Goblet cell metaplasia or hyperplasia is a prominent feature of chronic airway diseases associated with mucous hypersecretion, including chronic bronchitis, bronchiectasis, and bronchial asthma (reviewed in Ref. 29). Hypersecretion from an increased number of goblet cells has been considered to contribute to mucus plugging and airway obstruction (2).

In the airways of cigarette smokers, goblet cell metaplasia or hyperplasia is one of the morphological findings (32, 38). A wide variety of stimuli such as cigarette smoke (22), sulfur dioxide (21), ozone, and endotoxin (11) have been demonstrated to increase goblet cell number in the airways of experimental animals. The inhibitory effects of corticosteroid (7, 31), indomethacin (10), and N-acetylcysteine (30) on increases in goblet cell number induced by cigarette smoke have been reported and suggest that inflammatory mediators and reactive oxygen species play a role in goblet cell metaplasia or hyperplasia. However, the precise mechanisms underlying goblet cell metaplasia or hyperplasia induced by cigarette smoke in vivo remain unclear.

Platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), a phospholipid generated by activated platelets, leukocytes, and endothelial cells, is a potent proinflammatory autacoid with various biological effects that include activating eosinophils and neutrophils, bronchoconstriction, and enhancing vascular permeability (reviewed in Ref. 5). PAF has been implicated in the pathogenesis of airway inflammation and mucus hypersecretion. Previous in vitro studies have demonstrated the role of PAF as a potent airway mucin secretagogue in human and feline organ cultures (9, 25) and in cultured airway epithelial cells from guinea pigs (1).

PAF causes mucus secretion and may also affect the number of goblet cells in the airways. Levels of PAF-like lipids are reported to increase in plasma from humans and hamsters after exposure to cigarette smoke (14, 23). Focusing on goblet cell metaplasia, we hypothesized that endogenously generated PAF or PAF-like lipids might be involved in airway inflammation and in goblet cell metaplasia caused by cigarette smoke. In the present study, after repeated exposure of guinea pigs to cigarette smoke, we evaluated the number of goblet cells in the tracheal epithelium and PAF concentrations and cell profiles in bronchoalveolar lavage (BAL) fluid. We also examined the effect of a PAF receptor antagonist on goblet cell metaplasia and inflammatory cell accumulation in the airways after cigarette smoke exposure.
MATERIALS AND METHODS

Animals and study protocols. Pathogen-free male Hartley-strain guinea pigs weighing 450–550 g were used in this study. The animals were exposed repeatedly to cigarette smoke (six cigarettes for 1 h once a day) for 3, 7, or 14 consecutive days. BAL and histological assessments of tracheal tissues were performed 24 h after the last exposure. Sham-exposed animals were used as controls.

To study the effect of a PAF receptor antagonist, E-6123, on goblet cell metaplasia, E-6123 (1 mg kg\(^{-1}\) day\(^{-1}\)) or vehicle was administered orally 3 h before each cigarette smoke or sham exposure. To avoid the direct effects of cigarette smoke to the airway, such as bronchoconstriction (26), BAL and histological assessments were also performed 24 h after the last exposure. The dose of E-6123 was based on previous reports (17, 20).

E-6123 possesses a specific and potent PAF receptor-antagonistic effect on PAF-induced reactions in vitro and in vivo (33, 36). To confirm the selective effect of E-6123 on PAF-induced eosinophil infiltration in vivo, eosinophil chemotaxis assays were performed on guinea pig skin. Eosinophil infiltration was measured with eosinophil peroxidase activity as previously reported (28). E-6123 (1 mg·kg\(^{-1}\)·day\(^{-1}\)) or vehicle was administered orally 3 h before intradermal injections of 70 \(\mu\)l/site of vehicle, PAF (10\(^{-8}\) M), interleukin (IL)-5 (10\(^{-8}\) M), or leukotriene (LT) \(\text{B}_4\) (10\(^{-8}\) M) that were dissolved in saline containing 0.25% bovine serum albumin. Four hours after the injection, the animals were euthanized, the skin was removed, the sites were punched out with an 8-mm skin biopsy punch (Acupunch, Acuderm), and skin homogenates were prepared. Eosinophil peroxidase was assayed in diluted skin homogenates as previously described (8).

The experimental protocol was approved by the Committee on Animal Research (Faculty of Medicine, Kyushu University, Fukuoka, Japan).

