Airway oedema and obstruction in guinea pigs exposed to inhaled endotoxin

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Abstract

Protein extravasation and airway conductance (SGaw) were examined in awake guinea pigs exposed to inhaled endotoxin or saline for three hours. A significant increase in protein extravasation (as estimated by the leakage of protein bound Evans blue dye) was seen in the conducting airways of endotoxin exposed animals compared with saline exposed animals. Mean dye extravasation was significantly increased by one to threefold in the mainstem and hilar bronchi of endotoxin exposed animals. These changes in extravasation were accompanied by decrements in pulmonary function and by an influx of polymorphonuclear leucocytes into the airway wall. The SGaw decreased significantly by 60-90 minutes into exposure to endotoxin and had decreased by 22% and 34% at the end of exposure in the low and high dose endotoxin groups, respectively. Similar findings were obtained in animals exposed to cotton dust. Contrary to studies suggesting that platelet activating factor (PAF) is involved in the systemic and peripheral lung effects of endotoxin, pretreatment with the PAF antagonist WEB2086 did not prevent the conducting airway injury produced by inhaled endotoxin.

Bacterial endotoxin is an important component of the organic dusts generated by several agricultural and industrial processes.¹⁻³ Maximum airborne endotoxin concentrations greater than 10 $\mu\text{g}/\text{m}^3$ have been measured in the workplace.^{4,5} Inhalation of organic dusts has been reported to cause adverse respiratory effects, including cough, chest tightness,

and a reduction in the forced expiratory volume in one second, suggesting a toxic effect of these dusts on the conducting airways. Endotoxin, when administered intravenously, has profound systemic effects. Most prominent are effects on the pulmonary circulation, including sequestration of polymorphonuclear leucocytes (PMNs) in the pulmonary microvasculature and an increase in plasma protein extravasation into the pulmonary interstitium.⁶⁻⁸ By contrast with systemic administration of endotoxin, local administration in some sites (for example, into skin windows) can cause an inflammatory response, characterised by plasma protein extravasation and the local accumulation of PMNs, limited to the site of administration. Several investigators have shown that endotoxin or cotton dust contaminated with endotoxin can cause inflammation and an influx of PMNs in the lung parenchyma.^{9,10} Despite the fact that the conducting airways may be involved in some of the acute effects of inhaled endotoxin (for instance, airway obstruction), few studies have examined the functional and morphological effects of inhaled endotoxin in these airways. We hypothesised that an inhaled endotoxin aerosol would stimulate a local inflammatory response at the site of aerosol deposition within the conducting airways and that an increase in local plasma protein extravasation would be a prominent feature of this response. To test this hypothesis, we exposed guinea pigs to atmospheres containing saline or two different concentrations of endotoxin and examined airway mechanics, plasma protein extravasation, and airway morphology. The airborne endotoxin concentration used in the low dose exposure group in the present study was roughly equivalent, in terms of endotoxin concentration, to atmospheres generated in some dusty occupational settings.^{4,5}

In vitro, endotoxin has been shown to stimulate the release of platelet activating factor (PAF) from several cell types. Also, macrophages harvested from guinea pigs exposed to inhaled endotoxin produce increased quantities of PAF.¹¹ Furthermore, in vivo studies using PAF antagonists have suggested that PAF is an important mediator of the plasma protein extravasation that occurs in a variety of sites, including the conducting airways, after intravenous administration of endotoxin.¹² Recent studies have also

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found that PAF antagonists can inhibit the peripheral lung effects of inhaled endotoxin.¹³ To determine the relevance of these observations to the conducting airway responses to inhaled endotoxin, we examined the effects of the PAF antagonist WEB2086.

Methods

ANIMALS

Virus free, male, Hartley outbred guinea pigs (weight range 361 to 489 g, Charles River Breeding Laboratories, Wilmington, MA) were fed standard guinea pig chow (Purina Lab Chow) and water ad libitum and were housed in hanging steel mesh cages located within a positive pressure HEPA filtered isolator.

