Pseudomonas aeruginosa and sPLA$_2$ IB stimulate ABCA1-mediated phospholipid efflux via ERK-activation of PPARα–RXR

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Bacterial infection triggers an acute inflammatory response that might alter phospholipid metabolism. We have investigated the acute-phase response of murine lung epithelia to *Pseudomonas aeruginosa* infection. *Ps. aeruginosa* triggered secretion of the pro-inflammatory lipase, sPLA$_2$ IB (phospholipase A$_2$ IB), from lung epithelium. *Ps. aeruginosa* and sPLA$_2$ IB each stimulated basolateral PtdCho (phosphatidylcholine) efflux in lung epithelial cells. Pre-treatment of cells with glyburide, an inhibitor of the lipid-export pump, ABCA1 (ATP-binding cassette transporter A1), attenuated *Ps. aeruginosa* and sPLA$_2$ IB stimulation of PtdCho efflux. Effects of *Ps. aeruginosa* and sPLA$_2$ IB were completely abolished in human Tangier disease fibroblasts, cells that harbour an *ABCA1* genetic defect. *Ps. aeruginosa* and sPLA$_2$ IB induced the heterodimeric receptors, PPARα (peroxisome-proliferator-activated receptor-α) and RXR (retinoid X receptor), factors known to modulate *ABCA1* gene expression. *Ps. aeruginosa* and sPLA$_2$ IB stimulation of PtdCho efflux was blocked with PD98059, a p44/42 kinase inhibitor. Transfection with MEK1 (mitogen-activated protein kinase/extracellular-signal-regulated kinase kinase 1), a kinase upstream of p44/42, increased PPARα and RXR expression co-ordinately with increased ABCA1 protein. These results suggest that pro-inflammatory effects of *Ps. aeruginosa* involve release of an sPLA$_2$ of epithelial origin that, in part, via distinct signalling molecules, transactivates the *ABCA1* gene, leading to export of phospholipid.

Key words: ATP-binding cassette transporter A1 (ABCA1), murine lung epithelium, phosphatidylcholine, *Pseudomonas aeruginosa*, secretory phospholipase A$_2$ IB (sPLA$_2$ IB), surfactant.

INTRODUCTION

The alveolar epithelium provides a generous host epithelial air–surface interface that is continuously exposed to pathogens and inhaled particles. *Pseudomonas aeruginosa* is a respiratory pathogen in humans that infects epithelial cells of the airways and distal lung that leads to severe parenchymal injury [1]. The pathogenesis of acute and chronic *Ps. aeruginosa* infections involves both bacterial and host factors. Bacterial factors include attachment of bacteria to epithelial cells via expression of polar pili and flagella, and injection of effector proteins [ExoS, ExoT, ExoU and ExoY (exotoxin S, T, U and Y respectively)] elaborated by the type III secretory system [2,3]. These bacterial factors are responsible for triggering inflammatory responses, including secretion of pro-inflammatory cytokines that, via binding to specific receptors, activate signal transduction pathways [4–7]. One bacterial virulence factor, ExoU from *Ps. aeruginosa*, acts as a phospholipase as cytotoxicity of this toxin is blocked by inhibitors of cPLA$_2$ and iPLA$_2$ [cytosolic and Ca^{2+}-independent PLA$_2$ (phospholipase A$_2$) respectively] [8].

Ps. aeruginosa also increases sPLA$_2$ (secretory PLA$_2$) activity in bronchoalveolar lavage fluid and lung tissue [9]. sPLA$_2$ may cause alveolar epithelial cell damage and pulmonary surfactant dysfunction, and enhance production of mediators that induce tissue injury [10]. Thus highly virulent bacteria such as *Ps. aeruginosa* can compromise lung function by increasing the hydrolysis of phospholipids that serve as integral components of cell membranes or surfactant. The molecular mechanisms by which *Ps. aeruginosa* might alter phospholipid metabolism in host cells, however, remain largely unknown.

PLA$_2$ catalyses phospholipid hydrolysis at the *sn*-2 position of the glycerol backbone, with production of free fatty acids and lysophospholipids [11–13]. The superfamily of PLA$_2$s consists of two major classes, including low-molecular-mass secreted forms (sPLA$_2$s), containing ten groups, and high-molecular-mass cytosolic forms (cPLA$_2$s), containing three enzymes [14]. Both sPLA$_2$ and cPLA$_2$ initiate the production of bioactive lipid mediators, but their relative inflammatory potency is tissue- and cell-type-dependent [15]. sPLA$_2$s, in particular, play an important role in both host defence and induction of inflammatory reactions via generation of lipid mediators and pro-inflammatory cytokines in acute lung injury [9,12]. High levels of sPLA$_2$ IB and sPLA$_2$ IIA are observed in patients with the acute respiratory distress syndrome and other inflammatory disorders [16]. Aside from their enzymatic behaviour, sPLA$_2$s also trigger diverse biological responses via binding to specific receptors (M- or N-type) with different affinities [11]. Both sPLA$_2$ IB and sPLA$_2$ IIA act as high-affinity ligands to mouse M-type receptors, thereby activating signal transduction pathways such as MAPKs (mitogen-activated protein kinases) [17]. Both sPLA$_2$ IB and sPLA$_2$ IIA stimulate sPLA$_2$ IIA release from mesanglial cells, activate cPLA$_2$ and induce expression of the PPARα (peroxisome-proliferator-activated receptor α) independently of their enzymatic activity via interaction with M-type receptors or heparin sulfate proteoglycans

Abbreviations used: ABCA1, ATP-binding cassette transporter A1; apoAI/AII, apolipoprotein AI/AII; cfu, colony-forming units; DMEM, Dulbecco's minimal Eagle's medium; DPPC, dipalmitoyl phosphatidylcholine; DR, direct repeat; ERK, extracellular-signal-regulated kinase; Exo, exotoxin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL, high-density lipoprotein; HSF, human skin fibroblast; LPS, lipopolysaccharide; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MLE-12 cell, murine lung epithelium cell; MOI, multiplicity of infection; PLA$_2$, phospholipase A$_2$; cPLA$_2$, cytosolic PLA$_2$; sPLA$_2$, secretory PLA$_2$; PPARα, peroxisome-proliferator-activated receptor-α; PtdCho, phosphatidylcholine; RXR, retinoic X receptor; SM, sphingomyelin; TCA, trichloroacetic acid; VBM, Vogel–Bonnel medium.

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[18–20]. These observations indicate that $sPLA_2$s exhibit both catalytic and non-catalytic receptor-dependent properties that modulate cellular responses.

