Review

# Endotoxin in the environment – exposure and effects

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This review deals with endotoxin in the environment and its relation to disease among exposed persons. Data are presented on levels of endotoxin in different environments with maximum values of several $\mu g/m^3$. The cellular reactions of importance for inhalation exposure effects are attachment to lipopolysaccharide binding protein, CD14 cell surface protein and TLR-4 receptors. The internalisation of endotoxin in macrophages and endothelial cells results in local production of inflammatory cytokines with subsequent migration of inflammatory cells into the lung and the penetration of cytokines into the blood. These events orchestrate clinical effects in terms of toxic pneumonitis, airways' inflammation and systemic symptoms. Inhalation challenges with pure endotoxin and field studies confirm the relation between these effects and exposure to dusts containing endotoxin. It is possible that polymorphism in genes determining endotoxin reactivity, particularly TLR-4, influences the risk for disease after environmental exposures. Some data suggest that the inflammation caused by inhaled endotoxin may decrease the risk for atopic sensitisation among children and lung cancer among workers exposed to organic dust. Additional research is needed to clarify the role of other environmental agents that are present in connection with endotoxin, particularly (1→3)-β-D-glucan from mold cell walls.

## INTRODUCTION

It is often thought that agents in the environment that have an impact on human health generally have chemical, man-made origins. Thus the first reports suggesting that one of the most common natural environmental factors – Gram-negative bacteria present in soil, in water and on vegetation – was associated with lung disease among persons handling cotton fibres were received with disbelief.[1] The identification of bacterial endotoxin as a possible causative agent and supporting experimental evidence from animal experiments[2,3] did little to improve understanding. Additional difficulties in the endotoxin case were that there was initially no reliable method for the measurement of air-borne endotoxin and there was also a poor understanding of the pathogenesis of the symptoms among exposed persons. A number of animal inhalation studies and a few field studies were published in the 1980s and will be reviewed below.

With increasing understanding of the mechanisms behind the toxicity of inhaled endotoxin and how these fit with the pathophysiological mechanisms behind airways' inflammation, the ground-work was laid for the emergence of a large number of publications on endotoxin in the environment in the late 1990s. The purpose of this article is to review the present understanding of the presence of endotoxin in the environment and how exposure is related to disease in humans. Environmental exposure is usually through inhalation and because the cellular effects after inhalation are different from those seen *in vitro* and after injection, the focus will be on endotoxin administered via inhalation. The structure of endotoxin and the cellular reactions it induces have both been the subject of extensive reviews and will only be summarized in this context.

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## ENDOTOXIN IN THE ENVIRONMENT

### *Definition*

Gram-negative bacteria are ubiquitous in our environment – as examples, *Klebsiella* spp., Pseudomonads and

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Enterobacter are found in the soil, on vegetation and in water.[4] They carry and release a specific compound on their outer surfaces, which is a combination of polysaccharide chains, a lipid A unit responsible for the toxic effects in cells, and a connecting core molecule.[5]

This substance is referred to as endotoxin or lipopolysaccharide (LPS). However, the term 'lipopolysaccharide' should be reserved to denote the chemically pure substance, free from all other chemical compounds (an artificial compound not found in nature). The term 'endotoxin' should be used to refer to lipopolysaccharide as it appears in nature, on fragments of Gram-negative bacteria cell walls with other naturally occurring components of the cell wall. In water, endotoxin will detach from the cell wall and be present in the free form although it is still not a chemically pure LPS.[6]

### *Detection*

To detect endotoxin, the gold standard as of today is the *Limulus* amebocyte lysate (LAL) test.[7,8] This is a biological test that uses amebocytes from the hemolymph of the horseshoe crab. The amebocyte is a nucleated, granular cell that aggregates and forms a clot at the site of injury.

For the *Limulus* this is frequently caused by endotoxin from blue-green algae. The contents of the amebocytes' cytoplasmic granules can be isolated and used as the reagent in the LAL test for endotoxin. The activation of the enzymes in the clotting process can either be read as a clot formation (clot end-point version) or as the change in color over time of a dye that reacts with the enzyme (kinetic chromogenic version). The latter version is used by the majority of laboratories that determine endotoxin in environmental samples.[8,9]

The sample to be tested – air-borne particles collected on a filter, sedimented dust or a liquid – is elutriated in pyrogen-free water and diluted. The lysate is then added and the ensuing color reaction is recorded using a spectrophotometer and compared with a standard endotoxin. The test is sensitive and detects amounts down to picogram levels in environmental samples. There is often a variation in results between different laboratories, probably due to small and uncontrolled variations in the extraction procedures and the handling of the samples.[10] Within one laboratory, however, the results are highly reproducible.

