Antigen-induced Generation of Lyso-Phospholipids in Human Airways

By Floyd H. Chilton,*† Francis J. Averill,‖ Walter C. Hubbard,§ Alfred N. Fonteh,* Massimo Triggiani,‖ and Mark C. Liull

From the Departments of *Internal Medicine, Section on Pulmonary and Critical Care Medicine, and *Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27157; Department of Internal Medicine,‖Divisions of Pulmonary and Critical Care Medicine and ‖Allergy and Clinical Immunology, The Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland 21224; and †Division of Clinical Immunology and Allergy, University of Naples Federico II, School of Medicine, Via Pansini 5, 80131 Naples, Italy

Summary

The goal of the current study was to examine the formation of phospholipids, 1-radiyl-2-lyso-sn-glycero-phospholipids (lyso-PL) and 2-acetylated phospholipids (such as PAF) as well as mechanisms responsible for generating these phospholipids in bronchoalveolar lavage fluid (BALF) from allergic subjects challenged with antigen. Bronchoalveolar lavage was performed in normal and allergic subjects before, 5–30 min, 6 h, and 20 h after segmental antigen challenge via a wedged bronchoscope. Levels of 1-hexadecyl-2-lyso-phospholipids and 1-hexadecyl-2-acetyl-phospholipids were initially determined by negative ion chemical ionization gas chromatography/mass spectrometry (NICI-GC/MS). Antigen dramatically elevated quantities of 1-hexadecyl-2-lyso-phospholipids in allergic subjects 20 h after challenge when compared to non-allergic controls. In contrast, there was not a significant increase in levels of 1-hexadecyl-2-acetyl-phospholipids after antigen challenge. Closer examination of 1-palmitoyl-2-lyso-sn-glycero-3-phosphocholine (GPC) revealed that 1-palmitoyl-2-lyso-GPC, 1-myristoyl-2-lyso-GPC and 1-hexadecyl-2-lyso-GPC were three major molecular species produced after antigen challenge. 1-palmitoyl-2-lyso-GPC increased sevenfold to levels of 222 ± 75 ng/ml of BALF 20 h after antigen challenge. The elevated levels of lyso-PL correlated with levels of albumin used to assess plasma exudation induced by allergen challenge. In contrast, the time course of prostaglandin D2 (PGD2) or 9α, 11βPGF2 (11βPGF2) formation did not correlate with lyso-PL generation.

To examine the mechanism leading to lyso-phospholipid formation in antigen-challenged allergic subjects, secretory phospholipase A2 (PLA2) and acetyl hydrolase activities were measured. There was a significant increase in PLA2 activity found in BALF of allergic subjects challenged with antigen when compared to saline controls. This activity was neutralized by an antibody directed against low molecular mass, (14 kD) human synovial PLA2 and dithiothreitol. Acetyl hydrolase activity also markedly increased in BALF obtained after antigen challenge. This study indicates that high levels of lyso-PLs are present in airways of allergic subjects challenged with antigen and provides evidence for two distinct mechanisms that could induce lyso-PL formation. Future studies will be necessary to determine the ramifications of these high levels of lyso-phospholipids on airway function.

Asthma is a complex disease characterized by increased airway responsiveness to diverse stimuli and manifested by airway narrowing and inflammation (for review see reference 1). Evidence indicates that airway inflammation in asthma is characterized by an influx of inflammatory cells into airways and the release of inflammatory mediators, including biologically active phospholipids. One family of 2-acetylated phospholipids, collectively known as platelet-activating factor (PAF), is released by inflammatory cells in vitro in response antigenic and nonantigenic

*Abbreviations used in this paper: BAL, bronchoalveolar lavage; BALF, BAL fluid; DTT, dithiothreitol; FEV1, forced expiratory volume in 1 s; GPC, 1-radiyl-2-lyso-sn-glycero-3-phosphocholine; HG, hydrofluoric acid; lyso-PC, lyso-phosphatidylcholine; PAF, platelet-activating factor; PFB, pentfluorobenzoyl; PLA2, phospholipase A2; PNU, protein nitrogen units; TLC, thin layer chromatography.
challenge (2, 3). Demonstrated biological activities of PAF in vitro suggest that the release of PAF in vivo into the human airway may modulate certain characteristics of asthma such as bronchoconstriction, cellular infiltration, mucous hypersecretion, microvascular leakage, and bronchial hyperreactivity (4–16).

Lyso-phospholipids are another family of phospholipids which have been proposed to regulate tissue injury. Although this phospholipid family has received a little attention in lung injury, it has been recognized for some time that the heart generates large quantities of lyso-phospholipids during injury such as myocardial ischemia (17–20). Once formed, these lyso-phospholipids have been hypothesized to contribute to electrophysiologic alterations that lead to arrhythmogenesis (18, 19, 21–28). A few investigations in animal models have also suggested that lyso-phospholipids can participate in lung injury by increasing lung resistance, inducing capillary permeability and altering surfactant properties (29–31). However, the formation of large quantities of lyso-phospholipids in response to injury has not been clearly demonstrated in the human lung.

Lyso-phospholipids can be generated in tissues by several different mechanisms. The most direct mode of synthesis is the hydrolysis of long chain fatty acids from the sn-2 position of membrane glycerolipids of tissues (32, 33). A family of phospholipase A2 enzymes of relatively low molecular mass (~14 kD in size) classified as group I, group II and group III phospholipase A2 (PLA2) has been isolated as extracellular enzymes (for review see reference 34). In addition to their small sizes, they have high disulfide bond content and require millimolar calcium for catalysis. One member of this family, group II PLA2, has been implicated in the pathogenesis of many inflammatory diseases (35–38). This enzyme has been shown to be released from several cells including mast cells during immunologic challenge (39, 40). Group II PLA2 is present in high quantities in inflammatory sites such as peritoneal exudates from caseinatetreated animals, synovial fluids from patients with rheumatoid arthritis, and nasal lavage fluids from atopic subjects challenged with antigen (41–44). In addition, circulating levels of secretory PLA2 increase in several illnesses including sepsis, shock, severe injury, and pancreatitis (45, 46).

A more indirect pathway that can generate lyso-phospholipids is the hydrolysis of the acetyl moiety at the sn-2 position of 2-acetylated phospholipids that are generated during tissue injury. Both serum and cellular acetyl hydrolase have been described as enzymes that are clearly distinct from PLA2 isotypes (47–49). Recent studies by Tjoelker and colleagues suggest that the quantities of acetyl hydrolase that are found in tissues dramatically influence the response of that tissue to PAF (50).

In the current study, we examine the in vivo formation of phospholipid products, lyso-phospholipids, and PAF, from the PLA2 and/or acetyl hydrolase reactions in airways of allergic individuals challenged with antigen. This study indicates that high levels of lyso-phospholipids as well as secretory PLA2 and acetyl hydrolase are present in antigen-stimulated airways. Furthermore, they raise fundamental questions as to the role of lyso-phospholipids in lung injury.