PPARα is a nuclear receptor that functions as a key ligand-activated transcription factor that regulates genes involved in lipid metabolism. PPARα regulates these genes via heterodimerization with obligate partners, such as the RXR (retinoid X receptor) and LXR (liver X receptor), and subsequent binding to PPREs (PPAR-responsive elements) within target promoters [21–23]. PPARα regulates synthesis of apoAI (apolipoprotein AI) and apoAII (apolipoprotein AII) that participate in HDL (high-density lipoprotein) particle formation. PPARα and LXR heterodimerization also enhances expression of ABCAI (ATP-binding cassette transporter AI), a lipid pump that is involved in HDL synthesis that transports cellular phospholipids and cholesterol to apoAI [24–27]. Previous studies by us and others indicate that the ABCA1 transporter might play an important role in regulating lung phospholipid homoeostasis [28–30]. Mice harbouring targeted deletion of *ABCA1* exhibit respiratory distress and show abnormalities of surfactant phospholipid metabolism [30,31]. Furthermore, ABCA1 activation decreases surfactant PtdCho (phosphatidylcholine) levels, in part, via basolateral export of this phospholipid in alveolar type II epithelial cells [10,28]. These observations, together with prior studies showing that *Ps. aeruginosa* decreases PtdCho levels and increases lung phospholipase activity, led us to hypothesize that this bacterial pathogen might activate ABCA1 via distinct signalling mechanisms involving $sPLA_2$. Thus we tested effects of *Ps. aeruginosa* and $sPLA_2$ on ABCA1-mediated phospholipid export. Our results show that *Ps. aeruginosa* exploits an ABCA1 export pathway for PtdCho that appears to be driven by autocrine secretion of $sPLA_2$ in murine lung epithelia.

MATERIALS AND METHODS

Materials

Bovine pancreas $sPLA_2$ IB was purchased from Sigma Chemicals. *Ps. aeruginosa*, strain PA103, was obtained from Dr Timothy Yahr (University of Iowa, Iowa City, IA, U.S.A.). MLE-12 (murine lung epithelial) cells, H441 and HepG2 cell lines were obtained from A.T.C.C. (Manassas, VA, U.S.A.), and CaCo-2 cells were a gift from Dr E. Schaefer and Dr J. Ordovas (Tufts University School of Medicine, New England Medical Center, Boston, MA, U.S.A.). Culture medium was from the University of Iowa Tissue Culture and Hybridoma Facility. Immortalized HSFs (human skin fibroblasts) obtained from normal (EB86-282t) and Tangier disease patients (JKWt) were generously provided by Dr John Oram (Department of Medicine, University of Washington, Seattle, WA, U.S.A.). Glyburide was obtained from Calbiochem. MK 886 and MJ33 inhibitors were from Sigma Chemicals. Transwell plates with polyester membranes (0.4 μm pore size, 24 mm diameter) were obtained from Corning. The ECL® (enhanced chemiluminescence) Western blotting detection reagents were from Pierce Biotechnology and Amersham Biosciences. The rabbit polyclonal anti-ABCA1 antibody was purchased from Novus Biologicals. Anti-(phospho p44/p42) and anti-(total p44/p42) antibodies were from Cell Signaling Technologies. Anti-($sPLA_2$ IIA), anti-($sPLA_2$ X), anti-($sPLA_2$ V) and anti-PPARα antibodies were from Cayman Chemicals. Anti-($sPLA_2$ IB) antibodies were obtained from Upstate Biotechnology. Anti-RXR and anti-LXR antibodies were from Santa Cruz Biotechnology. Reverse-transcription reagents and SYBR Green master mix were from Invitrogen and Applied Biosystems respectively. HDL was obtained from Intracell, and Apo-AI was from Sigma Chemicals. The FuGENE6 transfection reagent was purchased from Roche Diagnostics. *Salmonella enterica* serovar Typhi and *Escherichia coli* LPS (lipopolysaccharide) were obtained from Sigma Chemicals. [^{3}H]Cholesterol (48.3 Ci/mmol) was purchased from PerkinElmer Life Sciences. [*methyl*-^{3}H]Choline chloride was obtained from American Radiolabeled Chemicals. For animal studies, 6–8 week-old male C57BL/6J mice (20–25 g) were obtained from Jackson Laboratories. All experimental procedures involving mice were performed in accordance with the protocols approved by the University of Iowa Animal Care and Use Committee.

Cell culture

MLE-12 cells were maintained in Hite's medium with 2 % (v/v) FBS (fetal bovine serum) at 37 °C in atmosphere containing 5 % CO_2. After reaching 80–100 % confluence, the cells were harvested using 0.25 % trypsin with 0.1 % EDTA and plated on to six-well, 12-well or 60-mm tissue culture dishes or on transwell dishes to study basolateral efflux [28]. After incubation overnight, the medium was changed to serum-free Hite's medium for $\sim$2 h, and then changed again to fresh serum-free medium. Cells were incubated with this serum-free medium alone (control medium) or in combination with various amounts of *Ps. aeruginosa* or $sPLA_2$ IB for various times. HSFs were grown in DMEM (Dulbecco's minimal Eagle's medium) with 10 % (v/v) FBS at 37 °C containing 5 % CO_2. HSF cells were plated on six-well plates and were grown to 70 % confluence. Subconfluent cells were exposed to *Ps. aeruginosa* [MOI (multiplicity of infection) of 5] or $sPLA_2$ IB (100 μg/ml).

CaCo-2 cells, a colonic cancer cell line, were cultured in T-75 flasks in DMEM with 10 % (v/v) FBS. Once the flasks reached 80 % confluence, the cells were split and plated at a density of 0.2×10^5 cells/well on polycarbonate micropore membranes (0.4 μm pore size, 24 mm diameter) inserted into transwells and cultured for 14 days to obtain a confluent monolayer of differentiated cells. The human hepatocellular liver carcinoma cell line (HepG2) was maintained in MEM (minimum essential medium) with Earle's BSS (balanced salt solution) and 10 % (v/v) FBS at 37 °C in atmosphere containing 5 % CO_2. H441 cells, a human lung cell line with characteristics of Clara cells, were maintained in RPMI 1640 medium with 5 % FBS at 37 °C in atmosphere containing 5 % CO_2.

Preparation of *Ps. aeruginosa*

Ps. aeruginosa (PA103) was maintained in VBM (Vogel–Bonnel medium). Bacteria originating from frozen stocks were inoculated on VBM plates overnight. The next day, colonies were scraped and grown by rotary shaking at 37 °C to reach exponential growth phase ($D_{540} = 0.65$–0.7) in tryptic soy broth supplemented with 100 mM monosodium glutamate and 1 % glycerol. Infection of cells was performed with an MOI of 5, an optimal PA103 concentration for cells as described previously [32].

