

Endotoxin and occupational airway disease

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Purpose of review

This review provides an update on the role of bacterial endotoxin in occupational airway disease, a problem of importance from diagnostic and preventive points of view.

Recent findings

Data from human inhalation studies have increased our understanding of the cell mechanisms underlying diseases related to endotoxin exposure. In addition, knowledge from molecular genetics may help us to identify individuals at risk. Several investigations have demonstrated that, apart from endotoxin, other microbial cell wall agents are also related to the risk for symptoms of occupational lung diseases, with pathogenic mechanisms different to those caused by endotoxin. Diagnostic methods have progressed from traditional lung function measurements to sampling of indicators of inflammation in the blood, nasal lavage and induced sputum. Investigations of a longitudinal design have provided important findings on the relationship between acute and chronic effects as well as exposures of risk and risk factors among individuals.

Summary

Endotoxin, as well as other agents derived from microbes, are important causative agents for occupational respiratory and other diseases, and exposure may occur in a large variety of occupational environments. Recent data from longitudinal studies provide important information on diagnostic and preventive measures.

Keywords

(1→3)- β -D-glucan, endotoxin, inflammation, lung function, microbial cell wall agents

Introduction

Respiratory discomfort and fully developed pulmonary disease are well known effects of occupational exposure to gases, vapours and particles. This review deals with another kind of causative agents – the microbial contamination of dusts – and describes recently published data on how specific microbial agents, particularly bacterial endotoxin, may cause inflammation in the airways and the body as a whole. The patho-physiology behind the inflammatory diseases will be described, as well as the tools for prevention in terms of exposure measurements and medical surveillance.

Historical perspective

The concept that bacterial endotoxin (lipopolysaccharide) is associated with symptoms and disease in occupational settings was first suggested in 1942 [1]. In the 1960s, Italian researchers published clinical as well as experimental studies suggesting that endotoxin is responsible for the peculiar disease called ‘byssinosis’ among workers exposed to cotton dust [2,3]. The development of a method to measure endotoxin in airborne dust samples and thus determine the exposure levels was a breakthrough for the subsequent experimental and field work. In two classic studies in experimental cotton card rooms, studying cotton workers and naïve individuals responsive to cotton dust, subjective reactions, lung function changes and clinical parameters after exposure were related to the amount of endotoxin in the dust but not to dust levels as such [4,5]. Subsequent investigations found that byssinosis and endotoxin were also present in other occupational settings such as wool-processing plants [6]. Endotoxin is now known to comprise an important part of all organic dusts, that is, particles of vegetable, animal or microbial origin.

Organic dust exposures

Organic dusts are present in a wide variety of occupational settings. From the original cotton dust environment, the exposure panorama has now expanded and covers a large variety of occupational settings. An increased prevalence of respiratory symptoms and lung function changes has been reported among operatives from paper factories [7], recycling plants [8], and workers involved in domestic waste collection [9], and mushroom farming [10]. Also the indoor environment has been found to be a source for organic dusts, mainly from microbes growing because of humidity problems or

Curr Opin Allergy Clin Immunol 6:62–66. © 2006 Lippincott Williams & Wilkins.

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Current Opinion in Allergy and Clinical Immunology 2006, 6:62–66

Abbreviations

MCWA microbial cell wall agent
TLR toll-like receptor

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1528-4050

due to storage of organic waste [11]. Even if the initial source of the organic dusts can be defined as vegetable or animal particles, there is generally a concomitant presence of microbes. The type of microbial growth that takes place during humid conditions is determined by the particular microenvironment.

Effects of endotoxin

The effects of endotoxin after inhalation are well known and reviews of the physiological responses and the cell toxicity have been presented [12,13^{••},14[•]]. Considering its inflammagenic effects, exposure to endotoxin can be associated with several different clinical aspects of occupational lung disease. Toxic pneumonitis is characterized by an acute increase in body temperature, shivering and influenza-like symptoms. It often develops in the afternoon or evening after a heavy exposure, such as cleaning a barn or repairing dusty equipment. The symptoms have usually gone by the next day. This clinical entity, previously referred to as the organic dust toxic syndrome, can be reproduced after inhaling pure endotoxin.

Irritation in the nose and airways and chronic airways inflammation with increased airway responsiveness can also be related to the inflammagenic properties of endotoxin [12]. Less recognized but well documented in experimental and epidemiological studies are the systemic effects in terms of fatigue, joint pains and gastrointestinal symptoms. The inflammatory reactions are less pronounced after repeated exposures, as illustrated in a study among swine confinement workers [15[•]]. This reflects the well known adaptation to endotoxin.

An important finding in our understanding of endotoxin toxicity has been the discovery of the toll-like receptor (TLR) family [16,17^{••}]. The presence of these receptors demonstrates that the body has a recognition and defence system against a variety of microbial molecular structures, often referred to as pathogen-associated molecular patterns. The TLR receptors trigger the secretion of inflammatory cytokines in the cell and inflammation develops at the site of action as well as in other parts of the body.

