PERSISTANCE OF GUINEA PIG PULMONARY RESPONSES TO A SINGLE COTTON DUST EXPOSURE.

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Abstract

The objective of this study was to determine the duration of the pulmonary response in guinea pigs to a single six hour cotton dust exposure. A total of twenty five guinea pigs were exposed to either an atmosphere of 10 mg/m^3 cotton dust (approximate mean, measured continuously) or clean air. The standard dust DB (5/89) was generated in a modified Pitt-3 acoustic dust generator. The breathing frequency of the animals was measured before the exposure, immediately post-exposure, and each day until sacrifice. Five animals were sacrificed at 1, 2, 3 and 4 days post-exposure along with five control animals that had been maintained in clean air. Upon sacrifice, pulmonary lavage was performed on the animals, with the supernatant of the first lavage frozen for detection of tumour necrosis factor, lactate dehydogenase, superoxide dismutase and N-acetyl β-d-glucosaminidase activities. Differential cell counts were determined on the cells harvested from the lavage. Chemiluminescence was measured to determine the degree of macrophage activation.

Introduction

The inflammatory response of guinea pigs to cotton dust has been well documented from this laboratory and others. This response is characterized by a dose dependent increase in breathing rate immediately after the exposure and persisting until sacrifice, usually at 18 hours post-exposure. A more sensitive indicator of inflammation is the influx of lymphocytes and polymorphonuclear leukocytes (PMN) into the air spaces.

Increased pulmonary edema, as measured by the wet weight/dry weight ratio of excised lungs, has also been observed following cotton dust exposure(Frazer *et al*, 1989). This indicates a failure at the air/blood barrier of the alveoli, allowing fluid from the blood to escape into the alveolus. This can also be seen by the increase in red blood cells (RBC) measured in differential cell counts from pulmonary lavage (Castranova *et al*, 1992) and by elevated plasma protein levels in the lavage fluid. Furthermore, increased levels of cytosolic enzymes in the lavage fluid are indicators of cellular lysis or leakage, thus damage at the cellular level in response to cotton dust exposure.

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Cytokine mediators have been implicated as chemotaxic factors inducing the influx of PMNs into the air spaces from the pulmonary capillaries. These chemotaxic factors may originate from macrophages, which become activated upon exposure to organic dusts or exposure to organic dust contaminants, such as endotoxin or FMLP.

The objective of this study was to determine how long following a single exposure to cotton dust the pulmonary responses would persist.

Methods & Materials

Specific pathogen free Dunkin Hartley male guinea pigs were purchased from Sprague Dawley, Indianapolis, IN. The animals were acclimated for one week in the NIOSH animal facility prior to dust exposure. The animals weighed an average of 312.8 +/- 7.4 grams at the time of exposure.

The exposure system used is similar to that described by Frazer *et al* (1987) and is shown in Figure 1. The animals were exposed for six hours to 10.6 +/- 1.0 mg/m^3 of DB 5/89 cotton dust. The airborne endotoxin was 2.52 +/- 0.17 x 10^3 EU/m^3 Control animals were maintained in a clean air environment for the duration of the experiment prior to sacrifice.

Breathing rate measurements were performed in a glass plethysmograph, with pressure fluctuations due to animal inhalation and exhalation detected with a B&K Spectral Analyzer. Breathing rates were measured prior to the exposure, immediately post-exposure, and each day prior to sacrifice. Measurements were made in both air and 10%CO_2

Exposed animals were sacrificed at 1, 2, 3, or 4 days post-exposure (n=5 for each time along with control guinea pigs). Lung cells were obtained by bronchoalveolar lavage after the method of Castranova *et al* (1990). Differential cell counts were obtained with an electronic cell counter equipped with a cell sizing attachment. Chemiluminescence, as an indicator of macrophage activation, was measured in the presence and absence of unopsonized zymosan and detected with a Berthold luminometer.

The first acellular lavagate of each animal was frozen (-80^{0}C) for later analysis after the cells had been removed. Tumor necrosis factor (TNF) was measured by the method of Shahan *et al*, (1994). An automated Cobas FARA II Analyzer was employed to determine plasma protein, lactate dehydrogenase (LDH) and N-acetyl B-d-glucosaminidase (B-NAG) levels (Vallyathan *et al, 1995).*

Results

The breathing rates of the guinea pigs in 10% CO_2 can be seen in Figure 2. While the rate immediately after the

exposure was significantly increased, the peak rate was not until the following day. By two days post-exposure, the animals were still breathing at a rate significantly above the control levels, but also significantly below the peak rate. By four days following the exposure the animals breathing rate had finally returned to control levels.

Figure 3 shows the yield of various cell types harvested by bronchoalveolar lavage. The significant increase in red blood cells indicates leakage at the air blood barrier between the alveoli and the pulmonary capillaries. As with the breathing rate, by the second day post exposure the yield of red blood cells had dropped significantly below that of the peak yield (seen at one day post exposure). This trend was also observed with lymphocytes and neutrophils, although it was not until the third day post exposure that these cell types decreased significantly below the peak yield at one day post exposure. At four days post exposure, none of these cell types had yet returned to control levels.