Materials and Methods

Study Population. Before the evaluation of subjects, protocols received Institutional Review Board approval, and informed consent was obtained. Four normal non-allergic, non-asthmatic subjects and three allergic asthmatic subjects underwent bronchoscopy and baseline bronchoalveolar lavage (BAL) without antigen challenge. 26 allergic subjects (9 with rhinitis alone and 17 with asthma and rhinitis) underwent segmental antigen challenge via a wedged bronroscope and were lavaged at various time intervals (from 5 min to 20 h) following challenge. Bronchoalveolar lavage fluid (BALF) were assayed for 2-acetylated phospholipids and lyso-phospholipids. Subjects were men and women between 20 and 33 yr of age with no history of smoking. Subjects were classified as allergic asthmatics on the basis of clinical criteria, methacholine reactivity, and immediate-type skin testing as previously described (51).

As allergic asthmatics reported a history of episodic wheezing, shortness of breath or chest tightness, and demonstrated a positive methacholine challenge test, defined as a 20% fall in the forced expiratory volume in 1 s (FEV1) to a methacholine concentration <25 mg/ml. Allergic rhinitic subjects had a history of asthmatic symptoms and no response to methacholine at concentrations of 25 mg/ml. Atopic status was determined by prick-puncture skin testing with a battery of nine common inhalant allergens. All allergic subjects had two or more positive skin test reactions and were responsive to ragweed, Timothy grass, or dust mite used for segmental allergen challenge. All normal subjects had no history of asthma, were unresponsive to methacholine, and had no positive skin test reactions. All subjects had a FEV1 >80% predicted, were asymptomatic, and required no medications for control of symptoms at the time of the study. No subject had a history of viral respiratory tract infection during the month before the study.

Bronchoalveolar Lavage and Segmental Antigen Challenge. Bronchoalveolar lavage was performed using a standardized protocol in accordance with guidelines established by a National Institutes of Health Workshop as previously described (52). Subjects were premedicated with atropine (0.6 mg IM). After inhalation of nebulized lidocaine (4%), an Olympus BF-10 fiberoptic bronchoscope was inserted into the airway. Local anesthesia was supplemented with lidocaine (2%) instilled via the bronchoscope. The bronchoscope was wedged in a subsegmental bronchus and a control sham challenge with 5 ml normal saline was performed. Antigen challenge was then performed by instilling 5 ml of antigen in a subsegment of the contralateral lung. Instillation of saline was always performed first to avoid contamination of the control subsegment by residual antigen via the bronchoscope. In this manner, each subject provided responses to saline control and to antigen. Most subjects were challenged with a total dose of 500 protein nitrogen units (PNU) of antigen, either short ragweed (Ambrosia elatior; Greer Laboratories, Inc., Lenoir, NC), dust mite (Derma-tophagoides farinae; Hollister-Stier, Miles Inc., Elkart, IN), or Timothy grass (Phleum pratense; Hollister-Stier, Miles). 1 PNU/ml contained 0.005 μg/ml of ragweed antigen E. The endotoxin concentration of a 100 PNU/ml ragweed dilution was 0.05 endotoxin units measured using a colorimetric Limulus lysate assay. Bronchoalveolar lavage was performed by instilling 5 X 20-ml aliquots of normal saline, preheated to 37°C, with immediate aspiration of each aliquot. Fluids returned form each segment were
processed separately for mediator measurements. BALF recovery ranged from 45–60%. Upon recovery, BALF were pooled and filtered through gauze. Aliquots were immediately acidified with 8.8% formic acid (20 μl/ml) to inactivate acid-labile acetylhydrolase. Fluids were then centrifuged (10 min, 400 g) to remove cells. 10 ng of the 1-0-16,16-16-trideuterohexadecyl-2-acetyl-GPC analogue of PAF (Biomol, Plymouth Meeting, PA), and two nanograms of 1-0-7,7,8,8-tetradeterohexadecyl-2-lyso-1-acyl-2-lyso-sn-glycero-3-phosphocholine (GPC), a tetradeterated analogue of lyso-PAF (Cayman Chemical, Ann Arbor, MI), were added to 2-ml aliquots of the cell-free supernatant fluids before sample extraction and derivatization procedures. BALF with internal standards were extracted and derivatized immediately or were stored at –70°C for subsequent extraction and derivatization.

Quantification of 2-acetylated Phospholipids and Lyso-phospholipids in BALF. BALF samples were extracted, and the pentafluorobenzoyl ester derivatives of 2-acetylated and lyso-phospholipids were synthesized and quantituated by combined capillary gas chromatography–mass spectrometry using modifications of methods previously described (53, 54). Briefly, 2-ml aliquots of BALF obtained and treated as described above were extracted with octadeclsilane cartridges (C18, Sep-Pack) (Waters Associates, Milford, MA) (55). Cartridges were conditioned with 10 ml of HPLC grade methanol (Fisher Chemical Co., Fairlawn, NJ) followed by 10 ml of HPLC grade water (Baker Chemical Co., Phillipsburg, NJ). 4 ml of methanol (Fisher Chemical Co.) and 4 ml of HPLC grade water (Fisher Chemical Co.) were added to 2-ml aliquots of acidified BALF containing the deuterated internal standards, creating a 40% (vol/vol) methanol/water mixture. The 40% methanol/water mixture was then loaded directly onto the conditioned cartridges, and the cartridge washed with 5 ml of water. The eluents from the loading and washing procedures were discarded. Phospholipid fractions containing 2-acetylated- and lyso-phospholipids were eluted from the cartridges with 5 ml of methanol and collected in Teflon-coated Oak-Ridge centrifuge tubes (Nalgene Co., Rochester, NY). The eluting solvent and residual water were evaporated under a nitrogen stream leaving a residue containing the eluted phospholipids. To remove the phosphate moiety of extracted phospholipids, the residue was treated with 500 μl of 49% hydrofluoric acid (HF) (Fisher Chemical Co.) and 1 ml hexane (Fisher Chemical Co.) with continuous shaking for 4 h at room temperature. The upper hexane layer was then removed, transferred to a clean silanized glass vial and dried under a stream of nitrogen. The pentafluorobenzoyl (PFB) esters of the resulting diglycerides were then synthesized by the addition of PFB esterification reagent. The pentafluorobenzoyl esterification reagent was prepared as follows: methylene chloride (Fisher Chemical Co.) was dried by passage of the solvent over anhydrous sodium sulfate (Aldrich Chemical Co., Milwaukee, WI). 2 mg of dimethyldimethylaminepyridine (Aldrich Chemical Co.) was added to 600 μl of anhydrous methylene chloride, followed by the addition of 4 μl of pentafluorobenzoyl chloride (Aldrich Chemical Co.). 50 μl of the resultant PFB esterification reagent was then placed onto the dried hexane residue and heated at 60°C for 5 min. The reaction solvent was evaporated under nitrogen, and the solid residue extracted twice with 0.5 ml of hexane and transferred to a clean silanized glass vial. The hexane-extracted product was then evaporated under nitrogen and dissolved in 30 μl of dodecane (Aldrich Chemical Co.) for injection into the capillary gas chromatograph. The recovery of analytes during solid-phase extraction and derivatization procedures was determined by the addition of radioabeled [3H]PAF and [3H]lyso-PAF (100,000 cpm) to each BALF. Recovery of HF hydrolysis products of [3H]PAF and [3H]lyso-PAF was 95%. The overall yield throughout the extraction and esterification procedures was >60% (data not shown).