In vivo infection with *Ps. aeruginosa*

Six-week-old male C57BL/6J mice weighing 20–25 g were deeply anaesthetized intraperitoneally with ketamine (60–100 mg/kg) and xylazine (10 mg/kg). Mice were then intratracheally injected with control agar particles or bacteria agar particles [10^4–10^5 cfu (colony-forming units) in 50 μl of control agar particles]. After 1 h, mice were killed with an intraperitoneal injection of pentobarbital (150 mg/kg), the lungs were lavaged, and surfactant pellets were isolated as described in [32]. Total RNA and lung homogenates were also harvested. Primary alveolar type II epithelial cells were isolated as described in [32].

Phospholipid efflux

Confluent monolayers on transwells were pulsed with 2 μCi of [*methyl*-^{3}H]choline chloride or 2.5 μCi of [*methyl*-^{3}H]cholesterol at the apical surface for 18–24 h, washed three times, and exposed apically to *Ps. aeruginosa*, $sPLA_2$ IB or LPS from *E. coli* or *Salmonella* Typhi in the presence of 20 μg/ml HDL or 10 μg/ml ApoAI acceptor. Lipids were extracted from basolateral and apical medium and analysed for PtdCho, SM (sphingomyelin) or lyso-PtdCho efflux using TLC in chloroform/methanol/light petroleum/ethanoic (acetic) acid/boric acid (40:20:30:10:1.8, by vol.) and liquid-scintillation counting [33]. For inhibitor studies, after pulsing with [*methyl*-^{3}H]choline chloride, cells were pre-incubated apically with glyburide (250 μM) for 1 h and then exposed to *Ps. aeruginosa* or $sPLA_2$ IB with HDL added to apical and basolateral medium. For determination of PtdCho efflux in HSFs, the subconfluent cells were labelled with 2 μCi of [*methyl*-^{3}H]choline chloride for 18–20 h, and unincorporated radiolabel was removed by washing cells three times before exposure to *Ps. aeruginosa* or $sPLA_2$ IB with Apo-AI (10 μg/ml) for 2 h. PtdCho efflux values were expressed as a percentage of total radiolabelled PtdCho recovered in the cells, basolateral and apical medium.

PtdCho and DPPC (dipalmitoyl phosphatidylcholine) analysis

PtdCho biosynthesis was measured as the rate of incorporation of [*methyl*-^{3}H]choline chloride into PtdCho. Cells were pulsed for the last 2 h of incubation with 2 μCi of [*methyl*-^{3}H]choline chloride, lipids were extracted and resolved by TLC, and PtdCho or DPPC was quantified by TLC scanning or liquid-scintillation counting [28]. Levels of DPPC mass was measured using a phosphorus assay as described [28].

Immunoblot analysis

Equal amounts of proteins in cell lysates were separated on 10 % polyacrylamide gels and probed with various antibodies [against ABCA1, $sPLA_2$, PPARα, RXR, LXR, ERK1/2 (extracellular-signal-regulated kinase 1/2) and β-actin] as described in [28]. Each antibody was used at a concentration as recommended by the manufacturer. For secreted proteins, 5 ml of the culture medium was precipitated with 60 % TCA (trichloroacetic acid) and 1.5 % sodium desoxycholate, and immunoblots were probed using a polyclonal antibody against $sPLA_2$ IB or $sPLA_2$ IIA.

Real-time PCR analysis

Total cellular RNA was isolated using Tri-Reagent. ABCA1 transcripts were detected by real-time PCR using mouse ABCA1 primers and rodent GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as an internal control as described in [28]. ABCA1 transcript detection primers were 5′-ACTAGTGCCAAGTTGCTCAG-3′ (forward) and 5′-TAAGTCCCTGCCCTTTGTACAAC-3′ (reverse). ABCG1 primers were 5′-GGGATCAGAACAGTCGCCTG-3′ (forward) and 5′-CGAGGTCTCTCTTATAGTCAGCGTC-3′ (reverse). ABCG5 primers were 5′-TGATTGGCAGCTATAATTTTGGG-3′ (forward) and 5′-GTTGGGCTGCGATGGAAA-3′.

Construction of *ABCA1* promoter-reporter plasmids

Human *ABCA1* promoter fragments were generated using human genomic DNA (GenBank® accession number AF287262) as a template by PCR with Pfu Turbo DNA polymerase (Stratagene). Forward and reverse primers contained restriction sites for EcoRV and HindIII respectively (see below): −949 forward, 5′-cggatatcAAAGGCAAACAGAGAAGTTG-3′, −205 forward, 5′-cggatatcTCCCTAGATGTGTCGTGG-3′, −77 forward, 5′-cggatatcACAGGCTTTGACCGATAG-3′, −49 forward, 5′-ggatatcGCTCGGTGCAGCCGAATC-3′, +274 reverse, 5′-gtacaagcttTGGCCTCGAAACAAAACAATAACG-3′, −77mut forward, 5′-AGGGAGAGCACAGGCTTT**A**AA**T**GATAGTA-**GTA**TCTGCGCTCGGTGCAGCCG-3′ and −77mut reverse, 5′-CGGCTGCACCGAGCGCAGA**TAC**TACTATC**ATTT**AAAGC-CTGTGCTCTCCCT-3′. Lower-case letters indicate added restriction sites, underlined letters indicate DR-4 (direct repeat 4) site and bold letters indicate mutated nucleotides.

PCR products were resolved on agarose gels, purified, and directionally cloned into pGL4-basic (Promega). The constructs were verified by restriction enzyme digestion and sequencing. A DR-4 mutant was prepared using QuikChange Site-Directed Mutagenesis kit (Stratagene) according to the manufacturer's instructions (see primers above) and verified by DNA sequencing.

Transfectional analysis

For analysis of *ABCA1* promoter activity, MLE-12 cells were plated into 12-well tissue culture dishes and allowed to reach ~50–60 % confluence. The medium was switched to serum-free conditions, and transient transfection was performed with 0.75 μg/well pGL4-ABCA1 plasmids in FuGENE6 transfection reagent overnight. Co-transfection with 0.25 μg/well pSV-β-galactosidase was used to control for transfection efficiency. Cells were lysed with reporter lysis buffer for analysis of luciferase and β-galactosidase activities. For MEK1 (MAPK/ERK kinase 1) effects, cells were transfected with MEK1 plasmid (4 μg/60-mm dish) overnight. The next day, cells lysates were processed for immunoblot analysis.

