

Fever and leukocytosis responses in goats to inhaled endotoxin are dose-dependent

Charles W. Purdy^{a,*}, David C. Straus^b, Mark D. Hoover^c

^a USDA, Agriculture Research Service, Conservation and Production Research Laboratory, P.O. Drawer 10, Bushland, TX 79012, USA

^b Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

^c Lovelace Respiratory Research Institute, P.O. Box 5890, Albuquerque, NM 87185, USA

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Abstract

Forty-five, weanling goats of mixed-sex, were randomly allotted to five treatment groups: no dust ($n=16$), raw organic dust exposure for 15 min ($n=6$), raw organic dust exposure for 1 h ($n=7$), raw organic dust exposure for 3 h ($n=7$), and raw organic dust exposure for 4 h ($n=9$). Inhalation exposures were performed in a closed tent for the allotted times. The amount of endotoxin calculated to be in the dust was shown to be $26.9 \mu\text{g/g}$. The amounts of dust introduced into the tent for each time period were 4.58 g/m^3 of air for 15 min; 7.46 g/m^3 of air for 1 h; 14.82 g/m^3 of air for 3 h; and 40.60 g/m^3 of air for 4 h. There was a significant increase in the white blood cell count in the animals dusted for 4 h, at 8 and 12 h following exposure. There was a significant decrease in the peripheral lymphocyte cell count following the 15 min exposure at 12 h post exposure, and there was a significant increase in the peripheral lymphocyte cell count following the 4 h exposure, 24 h after the exposure. There was a significant increase in the neutrophil cell count 8 and 12 h following the 4 h exposure, while there was a significant decrease in the neutrophil cell count 48 h following the 4 h exposure. There was a significant increase in the rectal temperatures of all goats receiving the 4 h dust exposure at all time periods (0 time, 4, 12, 24, and 48 h). There was a significant increase in the rectal temperatures of goats following the 1 h exposure, 8 h later. These results indicate that the larger the dose of inhaled endotoxin, the higher the resultant fever and leukocytosis. Published by Elsevier B.V.

Keywords: Feedyard dust; Aerosol application; Goat; Rectal temperature; Total white blood cell counts, neutrophil cell counts, and lymphocyte cell counts

1. Introduction

It is well known that the inhalation of small particles is directly related to higher respiratory death rates in the very old and the very young (Schwartz, 1997). It has also been shown that the total airborne parti-

cles are excellent predictors of daily mortality (Schwartz and Dockery, 1992). The mechanism of action of non-biological ultrafine particle lung toxicity has recently been elucidated (Salvi and Holgate, 1999). Their work showed that free radicals and toxic substances carried on the surface of respired particles cause tissue damage when they are deposited in the alveoli of the lungs. In addition, macrophages become activated due to oxidative damage caused by free radical exposure. These activated macrophages promote inflammation due to their release of pro-inflammatory cytokines via the activation

* Corresponding author. Tel.: +1 806 356 5764; fax: +1 806 356 5750.

E-mail address: cwpurdy@cpri.ars.usda.gov (C.W. Purdy).

of blood platelets and clotting factors. The result of this pro-inflammatory process directly affects the increase in plasma viscosity. This puts an added strain on the cardiovascular system, especially in those with pre-existing heart and lung problems.

Many agricultural processes put particulate matter into the air. The tilling of fields to prepare the ground for planting generates a great deal of airborne dust (Gillette and Blifford, 1972). In addition, animal production promotes airborne particulates via manure production.

High animal concentrations of any kind produce large amounts of manure. Probably the largest amount of manure produced by domestic animals is that produced by cattle. A large proportion of this manure is pulverized into dust (Sweeten et al., 1988), and certainly, high dust production is prevalent in cattle feedyards (Wilson et al., 2002).

There is known to be measurable quantities of endotoxin in this dust (Purdy et al., 2002a,b,c). This is because of the high concentrations of Gram-negative bacteria (GNB) in the fecal material of cattle, and all GNB are known to produce endotoxins (Purdy et al., 2002a). The outer members of GNB are composed of lipopolysaccharide molecules called endotoxin, with the toxicity residing in the lipid A portion (Luderitz et al., 1978). These endotoxin molecules are extremely heat stable and profoundly effect both humoral and cellular immunity in mammals (Burrell, 1990; Morrison and Ulevich, 1978). When these bacteria die in the soil, due either to desiccation or the bactericidal activity of ultraviolet light (Wilson et al., 2002), the endotoxin from their cell walls remain.

