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REVIEW ARTICLE

Health effects due to endotoxin inhalation (review)

V. Liebers · M. Raulf-Heimsoth · T. Brüning

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Abstract Endotoxins are ubiquitous in the environment and represent important components of bioaerosols. High exposure occurs in rural environment and at several workplaces (e.g. waste collecting, textile industry etc.). Adverse effects on human health induced by inhalation of endotoxin are described in several studies. Up to now the endotoxin levels are mainly measured using the *Limulus amoebocyte*-lysate (LAL) assay. This assay is well established, but for a suitable characterization of bioaerosols more parameters are necessary. Additional information, e.g. concerning the pyrogenic activity of organic dust samples may be delivered by whole blood assay. Whereas on the one hand protection measures at workplaces are demanded to avoid lung function impairment due to endotoxin exposure, on the other hand a protective effect of exposure to microbial agents like endotoxins with regard to allergy development has been observed. On the cellular level toll-like receptor 4 (TLR4) and IL-1 receptor as well as surface molecules like CD14 have been shown to play a pivotal role in the endotoxin activation cascade. In this review we summarize the mechanism of endotoxin recognition and its manifold effects on human health.

Keywords Endotoxin · Health effects · LAL-test · Whole blood assay-allergy

V. Liebers (✉) · M. Raulf-Heimsoth · T. Brüning
BGFA, Research Institute of Occupational Medicine,
German Social Accident Insurance, Ruhr-University Bochum,
Allergologie/Immunologie, Bürkle-de-la-Camp-Platz 1,
44793 Bochum, Germany
e-mail: liebers@bgfa.de

Introduction

Endotoxins are main constituents of organic dust and therefore inhalative exposure occurs in many different environments. High exposures are known for several workplaces, e.g. agriculture or cotton-textile industry (Liebers et al. 2006). In addition, indoor air concentrations increase due to tobacco smoking (Larsson et al. 2004; Sebastian et al. 2006).

Endotoxins are integral components of the outer membrane of Gram-negative bacteria like Enterobacteriaceae or Pseudomonadaceae. Endotoxins are composed of proteins, lipids, and lipopolysaccharides (LPS). LPS of Gram-negative bacteria is an amphiphilic, heat-stable and water-soluble macromolecule responsible for most of the biological properties of bacterial endotoxins. The hydrophilic polysaccharide moiety is composed of O-specific side chains and core sugars. Toxicity is more associated with the lipid component (lipid A, a phosphoglycolipid), whereas the immunogenicity of the LPS is associated with the polysaccharide component of the LPS-molecule (Fig. 1).

The immune response is dependent on specific chemical structures. The most potent lipid A is the hexaacylated lipid A from *E. coli* with a biphosphorylated diglucosamine backbone. In addition, only endotoxin aggregates are biological active; monomers fail to stimulate cytokine release of monocytes (Mueller et al. 2004).

The individual immune response to endotoxins is the result of a complex interaction between dose and timing of exposure, additive or synergistic effects and genetic predisposition (Vandenbulcke et al. 2005). Health effects of endotoxin exposure can best be described as paradoxical (Liu 2002; Radon 2006): positive as well as negative health effects have been described. The individual immunological response is determined by the interaction between the dose

