Goblet Cell Hyperplasia, Airway Function, and Leukocyte Infiltration after Chronic Lipopolysaccharide Exposure in Conscious Guinea Pigs: Effects of Rolipram and Dexamethasone

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ABSTRACT
The effects of chronic exposures (nine, 48 h apart) of conscious guinea pigs to lipopolysaccharide (LPS) (30 μg·ml⁻¹, 1 h) on airway function, airway histology (in particular, goblet cell numbers), and inflammatory cell infiltration of the lungs were examined as a model of chronic inflammatory lung disease, such as chronic obstructive pulmonary disease. The sensitivity of these parameters to treatment with the corticosteroid, dexamethasone, or the phosphodiesterase-4 (PDE4) inhibitor, rolipram, was determined. As the number of LPS exposures increased, there was a progressively persistent bronchoconstriction after each exposure. After nine LPS exposures, there was evidence on histological examination of airway infiltration of, predominantly, neutrophils in perivascular, peribronchial, and alveolar tissues. After chronic LPS exposure, the airway epithelium possessed a marked goblet cell hyperplasia and evidence of inflammatory edema, features contributory to reduced airway caliber. Treatment with dexamethasone (20 mg·kg⁻¹) or rolipram (1 mg·kg⁻¹), administered (i.p.) 24 and 0.5 h before exposure and 24 and 47 h after each subsequent exposure, attenuated the inflammatory cell infiltration into the airway, goblet cell hyperplasia, and inflammatory edema. Dexamethasone exacerbated, whereas rolipram reversed, the chronic LPS-induced bronchoconstrictions. This study demonstrates that chronic LPS causes persistent bronchoconstriction, neutrophilic airway inflammation, goblet cell hyperplasia, and edema. These rolipram-sensitive features suggest the potential of PDE4 inhibitors in chronic inflammatory lung diseases.

Chronic mucus hypersecretion is an important symptomatic and pathological feature of a heterogeneous group of chronic respiratory diseases that includes chronic bronchitis, chronic obstructive pulmonary disease (COPD), and asthma (Rogers, 1994; Jackson, 2001). Persistent mucus overproduction contributes to reduced airway caliber and the occlusion of small airways (reduced FEV₁), productive cough, and labored breathing (Jackson, 2001). Individuals with chronic mucus hypersecretion also suffer from an increased frequency and duration of respiratory infection, causing further exacerbation of their original respiratory pathology (Jackson, 2001).

The two major sources of mucus secretion in the respiratory tract are the surface epithelial goblet cells and mucus cells of the submucosal glands. In normal lungs, goblet cells are present in the large bronchi, becoming increasingly sparse toward the bronchioles. The submucosal glands are restricted to the large airways with their density decreasing with airway caliber, such that they are absent in the bronchioles. In chronic respiratory diseases, such as COPD and asthma, submucosal glands increase in size (hypertrophy), and the number of goblet cells is increased (hyperplasia), becoming more dense in the peripheral airways, via a phenotypic conversion of nongoblet epithelial cells (metaplasia) (Rogers, 1994; Jackson, 2001). The increased ratio of goblet cells to ciliated cells and the increased goblet cell density in terminal bronchioles, under conditions of hypersecretion, impairs clearance of mucus through mucociliary mechanisms or coughing, respectively. Lung histology from patients affected by COPD and asthma also shows the presence of edema, which can further reduce airway caliber and compromise...
lungs. A marked airway infiltration of macrophages and granulocytes is also present, principally neutrophils in COPD and eosinophils in asthma (Postma and Kerstjens, 1998). In clinical studies, these inflammatory parameters have been shown to correlate with a reduction in lung function (FEV₁) and an exaggerated bronchoconstriction [airway hyperreactivity (AHR)] to nonspecific stimuli (Postma and Kerstjens, 1998).