We have recently examined the effects of the inhalation of endotoxin-laden dust on ruminants. We first showed that the inhalation of feedyard dust induced a temporary febrile response and leukocytosis in sheep. Their physiological responses were thought to be due to the endotoxins in the dust (Purdy et al., 2002a). We confirmed this hypothesis in goats where we showed that it was the endotoxin in dust that was responsible for the observed rectal temperature increases as well as an increase in white blood cells (Purdy et al., 2002c). We also observed that repeated endotoxin/dust exposures induced a state of tolerance where the increased rectal temperatures were not observed. Interestingly, we observed that dust treated goats demonstrated pulmonary clearance of two potential bacterial pathogens (*Pasteurella multocida* and *Mannheimia haemolytica*) that was not significantly different from that shown in control (not dusted) animals (Purdy et al., 2003). All animal studies performed here were approved by the regional animal care committee.

1.1. Hypothesis

Goats exposed to increasing amounts of endotoxin/dust by inhalation will respond by demonstrating increased rectal temperature and leukocytosis.

1.2. Objectives

The data findings of the five treatment groups were compared by measuring rectal temperature and complete blood cell (CBC) counts. The amount of endotoxin dust inhaled by each goat was determined by the amount of time each animal spent in the tent which was being supplied with a constant amount of dust per unit of time.

2. Materials and methods

2.1. Goats

Forty-five Spanish weanling goats of both sexes were housed in a three-sided barn. They were treated for internal helminth parasites (Ivomec, MSD AGVET, Merck, Rahway, NJ) and coccidian (Amprolium, MSD AGVET, Merck).

2.2. Experimental design

The goats were randomly allotted to 12 pens (7420 cm³), three goats per pen. The goats were randomized into five treatment groups: control group, 0 min dust exposure in tent ($n = 7$); principal group, 15 min dust exposure in tent (plus 10 min for tent equilibration) $n = 6$; principal group, 1 h dust exposure in tent (plus 10 min for tent equilibration) $n = 7$; 3 h dust exposure in tent (plus 10 min for tent equilibration) $n = 7$; principal group, 4 h dust exposure in tent (plus 10 min for tent equilibration) $n = 9$. The goats were handled for 2 weeks prior to the start of the experiment to acclimate them to feeding, the taking of rectal temperatures, and jugular blood sampling.

Rectal temperatures were taken from all the goats prior to dust treatment and after their one dust treatment (at 4, 8, 12, 24 and 48 h). Total peripheral white blood cell (WBC) counts and differentials were taken prior to and after one dust treatment (for all appropriate times) at 4, 8, 12, 24, and 48 h on the control and principal goats. All data were subjected to the analysis of variance using the General Linear Models procedure of SAS (1996). Mean comparisons were performed by the least square means procedure of SAS (1996).

2.3. Sampling intervals

The rectal temperature and CBC counts were measured following a series relative to dust treatment; pre-dust treatment, and appropriate hour dust treatment, 4, 8, 12, 24 and 48 h post-dust treatment.

2.4. Tent

A semi-airtight dust tent (183 cm wide \times 244 cm long \times 213 cm tall) was used to contain the administered dust and goats (Purdy et al., 2002a,b,c).

2.5. Feedyard dust preparation

Dried feedyard manure was removed from a working feedyard, processed to a fine dust (Purdy et al., 2002a,b,c) and stored in plastic barrels.

2.6. Collection of dust aerosol

The dust aerosol was collected for 30 min with three Anderson two-stage microbial cascade impactors as previously described (Purdy et al., 2002b).

2.7. Challenge technique

The dust aerosol technique used to challenge the goats has been described (Purdy et al., 2002c).

2.8. Endotoxin assay

The endotoxin/dust was analyzed by using the kinetic chromogenic semi-quantitative Limulus amoebocyte lysate assay (Purdy et al., 2002c).

2.9. Determination of dust particle size

This was done as previously described (Purdy et al., 2002b).