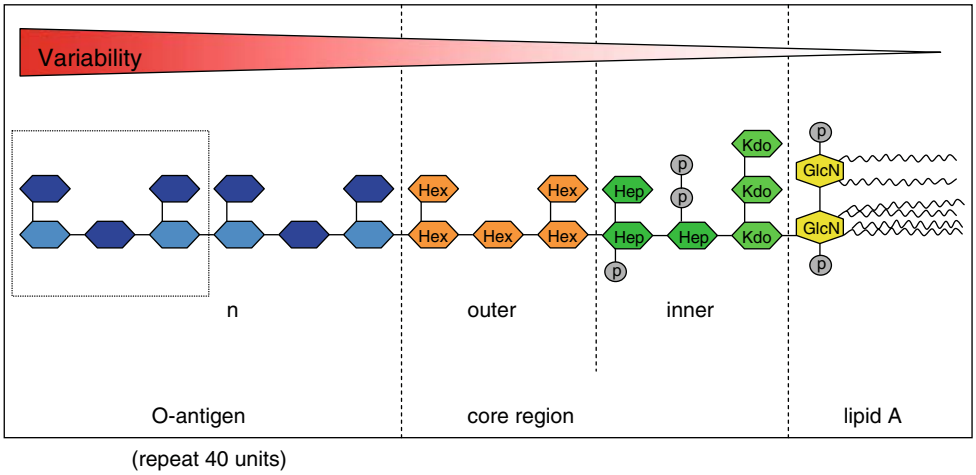

Fig. 1 Schematic structure of lipopolysaccharide

and timing of exposure to endotoxins, other environmental factors and genetic predisposition (Vandenbulcke et al. 2005). Organic dust toxic syndrome (ODTS), an acute non-allergic flu-like illness, may develop due to high heavy endotoxin exposure for example with agricultural seed (Smit et al. 2006). Furthermore, chronic inhalative endotoxin exposure of different degree at various workplaces has been linked to health impairments like fever, headache, nose and throat irritation, chest tightness, cough, shortness of breath, wheezing, acute airway flow restriction and inflammation (Rylander 2006; Douwes and Heederik 1997; Bakirci et al. 2007).

Positive effects have been described especially with respect to development of allergies (von Mutius et al. 2000; Braun-Fahrländer 2002; Eduard et al. 2004). Less well documented are positive effects with regard to cancer risk (Lange 2000; Mastrangelo et al. 2005). Mastrangelo et al. described significantly decreasing odds ratios for lung cancer in 2,561 Italian dairy farmers with increasing number of dairy cattle, assuming endotoxins as important factors stimulating the immune system. Laakkonen et al. (2007) reported a decreased risk of lung cancer among men with exposure to moulds and bacteria as well. It is assumed that endotoxin as a potent stimulator of endogeneous mediators may activate tumoricidal macrophages, cytototxic T cells and natural killer cells via TNF-α. In addition, exposure to certain microbial species may prime the immune system for a particular pattern of response, for example with respect to toll-like receptors (Mastrangelo et al. 2005). However, this is only hypothetical and so far it is not clear whether a causal relationship between endotoxin and cancer protection exists.

By all means, microbial components like endotoxin are necessary for the development of an intact immune system ("microbial pressure"). Not only the adaptive immune response but also innate immune activation is modified by prior events like infection. Due to this "innate imprinting" or "innate education" each human has an unique inflammatory profile (Goulding et al. 2007) Epidemiological studies especially with regard to asthma and infection have shown that exposure to microbial products in early life may prime an upregulation of T-helper 1 and a downregulation of T-helper 2 cells thereby reducing the risk for atopic disorders (Wong et al. 2008).

Environmental and occupational endotoxin exposure

Background levels of endotoxin are significantly higher in industrial areas with biofuel plants than in urban residential areas. In addition, the endotoxin background shows seasonal variations: in springtime higher endotoxin levels were measurable in towns than in autumn and winter (Madsen 2006). In general, background levels of endotoxin in the environment were below 10 EU/m^3 (see Table 1).

Compared to urban residents, exposure to endotoxin is greater among subjects living in rural areas with intensive livestock production (Schulze et al. 2006).

