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Something in the Air: Endotoxins and Glucans as Environmental Troublemakers*

R. S. YOUNG, A. M. JONES AND P. J. NICHOLLS

Division of Pharmacology, Welsh School of Pharmacy, Cardiff University of Wales, Redwood Building, King Edward VII Avenue, Cardiff CF1 3XF, UK

Abstract

This review provides a clear explanation of the current status of two common airborne contaminants, lipopolysaccharide and (1→3)-β-D-glucan, in the induction of indoor air-related disease.

A full description of the origin of these two products is given together with information of their structure and function. Details of the biochemical mechanisms by which they interact with human cells and the physiological consequences of these interactions are outlined.

Both compounds play a key role in the induction of airway inflammation and this paper highlights the environmental importance in the work place and home of these inhaled agents in terms of respiratory disease.

One of the current topics of interest in the scientific and popular press is the environment. Much publicity has surrounded increased incidences of diseases such as asthma and its possible link with air pollutants such as ozone and the oxides of nitrogen.

The two most important environments to man are our everyday environments; our work/school environment and our living/home environment. Two agents that are ever present in the air of these environments are endotoxin (also known as lipopolysaccharide) and glucan. These microbial products are present in the air at all times but appear in higher concentrations in work environments associated with organic dusts, such as cotton milling in the cotton industry. They pose a problem even in the office and home where humidifiers and dampness harbour Gram-negative bacteria and moulds. This presentation aims to explain the nature of these agents, examine the events that occur when they are inhaled, and define their importance in the induction of respiratory diseases.

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Correspondence: P. J. Nicholls, Division of Pharmacology, Welsh School of Pharmacy, Cardiff University of Wales, Redwood Building, King Edward VII Avenue, Cardiff CF1 3XF, UK

What are Endotoxin and Lipopolysaccharide (LPS)?

Definition

The terms lipopolysaccharide (LPS) and endotoxin are used to describe two natural products present in the outer cell membrane of all Gram-negative bacteria. Endotoxin may also be found in some blue-green algae. Organisms of this type are ubiquitous in the biosphere and hence endotoxin and lipo-polysaccharide can be regarded as common natural products (Young & Nicholls 1996).

The two terms essentially describe similar entities although they should not be used synonymously. Endotoxin is truly described as the toxin in the real-life situation i.e. it is the toxin present on whole bacteria and fragments of bacterial cell membranes. LPS is a chemically purified version of endotoxin. The essential difference between the two is that while the bacterial cell membrane proteins and other membrane components are present in the case of endotoxin, they are generally absent (or present to a lesser degree) in the case of LPS. The only exception to these definitions of endotoxin and LPS is in the use of the term 'control standard endotoxin'; this is essentially a chemically purified lipopolysaccharide which is used as a standard material for the limulus amoebocyte lysate (LAL) assay used in the quantification of endotoxins (Jacobs 1997).

It is important to realise that LPS is not a single homogenous molecule; rather it is a family of molecules. Each bacterial family typically produces LPSs which are similar in structure whilst bacteria of differing families tend to produce LPS with structural differences. In turn, each genus and species produces a structurally unique LPS (Jacobs 1997).

General structure and function

Endotoxins are major components of the external membrane of Gram-negative bacteria. They are essential for the physical organization and function of the outer membrane. The molecules are therefore important to the bacteria during processes such as growth and multiplication (Moran 1995). Endotoxins can be considered as complex lipopolysaccharide entities which consist of various oligosaccharide molecules connected by an anchor molecule to a lipid moiety. The anchor molecule is generally an eight-carbon sugar, 2-keto-3-deoxy-D-manno-octonic acid (KDO) and the lipid portion is known as lipid A (Figure 1). Endotoxins, and hence LPSs, can be regarded as essentially surface structures that play an important role in the interaction of Gram-negative bacteria with respect to their environment and their interaction with other organisms.

Lipopolysaccharides are immunoreactive surface antigens (O-antigens) of bacteria and accommodate binding sites for antibodies and for non-immunoglobulin serum factors (Reitschel & Brade 1992). This means that invading Gram-negative bacteria can be recognized by the host organism's immune system by interaction with LPS. In contrast to this, it has been demonstrated that certain endotoxin structures may prevent complement activation and the phagocytic intake of bacteria and aid the virulence of the invading organism (Reitschel & Brade 1992).