Anti-inflammatory steroids are the current mainstay of severe asthma treatment (British Thoracic Society et al., 1993), by inhibiting the transcription of proinflammatory mediators [e.g., eicosanoids, interleukins (IL), and tumor necrosis factor-α (TNF-α)], inducible enzymes [e.g., nitric-oxide synthase, and cyclooxygenase-2 (COX-2)], and adhesion molecules (Laitinen et al., 1992; Barnes and Adcock, 1993). However, little evidence exists of their clinical benefit on disease progression in COPD (Burge, 1999). Recently, attention has focused on the inhibition of phosphodiesterase isoenzyme-4 (PDE4) as a molecular target for COPD (and asthma) (Torphy et al., 1999). Evidence suggests that the subsequent intracellular elevation in cAMP induces airway smooth muscle relaxation, alleviates inflammatory edema, and suppresses immunocompetent cell activation and migration in models of acute pulmonary inflammation (Sekut et al., 1995; Torphy et al., 1999).

The acute symptoms of mucus hypersecretion, as in chronic bronchitis, can be modeled by exposure of rats to ozone or sodium metabisulfite (Murals and Roum, 1985; Shore et al., 1995). Features of severe asthma (goblet cell hyperplasia, AHR, and eosinophilic airway infiltration) have been mimicked by chronic antigen exposure of atopic mice (Rogers, 1994; Jackson, 2001). In this study, we therefore extend our previous research to this system.

The first aim of this study was to characterize the relationship between the previously described lung function and inflammatory cell influx and the lung morphology after single or chronic exposures to LPS. We regard the latter as more clinically relevant to chronic pulmonary inflammatory diseases, such as COPD. The second aim was to examine whether the corticosteroid, dexamethasone, or the PDE4 inhibitor, rolipram, affected the morphological changes as well as the functional parameters of acute and chronic LPS-induced inflammation.

**Materials and Methods**

**Animals.** Groups of six male Dunkin-Hartley guinea pigs, weighing 300 to 400 g, were used. Animals received food and water ad libitum, and room temperature (22 ± 2°C) and lighting (maintained on a 12-h cycle) were regulated. This work complied with the Guidelines for Care and Use of Laboratory Animals, according to the Animals (Scientific Procedures) Act of 1986 and GlaxoSmithKline policy.

**Measurement of Respiratory Function.** Airway function [specific airway conductance (sGaw)] was monitored in conscious guinea pigs, using whole body plethysmography as previously described by Griffiths-Johnson et al. (1988). A computerized data acquisition system replaced the original oscilloscope and angle resolver (Danahay and Broadley, 1997). Guinea pigs with a close-fitting face mask were placed in a restrainer that was then slid into the plethysmography chamber. A computer with a Biopac data acquisition system and AcqKnowledge software (Biopac Systems Inc., Santa Barbara, CA) acquired and stored data referring to the airflow across a pneumotachograph (Mercury FIL, GM Instruments, Ltd., Scotland, UK) as the animal breathed. The resulting change in box volume (pressure) was also simultaneously measured. Changes in airflow and box pressure were measured by two UP pressure transducers (Piden Controls Ltd., Canterbury, UK). The resultant waveforms could then be rapidly analyzed by comparing the gradients of the flow and the box pressure waves at a point where flow tended toward zero, i.e., in the first 30 ms of expiration. A function of these parameters, correcting for ambient pressure and the weight of the animal, determined a value for sGaw. At least five breaths were analyzed for each animal at each time point. Before all experiments, the animals were handled and familiarized with the apparatus to reduce stress.

**Inhalation Exposures and Administration of Anti-Inflammatory Compounds.** Groups (n = 6) of guinea pigs were exposed to LPS or the LPS vehicle (saline) with or without treatment with dexamethasone or rolipram as shown in Fig. 1 and as previously described (Toward and Broadley, 2001). In single exposure studies, guinea pigs were exposed in an exposure chamber (620 × 300 × 420 mm) for 1 h to an aerosolized solution of LPS (30 µg · ml⁻¹, endotoxin from *Escherichia coli* serotype O26:B6) (Sigma Chemical Co., Poole, Dorset, UK) or saline (NaCl for infusion British Pharmacopoea, 0.9% w/v) (Baxter Healthcare, Thetford, Norfolk, UK). The aerosol was generated by a Wright nebulizer driven by compressed air at 20 p.s.i., at a rate of 0.5 ml · min⁻¹. In chronic exposure studies, the animals received nine exposures, 48 h apart. The lethal dose of LPS (LD₅₀) within 24 h, 0.7 mg · kg⁻¹, i.p.) in guinea pigs was considered substantially higher than that administered in this study (Matsuda, 1998).