Epidemiological studies showed protective effects of environmental endotoxin exposure with regard to atopic asthma and allergy development in early childhood (von Mutius et al. 2000; Remes et al. 2003; Rylander and Michel 2005). This is consistent with one aspect of the "hygiene hypothesis", describing microbial exposures or infections associated with a lower incidence of atopic disease. The "hygiene hypothesis" has been proposed as a possible explanation for the increasing prevalence of allergic diseases in the western world over the last decades. Reduced microbial stimulation of toll-like receptors in early life may lead to a stronger allergen-induced Th2 response. However, even if children were protected from allergy development, they might develop other health impairments due to endotoxin exposure. Celedón et al. (2007) found among 500 children with risk of atopy an association of endotoxin

Table 1 Examples of endotoxin ranges (rounded off) measured with LAL-test in different environments

Location of sampling (airborne dust)	Endotoxin value	Published by
Outdoor background		
In towns	0.3 EU/m^3	Madsen (2006)
Near biofuel plants	5.3 EU/m^3	Madsen (2006)
In rural areas with intensive live stock production	<3 EU/m^3	Schulze et al. (2006)
Occupational settings		
Composting facility	8–3,000 EU/m^3	Bünger et al. (2007)
Dairy cattle	3–70 EU/m^3	Schierl et al. (2007)
Beef cattle	1,204–1,000 EU/m^3	Schierl et al. (2007)
Turkeys	500–5,200 EU/m^3	Schierl et al. (2007)
Pig cattle	40–7,500 EU/m^3	Schierl et al. (2007)
Pig farm	360–3,200 EU/m^3	Portengen et al. (2005)
Wood industry	40–3,700 EU/m^3	Rylander et al. (2005)
	4–90 EU/m^3	Mandryk et al. (1999)
Cotton mills	30–5,900 EU/m^3	Bakirci et al. (2007)
Environmental settings		
Room floor dust samples of apartments from atopic subjects	150–3,120,000 EU/m^2	Gehring et al. (2004)
Dust samples of mattresses from farming families	35,000–70,000 EU/m^2	von Mutius et al. (2000)

exposure with a reduced risk of atopy but increased risk of wheeze.

Nevertheless, a protective effect of farming with respect to allergy can be observed in adults as well. Eduard et al. (2004) described a lower asthma prevalence in 1,206 farmers compared to 727 urban subjects. Similarly a strong inverse relationship between endotoxin exposure and sensitization has been described for 162 pig farmers (Portengen et al. 2005). A slightly lower proportion of atopics among the operatives exposed to organic dust compared to controls was observed by Rylander and Michel (2005). Gehring et al. (2004) reported an association of house-dust mite exposure with decreased odds ratio of allergic sensitization to inhalant allergens in 350 adults as well.

Nevertheless, health impairments with respect to endotoxin exposure have been substantiated in several studies especially regarding occupational settings. These include for example agriculture work (Schierl et al. 2007), poultry farming (Donham et al. 2000), animal feeding (Heederick et al. 2007), waste collection (Bünger et al. 2007) or wood dust exposure in furniture industry (Jacobsen et al. 2007). Endotoxin values measured in the wood working industry are mostly low (<100 EU/m^3) compared to other workplaces (Schulze et al. 2003). There is evidence that levels of fungi in wood industry influence strongly the occurrence of bronchial symptoms (Rusca et al. 2007).

Endotoxins can increase disease severity acting on its own, causing lung function adverse effects and inflammatory responses or acting as a natural adjuvant to augment asthma and atopic inflammation.

Several studies reported a decrease of FEV_1 (forced expiratory volume in 1 s) and FVC (forced vital capacity) in association with endotoxin exposure (Donham et al. 2000; Jacobsen et al. 2007; Sigsgaard et al. 2004; Wang et al. 2002). It has to be considered that within a single workplace setting exposure to dust and endotoxin varies with working activity. For example, it was shown in a longitudinal study on ten Californian farms that highest endotoxin levels were measurable during cleaning of poultry houses and during machine harvest of vegetables (Nieuwenhuijsen et al. 1999).

Endotoxin exposure of compost workers did generally not exceed 200 EU/m^3 (Bünger et al. 2007). They described in a longitudinal cohort study of 218 compost workers a decline of FVC as well as a higher prevalence of conjunctivitis and mucosal membrane irritation. High concentrations of actinomycetes and other moulds may play a pivotal role for these health effects whereas endotoxin seems to be less important.

