Lysosomal Phospholipase A2 Is Selectively Expressed in Alveolar Macrophages*

Akira Abe‡, Miki Hiraoka‡, Susan Wild‡, Steven E. Wilcoxen§, Robert Paine III§, and James A. Shayman‡‡

From the ‡Division of Nephrology and §Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48109

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Lung surfactant is the surface-active agent comprised of phospholipids and proteins that lines pulmonary alveoli. Surfactant stabilizes the alveolar volume by reducing surface tension. Previously, we identified a lysosomal phospholipase A2, termed LPLA2, with specificity toward phosphatidylcholine and phosphatidylethanolamine. The phospholipase is localized to lysosomes, has calcium-independent activity, and transacylates ceramide. Here, we demonstrate that LPLA2 is selectively expressed in alveolar macrophages but not in peritoneal macrophages, peripheral blood monocytes, or other tissues. Other macroage-associated phospholipase A2s do not show a comparable distribution. LPLA2 is of high specific activity and recognizes disaturated phosphatidylcholine as a substrate. The lysosomal phospholipase A2 activity is six times lower in alveolar macrophages from mice with a targeted deletion of the granulocyte macrophage colony-stimulating factor (GM-CSF), a model of impaired surfactant catabolism, compared with those from wild-type mice. However, LPLA2 activity and protein levels are measured in GM-CSF null mice in which GM-CSF is expressed as a transgene under the control of the surfactant protein C promoter. Thus LPLA2 may be a major enzyme of pulmonary surfactant phospholipid degradation by alveolar macrophages and may be deficient in disorders of surfactant metabolism.

Previously, in an attempt to identify the enzyme that transacylates ceramide at the 1-hydroxyl position, a novel phospholipase A2 was characterized (1). The phospholipase A2, termed 1-O-acylceramide synthase or lysosomal phospholipase A2 (LPLA2)† has the following properties. In the presence of ceramide, the enzyme catalyzes the formation of 1-O-acylceramide by transacylation of fatty acids from the sn-2 position of phosphatidylcholine or phosphatidylethanolamine. In the absence of ceramide or other alcohols as acceptors, the enzyme acts as a traditional phospholipase A2. However, the phospholipase has a pH optimum of 4.5 and is mannose-rich and calcium-independent (2). The phospholipase amino acid sequence is 49% identical to human LCAT (3). The homology with LCAT is immediately apparent.

A role for an acidic phospholipase A2 activity has previously been suggested for the degradation of pulmonary surfactant phospholipids (4). The pulmonary acidic phospholipase A2 activity is also reported to be calcium-independent and inhibited by a transition state analog of arachidonate, MJ33 (5). In rats treated with MJ33 the surfactant phospholipid catabolism was inhibited by ~40–50%, suggesting that the drug-sensitive phospholipase A2 activity contributes significantly to total surfactant degradation (6). In the present paper LPLA2 was studied in alveolar macrophages to determine whether this enzyme might play a role in pulmonary surfactant catabolism.

MATERIALS AND METHODS

Reagents—Phosphatidylethanolamine, 1,2-dioleoyl-sn-glycero-3-phosphorylcholine (DOPC), and 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine (DPFC) were obtained from Avanti Polar Lipids (Alabaster, AL). Didetyl phosphate and monoclonal anti–Cyclo MoAb clone 9E10 mouse ascites fluid were purchased from Sigma, MJ33 was from Calbiochem, and N-Acetyl-N-erythro-sphingosine (NAS) was from Nevada (Pleasant Gap, PA). BCA protein assay reagent was obtained from Pierce.

Isolation of Rat Cells and Tissues—Respiratory disease-free female Wistar rats (125–150 g) were obtained from Charles River Laboratories, Inc. (Portage, MI) and housed under specific pathogen-free conditions. For isolation of alveolar macrophages by bronchoalveolar lavage, lavage buffer consisting of 0.15 M NaCl, 2.7 mM EDTA, 20 mM Hepes (pH 7.4), 5.5 mM D-glucose, and 1× antibiotic-antimycotic solution (Invitrogen) was used. Following anesthesia with subcutaneous sodium pentobarbital, lungs were surgically excised and lavaged as reported (7). Peritoneal macrophages were obtained by lavage of peritoneal spaces with RPMI 1640 medium containing 1× antibiotic-antimycotic solution.

