Secretory phospholipases A$_2$ stimulate mucus secretion, induce airway inflammation, and produce secretory hyperresponsiveness to neutrophil elastase in ferret trachea

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Okamoto K, Kim J-S, Rubin BK. Secretory phospholipases A$_2$ stimulate mucus secretion, induce airway inflammation, and produce secretory hyperresponsiveness to neutrophil elastase in ferret trachea. Am J Physiol Lung Cell Mol Physiol 292: L62–L67, 2007. First published September 1, 2006; doi:10.1152/ajplung.00181.2006.—Secretory phospholipases A$_2$ (sPLA$_2$s) are increased in the bronchoalveolar lavage fluid of patients with asthma and acute respiratory distress syndrome. Intratracheal sPLA$_2$ instillation induces acute lung injury in the rat and guinea pig. We hypothesized that sPLA$_2$ would stimulate mucus secretion in vitro and that intratracheal sPLA$_2$ exposure would induce mucus hypersecretion and airway inflammation in the ferret trachea in vivo. In vitro, porcine pancreatic sPLA$_2$ at a concentration of 0.5 or 5 U/ml significantly increased mucous glycoconjugate (MG) secretion from the excised ferret trachea. P-bromophenacylbromide (a sPLA$_2$ inhibitor), quercetin (a lipoxygenase inhibitor), or MK-886 (a 5-lipoxygenase inhibitor), each at 10$^{-5}$ M, significantly reduced sPLA$_2$-induced MG secretion. sPLA$_2$-stimulated MG secretion was decreased in Ca$^{2+}$-free medium. In vivo, ferrets were intubated for 30 min once per day for 3 days using an ETT coated with 20 units of porcine pancreatic sPLA$_2$ mixed in a water-soluble jelly. Constitutive MG secretion increased 1 day after sPLA$_2$ exposure and returned to control 5 days later. Human neutrophil elastase (HNE) at 10$^{-8}$ M increased MG secretion in the sPLA$_2$-exposed trachea compared with that in the control trachea, but methacholine at 10$^{-7}$ M did not. sPLA$_2$-induced secretory hyperresponsiveness continued for at least 5 days after sPLA$_2$ exposure ended. sPLA$_2$ increased tracheal inflammation, MG secretion, and secretory hyperresponsiveness to HNE probably through enzymatic action rather than by activation of its receptor.

Allergen challenge induces vasodilation, airway smooth muscle constriction, and increased mucus secretion, all characteristic of asthma (27). The intratracheal administration of LTs C$_4$ and D$_4$ can cause this same constellation of symptoms (5), and 5-lipoxygenase (5-LO) inhibitors decrease LT-stimulated mucous glycoconjugate (MG) secretion in cultured human lung tissue (5, 23).

We hypothesized that sPLA$_2$ would stimulate mucus secretion in vitro and that intratracheal sPLA$_2$ exposure would induce inflammation and mucus hypersecretion in vivo and make the airway more responsive to inflammatory mediators. We also evaluated potential mechanisms for sPLA$_2$-stimulated MG secretion.

METHODS

All reagents and supplies were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated. This study was approved by the Animal Care and Use Committee of Wake Forest University.

Animals and assay of tracheal secretion. Male adult ferrets (Marshall Farms, North Rose, NY) weighing ~2 kg were used in this study. The ferret tracheal airway is rich in submucosal glands and is a robust model for evaluating the regulation of MG secretion (12, 16–18, 21). For ex vivo studies, ferrets were killed by intraperitoneal injection of pentobarbital (120 mg/kg). Immediately after death, the trachea from larynx to carina was removed and divided into eight roughly equal segments, weighed, and immersed into Krebs-Henseleit solution (KHS; 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 3.4 mM CaCl$_2$, 2 g/l D-glucose, pH 7.4) at ferret body temperature, 38°C. After resting for 1 h in KHS, the segments were incubated for 30 min with KHS to measure constitutive secretion (period 1) and then incubated for 30 min with porcine pancreatic sPLA$_2$ at a concentration of 0.005, 0.05, 0.5, and 5 U/ml or with KHS alone to measure stimulated secretion (period 2). After incubation, the buffer was collected, and secreted MG was analyzed as described below. We randomized the administration of sPLA$_2$ and KHS to different segments in each experiment.