Following the recognition that the TLR-4 receptor plays an important role in endotoxin-induced inflammation, an increasing number of studies have been carried out dealing with polymorphisms in the gene controlling for TLR-4. In acute inhalation experiments using pure endotoxin, heterozygotes for either TLR-4/+896 or TLR-4/+1196 were found to have significantly lower numbers of white blood cells and reduced amounts of C-reactive protein compared with homozygotes with the common allele [18[•]]. Data from animal studies suggest that other genes apart from those regulating TLR-4, such as CD14 and MyD88, are important in the inflammatory response to endotoxin [19,20].

An interesting question for future studies is whether this blunted response to endotoxin indicates a lower or higher risk for respiratory disease. Conceptually, a lower degree of inflammation would seem beneficial, but on the other hand it implies that the body's capacity to respond to an inflammagenic agent is reduced, which may imply a higher risk. Support for this concept is found from studies on individuals with atopic sensitization. In acute inhalation experiments with pure endotoxin, atopics had a lower increase in temperature and blood neutrophilia as well as forced expiratory volume in 1 s (FEV₁) [21]. When atopics were exposed to organic dusts, however, the prevalence of lung function changes was higher, indicating an increased risk for occupational lung disease [22,23[•]]. Further longitudinal studies are thus required examining atopic sensitization and TLR-4 gene characteristics in newly hired employees exposed to endotoxin in occupational environments. As symptoms as well as acute changes in pulmonary function can develop within a year from starting work and as they are related to risk for chronic effects [23[•],24], such studies would be relatively easy to perform.

Several longitudinal studies have now demonstrated that the long-term pulmonary effects, for example a faster decrease in FEV₁ over the years, are related to the content of endotoxin in the dust, for example, from grain [25] and cotton [26^{••}]. There is also evidence that, apart from the acute effects on pulmonary function, respiratory symptoms are a good predictor for lung function changes that follow [27,28]. This implies that measurements of workplace exposure and medical surveillance of newly hired workers are suitable methods for identifying individuals at risk for chronic changes.

Apart from the direct inflammatory response to endotoxin, attention has been brought to the influence of this exposure on atopic sensitization. The origin of this concept was derived from studies on children growing up on farms, where it was found that they had a lower prevalence of atopic sensitization [29^{••}]. The underlying mechanism could be that, although inflammagenic properties of endotoxin cause symptoms of inflammation such as cough and bronchoconstriction, the inflammation causes a decrease in the reactivity to allergens by promoting a Th1 lymphocyte reaction pattern. As a consequence, the risk for sensitization, measured either as the skin prick test or as specific IgE antibodies in serum, would be less prevalent among persons exposed to endotoxin. Some data suggest that the same mechanisms may operate in occupational environments. In a study on 142 workers exposed to different organic dusts containing endotoxin and 53 controls, the proportion of individuals with atopic sensitization among the organic dust exposed group was 22% compared with 34% among the controls. In a study on Norwegian farmers, exposure to endotoxin

and fungal spores was negatively associated with atopic asthma [30^{*}]. These and similar data in previous studies have commonly been explained as due to the 'healthy worker effect'. However, one study on Chinese cotton workers reported that, among those leaving work, the proportion of atopics was no higher [24]. Further research is required to evaluate whether long-term occupational exposure to dusts containing endotoxin reduces the prevalence of atopic sensitization.

Other exposures

Although endotoxin is a common microbial constituent found in most organic dust environments, data accumulated over recent years have demonstrated that it is not the only microbial cell wall agent (MCWA) that has biological activities – there is a whole series of such agents in organic dusts. Muramic acid from bacteria, lipoteichoic acid and peptidoglycans from Gram-positive bacteria, mannan from yeasts, chitin (N-acetylglucosamine) from fungi and (1→3)- β -D-glucan from cell walls of fungi and certain bacteria are examples of such agents.

Of these agents, considerable interest has been directed towards the role of (1→3)- β -D-glucan in the development of occupationally related symptoms and disease. Inhalation toxicity studies with doses relevant for occupational exposures demonstrate that this agent does not cause a neutrophil invasion in the airways and persons inhaling pure (1→3)- β -D-glucan do not develop symptoms typical of endotoxin inhalation [31^{*}]. Although the information is as yet meagre, it is likely that (1→3)- β -D-glucan is a suppressor of the immune system, rendering the organism more vulnerable to exposure to inflammagenic agents or sensitization after exposure to antigens. High fungal concentrations (and thus (1→3)- β -D-glucan) were found in a fur factory where workers had an increased prevalence of symptoms of the airways and also radiograph abnormalities which would be consistent with exposure to mould and (1→3)- β -D-glucan but not to endotoxin exposure [32]. Workers exposed to (1→3)- β -D-glucan when collecting household waste had increased numbers of lymphocytes in the blood and subjective symptoms of airway irritation [33]. Among waste handlers, swelling of the nasal mucosa was associated with exposure to (1→3)- β -D-glucan [34].

