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MORPHOMETRIC ALTERATIONS IN THE LUNG INDUCED BY INHALED BACTERIAL ENDOTOXIN

R. Clark Lantz*, Robert Burrell** and David E. Hinton*

Departments of Anatomy (*) and Microbiology (**), West Virginia University Medical Center, Morgantown, WV 26506 USA.

ABSTRACT

Using lipopolysaccharide (LPS) derived from a bacterium found in cotton and cotton mill dust, aqueous aerosols (4 $\mu g/m^3$) were used to expose randomly selected hamsters to a 5 h aerosol of either saline or LPS. Additionally, groups received an i.p. injection of either LPS, to render them endotoxin tolerant, or cobra venom factor, for purposes of inactivating complement in vivo, prior to aerosol exposure. Quantitatively, septal capillary blood space showed increased V_v of polymorphonuclear leukocytes (PMNs) and platelets, decreased lung volume and increased N_v of endothelial pinocytotic vesicles following LPS inhalation. Prior tolerization abolished PMN and platelet recruitment while decomplementation abolished cell recruitment and changes in lung volume. Neither treatment abolished the increased pinocytotic vesicle N_v. These results implicate certain chemical mediators as being important in the response to inhaled LPS and demonstrate the effectiveness of using these techniques to gain a better understanding of the pathogenesis of disease.

Keywords: Inhaled bacterial endotoxin, lung alterations, morphometry.

INTRODUCTION

Morphometric analysis of lung alterations following exposure to pathogens is invaluable in illustrating subtle changes which might otherwise be overlooked. Results, from evaluation of initial structural alterations, support and guide subsequent nonmorphological techniques and, when coupled appropriately, can lead to a better understanding of the pathogenesis of a disease. To elucidate initial events in acute pulmonary inflammation from inhalation of bacterial-derived LPS, morphometric analysis, in conjunction with administration of modifying agents, was used to determine involvement of cell type(s) and possible chemical mediators.

MATERIALS AND METHODS

Male Syrian golden hamsters weighing 110-150 g, were used. Purified LPS was produced by the hot phenol-water extraction method from Enterobacter agglomerans, a bacteria commonly found growing on cotton plants. Aerosols of LPS were generated with a DeVilbiss #40 nebulizer and a pressure/vacuum pump at 2.5 psi. Exposures were carried out for 5 h. An LPS stock solution of 5 $\mu g/ml$ in pyrogen free saline under these conditions produced an effective aerosol concentration of 4$\mu g/m^3$. Decomplementation of some animals was accomplished by intraperitoneal injections of cobra venom factor (CVF), (250 U/kg divided into 4 doses over a 24 hour period).

Prior tolerization of some animals was carried out by i.p. injection of 0.1 LD_{50} LPS 48 h before aerosol exposure. Randomly selected hamsters were divided into groups containing six animals each. Individual groups were exposed to either saline or standard LPS aerosols. One group exposed to each aerosol was treated to tolerize or decomplement the animals. The exposure design is shown in Table 1.

Table 1. Experimental exposure and treatment groups.

AEROSOL	PRETREATMENT NONE	LPS-IP	CVF-IP
SALINE	6 animals	6 animals	6 animals
LPS	6 animals	6 animals	6 animals

After aerosol exposure and a six h waiting period, tissues were fixed by intratracheal instillation, removed and processed for morphometric analysis at both the light and electron microscopic levels.

Morphometric procedures using standard point counting techniques (Weibel, 1979) were used throughout these studies. Five blocks, selected at random from at least ten separate blocks/animal, were analyzed at the light and electron microscopic levels. Enough points were taken in each representative sample to assure a coefficient of variation of less than 10%. Cellular numerical densities were determined, using the method of Weibel and Gomez (1962) by enumerating nuclei. Pinocytotic vesicle number and volume were determined by using the method of moments of Bach as described in Weibel (1979) with corrections for section thickness and lost caps applied. Reconstruction of three dimensional size distributions of pinocytotic vesicles were performed using the tables of Rose (1980) to correct for section thickness. Differences between groups of animals were tested by use of two factor (aerosol exposure and type of pretreatment) analysis of variance.

RESULTS

Although no significant differences were seen in parameters measured at the light microscopic level, displacement volumes of fixed lungs from animals receiving LPS aerosol were significantly lower than volumes of lungs receiving saline aerosol (Table 2). Because the final volume of intratracheally fixed lungs depends on the rate of fixative entry (Hayatdavoudi et al., 1980), bronchoconstriction and alteration of lung mechanics prior to fixation may be the cause of lower fixed lung volumes. Decomplementation but not prior tolerization abolished this response.

At the electron microscopic level, recruitment of PMNs and platelets followed LPS inhalation. Increases in V_v of PMNs and platelets were increases in absolute volumes since the reference volume (distal lung capillary blood) did not change between groups (.257 ± .016 ml/animal). This response, typical of inflammatory processes, was inhibited if the animals were given either a prior tolerizing dose of LPS or were decomplemented with CVF pretreatment (Table 2).