Exposure to cigarette smoke. The animals were placed awake and unrestrained in a 125-liter chamber made of Plexiglas and exposed to diluted cigarette smoke. The chamber had three holes 3–5 mm in diameter. An exhaust hole in the top panel was connected to a vacuum system that could generate a constant vacuum flow (20 l/min). A cigarette was attached to an inlet hole in the front panel. The other hole, also in the front panel, was used as a fresh air inlet. With a constant vacuum flow, the smoke stream was drawn into the chamber and mixed with fresh air. Each cigarette was burned for 5 min and was then immediately detached from the chamber. The visible smoke disappeared over the next 5 min. The animals were exposed to six cigarettes for 1 h/day. The cigarettes were purchased from Japan Tobacco (Tokyo, Japan). According to the manufacturer’s specifications, each cigarette contained 2.7 mg of nicotine and 26 mg of tar.

Histological assessment. To avoid possible traumatic damage due to BAL, histological assessment of the tracheal tissue was done in separate animals. The animals were killed with an overdose of pentobarbital sodium. A cannula was introduced into the proximal portion of the trachea, and the lungs were inflated with buffered formalin applied at a constant...
were sectioned (3 mm thick) longitudinally from each sample were dissected out and embedded in paraffin. The tissues were made, and the cells were suspended in saline to obtain a suspension of 10^5 cells/ml. Cytospin preparations (Cytospin 3, Shandon, Pittsburgh, PA) were made, and the cells were determined under light microscopy with a standard hemacytometer (22). Goblet cells are defined as cells with large acid-Schiff (AB-PAS). Because it has been reported that cigarette smoke increased goblet cell numbers in both the tracheal epithelium from naive animals and control animals after 14 days exposure to cigarette smoke. Significantly different compared with sham-exposed control animals: *P < 0.05; **P < 0.01.

**RESULTS**

Goblet cell counts after exposure to cigarette smoke. In untreated naive animals, the tracheal epithelium contained goblet cells that stained with AB-PAS; there was no significant change in the number of tracheal goblet cells in naive animals and control animals after 14 days of sham exposure (Fig. 1). The tracheal epithelium of guinea pigs exposed to cigarette smoke for 14 days showed marked increases in the number of AB-PAS-positive cells compared with those in sham-exposed control animals (Fig. 2, A and B). There were visualized with a modified Wright-Giemsa stain (Diff-Quik, Baxter, McGaw Park, IL). Differential counts of 400 cells were performed under light microscopy in a single-blind manner.

**Measurement of PAF concentration.** The PAF content in BAL fluid was determined byRIA with a [3H]PAF scintillation proximity assay system (Amersham Pharmacia Biotech), which was based on a previous report (19). The lavage fluid was centrifuged at 100 g for 5 min at 4°C. The supernatant was removed and mixed with an equal volume of 20% acetic acid. The extract was applied to a bond-elute C18 extraction column (Sep-Pak Plus C18 cartridges, Waters, MA) previously equilibrated with 10-ml aliquots of 10% acetic acid. Most lipids were washed out with ethyl acetate. PAF was eluted by applying 6 ml of methanol and was then subjected to RIA according to the manufacturer’s protocol.

**Drugs.** Pentobarbital sodium was obtained from Abbott (North Chicago, IL). E-6123 ([S]-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4,3-'3';4']thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine) was provided by Eisai Pharmaceutical (Tokyo, Japan), dissolved in 100% ethanol at a concentration of 10 mg/ml, and then diluted with saline to a final concentration of 1 mg/ml.

**Data analysis.** Values are expressed as arithmetic means ± SE. The effects of cigarette smoke on goblet cell counts and BAL fluid cell counts were compared with ANOVA, and the significance of the differences between values was assessed with the Bonferroni correction for multiple comparisons. P values of <0.05 were considered significant.

![Fig. 3. Effect of repeated exposure to cigarette smoke on cell profiles in bronchoalveolar lavage fluid (BALF) from guinea pigs (n = 5). A: eosinophils and neutrophils. B: total and differential counts. Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils. Significant eosinophilia in BALF was observed after 14 days of exposure to cigarette smoke but not after 3 or 7 days of exposure. Neutrophilia had already been noted after 3 days of exposure, with no further increase after 3 days. The number of total cells, eosinophils, and neutrophils in BALF was significantly increased after 14 days of exposure to cigarette smoke. Significantly different compared with sham-exposed control animals: *P < 0.05; **P < 0.01.](http://ajplung.physiology.org/)