GC/MS analysis of derivatized phospholipids extracted from BALF was performed with a Varian 3400 GC interfaced with a Finnigan MAT TSQ-700 GC/MS/MS/DS operated as a single stage quadrapole. The mass spectrometer was operated in the negative ion chemical ionization mode using methane (Ultra High Purity; Matheson Gas Products, Bridgeport, NJ) at a pressure of 0.8–1.0 torr as the reagent gas. An emission current of 200 mA with an electron energy of 70V was employed for ionization. Characteristic molecular anions at m/z 552 (PAF) and 555 (2H3-PAF) were monitored for measurement of in vivo PAF levels. 1-hexadecyl-2-lyso-phospholipid levels were determined by monitoring of the characteristic molecular anion for the bispentafluorobenzoyl ester (PFBOE) of the monoglyceride (obtained after HF treatment) at m/z 704 using 2H4-lyso-PAF (signal at m/z 708) as an internal standard.

In a separate series of studies, levels of 1-myrstioylo-2-lyso-GPC, 1-hexadecyl-2-lyso-GPC and 1-palmitylo-2-lyso-GPC in BALF from seven atopic subjects obtained 20 h after antigen and saline challenges were determined. 2H4-1-hexadecyl-2-lyso-GPC (5 ng) served as the internal standard for the three lysophosphatidylcholines (lyso-PC) and was added to BALF before solid-phase extraction. A lyso-phosphatidylcholine fraction was obtained via thin-layer chromatography before HF hydrolysis of the polar head group. Briefly, the methanol eluate from solid-phase extraction was dried, dissolved in 100 μl of methanol and spotted onto silica gel G TLC plates and developed in a chloroform/methanol/acetic acid/water (50:28:8:3, vol/vol/vol/vol) solvent system. The resolved lyso-PC fraction of the TLC plate was scraped and extracted (3X) with 5-ml vol of methanol/water (3:1, vol/vol). The dried extract was then treated with HF for hydrolysis of the polar head group as described above. The monoglycerides generated via HF treatment of the lyso-phospholipids were extracted with hexane and transferred to a clean silanized glass vial. After solvent removal under a nitrogen stream, the monoglycerides were treated with pentafluoro-octylamine chloride in the presence of 4-dimethylaminopyridine and prepared for analysis as described above. The (PFBOE)2 derivative of the monoglycerides was detected by monitoring molecular anions at m/z 690, m/z 704, m/z 718, and m/z 708, for elution of 1-myrstioyl-(PFBOE)2, 1-0-hexadecyl-(PFBOE)2, 1-palmitylo-(PFBOE)2, and 2H4-1-0-hexadecyl-(PFBOE)2, respectively. Unfortunately, we were unable to measure concentrations of 1-stearoyl-2-lyso-GPC in BALF by GC/MS because of a major contamination peak found in BALF that migrated with the 1-stearoyl-(PFBOE)2.

Prostaglandin D2 and Albumin Measurements in BALF. PGD2 and its metabolite, 9α,11β-PGF2α(11β-PGF2α), were assayed in BALF samples to determine if release of these inflammatory lipid mediators had been initiated following antigen challenge. Both prostaglandins were assayed by combined GC/MS using previously published methods (56). Limits of detection of the prostaglandins were 0.1–0.2 pg/ml BALF. Albumin was also assayed in lavage fluids and served as an index of airway permeability after segmental antigen challenge. Albumin was quantified using a sensitive radiolmmunoassay (51).

Phospholipase A2 Activity in BALF. BALF for PLA2 activity measurements was obtained from five ragweed allergic, asthmatic subjects. These subjects were challenged with ragweed antigen at concentrations ranging between 20 and 500 PNU and were lavaged 20 h after challenge. Phospholipase A2 activity in BALF was
measured as described by Fonteh and colleagues (40). Briefly, PLA₂ activity was determined in 50 mM Tris HCL (pH 7.4) and 10 mM CaCl₂ in a total volume of 1.0 ml. This reaction was initiated by the addition of 0.1 µCi (9.7 nmol) [³H]AA-labeled Escherichia coli membranes (New England Nuclear, Boston, MA). After incubation (90 min at 37°C) in a water bath, the reaction was stopped by extracting lipids by the method of Bligh and Dyer (40). Free fatty acids were isolated from phospholipids by TLC on silica gel G developed in hexane/ethyl ether/formic acid (90:60:6, vol/vol). The radioactivity in lipids was located by using a radiochromatogram imaging system (Bioscan Inc., Washington, DC). Free AA and phospholipids were isolated by TLC zonal scraping and the quantity of radioactivity determined by liquid scintillation counting. PLA₂ activity was calculated and expressed as pmol AA released/h/m l of BALF.

In some experiments, the effects of antibody (F10-mAb) neutralization and dithiothreitol (DTT) on PLA₂ activity were examined. Portions of BALF were incubated (1 h, 37°C) with different concentrations of neutralizing antibody (F10-mAb) raised against recombinant human synovial fluid PLA₂. The antibody was a generous gift of Dr. Lisa Marshall (SmithKline Beecham, King of Prussia, PA). PLA₂ activity was determined in supernatant fluid fractions as described above. In other experiments, the effect of DTT on BALF PLA₂ activity was examined. Briefly, BALF was incubated with DTT on ice for 15 min. Subsequently, PLA₂ activity was measured as described above.

Acetyl Hydrolase Activity in BALF. The metabolism of PAF in BALF obtained from four allergic asthmatic subjects before and after antigen challenge was determined to ascertain acetyl hydrolase levels. [³H]PAF (1 µCi, 5 pmol; New England Nuclear, Boston, MA) was added (complexed to albumin [0.25 mg/ml]) to 5-ml aliquots of BALF obtained before, 5-30 min and 6 or 20 h after segmental antigen challenge via a wedged bronchoscope. BALF were maintained at 37°C and 1-ml aliquots of fluid were removed at 0, 10, 30, 60, and 90 min. Reactions were terminated by Bligh-Dyer extraction. The extracted phospholipid fractions were subjected to thin layer chromatography (TLC) using silica gel G plates with chloroform/methanol/acetic acid/water (50:28:8:3, vol/vol) as the developing system. The rate of metabolism of PAF to its [³H]lyso-PAF was determined by assessing the distribution of the radiolabel into the [³H]PAF and [³H]lyso-PAF fractions by liquid scintillation spectrometry.