Contaminated erythrocytes were removed by hypotonic lysis. Peripheral blood monocytes were isolated by centrifugation with Histopaque-1077 (Sigma).

After counting, cells were suspended in RPMI 1640 medium containing 1× antibiotic-antimycotic solution and plated in 100-mm culture containing SP-C and GM-CSF, LCAT, lecithin cholesterol acyltransferase; MJ33, 1-hexadecyl-3-trifluoroethylglycero-sn-2-phosphomethanol; Prdx6, 1-Cys-peroxiredoxin.
discharged through centrifugation at 37 °C in a humidified atmosphere of 5% CO2 in air. After 1 h, non-adherent cells were removed by washing with phosphate-buffered saline. Ninety-five percent of alveolar lavaged cells and 81% of peritoneal lavaged cells in the resultant adherent cell population were macrophages as confirmed by Wright-Giemsa staining (8). Greater than 90% of the adherent peripheral blood mononuclear cells were monocytes (meta).

Isolation of Cells and Tissues:—Wild-type C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). GM-CSF(−/−) was generated by Dranoff et al. (10). Bi-transgenic mice were generated from GM-CSF(−/−) mice by transgenic expression of a chimeric gene containing GM-CSF under the surfactant protein C (SP-C) promoter (SP-C-GM mice) (11). The specificity of the SP-C promoter results in transacylase activity being localized to lung and colon, but not to liver (9). Bi-transgenic mice were generated by Dr. J. Whitsett (Children’s Hospital, Cincinnati, OH). After anesthesia with intraperitoneal sodium pentobarbital, the trachea was cannulated and the lung was lavaged with phosphate-buffered saline containing 0.5 mM EDTA as described previously (12). The lavage fluid of each group was pooled, and the cell pellet was collected by centrifugation. All mice were housed in specific pathogen-free conditions. Mice were used at 3–5 months of age. All experiments were approved by the University of Michigan Committee on the Use and Care of Animals.

RNA Extraction and cDNA Synthesis—Total RNA was extracted from each rat organ using TRizol reagent (Invitrogen) followed by purification on RNaseasy kit (Qiagen, Valencia, CA). For isolated cells, total RNA was extracted using an RNasey kit. Total RNA was used to synthesize cDNA with oligo(dT)12–18 primers in the SuperScript First-Strand synthesis system (Invitrogen).

Primers and Standard Plasmid for Real Time PCR—Primers were designed from the LPLA2 (GenBank TM accession number AY490816), 1-Cys-peroxiredoxin 6 (Prdx6) (NM053576), and cytosolic calcium-independent phospholipase A2 (iPLA2) (NM_346803) gene sequences, respectively. The rat iPLA2 gene sequence was deduced from its amino acid sequence (P97570). The primer sets were as follows: LPLA2, 5′-AGAAGCACACAGGCTGGGC-3′ (forward) and 5′-AGAAAGCAGAC- CTTACATCTGGCCTGCCGCAA-3′ (reverse); Prdx6, 5′-CAGTGGTGGCACACAGAAGCTT- GAC-3′ (forward) and 5′-ACAGCTTCTTGGTCTGGGC-3′ (reverse); and iPLA2, 5′-ACTACATCGGCGCTGCGCAAA-3′ (forward) and 5′-AGAAAGCAGACAC- CTTACATCTGGCCTGCCGCAA-3′ (reverse). Standard plasmids were generated with the respective PCR products of LPLA2, Prdx6, and iPLA2 ligated into the pCR4-TOPO vector (Invitrogen) and followed by cloning, purification, quantification, and sequencing.

Preparation of the Soluble Fraction from Rat Alveolar and Peritoneal Macrophages, Peripheral Blood Mononuclear Cells, and Tissues.—For preparation of the soluble fractions of alveolar macrophages, peritoneal macrophages, and peripheral blood monocytes, the adherent cells on the culture dishes were washed three times with 8 ml of cold phosphate-buffered saline, scraped with a volume of phosphate-buffered saline, re-suspended into a 15-ml plastic tube. The cells were collected by centrifugation at 800 × g for 10 min at 4 °C, re-suspended with 0.4–1.0 ml of cold 0.25 M sucrose, 10 mM Hepes (pH 7.4), and 1 mM EDTA and disrupted by a probe-type sonicator for 10 × 5 s at 0 °C and centrifuged for 1 h at 100,000 × g at 4 °C. The supernatant was passed through a 0.2-μm filter and used as a soluble fraction.

