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In Vivo Effects of Endotoxin on Intraepithelial Mucosubstances in Rat Pulmonary Airways

Quantitative Histochemistry

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Bacteria-induced bronchopneumonias are often characterized by an influx of neutrophils and excess mucus in pulmonary airways. This study determined how endotoxin, a component of gram-negative bacteria and a potent inflammatory agent, affects the ultrastructure of the mucociliary apparatus and the amount of stored intraepithelial mucosubstances in the main axial airways within the lung. Rats were intranasally instilled, once a day for 3 days, with endotoxin or saline (controls). Animals were sacrificed 1, 2, or 7 days after the last instillation. Microdissected intrapulmonary axial airways (generations 8–11) from the right caudal lobes of infusion-fixed lungs were processed for light and electron microscopy. Morphometric techniques were used to determine the volume densities (Vs) of histochemically stained intraepithelial mucosubstances and numerical densities of airway epithelial cells. There were marked increases, compared with controls, in the amount of intraepithelial mucosubstances in the intrapulmonary axial airways at generations 8 and 11 in the right caudal lobes from endotoxin-instilled rats sacrificed 1, 2, and 7 days after the last instillation. There were significantly greater numbers of surface epithelial cells per length of basal lamina (i.e., hyperplasia) in endotoxin-exposed airways compared with airways from controls. This endotoxin-induced hyperplasia was due primarily to an increase in the number of mucus-secretory cells, which in endotoxin-exposed epithelium were columnar and contained numerous, large confluent, electron-lucent, secretory granules composed of acidic and neutral glycoproteins. In contrast, secretory cells in airway epithelium from controls were cuboidal and contained small discrete, electron-dense, granules composed of only neutral glycoproteins. The numbers of ciliated cells and basal cells were similar in both control and endotoxin-exposed epithelium. Only endotoxin-exposed epithelium, however, contained atypical epithelial cells with numerous basal bodies, few cilia, and few apical secretory granules. These results indicate that repeated airway instillations of endotoxin induce an increase in the amount of intraepithelial mucosubstances, secretory cell hyperplasia, and excess luminal mucus in pulmonary airways. Therefore, endotoxin released from gram-negative bacteria may be partially responsible for the structural alterations, in the airway surface epithelium, which result in the excess luminal mucus observed in bacteria-induced bronchopneumonias. (Am J Pathol 1992, 141:307–317)

Endotoxins are lipopolysaccharide-protein molecules released from the walls of gram-negative bacteria. Although they are not chemotactic for neutrophils,[1,2] they are more potent on a molar basis in inducing neutrophil emigration than all chemotactic factors tested.[3] Endotoxins are major bacterial constituents presumed to induce injury to the lungs in gram-negative sepsis and gram-negative, bacterial bronchopneumonia.[4] Pulmonary airway infections caused by gram-negative bacteria that contain endotoxin account for 40–60% of pneumonias acquired in hospitals, and mortality rate of these bacteria-induced pneumonias is approximately 50%.[5,6]

Inhalation exposure to endotoxin from gram-negative bacteria may also be an important factor in several occupational diseases, including byssinosis,[7] mill fever,[8] and bagassosis.[9] In addition, endotoxins are ubiquitous

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substances found in the domestic environment. They have been detected at biologically active concentrations in domestic water (0.04–1 μg/ml),[10] in air humidification systems (0.13–0.39 μg/mg^3),[11,12] and in commercial house dust extracts (0.45–500 μg/ml).[13] Therefore, large amounts of these potentially harmful substances could be inhaled in certain industrial and domestic environments to evoke deleterious airway responses. Asthmatic subjects may be particularly at risk, because they have more bronchial reactivity to inhaled endotoxin than normal subjects.[14]

Airway infections induced by endotoxin-laden bacteria are often characterized by increased production and secretion of respiratory mucus and influx of inflammatory cells (i.e., neutrophils). Both the inflammatory exudation and the hypersecretion of mucus are also believed to be important factors in the pathogenesis of obstructive pulmonary disorders like cystic fibrosis,[15–17] acute and chronic bronchitis,[18–20] and upper respiratory tract disorders, such as infectious rhinitis.[21] Although endotoxin inhalation is known to induce acute airway inflammation in laboratory animals,[22] its effects on the ciliated respiratory epithelium and the mucus-secretory apparatus lining the airway lumens within the lung have not been thoroughly investigated. We have recently reported that repeated intranasal instillations of endotoxin induce an increase in the amount of intraepithelial mucosubstances in the nasal respiratory epithelium when there is an absence of intranasal neutrophil influx and mucous hypersecretion when neutrophils are present.[23] Endotoxin may be a major etiologic agent responsible not only for the inflammatory cell influx, but also for the copious amounts of airway mucus often present in gram-negative, bacteria-induced, and endotoxin-related airway diseases.

This study determined what structural effect(s) endotoxin exposure has on the mucociliary apparatus of the large caliber, preterminal bronchioles in the rat lung. We were particularly interested in determining if endotoxin induces changes in the amount of intraepithelial mucosubstances in pulmonary airways as it does in the nasal airways of the rat. Although there has been one brief report that intratracheal instillations of *E. coli* lipopolysaccharide (endotoxin) induces secretory cell metaplasia in the lungs of Syrian hamsters,[24] there has been no reported study designed to morphometrically characterize the effects of endotoxin on the cellular populations and stored secretory products of the epithelium lining the large caliber, intrapulmonary airways. We elected to examine in detail the effects of endotoxin on these specific intrapulmonary airways in the rat for three reasons: 1) secretory cells in the epithelium lining these airways are predominantly serous cells[31] and the effect of endotoxin on this secretory cell type is unknown; 2) intrapulmonary airways at this level in the rat respiratory tract have been reported to exhibit the greatest degree of change in the number of secretory cells and the amount of intraepithelial mucosubstances, compared with other airway levels (e.g., trachea, terminal bronchioles) in rats exposed to other inhaled toxicants [i.e., SO_2,[38] tobacco smoke[39]]; 3) the large caliber intrapulmonary airways in the rat are at similar airway generations as human intrapulmonary bronchial airways that exhibit marked histologic alterations to the mucosal secretory apparatus in patients with chronic bronchitis.[18,19]