Cellular mechanisms induced by endotoxin contact

Studies which differentiated inhalable and respirable dust fractions, described that endotoxins were found in both fractions but with predominance in the inhalable fraction (Donham et al. 2000; Mandryk et al. 1999; Nieuwenhuijsen et al. 1999).

In consequence of respiration airways are permanently in contact with bacteria. Their highly conserved surface structures, called “pathogen-associated molecular patterns”

(PAMPs) are recognized by pathogen recognition receptors (PRRs). Organic dust contains high amounts of these factors like endotoxins and their components (Beutler and Rietschel 2003). After deposition in the airways, endotoxins may interact with macrophages (Fig. 2). This process is accelerated by the binding of LPS to lipopolysaccharid binding protein (LBP) via its lipid A component. LBP is present in the fluid on the airway surface and is part of the unspecific immune defence mechanisms. Together with other proteins, LBP has the task to transport foreign substances to the site of metabolism and destruction, e.g. monocytes and macrophages. Lung macrophages and blood monocytes carry the cell surface protein CD14 to which the LBP-LPS-complex attaches. In addition, supported by factors like CD 14 and the accessory protein MD2, the signal of LBP-LPS attachment is delivered to the toll-like receptor (TLR)4 (O'Neill 2004; Alexis et al. 2005). Furthermore soluble CD14 (sCD14) allows epithelial or dendritic cells to bind endotoxin in spite of the fact that they do not express CD14 constitutively. After internalization of endotoxin, a complex signal transduction cascade is started. A signal complex containing the three adaptor proteins MyD88 (myeloid differentiation factor), TIRAP (Toll/IL-2-Receptor-domain-containing-adaptor protein) and TOLLIP (Toll-Interacting protein) is formed. This complex stimulates phosphorylisation of IRAK (interleukin-receptor-associated kinase) and TRAF6 (TNF-receptor-associated factor). Phosphorylisation of IRAK and TRAF6 leads to activation of nuclear factor-κB (NF-κB) which migrates into the nucleus and induces finally the expression and release of a variety of inflammatory mediators, particularly

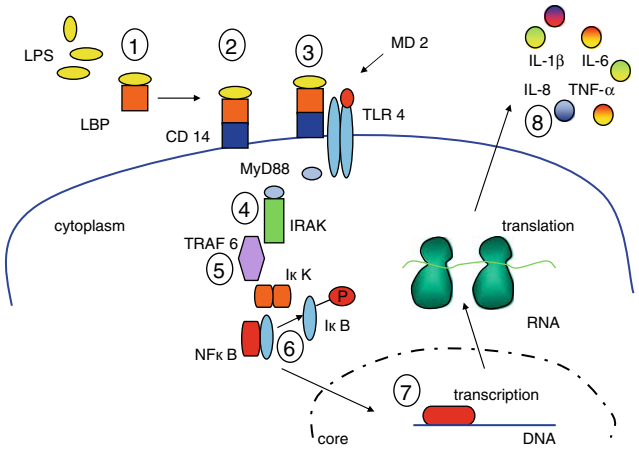

Fig. 2 Schematic view on the cellular endotoxin (LPS) signal transduction pathway. LPS-binding protein (LBP) solved in the plasma ligates at LPS **(1)**. This complex binds to the cell surface receptor CD 14 (**2**). Aggregation of LBP/LPS/CD14 complex with the protein MD2 and the transmembrane toll-like-Rezeptor (TLR) 4 **(3)** induces the signal transduction cascade in the cell **(4, 5)**. Finally transcription factor NFκB is activates **(6)** and starts translation of several genes to proteins, for examples proinflammatory mediators like IL-1β **(7, 8)**

