DNA synthesis and Bcl-2 expression during development of mucous cell metaplasia in airway epithelium of rats exposed to LPS

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EXPOSURE OF THE PULMONARY airways to inhaled toxins or allergens may cause injury to the airway epithelium that results in an acute inflammatory response, proliferation of surviving epithelial cells, and mucous cell metaplasia (MCM). MCM occurs in respiratory epithelia when nonproliferating, preexisting or proliferating epithelial cells differentiate into mucous cells in areas where no or few mucous cells are normally present. Increased numbers of airway mucous cells is a prominent feature of a number of bronchial diseases in humans, including chronic bronchitis (13), asthma (24), and cystic fibrosis (28). Proliferation of epithelial cells was reported in airways of subjects with chronic bronchitis (4); however, it is unknown to what extent existing or proliferating cells differentiate into mucus-storing cells and contribute to the development of MCM. Exposure to LPS (endotoxin) from the outer membrane of gram-negative bacteria induces MCM in pulmonary airways of laboratory rodents. This induction of MCM has been used as an experimental model for human chronic bronchitis (11, 33). In the present study, we used this in vivo animal model to investigate which portion of the MCM is derived from LPS-induced proliferating epithelial cells that differentiate into mucus-producing cells and the possible progenitors for these newly appearing secretory cells.

Programmed cell death plays a role in the development and maintenance of homeostasis (1). The Bcl-2 family of proteins includes both pro- and antiapoptotic molecules, and the ratio of these two subsets determines, in part, the susceptibility of cells to a death signal (23). Previous studies demonstrated that Bcl-2, an inhibitor of apoptosis with cell cycle regulatory functions, is expressed in metaplastic mucous cells. The purpose of the present study was to investigate the number of metaplastic mucous cells that are derived from proliferating epithelial cells and whether Bcl-2 has a role in cell cycle entry in these cells. Rats were intratracheally instilled with 100 μg of LPS from Pseudomonas aeruginosa in 500 μl of saline, and proliferating airway cells were labeled with bromodeoxyuridine (BrdU) by implanting a subcutaneous osmotic pump 24 h before instillation. The volume of stored mucous substance and the number of mucus cells were increased 10- and 3-fold, respectively, from 24–48 h after instillation. The number of total epithelial cells per millimeter of basal lamina increased, and the number of serous cells per millimeter of basal lamina decreased during this time. Approximately 50% of Alcian blue-periodic acid Schiff-stained mucous cells were labeled with BrdU at 48 h after instillation, suggesting that one-half of the secretory cells were derived from proliferating cells. Furthermore, 50% of the Bcl-2-positive mucous cells were BrdU negative and therefore derived from nonproliferating, preexisting cells. Our findings demonstrate that preexisting and proliferating cells differentiate into mucous cells and compose LPS-induced metaplasia and that Bcl-2 does not have cell cycle regulatory function in these cells.
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 12:12-h 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 four 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. All experiments were approved by the Lovelace Respiratory Research Institute’s Institutional Animal Care and Use Committee and were carried out at the Institute, a facility approved by the Association for the Assessment and Accreditation for Laboratory Animal Care International.

Intratracheal instillations, bromodeoxyuridine labeling, necropsy, and airway tissue selection. Twenty-four rats were anesthetized with 5% halothane in oxygen and intratracheally instilled with 100 μg of LPS from Pseudomonas aeruginosa serotype 10 (Sigma, St. Louis, MO) in 500 μl of pyrogen-free saline. The remaining 18 rats were not instilled and served as noninstilled controls. We used noninstilled rats as controls in this study because we were interested in the kinetics of various cell types after LPS instillation and in comparing those findings with findings in noninstilled rats. To identify airway cells that had undergone cell division, all rats received a continuous delivery of 10 μg/ml of bromodeoxyuridine (BrdU; 20 μg/ml) from a subcutaneously implanted osmotic pump that was implanted 24 h before the intratracheal instillations.