To avoid drawbacks related to biological tests, several attempts have been made to use chemical analysis. The most successful of these attempts has been the determination of fatty acids, using gas chromatography.[11] Reproducible results have been obtained, but unfortunately the sensitivity of the method is far below that of the *Limulus* test, causing limitations to its use in field studies.

The results of the *Limulus* test are dependent on the physical state of the endotoxin in the sample. If it is present in a water solution (*e.g.* as Gram-negative bacteria growing in water in a humidifier), the values represent all of the endotoxin present in the sample. If the analysis is made on a dust sample, where the endotoxin is still part of fragments of the bacterial cell wall, the results of the *Limulus* test do not represent the total amount of bioactive material. Some attempts have been made to calculate the relation between the amounts detected in the analysis and the bioactive amount, suggesting a ratio of 1:10.[12]

### *Measured values*

The first study of endotoxin in the environment reported the presence of endotoxin in house-dust[13] and water.[4] Interest later focused on occupational settings, and endotoxin has now been found in a large number of environments where organic dusts are present. A summary of some of these environments and the amounts detected is given in Table 1.

**Table 1**. Endotoxin in different general and occupational environments

| Location | ng/m$^3$ | References |
|---|---|---|
| **Home** | | |
| Dust | 18–50 | 21–24,27,83,110 |
| Humidifier | 130–3900 | 111 |
| Drinking water | 0.8–1250 | 112,113 |
| **Agriculture** | | |
| Farming | 50–2800 | 114,115 |
| Dairy barns | 25–3480 | 116 |
| Poultry | 33–301 | 7,117,118 |
| Swine confinement | 8–252 | 15,76–78,119 |
| Rice | 49–1341 | 120 |
| Animal feed | 0.2–1870 | 121,122 |
| Grain dust | 286–721 | 75 |
| **Waste** | | |
| Collecting | 0–5 | 17 |
| Composting | 7–53 | 18 |
| Fuel plant | 2–4 | 123 |
| Sewage | 1–32,170 | 19,20,68,124 |
| **Industry** | | |
| Wood handling | 0–375 | 125,126 |
| Saw mill | 0–4000 | 127–129 |
| Debarking | 108–375 | 16,64 |
| Metal cutting fluid | 0–600 | 130 |
| Potato processing | 37–82 | 131,132 |
| Cigarette manufacturing | 38–106 | 133 |
| Cotton mill | 2–314 | 3,10,72,73,79,134 |
| Brewery | 60–927 | 135 |
| Biotechnology | 0.1–12.8 | 136 |

Values are equalized to ng/m$^3$ (10 EU = ~1 ng).

The values reported should be regarded as examples – higher levels have been recorded in other studies and levels are very low in clean environments with organic dusts, such as where medical cotton is carded. The table is not intended to be complete – the number of studies is now so large that a complete table would be overwhelming. However, one major purpose of listing some of the studies is to show that studies were already being done in the 1970s and 1980s – a fact not always reflected in reference lists in publications of today.

Although the data are still incomplete, certain occupational exposures seem to involve particularly high levels. Such environments are cotton mills, particularly in carding areas, in swine confinement units and in the early stages of wood processing such as debarking.[14–16] The levels are generally low in the handling of household waste, except organic waste, and during composting.[17,18] The latter dust usually contains high amounts of molds. Sewage treatment plants represent a special case, as endotoxin levels are high at certain work sites and there is no contamination from molds.[19]

The sampling of environmental endotoxin usually involves stationary or personal air samplers and the amount of endotoxin is expressed as amounts per $m^3$. The field studies reviewed below show important differences in sampling strategies. Some studies use sampling periods over one or several days and others sample for shorter intervals or specify the work procedures during which sampling was done. This variation might affect the levels of endotoxin detected. As an example, it has been shown that the most important exposure to endotoxin in sewage treatment plants occurs in connection with specific work tasks that for the employee involve exposures during a limited time.[20] A long-term sampling would thus not detect a peak exposure, which is very relevant as a risk criterion for endotoxin.

Endotoxins are also found in indoor environments and their presence in house dust was first described some 40 years ago.[13] 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 on a farm, use of humidifiers with contaminated water, and storage of organic household waste.[21–24] Another source indoors is cigarette smoke.[25]

A seasonal variation with a lower amount during the summer and winter periods has also been reported, probably due to differences in the outdoor number of Gram-negative bacteria during different seasons.[26] Some studies have, however, not detected such seasonal variations.[27] Apart from these reasons for variation, the frequency of cleaning and life-style factors such as smoking and poor economical conditions have also been related to the amounts of endotoxin found indoors.