The specific MCWA exposure at a particular workplace is determined by the ecological characteristics. In cotton mills, endotoxin dominates because of the high number of Gram-negative bacteria growing on the cotton plant in the field and the rejection of mould-stained cotton bales from a quality control point of view. In sewage treatment plants there is a dominance of Gram-negative bacteria due to the normal microbial flora found on surfaces and in the soil, which mainly consist of *Enterobacter* and *Pseudomonas* species. In wood processing, the

microenvironment favours the growth of fungi, which suppresses the growth of Gram-negative bacteria. In organic waste, storage conditions determine the microflora – storage in paper sacks favours the growth of fungi whereas storage in plastic bins favours the growth of Gram-negative bacteria. Work site characteristics are also important. In sewage treatment plants high levels of endotoxin have been reported from sites where the water or the sludge is being agitated [35]. At other sites such as basins, the levels were found to be very low. For some occupational environments, there is also a seasonal variation in exposure. Household waste collectors in Sweden have a higher exposure to (1→3)- β -D-glucan during the summer months and a relation was found between daily temperature and exposure levels [36]. A relation between temperature and sunlight hours and farmer's lung disease was reported in a study from Japan [37]. These observations illustrate that the risk for symptoms and other exposure-related effects are higher at specific work sites or at certain times of the year, which is important when planning exposure measurements or medical surveillance.

The presence of different MCWAs means that it is difficult to compare relationships between various medical effects and endotoxin as measured in field studies. Endotoxin is only one of a multitude of biologically active agents that are present in organic dusts. Conclusions on causality can only be drawn if the observed reaction is unique for endotoxin or if other agents are controlled for. None of these conditions is usually present in field studies. Deductions on causality may be supported by performing repeated studies in different environments with precise determinations of the exposure dose or thorough dose-response relationships. These considerations do not, however, invalidate the possibility of using endotoxin as a marker for risk, whether as a surrogate or the true causative agent.

Diagnostic procedures

Early studies on organic dust-induced disease comprised mainly measurements of lung function. Symptoms also played an important role and Schilling's classification of byssinosis initially relied exclusively on symptoms typical among workers in cotton mills – Monday morning chest tightness [38]. With the increased understanding that the response to organic dusts and endotoxin is inflammation, indicators of this have been found in blood, nasal lavage, induced sputum, and broncho-alveolar lavage. Among waste handlers in Norway, the proportion of neutrophil leukocytes in nasal lavage during the working week correlated with increases in eosinophilic cationic protein, which was present among atopics as well as nonatopics, and myeloperoxidase and nasal swelling, measured by acoustic rhinometry [34]. On the final working day, levels of myeloperoxidase and IL-8 were associated with

exposure to endotoxin and fungal spores, measured the previous day. Similar findings were reported in a study on domestic waste collectors in the Netherlands in which the concentration of IL-8 in nasal lavage after a shift was related to the endotoxin exposure and the number of cells, and IL-8 levels were associated with respiratory symptoms [9]. Specific measurements of endotoxin binding protein CD14 have also been used as a marker of exposure and increased levels were found among cotton mill workers after a 6 h shift [39]. An evaluation of the different clinical measures to assess the presence of inflammation was carried out at a recent international workgroup meeting. It was concluded that many of these tests could, at present, only be used on a group basis or diagnostically for an evaluation during follow up of patients who received treatment or were removed from workplace exposure [40^{••}]. It was recommended that further work should focus on dose–response relationships, both for short-term and long-term effects.

Measurements and guidelines

The standard method for measuring endotoxin in the environment is the Limulus assay. This is a biological test with very high sensitivity. Although the reproducibility within the laboratory is quite good, the variation between laboratories can be rather high [41]. In view of this, there are at present no official guidelines, although values for no effect levels have been suggested (toxic pneumonitis = 200 ng/m³, airways inflammation = 10 ng/m³, both for 8 h exposures) [42]. Attempts have been made to use chemical methods to determine the levels of 3-hydroxy fatty acids, substances characteristic for endotoxin. Recent data have demonstrated that there is a good correlation between the Limulus-determined endotoxin levels and the amount of short-chain 3-hydroxy fatty acids; for longer chains, however, there was no relation or even an inverse relationship [43]. The major drawback for the chemical method is low sensitivity, which makes it difficult to use when analysing airborne samples, unless large sampling volumes are collected.

Conclusion

Endotoxin has been firmly established as a major causative agent for the inflammatory disease seen in occupational environments with organic dusts. The pathogenesis is relatively well known and the association to different clinical entities is supported by toxicity data. There is, however, increasing information that other MCWAs may also cause occupational lung disease. Although the clinical endpoints in terms of symptoms of inflammation may be the same, the underlying pathogenesis may be different, such as for (1→3)- β -D-glucan. For prevention, early diagnosis through medical surveillance using standardized symptom questionnaires and exposure determinations are essential tools.

Acknowledgements

Our work on microbial cell wall agents and disease in experiments and field studies has been supported by the Swedish Council for Working Life Research, FORMAS, Wilhelm and Martina Lundgren Scientific Research Fund, the Swedish National Foundation for Heart and Lung Research and Vårdalstiftelsen, Sweden.

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