At 24 or 48 h after instillation, animals were anesthetized by halothane inhalation and killed by exsanguination via the abdominal aorta or renal arteries (n = 12 rats/postinstillation time). Immediately after death, the trachea, extrapulmonary bronchi, and lungs were excised intact from the thoracic cavity. The excised lung lobes were intratracheally perfused with 10% neutral-buffered zinc formalin (half of the animals for light microscopic analysis) or 2% glutaraldehyde (other half of the animals for transmission electron microscopy) at 25 cm of constant fixative pressure for at least 4 h and then stored in a large quantity of the same fixative until further processing. The same procedures for killing, necropsy, and lung fixation were used for noninstilled control rats that were killed along with the LPS-instilled rats at 24 and 48 h after instillation (n = 9 noninstilled control rats/postinstillation time).

After fixation, the intrapulmonary airway axis of the left lung lobe from each rat was microdissected according to a modified version of the microdissection technique of Plopper et al. (27) previously described in detail (9). Dissections were performed under a high-resolution dissecting microscope (dual-view Wild M-8 stereomicroscope; Wild Heerbrugg, Switzerland). Beginning in 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). This system allowed us to assign each airway branch its own unique number, which also gave its branching history. Generation 5, large-diameter conducting airway along the main axial pathway, was excised and processed for light microscopic and ultrastructural (transmission electron microscopy) analyses (Fig. 1A). Generation 5 was chosen for all of the analyses because those airway regions do not contain Clara cells but predominantly serous and ciliated cells. In rats, epithelial cells lining the terminal bronchioles consist of primarily Clara cells (15). Furthermore, intratracheally instilled LPS readily reaches the generation 5 airways, which allows for consistent results over several rats, whereas more variability is observed in more distal airway epithelia.

Zinc-formalin-fixed airway tissues from generation 5 were stained with Alcian blue (pH 2.5)-hematoxylin and eosin for histopathological examination and with Alcian blue (pH 2.5)-periodic acid-Schiff sequence (AB-PAS) to detect acidic and neutral mucusubstances. In addition, these airway tissues were immunohistochemically stained to detect BrdU- and Bcl-2-labeled airway epithelial cells (see below).

![Fig. 1. A: diagrammatic representation of the site of tissue selection for morphometric analyses in the main axial airway of the left lung lobe of each rat. B–D: light photomicrographs of the airway epithelium (e) lining the main axial airway (airway generation 5) from noninstilled rats (B) and from rats intratracheally instilled with saline containing 50 μg of bacterial endotoxin (Pseudomonas aeruginosa, serotype 10) and killed 24 (C) or 48 (D) h after instillation. Note the presence of mucous (goblet) cells (arrows) in the bronchiolar epithelium lining the airway in D, but not in B or C. C: neutrophils and eosinophils can be seen on the luminal surface of the airways 24 h after instillation and at 48 h. D: neutrophils are found between the epithelia and smooth muscle layer. Hematoxylin, eosin, and Alcian blue (AB; pH = 2.5) stain. P, alveolar parenchyma; m, smooth muscle in airway wall; N, neutrophils. Bars = 50 μm.](http://ajplung.physiology.org/)