Statistical Analysis. Statistical analysis between groups was made using Student's paired t-test. Correlations were assessed using Spearman rank correlation analysis.

Results

Formation of 2-acetylated Phospholipids and Lyso-phospholipids in Human Airways. Initial experiments were designed to examine the quantities of PAF and closely related 2-acetylated analogs (i.e., 1-hexadecyl-2-acetyl-phospholipids) as well as lyso-phospholipids containing an 1-hexadecyl-linkage at the sn-1 position of the glycerol backbone in BALF fluids. Levels of 1-hexadecyl-2-acetyl-phospholipids as determined by GC/MS are depicted in Fig. 1. Quantities of 2-acetylated phospholipids in allergic subjects were near or below the detection limits (50 pg/ml) of the assay before antigen challenge. These values were not significantly different from those found in non-allergic subjects (data not shown). Antigen challenge did not significantly increase quantities of 2-acetylated phospholipids in BALF of allergic subjects 5–30 min, 6 or 20 h after challenge. We next measured the quantities of 1-hexadecyl-2-lyso-phospholipids in BALF of allergic subjects before challenge and found 22 ± 0.9 ng/ml, a value 20–30-fold higher than 2-acetylated phospholipids. These levels were not significantly different from those found in nonallergic subjects (data not shown).

Figure 1. 1-Hexadecyl-2-acetyl-phospholipid levels before and after antigen challenge. BALFs were obtained at the indicated times after antigen challenge. Internal standards were added and lipids extracted. 2-Acetylated phospholipids were converted to pentfluorobenzoyl derivatives and analyzed by NICI-GC/MS. 1-hexadecyl-2-acetyl-phospholipid levels in the absence and presence of antigen are shown. These data are the means ± SEM. ■, Normal saline; □, antigen.

Figure 2. 1-Hexadecyl-2-lyso-phospholipid levels before and after antigen challenge. BALFs were obtained at the indicated times after antigen challenge. Internal standards were added and lipids extracted. Lyso-phospholipids were converted to bispentafluorobenzoyl derivatives and analyzed by NICI-GC/MS. 1-hexadecyl-2-lyso-phospholipid levels in the absence and presence of antigen are shown. These data are the means ± SEM. ■, Normal saline; □, antigen. *P <0.05.
Data in Fig. 2 illustrates that there was no significant increase in quantities of 1-hexadecyl-2-lyso-phospholipids in BALF of allergic subjects at 5–30 min and 6 h after antigen challenge when compared to saline challenge. However, levels of 1-hexadecyl-2-lyso-phospholipid were significantly increased (compared to saline challenge or baseline levels) 20 h after antigen challenge ($P < 0.05$).

While these initial studies measured one select subclass of lyso-phospholipid, 1-hexadecyl-2-lyso-phospholipid, they provided no data regarding other lyso-phospholipid molecular species that may be produced during antigen challenge. To address this question, 1-radyl-2-lyso-GPC molecular species were isolated by TLC and analyzed by NICI-GC/MS. Fig. 3 illustrates the GC/MS analysis of several lyso-PC molecular species found in the BALF of seven allergic subjects 20 h after antigen challenge. Levels of 1-palmitoyl-2-lyso-GPC, 1-myristoyl-2-lyso-GPC and 1-hexadecyl-2-lyso-GPC were increased five- to ninefold in allergic subjects challenged for 20 h with antigen when compared to saline challenge controls. In particular, levels of 1-palmitoyl-2-lyso-GPC were high (222 ng/ml ± 75) in the late phase BALF. Fig. 4 illustrates the individual levels of 1-palmitoyl-2-lyso-GPC in seven subjects. While 1-palmitoyl-2-lyso-GPC levels increased in the BALF of all subjects after antigen challenge, the degree of change varied a great deal between individual subjects. The level of 1-palmitoyl-2-lyso-GPC in the BALF of one subject were extremely high approaching 700 ng/ml.

**Phospholipase A$_2$ Activity in Human Airways.** To gain some understanding into the mechanism(s) leading to the marked increases in 1-radyl-2-lyso-GPC molecular species in BALF, the activities of two potential enzymes that could regulate lyso-phospholipids levels were measured. PLA$_2$ activity was assayed by measuring the capacity of cell-free BALF to hydrolyze [H]AA from labeled $E.~coli$ membranes. Fig. 5 shows the PLA$_2$ activity in BALF from five allergic subjects challenged for 20 h with saline or antigen. BALF from saline challenged sites contained relatively high levels of PLA$_2$ activity (981 ± 121 pmol/h/ml BALF). In addition, there was a modest and significant increase in PLA$_2$ activity found in BALF after antigen challenge when compared to saline challenge. PLA$_2$ activity increased after antigen challenge in the BALF of all five subjects tested.

To better characterize this PLA$_2$ activity, BALF from allergic subjects (saline and antigen challenged) were exposed to either a neutralizing antibody (F10) directed against low

Figure 3. Influence of 20 h antigen challenge on levels of lyso-phosphatidylcholine molecular species. BALFs were obtained 20 h after antigen challenge. Lipids were extracted and lyso-phosphatidylcholine isolated by TLC. Lyso-phosphatidylcholine molecular species were then converted to bispentafluorobenzoyl derivatives and analyzed by NICI-GC/MS. These data are the means ± SEM of seven separate patients. ■, Normal saline; □, antigen. *$P<0.05$.

Figure 4. Influence of antigen challenge on levels of 1-palmitoyl-2-lyso-GPC in seven allergic subjects. BALFs were obtained 20 h after antigen challenge. Lipids were extracted and lyso-phosphatidylcholine isolated by TLC. Lyso-phospholipids were then converted to bispentafluorobenzoyl derivatives and analyzed by NICI-GC/MS. These are the individual measurements of 1-palmitoyl-2-lyso-GPC from seven separate patients.

Figure 5. PLA$_2$ activity before and after antigen challenge. BALFs were obtained 20 h after antigen challenge. PLA$_2$ activity was determined using labeled $E.~coli$ membranes as described in the methods. These are the measurements from five patients challenged with saline and antigen. *$P<0.05$. 

2239 Chilton et al.
molecular secretory PLA2, or a reducing agent, dithiothreitol (Fig. 6, A and B). Both the neutralizing antibody and dithiothreitol blocked the activity of PLA2 in BALF in a dose-dependent manner. These data reveal that the BALF of allergic subjects contain high levels of PLA2 activity with characteristics of low molecular weight, group II PLA2.