2.10. Statistical analyses

The mean results of measured variables were compared by analysis of variance using the General Linear Models procedures of SAS (1996). Means of rectal temperatures, WBC counts, and absolute neutrophil and lymphocyte counts were compared between principal and control groups during the experiment, and within any sample period of time. Significant differences between exposure groups were determined by Bonferroni's and Dunnett's adjusted paired *t*-test ($p \leq 0.05$) that allowed

pairwise comparisons of exposure group means and control means within any sample collection time.

3. Results

3.1. Endotoxin in dust aerosol

The amount of endotoxin in the raw organic dust was 26.9 $\mu\text{g/g}$. The amount of dust going into the tent was 4.58 g/m^3 for 15 min, 7.46 g/m^3 for 1 h, 14.82 g/m^3 for 3 h, and 40.60 g/m^3 for 4 h. Therefore, the amount of endotoxin per cubic meter of air in the tent was 123.20 μg in 4 h.

3.2. Clinical effects of endotoxin/dust inhalation

No panic or restlessness was observed in the goats during any of the data treatments. Occasionally some goats would cough during the dustings. Coughing in general seemed to increase for 1–2 h after the principal goats were returned to their pens. When the principals were removed from the dust tent, their hair was often dark brown with particulate dust matter. The 4 h exposure principal group's ($n=9$) mean rectal temperature significantly ($p \leq 0.005$) increased 8 h after the dust exposure within treatment group. The maximum mean rectal temperature following endotoxin/dust exposure was 40.5 °C (Table 1).

The mean total WBC counts can be seen in Table 1. The 3 h exposure group blood samples were lost from 8 to 24 h and no data during that time frame are reported for WBC, lymphocytes, and neutrophil counts. The 4 h exposure group had a significant ($p \leq 0.05$) increase in WBC counts at 8 and 12 h following the dust exposure. After 24 h, the WBC count was back to normal in this group.

The mean total lymphocyte cell counts can be seen in Table 1. A significant ($p \leq 0.05$) decrease occurred at 4 h in the 3 and 4 h dust exposure group. There was a significant ($p \leq 0.05$) decrease in total lymphocytes in the 15 min and 1 h dust exposure group at 12 h when compared to the control group. And finally, there was a significant ($p \leq 0.05$) increase in the lymphocyte cell count in the 4 h exposure group 24 h post-dust exposure.

The results of the total mean neutrophil cell counts can be seen in Table 1. There was a significant increase ($p \leq 0.05$) in total neutrophils for the 1 and 4 h exposure groups at 8 and 12 h following dust exposure. The neutrophil count of the 4 h exposure group dropped significantly ($p \leq 0.05$) below the control values at 48 h post-dust exposure.

Table 1
Temperature, total WBC, total lymphocyte, and total neutrophil count for control (no dust)^a and principal dust groups^b for varying times of exposure^c

Sample times (h) ^d	Rectal temperature (°C)		Total WBC (1000 cells/mm ³)	Total lymphocytes (1000 cells/mm ³)	Total neutrophils (1000 cells/mm ³)
	0	ND ^e (38.3); 15 min (38.3); 1 h (38.3); 3 h (38.3); 4 h (40.5) [*]	ND (15.4); 15 min (13.4); 1 h (13.6); 3 h (16.2); 4 h (16.4)	ND (9.3); 15 min (7.3); 1 h (7.3); 3 h (9.5); 4 h (11.5) [*]	ND (5.7); 15 min (5.3); 1 h (6.0); 3 h (6.5); 4 h (4.6)
4	ND (39.4); 15 min (39.4); 1 h (39.6); 3 h (39.6); 4 h (40.5) [*]	ND (15.4); 15 min (14.0); 1 h (13.6); 3 h (14.4); 4 h (14.8)	ND (8.8); 15 min (8.7); 1 h (7.4); 3 h (6.2) [*] ; 4 h (7.0) [†]	ND (9.4); 15 min (8.7); 1 h (7.1); 3 h (NP); 4 h (8.6)	ND (5.2); 15 min (7.6); 1 h (8.8); 3 h (NP); 4 h (9.8) [†]
8	ND (39.6); 15 min (39.8); 1 h (39.8); 3 h (40.4) [*] ; 4 h (40.0) [*]	ND (15.0); 15 min (15.4); 1 h (16.8); 3 h (NP); 4 h (18.8) [¶]	ND (15.0); 15 min (15.4); 1 h (16.8); 3 h (NP); 4 h (18.8) [¶]	ND (9.8); 15 min (6.8) [*] ; 1 h (6.9) [*] ; 3 h (NP); 4 h (10.0)	ND (5.4); 15 min (7.5); 1 h (9.2); 3 h (NP); 4 h (9.0) ^{§;†}
12	ND (39.8); 15 min (39.9); 1 h (40.0); 3 h (39.5); 4 h (40.3) [*]	ND (15.6); 15 min (15.0); 1 h (16.5); 3 h (NP); 4 h (19.4) [¶]	ND (8.3); 15 min (7.3) [*] ; 1 h (8.0) [*] ; 3 h (NP); 4 h (11.6)	ND (6.2); 15 min (6.2); 1 h (6.7); 3 h (NP); 4 h (5.8) ^{§;†}	ND (6.8); 15 min (5.8); 1 h (5.7); 3 h (6.3); 4 h (4.2) [§]
24	ND (38.4); 15 min (38.4); 1 h (38.4); 3 h (38.6); 4 h (39.7) [*]	ND (15.0); 15 min (14.0); 1 h (15.2); 3 h (NP); 4 h (17.9)	ND (8.5); 15 min (6.5); 1 h (7.8); 3 h (8.3); 4 h (9.8)	ND (6.8); 15 min (5.8); 1 h (5.7); 3 h (6.3); 4 h (4.2) [§]	
48	NP ^f	ND (15.6); 15 min (12.8); 1 h (14.0); 3 h (14.8); 4 h (14.4)			