The yield of macrophages (Figure 3), on the other hand, demonstrated a different pattern. There was a significant increase of macrophages at each day post-exposure, with the peak yield occuring after the fourth day post exposure. The activation of the macrophages (Figure 4) was elevated at one and two days post-exposure. However, these levels were not significantly elevated above control values. Higher dust or endotoxin levels are necessary for significantly higher activation of macrophages with a six hour cotton dust exposure (Robinson, 1995).

TNF levels from the first lavagate (data not shown) from exposed guinea pigs were no different than control levels. This is not surprising when considering the sacrifice times that were used in this study. According to Ryan *et al*, (1991), TNF levels increased rapidly from the onset of exposure, but had returned to control levels well before one day post-exposure, the earliest time point used in this study.

LDH (Figure 5) and albumin (Figure 6) levels measured in the first lavagate displayed the same profile as did the breathing rate response, ie. significantly elevated by one day post-exposure, significantly decreased from that peak level by two days, and back to control values by three (albumin) or four (LDH) days.

Summary

The data collectively indicate that pulmonary reactions to a single cotton dust exposure increase rapidly, peak approximately one day post-exposure and then decline towards control levels. Breathing frequency, macrophage activation, cytosolic enzymes, and protein levels all reach control levels by four days post-exposure.

However, it appears that the time course for the clearance of inflammatory cells from the air spaces is slightly longer than for the other parameters measured, indicating an increased time necessary for the clearance of cells from the air spaces.

The more puzzling piece of data remains the increase of alveolar macrophages. In past studies from this laboratory, at higher doses of cotton dust the number of lavagable macrophages showed a slight decline at one day post-exposure, but returned to control levels by two days, the longest post-exposure time point measured (Castranova *et al*, (1987). This reponse had been previously reported (Rylander *et al*, 1974) and was attributed to the increased adherance of the cells to the airways, thus making the cells more difficult to harvest by lavage. That finding was verified with an increase in macrophages seen in the air spaces by light microscopy (Lantz *et al*, 1985, Castranova *et al, 1987*), yet with a decrease in lavagable macrophages. However, in subsequent studies, at lower dust levels using cotton dust with high endotoxin levels, increases in lavagable alveolar macrophages were reported one day post-exposure (Robinson *et al*, 1995). It should be noted that the level of activation observed in this study was far below that measured in other studies with either higher endotoxin levels or higher dust concentrations. Thus, it may be that the macrophages were less activated in this study than in the previous studies, and therefore less adherent and more easily lavagable.

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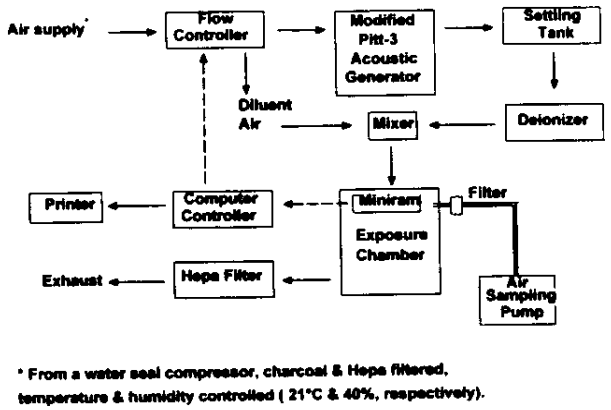

Figure 1. Schematic of the cotton dust generation and exposure system. The animals are caged within the exposure chamber, with the cotton dust concentration continuously controlled through computer feedback.

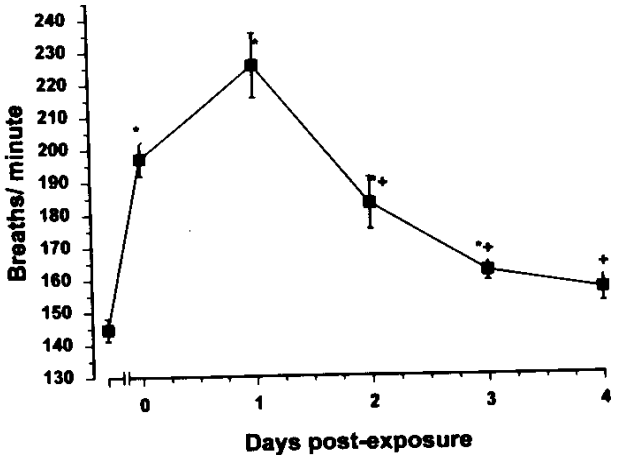

Figure 2. Breathing rates of guinea pigs in 10% CO_2 prior to dust exposure, and each day until sacrifice. The animals were exposed to approximately 10 mg/m^3 cotton dust for six hours and sacrificed at 1, 2, 3 or 4 days post exposure.