Materials and Methods

Animals and Nasal Instillations

Forty-eight female, F344/N, 12–14-week rats, from several different litters in the Institute's breeding colony, were used in this study. Rats were housed 2 or 3 per polycarbonate cage and supplied with sterilized hardwood chip bedding and filter tops. Animal rooms were maintained at 20–22°C with a relative humidity of 20–50% and a 12-hour light/dark cycle starting at 6:00 AM. Food (Lab Blox, Allied Mills, Chicago, IL) and water from bottles with sipper tubes were provided *ad libitum*. In addition, all rats used in the study were randomly assigned to one of six experimental groups according to body weight. The group assignments were adjusted to result in mean group body weights that were not significantly different from one another.

The rats were anesthetized with 5% halothane in oxygen, and 0.05 ml of saline containing 5 mg of lipopolysaccharide (endotoxin) from *Escherichia coli* 0111:B4 (Sigma Chemical Co., St. Louis, MO) per milliliter of pyrogen-free saline were instilled into each airway of the nasal cavity of 24 rats on three consecutive days. The other 24 rats were instilled with pyrogen-free saline as controls at similar times. Instillations were accomplished by allowing the rats to aspirate the instillate into the upper and lower respiratory airways after it was deposited as a bead of fluid on the external nares. All rats (i.e., endotoxin- and saline-instilled) were instilled on the same days and at similar times, between 8:00 and 10:00 AM.

Necropsy and Tissue Preparation for Histopathology

The rats were anesthetized by halothane inhalation and killed by exsanguination via the abdominal aorta or renal arteries 1, 2, or 7 days after the last instillation. Immediately after death, the trachea, extrapulmonary bronchi, and lungs were excised intact from the thoracic cavity and intratracheally perfused with fixative at 30 mm of fix-

ative pressure for 2 hours. Tracheobronchial and pulmonary airways from 36 of the rats (6 rats/group) were fixed with Carnoy's fixative (60% ethyl alcohol, 30% chloroform, and 10% glacial acetic acid), while the lungs from 12 animals (6 saline-instilled and 6 endotoxin-instilled rats), killed 2 days after the last instillation, were fixed with a modified Karnovsky's fixative (0.6% glutaraldehyde/0.45% paraformaldehyde in cacodylate buffer, pH 7.4). After a 2-hour perfusion-fixation, the trachea was ligated and the fully distended lung was immersed in a large volume of the same fixative until further processing.

After fixation, a midsagittal tissue section of the left lung lobe from each rat was excised and embedded in paraffin. Four to six micron-thick sections were cut, stained with hematoxylin and eosin, and alcian blue (pH 2.5), and microscopically examined for histopathology.

The intrapulmonary airways of the right caudal lung lobe from each animal were microdissected according to a modified version of the microdissection technique of Plopper et al.[25] Dissections were performed under a high-resolution dissecting microscope (dual-viewing Wild M-8 stereomicroscope; Wild Heerbrugg Ltd., Heerbrugg, Switzerland). Beginning at the lobar bronchus, the airways were split down the long axis of the largest daughter branches (i.e., main axial pathway) through the eleventh airway generation.

Each airway was numbered by a binary system originally used by Phalen et al.[26] For example, the trachea was numbered "1," and the right main stem bronchus, the larger of two branches from the trachea, was numbered "11." The smaller branch, the left extrapulmonary bronchus, was numbered "10." At every airway branch point, the smaller branch, or minor daughter, was numbered by adding a "0" to the number of the parent and the larger branch was numbered by adding a "1" to the number of the parent. This system allowed us to assign each airway branch its own unique number, which also gave its branching history. Lobar Airway 111111111 (generation 8) and airway 111111111111 (generation 11), in the main axial pathway of the right caudal lobe of Carnoy-fixed lungs, were excised and processed for light microscopy (Figure 1). The lobar airway 111111111 (generation 8) in the main axial pathway of the right caudal lobe of the Karnovsky's-fixed lungs was excised and processed for transmission electron microscopy (TEM).

Morphometry of Intraepithelial Mucosubstances

Airway tissues designated for light microscopy were embedded in glycol methacrylate, cut at 1 μm and stained with toluidine blue, for morphologic demonstration of epithelial cells, or alcian blue (pH 2.5)/periodic acid-Schiff sequence (AB/PAS) to demonstrate acidic and neutral mucosubstances.[27] Only the 1-μm–thick airway tissue sections stained with AB/PAS were used for morphometric analyses of intraepithelial mucosubstances. These analyses were made by light microscopy at a magnification of X400.

The volume density of AB/PAS-stained mucosubstance in the mucosal surface epithelium was determined using a semi-automatic imaging system, previously described in detail.[28] Briefly, the histochemically stained slides were imaged with a light microscope with an attached charge-coupled device camera and displayed on a color monitor. The area of stored, AB/PAS stained, mucosubstances within the septal surface epithelium was calculated using the image analysis software program from the manually or automatically circumscribed perimeter of the stained material. The length of the underlying basal lamina was determined by tracing the contour of the digitized image of the basal lamina that was projected on a videoscreen. The length of the digitized image was automatically determined by a computer software program (ImageMeasure, Phoenix Technology, Inc., Federal Way, WA) developed for morphometric analyses.