EXPOSURES

During a three hour exposure to aerosols of saline or endotoxin (dissolved in saline), animals were restrained in head out plethysmographs. Aerosols were generated with a Babington type nebuliser (Solosphere, Airlife Inc, Modesto, CA) driven by medical grade breathing air at 20 pounds per square inch. The output of the nebuliser (7.5 l/min) was diluted with 12.5 l/min air before entering the exposure chamber. Solutions of endotoxin of 1 and 10 µg/ml (*E coli* 0127:B8, Difco Laboratories, Detroit, MI) were made up with pyrogen free isotonic saline (Travenol Laboratories, Deerfield, IL) immediately before exposure. The reservoir of the nebuliser was emptied and refilled at around 45 minute intervals during exposure to minimise concentrating effects of the nebuliser. Exposure concentrations were determined by taking samples of the atmosphere of the chamber in the breathing zone of the animals two or three times during each exposure. Chamber air was sampled at 1.04 l/min through 47 mm filters (0.22 µm, Nuclepore, Pleasanton, CA) that were then soaked and agitated at room temperature in 30 ml of pyrogen free water. Endotoxin concentrations in the exposure chamber were calculated in two ways: by analysing random samples of the filter eluant for endotoxin with a chromogenic *Limulus* lysate assay (Whittaker Bioproducts, Walkersville, MD) and by multiplying a "loss factor" by the nominal endotoxin concentration (endotoxin output from the nebuliser divided by the total airflow). The "loss factor" was determined by dividing the actual sodium concentration (calculated by measuring sodium in the filter eluant with a flame photometer (IL-143, Instrumentation Laboratories, Lexington, MA)) by the nominal sodium concentration. Particle size measurements were made with a seven stage miniscascade impactor (Intox Products, Albuquerque, NM).

EXPERIMENTS

Animals were divided into four groups and exposed

to saline (n = 7), endotoxin (n = 6 for the low dose group and n = 5 for the high dose group), or cotton dust (n = 6) for three hours. Specific airway conductance (SGaw) was measured by the method of Agrawal¹⁴ in awake and spontaneously breathing guinea pigs at five minute intervals for 15 minutes before exposure and at 30 minute intervals during exposure. Animals were restrained by the posterior chamber of the Agrawal plethysmograph during the head only exposures and were removed for about two minutes for each measurement of SGaw. Immediately after exposure, each animal was anaesthetised with pentobarbital (30 mg/kg) and local lidocaine (subcutaneously) was used to surgically expose a jugular vein for injection of Evans blue dye (50 mg/kg, Sigma Chemicals, St Louis, MO), a marker of plasma protein extravasation.¹⁵ Rectal temperature was monitored and maintained between 37 and 38°C with a heat lamp. After 30 minutes of circulation, each animal was given a lethal injection of pentobarbital and perfused systemically via the aorta with phosphate buffered saline at a perfusion pressure of 95 mm Hg for two and a half minutes. A 1 cm section of the trachea (just proximal to the tracheal bifurcation), the mainstem bronchi, and the extrapulmonary hilar bronchi were removed, cleaned, and weighed before extraction of Evans blue dye in 1 ml of formamide (Sigma) at 60°C for 24 hours. The absorption intensity of each sample at 620 nm was measured with a spectrophotometer, compared with standard dilutions of Evans blue dye, and the dye content in each tissue section expressed as µg of dye/g of tissue (wet weight). A section of the left caudal lobe was saved and fixed in 1% paraformaldehyde/2% glutaraldehyde for morphological evaluation. To examine airway morphology, cross sections were dehydrated and embedded in glycol methacrylate. Sections 2.5 µm thick were stained for 30 to 45 minutes with Giemsa stain (original azure blend type; Harleco, Gibbstown, NJ) dissolved in 0.2 M TRIS-maleate buffer and adjusted to pH 5.5. Inflammatory cells were counted for each animal in two non-serial cross sections of a major intrapulmonary bronchus from the left caudal lobe on coded slides using a light microscope (magnification × 1000). Profiles of PMNs, eosinophils, and mast cells were counted in the mucosa (epithelium and lamina propria) around each bronchus and reported as cells/mm². Measurements of tissue area were determined by analysing 5 × 7 inch photographs of the mucosal region of the bronchus with a digitiser (Model 614B, Talos Systems, Scottsdale, AZ) linked to a computer (Model 9815S, Hewlett-Packard, Loveland, CO). The tissue area that was examined was calculated as the difference between the areas circumscribed by the outermost perimeter of the mucosal layer and the lumen of the bronchus.

