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Endotoxin and complement activation in an analysis of environmental dusts from a horse barn[1]

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Various work environments in agriculture naturally contain gram-negative bacteria and their endotoxins, which are heat stable, lipopolysaccharide-protein complexes that are integral parts of the outer membrane of gram-negative bacteria (1). Respiratory exposure to endotoxin-containing dusts has been associated with both an acute decline in pulmonary function (2) and chronic lung disease (3) in cotton dust-exposed subjects. Endotoxins can profoundly affect both humoral and cellular mediation systems in humans and experimental animals (4).

One biologically active humoral system that is associated with respiratory exposure to agricultural dusts is the complement cascade (5). Activation of complement by inhaled dusts results in the generation of active fragments which are chemotactic for leukocytes, cause vasoconstriction, and enhance vascular permeability. Such activities result in inflammation that could initiate or exacerbate events in the lung, which then could lead to acute or chronic pulmonary dysfunction and subsequent disease manifestation.

It is the purpose of this paper to quantify the presence of endotoxins in bulk dusts and material obtained from a work environment that was related to reports of respiratory problems in workers. The inflammatory potential of the samples was quantified by their activity against human serum complement in vitro.

Materials and methods

Horse barn samples. Bulk dusts were obtained from several areas within a horse barn that was associated with reports of respiratory disease in workers. Bulk dust samples (approximately 1 g) from the loft area (A), delivery chute (B), and exterior (C) of the feed bin, and bulk bedding material (D_1, D_2, D_3) were placed in sterile conical centrifuge tubes. They were extracted separately at room temperature with 25 ml of sterile, nonpyrogenic water (Travenol Laboratories, Deerfield, Illinois, United States) by rocking for 60 min (with occasional shaking). The extracts were centrifuged three times at 1000 *g* for 10 min, and the supernatant fluids were decanted and frozen at −85°C until assayed.

Endotoxin analysis. The endotoxin content of the extracts was quantified in duplicate by the kinetic chromogenic modification of the Limulus amebocyte lysate test (Kinetic-QCL, Whittaker Bioproducts, Walkersville, Maryland, United States). The endotoxin levels were defined in terms of endotoxin units (EU) per milligram of sample.

Complement activation. Each extract was analyzed for activity against human complement in vitro by reacting aliquots with normal human serum (NHS) and measuring hemolytic complement activity by the CH_{50} technique (5).

Results

Every sample of bulk material from the horse barn contained quantifiable contamination with endotoxins (table 1). The samples obtained from feed bin areas contained endotoxin levels which ranged from 58.20 EU · mg^{-1} at the delivery chute to 119.14 EU · mg^{-1} at the exterior of the feed bin. Markedly higher concentrations of endotoxins were detected in the bedding material. Three samples of bedding material ranged approximately 155-fold, from a low of 195.31 EU · mg^{-1} to the highest concentration of 30 273.44 EU · mg^{-1}.

All of the extracts, except C, showed inhibition or enhancement of the endotoxin assay. Validation studies were performed to overcome the inhibition/enhancement. The extracts demonstrated activity against human complement (CH_{50}) in a dose-dependent manner (table 2). The mean of seven saline-treated control levels was 73.6 (SE 1.63) CH_{50} U · ml^{-1}. Relatively similar complement consumption (%), when compared with saline-treated control levels, was observed with the three dust samples from the feed bin area. Two hundred microliters of extract resulted in approximately 100 % consumption of the available complement in 0.5 ml of NHS. By contrast, the bed-

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ding material was much more active against human complement. A lower concentration (25 µl) of extract resulted in the same level of complement consumption, and all three samples of bedding material were similar in their activity.

Discussion

The results of this study demonstrate that gram-negative bacterial endotoxins were present in all of the bulk dusts and materials tested, and the levels of contamination were similar to those in other agricultural dusts (1). It is apparent from the data, however, that all samples from the same barn do not contain the same level of endotoxin contamination. The endotoxin levels in the dusts associated with the feed bin were similar in magnitude, yet lower than in those found in the bedding material. By contrast to the similar range of endotoxin contamination in the feed bin dusts, the three samples from the bedding material varied markedly (155-fold). These results illustrate the difficulty of analyzing endotoxin contamination in bulk material. By routine, we analyze multiple samples of the bulk material because of the inherent variation from spot to spot in the sample.

Because endotoxins are known activators of complement, this study examined the hemolytic complement activity of the horse barn dusts. Complement activation is important in inflammatory events in the lung because complement components are present in human airways. The potential exists, therefore, for the direct interaction of dusts from the horse barn with complement after inhalation of dust-laden air.

Again, a difference between the bedding material extracts and those of the feed bin area was noted. While 25 µl of the bedding material extracts was required to consume 100% of the available complement, an eightfold greater quantity (200 µl) of the feed bin extracts was required to achieve the same level of complement consumption. In general, the lower endotoxin levels in the feed bin samples and their lower complement consumption when compared with higher endotoxin contamination and greater complement consumption with the bedding material suggest an association between the endotoxin levels and complement activation. When the data for endotoxin and complement consumption were examined for the bedding material alone, no such association was observed. For each bedding material extract, 25 µl of sample consumed 100% of the available complement; yet the three samples varied 155-fold in endotoxin contamination.

Although a potential contribution of endotoxins in activating complement is suggested by this study, the complete inflammatory potential of the samples cannot be attributed to endotoxins alone. Other unidentified complement-activating agents are likely to be present in the extracts. Likewise, endotoxins are known to demonstrate inflammatory activities beyond those against the complement system that could be expected to occur after the inhalation of endotoxin-containing dusts. Further studies on the description of other agents within agricultural dusts and their contributions to pulmonary inflammation and disease should increase the understanding of the response of the lung to these inhaled dusts.

Table 1. Endotoxin concentrations in bulk dust samples selected at various sites within a horse barn.

Sample and location	Endotoxin concentration ($EU \cdot mg^{-1}$)
Feed bin	
Loft area (A)	67.19[a]
Delivery chute (B)	58.20
Exterior (C)	119.14
Bedding material	
Sample (D_1)	30 273.44
Sample (D_2)	3 515.63
Sample (D_3)	195.31

[a] Average of results from duplicate assays.

Table 2. Percentage of reduction in hemolytic complement (CH_{50}) with increasing concentration of dust extracts from a horse barn (NHS = normal human serum)

Sample[a]	Extract concentration (µl · 0.5 ml^{-1} NHS)						
	5	10	25	50	100	200	300
A	0.0[b]	0.1	27.9	70.7	98.0	100	100
B	2.2	4.6	16.6	49.7	82.6	100	100
C	0.0	12.6	26.9	52.4	86.4	98.5	100
D_1	36.5	55.1	98.2	100	100	100	100
D_2	32.4	67.2	100	100	100	100	100
D_3	37.5	50.4	99.2	100	100	100	100

[a] Sample and location as shown in table 1.
[b] Percentage of reduction from saline control.

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