Evaluation of secretory pathways. Inhibitors were used to evaluate potential mechanisms for sPLA$_2$-induced MG secretion. Atropine (a muscarinic agonist) at 10$^{-4}$ M, p-bromophenacylbromide (p-BBP; an sPLA$_2$ inhibitor) at 10$^{-5}$ and 10$^{-4}$ M, 5,8,11,14-eicosatetraynoic acid (a lipoxygenase inhibitor) at 10$^{-4}$ M, quercetin (a lipoxygenase inhibitor, Sigma) at 10$^{-4}$ M, MK-886 (a specific 5-LO inhibitor; Calbiochem, San Diego, CA) at 5 × 10$^{-5}$ and 10$^{-4}$ M, and indo- methacin (a cyclooxygenase inhibitor, Sigma) at 10$^{-5}$ M were used. Ferret tracheal segments were rested in KHS. After resting for 1 h, the segments were incubated for 30 min in KHS with an inhibitor or KHS alone (period 1) and then incubated for another 30 min in KHS.

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containing 0.5 or 5 U/ml sPLA2 with or without an inhibitor or KHS alone (period 2).

Tracheal secretion in calcium-free medium. MG secretion in Ca²⁺-free medium was tested because sPLA2 requires millimolar concentrations of Ca²⁺ for enzymatic activity, whereas receptor-mediated sPLA2 activity has been reported to be calcium independent (13, 19). Ferret tracheal segments were first rested in KHS (1.3 mM Ca²⁺, pH 7.4). After resting for 1 h, the segments were incubated for 30 min with KHS (period 1) and then incubated for another 30 min in KHS containing porcine pancreatic sPLA2 at 5 U/ml or KHS alone in the presence (+) or absence (−) of 2 mM EGTA (period 2). Secreted MG was analyzed as described below.

Intratracheal sPLA2 exposure. Ferrets were anesthetized with 40 mg/kg ketamine and 5 mg/kg xylazine and intubated for 30 min once per day for 3 days using an endotracheal tube (ETT) coated with 20 units of porcine pancreatic sPLA2 mixed in 300 µl of water-soluble K-Y jelly (Johnson & Johnson, New Brunswick, NJ). The position of the ETT was checked with a neonatal bronchoscope. Trachea from larynx to carina were removed 1, 5, or 10 days after the sPLA2 exposure (sPLA2 day 1, sPLA2 day 5, sPLA2 day 10). Control animals were intubated with an ETT coated with 300 µl of K-Y jelly alone for 30 min per day for 3 days, and trachea were removed 1 day after the last intubation. Each trachea was divided into eight roughly equal segments, and the segments were rested in KHS. A middle segment was fixed in 10% formalin and processed for histological evaluation. After resting for 1 h, ferret tracheal segments were incubated for 30 min with KHS to measure constitutive MG secretion (period 1) and then incubated for 30 min in KHS with a secretagogue or KHS alone to measure stimulated MG secretion (period 2). To evaluate MG secretion in response to cholinergic or serine protease stimulation, methacholine (MCh) at 10⁻⁷ M or human neutrophil elastase (HNE; Elastin Products, Owensville, MO) at 10⁻⁸ M was used based on our previous studies (16).

MG secretion. Ferret tracheal mucus has high blood group titers, reflecting an abundance of galactose-N-acetyl-al–3 (fucose-al–2) galactose-R (21). These blood group antigens can be detected by Dolichos biflorus agglutinin (DBA) lectin. DBA immunohistochemistry has shown specific binding to goblet cells and submucosal glands in ferret trachea (16).

A sandwich enzyme-linked lectin assay was used to measure MG secretion as previously described (16–18). A 96-well microtiter plate was coated with 60 µl of DBA (6 µg/ml in PBS) and incubated at 4°C overnight. After rinsing five times with PBS with 0.5% Tween 20 (PBS-Tween), the plate was exposed to sample buffer and incubated at room temperature for 2 h. It was then incubated for 1.5 h with 50 µl of DBA conjugated with horseradish peroxidase (0.25 µg/ml) in PBS containing 1% bovine serum albumin. Before and after this step, the plate was washed five times with PBS-Tween. One hundred and fifty microliters of tetramethylbenzidine (0.42 mM) in citrate-acetate buffer (pH 6.0) was added to each well and incubated for 10 min. The reaction was stopped by adding 50 µl of 4.7 N H₂SO₄. Color development was read as the difference in absorbance at 450 and 650 nm. The concentration of MG was calculated by comparison with asialo ovine submaxillary mucus at 20–200 ng/ml. The amount of MG was expressed as mucin weight in the incubated buffer per trachea tissue weight (ng/mg tissue).