Cotton dust aerosols were produced by an acous-

tical generator modelled after the Pitt-3 dust generator.¹⁶ An eight inch speaker was driven at 31 Hz by a laboratory built sine wave signal generator (Jameco Inc, San Mateo, CA) and amplified by a 25 watt stereo receiver. Airflow through the dust generator (3–10 l/min) and the volume amplitude driving the speaker were adjusted manually throughout exposure to achieve a cotton dust concentration of about 30 mg/m³. Dust concentrations were monitored continuously with a light scattering device (RAM-1, GCA, Inc, Bedford, MA) and recorded at 15 minute intervals for calculations of time weighted averages (TWAs). A gravimetric filter sample was taken during each exposure for comparison with readings obtained with the light scattering device. The filter was then soaked and agitated at room temperature in 30 ml of pyrogen free water for one hour for determining endotoxin concentrations with the chromogenic assay. Bulk raw cotton dust of west Texas origin was provided by Cotton Incorporated (Raleigh, NC). Particle size measurements for cotton dust were done with a light scattering device (LAS-X, Particle Measuring Systems, Fremont, CA).

Further groups of animals were treated with intravenous (iv) saline ($n = 6$) or with iv ($n = 6$) or aerosolised ($n = 6$) WEB2086 (Boehringer Ingelheim, Ridgefield, CT) before and during exposure to endotoxin. For iv administration, a jugular catheter was surgically inserted and secured under anaesthesia by intramuscular injection of ketamine (25 mg/kg) and sparine (1.9 mg/kg) mixture. The animal was then allowed to recover for about two hours before exposure. Heparinised saline was used to keep the catheter patent and to flush the drug or placebo from the catheter dead space after administration. Pyrogen free saline and WEB2086 (10 mg/kg per injection and 2 ml/kg dissolved in saline) were given by iv bolus before and midway through a three hour exposure to endotoxin. For aerosol administration WEB2086 was dissolved in pyrogen free water (5 mg/ml) and nebulised with an ultrasonic nebuliser (Pulmo-Sonic, DeVilbiss, Somerset, PA) with an output of roughly 0.5 ml/min and a mass median aerodynamic diameter of approximately 5 μ m to ensure particle deposition in the airways. Medical grade breathing air carried the aerosol to the animal restrained in the Agrawal plethysmograph. The WEB2086 aerosol was administered to the animal for 10 minutes immediately before and midway through exposure. Aerosolised or iv WEB2086 alone had no effect on baseline SGaw. The efficacy of aerosolised WEB2086 was confirmed in a further group of animals ($n = 5$) treated with aerosolised WEB2086 before inhaling aerosols of doubling concentrations of PAF. To examine the baseline response to aerosolised PAF, animals were monitored for changes in SGaw at one, five, and 10 minutes after a five minute exposure to 0.1% albumin

followed by doubling concentrations of PAF (0.05% to 3.2% in 0.1% albumin) at 10 minute intervals until SGaw decreased by greater than 50%. The animals were allowed to recover for 90 minutes to two hours, treated with aerosolised WEB2086 or vehicle, and challenged again with 0.1% albumin, then by doubling concentrations of aerosolised PAF until SGaw decreased by 50%.