**Morphometry of intraepithelial mucusubstances.** The volume density (Vv) of AB-PAS-stained mucusubstances in the mucosal surface epithelium was estimated with a semiautomatic image analysis system previously described in detail (6). Briefly, histochemically stained slides were imaged with a ×40 planapo objective and a ×1.25 intermediate lens (Olympus Optical, Tokyo, Japan), a charge-coupled device camera (TM-840; Pulnix America, Sunnyvale, CA), an FG-100 digital image processing board (Imaging Technology, Woburn, MA), and a color monitor. The area of stained AB-PAS-stained mucusubstances within the surface epithelium was calculated from the automatically circumscribed perimeter of stained material with a personal computer and the public domain National Institutes of Health (NIH) Image program (22a). The linear length of the basal lamina under each analyzed region of the airway epithelium was determined by tracing the contour of the digitized image of the basal lamina that was projected on the video screen with the NIH image analysis software Scion Image on a Power Macintosh G4. The method used to estimate the volume of stored mucusubstance per unit surface area of epithelial basal lamina was described by Harkema et al. (9). The data were expressed as the mean ± SE Vv (nl/mm² basal lamina) of AB-PAS-positive mucusubstances within the epithelium. The images were also used to determine the height and width of at least eight ciliated and eight mucous cells chosen at random from each airway of rats from the three groups. These data are expressed as mean ± SE micrometers of length.

**Immunohistochemistry.** Airway tissue sections (4 μm in thickness) were placed on Probe-On Plus slides and deparaffinized in xylene. After incubation in 2% H2O2 in methanol to quench endogenous peroxidase, the tissues were hydrated in graded ethanol in distilled water and treated with a solution of 0.16% trypsin (Zymed, San Francisco, CA) for 10 min at 37°C. Washes were performed in

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Dulbecco’s PBS containing 0.05% Brij 35 (Sigma). After being blocked in (mM) 100 Tris, pH 7.7, 550 NaCl, and 10 KCl containing 1% normal goat serum, 2% BSA, and 0.1% Triton X-100, slides were incubated overnight in a Bcl-2 primary antibody (BD-PharMingen, San Diego, CA) at 1:1,000 dilution. We previously reported (31, 32) on the specificity of this antibody to the Bcl-2 protein. Tissues were fixed in 10% neutral-diaminobenzidine (Vector Laboratories, Burlingame, CA) as described by the manufacturer. Tissues were fixed in 10% neutral-buffered formalin and digested for 1 h min with 0.005% proteinase K (Sigma). After denaturation of tissue DNA with 1 M HCl, slides were incubated in automation buffer (Biomedica, Foster City, CA) with 1.5% horse serum. Primary BrdU antibody (BD-PharMingen) was added at a dilution of 1:25 for 1 h. BrdU-positive nuclei were visualized with the Vectastain ABC kit and the peroxidase substrate detected with the Vectastain ABC kit and the peroxidase substrate enzyme complex. Bcl-2 was stained with a polyclonal antibody (Santa Cruz Biotechnology, San Diego, CA) at 1:1,000 dilution. We previously reported (31, 32) on the specificity of this antibody to the Bcl-2 protein. Bcl-2 was stained with a polyclonal antibody (Santa Cruz Biotechnology, San Diego, CA) at 1:1,000 dilution. We previously reported (31, 32) on the specificity of this antibody to the Bcl-2 protein.

Results

Pulmonary histopathology and ultrastructural morphology of axial airway epithelium. Intratracheal instillation of LPS induced an acute bronchopneumonia involving the main axial airways and some of the preterminal bronchioles branching off of the axial airways and even extending into some of the more distal centriacinar regions of the lungs and adjacent alveolar parenchyma (multifocal bronchiolitis and alveolitis). The principal parenchymal features included an acute alveolitis with congestion of septal capillaries, interstitial edema, and flooding of alveolar air spaces with fibrinoid and eosinophilic exudate admixed with numerous neutrophils. Thickening of the alveolar septa in the affected regions was also due to mild to moderate hypertrophy and hyperplasia of type II pneumocytes. Mitotic figures were occasionally observed in the affected alveolar epithelium. There was also circumscribed cuffing of blood vessels with a mixed inflammatory infiltrate of mainly neutrophils and lesser numbers of mononuclear cells (monocytes and lymphocytes). In addition, preterminal and terminal bronchioles in the affected regions had thickened, hypertrophic surface epithelium along with a similar inflammatory cell infiltrate predominantly in the subepithelial interstitial walls but with some infiltration of neutrophils into the surface epithelium and airway lumen.