IL-1β, TNF-α and IL-6 (Liu 2002; Douwes et al. 2002). In addition, from animal models it is known that the IL-1 receptor is required for the development of chronic LPS-induced airway diseases although it is not essential for acute response (Brass et al. 2007). Furthermore, complement activation is part of the defence mechanism elicited by endotoxin contact: exposure to organic dust from swine confinements causes activation of human plasma complement factors C3 and B and the synthesis of factor C3 by lung epithelial cells in vitro (Acevedo et al. 2005). In vivo activation of complement could be detected in blood of exposed workers. Gases or ultra fine particles in the swine house may be involved in the activation of complement since wearing a halfmask did not abolish the response. Although LPS is able to activate complement factor C3, dust from swine barns is more potent in stimulation of complement. Endotoxin therefore is one of a variety of complement activators.

Gene polymorphisms

Bacterial signals play an important role in the maturation and development of the immune system. Polymorphisms in genes coding for receptors to bacterial components can modulate the immune responsiveness of the host to microbial agents.

CD14 is an important surface molecule for endotoxin transduction cascade, and its associated gene polymorphisms have been investigated several times without congruent results (Martinez et al. 2007).

Rylander and Michel (2005) studied 146 workers in industries with exposure to organic dust containing endotoxins. Compared to 53 controls they found lower IL-8 sera levels in atopic workers with CD14 (-550) polymorphism. In addition, IL-6 and Eosinophilic Cationic Protein (ECP) values were lower in endotoxin exposed subjects with TLR4/+896 AG polymorphism.

Eder et al. (2005) pointed out that phenotypic plasticity exists, which means that a genotype has the capacity to produce different phenotypes depending on environmental background. They described a CD14/-260 polymorphism in rural children which was associated with less specific IgE levels when high endotoxin exposure due to house dust was measurable. Relationship of endotoxin exposure, CD 14 polymorphism and allergy was highlighted by Simpson et al. (2006) as well. They confirmed that increasing endotoxin exposure is associated with risk of allegic sensitization and eczema but with increased risk of nonatopic wheeze in children with the CC genotype at -159 of the CD 14 gene.

Another important cell surface marker involved in endotoxin recognition is the toll-like receptor. For example

decreased LPS-induced IL-12 (p70) and IL-10 responses as well as a 4-fold higher prevalence of asthma were found to be associated with the TLR4 (Asp299Gly) polymorphism in 115 Swedish children (Fagerås Böttcher et al. 2004).

These data point out, that several polymorphisms play a role within the network of immunologic reactions to endotoxin. Epidemiologic studies may help to identify such risk factors and to design primary prevention strategies. However, successes in the identification of genetic polymorphisms that explain health effects due to endotoxin are limited so far. Results depend on experimental settings and studies which consider exposure, genetic factors and health effects within great study population are very complex. Nevertheless, it will be necessary to investigate gene-environment interaction more detailed rather than isolated genetic interactions to assess the impact of genetic factors on complex diseases.

Methods of bioaerosol description

Inhalative exposure to bioaerosols occur in dusty areas of occupational or environmental settings. Endotoxin is one important component of these exposures and one focus of measurement. In addition, endotoxin measurement plays an important role in the pharmaceutical industry since contamination with endotoxins may cause pyrogenic side effects (Trivedi et al. 2003).

It has to be considered that results of different test systems deliver distinct information: LAL test, whole blood assay and gas chromatography (GCMS) do not measure the same parameters. Whereas LAL test describes endotoxin activity, whole blood assay mirrors pyrogenic activity which may be due to endotoxins but also fungi or other microbial load. GCMS determines 3-hydroxy fatty acids from lipid A. Furthermore, it has to be emphasised, that for description of bioaerosols, first the collection of dust samples has to follow a standardized protocol. For example, usage of detergent, e.g. Tween for filter extraction or whether samples are frozen or not, changes results significantly (Liebers et al. 2007b; Spaan et al. 2007).