![Fig. 1. Protocol for single or chronic (nine exposures, 48 h apart) exposure to LPS (30 µg · ml⁻¹) or vehicle (pathogen-free saline) of conscious guinea pigs, with and without dexamethasone or rolipram dosing. Animals were terminated 24 h after the first or ninth exposure, bronchoalveolar lavage fluid was removed, and lung samples were taken for histology.](image)
et al., 1995). The average of two sGaw measurements was obtained prior to exposure (baseline or 47 h after the previous exposure) and then at regular intervals (0, 15, 30 min, and hourly) after exposure(s).

Dexamethasone (20 mg·kg\(^{-1}\)) or rolipram (1 mg·kg\(^{-1}\)) were administered (i.p.) 24 and 0.5 h before the first of the chronic exposures to LPS or saline and at 24 and 47 h after each subsequent exposure. Animals treated with rolipram during the chronic LPS study developed persistent bronchodilation, which would have interfered with an assessment of airway reactivity to histamine 24 h after the ninth exposure. Consequently, the last dose of rolipram was given 24 h after the eighth exposure to LPS or saline, which allowed sGaw to recover to baseline values, at 24 h after the ninth LPS exposure. Dexamethasone-21-phosphate, disodium salt (Sigma Chemical), and rolipram (Sigma Chemical) stock solutions were dissolved in 50% dimethyl sulfoxide (Sigma Chemical), 50% saline (Baxter Healthcare) and further diluted with saline for injection (1.0 ml). The final concentration of dimethyl sulfoxide was less than 5%, and this vehicle has previously been shown by this laboratory to have no effect on airway responses or inflammation in a similar chronic inflammatory model (Danahay and Broadley, 1998). Thus, control groups treated chronically with LPS and the vehicle for dexamethasone or rolipram were not included; comparisons were made between LPS-exposed animals with or without the drug treatments. Control groups receiving chronic saline and the drug treatments were, however, included. Doses of dexamethasone (Whelan et al., 1995; Toward and Broadley, 1999) and rolipram (Danahay and Broadley, 1997) were selected based upon the findings from other studies using similar models of inflammation and those that were without adverse effects. No animal appeared to be in respiratory distress or to exhibit other signs of discomfort during the exposure regimens or during any other part of the protocol described.

All animals underwent a bronchoalveolar lavage (BAL) 24 h after the last exposure to LPS or saline to determine total cell counts using a Neubauer hemocytometer and differential cell counts after staining with Trypan blue. The guinea pigs were overdosed with pentobarbitone sodium (400 mg·kg\(^{-1}\), i.p.; Euthatal; Rhone Merieux, Essex, UK), and the trachea was cannulated. A 1% solution of EDTA disodium salt (Sigma Chemical) was flushed through the cannula into the lungs (1 ml·100 g\(^{-1}\) of body weight), recovered 3 min later, and repeated once.

**Lung Histology.** After lavage, the lungs were fixed by slow immersion in neutral-buffered formalin (10%, pH 7.0) (1 ml·100 g\(^{-1}\) of body weight) via the tracheal cannula and, following immediate removal from the thoracic cavity, further immersed in neutral-buffered formalin for at least 72 h. After fixation, representative samples were cut through the large bronchi of the right and left lung (medial lobe), dehydrated in 70 to 100% ethanol/xylene, and embedded in paraffin wax. Sections were cut (6 µm), deparaffinized, and stained with hematoxylin and eosin or Masson’s trichrome for general morphology. Additional sections were stained with elastic van Gieson stain to differentiate elastic fibers and collagen, and Alcian Blue-periodic acid Schiff (ABPAS) was used for identification of mucin (neutral and acid)-containing cells.