The method we used to estimate the volume of stored mucosubstance per unit surface area of epithelial basal lamina has been described by Harkema et al.[29] The data were expressed as the mean volume density (Vs: nL/mm^2 basal lamina) of AB/PAS-positive mucosubstances within the epithelium ± the standard error of the mean (n = 6/experimental group).

Morphometry of Airway Epithelium

Tissues from axial airways (generation 8) in the right caudal lobes that were designated for TEM were postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohol solutions, infiltrated with propylene oxide solutions, embedded in epon araldite, and thin-sectioned with a diamond knife on an Ultracut E ultramicrotome (Reichert-Jung, Cambridge Instruments, Inc., Deerfield, IL). These sections were mounted on Formvar-coated, slotted grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi H7000 scanning/TEM (Hitachi Ltd., Tokyo, Japan). TEM montages of the entire airway epithelium within the section were prepared at a final magnification of x3900. Differential cell counts of the airway epithelium were determined by counting all nuclear profiles within the intact epithelium visible on these montages. We determined the number of cells per millimeter of lobar airway by counting the number of nuclear profiles per basal lamina length. We counted approximately 400–700 cells per montage, representing basal

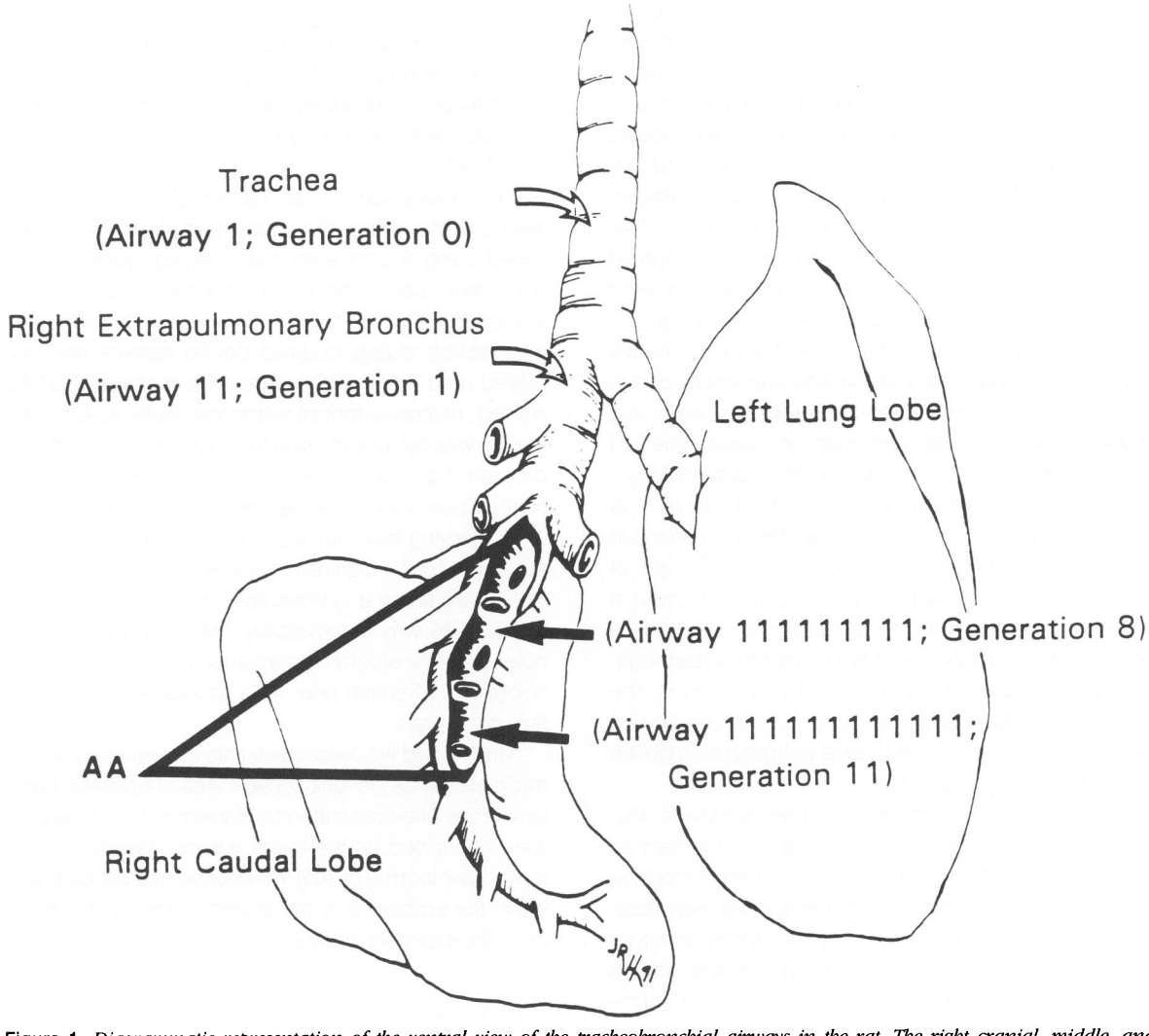

Figure 1. *Diagrammatic representation of the ventral view of the tracheobronchial airways in the rat. The right cranial, middle, and intermediate lung lobes have been removed. AA = region of the intrapulmonary main axial airway in the right caudal lobe from which tissues were taken for morphometric analysis. The ventral wall of the airway segment has been removed exposing the luminal surface and the entrances to the smaller airway branches. G = airway generation (e.g., G8 = eighth airway generation); airway numbers were determined by Phalen's binomial numbering system (see text for details). Only tissues from G8 were examined by electron microscopy as well as light microscopy.*

lamina lengths of 45–125 microns per montage. The length of basal lamina for each montage was determined using the image analysis system and the computer software program for morphometric analyses described earlier.

