ACTA STEREOL 1984; 3/1: 75-78
ORIGINAL PAPER

QUANTITATIVE MORPHOLOGICAL STUDIES OF THE LUNG FOLLOWING INHALATION OF BACTERIAL ENDOTOXIN

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ABSTRACT

To define the possible protective effect(s) of prior i.p. injection (tolerization) of an endotoxin preparation (LPS) against inhaled LPS, morphometric analysis of Syrian golden hamster lung was undertaken. Vv of PMNs and platelets in lung septal capillary blood was significantly increased in the nontolerized but LPS aerosol-exposed animals vs nontolerized control and tolerized, LPS aerosolized animals. While tolerization protected against cellular influx, lungs from tolerized, LPS aerosolized animals showed increased mean thickness of air-blood barrier. Although tolerization protected the animal from some effects of LPS inhalation, the process itself was associated with alteration in lung septum.

INTRODUCTION

Endotoxin from gram negative bacteria associated with cotton dust is a possible etiological agent in byssinosis (Rylander and Lundholm, 1977). Evidence suggests that cotton mill workers may become tolerized to the effects of inhaled cotton dust. Workers exposed to cotton dust demonstrate acute respiratory symptoms on the first work day of the week but symptoms subsequently decrease throughout the remainder of the week. Prior exposure to a sublethal dose of LPS may afford a method of tolerizing experimental animals against the effects of inhaled endotoxin and create an experimental situation in which tolerized and nontolerized animals may be studied for pulmonary response to cotton dusts. This study was undertaken to define, quantitatively, changes caused by tolerization and to evaluate its protective effect(s) against inhaled LPS.

METHODS AND MATERIALS

Protein free LPS was isolated by recognized phenol-water procedures (Westphal and Jann, 1965) from Erwinia herbicola for aerosol studies of control, nontolerized and previously tolerized (single i.p. injection of 0.1 LD50 LPS 48 hrs prior to aerosol) male Syrian golden hamsters. LPS aerosol (effective concentration = 0.004 mg/m^3)was administered for 5 hrs. Six hrs post exposure, lungs were fixed by tracheal infusion of 1/2 strength Karnovsky's fixative (containing 0.01% picric acid in 0.1 M phosphate buffer, pH 7.4) held at a constant pressure of 20 cm H2O for 1 hr. Excised lungs were placed in additional fixative overnight (4^{O} C). After lung volumes and weights were recorded, tissues were processed for stratified analysis at light and electron microscopic levels. Using standard point count morphometry (Weibel, 1979), distal lung structure was analyzed in control (5 hrs of saline aerosol, with or without LPS i.p. injection) and LPS aerosol-exposed (tolerized and nontolerized) hamsters. Using H & E stained, paraffin sections, volume densities of large airways, large blood vessels and distal lung were determined. Lung parenchyma was evaluated using toluidine blue- stained Epon-embedded material (1 um) to determine volume densities of septa and distal lung air space. At the electron microscopic level, a square lattice (12 X 16 = 192 points/field, distance between points = 0.6 µm) was used to determine volume densities of epithelium, endothelium and interstitial space, using tissue volume as the reference space, and volume densities of leukocytes and platelets in the septal capillary blood. Surface densities of epithelium and endothelium were determined by counting intersections of lines between points. Eight fields/ animal, six animals/ group were analyzed. Harmonic mean thickness of the tissue and of the plasma, used to calculate maximum oxygen diffusion capacity, was obtained by use of a microcomputer and a digitizing pad. All data were collected and analyzed using a semi-automatic computer assisted device (Micrographics, Inc., Cuyahoga Falls, Ohio). Factorial analysis ($P<0.05$ being significant) was used to differentiate between the effects of tolerization versus the effects of LPS aerosol exposure.

RESULTS

Analysis of total lung volume and light microscopic (paraffin and semithin Epon sections) morphometric parameters did not show significant differences between groups.

Morphometric parameters determined at the electron microscopic level showed the following significant alterations

(Table 1). Inhalation of LPS alone caused a significant increase in Vv of polymorphonuclear leukocytes (PMN) and platelets seen in the septal capillary blood. These increases were eliminated in tolerized, LPS aerosolized animals. Conversely, Vv of leukocytes other than PMNs was significantly increased in tolerized animals given saline aerosol but was absent in tolerized, LPS aerosolized animals. In addition, the combination of LPS and tolerization lead to a significant increase in arithmetic mean barrier thickness of septal tissue.

TABLE 1
EFFECTS OF LPS AEROSOL AND TOLERIZATION ON DISTAL LUNG ELEMENTS OF SYRIAN GOLDEN HAMSTER

	SALINE AEROSOL		LPS AEROSOL	
	NT	TOL	NT	TOL
Vv PMN	4.6±2.6	4.2±1.4	16.4±3.1[a]	4.1±1.2
Vv platelets	1.5±0.3	0.5±0.3	5.1±1.0[a]	0.3±0.2
Vv other leuk.	0.8±0.5	4.9±0.8[a]	1.8±0.8	1.3±1.0
$\bar{\tau}$ total	0.96±0.06	0.84±0.09	0.98±0.05	1.39±0.14[b]
$\bar{\tau}$ epithelium	0.24±0.02	0.19±0.01	0.22±0.02	0.32±0.02[b]

Volume densities are in % and are referenced to septal capillary blood volume.
$\bar{\tau}$ = arithmetic mean barrier thickness (μm)
a - significantly different from LPS-TOL and Saline-NT
b - significantly different from LPS-NT and Saline-TOL

In order to determine region(s) of the septum where increase(s) in barrier thickness were occurring, the tissue compartment was divided into epithelium, interstitial space and endothelium. Mean barrier thickness of each compartment was calculated by dividing volume density of each by the average of the epithelial and endothelial surface densities (Bachofen and Weibel, 1977). Through this analysis, the site of mean barrier thickness increase in tolerized, LPS aerosolized animals was found to be epithelium (TABLE 1).

DISCUSSION

Five hrs following inhalation of LPS aerosol significant increases are seen in volume density of PMNs and platelets in septal capillary blood. These increases are not seen when animals receive an i.p. injection of 0.1 LD50 LPS 48 hours before the aerosol exposure. Thus some of the effects of LPS inhalation are removed by tolerization. However, additional

changes occur due to tolerization itself or to combined LPS aerosol exposure plus tolerization. These changes include a difference in the type of leukocytes which respond and an increase in the arithmetic mean barrier thickness of the tissue, which occurs predominately in epithelium.

It is interesting that neither a significant increase in volume density nor mean barrier thickness occurred in endothelium. Previous experiments in which endotoxin has been injected i.v. have shown that the endothelial cell is the site of damage in the lung (Meyrick and Brigham, 1983). The route of administration and/or the concentration of LPS administered in our studies differ from previous endotoxin studies which may account for the differences.

Tolerization brought about through prior i.p. injection may work by sequestration of potential responder cells (PMNs) (Leak, 1983) in peritoneal cavity. Increased epithelial mean barrier thickness of tolerized and subsequently LPS aerosolized animals, may be explained on the basis of differences in responding cells. When tolerized animals were given saline aerosol, a significant increase in Vv of "other" leukocytes (monocytes and lymphocytes) in the septal capillary blood was seen. Should these cells be present in the lung at the time of LPS aerosol challange, recruitment into alveoli and release of superoxide could account for the epithelial damage. These and other factors are being investigated.

This work was supported by Cotton Research Inc. Project 82-521.

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