The relative increase of MG in each tracheal segment was calculated as the ratio of the actual amount of secreted MG in period 2 incubation divided into that of period 1. A secretory index (SI) for stimulated MG secretion was calculated using the ratio of stimulated MG secretion divided by that of unstimulated secretion (both period 1 and 2: KHS) in each animal. A percent (%) change of SI was calculated using the SI of stimulated MG secretion divided by that of unstimulated (KHS) secretion in each animal.

Histological evaluation. A middle segment of trachea was fixed in 10% formalin, embedded in paraffin, and processed for histological analysis using light microscopy. The tissue was cut in 4-µm sections, and the slides were stained with hematoxylin and eosin to measure the accumulation of inflammatory cells and overall severity of inflammation. The accumulation of inflammatory cells was variable, possibly related to the degree of tissue exposure to the sPLA2-coated tubes. Thus we could not accurately quantify the amount of inflammation by cell counts so we determined the type of cellular infiltrate and how long the inflammatory cells and loss of epithelial integrity persisted after sPLA2 exposure ceased.

Statistical analysis. Statistical analysis of data was performed using the StatView 5.0 statistics package (SAS, Cary, NC). After confirming that data were normally distributed, data were analyzed using ANOVA Scheffe’s F test or two-tailed unpaired t-test. P values of ≤ 0.05 were considered statistically significant. Data are presented as means ± SE.

RESULTS

sPLA2 stimulates MG secretion in the ferret trachea in vitro. sPLA2 rapidly stimulated MG secretion in the excised ferret trachea in a dose- and a time-dependent manner (Fig. 1). sPLA2 at a concentration of 0.5 (P < 0.05) and 5 (P < 0.0001) U/ml significantly increased MG secretion compared with KHS control.

sPLA2 and LO inhibitors decrease sPLA2-induced MG secretion. Atropine at 10⁻⁴ M had no effect on sPLA2-induced MG secretion. sPLA2-induced MG secretion was significantly decreased by p-BPB (an sPLA2 inhibitor) at 10⁻⁴ M (P < 0.01), quercetin (a LO inhibitor) at 10⁻⁴ M (P < 0.01), or MK-886 (a specific 5-LO inhibitor) at 5 × 10⁻³ M (P < 0.01) (Fig. 2). MK-886 at 10⁻⁴ M also inhibited sPLA2-induced MG secretion (P < 0.005) as shown in Fig. 3, top. Indomethacin (a cyclooxygenase inhibitor) at 10⁻⁴ M did not affect sPLA2-induced MG secretion (Fig. 2).

Mucin secretion in calcium-free KHS. sPLA2 at 5 U/ml significantly increased MG secretion in the ferret trachea (KHS in 1.3 mM Ca²⁺), and secretion was significantly decreased in Ca²⁺-free medium (KHS in 1.3 mM Ca²⁺ + 2.0 mM EGTA) as shown in Fig. 3. Constitutive MG secretion was also decreased in Ca²⁺-free medium compared with KHS control (P < 0.01).

Intratracheal sPLA2 exposure induced MG hypersecretion. Ferrets were intubated once per day for 3 days with an ETT coated with 20 units of sPLA2 mixed in water-soluble K-Y jelly. Constitutive MG secretion significantly increased 1 day after the last sPLA2 exposure compared with control (P < 0.0005) and returned to control levels at 5 days (sPLA2 day 5), as shown in Fig. 4, HNE at 10⁻⁸ M markedly and synergistically stimulated MG secretion in the sPLA2-exposed trachea (P < 0.01), and this hyperresponsiveness to HNE continued for 5 days (P < 0.01) and returned to control by day 10 (Fig. 5). MCH at 10⁻⁷ M increased MG secretion in the sPLA2-exposed trachea no more than from the nonexposed trachea.