Statistical analysis

Statistical analysis was performed using Student's *t* test [34]. Results are presented as means ± S.E.M. for three to six independent experiments.

RESULTS

Ps. aeruginosa induces PtdCho efflux in lung epithelia

MLE-12 cells were cultured in plastic dishes or in transwell dishes to assess phospholipid efflux (Figure 1). After exposure to *Ps. aeruginosa* (MOI = 5) or $sPLA_2$ IB (100 μg/ml), medium was harvested, and lipids were extracted and processed for PtdCho efflux. *Ps. aeruginosa* triggered a 4–5-fold increase in [*methyl*-^{3}H]PtdCho efflux on plastic dishes (Figure 1A) and produced a 2-fold induction of basolateral PtdCho efflux in transwell dishes at 2 and 4 h compared with control (Figure 1B). Similarly to effects of the bacteria, exogenous $sPLA_2$ IB produced a ~2-fold increase in PtdCho export (Figures 1C and 1D) and yet inhibited apical PtdCho secretion (Figure 1E). Of note, the kinetics of increased basal secretion of phospholipid by $sPLA_2$ preceded inhibition of apical export, suggesting that these routes for lipid export are linked. Lastly, in preliminary results, these effects on export by $sPLA_2$ IB were also associated with a ~60 % decrease in choline incorporation into PtdCho, suggesting that phospholipid synthesis is also reduced (results not shown).

To determine the specificity of lipid efflux, MLE-12 cells were cultured in transwells, labelled with [^{3}H]cholesterol, and subsequently exposed to *Ps. aeruginosa* (MOI = 5) or $sPLA_2$ IB (100 μg/ml) (Figure 2). The results demonstrate that there was no significant induction of cholesterol efflux by *Ps. aeruginosa* and a tendency for down-regulation of cholesterol efflux by $sPLA_2$ IB (Figure 2A). We next assessed the specificity of responses

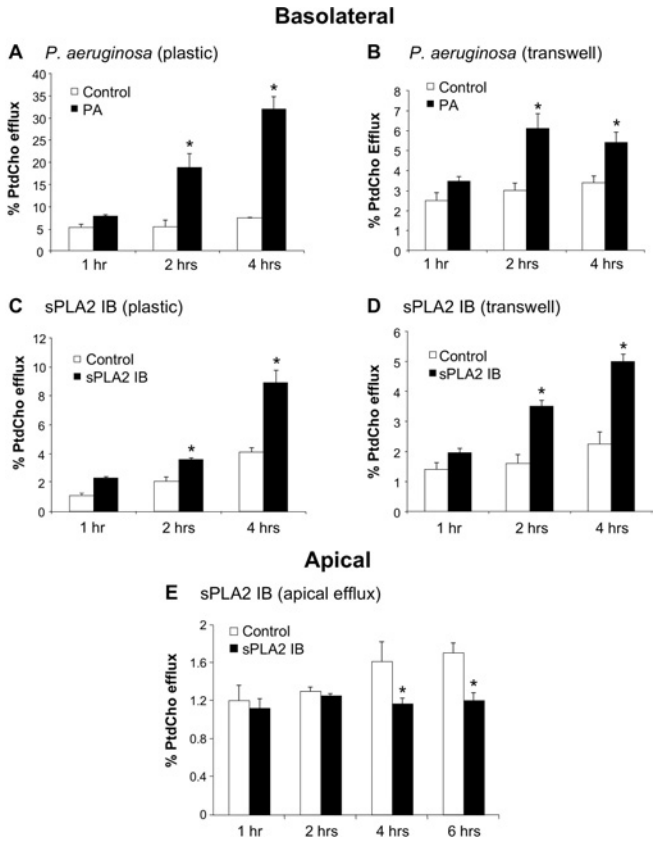

Figure 1 ***Ps. aeruginosa*** **and sPLA$_2$ IB induce PtdCho efflux in lung epithelia**

Effect of *Ps. aeruginosa* (PA) on basolateral PtdCho efflux was assayed in MLE-12 cells plated in 60 mm plastic and transwell dishes, labelled with [*methyl*-^{3}H]choline and in the presence of 20 μg/ml HDL as a PtdCho acceptor. Cells were exposed to *Ps. aeruginosa* (MOI = 5) for various times (**A**, **B**). Effect of 100 μg/ml sPLA$_2$ IB on basolateral (**C**, **D**) and apical (**E**) efflux was measured under similar conditions. The cells and basolateral and apical medium were harvested separately after exposure to *Ps. aeruginosa* and sPLA$_2$ IB and processed for PtdCho analysis. $^*P < 0.05$ compared with control. Results are means $\pm$ S.E.M. for three to five independent experiments.

to *Ps. aeruginosa* in other epithelia and to other phospholipids (Figures 2B and 2C). Significant effects on PtdCho efflux were observed in MLE-12 cells and HepG2 cells, the latter only after prolonged exposure to the pathogen (Figure 2B). *Ps. aeruginosa* and sPLA$_2$ IB did not trigger export of SM or lyso-PtdCho (Figure 2C). To investigate whether effects were unique to *Ps. aeruginosa*, LPS from other Gram-negative bacteria were tested. Indeed, *E. coli* did not induce PtdCho efflux from cells, and *Salmonella* Typhi just slightly induced PtdCho efflux (Figure 2D). Thus *Ps. aeruginosa* and sPLA$_2$ IB selectively stimulate export of the major phospholipid in lung epithelia. Bacterial effects on PtdCho export, however, were not restricted to MLE-12 cells.

Ps. aeruginosa and sPLA$_2$ IB up-regulate ABCA1 expression in lung epithelia

Our previous studies demonstrated that the lipid pump, ABCA1, serves as a basolateral PtdCho exporter in lung epithelial cells [28]. To examine whether *Ps. aeruginosa* increases PtdCho efflux by modulating ABCA1, we first investigated immunoreactive levels of ABCA1 after bacterial infection. For these experiments, we used a commercially available anti-ABCA1 antibody that cross-reacts with murine ABCA1 with a predicted molecular mass of $\sim$200 kDa. Immunoblotting revealed that *Ps. aeruginosa* increased ABCA1 protein levels (Figure 3A). Next, changes in ABCA1 were correlated with levels of steady-state ABCA1 transcripts after *Ps. aeruginosa* infection (Figure 3B). Quantitative PCR analysis revealed that *Ps. aeruginosa* increased expression of ABCA1 transcripts ($\sim$1.5–2.5-fold) (Figure 3B). Of note, changes in ABCA1 mass and mRNA in response to bacterial infection temporally preceded export of PtdCho from cells. These data suggest that *Ps. aeruginosa* transcriptionally activates the *ABCA1* gene and that this transporter might mediate functional effects of this pathogen on phospholipid export.