![Fig. 4. Effect of repeated exposure to cigarette smoke on platelet-activating factor (PAF) concentration in BALF from guinea pigs (n = 5). PAF levels in BALF were increased in a time-dependent fashion after repeated exposure to cigarette smoke. PAF levels in BALF from animals after 7 days exposure to cigarette smoke were significantly higher than those after sham exposure. Significantly different compared with sham exposed-control animals: *P < 0.05; **P < 0.01.](http://ajplung.physiology.org/)
significant time-dependent increases in the number of goblet cells in the tracheal epithelium after repeated exposure to cigarette smoke (Fig. 1). Compared with those in sham-exposed animals, there were significant increases in goblet cell counts after 14 consecutive days of cigarette smoke exposure; the count of total epithelial cells after 14 consecutive days of exposure. There was no significant change in the number of total epithelial cells after 14 consecutive days of cigarette smoke exposure but not after 3 or 7 days of exposure. There was no significant change in the number of total epithelial cells after 14 consecutive days of cigarette smoke exposure but not after 3 or 7 days of exposure.

**Cell profiles and PAF levels in BAL fluid after smoke exposure.** Repeated exposure to cigarette smoke also caused an increase in the number of eosinophils and neutrophils in BAL fluid (Fig. 3). Significant eosinophilia in BAL fluid was observed after 14 days of repeated exposure to cigarette smoke but not after 3 or 7 days of exposure. Neutrophilia had already been noted after 3 days of exposure, with no further increase after 3 days. Histologically, a predominant accumulation of eosinophils and neutrophils was observed in the airways but not in the alveoli after 14 days of exposure to cigarette smoke. There was no significant change in the number of macrophages and lymphocytes in BAL fluid after cigarette smoke exposure. There was no difference between cell counts in BAL fluid from naive animals and from control animals after 14 days of sham exposure (data not shown).

Repeated exposure to cigarette smoke increased the concentration of PAF in BAL fluid time dependently (Fig. 4). The concentration of PAF in the BAL fluid from the animals exposed for 7 days was significantly higher compared with that in sham-exposed control animals. The recovery rate of BAL fluid did not differ significantly between groups; the recovery rate was 96.9 ± 1.1% in control animals and 93.1 ± 3.3% in cigarette smoke-exposed animals.

**Effect of E-6123 on goblet cell counts and on cell profiles in BAL fluid.** To examine the role of endogenous PAF in the increase in goblet cells after exposure to cigarette smoke, we administered E-6123 to animals before cigarette smoke exposure each day. Pretreatment with E-6123 significantly suppressed the increase in goblet cell number observed after 14 days of exposure (Figs. 2C and 5A). E-6123 treatment also significantly inhibited the cigarette smoke-induced eosinophilia and neutrophilia in BAL fluid (Fig. 5B). Treatment with E-6123 had no effect on the number of tracheal goblet cells or on eosinophil and neutrophil counts in BAL fluid in sham-exposed animals (data not shown).

**Effect of E-6123 on PAF-induced eosinophil infiltration in guinea pig skin.** Eosinophil peroxidase activity in diluted skin homogenates was significantly increased after intradermal injections of PAF, IL-5, and LTB4. Eosinophil peroxidase levels after PAF, IL-5, and LTB4 were 258.0 ± 14.1, 185.8 ± 8.3, and 320.9 ± 13.9%, respectively, of control values. Pretreatment with E-6123 significantly increased the number of eosinophils and neutrophils in BAL fluid induced by repeated exposure to cigarette smoke. These findings suggest that endogenously generated PAF facilitates airway inflammation and goblet cell metaplasia after exposure to cigarette smoke.

**DISCUSSION**

The present study demonstrates that repeated exposure to cigarette smoke increases the number of goblet cells in the tracheal epithelium of guinea pigs in vivo. Cigarette smoke also causes significant airway eosinophilia and neutrophilia and a marked increase in PAF concentration in BAL fluid. Treatment with a specific PAF receptor antagonist, E-6123, significantly attenuates the increase in airway goblet cells and in eosinophils and neutrophils in BAL fluid induced by repeated exposure to cigarette smoke. These findings suggest that endogenously generated PAF facilitates airway inflammation and goblet cell metaplasia after exposure to cigarette smoke.