The number of goblet cells in the epithelium of large airways was determined using light microscopy. Two slide sections (left and right lobe) from each animal were coded and assessed “blind” to prevent bias. Only cells that were stained purple/magenta with ABPAS and were morphologically typical of goblet cells were counted. To reduce intra- or intergroup variation between the sections derived from different locations in the bronchial tree, all the airways measured possessed a similar degree of cartilaginous plating, indicative of the lower large bronchial region. The internal airway perimeter has been shown to be unaltered by smooth muscle contraction or lung inflation (Pare and Hogg, 1989) and was, therefore, measured using Image Acquisition software (Qwin standard V2.2; Leica, Heidelberg, Germany) to determine the epithelial perimeter. The mucin-containing cells were then expressed as goblet cells per millimeter of airway epithelium.

Data Analysis. To reduce intersubject variability, changes in sGaw from the baseline sGaw values taken before a procedure are presented as a percentage of the mean baseline value preceding the
first LPS or saline challenge. Absolute values of baseline sGaw are stated in the figure legends. Significance of differences in the number of airway epithelial goblet cell was compared using analysis of variance, followed by Scheffe’s post hoc analysis. Changes in airway function were compared using analysis of variance followed by the appropriate paired or unpaired Student’s (two-tailed) t test. Differences were considered statistically significant at p < 0.05 (Motulsky, 1995).

Results

Airway Function

Effects of Chronic Exposure to LPS on Airway Function. The first exposure to LPS in the chronic exposure study caused an immediate bronchoconstriction (−12.4 ± 10.7% decrease from baseline sGaw values), which recovered 15 to 30 min later but was not significantly different (p > 0.05) from the response to saline (−20.5 ± 3.6%) (Fig. 2). The seventh, eighth, and ninth exposures to LPS caused a decline in sGaw (−16.9 ± 5.9, −20.6 ± 2.8, and −23.1 ± 3.6 peak percentage decrease from baseline sGaw values), with a progressive increase in duration of bronchoconstriction. After the eighth exposure, there was still significant bronchoconstriction at 30 min (Fig. 2D), whereas after the ninth exposure, the bronchoconstriction remained until 19 h after exposure (Fig. 2B).

Effects of Dexamethasone or Rolipram Treatment on the Airway Function Responses to Chronic LPS Exposures. Dexamethasone or rolipram treatment did not significantly affect (p > 0.05) the initial LPS-induced bronchoconstriction after the first exposure. However, dexamethasone exaggerated the duration of prolonged bronchoconstriction after the ninth LPS exposure, from 19 h to at least 24 h after exposure (Fig. 2C). In contrast, rolipram-treated animals developed a significant (p < 0.001) and persistent bronchodilation at 24 and 47 h after the seventh exposure to LPS (Fig. 2D). When rolipram was withdrawn 24 h after the eighth exposure to LPS, bronchodilation returned to baseline sGaw values at 24 h after the ninth exposure. No bronchodilator activity occurred in rolipram-treated animals exposed to chronic saline (data not shown). Lung function did not differ in the absence or presence of dexamethasone or rolipram treatment in saline-exposed animals (data not shown).

Lung Morphology

Effects of Single or Chronic Exposures to LPS on the Upper Airways. Large bronchial sections, stained with ABPAS, from the lungs of naive animals or those removed 24 h after a single or chronic saline exposure (Fig. 3A), appeared to possess a normal composition of epithelial cells with an occasional darkly stained goblet cell. Compared with naive animals, at 24 h after a single LPS exposure, the number of goblet cells increased 107% (p > 0.05) (Fig. 4). However, at 24 h after chronic LPS exposure, the ratio of goblet cells containing both acid (purple) and neutral (magenta) mucus (neutral and acid mucins)-containing goblet cells (G) and depict airway smooth muscle (ASM), alveoli (Al), and cartilaginous plating (C). In chronic LPS-exposed animals (B), goblet cell hyperplasia was attenuated with both dexamethasone (C) and rolipram (D) treatment. The airway epithelium from naive animals or those 24 h after a single saline exposure did not appear to be different from that of animals chronically exposed to saline (A). Bar = 50 μm.
were determined by analysis of variance (single factor), followed by Scheffé’s post hoc analysis.