For the preparation of rat tissue soluble fractions, each tissue was washed with cold phosphate-buffered saline, weighed, and homogenized by a Potter-Elvehjem-type homogenizer with cold 0.25 M sucrose, 10 mM Hepes (pH 7.4), and 1 mM EDTA to obtain 10% homogenate. The homogenate was centrifuged for 10 min at 600 × g at 4 °C. The resultant supernatant was sonicated by a probe-type sonicator for 10 × 5 s at 0 °C and centrifuged for 1 h at 100,000 × g at 4 °C. The supernatant was passed through a 0.2-μm filter and used as a soluble fraction.

For the preparation of the soluble fraction of mouse alveolar macrophages, the macrophages were collected from wild-type (C57BL/6), and GM-CSF(−/−) mice by whole lung lavage and pooled as described above. The cell pellets were washed three times with cold phosphate-buffered saline and resuspended in cold 0.25 M sucrose, 10 mM Hepes (pH 7.4), and 1 mM EDTA. The suspension was disrupted by a probe-type sonicator and followed the same procedure as described above.

Enzyme Assay (Transacylase Activity)—The phospholipids DOPC and phosphatidylethanolamine (PE) along with NAS were used in the assay system as donor and acceptor, respectively, of an acyl group. The transacylase activity was determined by analysis of the 1-O-acyl-N-acetylsphingosine formation rate. The reaction mixture consisted of 45 mM sodium citrate (pH 4.5), 10 μg/ml bovine serum albumin, 40 μM NAS incorporated into phospholipid liposomes (DOPC/PE/diethyl phos- phatidylcholine, 2:1:1 molar ratio), an equal soluble fraction (0.7–10 μg) in a total volume of 500 μl. The reaction was initiated by adding the soluble fraction, kept for 5–60 min at 37 °C, and terminated by adding 3 ml of chloroform/methanol (2:1) plus 0.3 ml of 0.9% (w/v) NaCl. The mixture was centrifuged for 5 min at room temperature. The resultant lower layer was transferred into another glass tube and dried down under a stream of nitrogen gas. The dried lipid, dissolved in 40 μl of chloroform/methanol (2:1), was applied on a high performance thin layer chromatography plate and developed in a solvent system consisting of chloroform/acetone (9:1). The plate was dried and soaked in 8% (w/v) CuSO4, SHO, 6.8% (w/v) H3PO4, and 32% (v/v) methanol. The uniformly white wet plate was then dried by a hair dryer and chlороform/methanol (1:1). The spot of 1-O-acyl-N-acetylsphingosine was visualized with an ultraviolet lamp (254 nm) and quantified using a NIH Image program version 1.62.

Dipalmitoylphosphatidylcholine Degradation Studies.—The reaction mixture consisted of 47 mM sodium citrate (pH 4.5), 10 μg/ml bovine serum albumin, liposomes (130 μM phospholipids), and 2.58 μg/ml of the soluble fraction of rat alveolar macrophages in a total volume of 500 μl. The liposomes consisted of dipalmitoylphosphatidylcholine, dioleylphosphatidylcholine, and dicetyl phosphate at a molar ratio of 50:50:16. Trace 1-palmitoyl-2-[1-C14]palmitoyl-t-3-phosphatidylcholine (2.5 × 108 cpm/assay) was added. The reaction was initiated by the addition of 15 μl of the soluble macrophage fraction or sucrose buffer (1 μg/ml) pretreated for 40, 80, and 120 min at 37 °C. The reaction was terminated by the addition of 3 ml of chloroform/methanol (2:1) plus 300 μl of 0.9% NaCl. The resultant lower layer was transferred into a small glass tube and dried down under a stream of nitrogen gas. The dried lipid was redissolved with chloroform/methanol (2:1) and applied to high performance thin layer chromatography plates. The free fatty acid and lysophosphatidylcholine were separated in solvent systems consisting of chloroform/methanol/acetic acid (98:2:1) and chloroform/methanol/water (60:35:8). The products were detected under ultraviolet light, sprayed, and transferred into scintillation vials. The silica gel was dispersed in 5 ml of ECOLOMGE (ICN Biomedicals) by a sonic sonicator. After sonication, it was transferred to a polyvinylidine difluoride membrane using the filter (20 μm Trisc and 150 mM glycine in 20% methanol) at a constant voltage of 100 volts for 3 h at 4 °C. The membrane was incubated with an anti-mouse LPLA2 peptide (100RTSRATQFPD-109) rabbit serum and monoclonal anti-c-Myc mouse ascites fluid. The anti-Myb antibody complex on the membrane was visualized with an anti-rabbit IgG horseradish peroxidase-conjugated goat antibody or an anti-mouse IgG horseradish peroxidase-conjugated goat antibody using diaminobenzidine and hydrogen peroxide.