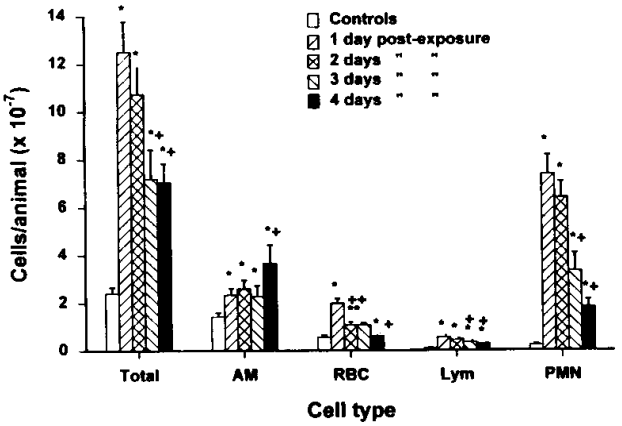

Figure 3. Yields of total cells, alveolar macrophages (AM), red blood cells (RBC), lymphocytes (Lym) and polymorphonuclear leukocytes (PMN)from bronchoalveolar lavage of guinea pigs at 1, 2, 3, or 4 days following exposure to approximately 10 mg/m^3 cotton dust. * indicates a significant increase above control levels, + indicates a significant decrease from the peak level.

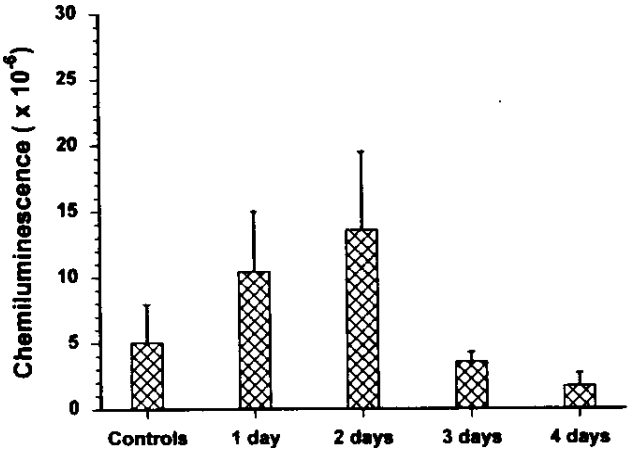

Figure 4. Difference in chemiluminescence levels (zymosan stimulated rate minus resting rate) from guinea pig macrophages harvested by bronchoalveolar lavage at 1, 2, 3, or 4 days post exposure to cotton dust inhalation.

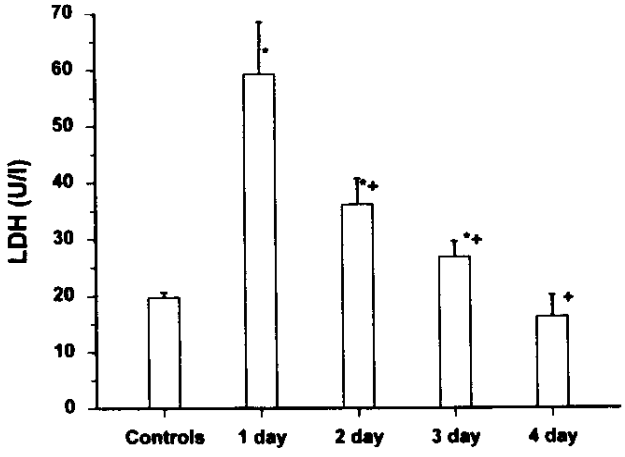

Figure 5. Cytosolic enzyme levels (lactate dehydrogenase) measured with clinical diagnostic methods (Cobas Fara II Analyzer, Roche)from the first lavagate of guinea pigs sacrificed 1, 2, 3 or 4 days post cotton dust inhalation (10.6 mg/m^3)

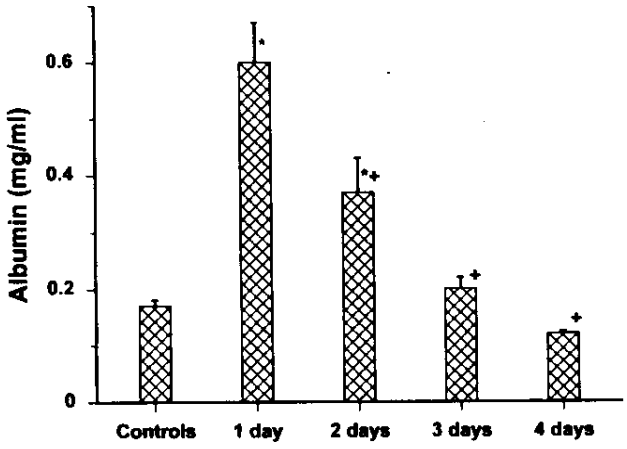

Figure 6. Protein (albumin) levels measured in the first lavagate of guinea pigs exposed to cotton dust inhalation and sacrificed at 1, 2, 3, or 4 days post-exposure