For sampling in homes, both air-borne samples and sedimented dust, obtained by vacuum cleaning, have been used. Whereas endotoxin can be found in most samples of vacuumed dust, air-borne dust, even with agitation, usually contains no, or very small amounts of, endotoxin (unpublished data). There is, as yet, no agreement on which fraction of the indoor dust is the most relevant for respiratory effects.

In summary, bacterial endotoxin is a part of our normal environment and high amounts can be found, particularly when organic dusts contaminated with Gram-negative bacteria are agitated or when humidifiers are operated with contaminated water. What, then, are the effects when these aerosols are inhaled?

## CELL REACTIONS AND ENDOTOXIN

To understand the disease that develops in man after inhalation of endotoxin or dusts that contain endotoxin, a short description of the cellular reactions after inhalation provides a useful background. Detailed reviews on the molecular mechanisms involved have recently been published.[28,29]

After deposition in the airways, the lipid A part of the endotoxin is opsonized by a lipopolysaccharide binding protein (LBP) present in the fluid on the airway surface, probably produced both by type II epithelial cells and exudation from the vascular compartment.[30] This type 1 acute-phase protein is, among other proteins, a part of the body's defense against external agents. Their role is to transport the foreign substance to a site of metabolism and destruction. For endotoxin in the lung, these sites are macrophages and epithelial cells. Macrophages carry a surface protein, CD14, to which the LBP attaches. CD14 is also present in a free, soluble form in normal alveolar fluid, where it facilitates the attachment of endotoxin to cells without CD14 on their cell membranes such as epithelial and dendritic cells.[31]

Although the endotoxin is now attached to the reactor cell surface, an additional step is required before cell activation can take place. Data collected in recent years rather convincingly demonstrate that, for macrophages, this 'missing link' is a Toll-like receptor and that the TLR-4 form is particularly important for cellular activation by endotoxin.[32,33] TLR-3 may play a role in the activation of dendritic cells.[34]

When the endotoxin is internalised, NF-$\kappa$B initiates the production of a variety of inflammatory cytokines, particularly IL-1$\beta$, TNF-$\alpha$ and IL-6.[35] The ensuing tissue reactions have been studied in a number of inhalation models using animals and humans.

## THE LUNG AND ENDOTOXIN

The first study on inhaled endotoxin used an animal model with rabbits.[36] The results described cell infiltration

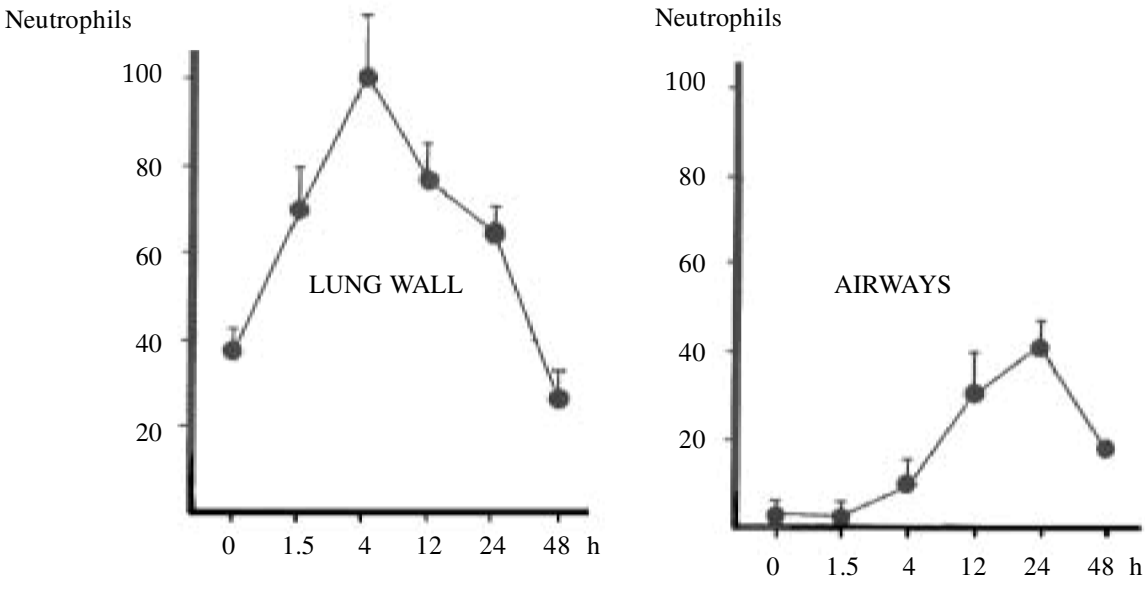


**Fig. 1**. Neutrophils ($10^6$) in lung wall and airways of guinea pigs at different times after a 40 min inhalation exposure to LPS.[35]