Statistics

The natural logarithms of the data were used for statistical analyses. Data were tested for equality of group means by using an unpaired Student's *t*-test with Bonferroni correction for multiple comparisons. The criterion for statistical significance was set at $P \leq 0.05$.

Results

Pulmonary Histopathology

Intranasal instillations of endotoxin in rats sacrificed 1 or 2 days after the last instillation induced several focal areas of reddening and consolidation of the pulmonary parenchyma surrounding large intrapulmonary airways, usually located near the hilar region of the right and left lung lobes, but occasionally involving the mid and/or distal aspects of the lobes. These gross alterations involved approximately 25–50% of the pulmonary parenchyma in the affected lobes. Microscopically, these reddened and

consolidated areas were characteristic of an acute bronchopneumonia. The principal parenchymal features included congested alveolar capillaries, interstitial edema within alveolar septa, flooding of alveolar airspaces with fibrinous and eosinophilic exudate admixed with numerous polymorphonuclear leukocytes (neutrophils), and lesser numbers of mononuclear leukocytes. Marked thickening of the alveolar septa was due not only to interstitial edema, neutrophilic influx, and fibrin accumulation, but also to marked hypertrophy and hyperplasia of type 2 pneumocytes. Mitotic figures were also common features in the affected alveolar epithelium. Pulmonary venules within the areas of pneumonia were often cuffed by an interstitial accumulation of mononuclear leukocytes (i.e., monocyte/macrophages and lymphocytes) and lesser numbers of neutrophils. In addition, preterminal and terminal bronchioles in the affected regions had hypertrophic surface epithelium and often conspicuous clumps of catarrhal exudate within their lumens. Marked interstitial edema accompanied by an accumulation of mononuclear leukocytes and neutrophils surrounded these distal airways and adjacent pulmonary arteries and arterioles.

Principal lesions in the main intrapulmonary axial airways and several of the other large caliber, preterminal bronchioles consisted of a markedly thickened, columnar surface epithelium with numerous secretory cells containing copious amounts of AB/PAS-stained material, varying amounts of catarrhal exudate scattered along the luminal surface, peribronchiolar edema, and an accumulation of polymorphonuclear and mononuclear leukocytes in the airway interstitium (Figure 2). Similarly sized bronchiolar airways in saline-instilled rats (controls) had a thin, cuboidal, surface epithelium containing only a scant amount of AB/PAS-stained mucosubstances and no inflammatory cell infiltrates. No intraepithelially AB/PAS-stained material was evident in the terminal bronchioles of saline- or endotoxin-instilled rats at any time after instillation. In addition, control rats had no gross or microscopic lesions in the pulmonary parenchyma.

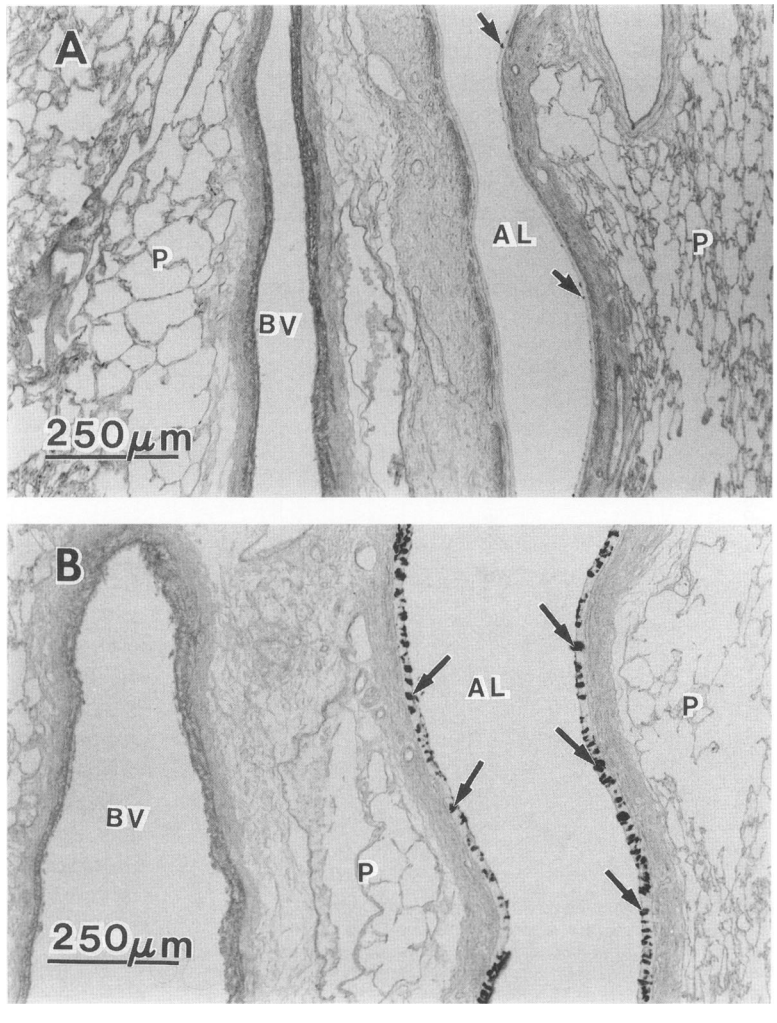

Figure 2. *Light photomicrograph of the main axial pathway in the left lung lobe of a rat repeatedly instilled with saline* (**A**) *or endotoxin* (**B**) *and sacrificed 2 days after the last instillation. AL = axial airway lumen; arrow = alcian blue-periodic acid Schiff (AB/PAS) stained-intraepithelial mucosubstances; P = parenchyma of lung; BV = blood vessel. Section stained with AB/PAS.*

Rats instilled with endotoxin and sacrificed 7 days after the last instillation had no gross lesions in any of the lung lobes. The only consistent histologic alteration in the sections examined was a moderate thickening of the surface epithelium containing secretory cells with AB/PAS-stained material lining the main intrapulmonary axial airway and a few preterminal bronchioles. No inflammatory or alveolar epithelial responses similar to those in the endotoxin-instilled rats sacrificed at 1 or 2 days after the last instillation were present in the endotoxin-instilled rats that were sacrificed 7 days after the last instillation.

