RELATIONSHIP BETWEEN CD14 EXPRESSION ON MONOCYTES, SPIROMETRY AND SYMPTOMS ACROSS THE WORKING WEEK IN ENDOTOXIN EXPOSED COTTON WORKERS.

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Abstract

We studied the relationship between CD14 expression on monocytes and lower respiratory tract symptoms and lung spirometry in endotoxin exposed cotton workers. Spirometry and CD14 monocyte expression were measured in 39 workers on Monday (pre and 5 hours into shift) and Thursday (mid-shift) of the working week. Eight (20%) were classified as symptomatic (one or more work related lower respiratory tract symptoms). Twelve operatives (35%) had a fall of Fev1 and 11 workers (31%) of FVC of more than 5% during the working week. The percent change in CD14 expression (0-5 hours and 0-96 hours) was smaller in symptomatic workers but did not reach statistical significance compared to asymptomatic workers. Pearson's correlation coefficient between percent change in FVC (but not Fev1) and percent change in CD14 expression (0-96 hours) was significant (r=0.36, 0=0.046). Workers with FVC (but not Fev1) decline greater than 5% were more likely to have a smaller increase in CD14 expression at 5 hours (r=-0.89; p=0.017). A smaller change in across shift (but not across week) CD 14 expression on monocytes was correlated with greater decline in across week FVC. The trend was similar but did not reach statistical significance for both Fev1 across week change and symptomatic status.

The results of this small pilot study suggest that CD14 as a marker of endotoxin exposure may relate to the physiological change occurring in workers effected by their working environment.

Introduction

Endotoxin is one of the contaminants of cotton dust that may be responsible for respiratory illness within textile workers. Currently occupational exposure standards in both the UK and USA are set on dust levels rather than endotoxin, although consideration is being given in certain groups with regard to the possibility of setting an exposure standard for endotoxin. However endotoxin measurement is fraught with problems and there is a wide variation in inter laboratory values even when assaying identical samples (Chun 1999).

In addition the mechanism by which endotoxin exerts its physiological response is unknown. It appears to have only weak immunological properties, but is powerfully pro-inflammatory.

CD14 is a cell receptor expressed predominantly on monocytes and macrophages, but may be weakly present in neutrophils and other cells. It is thought to be the functional cell activating receptor for endotoxin, binding it with a high affinity.

Preliminary work has shown that CD14 expression is increased following endotoxin exposure on human alveolar macrophages in-vitro (Swann 1998) and the release of endotoxin induced mediators can be blocked by anti-CD14 antibodies. Little work has been done on the response of CD14 in the in-vivo situation and the purpose of the current study was to examine the relationship between endotoxin exposure in the working environment of cotton mills in the UK, with CD14 expression on monocytes. Workers who experienced respiratory symptoms and who demonstrated a physiological response were compared to see if they behaved differently from workers with no symptoms or physiological response in terms of the extent of change of CD14 expression following exposure. The study is a pilot study to a proposed more definitive study of other organic dust exposed occupational groups

Methodology

Target Population

3 cotton mills involved in a 5 year epidemiological study of respiratory health were approached to participate (Fletcher 1999). Volunteers were recruited, to provide blood samples, pulmonary function tests and measurements of dust and endotoxin exposure.

These workers represent a range of dust and endotoxin exposed workers from a waste cotton mill, a medium cotton mill and one mill producing cotton blend. As a result a wide distribution of endotoxin exposure was included.

The recruits were random volunteers in this pilot study. At the time of sampling their previous responses to respiratory health questions and lung physiology was unknown.

Blood Samples

Blood samples were collected at the start of the first working shift, 5 hours into the first shift and 5 hours into the fourth working shift of the working week. 10 mls of whole blood

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and 12 mls of serum were collected on each occasion. Samples were transported to the Health and Safety Laboratory at Sheffield for same day processing.

Lung Physiology

Lung spirometry was performed at the start of the first working shift and 5 hours into the fourth working shift. For the purpose of this small pilot study, a fall in Fev1 of 5% or more was classified as a respiratory response. As only workers on the early (morning) or day shift were included, the influence of diurnal variation was minimised. The best of 3 forced expiratory manoeuvres with a variation of less than 5% of Fev1 was accepted as satisfactory technique.