of alveolar cell walls and oedema. The changes regressed with increases in exposure time, suggesting habituation. A guinea pig model was later developed and used both for studies of LPS and of different organic dusts containing endotoxin, particularly cotton dust.[2,37] A major finding in these studies was the invasion of neutrophils into the lung and the airways as illustrated in Figure 1.

Neutrophil invasion into the lung tissue after inhalation of LPS is rapid, taking place within hours of exposure.[38]

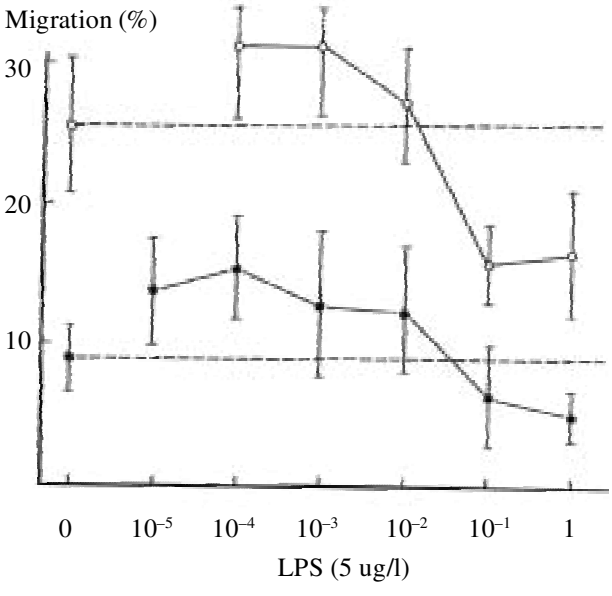


**Fig. 2**. Migration *in vitro* of neutrophils from blood of guinea pigs at different concentrations of LPS in migration medium. Results are percent of migration against zymosan. Open circles, neutrophils were pre-incubated in fetal calf serum containing LPS; filled circles, neutrophils were incubated in LPS-free fetal calf serum.[37]

This is followed by a later penetration into the airways with a maximum at 24 h after exposure. The invasion into the lung and into the airways is probably determined by different mechanisms, as corticoid pretreatment prevents the invasion into the airways but not the migration into the lungs.[39]

Other animal studies demonstrated that neutrophil migration into the airways was caused by chemotactic factors secreted by alveolar macrophages.[40] It was also shown that the secretion of chemotactic factors was influenced by a pre-incubation with LPS, as illustrated in Figure 2.

At low levels up to 0.1 μg/ml, the pre-incubation stimulated the secretion of chemotactic factors while concentrations above this value inhibited the secretion. The finding that the initiation of neutrophil migration requires only a very small amount of LPS, and the knowledge that LBP is required for the effect of inhaled endotoxin, limit the usefulness of results of studies *in vitro* with isolated cells incubated in high concentrations of LPS.

There is also information on the effects of inhaled LPS on the immune system. It has long been known that endotoxin is an adjuvant. As an example, a 5-week inhalation exposure to ovalbumin did not affect the level of IgG antibodies in the blood.[41] When LPS was given together with ovalbumin, the result was a large increase in antibodies. The timing of the LPS exposure in relation to the development of IgE antibodies was studied in a rat model where it was shown that a single inhalation of LPS 1 day before and up to 4 days after an intraperitoneal injection of ovalbumin decreased the production of IgE ovalbumin antibodies.[42]

In summary, the results of these and a number of other animal studies clearly demonstrate that inhalation of

LPS and dust containing endotoxin initiates the secretion of inflammatory cytokines that produce an inflammatory response in the airways. This effect is present at levels of exposure as low as nanograms/$m^3$, which is a level encountered and exceeded in a variety of environments. Against this background, it is of interest to review the evidence for an inflammatory response in the airways of humans after an inhalation exposure to endotoxin.