STATISTICAL ANALYSES

The effect of each exposure on SGaw was analysed by one way analysis of variance and a Dunnett's t test comparing pre-exposure SGaw values with values at each time point during exposure. Group differences in tissue Evans blue dye content and inflammatory cell counts were analysed by a one way analysis of variance and a Dunnett's t test where indicated. The provocative concentration of PAF that decreased SGaw by 50% (PC_{50}) was calculated by log₂ linear interpolation and used for comparison (paired Student's t test) of the response to PAF before and after treatment with WEB2086. Levels of $p \leq 0.05$ were accepted as evidence of significant group differences.

Results

The content of Evans blue dye in the airways was increased by exposure to inhaled endotoxin. In each generation of bronchi examined, significant dye extravasation occurred in both endotoxin groups (fig 1). Mean dye extravasation was increased by 106% and 314% in the mainstem bronchi and by 124% and 247% in the hilar bronchi of the animals treated with low and high dose endotoxin, respectively (all p values < 0.05). In the trachea, mean Evans blue dye extravasation was significantly increased only in the

Figure 1 Effect of a three hour exposure to saline, low or high dose endotoxin, or cotton dust on airway protein extravasation in the trachea, mainstem bronchi, and hilar bronchi. The columns and bars represent the mean $\pm$ SE for each group. For comparison of saline v endotoxin and cotton dust exposure groups * $p < 0.05$, ** $p < 0.01$.

Figure 2 The effect of inhaled endotoxin and cotton dust on specific airway conductance (SGaw, mean $\pm$ SE). The axis is time (min) during a three hour exposure to saline ($n = 7$), low dose endotoxin ($n = 6$), high dose endotoxin ($n = 5$), or cotton dust (32 mg/m^3 ($n = 6$)). The axis is the mean specific airway conductance (SGaw) during exposure expressed as a % of the mean baseline SGaw.

high dose endotoxin animals (136% increase, $p < 0.01$).

These increases in protein extravasation were accompanied by a significant decrease in SGaw. Exposure to endotoxin produced significant changes in SGaw by 60 to 90 minutes into exposure (fig 2). At the end of exposure, mean SGaw had decreased by 22 and 34% for animals exposed to the low and high dose endotoxin, respectively.

Morphological examination of airway sections demonstrated major differences in the number of PMNs, but not eosinophils or mast cells, in the intrapulmonary bronchi of animals exposed to

Figure 3 The effect of a three hour exposure to saline, low or high dose endotoxin, or cotton dust on the number of polymorphonuclear leucocytes (PMNs), eosinophils (EOs), and mast cells in the bronchi. Cell profiles were counted in the epithelium and lamina propria in a major bronchus from the left caudal lobe and are expressed as the number of cells/mm² of airway wall. The columns and bars represent the mean $\pm$ SE for each group. For comparison of saline v endotoxin and cotton dust exposure groups $**p < 0.01$.

endotoxin compared with animals exposed to saline. A statistically significant dose dependent influx of PMNs was seen in the mucosal wall of bronchi from animals exposed to endotoxin (fig 3).

Exposure to cotton dust caused a similar spectrum of changes in the airways. Inhaled cotton dust produced a significant increase in the Evans blue dye content of the trachea, mainstem bronchi, and extrapulmonary hilar bronchi (138%, 325%, and 276% greater in animals exposed to cotton dust than in animals exposed to saline aerosols (fig 1)). The SGaw was significantly decreased by 60 minutes into exposure and was 43% below mean baseline SGaw by the end of exposure (fig 2). These changes were accompanied by a significant increase in the number of airway PMNs (fig 3).