Acetyl Hydrolase Activity in Human Airways. An increase in lyso-phospholipid levels could also result from the hydrolysis of an acetyl moiety from the sn-2 position of the glycerol backbone. Both cellular and serum acetyl hydrolase activities which are distinct from classical PLA2 have been described (47-50). Acetyl hydrolase activity was determined in BALF by monitoring the catabolism of [3H]PAF. [3H]PAF was added to BALF recovered before and 5-30 min and 20 h after antigen challenge. Fig. 7 indicates that acetyl hydrolase activity in BALF varies significantly depending on whether the antigenic response has been initiated. Top, middle, and bottom panels illustrate acetyl hydrolase activity before, 5-30 min and 20 h after antigen challenge. Whereas acetyl hydrolase activity is present before antigen challenge, its activity is increased significantly 5-30 min after antigen challenge. Moreover, whereas acetyl hydrolase activity is present before antigen challenge, its activity is decreased significantly 20 h after antigen challenge.

Figure 6. Effect of F-10 antibody and dithiothreitol on BALF PLA2 activity. BALFs were obtained 20 h after antigen challenge. The fluids were treated with an antibody (F10) that neutralizes human synovial PLA2 and DTT. PLA2 activity was determined using labeled E. coli membranes as described in the methods. These are the measurements from three patients challenged with antigen. *P <0.05.

Figure 7. Acetyl hydrolase activity in BALF before and after antigen challenge. BALFs were obtained at the indicated times after antigen challenge. Labeled PAF was incubated in BALF and metabolites were isolated by TLC. These data show the conversion of PAF to lyso-PAF and are means ± SEM of four patients at each time point. —, PAF; —O—, lyso-PAF.
Correlation of Lysophospholipid Levels with Changes in Prostaglandin D2 and Albumin in Human Airways. The next series of experiments were designed to examine whether changes in lysophospholipid levels could be correlated with other markers of antigen activation. Levels of PGD2 and its metabolite 11β-PGF2 were measured by NICI-GC/MS in BALF obtained before, 5–30 min, 6 and 20 h after antigen challenge. Levels of both PGD2 and 11β-PGF2 increased significantly 5–30 min (Fig. 8) after challenge when compared to saline challenge levels. PGD2 levels were much lower but remained slightly elevated over saline challenge levels at 6 and 20 h. These data demonstrated that the time course of formation of PGD2 and 11β-PGF2 was clearly different than that seen for lyso-phospholipids. In contrast to these prostaglandins, albumin levels were markedly elevated 20 h after antigen challenge (Fig. 9). When these levels were compared to levels of 1-hexadecyl-2-lyso-phospholipids in individual subjects, there was a significant correlation between albumin and 1-hexadecyl-2-lyso-phospholipid levels.

Discussion

Studies in a variety of tissues and the heart, in particular, have shown that high concentrations of lyso-phospholipids are generated during injury. This can be demonstrated in vitro with isolated myocytes, in models of ischemia–perfusion injury, or in vivo during myocardial infarction. Whereas the exact role of these lyso-phospholipids in heart injury is not clear, it is known that they can contribute to membrane dysfunction, electrophysiological alterations, lethal arrhythmias, and cellular injury of the ischemic myocardium. In contrast to the heart, there have been few studies examining the role of lyso-phospholipids in lung injury. This may be due, in large part, to the fact that it is difficult to measure lyso-phospholipids in tissues and that there have been few studies that have reported lyso-phospholipid formation at significant levels in the lung. It has been shown that measurable quantities of lyso-PAF are present in BALF of both normal, rhinitic and asthmatic subjects (12, 57). Results in the current study reveal that relatively high concentration of lyso-phospholipids reside in the epithelial lining fluid of allergic subjects and levels dramatically increase during
challenged with antigen. Whereas all potential molecular species of lyso-phospholipids were not examined, these data indicate that at least three major molecular species, 1-myristoyl-2-lyso-GPC, 1-hexadecyl-2-lyso-GPC and 1-palmitoyl-2-lyso-GPC are found in BALF of human airways.

At least two fundamental questions arise from these data: (a) what are the roles of these high concentrations of lyso-phospholipids in lung injury? and (b) what are the enzymatic mechanisms which generate lyso-phospholipids in human airways? In regard to the former, the role of lyso-phospholipids in lung injury has not been clearly elucidated. However, mean BALF concentrations of 1-palmitoyl-2-lyso-GPC at 20 h were 222 ng/ml and reached levels of 685 ng/ml in one subject. Since the amount of saline injected into the bronchus could dilute the alveolar lining fluid from 50- to 100-fold (58), the mean concentration of 1-palmitoyl-2-lyso-GPC in lining fluid may range from 25 to 50 μM after antigen challenge and could reach 150 μM in some subjects. Lyso-phospholipids levels this high affect several biological processes that could alter lung function. These concentrations are 10–20-fold higher than the critical micelle (aggregate) concentration for lyso-PC, and this physical form of lyso-phospholipids can disrupt cellular membranes via detergent effects resulting in cytolyis. For example, PL-A2–induced lyso-PC formation in isolated hamster lungs has been correlated with type I epithelial cell toxicity (29, 33). In addition to detergent effects, lyso-PC can irreversibly inactivate surfactant (30) by its capacity to disorder the surface film thereby reducing the ability to lower surface tension. Lyso-phospholipids have also been shown to increase airway and capillary permeability in isolated perfused rat lungs (31). The fact that albumin concentrations increase as a function of the concentration of lyso-phospholipids in BALF further emphasizes the potential role of lyso-phospholipid in inducing cell injury and changes in airway permeability. There is also some question as to whether the lyso-phospholipids could serve as precursors for the biosynthesis of 2-acetylated phospholipids such as PAF. For example, we have demonstrated that activated human neutrophils can readily utilize exogenously–provided 1-hexadecyl-2-lyso-GPC for the synthesis of 1-hexadecyl-2-acyl-GPC (59). Finally, very recent studies have shown that relatively low concentrations of lyso-PC transcriptionally regulate growth factor gene expression in human monocytes and endothelial cells (60, 61). Taken together, these data imply that lyso-phospholipids have great potential to affect lung injury.

In contrast to the potential deleterious impact of lyso-phospholipids, these lyso-phospholipids may represent key intermediates in pathways for generating new surfactant or for membrane lipid remodeling. Alternatively, several studies indicate that polyunsaturated acyl residues of phospholipids can undergo oxidative damage and fragmentation in the presence of free radicals generated by activated inflammatory cells (for a review see reference 62). Watson and colleagues have further demonstrated that acetyl hydrolase has the capacity to cleave many of these oxidatively fragmented phospholipids to form lyso-phospholipids (63). Therefore, lyso-phospholipids observed in our studies may represent the product of clearance of oxidatively damaged phospholipids by lipases such as acetyl hydrolase.