^a $n = 7$ for no dust group.

^b $n = 6$ for 15 min group; $n = 7$ for 1 h group; $n = 7$ for 3 h group; $n = 9$ for 4 h group.

^c Time exposed to dust.

^d Time after dusting.

^e No dust.

^f Not performed.

^{*} Significantly different than control group ($p \leq 0.0001$).

[#] Significantly different than control group ($p \leq 0.004$).

[†] Significantly different than at 0 h for treatment group ($p \leq 0.05$).

[§] Significantly different than control group ($p \leq 0.05$).

4. Discussion

Organic dust from feedyards contains endotoxin, bacteria and fungal spores attached to a variety of different particles or as free entities. We have shown that all of those fractions, either attached to dust particles or free can cause pulmonary inflammation (Purdy et al., 2002a,c). We have also shown that it is the endotoxin component of dust that is responsible for the rectal temperature increase and leukocytosis seen in ruminants (sheep and goats) inhaling this dust (Purdy et al., 2002a,c).

While there is a great deal known about the consequences of the inhalation of endotoxin by humans (Edward, 1997; Rylander and Morey, 1982; Jagielo et al., 1996), there is a paucity of literature on its effects on animals. Burrell (1990) concluded that endotoxin was the most biologically important component of organic dust inhaled by man. We have shown similar results in ruminants (Purdy et al., 2002a,b,c). We showed that the inhalation of endotoxin in dust induces in goats a rapid and transient significant increase in body temperature between 4 and 12 h and a leukocytosis between 12 and 24 h. These changes followed a 4 h aerosol dust exposure in a closed tent (Purdy et al., 2002b,c). This leukocytosis was associated with an increase in neutrophils between 4 and 8 h, and a decrease in lymphocytes in the same time period following dust exposure. The body temperature response had abated by 24 h after dusting and the increase in WBC was gone by 48 h post endotoxin/dust exposure. We also found that repeated daily 4 h endotoxin/dust exposures induced in goats a state of tolerance to both the rectal temperature spikes and the increase in total WBCs that were characteristically seen following a solitary dust exposure.

In this study we wanted to examine whether the endotoxin induced fever and leukocytosis required a specific amount of endotoxin/dust and that increasing the threshold of endotoxin/dust in excess of this amount would not result in an increase in fever and leukocytosis. The obvious alternative hypothesis would be that increased doses of endotoxin would correlate with increased fever and leukocytosis. The way in which we examined this question was by incrementally increasing the amount of inhaled endotoxin in goats. Our results show that the latter hypothesis is true. Table 1 shows that the more endotoxin that is inhaled, the higher the fever and the leukocytosis. It is thought that dust particles, and therefore endotoxin, play some part in the causing acute bovine respiratory disease in cattle housed in feedyards (MacVean et al., 1986). The exact role of feedyard dust in this phenomenon remains to be determined.

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