RESULTS

A series of tissues from the Wistar rat were isolated and assayed for lysosomal phospholipase A2 activity (Fig. 1A). Because the lysosomal phospholipase A2 can transacylate ceramide at the 1-hydroxy position, the transacylase activity was determined as the formation of 1-O-acyl-N-acetylsphingosine (1-O-acyl-NAS). The enzyme activity was comparable in a wide range of tissues including brain, kidney, spleen, thymus, and lung. Of the tissues assayed, the specific activity was highest in thymus and spleen, suggesting that hematopoietic cells might...
be a source of higher enzyme activity. Pulmonary alveolar macrophages were studied next. When pulmonary alveolar macrophages were isolated by bronchoalveolar lavage and assayed for phospholipase A2 activity, a 40-fold higher activity of the lipase was observed compared with that of other tissues. This difference was not present in either peritoneal macrophages or peripheral blood monocytes.

A comparison of the enzyme activity between peritoneal macrophages, peripheral blood monocytes, and alveolar macrophages was made. The higher LPLA2 activity in alveolar macrophages compared with that in peritoneal macrophages and monocytes was evidenced by the formation of 1-O-acyl-NAS and free fatty acid (Fig. 1B). Because the initial velocity of the reaction in the alveolar macrophage was significantly greater than that observed in other cells and tissues, the reaction time was shortened to 5 min from 30 min, and the assay protein was lowered to 0.9 g from 10 g (Fig. 1C). Under these conditions, the phospholipase A2 activity was linear. The enzyme activities in the peritoneal macrophages and monocytes were slightly higher but comparable with those observed in the other tissues and significantly less than that measured in the alveolar macrophages. These data suggest that elevated lysosomal phospholipase A2 is a marker of the terminally differentiated alveolar macrophage.

We also evaluated the mRNA expression of LPLA2 in the tissues and macrophages using real time PCR (Fig. 1D). A good correspondence was observed in the mRNA levels normalized to total RNA and the transacylase activity. A comparison was also made between LPLA2 and another reported acidic phospholipase A2. This phospholipase, termed aiPLA2, was identified as the same protein as Prdx6, a non-selenium glutathione peroxidase (14, 15). The mRNA levels of Prdx6 were not significantly greater in the alveolar macrophage as compared with other tissues. Another macrophage-associated phospholipase A2 is the calcium-independent group VIA enzyme termed iPLA2 (16). mRNA levels of iPLA2 were also not significantly greater in the alveolar macrophage compared with other tissues.

We next sought to demonstrate that the high transacylase/
phospholipase A2 activity present in the alveolar macrophage was, in fact, LPLA2. A polyclonal antibody was raised to a peptide corresponding to the sequence 100RTSRATQFPD109 of the mouse LPLA2 protein. An immunoblot of the soluble protein fractions of rat peritoneal and alveolar macrophages was compared with that of c-Myc-tagged mouse LPLA2 expressed in COS-7 cells (mLPLA2) were separated by SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting with a rabbit polyclonal antibody raised to mouse LPLA2 peptide 100RTSRATQFPD109 and a monoclonal antibody to c-Myc. LPLA2 and c-Myc-tagged LPLA2 were detected as described under “Materials and Methods.” Std, standard. B, the densitometric profile of the alveolar macrophage (AM) and mouse LPLA2 (mLPLA2) lanes from panel A. The ratio of the alveolar macrophage band area to the mouse LPLA2 band area was 1:0.57. C, the transacylase activity of the soluble fraction of alveolar macrophage (AM) compared with that of mouse LPLA2 (mLPLA2). The ratio of the initial velocity of 1-O-acyl-N-AS formation in the alveolar macrophage soluble fraction to that of the mouse LPLA2 soluble fraction was 1:0.58.