**FIG. 4.** Goblet cells per millimeter of airway epithelium from guinea pigs before (naive) and 24 h after a single or chronic (nine, 24 h apart) exposure (60 min) to nebulized LPS (30 μg · ml⁻¹) or vehicle (pathogen-free saline), in the absence and presence of dexamethasone (20 mg · kg⁻¹) or rolipram (1 mg · kg⁻¹) treatment. Treatment was administered (i.p.) 24 and 0.5 h before exposure and 24 and 47 h after each subsequent exposure. The last dexamethasone and rolipram doses were administered 47 and 24 h after the eighth exposure, respectively. Two representative sections of large bronchi, from right and left medial lobes, were stained purple/magenta with ABPAS to identify and count blind morphologically typical goblet cells. The internal airway perimeter was measured using Image Acquisition software (Leica), and the goblet cell number was expressed per millimeter (GC mm⁻¹) of airway epithelium. Each point represents the mean ± S.E.M. (n = 6) of two GC mm⁻¹ of airway epithelium determinations per animal. Significance of differences in the GC mm⁻¹ of airway epithelium was compared with those of animals exposed to chronic saline (+, p < 0.05) or chronic LPS (†, p < 0.05) and were determined by analysis of variance (single factor), followed by Scheffé’s post hoc analysis.

**Discussion**

Chronic airflow obstruction and AHR are characteristic features of patients with COPD and severe asthma (Laitinen et al., 1992; Postma and Kerstjens, 1998). In COPD, there are increased numbers of inflammatory cells (predominantly neutrophils) in the airway wall, particularly in the epithelial layer and around the submucosal glands (Postma and Kerstjens, 1998). Persistent airway inflammation in these patients results in airway wall edema, deposition, and remodeling of connective tissue components (e.g., submucosal and adventitial collagen disposition), together with hypertrophy and hyperplasia of submucosal glands or the goblet cell phenotype, respectively (Barnes, 1998; Jackson, 2001). These pathological features reduce airway caliber and are thought to contribute to the heightened constrictor response from spasmodic stimuli (AHR) and airflow obstruction in COPD (Pare and Hogg, 1989). In this study, we examined the functional and morphological effects of chronic pulmonary inflammation derived from repeated exposure of guinea pigs to LPS as a model for the progressive inflammatory processes of COPD and the ability of dexamethasone and rolipram to suppress these changes.

The first exposure to LPS caused a small bronchoconstriction that was no different from that observed after saline inhalation (Toward and Broadley, 2000), which we have previously attributed to the saline condensing in the airways or to obstructed airway conductance (Toward and Broadley, 2001). By the ninth exposure to LPS, there was a prolonged period of bronchoconstriction, which did not occur with repeated saline exposure. Previously, we have reported that the influx of inflammatory cells into the lungs as measured by BAL, particularly neutrophils, increases with the number of exposures. Also, AHR to histamine was prolonged from 2 h after a single exposure to at least 24 h after the eighth exposure (Toward and Broadley, 2001). In the present study, we further demonstrate infiltration of neutrophils and macrophages into the alveolar, perivascular, and peribronchial spaces after chronic LPS exposure.

The increased infiltration of neutrophils into the BAL fluid and lung tissues was likely to be initially orchestrated by chemotactic factors, such as TNF-α and IL-8, released into the airways by resident macrophages, epithelial cells, and lymphocytes (Snella and Rylander, 1985; Brigham and Mey-
The early synthesis of TNF-α (Brigham and Meyrick, 1986) in response to LPS, activates other proinflammatory mediators, including arachidonic acid metabolites, deleterious cytotoxins (proteases and reactive oxygen species), and cytokines. Bronchial biopsies from patients with COPD show similar inflammatory processes, and sputum samples have elevated TNF-α, IL-8, reactive oxygen species, and proteolytic enzyme levels (Barnes, 1998). The eicosanoid products of arachidonic acid, namely leukotrienes (B₄, C₄, D₄, and E₄), platelet-activating factor (PAF), and leukotrienes species, and proteolytic enzyme levels (Barnes et al., 1999). Edematous swelling of the trachea, eicosanoids, or proteolytic enzymes (Brigham and Meyrick, 1986) in response to LPS, activates other proinflammatory mediators, including arachidonic acid metabolites, deleterious cytotoxins (proteases and reactive oxygen species), and cytokines. Bronchial biopsies from patients with COPD show similar inflammatory processes, and sputum samples have elevated TNF-α, IL-8, reactive oxygen species, and proteolytic enzyme levels (Barnes, 1998; Barnes et al., 1999; Torphy et al., 1999).