The presence of an inflammatory agent on the epithelial side of the air-blood barrier has led to recruitment of PMNs and platelets from the capillary blood. For this to occur, some signal or mediator must cross the septal tissue barrier. Alteration in one or more elements of this barrier might occur due to presence of mediator. As a first attempt to determine site(s) of alteration following LPS inhalation, V_v, N_v and average cellular volumes of epithelial and endothelial cells were determined. The only

Table 2. Effects of aerosol exposure and pretreatment on fixed lung volume, volume density of capillary blood leukocytes and endothelial pinocytotic vesicles in hamster distal lung.

PARAMETER	AEROSOL	PRETREATMENT NONE	LPS-IP	CVF-IP
LUNG VOL (cm^3)	SALINE	3.37±0.11	3.95±0.13	3.22±0.16
	LPS	2.65±0.15[A]	3.36±0.16[B]	3.57±0.16
V_v PMN	SALINE	0.020±0.010	0.042±0.010	0.029±0.012
	LPS	0.164±0.030[C]	0.032±0.010	0.051±0.016
V_v PLATELET	SALINE	0.007±0.003	0.005±0.003	0.018±0.006
	LPS	0.051±0.010[C]	0.002±0.001	0.026±0.005
N_v VES (μm^{-3})	SALINE	155±16	113±9[E]	135±15
	LPS[D]	296±30	175±5[E]	277±52
V VES (10^{-4} μm^3)	SALINE	1.51±0.16	1.17±0.07	0.99±0.13
	LPS	1.27±0.10	1.01±0.05	1.23±0.08

Values are means ± SEM.
PMN = polymorphonuclear leukocytes.
Reference volume for volume density of leukocytes is distal lung capillary blood.
VES = Vesicles.
Reference volume for numerical density of vesicles is distal lung capillary endothelial cytoplasm.
V is average volume of endothelial vesicles.

A = LPS aerosol - no pretreatment significantly different from saline aerosol - no pretreatment and from LPS aerosol - CVF-IP pretreatment ($P<0.05$).
B = LPS aerosol - LPS-IP pretreatment significantly different from saline aerosol - LPS-IP pretreatment and from LPS aerosol - CVF-IP pretreatment ($P<0.05$).
C = LPS aerosol - no pretreatment significantly different from saline aerosol no pretreatment and all other LPS aerosol groups ($P<0.05$).
D = All LPS groups different from saline aerosol groups ($P<0.05$).
E = LPS-IP groups are significantly different from all other pretreatment groups ($P<0.05$).

significant difference was an increase in average cellular volume of epithelial type I cells in LPS aerosol tolerized animals. This change was absent in animals receiving LPS aersol exposure alone.

Although volume and number of endothelial cells did not change, more subtle changes might be present. Lewis et al. (1983) have shown that vascular infusion of platelet activating factor, a mediator which could be present following LPS inhalation, caused increases in the number and volume of endothelial pinocytotic vesicles. Increases in the number of vesicles have also been seen in pulmonary capillary endothelium following either increases in hydrostatic or oncotic pressures (DeFouw, 1980). Thus, changes in the number and/or volume of pinocytotic vesicles may be indicative of direct or indirect (vasoactive) action of mediators on

endothelium. Table 2 shows estimates for the numerical density and average volume of pinocytotic vesicles. Statistical analysis showed that LPS aerosol leads to increases and tolerization to decreases in the numerical density of vesicles. Increases in the numerical density is indicative of increases in absolute number of vesicles since the reference volume (endothelial cytoplasm) was not different between the groups (0.066 $\pm$ 0.01 ml/animal). Prior tolerization or complement inactivaction had no effect on the increased numerical density of vesicles following LPS inhalation. No change in the average volume or three dimensional size distributions of the vesicles was seen.

Among the various mediators which have been implicated in inflammation are complement and prostaglandins. Wedmore and Williams (1981) have postulated that both an activated complement product and prostaglandins are necessary for exudation during inflammation. In the present study, the role of complement in the response following LPS inhalation was tested by blocking complement activation through use of cobra venom factor prior to LPS aerosol exposure. Removal of complement activation abolished changes in fixed lung volume and recruitment of PMN and platelets following subsequent LPS inhalation. These findings indicate at least a partial role for complement in the response.

DISCUSSION

The purpose of this study has been to use morphometric methods to identify the responses to LPS inhalation. Once the responses were identified (changes in fixed lung volume, recruitment of leukocytes and increased N_v of endothelial pinocytotic vesicles), certain mediators were implicated. Pharmocological manipulation (decomplementation) in conjunction with additional morphometric analysis supplied further evidence of the role of complement in the response. The results demonstrate the effectiveness of combining pharmacological and morphometric techniques to gain a better understanding of the pathogenesis of disease.

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