Fig. 2. Expression of LPLA2 protein in rat macrophages. A, immunoblot analysis of the soluble fraction of alveolar macrophages and peritoneal macrophages. The soluble cell fraction (20 μg of total protein) obtained from alveolar macrophages (AM), peritoneal macrophages (PM), and c-Myc-tagged mouse LPLA2 over-expressed in COS7 cells (mLPLA2) were separated by SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting with a rabbit polyclonal antibody raised to mouse LPLA2 peptide 100RTSRATQFPD109 and a monoclonal antibody to c-Myc. LPLA2 and c-Myc-tagged LPLA2 were detected as described under “Materials and Methods.” Std, standard. B, the densitometric profile of the alveolar macrophage (AM) and mouse LPLA2 (mLPLA2) lanes from panel A. The ratio of the alveolar macrophage band area to the mouse LPLA2 band area was 1:0.57. C, the transacylase activity of the soluble fraction of alveolar macrophage (AM) compared with that of mouse LPLA2 (mLPLA2). The ratio of the initial velocity of 1-O-acyl-N-AS formation in the alveolar macrophage soluble fraction to that of the mouse LPLA2 soluble fraction was 1:0.58.

phospholipase A2 activity present in the alveolar macrophage was, in fact, LPLA2. A polyclonal antibody was raised to a peptide corresponding to the sequence 100RTSRATQFPD109 of the mouse LPLA2 protein. An immunoblot of the soluble protein fractions of rat peritoneal and alveolar macrophages was compared with that of c-Myc-tagged mouse LPLA2 expressed in COS-7 cells (Fig 2A). The immunoblot identified a major band in the alveolar macrophage protein fraction of the predicted molecular mass. No corresponding band was detected in the peritoneal macrophage fraction or when preimmune serum was used. The antibody recognized the c-Myc-tagged protein as well. The identity of the c-Myc-LPLA2 was confirmed with an anti-c-Myc antibody. Densitometric measurements of the detected bands demonstrated a ratio of 1:0.57 between the alveolar macrophage LPLA2 and the mouse LPLA2 (Fig. 2B). A comparison of reaction velocities was also made between the endogenous enzyme and the expressed LPLA2 (Fig. 2C). The ratio of the reaction velocities was 1:0.58. These data suggest that the transacylase/phospholipase A2 activity measured in the alveolar macrophage was due to LPLA2.

The transacylase and phospholipase A2 activities in the alveolar macrophage were further evaluated for their calcium dependence and pH optima (Fig. 3A). No phospholipase A2 or transacylase activities were observed at pH 7.4 in the presence or absence of calcium. The absence of activity persisted even when the reaction was followed for up to 60 min. At pH 4.5 the formation of both 1-O-acyl-N-acetylsphingosine and free fatty acid was observed.

The majority of pulmonary surfactant phospholipid is in the form of dipalmitoylphosphatidylcholine. Thus, it should be demonstrable that this disaturated lipid is a suitable substrate for LPLA2. Dipalmitoylphosphatidylcholine has a phase transition temperature of 41 °C. Therefore, liposomes containing dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and dicetyl phosphate were used (Fig. 3B). Under these conditions and in the absence of N-acetylsphingosine as an acceptor, the release of both palmitic acid and oleic acid was observed (Fig. 3C). To ascertain the relative contributions of phospholipase A1 and A2 activities in the degradation of the disaturated phosphatidylcholine, a liposomal mixture containing sn-2-labeled phosphatidylcholine was incubated with a soluble fraction obtained from the alveolar macrophages (Fig. 3D). In the absence of the soluble fraction, the spontaneous but significant release of radiolabeled palmitic acid and lysophosphatidylcholine occurred in a time-dependent manner (42 cpm/min and 26 cpm/min for palmitic acid and lysophosphatidylcholine, respectively). In the presence of the alveolar macrophage fraction, significant amounts of radioactive palmitic acid and lysophosphatidylcholine were produced. Because the dipalmitoylphosphatidylcholine was labeled at the C2 position, the
Radiolabeled lysophosphatidylcholine detected represented sn-2-lysophosphatidylcholine, the product of phospholipase A1 activity. Under these conditions, the rate of formation of sn-2-lysophosphatidylcholine was two times that of palmitic acid formation. Therefore, 33 and 66% of the palmitic acid released from dipalmitoylphosphatidylcholine in this assay system is due to the respective activities of phospholipase A2 and phospholipase A1.