Principal LPS-induced lesions in the intrapulmonary, noncartilaginous, main axial airways and several of the other large-caliber, preterminal bronchioles consisted of an acute neutrophilic bronchiolitis with some of the airway epithelium showing a thick columnar respiratory epithelium at 48 h after instillation. The surface epithelium lining similar large-diameter airways in the lungs of noninstilled control rats was a thin, cuboidal respiratory epithelium composed only of ciliated and nonciliated serous cells (Fig. 1B). The average height of epithelial cells was not significantly increased at 24 h but increased from 7.3 ± 0.4 μm in noninstilled control rats to 12.1 ± 0.6 μm in airways of rats at 48 h after instillation. However, no change in the width of epithelial cells was observed among any of the groups. Mucous cells were rarely found in the main axial airways of noninstilled control rats or in the LPS-instilled rats killed 24 h after instillation (Fig. 1C). However, at 48 h after instillation the increase in airway epithelial thickness in the LPS-instilled rats was due to both an increase in epithelial cell size (hypertrophic columnar ciliated and nonciliated cells) and an increase in the number of nonciliated epithelial cells (hyperplasia). In addition, there was a conspicuous MCM characterized by the appearance of numerous tall, columnar mucous cells containing copious amounts of AB-PAS-stained mucosubstances (Fig. 1D).

Morphometry of intraepithelial mucosubstances and mucous cells. To determine whether the increase in epithelial mucosubstances was due to metaplasia, both Vₐ and numbers of mucous cells per millimeter of basal lamina were morphometrically estimated. At 24 h after LPS instillation, the Vₐ of intraepithelial AB/PAS-stained mucosubstances was reduced by one-half of that found in noninstilled control rats (Fig. 2). However, at 48 h after LPS instillation the Vₐ of mucosubstances was increased by ~10-fold (Fig. 2). The number of mucous cells per millimeter of basal lamina had increased from 17 in control rats to 19 (4 BrdU labeled and 15 unlabeled) in LPS-instilled rats at 24 h after LPS instillation. At 48 h after LPS instillation, the number of mucous cells per millimeter of basal lamina increased to 46 (24 BrdU labeled and 22 unlabeled) (Fig. 3A).

Proliferation of airway epithelial cells. BrdU-positive mucous cells were essentially absent in noninstilled control rats but were increased to 4/mm basal lamina at 24 h after LPS instillation (Fig. 3A). At 48 h after instillation, BrdU-positive mucous cells were increased to ~24, suggesting that the
majority of epithelial cells proliferated between 24 and 48 h after LPS instillation (Fig. 3A). Whereas the ratio of BrdU-positive and -negative mucous cells in LPS-instilled rats was 1:4 at 24 h, the ratio was increased to 1:1 at 48 h after instillation (Fig. 3A). Adjacent BrdU-positive mucous cells were observed throughout the epithelia (see example in Fig. 3B).

Airway epithelial morphometry: differential cell densities. To further determine which types of epithelial cells were increased in number after LPS-induced proliferation, the number of the various epithelial cell types was quantified from the montage of electron micrographs taken from the main axial airway (generation 5) of the glutaraldehyde-fixed left lung lobe. Ultrastructurally the thin surface epithelium lining the intrapulmonary axial airway of noninstilled control rats was composed mainly of low cuboidal ciliated cells and nonciliated serous cells (Fig. 4, A and A1). Basal cells were rarely seen at this airway generation. Serous cells were cuboidal with protruding apical surfaces lined by microvilli, electron-dense cytoplasm, abundant supranuclear granular endoplasmic reticulum, and discrete, membrane-bound, electron-dense secretory granules. Ciliated cells in the airway epithelium of these control rats were also cuboidal and had moderately electron-lucent cytoplasms with abundant mitochondria and basal bodies in their apices underlying the cilia-lined luminal surfaces (Fig. 4A1).