With regard to the question of mechanism of formation, again it is difficult to unequivocally predict how lyso-phospholipids are formed during antigen challenge. Acute respiratory failure associated with disorders such as pancreatitis correlates with serum levels of PL-A2 (46). We recently demonstrated that nasal lavage of allergic subjects contains PL-A2 activity and lyso-phospholipids and that both are enhanced markedly following nasal challenge with antigen or histamine (43, 57). The nasal lavage PL-A2 was purified and found to be similar to a recombinant form of human PL-A2 identified in synovial fluids from patients with arthritis (43). The current study shows that a secretory PL-A2 is present in BALF of normal and allergic subjects and that this activity was increased significantly in allergic subjects after antigen challenge. This activity was also blocked by a neutralizing antibody directed against synovial fluid PL-A2 and dithiothreitol, consistent with characteristics of a low molecular mass secretory PL-A2. Although there is no direct evidence that secretory PL-A2 contributes to lyso-phospholipid generation in human airways, group I PL-A2 has been shown to induce lyso-phospholipid formation in hamster lung preparations (33). Other studies have demonstrated that mast cells contain group II PL-A2 and release this enzyme during the antigen-induced cross-linking of IgE (39, 40). Therefore, it may be that human mast cells release secretory PL-A2 which, in turn, hydrolyze lung phospholipids to form lyso-phospholipids.

The lung source of the phospholipid substrate for the PL-A2 reaction is unknown. In the hamster lung, surfactant phospholipids were suggested to be the preferred substrate for secretory PL-A2. As mentioned above, PL-A2 has been shown to destroy the activity of isolated lung surfactant and this loss of activity has been postulated to be due to the formation of lyso-phospholipids. Interestingly, the ratio of 1-myristoyl-2-lyso-GPC to 1-palmitoyl-2-lyso-GPC observed in these studies correlates well with the 1-myristoyl-2-palmitoyl-GPC to 1-palmitoyl-2-palmitoyl-GPC ratio in human lung surfactant. However, the presence of relatively large amounts 1-hexadecyl-2-lyso-GPC suggest that cellular phospholipids in the lung may also serve as a substrate for secreted PL-A2. Group I and Group II PL-A2 can interact with various cells through specific receptors on their plasma membranes (64, 65) and this may account for high levels of other lyosphospholipids in BALF of antigen-challenge individuals.

As mentioned above, it is also possible that lyso-phospholipids are formed by the hydrolysis of 2-acetylated or oxidatively damaged phospholipids released into the lung. This hypothesis is supported by the fact that acetyl hydrolase levels in BALF increase in a time-dependent manner following antigen challenge. However, two lines of reasoning argue against acetyl hydrolase generating the bulk of the observed lyso-phospholipids. First, the ratios of 1-myristoyl-, 1-palmitoyl- and 1-hexadecyl-2-lyso-GPC, do not fit the pattern of 2-acetylated phospholipids thought to be
synthesized during antigen activation in the lung. For example, previous studies suggest little 1-myristoyl-2-acetyl-GPC and more 1-hexadecyl-2-acetyl-GPC are synthesized by mast cells within the lung (66). Second, high levels of 2-acetylated phospholipids such as PAF would be required in airways to serve as a substrate for acetyl hydrolase. This would seem unlikely since we were unable to measure levels much higher than 50 pg/ml of BALF at any time point after antigen challenge. However, this does not rule out the possibility that large quantities of oxidatively-damaged phospholipids are formed during antigen challenge and lyso-phospholipids observed in the late phase are a result, in part, of acetyl hydrolase-catalyzed cleavage of these phospholipids.

In conclusion, lyso-phosphatidylcholine levels are markedly elevated in BALF obtained 20 h after antigen challenge from allergic subjects. The elevated lyso-phospholipids are likely a result of elevated secretory PLA2 or acetyl hydrolase levels. This antigen-dependent accumulation of lyso-phospholipids in the lung may play an important role in the disrupted airways permeability, barrier function and surfactant properties characteristic of late inflammatory responses.

These studies were supported, in part, by grants from the National Institutes of Health AI 24985, HL 50395 (F.H. Chilton), AI 31867 (M.C. Liu), and HL 49545 (M.C. Liu).

Address correspondence to Floyd H. Chilton, Section on Pulmonary and Critical Care Medicine, Bowman Gray School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1054.

Received for publication 12 December 1995 and in revised form 23 February 1996.