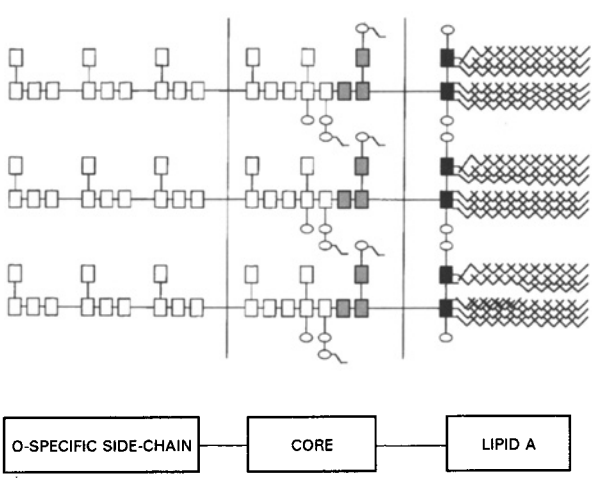

Figure 1. A schematic representation of the general structure of wild type lipopolysaccharides. Phosphate, ○; ethanolamine ⟍, monosaccharide units, □, ▩, ■; long chain fatty acids, ⋋⋌.

Structural regions

Lipopolysaccharide can be considered to consist of three general regions; the lipid A region, the inner and outer core or core regions, and the O-specific side chain.

The lipid A region, as its name suggests, contains a major lipid component. The other two regions are polysaccharide chains composed of varying sugar units.

Non-synthetic LPS contains many other components e.g. cations such as Ca^{2+}, Mg^{2+} and Na^{+} and amines such as spermine and putrescine (Galanos et al 1977). Studies using synthetic lipid A analogues and endotoxin preparations suggest that such components do not play a major role in the toxicological functioning of LPS and endotoxin. However, they may have an important role in the normal biochemical functioning of the bacterial cell.

How Do Endotoxins/lipopolysaccharides Interact with Cells?

LPS receptors and binding proteins

If the cells of the body are to respond to LPS, then sites of molecular recognition i.e. receptors, must exist for at least part of the LPS molecule. There is also the possibility that, in view of the amphiphilic and amphoteric nature of LPS, it may interact with cell membranes and cellular sites in a non-specific fashion. The effects of LPS are recognized as being mediated through three general types of interaction; binding to cell surface receptors (e.g. CD14 and CD11c-CD18 binding on leukocytes), binding to soluble LPS binding proteins (e.g. lipopolysaccharide binding protein (LBP) which modifies cellular interaction with LPS), and non-specific interactions with membranes.

The binding of lipolysaccharide to these receptors triggers a range of secondary molecular events including the activation of various protein kinases mediating protein phosphorylation, release of arachidonic acid and its many metabolites and the alteration of gene expression (Hewett & Roth 1993). The result of these molecular events is the production of mediators such as cytokines (e.g. tumour necrosis factor-α (TNF-α) and interleukin 1 (IL-1), arachidonic acid metabolites such as prostacyclin and thromboxane A_2 and nitric oxide (NO). All of these mediators are capable of inducing physiological changes such as inflammation, smooth muscle constriction and vasodilatation.

Other interactions

In addition to responses mediated via LPS receptors and binding proteins, it has been shown that LPS also has other effects including cytotoxicity (e.g. LPS has been shown to be directly toxic to cells in-vitro under certain conditions (Ryan & McAdam 1977)) and direct stimulation of mediator release. Examples of the latter include the release of arachidonic acid metabolites from macrophages via phospholipase A_2 (Mohri et al 1990) and alteration of gene expression in macrophages to induce the expression of TNFα (Zuckerman et al 1989).

Why is the Study and a Knowledge of the Actions of Endotoxin and Lipopolysaccharide Important to Man?

Despite having some unique chemical features and being formed in only Gram-negative bacteria and blue-green algae, the widespread nature of these organisms (especially the former) make endotoxin and LPS common features of many environments. Wherever there is potential for the growth and division of bacteria there is likely to be endotoxin in that environment, in either a free or bound form. Endotoxin and lipopolysaccharide may interact with many other organisms via several routes e.g. through inhalation, oral ingestion or via the circulatory system.

Endotoxin is also an environmental problem for the pharmaceutical industry in the manufacture of parenteral products. It is a potent pyrogen and the requirement for pyrogen-free water for parenteral dosage forms is a costly exercise.

Is LPS an Environmental Hazard? Why is a Study of Inhaled Endotoxins and Lipopolysaccharide Important? In What Type of Environments is Airborne Endotoxin Found?