Limulus amoebocyte lysate (LAL)-test

At present the *Limulus amoebocyte* lysate (LAL) test is the most accepted assay for endotoxin measurements. This assay is based on the activation of a clotting enzyme present in the lysate of haemolymph of the *Limulus polyphemus* (horseshoe crab). The LAL-assay is adapted as the standard assay for endotoxin detection by the American Food and Drug Administration in 1980. Three methodic types of the LAL-test for a quantitative endotoxin measurement exist: turbidimetric (measuring turbidity), chromogen-kinetic (measuring colour change over time due to enzymatic turnover of substrate) and the endpoint assay (measuring gel clotting). Especially the chromogen kinetic versions of the LAL-assay are very sensitive and have a broad measurement range (appr. 0.01–100 endotoxin units (EU)/mL ≈ 1 pg/mL to 10 ng/mL). A modified LAL-test may be used for β-glucan determination ("Glucatell"). Attention has to be paid, since minute variations in the test instructions may cause changes in the results of this sensitive test already (Chun et al. 2006; Liebers et al. 2007b).

Whole blood assay

Diseases caused by organic dust are mainly of inflammatory nature and the pyrogenic activity of the dust is a characteristic feature (Thorn 2001). This aspect is mirrored using whole blood assay: human peripheral blood cells are incubated with aliquots of sample material potentially containing endotoxin, e.g. dust filter extracts. The amount of cytokines (e.g. IL-1β, IL-6, IL-8, MCP-1) released from the activated blood cells describes the pyrogenic activity of the sample. Generally, IL-1β is measured since this cytokine delivers highest robustness. Using fresh human blood the test is difficult to standardize since the ability to release cytokines is individually different and varies from donor to donor due to gender (Aulock et al. 2006) and genetic predisposition (Wouters et al. 2002; Wurfel et al. 2005). Therefore, the development of cryoconserved blood pools is an important step towards standardization of this test (Kindinger et al. 2005).

Lipopolysaccharides from different bacterial sources elicit different cytokine responses in the whole blood assay (Mathiak et al. 2003). Perhaps, this phenomenon may be used to characterize the different dust composition of bioaerosol samples. However, so far whole blood assay is still a topic of research and not routinely used for description of dust samples.

Gas chromatography and mass spectrometry (GCMS)

Quantitative information about the endotoxin content of dust samples may be provided by GCMS determination of 3-hydroxy fatty acids from lipid A. In contrast to LAL-test these measurements do not reflect the biological activity of a sample. Since different species of bacteria reveal different 3-OH fatty acids, the absolute amount of LPS is not determined with this method as well (Binding et al. 2004). A good correlation between endotoxin determined with LAL-test and the amount of short-chain 3-hydroxy fatty acids exists. However, for long chain fatty acids there was no correlation. In addition this chemical method has a low sensitivity, which makes application limited (Rylander 2006).

Miscellaneous properties of endotoxin exposure and effects

Dust and endotoxin exposure occur in many environments. Inhaled endotoxins contribute significantly to the induction of airway inflammation and dysfunction. Highest exposures from up to 6,000 EU/m^3 are found in agricultural workplaces with animal breeding or handling and during cleaning activities. However, only in few working facilities continuous high airborne exposures are measurable. Furthermore, personal endotoxin exposure varies with kind of activity at the workplace. It has to be considered that within one workplace, e.g. cotton mills the variation in endotoxin exposure is high depending on the working process (Liebers et al. 2007a, see Table 1). Therefore measurements are only suitable to describe endotoxin exposure in clearly circumscribed areas and with regard to the actual working activity.