Histopathology of the sPLA2-exposed trachea. One day after the last sPLA2 exposure (sPLA2 day 1), there was an influx of inflammatory cells in both the epithelium and the lamina propria. The inflammatory cells comprised a mix of polymorphonuclear and mononuclear cells. Five days after the last sPLA2 exposure (sPLA2 day 5), fewer inflammatory cells were in the epithelium, but there was increased airway mucus secretion. The lamina propria was relatively free of inflammation. Ten days after the last sPLA2 exposure (sPLA2 day 10), inflammatory cell numbers were similar to the airway not exposed to sPLA2. The lamina propria resembled control

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The kinetics of sPLA₂-induced MG secretion. Tracheal segments were incubated in Krebs-Henseleit solution (KHS). After resting for 1 h, segments were incubated in KHS for 30 min (period 1) and then for another 30 min in KHS with sPLA₂ at a concentration of 0.005 (n = 7), 0.05 (n = 7), 0.5 (n = 31), or 5 (n = 14) U/ml or KHS alone (n = 35) (period 2). Secreted MG was measured by enzyme-linked lectin assay (ELLA). The relative increase of MG in each tracheal segment was calculated as the ratio of the amount of secreted MG in period 2 incubation divided into that of period 1. A secretory index (SI) for stimulated MG secretion was calculated as the ratio of stimulated MG secretion divided by that of unstimulated secretion. All data are expressed as means ± SE.

**DISCUSSION**

sPLA₂ activity is increased in the serum or BALF of patients with asthma (2) or ARDS (14, 37). We show here that sPLA₂ is a rapid and potent MG secretagogue, this effect is dose dependent, and it can be blocked by Ca²⁺ depletion or by LT inhibitors but not by cholinergic inhibitors. In vivo, sPLA₂ also induces a state of “secretory hyperresponsiveness” to HNE but not by cholinergic inhibitors. In vivo, sPLA₂ at a concentration of 0.005 (n = 7), 0.05 (n = 7), 0.5 (n = 31), or 5 (n = 14) U/ml or KHS alone (n = 35) (period 2). Secreted MG was measured by enzyme-linked lectin assay (ELLA). The relative increase of MG in each tracheal segment was calculated as the ratio of the amount of secreted MG in period 2 incubation divided into that of period 1. A secretory index (SI) for stimulated MG secretion was calculated as the ratio of stimulated MG secretion divided by that of unstimulated secretion. All data are expressed as means ± SE.

**Fig. 1.** Secretory PLA₂ (sPLA₂) stimulates mucous glycoconjugate (MG) secretion in the ferret trachea. *Top:* tracheal segments were immersed in Krebs-Henseleit solution (KHS). After resting for 1 h, segments were incubated for 30 min in KHS with an inhibitor or with KHS alone (period 1). Secreted MG was measured by enzyme-linked lectin assay (ELLA). The relative increase of MG in each tracheal segment was calculated as the ratio of the amount of secreted MG in period 2 incubation divided into that of period 1. A secretory index (SI) for stimulated MG secretion was calculated as the ratio of stimulated MG secretion divided by that of unstimulated secretion. All data are expressed as means ± SE. *P < 0.05 or **P < 0.0001 compared with KHS. *Bottom:* kinetics of sPLA₂-induced MG secretion. Tracheal segments were incubated in KHS with porcine pancreatic sPLA₂ at a concentration of 0.5 or 5 U/ml sPLA₂ or KHS alone (each; n = 6), and the buffers were collected at 5, 10, 15, and 30 min after incubation. All data are shown as concentration of secreted MG and expressed as means ± SE. *P < 0.0001 or **P < 0.0005 compared with each KHS group; +P < 0.01 compared with KHS group.

**Fig. 2.** Inhibition of sPLA₂-induced MG secretion. Tracheal segments were incubated for 30 min in KHS with an inhibitor or with KHS alone (period 1) and then for another 30 min in KHS alone or KHS containing sPLA₂ at 0.5 U/ml with or without an inhibitor (period 2). Shown are sPLA₂ at 0.5 U/ml alone (n = 23), atropine at 10⁻⁴ M (n = 3), p-bromophenacylbromide (p-BPB) at 10⁻⁵ (n = 6) and 10⁻⁴ M (n = 6), quercetin at 10⁻⁴ M (n = 4), eicosatetraenoic acid (ETYA) at 10⁻⁴ M (n = 4), MK-886 at 5 × 10⁻⁵ M (n = 5), and indomethacin at 10⁻⁵ M (n = 6). Data are shown as percent change of SI compared with KHS group and expressed as means ± SE. MG secretion stimulated by sPLA₂ at 0.5 U/ml was significantly decreased by p-BPB at 10⁻⁴ M, quercetin at 10⁻⁴ M, and MK-886 at 5 × 10⁻⁵ M, compared with sPLA₂ at 0.5 U/ml alone (*P < 0.01).