To determine whether effects of *Ps. aeruginosa* are recapitulated with another pro-inflammatory molecule activated by the pathogen, cells were incubated with sPLA$_2$ IB, and then cellular lysates were processed for ABCA1 immunoblotting and mRNA analysis (Figures 3C–3D). Immunoblotting demonstrated induction of ABCA1 levels by sPLA$_2$ IB over a 1–4 h period of analysis (Figure 3C). The phospholipase also increased *ABCA1* mRNA levels in murine lung epithelium (Figure 3D). In addition to ABCA1, other ATP-binding cassette transporters might mediate cellular efflux of phospholipids. Quantitative PCR revealed that *Ps. aeruginosa* stimulated expression of ABCA1 transcripts, but not ABCG1 and ABCG5 (Figure 3E).

To examine effects of *Ps. aeruginosa in vivo*, mice were infected with PA103. After 1 h, levels of DPPC, the major surface-active phospholipid in lavage, decreased substantially compared with the control (Figure 4A). Immunoblot analysis and quantitative PCR demonstrated induction of ABCA1 protein and mRNA levels in lung (but not liver) with PA103 infection that varied as a function of inoculum size (Figures 4B and 4C). PtdCho efflux was also determined in freshly isolated type II epithelial cells after *Ps. aeruginosa* (MOI = 5) infection. Indeed, PA103 increased PtdCho efflux in these cells, an effect attenuated using glyburide, an ABCA1 inhibitor (Figure 4D). Thus *Ps. aeruginosa* effects on PtdCho efflux *in vitro* are also observed *in vivo* and in primary murine lung epithelial cells.

To evaluate further a mechanistic role for ABCA1 in mediating functional responses to *Ps. aeruginosa* and sPLA$_2$ IB, we used HSFs obtained from normal and Tangier disease patients that express a defective *ABCA1* gene, and glyburide (Figure 5). Indeed, *Ps. aeruginosa* and sPLA$_2$ IB stimulation of PtdCho efflux that was detected in HSFs was not observed in Tangier disease cells (Figures 5A and 5C). Furthermore, stimulation of cellular basolateral transport of the phospholipid by *Ps. aeruginosa* and sPLA$_2$ IB in MLE-12 cells was blocked with glyburide (Figures 5B and 5D). Thus these studies indicate that effects of *Ps. aeruginosa* and sPLA$_2$ IB on PtdCho efflux in lung epithelia are mediated, in part, by ABCA1.

Ps. aeruginosa and sPLA$_2$ IB stimulate phospholipase secretion from lung epithelial cells

Ps. aeruginosa and sPLA$_2$ IB each triggered release of secretory phospholipases from lung epithelia into the culture medium (Figure 6). First, to determine whether these phospholipases were released from *Ps. aeruginosa* or host cells, we probed for sPLA$_2$ IB in medium cultured with bacteria in the presence or absence of lung epithelial cells. As shown in Figure 6A (lanes 1–3), sPLA$_2$ IB was not detected in bacterially infected culture medium devoid of lung epithelial cells; sPLA$_2$ IB was detected, however, when bacteria were co-cultured with cells, indicating that the origin of the sPLA$_2$ IB was likely to be derived from epithelial cells. This was observed regardless of whether bacteria were incubated with MLE-12 cells or primary murine type II cells that synthesize surfactant (Figures 6B and 6C).

Ps. aeruginosa and exogenously applied sPLA$_2$ IB also produced a robust increase in secretion of another related

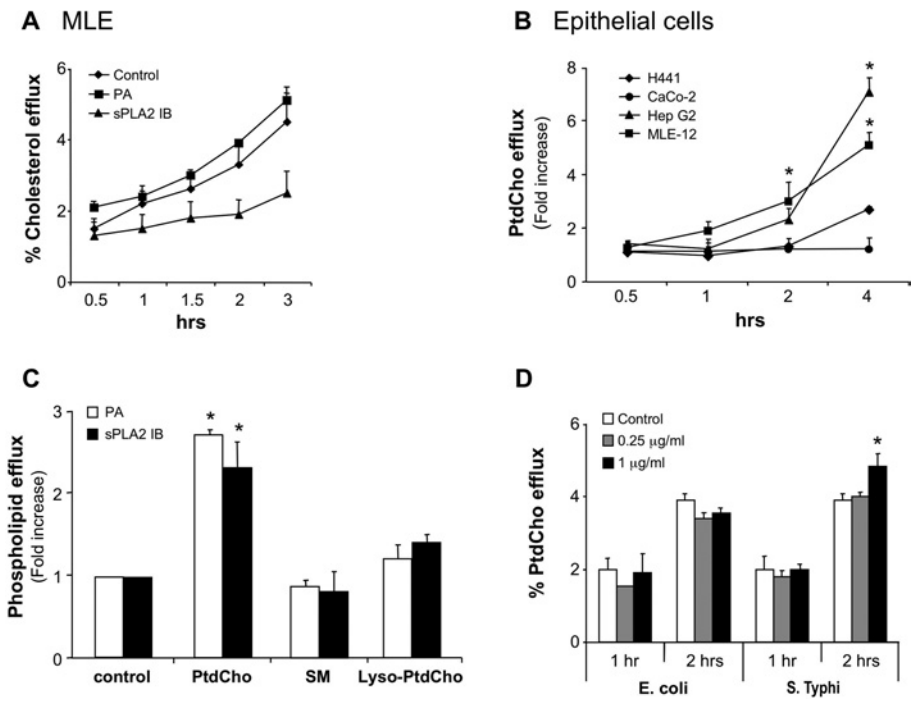

Figure 2 ***Ps. aeruginosa*** **triggers phospholipid efflux selectively**

(**A**) Effect of *Ps. aeruginosa* (PA) and $sPLA_2$ IB on cholesterol efflux in MLE-12 cells plated in transwells and labelled with [^{3}H]cholesterol. Cells were exposed to *Ps. aeruginosa* (MOI = 5) and $sPLA_2$ IB (100 μg/ml) for various times after labelling and processed for cholesterol efflux. (**B**) Effect of *Ps. aeruginosa* on PtdCho efflux in different types of epithelial cells. (**C**) Effect of *Ps. aeruginosa* (PA) and $sPLA_2$ IB on efflux of SM and lyso-PtdCho in MLE-12 cells after 2 h of exposure to agonists. (**D**) Effect of LPS from *E. coli* and *Salmonella* Typhi (S. Typhi) on PtdCho efflux in MLE-12 cells. $^*P < 0.05$ compared with control. Results are means $\pm$ S.E.M. for three independent experiments.