Morphometry of Intraepithelial Mucosubstances

The quantitative changes in the volume of AB/PAS-stained mucosubstances per square millimeter of basal lamina (Vs) during the postinstillation periods are illustrated in Figures 3 and 4. Because there were no significant differences in the amount of intraepithelial mucosubstances among any of the saline-instilled controls, data from these animals were combined and presented as a single control group (mean ± standard error). Compared with the saline-instilled controls, there were significantly greater amounts of stained mucosubstances in the surface epithelia lining generations 8 and 11 along the main axial airways of endotoxin-instilled rats sacrificed 1 day after the last instillation (approximately 1200 and 1000% more than controls, respectively). Rats instilled with endotoxin and killed 2 days after the last instillation had 1800 and 300% more mucosubstances in generations 8 and 11, respectively, than saline-instilled controls. Rats killed 7 days after the last endotoxin instillation still had approximately 5 and 4 times more intraepithelial mucosubstances in airway generations 8 and 11 of the main axial pathway, respectively, compared with saline-instilled controls.

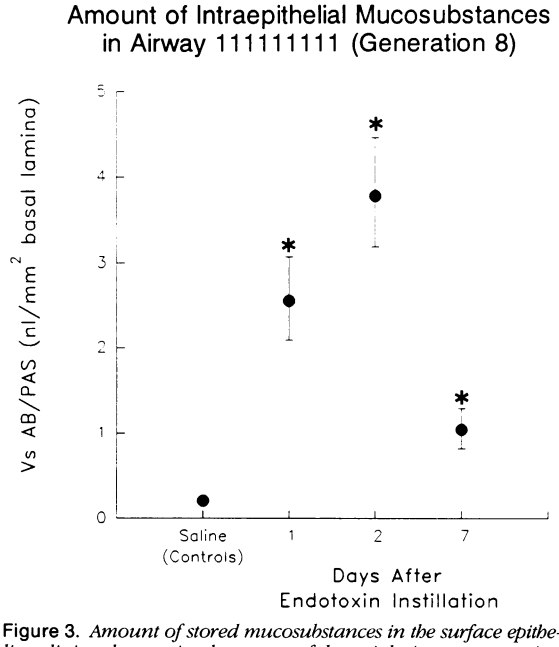

Figure 3. *Amount of stored mucosubstances in the surface epithelium lining the proximal segment of the axial airway, generation 8, in the right caudal lobe at 1, 2, and 7 days after the last instillation of endotoxin. Vs = volume of intraepithelial mucosubstance per surface area of underlying basal lamina.*

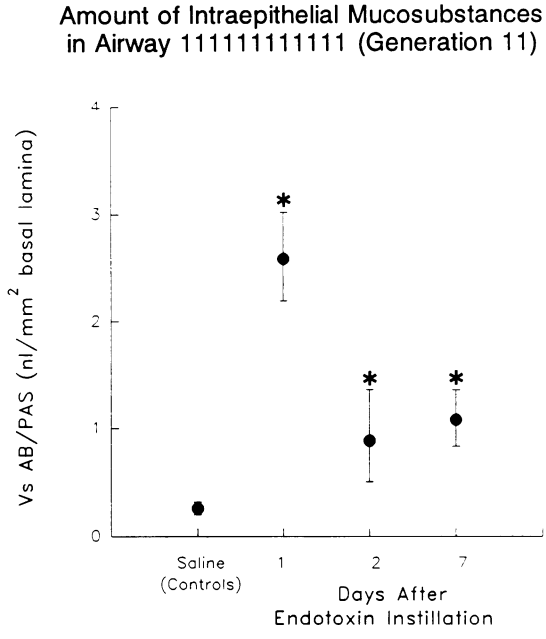

Figure 4. *Amount of stored mucosubstances in the surface epithelium lining the distal segment of the axial airway, generation 11, in the right caudal lobe at 1, 2, and 7 days after the last instillation of endotoxin. Vs = volume of intraepithelial mucosubstance per surface area of underlying basal lamina.*

Ultrastructure and Morphometry of Airway Epithelium

Saline-instilled control rats that were designated for TEM and sacrificed 2 days after the last instillation had a thin, simple, cuboidal, respiratory epithelium composed of ciliated and secretory cells lining the main axial pathway at generation 8. Basal cells were rare and represented only 2–3% of the epithelial cell population. Secretory cells were cuboidal with protruding apical surfaces lined by a few microvilli, electron-dense cytoplasm, abundant supranuclear granular endoplasmic reticulum, and discrete, membrane-bound, moderately electron-dense secretory granules with granular matrices and varying-sized, electron-dense cores (Figure 5). In addition, there

