EXPOSURE TO COTTON DUST AND ENDOTOXIN CAUSES A SIGNIFICANT CHANGE IN MONOCYTE CD14

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Abstract

We have studied changes in the mean linear fluorescence of monocyte CD14, the endotoxin receptor, in a group of 25 cotton workers (mean age 41 years). Workers were placed into one of 4 exposure categories. Monocyte CD14 fluorescence was measured by flow cytometry, on samples taken on Monday morning (CD14 pre), after 6 hours (CD14 +6) and after 72 hours (CD14 +72). Workers in the higher exposure categories 3 (between 10-100 EU/ m^3) and 4 (between 100-400 EU/ m^3) showed significantly increased CD14 expression after 6 hours, rising from a mean of 5.58 at baseline to 8.76 at 6 hours (p=0.01) in group 3, the corresponding figures for the highest exposure group being 6.61 and 9.82. All CD14 values returned to near baseline levels when measured after shift on the fourth working day. We propose that CD14 expression on monocytes may be used to monitor workers exposed to endotoxin and may help to determine the mechanism of action of lipopolysaccharide in producing respiratory ill health.

Introduction

Endotoxin is ubiquitous, and exposure occurs at high levels in certain defined occupations (Simpson *et al*, 1998). Endotoxin comprises the lipopolysaccharide (LPS) component of the cell walls of gram negative bacteria, and it is the lipid A component of this molecule which appears to be the most important with respect to biological activity and associated disease (Jacobs *et al*., 1997). Many of the occupations where there are high levels of endotoxin exposure are associated with organic dust exposure (Simpson *et al*, 1998), and include cotton textile workers, hemp textile workers, farmers, swine workers, grain handlers, poultry farmers, sewage processing, and waste handling. In addition, high level exposure is associated with the condition organic dust toxic syndrome. The levels of airborne endotoxin measured in these differing and diverse occupations range from 0.1-75000 ng/m^3 (Swan *et al*., 1999). Ideally, endotoxin exposure should be limited to the lowest reasonably practical level, and certain authorities have recently suggested the establishment of occupation exposure standards (Heederick and Douwes, 1997). Whilst exposure level setting may lead to a reduction in workplace levels, it is important to gain understanding of how endotoxin exerts its effect in humans. Acute exposure may result in a range of health effects such as fever, chest tightness and cough (Zock *et al*., 1999). The importance of longer-term low dose exposure is not known, but short-term exposure studies have demonstrated that endotoxin is able to produce an inflammatory response in the airway, associated with neutrophil and sputum IL-8 production (Nightingale *et al*., 1998). Therefore, it is likely that repeated exposures over longer periods of time could be associated with chronic adverse health effects. With this in mind, endotoxin has been implicated in the aetiology of byssinosis, a respiratory condition characterised by Monday chest tightness which in some cases may lead to permanent lung damage in workers exposed to cotton, hemp, sisal and jute (Parkes, 1994; Fishwick *et al*., 1992).

Previous work by our group has shown that CD14, the lipopolysaccharide cell surface receptor, is upregulated on monocytes, in response to in vitro challenge with LPS (Swan *et al*., 1998) and that expression of CD14 peaked between four and six hours after exposure to endotoxin in a dose dependent fashion. In this study we describe a workplace investigation of cell surface molecule changes in a group of cotton workers exposed to endotoxin, and a group of non exposed control workers.

Methods

Study Population

Twenty-five current cotton workers from two spinning mills and nine non-exposed laboratory scientists were recruited to participate in the study. All volunteers in the study were asked to give a blood sample prior to the working shift on Monday, after six hours of work on that Monday and again on the following Thursday, six hours into the working shift. In the case of the cotton workers, all carried out their normal tasks, and all were exposed to their "normal" work environment.

Endotoxin Exposure. Previous data from each of the two cotton mills (personal breathing zone dust estimations) were available for all the cotton workers in the study. Each worker was then placed into one of four *a priori* defined endotoxin exposure groups according to current exposure; group 1 – less than 1 EU/m^3, group 2 – between 1 and 10 EU/m^3 , group 3 – between 10 and 100 EU/m^3 and group 4 – between 100 and 400 EU/m^3. The control group is subsequently referred to as group 0.