The mean (SD) chamber temperature for all groups was $23.8 (1.7)^{\circ}\text{C}$. The mean (SD) concentrations of endotoxin as measured by the chromogenic *Limulus* amoebocyte lysate assay were $6.1 (2.3) \mu\text{g/m}^3$ and $71.7 (13.2) \mu\text{g/m}^3$ for the low and high dose endotoxin groups. The concentrations calculated using the sodium concentrations measured with the flame photometer were $9.8 (0.5) \mu\text{g/m}^3$ and $99.0 (9.0) \mu\text{g/m}^3$ for the low and high dose endotoxin groups. The mass median aerodynamic diameter of the saline and endotoxin particles produced by the nebuliser was $1.4 \mu\text{m}$ with a geometric standard deviation (GSD) of 1.8. The mean (SD) cotton dust concentration measured by the RAM-1 light scattering device during the three hour exposures was $31.6 (1.0) \text{ mg/m}^3$. Gravimetric analysis of the chamber concentration of cotton dust agreed with the results obtained with the RAM-1. A mean (SD) dust concentration of $27.2 (3.8) \text{ mg/m}^3$ was measured by the RAM-1 during the same intervals in which gravimetric analyses indicated $27.5 (4.6) \text{ mg/m}^3$. As measured by the chromogenic lysate assay, the airborne endotoxin concentration during exposures to cotton dust was $5.1 (1.6) \mu\text{g/m}^3$. The count median diameter of the cotton dust particles produced by the acoustical generation system was $1.8 \mu\text{m}$ with a GSD of 2.6.

Intravenous treatment with a concentration of the PAF antagonist WEB2086 that had previously been shown to block the airway effects of PAF¹⁷ and the systemic effects of iv endotoxin¹⁸ did not prevent the decrease in SGaw or the protein extravasation caused by inhaled endotoxin. Delivery of the PAF antagonist to the airways also did not prevent the airway effects of inhaled endotoxin (table) although the dose of WEB2086 delivered to the animals was sufficient to cause a sixfold shift in the bronchoconstrictor response to inhaled PAF. The PC₅₀ (mean $\pm$ SE) for inhaled PAF was $0.27 \pm 0.10 \text{ mg/ml}$ before and $1.64 \pm 0.29 \text{ mg/ml}$ after treatment with aerosolised WEB2086. The PC₅₀ for inhaled PAF was 0.29 ± 0.16 before and 0.38 ± 0.48 after treatment with the aerosolised vehicle for WEB2086 (water).

The effect of WEB2086 on the airway effects of inhaled endotoxin*

Treatment	No	Baseline SGaw at end of exposure (%)	Evans blue dye content ($\mu\text{g dye/g tissue}$)		
			Trachea	Mainstem bronchi	Hilar bronchi
Intravenous saline	6	86 $\pm$ 7	38.1 $\pm$ 3.8	82.8 $\pm$ 8.3	86.7 $\pm$ 8.8
Intravenous WEB2086	6	74 $\pm$ 6	32.2 $\pm$ 3.6	56.5 $\pm$ 7.6	74.3 $\pm$ 10.2
Aerosol WEB2086	6	63 $\pm$ 5	32.3 $\pm$ 5.7	71.4 $\pm$ 8.4	81.0 $\pm$ 13.6

*Data are expressed as mean $\pm$ SE.
Animals were exposed to the high dose of endotoxin.

Discussion

The present study has shown that inhaled endotoxin in guinea pigs produces an increase in protein extravasation in the conducting airways in addition to the well established production of airway obstruction.^{19,20} The increase in extravasation of plasma protein bound dye in the trachea and extrapulmonary bronchi after a single three hour exposure to endotoxin was accompanied by an influx of PMNs into the airway wall. The magnitude of protein leakage appeared to be greater in the bronchi than in the trachea, but it is not clear whether this effect was due to greater deposition of the inhaled particles in the bronchi or to a difference in the physiological response of the airways. Plasma protein extravasation could contribute to airway obstruction by either a direct decrease in airway calibre due to the leakage of protein and the accompanying fluid into the airway wall or by the extravasation of blood borne elements or mediators which induce a change in airway smooth muscle tone. Unrelated to the effects on airway conductance, the leakage of fluid and protein into the airway wall may contribute to the symptoms of chest tightness reported by workers exposed to organic dusts contaminated with endotoxin.