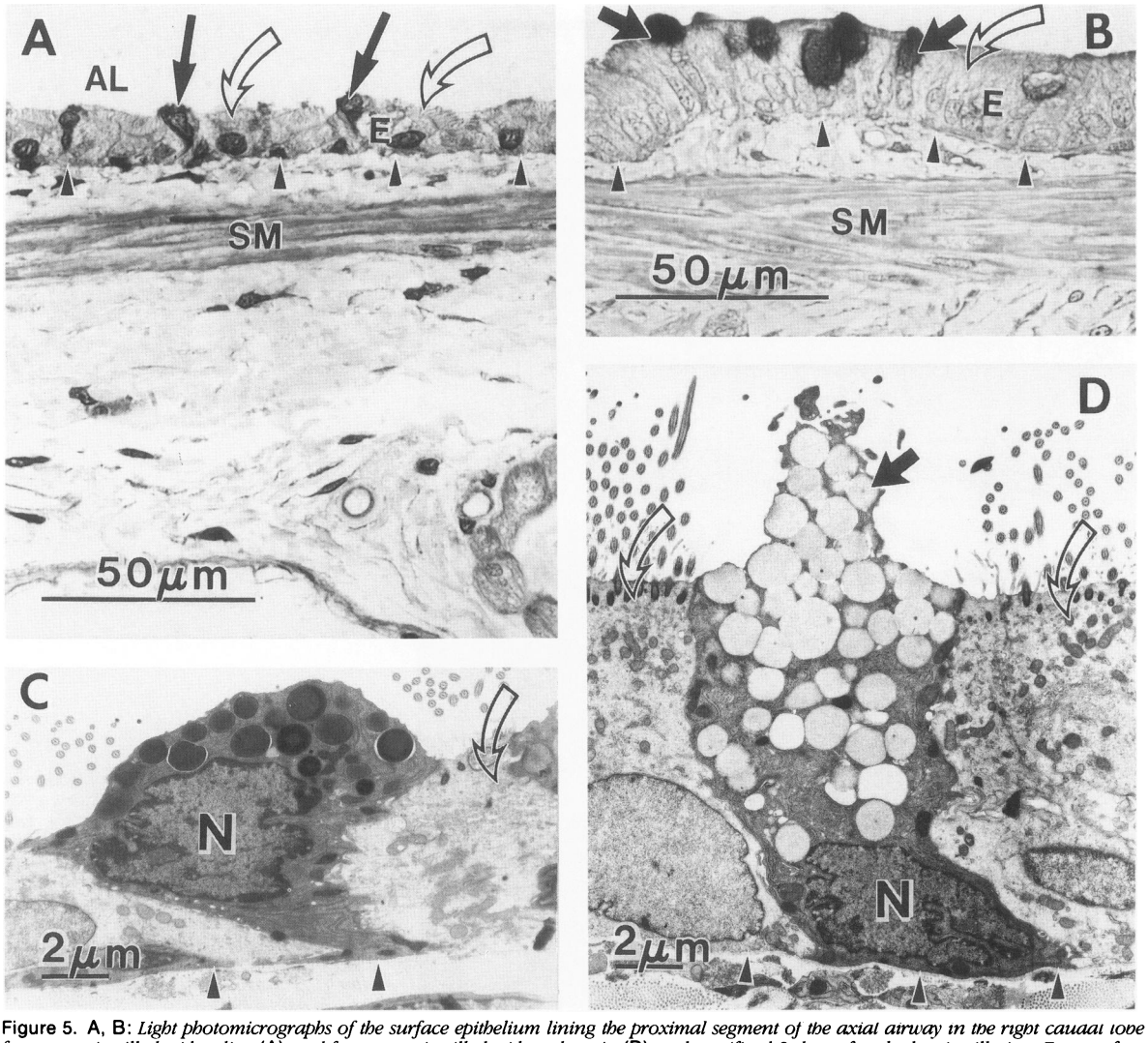

Figure 5. A, B: *Light photomicrographs of the surface epithelium lining the proximal segment of the axial airway in the right caudal lobe from a rat instilled with saline* (A) *and from a rat instilled with endotoxin* (B) *and sacrificed 2 days after the last instillation. E = surface epithelium; closed arrow = secretory cell; open arrow = ciliated cell; AL = airway lumen; arrowhead = basal lamina; SM = smooth muscle in lamina propria. Sections stained with toluidine blue.* C, D: *Electron photomicrographs of secretory cells in the axial airway epithelium from a rat instilled with saline* (C) *and from a rat instilled with endotoxin* (D) *and sacrificed 2 days after the last instillation. Closed arrow = secretory granules; open arrows = ciliated cells; N = nucleus; arrowhead = basal lamina.*

were secretory cells with discrete, apical, granules that were homogeneously electron-dense.

Ciliary cells in airway epithelium from saline-instilled controls were cuboidal and had electron-lucent cytoplasms with abundant mitochondria and basal bodies in their apices underlying the cilia-lined luminal surfaces.

In contrast, endotoxin-instilled rats sacrificed 2 days after the last instillation had a thick respiratory surface epithelium along the main axial pathway at the eight airway generation. The epithelium in these endotoxin-instilled animals was composed of predominantly columnar secretory and ciliated cells. Like the airway epithelium in controls, basal cells were rarely evident. Secretory cells in the airway epithelium after endotoxin instillations were columnar with moderately electron-dense cytoplasm and numerous electron-lucent, partially membrane-bound, secretory granules in the supranuclear cytoplasm (Figure 5). Both discrete and confluent granules were present, and occasionally a granule contained a small electron-dense core.

Ciliated cells from these endotoxin-instilled rats were also columnar and had hypertrophic nuclei and abundant electron-lucent cytoplasm. There were some ciliated cells with only a few and/or shortened cilia, but containing numerous basal bodies, deuterosomes, and other precursor bodies of ciliogenesis in their apical cytoplasms. Sometimes these atypical ciliated cells also contained a few, moderately electron-dense, secretory granules with or without electron-dense cores (Figure 6).