## INHALED ENDOTOXIN IN HUMANS

The first inhalation challenge in humans using purified endotoxin appeared in 1969[1] and in the 1980s[43–45] and were followed by a series of studies using LPS evaluating the reaction in normal persons and persons with clinical asthma and studying the cellular reactions.[46–49] One study used intratracheal instillation.[50] The most widely used end-points were lung function, airway responsiveness, CO diffusion and the amount of inflammatory mediators and inflammatory markers in lung lavage and blood. A summary of an evaluation of different inflammatory parameters at different times after exposure is illustrated in Figure 3.

The number of polymorphonuclear cells increases a few hours after acute inhalation exposure.[51] This increase reflects the previously described secretion of chemotactic factors from macrophages and a mobilisation of neutrophils in the blood, following the rapid migration into the lung (Fig. 1). The amount of E-selectin is also significantly increased, indicating an activation of endothelial cells. C-reactive protein is increased at 24 h after inhalation, reflecting the activation of liver cells by IL-6, IL-1 and TNF-α. There is also an increase in the amount of LBP. Other studies have shown an increase in the amount of TNF-α in blood a few hours after inhalation[52] and an increase in myeloperoxidase and eosinophilic cationic protein in sputum at 24 h after inhalation.[53] Inflammatory cytokines are generally elevated 2–6 h after exposure, together with neutrophils, whereas other inflammatory cells such as macrophages, monocytes and lymphocytes are increased at 24 h, a time when cytokines have returned to normal levels.[50,51]

LPS inhalation studies have also evaluated the effect in terms of functional changes such as pulmonary function and airway responsiveness.[45,46,54] These changes are relatively small in normal subjects but more pronounced among asthmatic subjects, suggesting that the inflammation induced by endotoxin further aggravates the asthmatic inflammatory response. Unexpectedly, non-symptomatic atopics, as defined by a positive skin prick test, are less reactive to inhaled LPS.[51]

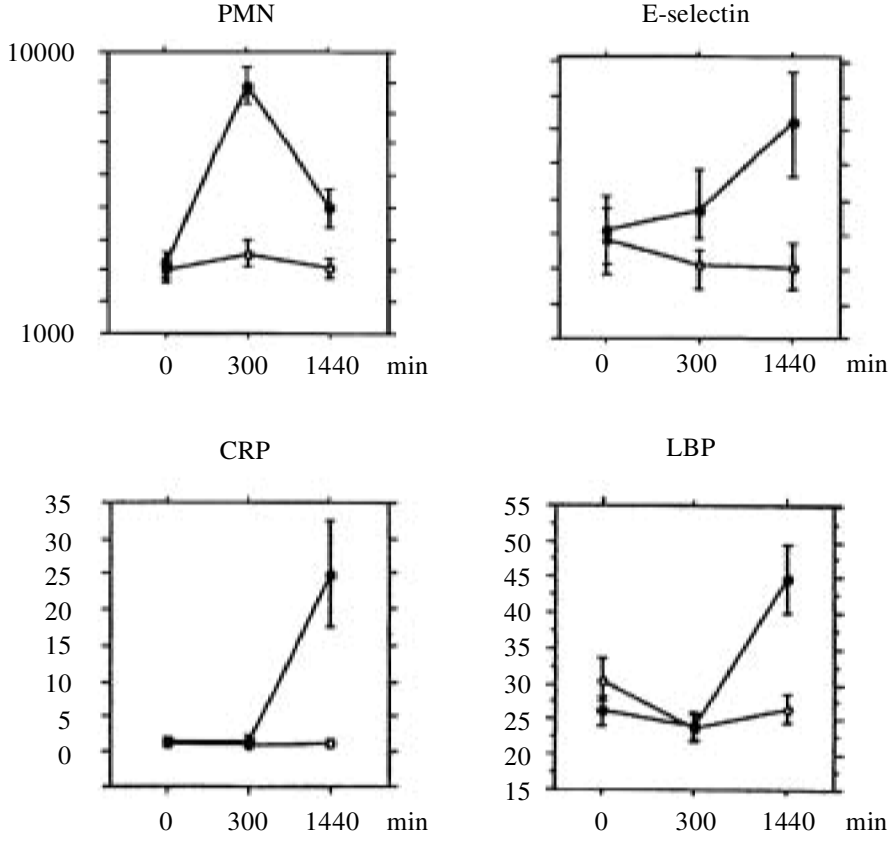


**Fig. 3**. Inflammatory parameters in blood of human subjects at different times after an acute inhalation of LPS. PMN, polymorphonuclear leukocytes; CRP, C-reactive protein; LBP, lipopolysaccharide binding protein.[48]

In the intact animal, LPS does not penetrate to the blood.[55] This suggests that the defense system in the lung is capable of preventing penetration of inhaled endotoxin into the blood under normal conditions. It is clear from the above, however, that the inhalation of endotoxin may lead to the release of different inflammatory cytokines in the blood, which may provoke systemic effects. Apart from inflammatory mediators, cerebral catecholamine has also been shown to become activated, and serotonin metabolism is affected by endotoxin exposure.[56]

How then does the experience gained in inhalation studies of LPS translate into clinical disease?

