and their role for the development of symptoms among persons in buildings with humidity problem. The inflammatory and immune suppressive effects and field studies on the relation between exposures to these MCWA and effects among exposed persons will be discussed. It is concluded that both agents play an important role for symptoms and disease among persons living in humid buildings.

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

INTRODUCTION

A major building characteristic, which in many studies has been related to respiratory disease in general and symptoms of asthma in particular, is home dampness. Home dampness is associated with an increased risk for growth of microorganisms. These contain a variety of specific agents on or in their cell wall (microbial cell wall agents or MCWA). This presentation will discuss two important MCWA in indoor air—endotoxin from Gram-negative bacteria and (1→3)-β-D-glucan from moulds. It will describe how exposure to these agents may cause an inflammatory response in the airways and review the results from field studies, where the relation has been studied between exposure to these agents and the risk for atopy and asthma.

MICROBIAL CELL WALL AGENTS—EXPOSURES AND EFFECTS

Endotoxin

Gram-negative bacteria are ubiquitous in our environment—species of Klebsiella, Pseudomonas and Enterobacter are found in the soil, on vegetation and in water. They carry a specific compound on their cell surfaces that is a combination of polysaccharide chains, a lipid A unit and a connecting core molecule. This substance is referred to as ‘endotoxin’ or ‘lipopolysaccharide’ (LPS).

Endotoxins are present in indoor environments and their presence in house dust was first described some 25 years ago. Data are now available from a number of studies around the world and several of these have evaluated factors that determine the amount of endotoxin found indoors. Examples of such factors are the presence of pets, living in a farm and storage of biological household waste.

The mechanisms behind the effects of endotoxin have been extensively studied. Inhalation of endotoxin will stimulate alveolar macrophages and epithelial cells to produce a variety of cytokines such as interleukin-1 (IL-1), IL-6, IL-8 and tumour necrosis factor alpha (TNFα). The cytokine profile corresponds to a Th1-like pattern. The cytokines activate a number of different cells in the body including hepatocytes that secrete C-reactive protein (CRP).

The release of cytokines in moderate amounts initiates beneficial inflammatory reactions, for example moderate fever, activation of defence cells and microbiocidal mechanisms and

acute phase reactions. Large amounts of these products, however, have harmful effects and cause cell damage and functional collapse.

After inhalation of endotoxin, a major cell reaction is a migration of neutrophil leukocytes from the blood into the lung parenchyma and later into the airways. This hallmark of endotoxin exposure has been used in a variety of animal and human studies, assessing the inflammatory potency of pure endotoxin and endotoxin-containing organic dusts. The inflammation in the airways can lead to a decrease in pulmonary function, measured as a decrease in the forced expiratory volume in 1 s (FEV_1) and increased airway responsiveness, as measured with methacholine or histamine challenges.

Due to the distribution of inflammatory cytokines from the lung, there may also be systemic symptoms in terms of joint pains, fatigue and headache and increases in body temperature.

Moulds

Moulds are ubiquitous in the environment and will grow on a variety of materials, provided that there is enough environmental or material humidity. The layman's term 'mould(s)' refers to growing colonies of a mixture of different species of fungi. The presence of specific species depends on the growth conditions, which are determined by a variety of factors such as characteristics of the surface, humidity and temperature.

The lung diseases, which develop after mould exposure, have cellular characteristics that are different from those caused by endotoxin-containing dusts. There is an effect on lymphocytes, probably mediated by macrophages, with alterations in numbers and function. It is thus of interest to evaluate the effect of MCWA present in the mould cells. The most widely researched of such agents is (1→3)-β-D-glucan, a polyglucose compound.

In *in vitro* models and after i.p. injection, (1→3)-β-D-glucan has been found to cause secretion of TNFα and other inflammatory cytokines. The effects after inhalation are different. In acute exposures, (1→3)-β-D-glucan will not elicit the type of inflammatory response that occurs after exposure to endotoxin. The exposure will, however, modulate the response to a simultaneous exposure or following exposure to other agents. As an example, mice were exposed to an aerosol with or without (1→3)-β-D-glucan and simultaneously sensitized with aerosolized OVA antigen. The OVA-specific IgE response was higher in (1→3)-β-D-glucan pre-exposed mice than those exposed to OVA only. Furthermore, it was also shown that (1→3)-β-D-glucan suppressed the antigen-induced IgE specific tolerance. (1→3)-β-D-glucan also potentiated the OVA-induced infiltration of eosinophils in the airways, which is generally regarded as an indicator of an airway allergic inflammation.