Numerical densities of the different epithelial cells lin-

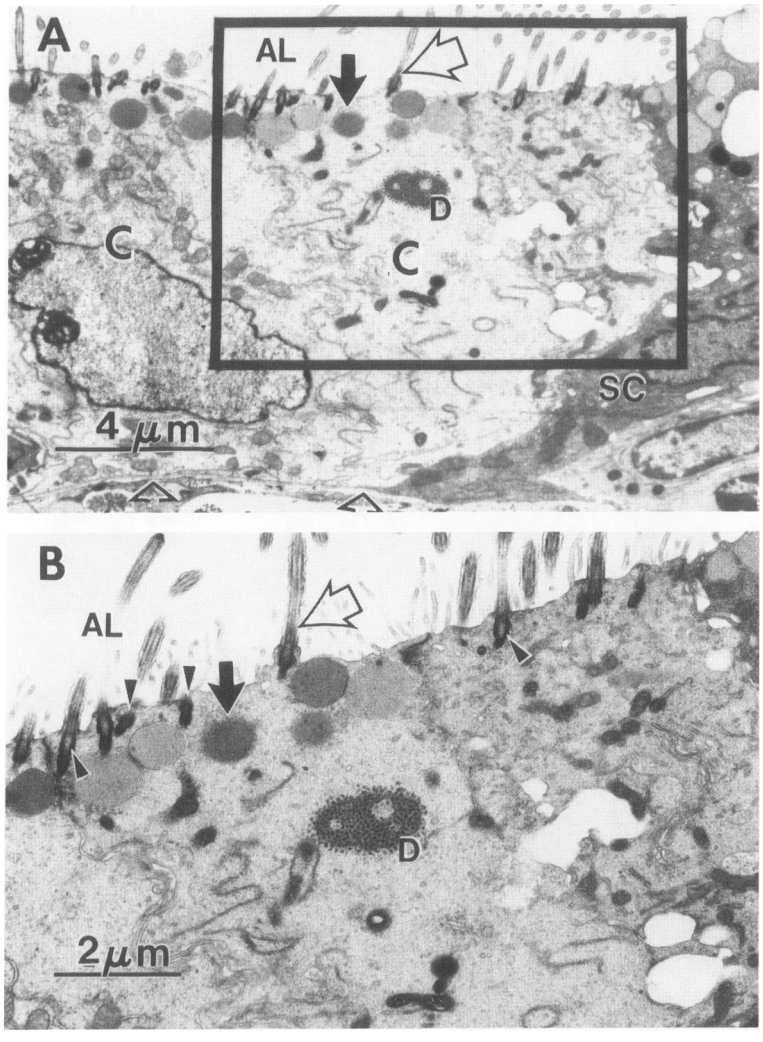

Figure 6. A: *Transmission electron photomicrograph of surface epithelial cells lining the main axial airway (generation 8) in the right caudal lobe of a rat sacrificed 2 days after the last instillation of endotoxin.* B. *Higher magnification of the area within box in* (A). *C = unusual ciliated cells with secretory granules (closed arrows) in their apical cytoplasm; AL = airway lumen; SC = secretory cell; open arrowhead = basal lamina; open arrow = cilia; closed arrowhead = basal body; D = large deuterosome.*

ing the main axial pathway at generation 8 from saline-instilled and endotoxin-instilled groups, sacrificed 2 days after the last instillation, are presented in Table 1. Endotoxin-instilled rats had approximately 30% more epithelial cells per mm of basal lamina than saline-instilled controls. This increase was predominantly due to an increase in the numbers of secretory cells (63 ± 5 cells per mm/basal lamina in the endotoxin-instilled group compared with 37 ± 3 cells/mm basal lamina in the control group). There were no statistical differences in the number of ciliated or basal cells/mm of basal lamina between control and endotoxin-instilled groups. The epithelial cell population in controls consisted of 61% ciliated cells, 36% secretory cells, and 3% basal cells. In contrast, the cellular population of airway epithelium in endotoxin-instilled rats was composed of 54% ciliated cells, 43% secretory cells, and 3% basal cells. In addition, the number of intraepithelial inflammatory cells were negligible in both control and endotoxin-instilled rats.

Table 1. *Endotoxin-induced Changes in the Abundance of Airway Epithelial Cells† (Mean ± Standard Error of Mean)*

Experimental group	Ciliated cells	Secretory cells	Basal cells	Total epithelial cells
Saline-instilled	64 ± 5	37 ± 3	3 ± 1	104 ± 5
Endotoxin-instilled	78 ± 6	63 ± 5*	4 ± 1	145 ± 10*

* Significantly different ($P \leq 0.05$) from saline-instilled (control) group.
† Cells/mm of basal lamina in axial airway (generation 8) of right caudal lobe.

Discussion

The results of this study indicate that repeated instillations of endotoxin induce a persistent increase in the amount

of intraepithelial mucosubstances and marked hypertrophy/hyperplasia of epithelial cells lining the main axial pathways within the rat lung lobes. These changes are similar to secretory epithelial alterations in bronchial airways of human patients with chronic bronchitis,[18–20] bronchopneumonia,[5,6] and cystic fibrosis.[15,16] In addition, the gross and microscopic observations of excess mucus in the lumens of axial airways in endotoxin-instilled rats are also similar to the copious amounts of mucus that accumulate in the bronchial airways of human patients with chronic bronchitis. This excessive production and secretion of airway mucus cause patients with bronchitis to have chronic sputum production that is usually associated with a chronic cough.[18] Similar clinical signs were not observed in the endotoxin-instilled rats; however, the animals at 1 and 2 days after the last instillation appeared to have varying degrees of difficulty in breathing.