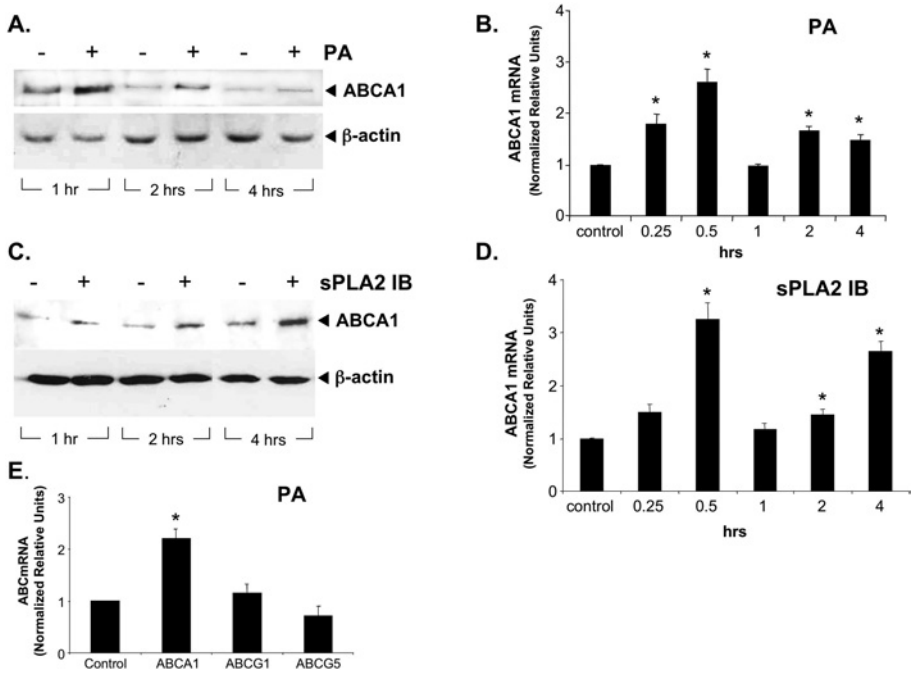

Figure 3 ***Ps. aeruginosa*** **and $sPLA_2$ IB stimulate expression of ABCA1 in lung epithelial cells**

MLE-12 cells were incubated with (+) or without (−) *Ps. aeruginosa* (MOI = 5) (PA) (**A**, **B**) or $sPLA_2$ (100 μg/ml) (**C**, **D**) for various times. (**A**, **C**) ABCA1 and β-actin levels were determined by immunoblotting using ~100 μg of cell lysate protein, resolution by SDS/10 % PAGE and probing with anti-ABCA1 or β-actin polyclonal antibodies. (**B**, **D**) *Ps. aeruginosa* and $sPLA_2$ induce *ABCA1* mRNA levels in MLE-12 cells. Cultured cells were infected with or without *Ps. aeruginosa* for various times or cultured with $sPLA_2$, and total cellular RNA was harvested for analysis of *ABCA1* mRNA by real-time PCR. (**E**) MLE-12 cells were exposed to *Ps. aeruginosa* (MOI = 5) (PA) for 2 h, and total cellular RNA was harvested for analysis of *ABCG1* and *ABCG5* mRNA by real-time PCR. Results are mean $\pm$ S.E.M. relative units, which were normalized to murine GAPDH, for three independent experiments. $^*P < 0.05$ compared with control.

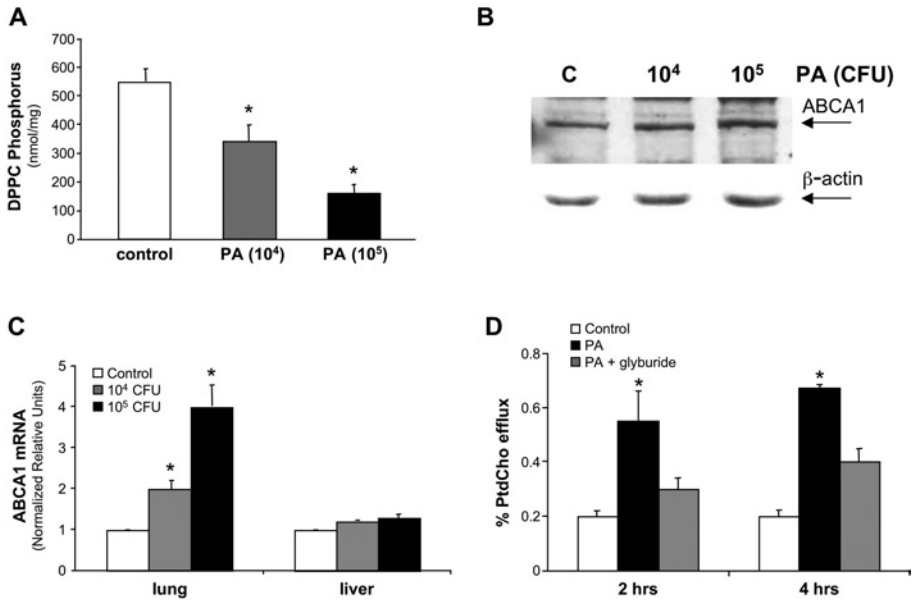

Figure 4 ***Ps. aeruginosa*** **regulates PtdCho export** ***in vivo***

C57BL/6J mice were intratracheally administrated with *Ps. aeruginosa* strain PA103 (PA) using either control agar particles or bacterial agar particles (10^4–10^5 cfu). After 1 h, mice were killed, the lungs were lavaged, and surfactant pellets were isolated. (**A**) Surfactant pellets were analysed for DPPC levels, expressed as nmol of lipid phosphorus/mg of protein. (**B**) The lungs were processed for ABCA1 and β-actin levels by immunoblot analysis. (**C**) Total mRNA was harvested from lung and liver for analysis of *ABCA1* mRNA by real-time PCR. (**D**) Primary mouse type II cells were isolated and labelled with [*methyl*-^{3}H]choline for 20 h, exposed to glyburide (250 μM) for 1 h, and then incubated with or without PA103 (MOI = 5) in the presence of HDL (20 μg/ml). Medium and cells were harvested for PtdCho analysis. $^*P < 0.05$ compared with control. Results are means $\pm$ S.E.M. for three independent experiments.