In our original characterization of LPLA2 we noted that the enzyme was insensitive to the phospholipase A2 inhibitors bromoenol lactone and nonadecyltetraenyl trifluoromethyl ketone. However, MJ33, an inhibitor demonstrated to block surfactant phosphatidylcholine catabolism in vivo, was not evaluated. The LPLA2 activity was measured in the presence of this compound. A concentration-dependent inhibition of the transacylase activity was observed (Fig. 4). A comparable response was noted for the expressed c-Myc-tagged mouse LPLA2.

Pulmonary alveolar proteinosis is a disorder of the impaired catabolism of surfactant phospholipids and proteins. The GM-CSF null mouse was discovered to exhibit a phenotype consistent with this human disease. These mice display excess surfactant accumulation in the lungs associated with the engorgement of lipids within alveolar macrophages (10). This phenotype is reversed in bi-transgenic mice that are GM-CSF null but express GM-CSF under the control of the surfactant protein C promoter (11). LPLA2 activity was measured in the alveolar macrophages of C57BL/6 mice, GM-CSF null mice, and bi-transgenic mice that express GM-CSF under the control of the surfactant protein C promoter (Fig. 5).

LPLA2 levels in the bi-transgenic macrophages were slightly

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**Fig. 3. Characterization of alveolar macrophage LPLA2 activity.** A, effect of pH and calcium on the transacylase activity in rat alveolar macrophages. The soluble fraction (1.50 µg of protein/ml) of rat alveolar macrophages was assayed in 47 mM sodium citrate (pH 4.5) or in 150 mM NaCl and 10 mM Tris-HCl (pH 7.4) with 40 µM NAS at 37 °C for the indicated time periods. Under neutral conditions, the reaction mixture containing 1 mM EDTA or 1 mM CaCl2 was used for the assay. The reaction products were separated by thin layer chromatography as described under “Materials and Methods.” B and C, degradation of dipalmitoylphosphatidylcholine by rat alveolar macrophages. The soluble fraction (3.14 µg of protein/mg) of rat alveolar macrophages was incubated in citrate buffer, pH 4.5, with 130 µM phospholipid in liposomes consisting of DOPC/DPPC/dicetyl phosphate (the molar ratio of 3.07:3.07:1) at 37 °C for indicated time period. The released free fatty acids in the reaction were separated by a silver nitrate-impregnated high performance thin layer chromatography plate that was developed in a solvent system consisting of chloroform/acetic acid (95:5). In panel C the released free fatty acid by the soluble fraction was corrected by subtracting the fatty acid released in the absence of the soluble fraction at each time point and plotting against incubation time. D, metabolism of 1-palmitoyl-2-[1-14C]palmitoyl-L-3-phosphatidylcholine by rat alveolar macrophages. The liposomes consisted of dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, and dicetyl phosphate in a molar ratio of 50:50:16 with the addition of trace 1-palmitoyl-2-[1-14C]palmitoyl-L-3-phosphatidylcholine (2.55 × 105 cpm/assay). The reaction was initiated by the addition of 15 µl of the soluble macrophage fraction or sucrose buffer and incubated for 40, 80, and 120 min at 37 °C. The products were extracted, separated, and quantified as detailed under “Materials and Methods.” sn-2-Lyso-PC, sn-2-lysophosphatidylcholine.
rat alveolar macrophages and c-Myc-tagged mouse LPLA2-transfected COS 7 cells. The soluble fraction (2.42 μg of protein/ml) prepared from rat alveolar macrophage (AM) and the fraction (2.26 μg of protein per milliliter) from the LPLA2 (mLPLA2)-transfected cells were assayed in citrate buffer, pH 4.5, with 40 μMNAS in liposomal form with different concentrations of MJ33. The enzyme activity was determined as described under “Materials and Methods.” The enzyme activity in the absence of MJ33 was used as the control.

higher than in the wild-type mice.

We next attempted to determine whether GM-CSF alone was sufficient to result in LPLA2 expression in the alveolar macrophages from the null mice. GM-CSF−/− alveolar macrophages were isolated and cultured for up to 5 days in the presence or absence of GM-CSF (10 ng/ml). No change in LPLA2 activity was observed in the treated macrophages even when the reaction was followed for 60 min (Fig. 6).