PAF is known to cause airway mucus secretion in vitro (1, 9, 25) and to affect the number of goblet cells in the airways. PAF causes a reduction in goblet cell number in the conjunctiva of guinea pigs in vivo (37). In contrast, our study shows that treatment with a specific PAF receptor antagonist significantly attenuates the goblet cell metaplasia induced by exposure to

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Fig. 5. Effect of E-6123 on tracheal goblet cell count and cell count in BALF after 14 days exposure to cigarette smoke (n = 5 animals/group). Increases in goblet cell count in the tracheal epithelium (A) and eosinophils and neutrophils in BALF (B) after 14 days exposure to cigarette smoke were reduced significantly by treatment with E-6123. **P < 0.01 compared with sham-exposed control animals.
cigarette smoke. The evidence that intratracheal instillation of PAF increases goblet cell number in the trachea of guinea pigs and rats (24) and that airway epithelial cells in humans and guinea pigs have specific binding sites for PAF supports our findings (12, 18). The previous findings of PAF-induced depletion of goblet cells in the conjunctiva (37) suggest that PAF acts as a mucus secretagogue but does not reduce goblet cell number.

The level of PAF in tissues is determined by the balance of synthesis and degradation (34). A key mechanism for the reduction in PAF is hydrolysis catalyzed by a family of PAF acetylhydrolases. Cigarette smoke increases the levels of PAF-like lipids in plasma (14, 23). PAF is known to be produced by various cells, including neutrophils and eosinophils (5), and exposure to cigarette smoke induces airway plasma exudation in guinea pigs (13). It has been reported that treatment with an inhibitor of leukocyte recruitment prevented PAF-induced eosinophil recruitment but had no effect on the increase in goblet cells induced by PAF (24). From these previous reports and the present findings, we speculate that high PAF levels in BAL fluid after exposure to cigarette smoke may be attributed to increased plasma leakage and/or to the increased production of PAF by accumulated leukocytes. PAF then binds to the PAF receptor on airway epithelial cells and causes goblet cell metaplasia. Alternatively, impaired enzymatic activity may explain the increased PAF concentration. Cigarette smoke extract has been reported to inhibit the activity of PAF acetylhydrolase in human plasma (27).

In the present study, cigarette smoke increased goblet cell number in the guinea pig trachea. The relative contributions of cell division (hyperplasia) and cell differentiation (metaplasia) to this increase in goblet cell number remain unclear. An increased mitotic index in rat airway epithelium was found after exposure to cigarette smoke, suggesting hyperplasia (16). However, a study (3) tracing the course of radiolabeled thymidine through the changing epithelial cell population demonstrated that the increased number of goblet cells is due to both hyperplasia and metaplasia processes in rat airways exposed to cigarette smoke for 2 wk. In the present study, repeated exposure to cigarette smoke increased the number of goblet cells without affecting the numbers of total epithelial cells in the tracheal epithelium. These findings indicate that cigarette smoke induces goblet cell metaplasia in the airways.

Mucin genes are believed to be expressed during goblet cell growth (6). Cigarette smoke increases airway goblet cell numbers and PAF levels, and treatment with a specific PAF receptor antagonist inhibits these increases in goblet cell number. In the tracheal epithelium, exogenously applied PAF induces the expression of the mucin gene MUC5AC (24), a major mucin in the airways. Recently, it has been shown that goblet cell metaplasia correlates with induction of MUC5 mRNA and MUC5 protein in the airways of allergen-exposed mice (39). These findings strongly suggest that cigarette smoke increases PAF levels in airways and that this, in turn, triggers a signaling cascade that activates mucin gene transcription. This PAF signaling pathway in epithelial cells remains to be investigated. Because PAF causes mucus secretion in the airways (1, 9, 25), PAF might stimulate mucin synthesis indirectly through increasing mucus secretion (29).

Chronic obstructive pulmonary diseases are known to be associated with cigarette smoking. In the present study, cigarette smoke caused airway eosinophilia and neutrophilia. In addition to neutrophils, eosinophils have been reported to be involved in the airway inflammation observed in chronic obstructive pulmonary disease (4, 35). Furthermore, goblet cell metaplasia or hyperplasia is a prominent feature of chronic airway diseases that are associated with mucus hypersecretion, including chronic bronchitis and bronchial asthma. The present data suggest that PAF or PAF-like lipids play an important role in goblet cell metaplasia or hyperplasia and hypersecretion in the airways of patients with chronic obstructive pulmonary diseases.

In summary, repeated exposure to cigarette smoke induces metaplasia of airway goblet cells and airway inflammation associated with elevated levels of PAF in BAL fluid. This airway inflammation and goblet cell metaplasia are attenuated significantly by a specific PAF receptor antagonist. Our findings suggest that endogenous PAF plays an important role in airway goblet cell metaplasia induced by cigarette smoke. This provides a plausible mechanism for the hypersecretion that occurs in cigarette smoke-related pulmonary diseases and suggests potential roles for PAF receptor inhibitors in the treatment of diseases with airway hypersecretion.

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