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Flow Cytometry

Ten millilitres of whole blood was collected at each time point from the volunteers EDTA as an anticoagulant (Becton Dickinson Vacutainer systems, USA). One hundred microlitres of EDTA treated blood was added to LP4 tubes containing the appropriate amount of each labelled antibody; appropriate isotype control antibody conjugates were included to establish background fluorescence. Each tube was then incubated for 25 minutes at room temperature in the dark. Following red cell lysis, samples were analysed on a calibrated Epics-XL flow cytometer (Coulter Electronics). Lymphocytes and monocytes were distinguished from cell debris and other cell types by CD14/CD45 gating. 10,000 events were collected for each lymphocyte sample, 5,000 events for monocytes. Data for CD14 was expressed as mean linear fluorescence.

Data Analysis

All data were cleaned and analysed using SPSS (Base 8 for Windows) statistical package. Arithmetic means have been compared between groups. The differences between group means have been explored using both independent samples t testing and one way analysis of variance (ANOVA), and changes within each group with time have been expressed using two tailed paired sample t tests. Underlying normality of the data has been assumed for comparisons of these groups.

Ethics

Ethical approval for this study was obtained through the Local Regional Ethics Committee of South Manchester and all subjects gave a written informed consent to participate.

Results

Study Population

Twenty five cotton workers, with a mean age of 41 years, took part in the study. In addition, 9 laboratory scientists, with a mean age of 32 served as the non exposed group 0. Thirteen of the cotton workers (52%) and 5 of the non exposed controls (56 %) were male. Six of the cotton workers (24%) had previously complained of at least one work related respiratory symptom (worse at work or improving on rest days) and one worker (4%) currently complained of symptoms suggestive of byssinosis. Three workers were, on the basis of current exposure placed in exposure group 1, six in group 2, twelve in group 3 and four in group 4.

Changes in Cell Surface Molecule Expression

Figure 1 shows that the expression of CD14 was not significantly different in exposure groups 1-4 compared to the control (Group 0) population, except for group 1 ($p=0.02$) where the expression was noted to be lower as judged by t testing. One way ANOVA noted a significant difference between all baseline means ($p=0.045$).

There was a non-significant increase in CD14 after 6 hours for workers in exposure groups 1 and 2 (up to an estimated exposure level of 10EU/ m^3). Workers in group 3 showed a significant increase in CD14 at 6 hours ($p=0.01$), and a non significant increase in CD14 was seen in exposure group 4 ($p=0.08$) after 6 hours. If those in groups three and four were combined, the CD14 MLF seen at 6 hours was significantly increased in comparison to baseline ($p=0.001$). After 72 hours, CD14 expression returned to near baseline levels in all groups, with exposure groups 0 and 4 showing a significant drop below the baseline levels ($p<0.001$ and $p=0.01$ respectively). To further investigate the relationship between CD14 and cotton dust exposure, the percentage increase in CD14 at 6 hours (CD14PIN), in comparison to baseline, was calculated for all cotton workers. There was no significant relationship between time in years exposed to cotton and CD14PIN ($r= - 0.345$, $p=0.161$), although there was a tendency for CD14PIN to decrease with increasing total time spent in the cotton industry. Again, the mean CD14PIN was lower in those cotton workers with current work related respiratory symptoms (n=6, 34.8%, SD 39.5) in comparison to those with no current work related symptoms (69.1%, SD 59.7), although this difference did not reach statistical significance ($t=-1.29$, $p=0.213$).

Discussion

We have chosen to study various candidate cell surface markers that are associated with airway inflammation, and a putative endotoxin receptor in a group of workers exposed to varying concentrations of cotton dust and endotoxin, and compared these markers to those found in a non exposed working population. To our knowledge, this has not been previously reported.

The clinical conditions associated with organic dust exposure are complex, although byssinosis and organic dust toxic syndrome are the two best characterised. Byssinosis is a lung disease associated with organic dust exposure, and is characterised by the presence of chest tightness on the first working day, reducing in intensity as the week progresses. Physiologically, this condition is characterised by airway inflammation and airways obstruction, with variable amounts of sputum production (Parkes, 1994).

The periodicity of these symptoms, being most severe on the first working day after a break in exposure during rest days, is not currently explained by any model of exposure or known mechanism. Approximately one quarter of the cotton workers in this study had recently reported work related lower respiratory symptoms and one had byssinosis. These figures are consistent with the current expected levels in this industry.