Respiratory Symptoms

Once the lung function and sampling procedure was complete, those individuals who participated, were identified from the 5 year longitudinal study previously reported (Fletcher 1999). Any individual who had reported one of 5 possible work related respiratory symptoms when they were last interviewed as part of the study, were highlighted for comparison with those workers who had experienced no respiratory symptoms. The symptoms under investigation were cough, sputum production, wheeze chest tightness, and breathlessness. A symptom was categorised as work related if the individual worker reported the symptom as experienced as being more severe on one or more work days, during a shift or as improving on rest days.

CD14 Expression on Monocytes

The whole blood samples in EDTA were labelled with Phycoerythrin conjugated mouse monoclonal anti-human CD14 and control antibodies. Red cells were lysed and remaining cells fixed. Mean linear fluorescence was measured using a Coulter Epics XL flow cytometer. Details of the technique are reported in the same Proceedings (Curran 2000).

Analysis

The percent change of CD14 expression across the first shift and across the working week was calculated for each individual. Mean and confidence intervals of the percent change of CD14 expression was compared between individuals who were identified as having experienced work related respiratory symptoms with those who did not experience symptoms. Pearson's coefficients of correlation were used to compare across shift and across week change in CD14 expression and across week change in lung function indices, both in the group as a whole and in the sub-group of workers who were identified as having a 5% or more change in lung function parameter.

Results

25 workers provided 3 blood samples for analysis, while a further 14 workers gave 2 of the 3 samples. Eight of these 39 workers were identified as having experienced work related respiratory symptoms and 12 were identified as having had a cross working week fall of Fev1 of 5% or more. CD14 rose from a mean of 5.8% at pre-shift, to 7.7% at 5 hours in to the first working shift. It had fallen to a group mean of 5.0% by the middle of the fourth working shift.

Table 1 presents the across shift and across week change in CD14 expression in symptomatic compared to asymptomatic workers. The workers with respiratory symptoms have a smaller across shift rise in CD14 expression, and a greater across week fall. However the differences are not significant, partly in response to the small numbers.

Table 2 shows the correlation coefficients between change in Fev1 and FVC across the working week with the percent change in CD14 expression in the group as a whole. Table 3 presnts the same data for the subgroup of workers who had a greater than 5% fall in Fev1 or FVC across the working week only. Significant associations are seen between the percent change in FVC (but not Fev1) and the across week change in CD14 expression for the whole group, and between the percent change in FVC and across shift change in CD14 expression in the subgroup of those workers who did have a fall in their FVC. The direction of the correlation is as for those with symptoms, in that those with a greater fall in FVC across the week, have a lower across shift rise in CD14 expression, but a larger across week fall in CD14 expression.

Conclusions

This pilot study has demonstrated that the measurement of CD14 expression on monocytes can successfully be done in endotoxin exposed workers. While the pilot numbers are small the parallel publication (Curran 2000) has demonstrated a correlation with exposure. The analysis presented in this paper has shown that the changes in CD14 expression following exposure to endotoxin in the workplace can be related to pulmonary function change, and though not statistically significant, the changes in workers who experience work related respiratory symptoms may be different from those who do experience symptoms. A more definitive study with a larger population would be required to investigate this further.

This pilot study has highlighted the need for more work in the area of cellular response in vivo, that may lead to a greater understanding of the mechanisms of action of endotoxin in both acute and chronic exposure.

References

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Table 1. Percent (%) change in CD14 expression on monocytes (across the first working shift and across the working week) in workers with work related respiratory symptoms and asymptomatic workers

	Across Shift	**Across Week**
Symptomatic workers	+ 34.8	- 23.1
Asymptomatic workers	+ 65.9 n.s	- 2.6 n.s

n.s: no significant difference between symptomatic and asymptomatic workers.

Table 2. Correlation between change in CD14 expression (across the first working shift and across the working week) and percent change in lung function (Fev1 and FVC) in all workers studied.

	% change in CD14 expression	
	Across Shift	**Across Week**
Across week change in Fev1	- 0.26 (p=0.25)	0.21 (p=0.25)
Across week change in FVC	- 0.05 (p=0.82)	**0.36 (p<0.05)**

Table 3. Correlation between change in CD14 expression (across the first working shift and across the working week) and percent change in lung function (Fev1 and FVC) in workers with a 5% fall in Fev1 or FVC across the working week only.

	% change in CD14 expression	
	Across Shift	**Across Week**
Across week change in Fev1	- 0.44 (p=0.39)	0.15 (p=0.67)
Across week change in FVC	**- 0.89 (p<0.05)**	0.37 (p=-0.29)