References

1. Djukanovic, R., W.R. Roche, J.W. Wilson, C.R. Beasley, O.P. Twentyman, R.H. Howarth, and S.T. Holgate. 1990. Mucosal inflammation in asthma. Am. Rev. Respir. Dis. 142:434-457.
2. Triggiani, M., R.P. Schleimer, J.A. Warner, and F.H. Chilton. 1991. Differential synthesis of 1-acyl-2-acyl-sn-glycero-3-phosphocholine and platelet-activating factor by human inflammatory cells. J. Immunol. 147:660-666.
3. Mencia-Huerta, J.M., R.A. Lewis, E. Razin, and K.F. Austen. 1983. Antigen-initiated release of platelet-activating factor (PAF-acether) from mouse bone marrow-derived mast cells sensitized with monoclonal IgE. J. Immunol. 131:2958-2964.
4. Wasserman, S.I. 1988. Platelet-activating factor as a mediator of bronchial asthma [published erratum appears in Hosp Pract (Off Ed) 1989 Jan 15; 24(1):17]. Hosp. Pract. (Off. Ed.). 23:49-58.
5. Rubin, A.H., I.J. Smith, and E. Patterson. 1987. The bronchoconstrictor properties of platelet-activating factor in humans. Am. Rev. Respir. Dis. 136:1145-1151.
6. Stmler, N.P., and J.T. O'Flaherty. 1983. Spasmogenic properties of platelet-activating factor: evidence for a direct mechanism in the contractile response of pulmonary tissues. Am. J. Pathol. 113:75-84.
7. Smith, L.J. 1991. The role of platelet-activating factor in asthma. Am. Rev. Respir. Dis. 143:S100-102.
8. Henson, P.M., P.J. Barnes, and S.P. Banks-Schlegel. 1992. NHLBI workshop summary. Platelet-activating factor: role in pulmonary injury and dysfunction and blood abnormalities. Am. Rev. Respir. Dis. 145:726-731.
9. Derjep, A., B. Amoux, R. Mase, A. Lockhart, and J. Benveniste. 1983. Acute effects of intratracheal administration of platelet-activating factor in baboons. J. Appl. Physiol. Respir. Environ. Exercise Physiol. 55:799-804.
10. Patterson, R., P.R. Bernstein, K.E. Harris, and R.D. Krell. 1984. Airway responses to sequential challenges with platelet-activating factor and leukotriene D4 in rhesus monkeys. J. Lab. Clin. Med. 104:340-345.
11. Nakamura, T., Y. Morita, M. Kuriyama, K. Ishihara, K. Ito, and T. Miyamoto. 1987. Platelet-activating factor in late asthmatic response. Int. Arch. Allergy Appl. Immunol. 82:57-61.
12. Stenton, S.C., E.N. Court, W.P. Kingston, P. Goadby, C.A. Kelly, M. Duddridge, C. Ward, D.J. Hendrick, and E.H. Walters. 1990. Platelet-activating factor in bronchoalveolar lavage fluid from asthmatic subjects. Eur. Respir. J. 3:408-413.
13. Cuss, F.M., C.M. Dixon, and P.J. Barnes. 1986. Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. Lancet. 2:189-192.
14. Chung, K.F., H. Aizawa, M. Kuriyama, K. Ishihara, K. Ito, and T. Miyamoto. 1987. Lysophosphoglycerides and their accumulation in cardiac lymph effluents and potentiation of their arachidonic acid metabolism: role of thromboxane generation. J. Pharmacol. Exp. Ther. 236:580-584.
15. Smith, L.J., A.H. Rubin, and R. Patterson. 1988. Mechanism of platelet activating factor-induced bronchoconstriction in humans. Am. Rev. Respir. Dis. 137:1015-1019.
16. Vargaftig, B.B., J. Lefort, M. Chignard, and J. Benveniste. 1980. Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. Eur. J. Pharmacol. 65:185-192.
17. Akita, H., M.H. Creer, K.A. Yamada, B.E. Sobel, and P.B. Corr. 1986. Electrophysiologic effects of intracellular lysophosphoglycerides and their accumulation in cardiac lymph with myocardial ischemia in dogs. J. Clin. Invest. 78:271-280.
18. Snyder, D.W., W.A. Craford, Jr., J.L. Gishow, D. Rankin, B.E. Sobel, and P.B. Corr. 1981. Lysophosphoglycerides in ischemic myocardium effluents and potentiation of their arachidonic acid metabolism. J. Physiol. 241:1:700-1:707.
19. Goevi, W.C., and M.C. Boadle. 1967. The cardiac action of lysolecithin. J. Pharmacol. Exp. Ther. 156:339-344.
20. Seldis, S.P., M. Horn, J.M. Sequeira, and R. Esposito. 1993.
Lysophosphatidylcholine accumulation in ischemic human myocardium. J. Lab. Clin. Med. 121:111–117.
21. Man, R.Y. 1988. Lysophosphatidylcholine-induced arrhythmias and its accumulation in the rat perfused heart. Br. J. Pharmacol. 93:412–416.
22. Clarkson, C.W., and R.E. Ten Eick. 1983. On the mechanism of lysophosphatidylcholine-induced depolarization of cat ventricular myocardium. Circ. Res. 52:543–556.
23. Sedlis, S.P., J.M. Sequeira, G.G. Ahumada, and N. el Sherif. 1988. Effects of lysophosphatidylcholine on cultured heart cells: correlation of rate of uptake and extent of accumulation with cell injury. J. Lab. Clin. Med. 112:745–754.
24. Weltzien, H.U. 1979. Cytolytic and membrane-perturbing properties of lysophosphatidylcholine. Biochim. Biophys. Acta. 559:259–287.
25. Sargent, C.A., O. Vesterqvist, M.L. Ogletree, and G.J. Grover. 1993. Effects of endogenous and exogenous lysophosphatidylcholine in isolated perfused rat hearts. J. Mol. Cell. Cardiol. 25:905–913.
26. Boachie-Ansah, G., K.A. Kane, and A.C. Rankin. 1992. Effects of a combination of acidosis, lactate, and lysophosphatidylcholine on action potentials and ionic currents in guinea pig ventricular myocytes. J. Cardiovasc. Pharmacol. 20:538–546.
27. Woodley, S.L., H. Ikenouchi, and W. H. Barry. 1991. Lysophosphatidylcholine increases cytosolic calcium in ventricular myocytes by direct action on the sarcocenema. J. Mol. Cell. Cardiol. 23:671–680.
28. Liou, E., I.J. Goldhaber, and J.N. Weiss. 1991. Effects of lyso-phosphatidylcholine on electrophysiological properties and excitation-contraction coupling in isolated guinea pig ventricular myocytes. J. Clin. Invest. 88:1819–1832.
29. Niewoehner, D.E., K. Rice, A.A. Sinha, and D. Wangerstein. 1987. Injurious effects of lysophosphatidylcholine on barrier properties of alveolar epithelium. J. Appl. Physiol. 63:1979–1986.
30. Amirkhanian, J.D., and H.W. Taeusch. 1993. Reversible and irreversible inactivation of preformed pulmonary surfactant surface films by changes in subphase constituents. Biochim. Biophys. Acta. 1165:321–326.
31. Lindahl, M., A.R. Hede, and C. Tagesson. 1986. Lysophosphatidylcholine increases airway and capillary permeability in the isolated perfused rat lung. Exp. Lung Res. 11:1–12.
32. Rice, K.L., P.G. Duane, and D.E. Niewoehner. 1988. Effects of phospholipase A2 and its hydrolytic products on alveolar epithelial permeability and elastase-induced emphysema. Am. Rev. Respir. Dis. 138:1196–1200.
33. Niewoehner, D.E., K. Rice, P. Duane, A.A. Sinha, R. Gebhard, and D. Wangerstein. 1989. Induction of alveolar epithelial injury by phospholipase A2. J. Appl. Physiol. 66:261–267.
34. Dennis, E.A. 1994. Diversity of group types, regulation, and function of phospholipase A2. J. Biol. Chem. 269:13057–13060.
35. Chen, J., S.J. Engle, J.J. Seilhamer, and J.A. Tischfield. 1994. Cloning and recombinant expression of a novel human low molecular weight Ca2+-dependent phospholipase A2. J. Biol. Chem. 269:2365–2368.
36. Seilhamer, J.J., W. Pruzanis, P. Vadas, S. Plant, J.A. Miller, J. Kloss, and L.K. Johnson. 1989. Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. J. Biol. Chem. 264:5335–5338.
37. Kramer, R.M., C. Hession, B. Johansen, G. Hayes, P. McGray, E.P. Chow, R. Tizard, and R.B. Pepinsky. 1989. Structure and properties of a human non-pancreatic phospholipase A2. J. Biol. Chem. 264:5768–5775.
38. Mizushima, H., I. Kudo, K. Horigome, M. Murakami, M. Hayakawa, D.K. Kim, E. Kondo, M. Tomita, and K. Inoue. 1989. Purification of rabbit platelet secretory phospholipase A2 and its characteristics. J. Biochem. 105:520–525.
39. Murakami, M., I. Kudo, Y. Suwa, and K. Insusboue. 1992. Release of 14-kDa group II phospholipase A2 from activated mast cells and its possible involvement in the regulation of the degranulation process. Eur. J. Biochem. 209:257–265.
40. Fonteh, A.N., D.A. Bass, I.A. Marshall, M. Seeds, J.M. Samet, and F.H. Chilton. 1994. Evidence that secretory phospholipase A2 plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. J. Immunol. 152:5438–5446.
41. Forst, S., J. Weiss, P. Elsbach, J.M. Maragone, I. Reardon, and R.L. Heinrikson. 1986. Structural and functional properties of a phospholipase A2 purified from an inflammatory exudate. Biochemistry. 25:8381–8385.
42. Pruzanski, W., P. Vadas, E. Stefanski, and M.B. Urowitz. 1985. Phospholipase A2 activity in sera and synovial fluids in rheumatoid arthritis and osteoarthritis. Its possible role as a proinflammatory enzyme. J. Rheumatol. 12:211–216.
43. Stadel, J.M., K. Hoyle, R.M. Naclerio, A. Roschak, and F.H. Chilton. 1994. Characterization of phospholipase A2 from human nasal lavage. Am. J. Respir. Cell Mol. Biol. 11:108–113.
44. Touqui, L., N. Herpin-Richard, R.-M. Gene, E. Jullian, D. Aljabi, C. Hamberger, B.B. Vargaftig, and J.-F. Dessanger. 1994. Excretion of platelet activating factor-acetylhydrolase and phospholipase A2 into nasal fluids after allergenic challenge: possible role in the regulation of platelet activating factor release. J. Allergy Clin. Immunol. 94:109–119.
45. Nevalainen, T.J. 1993. Serum phospholipases A2 in inflammatory diseases. Clin. Chem. 39:2453–2459.
46. Anderson, B.O., E.E. Moore, and A. Banerjee. 1994. Phospholipase A2 regulates critical inflammatory mediators of multiple organ failure. J. Surg. Res. 56:199–205.
47. Farr, R.S., M.L. Wardlow, C.P. Cox, K.E. Meng, and J.-F. Dessanger. 1994. Excretion of platelet activating factor-acetylhydrolase and phospholipase A2 into nasal fluids after allergenic challenge: possible role in the regulation of platelet activating factor release. J. Allergy Clin. Immunol. 94:109–119.
48. Ehst, M.R., D.M. Stafforini, S.M. Prescott, and T.M. Mcintyre. 1991. Human macrophages secrete platelet-activating factor acetylhydrolase. A mechanism for resolution of pulmonary inflammation. Chest. 99:95–108.
49. Stafforini, D.M., E.N. Rollins, S.M. Prescott, and T.M. Mcintyre. 1993. The platelet-activating factor acetylhydrolase from human erythrocytes. Purification and properties. J. Biol. Chem. 268:3857–3865.
50. Tjoelker, L.W., C. Wilder, C. Eberhardt, D.M. Stafforini, G. Greene, and D.E. Greene. 1983. Human serum acid-labile factor is an acylhydrolase that inactivates platelet-activating factor. Fed. Proc. 42:3120–3122.
51. Lai, M.S., D.M. Stafforini, S.M. Prescott, and T.M. Mcintyre. 1993. The platelet-activating factor acetylhydrolase from human erythrocytes. Purification and properties. J. Biol. Chem. 268:3857–3865.
workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am. Rev. Respir. Dis.* 132:180-185.