In studies on humans, persons were divided according to their levels of airborne (1→3)-β-D-glucan in their homes. They were exposed to an aerosol of saline or saline with (1→3)-β-D-glucan. After exposure to saline alone, there was a small increase in the production of TNFα in blood mononuclear cells, reflecting the slight inflammatory response to saline. This increase was not seen after inhalation of saline with (1→3)-β-D-glucan among persons with high levels of (1→3)-β-D-glucan in their homes. This blunting of an inflammatory response by (1→3)-β-D-glucan is similar to what has earlier been reported in animal models.

Experience from Field Studies

Regarding field studies on disease and the relation to different MCWA, some methodological questions are important. Most of the studies on relationship between microbes in indoor environments and airway symptoms have estimated the exposure as the number of viable bacteria and moulds. That is no longer considered an adequate measure of exposure as the inflammagenic and allergenic properties of the microbes are not related to viability. Better

estimates of exposure are determinations of MCWA such as endotoxin and (1→3)-β-D-glucan. These measures give information on the total cell biomass present (alive and dead cells).

There are an increasing number of studies in homes showing a relation between endotoxin exposure and respiratory symptoms. In real life conditions, however, moulds and Gram-negative bacteria often occur together and conclusions on causality for any one of these agents are difficult to draw. This requires studies in different environments, where both agents are measured and the relative amount of the two agents varies.

Some field studies have evaluated the relation between mould biomass exposure and atopy and symptoms among children. One investigation comprised two schools—one with mould problems and another without problems. The extent of respiratory symptoms was significantly higher in the school with higher levels of (1→3)-β-D-glucan (15.3 ng/m^3) as compared to the school with low levels (2.9 ng/m^3). Among atopic children, the extent of symptoms of dry cough, cough with phlegm and hoarseness was similar to the non-atopics in the low (1→3)-β-D-glucan school, but significantly higher than non-atopics in the high (1→3)-β-D-glucan school, suggesting that atopy predisposes for a more severe airways inflammation.

Another study investigated the relation between children's asthma and the amount of (1→3)-β-D-glucan, endotoxin and measures of house dust mite antigen in the dust from the children's dwellings. A significant relationship to variability in peak flow was found for (1→3)-β-D-glucan but not for the other agents.

Several studies have reported an increased risk of respiratory infections among children exposed to moulds indoors. An underlying mechanism may be a reduced defence against infectious agents, induced by (1→3)-β-D-glucan as discussed previously. Recent studies have found relationships between mould exposure and increase in total levels of IgE and a threshold value of 25 ng (1→3)-β-D-glucan/mg floor dust has been suggested.

Regarding endotoxin, a relation has been found between the amount of endotoxin in the dust and the severity of asthma. Among children, a study from Brazil reported a significant relationship between clinical asthma scores among children and levels of endotoxin in their homes. In a cohort of 499 infants, a relation was found between house dust endotoxin levels and wheeze among the children. The underlying effect in these studies is probably an airways inflammation induced by endotoxin and involving the kind of inflammatory response described previously.

In contrast to the negative effects of endotoxin on normal as well as asthmatic subjects, some data suggest that the inflammation induced by endotoxin might have beneficial effects on the risk for atopic sensitization. In a study among 61 infants, 9–24 months old with a high risk for sensitization, it was found that the risk for atopic sensitization was inversely related to the amount of endotoxin in house dust. Among children living on farms where the prevalence of atopic sensitization is known to be low, indoor endotoxin levels were higher than in a control group. In another study, the amount of endotoxin in the house dust was inversely related to the presence of symptoms of shortness of breath, skin rash and cough. These data suggest that a certain exposure to endotoxin, with the subsequent secretion of inflammatory cytokines, might be beneficial for the maturation of the immune system, suppressing the risk for atopic sensitisation. An important caveat is that the real-life exposure comprised a number of other agents as well.

SUMMARY

The data on endotoxin, moulds and (1→3)-β-D-glucan for symptoms experienced indoors, atopy and asthma can be summarized as follows:

1. Exposure to endotoxin causes an inflammatory response in the airways, characterized by a secretion of Th1 type cytokines and an invasion of neutrophils. Clinically, it is recognizable as an irritation in the airways due to inflammation and may be misdiagnosed as an allergic asthma.
2. (1→3)-β-D-glucan in the cell wall of moulds may affect the normal defence reactions to environmental agents with an increased risk for infections and development of atopy. The latter is not to the (1→3)-β-D-glucan itself but to environmental allergens in general.
3. In persons with an allergic asthma, due to usual environmental allergens, an endotoxin exposure will induce a more serious inflammatory response than among non-asthmatics.

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