Monocyte cell surface CD14 was chosen as the main endpoint for investigation as this has been shown to be increased following in vitro challenge of peripheral blood with endotoxin (Swan *et al*., 1998). This study has shown that monocyte cell surface CD14 expression is upregulated in response to 6 hours work exposed to levels of endotoxin above 10EU/m^3. This upregulation does not persist at 72 hours during the working week, and was not seen in non-exposed individuals. In fact, CD14 expression fell at 6 hours in the non exposed group. At 72 hours, CD14 levels were significantly lower than preshift Monday values in the lower exposed groups and this was also true, although not statistically significant, for those in the higher exposed categories.

Interestingly, the baseline measure of CD14 in the non-exposed group was higher than any of the exposed groups of workers. The small size of this study limits interpretation of this finding, although it might be hypothesised that continuous chronic exposure to endotoxin may down regulate basal CD14 expression, with upregulation occurring rapidly after exposure, following a break in exposure.

The changes seen in CD14 expression appear to mirror the changes in symptoms seen classically in byssinosis, and although the study is small, suggest that LPS interaction with CD14 may be an important part of the mechanism responsible for this disease. As upregulation may lead to the production of pro-inflammatory cytokines (Swan *et al.*, 1998), chronic exposure may lead in certain individuals to the disease state, characterised by airway inflammation. Once initiated, further exposure to LPS may increase airway inflammation mediated by CD14, and cause an increase in symptoms, seen classically and maximally through the first working day, attenuating as the working week progresses.

This study was unable to show any significant relationship between the magnitude of CD14 response at 6 hours and either duration of cotton dust exposure or the presence of work related symptoms, although the data suggest that upregulation relates to short term exposure and the absence of symptoms. This finding may well be explained by the fact that these "susceptible workers" may leave the industry early, leaving a survivor population of workers, able to tolerate the working environment long enough to subsequently develop respiratory symptoms.

Whilst these data are the result of a preliminary study to assess changes in cellular responses, they suggest that CD14 expression may be a useful tool to assess individual responses to endotoxin exposure in the workplace.

References

Fishwick D, Fletcher AM, Pickering CAC, Niven R, Faragher EB. Lung function, bronchial reactivity, atopic status and dust exposure in Lancashire cotton mill operatives. *American Review of Respiratory Disease* 1992; **145**: 1103-1108.

Heederik D and Doues J. Towards an occupational exposure limit for endotoxins? Ann Agric Environ Med **4**:17-19

Jacobs RR, Heederik D, Douwes J, Zahringer U. Endotoxin Structure. Int J *Occ Environ Health* 1997;**3**:S6-S7.

Nightingale JA, Rogers DF, Hart LA, Kharitonov SA, Chung KF, Barnes PJ. Effect of inhaled endotoxin on induced sputum in normal, atopic and atopic asthmatic subjects. *Thorax* 1998;**53**:563-571.

Parkes WR. Occupational Lung Disorders. 3rd Edition. Butterworth-Heinemann Ltd, Oxford 1994.

Simpson JCG, Niven R, Pickering CAC, Oldham LA, Francis HC. Prevalence and predictors of work related respiratory symptoms in workers exposed to organic dusts. *Occup Environ Med* 1998:**55(10)**:668-72.

Swan JRM, Crook B. Review. Occupational exposure to endotoxin. HSL internal report 1999.

Swan JRM, Curran AD, Beckett P. The potential of a monocyte cell surface marker as an indicator of endotoxin exposure. *Biomarkers* 1998;**3**:73-79.

Zock JP, Heederik D, Brunekreef B. Influence of shift work and host factors on endotoxin-related acute peak flow changes. *Am J Respir Crit Care Med* 1999;**159**:137-142.

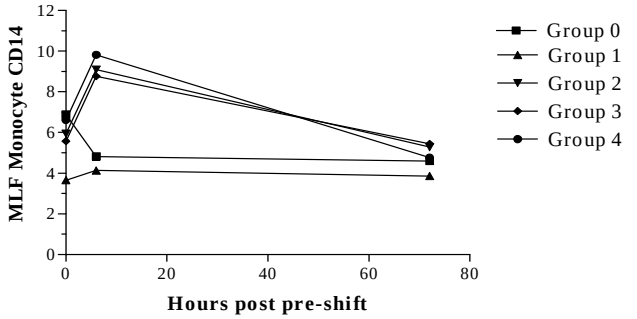

Figure 1. Mean CD14 MLF in each exposure group. Baseline, 6 hour and 72 hour data. All p values relate to change from baseline at 6 hours. Group 0 p=0.002, Group 1 p=0.38, Group 2 p=0.15, Group 3 p=0.01, Group 4 p=0.08.