Interest in the role of inhaled endotoxins in pulmonary disease has been focused on several areas especially in relation to pulmonary disease associated with working environments where airborne organic dusts are generated. In the search for an agent that is responsible for the induction of byssinosis, a pulmonary disease that is primarily associated with workers in the cotton-mill industry, the role of bacterial endotoxins has been subject to much investigation. More recently, endotoxin has come to prominence in the field of respiratory toxicology where extensive study has taken place into the home and other environments not usually associated with organic dusts and organic dust-induced disease (Rylander 1994a). Previously research efforts linked to working environments where organic dusts are present have focused on the effects of the whole dust on the airways. However, clinical symptoms have shown better correlation with elevated concentrations of airborne endotoxin than with the dust itself (Rylander & Vesterlund 1982; Rylander & Bergstrom 1993).

There are many working environments where high levels of endotoxin are believed to cause respiratory symptoms (Jacobs 1997) (Table 1). In cotton mills, workers are known to experience conditions such as fever, chills and malaise (Rylander 1994a, b), as well as more specific respiratory symptoms such as cough, wheeze and specific clinical conditions such as byssinosis. Another important response seen after exposure to LPS is the phenomenon of bronchial hyperresponsiveness as observed in asthma. This syndrome has been witnessed both in man (Rylander & Bergstrom 1993) and experimental animals (Young & Nicholls 1996). Byssinosis is characterized by a phenomenon known as 'Monday dyspnoea' – a cycle whereby workers feel the worst respiratory symptoms at the beginning of the week after a weekend break from the dusty environment followed by a general reduction of symptoms as the working week proceeds. The cycle is repeated in subsequent working weeks. Symptoms include wheezing, chest tightness and shortness of breath accompanied by changes in lung function (Nicholls 1992). These symptoms may progress to the full byssinotic state which involves severe respiratory disability (Nicholls 1992). Variable worksite con-

Table 1. Examples of environments associated with exposure to endotoxin.

Agricultural environments
Animal confinement buildings
Grain and hay handling
Transportation of animal waste
Transportation of agricultural produce
Industrial environments
Animal feed production
Biotechnology e.g. enzyme and antibody production
Cotton processing
Flax processing
Laboratory animal confinement
Machine oils
Paper production
Pharmaceutical industry
Wood processing
Waste processing
Composting
Recycling
Rubbish collection
Water (sewage) treatment works
Others
Schools, offices and other environments where humidifiers and ventilation ducts are present

ditions lead to variable airborne concentrations of endotoxin. Workers have been estimated to inhale approximately 40–100 μg endotoxins per day (Jacobs 1997) in addition to the other various components of the dust. Inhalation of other organic dusts containing high concentrations of endotoxin and achieving airborne dispersions with a high (40%) proportion of respirable ($< 5\,\mu$m) particles can also cause respiratory symptoms somewhat similar to those to those of byssinosis. Occupational examples include carpet weavers who use wool almost exclusively, grain handlers from various work environments including farmers, grain-elevator operators and dock workers, workers who handle animal feed, flour and seed-mill workers.

Exposure to endotoxin shows strong epidemiological correlation with the symptoms of organic dust toxic syndrome (ODTS), an acute fever accompanied by chills, joint pains and other symptoms typical of influenza (Rylander 1994a; Jacobs 1997). The farming industry presents a wide range of settings which are potential environments for the induction of respiratory disorders. Organic raw materials used in farming such as mouldy hay, straw, grain dusts, manure, silage, swine and poultry dusts have all been demonstrated to produce adverse lung reactions. The endotoxin here is derived from the external surfaces of plants and animals, animal intestines/faecal matter and decomposing organic matter. Activities such as harvesting, threshing, transportation, cleaning, grinding, feeding and other aspects of animal husbandry release dusts that can carry organisms into the air. Endotoxin is therefore one of the potential causative agents of lung disease within the farming environment. An important point to note is that as well as endotoxin present in the atmosphere of these workplaces other irritants capable of altering airway function are present. In the case of cotton workers, these include inhaled tannins (Rohrbach 1994), antigenic proteins and other chemical agents (Nicholls 1992). Swine workers are exposed to noxious gases such as ammonia, hydrogen sulphide and other micro-organisms such as fungi and moulds (Nicholls 1992).

As well as relating to the older disease states, endotoxin may be linked to more modern disease states, for example in the case of humidifier fever and sick-building syndrome (Teeuw 1993). The two latter conditions are often inextricably linked, in that contamination of a humidifier is found to be the cause of the so-called sickness of the building (Teeuw 1993). Symptoms experienced include fever, chills, muscular pain, fatigue, respiratory and skin disorders. Several studies have linked the presence of endotoxin in contaminated water to these symptoms (Rylander 1994a; Rylander & Vesterlund 1982). However, there is no convincing evidence to show that endotoxin alone is responsible for the induction of these conditions (Jacobs 1997).

