IS BYSSINOSIS RELATED TO CD-14 GENEPOLYMORPHISM?

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Abstract

Exposure to cotton dust causes an airways inflammation with Monday morning chest tightness and decreases in spirometry as hallmark effects. The response has been related to the content of endotoxin in the dust and symptoms of byssinosis have been reproduced after exposure to pure endotoxin. An important prerequisite for the effects of endotoxin is the binding to the cell surface. This takes place through interaction between endotoxin and the receptor CD14. A recent study described a polymorphism in the CD14 gene in terms of a single C: T substitution in the flanking region of the gene. The frequency of allele C was about 50% in a Caucasian population. Homozygotes for the C allele (C:C) and C:T heterozygotes had lower than normal serum levels of soluble CD14. Blood cells from these persons were also low producers of IFNg and high producers of IL-4 when stimulated *in vitro*. It is suggested that symptoms after inhalation of endotoxin and cotton dust are related to sCD14 polymorphism. Studies to test this hypothesis are in progress.

Introduction

Byssinosis is an airways inflammation which may develop after the exposure to cotton dust. The frequency of acute as well as chronic effects is related to the amount of endotoxin in the dust. Even at high exposure levels there are always a significant proportion of the workers that have no symptoms or no changes in spirometry. This paper will present the hypothesis that the absence or presence of effects and disease could be related to a polymorphism in the gene coding for the protein CD-14 – an important part of the mechanism behind endotoxin toxicity.

Byssinosis

It is almost 200 years ago when a peculiar disease among workers processing cotton was described by Patissier in France [Patissier 1822]. Based on a number of epidemiological and experimental investigations over the following century, the characteristics of the disease were defined in the so called Manchester criteria [Rylander *et al* 1987]. According to these, exposure to cotton dust may cause mill fever, an acute reaction with influenza like symptoms and respiratory symptoms, nowadays referred to as toxic pneumonitis. Furthermore, pulmonary function may be altered, most often measured as a decrease in the forced expiratory volume in one second (FEV_1), either over the shift or permanently in relation to the predicted value. An important symptom is chest tightness, which is the hallmark of byssinosis as originally described in epidemiological studies, particularly by Schilling and co-workers [Schilling 1956; Roach and Schilling 1960].

This symptom comprises a pressure over the chest but without the air hunger in asthma and occurs on Mondays or the first day after return from an absence from work; nowadays more often after exposure during a particularly dusty operation such as cleaning of machines. Chest tightness is not directly related to bronchoconstriction. The airway responsiveness is increased and the prevalence of chronic bronchitis may be increased among the exposed workers.

Studies during the 1980s and 1990s show that a similar symptomatology is caused by exposure to a variety of organic dusts such as in swine confinement buildings, when handling grain or during farming.

It is now increasingly realized that the symptoms described above are caused by a cell-mediated inflammation. No evidence for an allergic reaction or an IgE mediated mechanism has been presented. Some studies have evaluated the inflammatory hypothesis using cotton dust but most of the information on humans is available from exposures to other organic dusts. Some early animal studies demonstrated an increase in the cytokine TNFa in guinea pigs exposed to cotton dust [Ryan and Karol 1991]. Other studies evaluated the activation of blood coagulation in terms of procoagulant factor and it was shown that blood monocytes from cotton workers had a higher activity of procoagulant factor (PCA) [Beijer *et al* 1990]. The degree of PCA was related to the decrease in FEV_1 over the work shift. Several studies have demonstrated that exposure to organic dusts cause an increase in the amount of inflammatory markers such as myeloperoxidase and eosinophilic cationic protein as well as inflammatory cytokines such as TNFa, IL-8, IL-10 and IL-12, measured in blood, lung lavage or induced sputum [Sandström *et al* 1992; Schwartz *et al* 1985; Rylander *et al* 1999].

It is common experience from field as well as experimental studies that there are large variations in the reactions between individuals. At the same dust levels certain workers may report chest tightness and respiratory symptoms and experience decreases in FEV_1 whereas others will be completely without symptoms [e.g. Haglind *et al* 1981; Haglind and Rylander 1984; Fishwick *et al* 1996].

Further information on the characteristics of reactive persons is found in some experimental studies. Atopic individuals had

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a more pronounced lung function response after exposure to cotton dust than non-atopics [Sepulveda *et al* 1984]. Changes in spirometry after cotton dust exposure have been related to atopy among exposed subjects and the presence of cytotoxic T-lymphocytes [Beijer *et al* 1995].

Relation to Endotoxin

A role of endotoxin in disease caused by cotton dust was first suggested by Pernis and colleagues in Italy [Pernis *et al* 1961] and Cavagna and colleagues [Cavagna *et al* 1969]. Further work was facilitated when the Limulus test to measure the content of endotoxin in cotton dust became available in the 1980s.

Dose-response relationships with endotoxin and spirometry among cotton workers have been shown. In one classic experimental design, five cottons of different origins and with different amounts of Gramnegative bacteria and hence endotoxin were used. In one set of experiments, cotton workers were exposed to dust from the different cottons during a 6 hour simulated shift [Rylander *et al* 1985]. There was a relation between the amount of airborne endotoxin and the decrease in FEV_1 over the work shift as well as the increase in blood neutrophils. The FEV_1 decrease was more pronounced among the smokers.