The hypertrophic airway epithelium of the rats instilled with LPS and killed 24 h after instillation was composed predominantly of tall cuboidal or columnar ciliated cells and serous cells with few electron-dense secretory granules (Fig. 4, B and B1). An occasional serous cell also contained a few small electron-lucent granules (Fig. 4B1). Individual neutrophils were commonly found scattered throughout the surface epithelium, underlying subepithelial tissues, and luminal surface in the airways of these LPS-instilled rats (Fig. 4B1). Mucous cells, however, were not observed in the axial airway epithelium of these animals.

In contrast, the hypertrophic-hyperplastic respiratory epithelium lining the main axial airways in the lungs of LPS-instilled rats killed 48 h after instillation was composed predominantly of tall cuboidal or columnar mucous and ciliated cells (Fig. 4, C and C1). Mucous cells had moderately electron-dense cytoplasm and numerous electron-lucent secretory granules. Both discrete and confluent granules were present in the supranuclear cytoplasm of these newly appearing secretory cells (Fig. 4Cl). In some of the mucous cells there were occasional electron-lucent secretory granules with an electron-dense core, the latter resembling the electron-dense matrix found in the secretory granules of serous cells in the control rats. Interestingly, serous cells were not frequently observed in the axial airways of these LPS-instilled rats killed at 48 h after instillation (see the results of epithelial cell morphometry described below).

The numbers of ciliated and undefined cells remained unchanged in LPS-instilled rats at 24 and 48 h after LPS instillation compared with noninstilled control rats (Fig. 4D). The numbers of serous cells increased by 13 cells/mm basal lamina at 24 h but decreased significantly by 39 cells/mm basal lamina 48 h after LPS instillation. In contrast, the numbers of mucous cells increased from ~1 to 55 from 24 to 48 h after instillation. The total numbers of epithelial cells remained essentially unchanged at 24 h but increased by 30 (an increase of 24%) at 48 h after LPS instillation (Fig. 4D).

Expression of Bcl-2 in proliferating and nonproliferating mucous cells. To determine whether Bcl-2 expression occurs in mucous cells that were derived from existing or proliferating...
epithelial cells, lung tissue sections were subjected to immunostaining for Bcl-2 and BrdU followed by staining with Alcian blue (Fig. 5). The percentage of Bcl-2-positive mucous cells in noninstilled rats or in rats at 24 h after LPS instillation was mostly 44% (data not shown). From the total numbers of metaplastic mucous cells at 48 h after LPS instillation, 81% of mucous cells did not express Bcl-2 and the remaining 19% displayed immunoreactivity to Bcl-2 (Fig. 5B). From the Bcl-2-negative mucous cells, 44% were BrdU negative and 56% were BrdU positive (Fig. 5B), suggesting that only half of the metaplastic mucous cells had differentiated from proliferating epithelial cells and the rest had differentiated from nonproliferating, preexisting epithelial cells. From the Bcl-2-positive mucous cells, 47% were BrdU negative and 53% were BrdU positive (Fig. 5B). These results indicate that only half of the Bcl-2-positive mucous cells were derived from proliferating epithelial cells.

DISCUSSION

The results of the present study demonstrate that mucous cells account for all of the LPS-induced increases in epithelial cell numbers in airways. In total, approximately half of the metaplastic mucous cells were derived from proliferating cells and the other half from nonproliferating, preexisting epithelial cells differentiating into mucous cells. In addition, only half of the Bcl-2-positive mucous cells had BrdU labeling, whereas the other half were nonproliferating, preexisting epithelial cells.
cells, suggesting that Bcl-2 expression in mucous cells is not associated with regulating entry into the cell cycle.