In the guinea pig, intratracheal instillation of 10 or 30 units of sPLA₂ rapidly increases the thickness of interalveolar septa by edema and epithelial cell swelling (7, 8). We applied sPLA₂ directly to the tracheal epithelium by intubating ferrets with an ETT coated with sPLA₂ mixed in water-soluble jelly for 30 min once per day for 3 days. This technique was described previously by our group for exposure of the airway to bacterial endotoxin (18). Our results in vivo suggest that sPLA₂ induces local airway tissue injury and stimulates mucus hypersecretion.

sPLA₂ type IB is abundant in pancreatic fluid of many species and is sometimes referred to as pancreatic type sPLA₂. sPLA₂ type IIA is sometimes called inflammatory type, because it is highly expressed in the plasma or synovial fluids of patients with inflammatory diseases such as rheumatoid arthritis (30) and septic shock (38). sPLA₂ type IB is produced as an inactive proenzyme and activated by proteolytic enzymes (25, 26). sPLA₂ type IB was used in this study because it has similar enzymatic characteristics to sPLA₂ type IIA, it has a widespread tissue distribution including in the lung (31, 33, 36), and it has potent pathophysiological activity (1, 11, 15). Both sPLA₂ type IB and IIA are 14-kDa proteins (35) and require millimolar amounts of Ca²⁺ for enzymatic activity. Activated sPLA₂ type IB is detected in the serum and urine of subjects with acute lung injury but not in healthy controls (28).

sPLA₂ type IB has also been shown to regulate cellular function in vitro. It stimulates cell proliferation in Swiss 3T3 cells (1) and pancreatic cancer cells (11) and prostaglandin production and sPLA₂ type IIA expression in rat mesangial cells (15). These actions of sPLA₂ type IB are thought to be receptor mediated. A sPLA₂ type IB specific receptor has been identified (20). The receptor-mediated actions of this enzyme
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Fig. 3. Top: MK-886, an (5-lipoxygenase) inhibitor, inhibited sPLA2-induced MG secretion. Tracheal segments (each group; n = 8) were incubated for 30 min in KHS with MK-886 at 10⁻⁴ M or KHS alone (period 1) and then for another 30 min in KHS alone or KHS containing sPLA2 at 5 U/ml with or without MK-886 at 10⁻⁴ M (period 2). Data are shown as SI and expressed as means ± SE. *P < 0.0001 and **P < 0.005. Bottom: MG secretion in calcium-free medium. Tracheal segments were incubated for 30 min in KHS (1.3 mM Ca²⁺) (period 1) and then for another 30 min in KHS alone, KHS containing sPLA2 at 5 U/ml with or without EGTA at 2 mM (each group; n = 4) (period 2). Data are shown as SI and expressed as means ± SE. *P < 0.0001, **P < 0.01, and ***P < 0.0005.

Fig. 4. Constitutive MG secretion in the sPLA2-exposed trachea. Ferrets were intubated with an endotracheal tube (ETT) coated with 20 units of sPLA2 mixed in water-soluble K-Y jelly for 30 min once daily for 3 days. One (sPLA2 days 1), 5 (sPLA2 days 5), or 10 days (sPLA2 days 10) days after the last sPLA2 exposure, tracheae were removed. Control animals were intubated with an ETT coated with jelly alone, and tracheae were removed 1 day after the third and last intubation (control intubated). After resting for 1 h in KHS, segments were placed in fresh KHS for 30 min. Secreted MG was measured by ELLA. Data are shown as secreted MG. Shown are control unintubated: no treatment (n = 36), control intubated (n = 40), sPLA2 day 1 (n = 40), sPLA2 day 5 (n = 32), and sPLA2 day 10 (n = 32). Data are shown as absolute amount of secreted MG and expressed as means ± SE. *P < 0.0005 compared with control unintubated.

sPLA2-induced MG secretion, supporting the hypothesis that cyclooxygenase pathway products (e.g., prostaglandins) do not play an important role in sPLA2-induced MG secretion. Quercetin, an LO inhibitor, decreased sPLA2-induced MG secretion, suggesting that sPLA2 may stimulate MG secretion by activating the LO pathway. MK-886, a specific 5-LO inhibitor, decreased sPLA2-induced MG secretion in the ferret. MK-886 binds a membrane-bound 5-LO-activating protein, thereby inhibiting the translocation and activation of 5-LO (6, 29). MK-886 at 5 × 10⁻⁵ M also significantly decreased sPLA2-induced MG secretion. Thus sPLA2 probably stimulates tra-