In another cardroom experiment, naïve subjects were recruited and pre-screened for reactivity to cotton dust [Castellan *et al* 1987]. Those who responded with a decrease of 5% or more in FEV_1 were selected for the study, involving a 2-hour exposure to the different dusts. It was found that the decrease in FEV_1 related to the amount of airborne endotoxin but not to dust levels.

Endotoxin Toxicity

The mechanisms behind the effect of endotoxin have been extensively studied and several reviews have been presented [e.g. Ulmer 1997]. Regarding the lung, the most widely researched cell population that responds to endotoxins is the monocyte/macrophage group although epithelial cells and dendritic cells are also known to respond.

Inhalation of endotoxin or endotoxin containing dusts will stimulate alveolar macrophages to produce a variety of cytokines such as interleukin-1, IL-6, IL-8, tumour necrosis factor alpha (TNFa) and others. These cytokines, when produced in moderate amounts, initiate beneficial inflammatory reactions e g moderate fever, activation of defense cells and microbicidal mechanisms and initiation of acute phase reactions. Large amounts of these products, however, have harmful effects and cause cell damage and functional collapse.

The endotoxin activation of cells takes place by membrane proteins. CD-14 is the prominent endotoxin binding structure on monocytes/macrophages. Lipopolysaccharide binding protein (LBP) plays an important role in forming a complex with LPS that facilitates attachment to CD14. In addition to membrane bound CD14, there is also a soluble form (sCD14). sCD14 is present in normal serum but in increased amounts during an acute phase reaction. It is likely that sCD14 is particularly important for activation of cells lacking membrane bound CD14 such as endothelial and denditric cells (DC).

After repeated exposure to endotoxin, there is an adaptation process, characterized by a decreased infiltration of neutrophils and a limitation of the response to the airway epithelium, in contrast to the lung tissue engagement that is present in toxic pneumonitis. The underlying mechanisms are probably secretion of inflammatory inhibitors such as IL-10.

A cessation in the exposure will lead to the reappearance of the acute reaction in a short time, either by cessation of secretion of the inflammatory inhibitors or the accumulation of activators that were previously depleted by the continuous exposure. Clinically, the reappearance of the acute reaction takes the form of Monday fever or of chest tightness, present among cotton workers after the weekend absence from work. In work conditions where no weekend break was taken such as in Hong Kong in the 1960s, Monday morning symptoms were not present.

In summary, the field studies on the effects of cotton dust as well as experimental studies on inhaled endotoxin demonstrate that there are differences in susceptibility between individuals in acute as well as chronic exposure conditions. Persons with a pre-existing inflammation in the airways, either induced by smoking, asthma or atopy generally have a higher response. It is possible that the variations between individuals are related to functional differences in the mechanisms behind the inflammatory response induced by endotoxin.

The CD-14 Gene

A recent study among children in the US described a polymorphism in the CD14 gene in terms of a single C-to-T transition at base pair –159 from the major transcription start site [Baldini *et al* 1999]. The frequency of allele C was about 50% in a sample of 513 children. TT homozygotes had significantly higher sCD14 levels than carriers of CC and CT genotypes. TT homozygotes who were atopic, had significantly lower levels of IgE. Subjects with high INFg secretion from stimulated blood cells had higher levels of sCD14. Subjects with high IL-4 secretion had lower levels of sCD14.

Hypothesis

Against the above background, the following hypothesis can be formed.

There is a relation between CD14 gene polymorphism and the reactions after exposure to cotton dust because:

- Persons with a CD14/-159 T allele have a lower amount of sCD14 which is important in the initiation of the inflammatory response brought about by endotoxin.
- Persons with a CD14 –159 T allele have a smaller proportion of atopics, which is related to reactivity to cotton dust and endotoxin.

How the polymorphism might affect the reaction is uncertain. Michel *et al* [2000] have recently shown that the systemic response after inhalation of endotoxin is more marked among non-atopics. As a consequence persons with a CD14-159 allele would have a more pronounced toxic reaction in terms of fever, leukocytosis and inflammation in the airways after exposure, initiated by lack of sCD14. Alternatively, a lack of sCD14 could imply a less severe initiation of inflammation and fever.

Endotoxin stimulation of DC results in substantial IL-12 production, an important cytokine for the differentiation of T lymphocytes into Th1 type [Snijders *et al* 1998]. On this base, an improper ability of endotoxin to initiate activation of DC as well as macrophages could result in the development of Th2-type lymphocytes. These excrete IL-4 and switch the B cell immunoglobulin production towards an IgE-response to environmental allergens. CC and CT genotypes had a lower excretion of sCD14 and persons with a low sCD14 had higher secretion of IL-4. Gene polymorphism could thus be a risk factor for atopy and possibly allergy.

The above concepts can be tested in field and experimental studies by comparing the effects of exposure in persons with different gene structures. If the hypothesis can be proven, it would have implications also for the understanding of disease caused by other kinds of organic dusts such as during farming, garbage collection, working in sewage treatment stations and saw mills.

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