Considering the potential damage that appears to be caused by inhalation of LPS, it would be prudent to have an exposure limit for LPS. To date, no definitive limit exists. Rylander and Jacobs (Jacobs 1997), in the criteria document, Endotoxin in the Environment, attempt a classification based on a no-effect level principle e.g. toxic pnemonitis is not seen at environmental endotoxin levels of below 200 ng m^{-3}. Unfortunately, the signs of airway inflammation appear at approximately 10 ng m^{-3}. The value above (and others in the criteria document) is based on persons who have no history of atopy or asthma. In addition, these values do not account for the wide inter-laboratory variation of LPS concentrations measured using the LAL assay (quantitative values for LPS concentration in dust samples are subject to wide variation, even using the same assay system) and only partially account for the wide personal variation in response to inhaled endotoxin/LPS. Further investigation and refinement are required before a full understanding of the harm that inhaled LPS can cause is obtained.

What are Glucans?

Glucans are natural products found in the cell walls of plants, fungi and bacteria. They are glucose polymers, that consist of glucopyranosyl subunits connected by α or β linkages (Williams 1996). The most potent glucans have a (1→3)-β-D backbone and are commonly isolated from fungi. Their basic structure is shown in Figure 2. Branching of the polymer is also possible, generating a (1→6)-β side

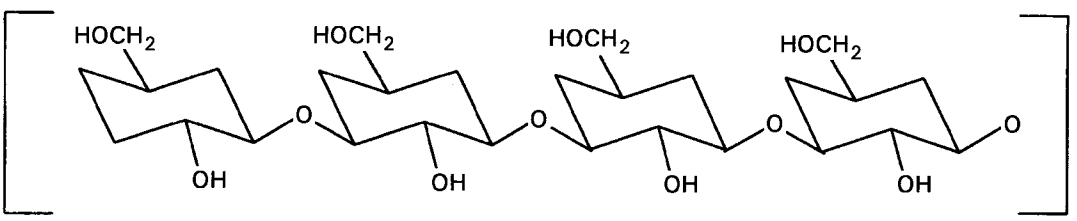

Figure 2. Basic structure of the (1→3)-β-D-glucans.

chain. The ultrastucture of the (1→3)-β-D-glucans is highly variable, from a random coil arrangement through to single helices and finally stable triple helices. The physical characterization of this family of glucans is important, as it appears that their activity is dependent on their molecular weight, degree of branching, ultrastructure and source.

What Biochemical Actions Do Glucans Possess? Where can Glucans be Found?

The (1→3)-β-D-glucans were first investigated due to their systemic immunomodulating effects. The glucans, lentinan and schizophyllan, are available in Japan as cancer therapies due to their anti-tumour activity (Ohno et al 1995). They were also discovered to increase resistance to challenge with Gram-negative bacteria and this led to a water soluble glucan entering trials as a prophylactic treatment of septic morbidity (Williams et al 1996).

More recently glucans have been discovered in a whole host of different materials and settings. These include bird droppings, mould in damp houses, and the tartar coating on teeth. Of particular interest was the discovery of (1→3)-β-D-glucans in organic dusts, such as cotton and grain. Quantities up to 1 $\mu g\,mL^{-1}$ have been measured in the air of an experimental cotton cardroom (Rylander et al 1989). These glucans are regarded as a potential novel component in the respiratory diseases associated with such dusts.

There is believed to be some relationship between the airborne (1→3)-β-D-glucan levels and the extent of respiratory sick-building symptoms, particularly nasal and throat irritation (Rylander 1996). These same symptoms were noted after human subjects were exposed to an aerosol of (1→3)-β-D-glucan (Rylander 1996). These symptoms are mainly related to airway inflammation.

What Effects Do Glucans Have on Inflammation?

In-vivo experiments have been performed to determine whether glucans influence respiratory tract inflammation. Guinea-pigs were exposed to aerosols of various glucans. After both acute and chronic exposure to an insoluble glucan (curdulan) there is very little change in the number of inflammatory cells found within the lungs (Fogelmark et al 1992, 1994; Rylander 1994a, b) However, when curdulan is rendered soluble (by treatment with NaOH) there is a significant increase in the number of neutrophils in the lungs (Rylander 1994a, b). This effect is reproduced when using a naturally water soluble glucan, schizophyllan (Fogelmark et al 1992; Rylander 1994a, b). There is opposing evidence on the effects of particulate (1→3)-β-D-glucan e.g. from baker's yeast on the inflammatory process. One study indicates that exposure to baker's yeast causes a significant increase in the number of neutrophils and lymphocytes (Milanowski 1997), whereas another shows no evidence of an inflammatory reaction (Fogelmark et al 1992; Rylander 1994a, b).