Fig. 5. Human neutrophil elastase (HNE) and methacholine (MCh) stimulate MG secretion in the sPLA2-exposed trachea. Ferret tracheal segments were placed in KHS for 30 min to measure constitutive MG secretion (period 1) and then were incubated for another 30 min in KHS with HNE at 10⁻⁸ M, MCh at 10⁻⁷ M, or KHS alone (period 2). Shown are control unintubated (period 2); KHS and HNE at 10⁻⁸ M, MCh at 10⁻⁷ M; n = 15, 11, 10), control intubated (n = 14, 13, 13), sPLA2 day 1 (n = 14, 13, 13), sPLA2 day 5 (n = 10, 11, 11), and sPLA2 day 10 (n = 9, 12, 11). All data are shown as SI for MG secretion and expressed as means ± SE. *P < 0.01 compared with control unintubated.

Consistent with this, we demonstrate that p-BPB decreased sPLA2 type IB-stimulated MG secretion. These results suggest that sPLA2 type IB may stimulate MG secretion in the ferret trachea via its enzyme activity and indirectly through LT synthesis rather than by a receptor-mediated action. The results of MG secretion in Ca²⁺-free KHS support this because Ca²⁺-free KHS decreased sPLA2-induced MG secretion, and Ca²⁺-free medium also decreased constitutive MG secretion.

AA is a substrate for LO in the AA cascade generating eicosanoids, including LTs that are potent secretagogues (5, 23). Indomethacin, a cyclooxygenase inhibitor, did not affect appear to be independent of Ca²⁺ (13, 19), whereas the enzymatic activity is Ca²⁺ dependent. p-BPB can inhibit sPLA2 enzymatic activity but not its receptor-mediated action (4).
cough MG secretion through the activation of LO and the 5-LO pathway.

In persons with asthma, bronchial hyperresponsiveness is defined by the decrease in airflow, generally FEV1, to a variety of stimuli including MCh, histamine, hyperosmolar challenge, exercise, or cold air. In this study, we show that local tracheal exposure to sPLA2 exposure induced tracheal inflammation, producing a long-lasting enhanced secretory response to HNE but not to MCh stimulation (Fig. 5). We have termed this enhanced and synergistic secretory response secretory hyper-

responsiveness. This suggests that sPLA2 causes hypertrophy or hyperplasia of secretory cells or glands (analogous to asthma increasing the amount of smooth muscle in the airway) and/or that the secretory apparatus is sensitized after sPLA2 exposure to produce a greater secretory response to HNE, analogous to the concept of “twitchy airways.” Because secretory hyper-

responsiveness to NHE returned to control levels 10 days after the last sPLA2 exposure, we hypothesize that in this model, the airway has been primed to respond to NHE in an exaggerated manner.

Bowton and colleagues (3) studied the effect of LY-333013, a potent inhibitor of group II sPLA2, on allergen-induced bronchoconstriction following inhaled allergen challenge in atopic asthmatics. Compared with placebo, LY-333013 had no impact on bronchial hyperresponsiveness defined by pulmonary function changes following inhaled allergen challenge (3). However, when Masuda and colleagues (24) examined the expression and function of sPLA2 in human lung-derived cells and in human lungs with pneumonia, they showed that whereas groups V and X sPLA2 were widely expressed in the airway epithelium, interstitium, and alveolar macrophages, group IIA sPLA2 was restricted to the pulmonary arterial smooth muscle layers and bronchial chondrocytes, and groups IIE and IIF sPLA2 were minimally detected. These results suggest that groups V and X sPLA2 are involved in disease pathogenesis in the human lung to a greater extent than the group II sPLA2 enzymes.

Inflammatory cells consisting of polymorphonuclear and mononuclear cells rapidly accumulated in the sPLA2-exposed trachea by day 1, but by day 10 after exposure, the sPLA2-exposed trachea appeared the same as control tracheae. Neutrophils release chemical mediators that are potent airway secretagogues (5, 34). The number of neutrophils and the amount of neutrophil elastase is increased in sputum of patients with cystic fibrosis or severe asthma (10, 32). HNE enhanced amount of neutrophil elastase is increased in sputum of patients with cystic fibrosis or severe asthma (5, 34). The number of neutrophils and the

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both models. It has been reported that bacterial endotoxin

stimulates sPLA2 mRNA expression and enzymatic activity in the rat lung (22). Although endotoxin is known to trigger

inflammation both through CD14 and CD137 and Toll-like receptors 4 and 2 signaling, it is possible that some of this airway hypersecretory response to endotoxin occurs through a sPLA2-dependent pathway.

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