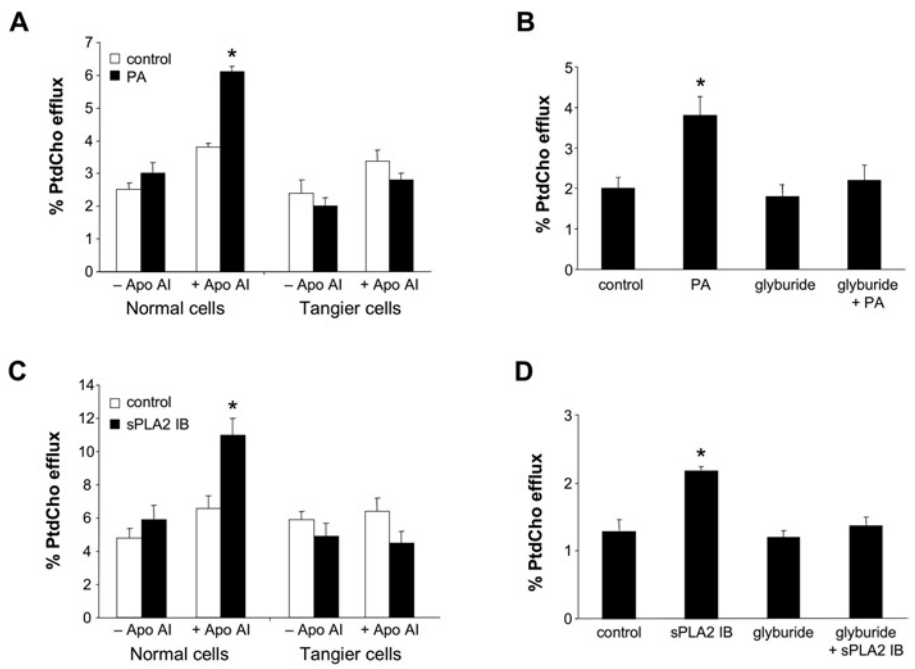

Figure 5 ***Ps. aeruginosa*** **and sPLA$_2$ IB regulate PtdCho export via ABCA1 in lung epithelia**

(**A**, **C**) Immortalized HSFs obtained from normal and Tangier disease patients were plated on plastic dishes, labelled with [*methyl*-^{3}H]choline for 20 h, and then incubated with or without *Ps. aeruginosa* (MOI = 5) (PA) (**A**) or sPLA IB (100 μg/ml) (**C**) using apoAI (10 μg/ml) as a PtdCho acceptor. Medium and cells were harvested after 2 h of exposure to *Ps. aeruginosa* or sPLA$_2$ IB and processed for PtdCho analysis. (**B**, **D**) Effect of pharmacological ABCA1 inhibition on *Ps. aeruginosa* (**B**) or sPLA$_2$ IB (**D**) -induced basolateral efflux was assayed in transwell dishes. Cells were labelled with [*methyl*-^{3}H]choline for 20 h, exposed to glyburide (250 μM) and then incubated with or without *Ps. aeruginosa* (MOI = 5) or sPLA$_2$ IB (100 μg/ml) for 2 h in the presence of HDL (20 μg/ml). Medium and cells were harvested for PtdCho analysis. $^*P < 0.05$ compared with control. Results are means $\pm$ S.E.M. for three to four independent experiments.

phospholipase, sPLA$_2$ IIA, rapidly within a few minutes and the effect persisted for up to 2–4 h (Figures 6D–6G). In contrast, other sPLA$_2$ isoforms previously identified within type II alveolar cells, including sPLA$_2$ X and sPLA$_2$ V, were not secreted into the medium after agonist stimulation (results not shown) [11,35,36].

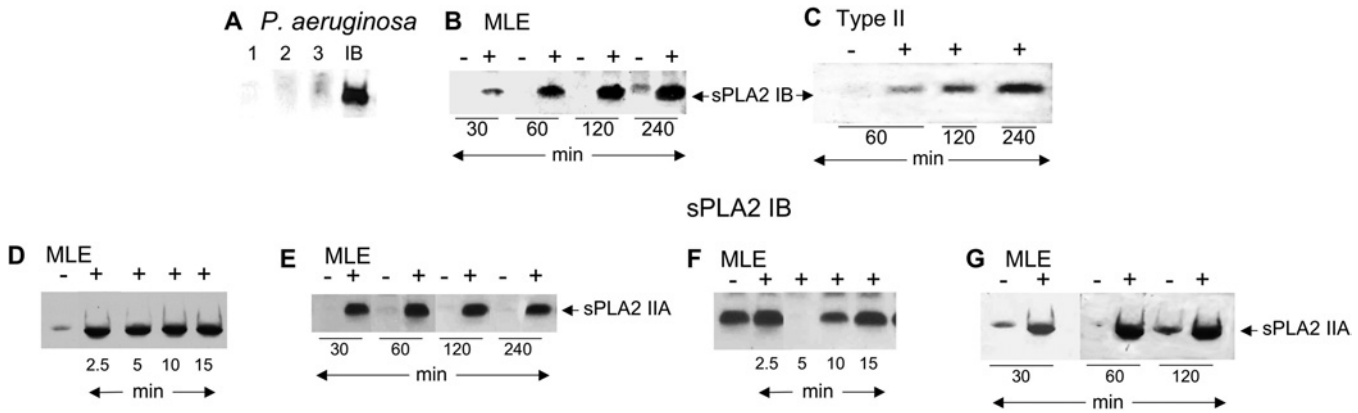

Figure 6 ***Ps. aeruginosa*** **and $sPLA_2$ IB stimulate secretion of sPLA IIA and $sPLA_2$ IB in lung epithelia**

(**A**) *Ps. aeruginosa* was incubated in serum-free Hite's medium without antibiotics and in the absence of MLE-12 cells for 2 h. Proteins from 5 ml of the medium were precipitated with 60 % TCA and 1.5 % sodium desoxycholate and applied on SDS/PAGE at various concentrations: lane 1, 50 μg; lane 2, 100 μg; lane 3, 150 μg. Gels were then processed for $sPLA_2$ IB immunoblotting. In lane 4, bovine pancreas $sPLA_2$ IB (5 μg) was loaded on to the gel as a positive control. MLE-12 (**B**, **D**–**G**) and mouse primary type II cells (**C**) were incubated with (+) or without (−) *Ps. aeruginosa* (MOI = 5) (**B**–**E**) or $sPLA_2$ IB (100 μg/ml) (**F**, **G**) for various times, and medium and cell lysates were harvested. Proteins from 5 ml of the medium was precipitated with 60 % TCA and 1.5 % sodium desoxycholate, and immunoblot analysis was performed with anti-($sPLA_2$ IB) and anti-($sPLA_2$ IIA) polyclonal antibodies. Bovine pancreas $sPLA_2$ (5 μg) was loaded on the gel as a positive control. Immunoblots are representative of three independent experiments.