## DISEASES AND ENDOTOXIN

Early observations reported that persons exposed to a high concentration of organic dust contaminated with Gram-negative bacteria developed a flu-like syndrome with fever, chills, muscle and joint pains and fatigue.[57] Subsequent studies on workers in other environments with organic dusts contaminated with endotoxin and results of human inhalation studies using LPS have convincingly shown that this syndrome is caused by an acute inflammation in the airways. The terminology has changed over the years and location-related names such as printer's fever, mill fever and Monday fever have been used. In view of the underlying pathology, the disease should be referred to as 'toxic pneumonitis', indicating its character as an inflammation caused by a toxic, non-infectious agent. Classic symptoms are an increase in body temperature, chills and symptoms in the respiratory tract, tiredness and headache. All these symptoms can be reproduced by inhalation of LPS and dusts containing endotoxin.[49,53,58] The symptoms generally disappear the following day.

Toxic pneumonitis is not unique for endotoxin – it can also be produced by inhalation of toxic, chemical fumes and by mold aerosols. The latter exposure is also related to a risk for hypersensitivity pneumonitis, which has caused some confusion and the term 'acute allergic alveolitis' has been suggested. It is clear, however, that toxic pneumonitis is one specific entity, related to a simple inflammatory response. The inhalation of an aerosol of molds in sufficiently high concentration can give rise to an inflammatory response similar to the one induced by endotoxin.[59] Hypersensitivity pneumonitis requires repeated inhalations resulting in a sensitisation related to T-cell activation and invasion of lymphocytes into the lung tissue.[60] The mold cell contains (1→3)-β-D-glucan, that has the ability to induce changes in lymphocytes and, in high concentrations, cause the formation of granulomas, a typical marker of hypersensitivity pneumonitis.[61,62] The pathogenesis of hypersensitivity pneumonitis is thus not related to endotoxin exposure, nor does toxic pneumonitis comprise part of the clinical picture for this disease.[58]

Persons chronically exposed to LPS or dusts containing endotoxin may develop an inflammation in the airways.[47,63,64] This is clinically recognisable as an increase in airway and general symptoms, increased airway responsiveness and, at more advanced stages of the inflammation, a decrease in lung function. The inflammation can be diagnosed by an increased level of inflammatory cytokines in blood, broncho-alveolar lavage or nasal lavage.[64–66] Diagnostic instruments are questionnaires and blood, and sputum or lung lavage samples for determining inflammatory markers or cytokines. While these end-points can be used to differentiate between exposed and non-exposed groups and dose-response relationships can be found, the methods are not as yet standardised to be used at the individual level.

While it is clear that the airway inflammation caused by endotoxin may produce a clinical picture that in severe cases can be diagnosed as asthma, the underlying pathogenesis is a non-specific inflammation with no relation to IgE-mediated reactions or antibodies to endotoxin.

The previously described dissemination of inflammatory cytokines from the lung throughout the blood system has as a practical consequence that symptoms of fatigue, joint pains and fatigue among persons exposed to LPS or dusts containing endotoxin are a sign of exposure and should be taken into account in health examinations. Symptoms in the joints and tiredness have been reported by persons working in swine confinement buildings, in a wood debarking area in a paper industry,[64,67] and in sewage treatment plants, where the exposure agent is an almost pure aerosol of endotoxin.[19,68] These findings are consistent with the presence and amplitude of systemic effects found after inhalation of endotoxin.[51]

Another symptom reported in relation to endotoxin exposure is gastrointestinal disturbances. These are present among persons that have inhaled LPS and particularly among waste collectors and persons working in sewage treatment plants.[69,70] The reason for these symptoms may be the exposure to endotoxins with a new polysaccharide structure, in comparison to the types that usually inhabit the intestinal tract.

Several studies show a relation between the risk for septic shock and endotoxin exposure.[71] As the source of the endotoxin in this case is not the environment, this disease complex is not dealt with in this review.