53. Ramesha, C.S., and W.C. Pickett. 1986. Measurement of sub-picogram quantities of platelet activating factor (AGEPC) by gas chromatography/negative ion chemical ionization mass spectrometry. *Biomed. Environ. Mass Spectrom.* 13:107–111.

54. Murphy, R.C., and K.L. Clay. 1987. Measurement of platelet-activating factor by physicochemical techniques. *Am. Rev. Respir. Dis.* 136:207–210.

55. Janero, D.R., and C. Burghardt. 1990. Solid-phase extraction on silica cartridges as an aid to platelet-activating factor enrichment and analysis. *J. Chromatogr.* 526:11–24.

56. Liu, M.C., E.R. Bleecker, L.M. Lichtenstein, A. Kagey-Sobotka, Y. Niv, T.L. McLemore, S. Permutt, D. Proud, and W.C. Hubbard. 1990. Evidence for elevated levels of histamine, prostaglandin D$_2$, and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. *Am. Rev. Respir. Dis.* 142:126–132.

57. Shin, M.-H., F.J. Averill, W.C. Hubbard, F.H. Chilton, F.M. Baroody, M.C. Liu, and R.M. Naclerio. 1994. 1-0-hexadecyl-2-lyso-sn-glycero-3-phospholipid generation after nasal challenge with allergen. *Am. J. Respir. Crit. Care Med.* 149:660–666.

58. Rennard, S.I, G. Basset, D. Lecossier, K.M. O'Donnell, P. Pinkston, P.G. Martin, and R.G. Crystal. 1986. Estimation of volume of epithelial lining fluid recovered by lavage using urea as a marker of dilution. *J. Appl. Physiol.* 60:532–538.

59. Chilton, F.H., J.M. Ellis, S.C. Olson, and R.L. Wykle. 1984. 1-0-alkyl-2-arachidonoyl-sn-glycero-3-phosphocholine. A common source of platelet-activating factor and arachidonate in human polymorphonuclear leukocytes. *J. Biol. Chem.* 259:12014–12019.

60. Nakano, T., E.W. Raines, J.A. Abraham, M. Klagsbrun, and R. Ross. 1994. Lysophosphatidylcholine upregulates the level of heparin-binding epidermal growth factor-like growth factor mRNA in human monocytes. *Proc. Natl. Acad. Sci. USA.* 91:1069–1073.

61. Kume, N., and M.A. Gimbrone, Jr. 1994. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J. Clin. Invest.* 93:907–911.

62. Zimmerman, G.A., S.M. Prescott, and T.M. McIntyre. 1995. Oxidatively fragmented phospholipids as inflammatory mediators: the dark side of polyunsaturated lipids. [Review]. *J. Nutrition.* 125(6 Supp.):1661S–1665S.

63. Watson, A.D., M. Navab, S.Y. Hama, A. Sevanian, S.M. Prescott, D.M. Stafforini, T.M. McIntyre, B.N. Du, A.M. Fogelman, and J.A. Berliner. 1995. Effect of platelet-activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. *J. Clin. Invest.* 95:774–782.

64. Ishizaki, J., K. Hansaki, K. Higashino, J. Kishino, N. Kikuchi, O. Ohara, and H. Arita. 1994. Molecular cloning of pancreatic group I phospholipase A$_2$ receptor. *J. Biol. Chem.* 269:5897–5904.

65. Lambeau, G., P. Ancian, J. Barhanin, and M. Lazdunski. 1994. Cloning and expression of a membrane receptor for secretory phospholipases A$_2$. *J. Biol. Chem.* 269:1575–1578.

66. Triggiani, M., W.C. Hubbard, and F.H. Chilton. 1990. Synthesis of 1-acyl-2-acetyl-sn-glycero-3-phosphocholine by an enriched preparation of the human lung mast cell. *J. Immunol.* 144:4773–4780.