$V_s$ of stored mucous substances was reduced 24 h after LPS instillation. This reduction is probably due to induced hypersecretion of stored mucous substances, as observed in other studies (30, 32). However, 48 h after instillation, $V_s$ was increased by 10-fold and the number of mucous cells by 2.7-fold compared with noninstilled controls. Similar increases in $V_s$ of stored mucous substances have been reported (7); however, previous studies have not reported the increases in epithelial cell number. The present results show that in addition to increases in the number of epithelial cells that synthesize and store mucous substances, the stored product per cell had also increased by approximately threefold.

**Mucous cells derived from existing serous cells and from proliferating epithelial cells.** Studies have established that exposure to allergens causes proliferation and extensive MCM in pulmonary airways (3, 29). Furthermore, exposure of rats to ozone induces MCM in nasal airways (5) and intranasal instillation of LPS causes a four- to sixfold increase in the number of proliferating epithelial cells in epithelia lining the nasal turbinates (8). However, these studies were not designed to determine whether existing or proliferating epithelial cells differentiate into mucous cells resulting in MCM. The dramatic increase of BrdU-positive mucous cells per millimeter of basal lamina from only 4 at 24 h after instillation to 25 at 48 h clearly shows that the majority of proliferation did not occur until after 24 h after LPS instillation. The presence of BrdU-positive mucous cells adjacent to each other suggests that these cells may be proliferating as differentiated mucous cells. Only half of the mucous cells in the metaplasia were derived from LPS-induced cell proliferation, and the other half were mucous cells that differentiated from nonproliferating, preexisting cells.

Quantification of the numbers of various cell types that constitute the epithelium before and 24 and 48 h after LPS instillation suggests that the mucin-storing cell is the cell type that constitutes the increase in number of epithelial cells after LPS-induced injury. At 24 h after instillation, the number of serous cells was increased by $\sim 15$ cells/mm basal lamina. We assume that this increase was due to differentiation of the proliferating, preexisting cells and of unidentified cells, designated as "other" (few basal cells) into serous cells. However, basal cells were rarely found at the airway generation selected for our analysis, and therefore we believe that these cells did not play a major role in the LPS-induced epithelial hyperplasia.

At 48 h after instillation, the number of serous cells decreased by $\sim 38$ cells/mm basal lamina. During this period, epithelial cell number increased by $\sim 30$ cells/mm basal lamina. There was no evidence of morphological apoptosis, necrosis, or oncosis. In addition, there was no change in the width of the cells, suggesting that unknown mechanisms must account for accommodating the increased number of epithelial cells within the same area of basal lamina. The sum of the decrease in serous cells and the increase in the total numbers of epithelial cells slightly exceeds but closely approximates the numbers of metastatic mucous cells at 48 h after instillation. The hypothesis that the increase in epithelial cell number consists of metastatic mucous cells is further supported by the fact that the number of BrdU-positive mucous cells at 48 h was similar to the increase in total epithelial cells. The major increase in mucous cells in LPS-instilled rats consists of BrdU-positive mucous cells (from 0 in control to 24 cells/mm basal lamina in LPS-instilled rats), whereas the increase in BrdU-negative mucous cells was minimal (from 16 in controls to 22 cells/mm basal lamina in LPS-instilled rats). The presence of occasional serous cells containing a few small electron-lucent granules at 24 h after LPS instillation suggests that serous cells differentiate into mucous cells. Furthermore, the similarity of the numbers representing the decrease of serous cells and the BrdU-negative metaplastic mucous cells led to the conclusion that serous cells must have differentiated into mucous cells, constituting the portion of mucous cells that was derived from nonproliferating, preexisting epithelial cells.