Endotoxin is present in significant quantities in airborne organic dusts encountered in agricultural and textile workplaces throughout the world. Several studies have previously reported that inhaled endotoxin can alter pulmonary function in man and animals.¹⁹⁻²¹ The present study examined the actual time course of development of this airway obstruction in awake, spontaneously breathing animals during acute exposures to endotoxin. Previous studies examining the pulmonary effects of inhaled endotoxin and cotton dust in animals have focused on the irritant effects of these agents.^{21,22} In those studies, changes in tidal volume and frequency, but not airway obstruction, were monitored non-invasively. Because studies have shown that airway obstruction and chest tightness develop reproducibly in textile workers or naive human volunteers within the first few hours of exposure to endotoxin or organic dusts,^{1,20} we chose to monitor specific airway conductance (SGaw) using the non-invasive Agrawal method. In the present study, inhaled endotoxin

produced significant decreases in SGaw at 60 to 90 minutes into exposure. Although the similarity in the time course of development of airway obstruction during exposure to endotoxin or cotton dust is intriguing and consistent with the hypothesis that endotoxin plays a major part in the airway obstruction produced by inhaled cotton dust, the participation of other bioactive agents present in cotton dust²³ was not examined. In the present study, exposure to 31.6 mg/m³ cotton dust produced nearly twice the decrease in SGaw (43% *v* 22%) and twice the increase in protein extravasation (for example, 140.4 *v* 66.1 $\mu\text{g dye/g tissue}$ in the hilar bronchi) that occurred in animals exposed to a similar concentration of endotoxin nebulised in isotonic saline (5.1 $\mu\text{g/m}^3$ endotoxin during exposure to cotton dust and 6.1 $\mu\text{g/m}^3$ during the low dose endotoxin exposure as measured by the *Limulus* lysate assay). Thus other agents in cotton dust may have contributed to the protein extravasation and the airway obstruction. Also, a non-specific stimulation due to the high level of cotton dust particles themselves may have occurred in combination with the specific effects of endotoxin. Alternatively, the concentration of endotoxin measured during the exposure to cotton dust may have been underestimated by the *Limulus* lysate assay or by the extraction technique used in the present study. Measurements of the endotoxin concentration in air samples have been notoriously difficult. Recently, data have been presented suggesting that this commonly used extraction technique (room temperature agitation of filters in pyrogen free water) may underestimate the amount of endotoxin on filters loaded with cotton dust.²⁴ Also, evidence has been presented suggesting that the physical form of endotoxin being studied can significantly affect the results of the *Limulus* amoebocyte lysate assay. Under typical assay conditions, the assay does not measure the same amount of endotoxin when the endotoxin is in a soluble form compared with a cell wall bound form that is present in nature.²⁵ The actual concentration of endotoxin in atmospheres generated by industrial handling of organic dusts is thus uncertain. Despite the likely underestimation of endotoxin concentrations from the most widely used methods of extraction and analysis, concentrations

greater than 10 $\mu\text{g}/\text{m}^3$ have been reported in work settings.^{4,5} This concentration of endotoxin is similar to that measured in the present study during exposures to low dose endotoxin and to cotton dust. The small difference between the endotoxin concentrations we measured with the *Limulus* amoebocyte lysate assay (6.1 and 71.7 $\mu\text{g}/\text{m}^3$) and the nominal concentrations we calculated from measurements of filter concentration of sodium (9.8 and 99.0 $\mu\text{g}/\text{m}^3$) could be due to underestimation of the true concentration by the *Limulus* amoebocyte lysate assay or to selective loss of endotoxin in our aerosol generation system. None the less, whereas the concentration of dust used in the present study was significantly higher than that currently found in the textile industries of the United Kingdom and the United States, organic dust concentrations in agricultural settings in these countries as well as textile mills of developing countries may be similar to this high level of organic dust.

As shown by other investigators, one possible problem in interpreting the significance of airway obstruction in awake guinea pigs exposed to endotoxin or cotton dust is the contribution of extrathoracic *v* intrathoracic airways in the observed changes in airway conductance.²⁶ Because guinea pigs are obligatory nose breathers, the airway obstruction seen in the present study may have been due to the effects of inhaled endotoxin on the upper respiratory tract or larynx. The possible relevance of the observed airway obstruction in this and other small animal studies must be viewed in terms of the intrathoracic airway effects and symptoms found in workers exposed to organic dusts contaminated with endotoxin. Experimental evidence of intrathoracic airway obstruction in tracheostomised animals would be necessary to clarify this issue.