What Effects Do Combined LPS/endotoxin and Glucan Have on Inflammation?

Exposure to organic dusts involves exposure to a variety of agents, not just a single component. Therefore, the combined effect of glucan and endotoxin on the airways has been investigated. There is synergy (a significant increase in airway neutrophils, macrophages and lymphocytes), compared with the effect of endotoxin alone, when guinea-pigs inhale a mixture of curdlan and endotoxin chronically (Fogelmark et al 1994; Rylander 1994a, b). There are also more pronounced histological changes with the combined exposure (Fogelmark et al 1994). A possible explanation is that glucan causes sensitization of the animals to the effects of endotoxin, by decreasing macrophage function (Fogelmark et al 1994).

The inflammatory process is heavily dependent on various signalling molecules, particularly cytokines, for chemoattraction and activation of inflammatory cells. In-vitro experiments with murine macrophages indicate that glucans may well have a role in the production and secretion of cytokines. When murine macrophages are stimulated with grifolan (a gel-forming, branched (1→3)-β-D-glucan) an increase in the release of TNF-α, IL-1 and IL-6 is observed (Adachi et al 1994b). TNF-α and IL-1 are two prominent cytokines in the development of an inflammatory reaction. However, when the same experiment is carried out using different glucans, such as schizophyllan and laminarin, there is no induction of cytokine production (Adachi et al 1994a). Further studies indicate that the ability of the glucan to stimulate TNF-α release is dependent on the molecular weight and degree of branching of the glucan (Okazaki et al 1995).

The induction of cytokine production has also been investigated in-vivo. In this case, grifolan is unable to directly release TNF-α into mouse serum (Ohno et al 1995). However, it does promote an elevated release after stimulation with endotoxin (Ohno et al 1995). This suggests that the glucan primes the macrophages, rendering them more sensitive to the effects of endotoxin.

The pleiotropic actions of various glucans is highlighted by another inflammatory marker-reactive oxygen species generation (Okazaki et al 1996; Rylander 1994a, b). Reactive oxygen species, such as superoxide anion (O_2^-), hydroxy radicals ($OH^\bullet$) and hydrogen peroxide (H_2O_2), are released during the inflammatory process. Their main function is to destroy micro-organisms. However, they can have detrimental effects such as epithelial damage. In-vitro experiments using murine and guinea-pig macrophages have shown that the particulate (1→3)-β-D-glucans can trigger O_2^- and H_2O_2 production (Okazaki et al 1996; Milanowski 1997). The soluble glucans however, can not enhance the synthesis of these oxygen species (Okazaki et al 1996).

How Do Glucans Interact with Cells to Modulate Inflammation?

It has been reported that (1→3)-β-D-glucans can bind to a specific receptor on the macrophage, demonstrated in both murine and human cell lines (Williams et al 1997). A specific (1→3)-β-D-glucan receptor has also been identified on monocytes, natural killer cells and neutrophils, and is believed to be complement receptor type 3 (CD11b/CD18) (Thornton et al 1996; Vetvicka et al 1996).

The macrophage glucan receptor appears to have differing affinity depending on the (1→3)-β-D-glucan that binds. It has a much higher affinity for the branched (1→3)-β-D-glucans and this may help to explain some of the differing activities of the various glucans. Radiolabelling studies using [^{3}H]glucan phosphate suggest that after binding of the glucan there is a rapid uptake process, and internalization of the glucan (Williams et al 1997).

How Can We Further Our Knowledge of the Actions of (1→3)-β-D-Glucan?

(1→3)-β-D-Glucans certainly have a role in respiratory inflammation. Their pharmacology, as yet, appears complicated and further research into their actions and interactions needs to be carried out. Work is currently underway to examine their effect on airway smooth muscle, and preliminary studies indicate that glucans may cause airway hyporesponsiveness (Jones et al 1997). A significant development is the production of specific (1→3)-β-D-glucan antibodies, which can be used as immunological probes to further our understanding of glucan activity (Adachi et al 1994b).

Conclusions

There is mounting evidence that both endotoxins and glucans are potent biologically active agents possessing marked adverse effects on the airway following inhalation. The demonstration of synergy between these agents has important implications in respect of air quality in a range of varied environments not least of which will be the difficulty of establishing meaningful safety threshold levels. However, efforts should be directed to reduction of airborne concentrations of these contaminants particularly in dusty work environments and damp dwellings.

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