In this study, histological examination of the lungs after a single LPS exposure showed no morphological features to support a geometric reduction in airway caliber that would potentiate a spasmogen-induced airway narrowing and explain the AHR seen previously with this model (Pare and Hogg, 1989). However, in chronically LPS-exposed animals, the airway histology showed extensive migration of neutrophils but little evidence of airway collagen or elastic fiber remodeling, smooth muscle hypertrophy, or epithelial shedding. Both dexamethasone and rolipram were equieffective in animal models, COPD, and asthma as causes of AHR, bronchoconstriction, increased airway permeability or leukocyte influx (Brigham and Meyrick, 1986; Laitinen et al., 1992; Barnes, 1998; Barnes et al., 1999).

Histological examination after chronic LPS exposure also revealed increased alveolar wall thickness and evidence of edema and plasma exudation. The inflammatory edema observed after chronic LPS exposure may be a result of an increased capillary blood pressure by vasoconstrictor eicosanoids or an increased permeability of the capillary wall from the release of reactive oxygen species (including NO and peroxynitrite), eicosanoids, or proteolytic enzymes (Brigham and Meyrick, 1986; Barnes et al., 1999). Edematous swelling of the airway wall and increased airway exudate in the lung have been shown to reduce airway caliber and correlate with AHR in sheep (Hwang et al., 2001), and may contribute to the AHR observed in the present chronic LPS model. Edema is also a probable contributor to the prolonged bronchoconstriction seen in this study after chronic LPS exposure. The edema and accumulation of proteinaceous fluid in the alveolus was inhibited by rolipram to a greater extent than by dexamethasone. This may indicate an increased potency of rolipram on granulocyte infiltration into the airway and a subsequent release of edema-inducing mediators. It may also explain why the prolonged bronchoconstriction following the final LPS challenge was attenuated by rolipram but not by dexamethasone and adds weight to the conclusion that the prolonged bronchoconstriction was associated with the edema. The generation of inducible COX-2-derived prostanoids, PAF, and leukotrienes may also contribute to the prolonged chronic LPS-induced bronchoconstrictions. In airway epithelial and smooth muscle cell cultures, dexamethasone inhibits COX-2 expression and the subsequent release of bronchoconstrictor prostanoids (Barnes et al., 1999). However, in this study, dexamethasone exacerbated the later LPS-induced bronchoconstrictions. This may be due to inhibition of expression of the functionally antagonistic COX-2-derived bronchodilator, PGE₂ (Barnes et al., 1999). The persistent bronchodilation in rolipram-treated animals during later LPS challenges, but not saline exposures, may be due to an induction of the COX-2-derived PGE₂ by rolipram. The bronchodilatory second messenger of PGE₂ is cAMP, the levels of which will be elevated by PDE4 inhibition with rolipram (Uhlig et al., 1995; Barnes et al., 1999).