Investigators have previously found significant increases in the number of PMNs as well as chemotactic factors for PMNs in the lavage fluid from animals exposed to endotoxin or extracts of cotton dust.¹⁰ Evidence also indicates that a crude extract of cotton dust attracts PMNs to the conducting airways of hamsters.⁹ The results of our present study confirm the influx of PMNs to the site of the airway injury produced by inhaled endotoxin and cotton dust. The presence of PMNs at the site of the plasma protein leakage (and the possible site of airway obstruction) suggests a role for PMNs in the adverse effects of inhaled endotoxin and cotton dust. Such a role for PMNs has recently been reported for some of the acute pulmonary responses to inhaled cotton dust. Partial depletion of circulating PMNs by treatment of cotton dust exposed guinea pigs with hydroxyurea did not alter the magnitude of the increase in frequency produced in response to a post-exposure CO_2 challenge.²⁷ Complete depletion of circulating PMNs with cyclophosphamide, however,

significantly inhibited both the response to CO_2 and the zymosan activation of alveolar macrophages obtained by lavage.

Our findings provide a suitable model for studying the role of inhaled endotoxin in the acute respiratory effects of organic dusts. To date, endotoxin has been associated with, rather than causally linked to, various organic dust diseases. It has been shown that biologically significant quantities of endotoxin are present in the respirable fractions of several organic dusts and that the acute symptoms of byssinosis are better correlated with airborne endotoxin concentrations than respirable cotton dust concentrations.¹ These types of studies, however, cannot rule out the importance of other biological agents present in organic dusts. The growth and storage conditions that lead to increases in Gram negative bacterial contamination of cotton, grain, and confinement buildings for farm animals could result in the presence of other contaminants such as moulds, fungi, antigenic material, and tannins, which may also become airborne during manufacturing or handling processes. Therefore, the results from these types of experiments can be ambiguous, at best, in determining the aetiological agent(s).

Among the most prominent effects of systemically administered endotoxin is extravasation of plasma protein into a variety of tissue beds,²⁸ including the conducting airways.¹² Because PAF is known to cause both plasma protein extravasation and airway obstruction, this mediator is a likely candidate for involvement in the effects of endotoxin on airways. Indeed, in a recent study, treatment with a PAF antagonist was shown to inhibit the extravasation of plasma protein into airways caused by intravenous administration of endotoxin in guinea pigs.¹² Other investigators have also shown that the PAF antagonist RP48740 inhibits the protein leakage in the alveolar region after exposure to inhaled endotoxin.²⁴ In the present study, however, we examined the role of PAF in the conducting airway injury produced by inhaled endotoxin. Intravenous treatment with the PAF antagonist WEB2086, at a dose shown by other investigators to inhibit the systemic effects of iv endotoxin¹⁸ and the airway effects of PAF,¹⁷ did not block the decrease in SGaw or the extravasation of protein caused by inhaled endotoxin. To show that this apparent lack of a role for PAF was not due to an insufficient concentration of the antagonist in the cells lining the lumen of the airways, presumably the initial site of airway injury, we also treated animals with WEB2086 by the aerosol route. Despite its effectiveness in blocking the decrease in SGaw produced by inhaled PAF itself in this study and airway oedema in other studies, inhaled WEB2086 did not block the airway effects of inhaled endotoxin. These findings suggest that the mediators of the local inflammatory effects of endotoxin deposited within

the lumen of the conducting airway may be different from the mediators involved in the local effects of endotoxin deposited in the alveoli and in the airway effects of endotoxin administered systemically. This difference appears to exist even for the same endpoint (plasma protein extravasation) in a single species (the guinea pig). Thus, whereas the airway effects of systemic sepsis can be examined by studying systemically administered endotoxin, the airway effects of endotoxin contamination of inhaled organic dusts require administration of endotoxin by inhalation.

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