DISCUSSION

There are three significant findings in the present study. First, LPLA2, an acidic phospholipase of unknown function, is highly expressed in alveolar macrophages. Although LPLA2 expression and activity is ubiquitously found in all tissues assayed, the mRNA levels and enzyme activity are robustly present in the pulmonary alveolar macrophage. The LPLA2 activity in peripheral blood monocytes and peritoneal macrophages is comparably low. These data are consistent with the interpretation that LPLA2 is a marker of the differentiated alveolar macrophage.

Second, LPLA2 recognizes disaturated phosphatidylycerolines as substrates. Dipalmitoylphosphatidylethanolamine is the major component of the pulmonary surfactant. Phosphatidylethanolamine and phosphatidylethanolamine account for 84.1 and 1.9%, respectively, of the surfactant lipid in the C57BL/6 mouse (17). Therefore, LPLA2 may potentially serve as the primary catalytic enzyme of surfactant phospholipids.

Third, LPLA2 activity is very low in alveolar macrophages from GM-CSF−/− mice. These mice have been characterized as a model of pulmonary alveolar proteinosis, a disorder of impaired surfactant catabolism. The activity is restored in GM-CSF−/− mice in which GM-CSF is expressed in type II alveolar epithelial cells under the control of the SP-C promoter. Thus LPLA2 may be a link between GM-CSF deficiency and impaired surfactant lipid degradation.

The enzyme or enzymes responsible for the catabolism of surfactant phospholipids have been the object of prior studies. These reports have suggested that degradation of dipalmitoylphosphatidylethanolamine occurs either through the activity of an undefined calcium-independent phospholipase A1 or through the activity of a calcium-independent phospholipase A2 that has optimal activity at an acidic pH (18). Our data indicate that both activities are present in the alveolar macrophage.

The existence of an acidic phospholipase A2 activity was first described over 25 years ago (19, 20). Such an activity was subsequently described in rabbit lung and in rat and human alveolar macrophages. Fisher and colleagues identified such an activity in lung secretory lamellar bodies and in lysosomes (21). They further reported that this activity was inhibited by the phospholipid transition state analogue MJ33 (6). In support of this observation, they demonstrated that MJ33 inhibited DPPC degradation by 50% in isolated perfused rat lungs. Using MJ33 they isolated a protein with a molecular mass of 15 kDa (21) and, subsequently, at 26 kDa (14) as the putative lysosomal phospholipase A2.

There are several reasons why aiPLA2 is unlikely to be the enzyme accounting for acidic phospholipase A2 activity that catalyzes the degradation of surfactant phospholipids. First, aiPLA2 has low specific activity, it is a cytosolic enzyme, and it lacks the mannose groups typically seen in lysosomally targeted proteins. The tissue distribution of aiPLA2 does not favor the lung, monocytes, or macrophages. For aiPLA2 to function as a phospholipase it would have to be a dual function enzyme with two separate catalytic domains, one for the lipase and one for glutathione peroxidase. Finally, gene targeting of aiPLA2 does not result in a pulmonary phenotype consistent with aberrant surfactant metabolism (22, 23).
clearly, GM-CSF is necessary but not sufficient to induce alveolar macrophage differentiation.

An additional unexplained finding was the presence of high cholesterol levels in the alveolar macrophages of the GM-CSF null mice. This observation does not appear to have been reported previously in the phenotypic characterization of these mice. Although the mechanism for this accumulation is not apparent, an association between GM-CSF and cholesterol metabolism has been reported previously (25).

This observation is also consistent with a recently reported study in which our group evaluated the role of agonists that induce macrophage differentiation on LPLA2 expression in THP-1 cells (26). In this study we observed that phorbol ester and all-trans retinoic acid induced LPLA2 transcription, but that GM-CSF was without effect. Sorting out those signals that regulate both alveolar macrophage differentiation and LPLA2 expression will require additional work.

Thus, we have demonstrated the robust expression of an acidic lysosomal phospholipase A2 within the alveolar macrophage, the primary site of surfactant degradation. The low expression and activity of this phospholipase A2 in a model of pulmonary alveolar proteinosis raises the possibility that this phospholipase may mediate human disorders associated with abnormal surfactant metabolism.

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