## FIELD STUDIES

The most commonly used end-point in diagnoses of airways' inflammation in field studies has been measurements of the forced expiratory volume in one second ($FEV_1$) and other parameters of lung function. Two studies in experimental cardrooms used a design in which

the same subjects were exposed to dust from several cottons with different amounts of endotoxin contamination but at the same dust level. One of these studies used cotton workers.[72] There was no relation between the decrease in $FEV_1$ after exposure and the amount of dust but a significant dose-response relationship for endotoxin levels that were also related to blood neutrophilia and symptoms of byssinosis. The other study used naïve subjects, selected for their airway reactivity to cotton dust.[73] Again, there was no relation between the decrease in $FEV_1$ and dust but a significant correlation for endotoxin. Subsequent studies on workers exposed to grain dust, farmers and swine confinement building workers have confirmed that the extent of symptoms and respiratory function declines are related to the amount of endotoxin in the different dusts.[14,74,75]

While decreases in $FEV_1$ have been demonstrated after acute inhalation of LPS and in cross-sectional occupational studies, longitudinal studies are a better instrument for determining the risk for chronic disease. In a study on randomly selected swine farmers in Iowa, cross-shift changes in lung function as well as the longitudinal decline in lung function over a 2-year period were related to the amount of endotoxin in the dust.[76] Similar findings were reported in studies in Canada[77] and in The Netherlands.[78] Cotton and silk textile workers in Shanghai were followed for a 15-year period, and the longitudinal changes in $FEV_1$ were found to be related to a high level of exposure to endotoxin.[79]

Although measurements of $FEV_1$ represent a traditional method for estimating the impact of inhaled environmental agents such as endotoxin, it is now clear that it is a relatively insensitive measure of airways' inflammation and that wide-spread symptoms of inflammation can be present even when the changes in spirometry are rather small.[51,67,80] Some studies have used airway responsiveness as an end-point for inhalation-induced effects. In a study on cotton workers, a dose-response relationship was found between the exposure to air-borne endotoxin at the different work sites and the decrease in $FEV_1$ after inhalation of a standardized dose of methacholine.[81] In a follow-up study in 171 pig farmers in The Netherlands, however, a relationship with the increase in responsiveness over 3 years was found only with the amount of ammonia in the air.[78]

In the home environment, which implies a chronic exposure, a relation has been found between the amount of endotoxin in house-dust and the severity of asthma.[82,83] A study in Brazil reported a significant relationship between clinical asthma scores among children and levels of endotoxin in their homes.[84] In a cohort of 499 infants, a relation was found between house-dust endotoxin levels and wheeze among the children.[85]

On the basis of toxicological data and experience from field studies, proposals have been made for guidelines for non-effect levels for endotoxin.[12] They suggest that the non-effect level for airways inflammation is 10 $ng/m^3$, for general symptoms 100 $ng/m^3$, and for toxic pneumonitis 200 $ng/m^3$. In The Netherlands, it was later suggested that 4.5 $ng/m^3$ be the limit based on personal inhalable dust during 8 h.[86]

## ENDOTOXIN AND BENEFICIAL EFFECTS

Some data suggest that the inflammation caused by endotoxin might have beneficial effects on the risk for atopic sensitisation. In a study of 61 infants with a high risk for sensitisation, it was found that the risk for atopic sensitisation was inversely related to the amount of endotoxin in house-dust.[87] Among children living on farms where the prevalence of atopic sensitisation is known to be low, indoor endotoxin levels were higher than in a control group.[88,89] 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.[90] 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 in the Th1 direction, suppressing the risk for atopic sensitisation.[91,92]

Certain studies have shown an inverse relationship between exposure to endotoxin and the risk for lung cancer. An investigation of the mortality of cotton workers reported a lower than expected mortality for lung cancer.[93] This observation was followed by a series of reports on an animal model in which the growth of metastasis in the lung was reduced by inhalation of LPS.[94] Studies from Sweden and Italy found the risk for lung cancer to be lower than expected among farmers exposed to organic dusts.[95,96] These data were corrected for smoking.

These studies support early observations that exposure to a 'fever-causing agent' derived from Gram-negative bacteria could arrest the growth of tumors.[97] The underlying mechanisms may be an activation of the immune system with macrophage surveillance and increased secretion of tumorogenic cytokines (*e.g.* TNF-$\alpha$).[98] A recent review paper suggested a need for further research in this important area.[99]

## CAVEATS

Thus far, this review has produced evidence for a relationship between exposure to LPS and dusts containing endotoxin and inflammatory disease in the airways and the body as a whole. However, the risk evaluation in an environment is complicated by factors related to the exposure and to individual susceptibility.