The morphologic counterpart of chronic bronchitis in humans is an increase in the size of the mucus-secreting apparatus as it was in the endotoxin-instilled rats of the present study. In humans with chronic bronchitis, it is assumed that most of the luminal mucus originates from abnormally enlarged submucosal glands composed of hyperplastic and hypertrophic mucus-secreting cells, although increased numbers of mucus-secreting cells lining the airway lumen also contribute to the exaggerated amount of luminal mucus. In contrast, the endotoxin-induced increase in the size of the mucus-secreting apparatus was restricted to the surface epithelium lining the rat pulmonary airway, because submucosal glands are not present in the distal tracheobronchial airways of this animal species.[30,31]

Secretory cell hyperplasia is also a common regenerative response of tracheobronchial epithelium in laboratory rodents after mechanical and chemical injury, or vitamin A deficiency. Numerous studies of tracheobronchial injury and repair have demonstrated that secretory cells are the primary progenitor cells in the repair process in both rodents[32–36] and in monkeys.[37] In the present study, the increase in the number of epithelial cells after endotoxin instillations was due to an increase in the number only of secretory cells. There was no increase in the number of basal cells. This may suggest that these cells did not play a primary role in the development of the hyperplastic state. In addition, there was no decrease in the numbers of any of the types of epithelial cells, which indicates that the cellular response to endotoxin-exposure was not a substitution of one epithelial cell type for another. Additionally, there was a marked change in the morphology of the secretory cell population after endotoxin instillations. Secretory cells were cuboidal serous cells with small electron-dense secretory granules in saline-control rats and columnar mucous goblet cells with large, confluent, electron-lucent secretory granules in endotoxin-instilled animals.

Repeated instillations of endotoxin induced conspicuous changes in the histochemical character of the mucosubstances within the airway epithelial cells. Instead of the predominantly PAS-positively stained secretory material in the airway epithelium of saline-controls, endotoxin-exposed airway epithelium contained secretory cells with both AB- and PAS-positively stained material. This striking increase in the AB-staining of acidic mucosubstances after endotoxin instillations is similar to the increases in acidic mucosubstances reported in tracheobronchial and intrapulmonary axial airways of rats exposed to sulfur dioxide[38] or tobacco smoke,[39] and nasal epithelium of rats or monkeys repeatedly exposed to ozone.[40,41] The secretory granules in the secretory cells of the airway epithelium of our endotoxin-instilled rats were also ultrastructurally characteristic (i.e., large confluent granules with an electron-lucent matrix) of granules containing much acidic and little neutral glycoproteins. This type of secretory granule is a characteristic feature of mucous goblet cells.[42,43] This was in contrast to the serous-like secretory cells in the saline-controls that had small, electron-dense, secretory granules that were PAS-positively stained, indicating a neutral glycoprotein composition.[42,43]

Although both the proximal (generation 8) and distal (generation 11) airway segments had similar endotoxin-induced increases in intraepithelial mucosubstances, there was a significant drop in the amount of stored mucosubstances between 1 and 2 days after the last instillation only in the distal segment. A similar drop in intraepithelial mucosubstances was not observed in the proximal airway segment until 1 week after the last instillation. The reasons for the difference in response between the two regions at day 2 postinstillation is unknown. It may be due to inherent differences in epithelial sensitivity and/or differences in the amount and duration of endotoxin exposure to those specific airway segments. However, both the proximal and distal segments had 4–5 times more stored mucosubstances, compared with controls, 1 week after the last endotoxin instillation.

The fact that no serous secretory cells were found in endotoxin-exposed airways in the present study indicates that serous cells were either killed by endotoxin and replaced by mucous secretory cells, or endotoxin-exposure stimulated serous cells to transform into mucous secretory cells. Because no epithelial necrosis or exfoliation was evident in any of the examined endotoxin-exposed tissue and the fact that previous studies conducted at our institute have found no acute cytotoxic effects of endotoxin on rat nasal airway epithelium,[23,45] we believe that most of the endotoxin-induced increase in

mucous secretory cells was due to transformation of serous cells already present in the epithelium before exposure.

Interestingly, secretory granules with an electron-lucent matrix were also present in some of the ciliated cells in rats instilled with endotoxin. Some investigators have labeled airway epithelial cells with these features as cilia-secretory cells or preciliated cells and have identified them in rats after injections of isoprenaline sulphate, a β-adrenergic agonist[46] or mechanical injury[33–35] and occasionally in resected human lung.[47] In the present study, these "transitional" cells with electron-lucent cytoplasm, basal bodies, few or no cilia, and small numbers of secretory granules were a consistent feature in the airway epithelium of endotoxin-instilled rats, but were absent in the airway epithelium of saline-instilled controls. The cellular origin of these unusual epithelial cells with features of both ciliated and secretory cells is unknown.

In summary, this study has demonstrated that repeated intranasal instillations of endotoxin induces significant alterations to the mucous apparatus lining the main axial airways in the lung of the rat. Our results suggest that endotoxin exposure provokes an increase in intraepithelial stores of mucosubstances, epithelial cell hyperplasia and secretory cell metaplasia, and excess mucus in the lumens of intrapulmonary airways. Therefore, the endotoxin component of gram-negative bacteria could be a major etiologic agent responsible for the copious amounts of airway mucus often present in gram-negative bacteria-induced airway disease. In addition, this specific animal model of bronchitis and bronchopneumonia may be used to understand the cellular and molecular pathogenesis of secretory cell hyperplasia, a common airway epithelial alteration in several chronic airway diseases of humans.

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