It is not clear which type of cell(s) may have undergone proliferation after LPS instillation. It is possible, as mentioned above, that the few mucous cells that existed in control rats and/or some of the basal cells that were categorized as unidentified cells entered several proliferation cycles. Proliferation and cell differentiation in airway epithelia before and after exposure to various agents or mechanical injury has been reviewed previously (2). A cell kinetic study of hamster tracheal epithelium suggested a three-compartment model of cell renewal. The first compartment comprises a self-renewing compartment of proliferating basal cells that gives rise to a compartment consisting of mucous cells. Some mucous cells retain the ability to divide while others lose it and become fully differentiated. The third compartment consists of fully differentiated cells only, which do not divide and have a finite lifespan. Keenan et al. (16–19) found dividing mucous and basal cells at a ratio of 2:1 after mechanical injury of hamster trachea. In studies in which proliferating cells were arrested with colchicine, these investigators found that a larger proportion of secretory cells compared with basal cells were found in metaphase. Jeffery and Reid (15) observed that, after exposure to tobacco smoke, mucous cells arise mainly by self-replication and transformation of existing serous cells, whereas the production of ciliated cells appears to be mainly due to the differentiation of basal cells. They also reported cells with transitional features of both serous and mucous cells, suggesting that the increase in mucous cell numbers is due to transformation of preexisting serous cells. In addition, Harkema and Hotchkiss (7) previously reported that endotoxin-instilled rats had $\sim 30\%$ more epithelial cells per millimeter of basal lamina than saline-instilled rats at 48 h after instillation. They reported that epithelial cell numbers increase after endotoxin instillation because of the increase in mucous cell numbers only and that the number of basal cells remain constant, suggesting that mucous cells are the primary mediators in the development of the hyperplastic stage (7).

Bcl-2 does not regulate entry into cell cycle. Previous reports demonstrated that Bcl-2 may function as an inhibitor of apoptosis as well as an inhibitor of cell cycle progression (12, 20, 22, 25). Our data show that Bcl-2 is expressed in BrdU-negative mucous cells. Only about half of the Bcl-2-positive mucous cells had incorporated BrdU, representing newly formed cells, whereas the other Bcl-2-positive cells were BrdU-negative; therefore, these cells must have been nonproliferating, preexisting cells that were present before LPS injury. Our data indicate that the presence of Bcl-2 is not required for mucous cells to proliferate and that Bcl-2 expression is not associated with entry into the cell cycle. We have shown (32) that mucous cell numbers are decreased to background levels at
least 2 days after the percentage of Bcl-2-positive cells has decreased to levels found in control animals. These data support the hypothesis that Bcl-2, which is an inhibitor of apoptosis, must be downregulated before there is a reduction in the numbers of mucous cells. Together, these data indicate that in airway epithelia, Bcl-2 may regulate the life span of metastatic mucous cells rather than regulating the entry into the cell cycle. How adjacent mucous cells are differentiated to either express or not express Bcl-2 is not known, but it does not appear to be associated with the age of the cell.

In our study, the number of epithelial cells per millimeter of basal lamina was increased after LPS exposure from \( \sim 125 \) to 155, which represents an increase of \( \sim 24\% \). These newly formed cells consisted primarily of mucous cells. Interestingly, the percentage of Bcl-2-expressing mucous cells (20–23%) approximates the percentage increase in epithelial cell numbers as a result of LPS instillation. The percentage of cells that must be eliminated to restore the conditions before injury and the percentage of mucous cells that express Bcl-2 are strikingly similar and may indicate that Bcl-2 expression is an integral part of the recovery process. Further mechanistic studies to elucidate the role of Bcl-2 in metastatic mucous cells will be crucial for designing novel therapeutic tools that reduce the numbers of mucous cells in airways of patients with chronic bronchitis and possibly after airway diseases.

ACKNOWLEDGMENTS

The authors thank Catherine Bressee (Michigan State University) for technical assistance in the morphometric analyses, Yoneko Knighton (Lovelace Respiratory Research Institute) for preparing tissue samples for light microscopic analyses, and Pat Cossey (Lovelace Respiratory Research Institute) for technical assistance in the transmission electron microscopy and the construction of the montages of the electron photomicrographs of the airway epithelium.

GRANTS

This research was funded in part by NIH Grants HL-59391, HL-068111, and ES-09237.

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