Although measurement of endotoxins is not completely standardized so far, it is clear that endotoxin exposure can cause acute and chronic health effects (Rylander 2006). Important for individual endotoxin effects are time point, level and duration of exposure as well as individual susceptibility, including for example the individually different expression of CD14 (Alexis et al. 2001; Vercelli 2003). Alexis et al. (2004) showed that healthy people reveal increased IL-13 and decreased IFN-γ levels in sputum after inhalation of 10,000 EU endotoxin, suggesting that low-dose endotoxin challenge skews airway inflammation in a Th2 response in vivo. Nevertheless, lung function decrease following endotoxin exposure is usually not due to allergic sensitization (Walusiak et al. 2004). In fact, exposure especially in childhood may aid to protection for asthma and atopy (Braun-Fahrländer et al. 2002; von Mutius et al. 2000). However, even if atopic asthma has been prevented, endotoxin exposure may contribute to the development of non-atopic asthma (Eduard et al. 2004). Furthermore, studies of metropolitan home living conditions showed that households with detectable allergen levels but relatively low endotoxin levels may provide a predisposing environment for animal allergen sensitization (Gereda et al. 2001). This points out that the impact of endotoxins for humans is manifold and depends on the context of exposure. So far, it has not been clarified whether endotoxin itself is responsible for protective effects regarding allergy or whether it is a surrogate marker which has been spotlighted because measurement is established. However, there is growing evidence from animal studies that bacteria are able to induce a TH1-polarizing program. Isolates from the Gram-negative bacteria *Acinetobacter lwoffii* and the Gram-positive *Lactococcus lactis* activated human dendritic cells in vitro leading to upregulation of the TH1-polarizing cytokine IL-12 p70. Furthermore, suppression of local allergy inflammation could be demonstrated in the mouse model (Debarry et al. 2007). Further investigations are necessary to clarify which microorganisms are predominant in different kinds of dust. Whereas *Lactococcus* and *Acinetobacter* were found in cows shed, airborne bacteria in Chinese outdoor environments were mainly *Micrococcus*, *Staphylococcus*, *Bacillus* and *Pseudomonas* (Fang et al. 2007).

Acute and short time effects have to be distinguished from reactions due to chronic exposure. Synergistic effects, e.g. due to tobacco smoke have to be considered (Sebastian et al. 2006). Muramic acids from bacteria, lipoteichic acid and peptidoglykans from Gram-positive bacteria and (1→3)-β-D-glucan from cell walls of fungi represent further microbial constituents with inflammatory properties. Symptoms due to, e.g. glucan inhalation are different from that measured due to endotoxin inhalation (Rylander 2006), nevertheless effects are overlapping and causative agents not clearly circumscribed.

Field studies usually do not differentiate between effects of single components. However, FEV_1 decrease and inflammatory response due to endotoxin inhalation has been shown in clinical challenge experiments (Kitz et al. 2006) verifying that endotoxin is a potential inducer of adverse health effects.

However, with respect to work-related studies of endotoxin effects it has to be kept in mind that the healthy worker effect may falsify investigations. People with acute health effects due to endotoxin exposure usually change their workplace prematurely, e.g. during their education. Workers who arrange with workplace conditions may develop endotoxin tolerance (Bakirci et al. 2007). Even if detailed cellular mechanisms are not yet clarified, it is clear that human monocytes and macrophages can be induced to become tolerant, showing altered TNF, IL-1 and IL-6 production due to LPS stimulation (West and Heagy 2002). Therefore under special conditions, like continuous exposure, LPS-dependent signal transduction pathway may be altered.

Conclusions

Endotoxin is Janus-faced with regard to human health effects. To clarify its role in various occupational and environmental settings measurement of endotoxins is important. To obtain comparable endotoxin values from different measure settings sampling strategies, extraction protocols and test systems have to be internationally harmonized and standardized (Spaan et al. 2007; Liebers et al. 2006). In spite of the fact, that positive effects of endotoxin have been reported with respect to allergy, health impairments in occupational settings are undoubtful. Therefore preventive measures are highly recommended in areas with estimated high endotoxin exposures, even if accurate measurements

are missing. However, personal susceptibility and individual exposure settings result in non-consistent health effects.

Further research is needed to highlight exposure conditions and synergistic effects of endotoxin and other environmental factors within the human immunological network.

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