AHR after chronic LPS exposures may have been due to the formation of the powerful oxidant peroxynitrite from the interaction of inflammatory-derived superoxide with NO (Beckman, 1996; Barnes et al., 1999), excessive airway levels of which occur in chronic LPS-exposed animals (Toward and Broadley, 2001). Peroxynitrite can induce AHR in guinea pigs (Sadeghi-Hashijin et al., 1996), possibly through cytotoxic damage of the airway epithelium to expose sensory...
nerves (Barnes et al., 1999) or an impairment of β-adrenoceptors (Kanazawa et al., 1999). Levels of peroxynitrite or the peroxynitrite-induced nitration product, nitrotyrosine, were not, however, determined in the current study. The major histological change observed after chronic LPS exposures was an increase in the density of goblet cells in the epithelial layer. The close proximity of inflammatory cells to the epithelial goblet cells and submucosal glands in the histology of patients with COPD suggests a causative association between leukocytes and the hypersecretory mucus phenotype (Postma and Kerstjens, 1998). Human airways possess a large number of submucosal glands and goblet cells, whereas in guinea pig airways, goblet cells are the predominant source of mucus secretion (Jackson, 2001). In guinea pigs, neuronal (cholinergic, adrenergic, and peptidergic) control of mucus secretion appears to be via the goblet cells, whereas in humans, it is the submucosal glands that are predominantly innervated. However, in both guinea pigs and humans, inflammatory mediators act on goblet cells directly (and also via neuronal mechanisms in guinea pigs) to influence mucus secretion. Consequently, despite these species differences in the mechanistic regulation and source of mucus secretion, this model of LPS-induced goblet cell hyperplasia has clinical relevance, as the majority of airflow obstruction in COPD and asthma occurs in the smaller airways, where goblet cells but not submucosal glands are expressed. In this study, a single exposure to LPS caused only a slight increase in goblet cells 24 h later. However, after chronic LPS exposure, the epithelial goblet cells were greatly increased. The peribronchial migration of activated leukocytes into the airway lumen and persistent exposure to proinflammatory stimuli are likely to contribute to goblet cell up-regulation, although the degree of hyperplastic and metaplastic mechanisms involved in the derivation of this phenotype are unclear (Rogers, 1994). Contrary to the T-helper 2 (T_{H2})-derived inflammation in asthmatics, LPS causes a predominantly T_{H1}-favored cytokine response (Blyth et al., 1998; Barnes et al., 1999). This imbalance in T_{H} expression appears to affect the mechanism of goblet cell induction. Shimizu et al. (2000) showed in atopic rats exposed to antigen that leukotrienes (C_{4}, D_{4}, and E_{4}) are potent secretagogues and inhibitors of ciliary beat frequency, and play an important role in goblet cell hyperplasia, whereas in LPS-inoculated animals, neutrophil- and COX-derived products were important (Barnes et al., 1999). Neutrophil-derived reactive oxygen species have been shown to enhance mucin
release in guinea pig tracheal and human bronchial epithelial cells via a NO-dependent pathway, an effect blocked by COX inhibition (Wright et al., 1996; Barnes et al., 1999). In the current study, neutrophil-derived superoxide, the excess airflow NO after chronic LPS inhalation (shown in our previous study, Toward and Broadley, 2001) and their combined product, peroxynitrite, could stimulate mucus secretion and goblet cell hyperplasia (Beckman, 1996; Wright et al., 1996; Barnes et al., 1999). Inflammation-derived prostanooids, PAF, and proteolytic enzymes are also capable of stimulating goblet cell mucus secretion and hyperplasia, exacerbating the reduction in airflow caliber after chronic LPS exposure (Barnes et al., 1999). Both dexamethasone and rolipram attenuated goblet cell hyperplasia after chronic LPS exposure. Suppression of inflammatory cell activity with rolipram or dexamethasone reduces the production of reactive oxygen species, eicosanoids, and protease, which stimulate goblet cell secretion and contribute to the etiology that induces a hypersecretory phenotype and edema after chronic LPS (Barnes and Adcock, 1993; Torphy et al., 1999).

In conclusion, this study demonstrates that morphological changes to the airways, including neutrophil infiltration, edema, and goblet cell hyperplasia, following chronic LPS exposure of conscious guinea pigs are associated with functional changes of persistent bronchocstriction. These changes, along with the persistent AHR observed in our previous study, are characteristic features of COPD. Both dexamethasone and rolipram attenuated goblet cell hyperplasia and edema, probable contributors of the reduced airflow caliber and AHR, via attenuation of LPS and inflammatory cell-derived proinflammatory mediators. In common with COPD, in which steroids have only modest beneficial effects, primarily on quality of life, in this study, dexamethasone failed to improve a deficit in lung function. Rolipram, however, improved lung function. The conscious guinea pig chronically exposed to LPS may therefore prove a useful model of COPD, and the results support the further development of PDE4 inhibitors for the treatment of COPD or severe asthma.

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