*Chronic exposure*

Although a substantial body of information is available on the acute effects of inhalation of LPS or dusts containing endotoxin, far less is known about chronic exposure, which is of greater relevance in terms of environmental exposures. A phenomenon of adaptation regarding the clinical outcomes, particularly fever, is well known in the case of LPS.

With respect to neutrophils, repeated inhalations of LPS would result in a lower number of neutrophils in the airways and a lower secretion of TNF-α as compared to the acute reaction.[36,100] A pre-exposure to endotoxin decreased the response to inhaled grain dust that contained endotoxin.[101] The mechanisms of endotoxin tolerance was studied in animal models, demonstrating a decreased production of TNF-α following pre-treatment with endotoxin.[102] However, this habituation could be overcome by a very large dose of endotoxin or by another agent interfering with the defence mechanism – the Cottesloe principle.[103]

Other data suggest that repeated exposures to LPS would increase the reactivity of platelets to cell stimuli.[104] The production of procoagulant factor by blood monocytes from cotton workers was increased after stimulation with LPS as compared to non-exposed persons, suggesting a cellular sensitisation after chronic exposure to dust containing endotoxin.[105] There was also a relation between procoagulant activity and the decrease in $FEV_1$ after inhalation of cotton dust.[106] These results demonstrate that the alterations induced by repeated exposures to LPS are complex and differ according to the effect system studied. For humans, the adaptation to the fever response could be interpreted as beneficial but the consequences of a possible sensitisation of different inflammatory cell systems are not known.

*Other agents*

A large number of investigations have used the amount of air-borne endotoxin to characterize the exposure, particularly in studies on organic dusts. The real-life situation involves a combined exposure to a variety of agents, however, several of which have inflammatory properties and others the capacity to influence the immune system. It has been shown that (1→3)-β-D-glucan, a polyglucose compound in the cell wall of fungi and some plants and bacteria, can induce an inflammatory response and influence the inflammatory response due to endotoxin and to a modulation of the development of antibodies.[107] Even though the case for endotoxin is strong and experience tells us that it can serve as an indicator of risk, there is still a need to investigate other potentially active agents in environments with endotoxin exposure.

*Genetic effect determinators*

The results of studies in the occupational organic dust environment and of LPS inhalation studies demonstrate a considerable inter-individual variability in the response. Even at high dust exposure levels, a proportion of the exposed workers do not experience any symptoms or disease. In challenges with LPS, certain subjects develop fever and a systemic inflammatory response (*i.e.* fever, increased blood neutrophils and CRP) and others will not react.[51]

Conceptually, differences in the response to organic dusts or LPS may be due to differences in the mechanism responsible for the toxicity of the agent. One study showed that the $FEV_1$ response after inhaled LPS was blunted among persons with mutants in the extracellular domain of the TLR-4 receptor gene.[108] The mutant variants were present in 5.8% of endotoxin-responsive persons and 22.6% of those who were hyporesponsive.

The results of an endotoxin inhalation study on European adults demonstrated a difference in reactivity to inhaled LPS related to TLR-4 gene polymorphism.[109] Subjects with a mutant allele +896 AG had a significantly lower increase in white blood cells and lower amounts of CRP after an inhalation of LPS. Based on the CRP value at 24 h, all +896 AG subjects except one (94%) were classified as LPS low-responders in comparison with 27% of the +896 AA subjects.

These observations support a concept that differences in reactivity to LPS could be a result of functional differences in the cellular mechanism responsible for the effects of endotoxin and of genetic characteristics determining the degree of reactivity to endotoxin. This concept could have important applications in the prediction and understanding of the risk for disease – not only after environmental and occupational exposures but also in septic shock,[71] the maturation of the immune system among children,[92] and perhaps for the risk for lung cancer.[99]

## CONCLUSIONS

This review of the environmental agent endotoxin and its purified form, lipopolysaccharide, shows important relations between exposure and disease, particularly in the airways but also of a systemic nature. The major response is an inflammation, triggered by defense cells, particularly macrophages but also epithelial cells and dendritic cells. The production of inflammatory cytokines initiates reactions both in the lung and in the body as a whole. At certain exposure levels, and/or at certain periods during life, the exposure may be beneficial and may reduce the risk for disease. In the real-life environment, it is still an open question whether the outcome depends solely on the dose of endotoxin or on the simultaneous presence of other agents. Some data suggest that genetic determinants may

be of importance for the effect outcome. The need for further research is obvious.

## Acknowledgements

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