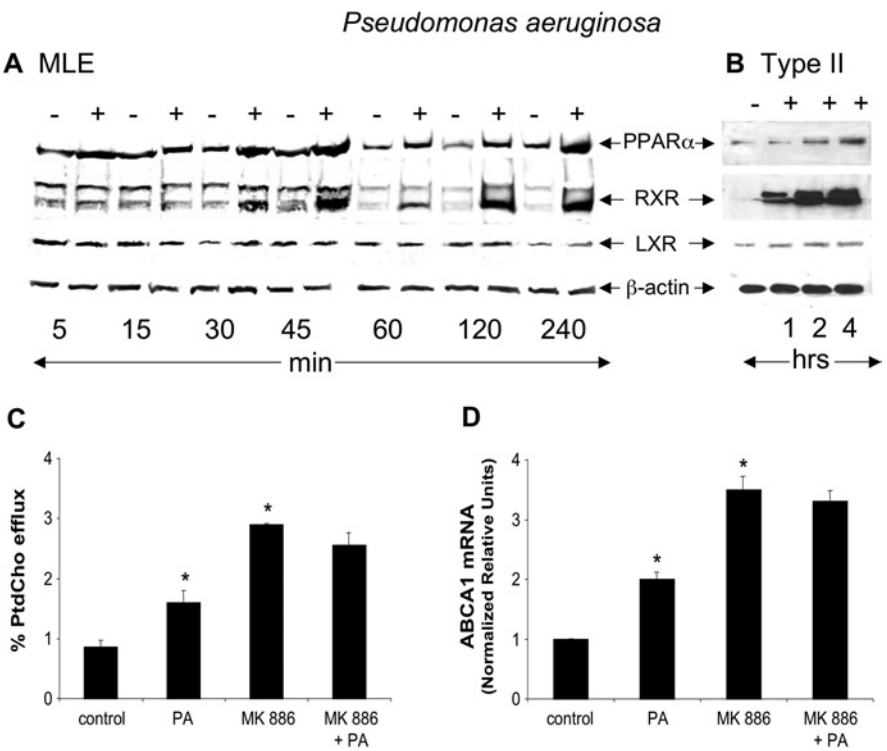

Figure 7 ***Ps. aeruginosa*** **up-regulates PPARα and RXR in MLE-12 cells**

MLE-12 (**A**) and primary mouse type II (**B**) cells were incubated with (+) or without (−) *Ps. aeruginosa* (MOI = 5) for various times. Cell lysates were harvested, separated by SDS/10 % PAGE, and probed with anti-ABCA1, anti-PPARα, anti-RXR, anti-LXR and anti-β-actin antibodies. Relative protein levels in each experiment were detected by stripping one nitrocellulose membrane and re-probing with various antibodies. (**C**) Basolateral PtdCho efflux. MLE-12 cells were plated on transwells, labelled with [*methyl*-^{3}H]choline for 20 h, incubated with MK 886 (5 μM) for 1 h, and then infected with *Ps. aeruginosa* (MOI = 5) for 2 h using HDL (20 μg/ml) as a PtdCho acceptor. Basolateral medium and cells were harvested for PtdCho analysis. (**D**) MLE-12 cells were incubated with MK 886 and *Ps. aeruginosa* as described above, and total RNA was harvested for *ABCA1* mRNA analysis by real-time PCR using GAPDH transcripts as an internal control. $^*P < 0.05$ compared with control. Results in histograms are means ± S.E.M. for three independent experiments.

Ps. aeruginosa and $sPLA_2$ IB increase ABCA1 expression via PPARα–RXR heterodimerization

$sPLA_2$ IB and $sPLA_2$ IIA can activate PPARα [18]. PPARα in turn heterodimerizes with RXR or LXR nuclear transcription factors that potently increase ABCA1 expression [37]. Thus we examined whether *Ps. aeruginosa* modulates heterodimerization with these receptor-binding partners. Immunoblot analysis of cells infected with *Ps. aeruginosa* (Figures 7A and 7B) revealed 3–4-fold induction of PPARα from 30 min to 4 h in cells, and at 2 h and 4 h

in primary mouse type II cells compared with control, as determined by densitometric analysis. In addition, *Ps. aeruginosa* also increased RXR expression from 2- to 20-fold co-ordinately with PPARα stimulation without altering LXR or β-actin levels (Figures 7A and 7B).

Previously, it has been shown that activation of PPARα reduced LXR–RXR formation, while LXR activation inhibited formation of PPARα–RXR [38,39]. Thus heterodimeric association between individual dimeric receptor pairs can be manipulated that could potentially have an impact on ABCA1 expression [40]. To examine the functional relationships of these receptors to PtdCho efflux, cells were exposed to the PPARα inhibitor MK 886 that surprisingly increased PtdCho efflux from cells independently of the effect of *Ps. aeruginosa* (Figure 7C). MK 886 also increased *ABCA1* mRNA levels (Figure 7D). Thus these data suggest that, although PPARα might partake in ABCA1 activation in response to *Ps. aeruginosa* infection, it can act as a relative endogenous repressor or competitor of LXR–RXR complex formation that potently transactivates the *ABCA1* gene [24,40,41]. Moreover, the effects of *Ps. aeruginosa* on PPARα and RXR expression were recapitulated with exogenous $sPLA_2$ IB, which produced a 2–4-fold increase in expression of these receptors over 5–240 min of analysis (Figure 8A). As with *Ps. aeruginosa,* PPARα inhibition led to activation of PtdCho efflux and increased *ABCA1* mRNA expression, a process that was not additive or synergistic with actions of $sPLA_2$ IB (Figures 8B and 8C). These data suggest that *Ps. aeruginosa* and $sPLA_2$ IB can stimulate ABCA1 phospholipid export using similar mechanisms.

Ps. aeruginosa and $sPLA_2$ IB stimulate PtdCho efflux via activation of p44/42 kinase

The MAPK p44/42 phosphorylates PPARα and increases its expression [42]. Indeed, *Ps. aeruginosa* and $sPLA_2$ IB each triggered an increase in p44/42 activity in cells (Figures 9A and 9C), which was determined to be 2–3-fold by densitometric analysis (results not shown). These changes in p44/42 activity were observed in response to agonist stimulation within 2.5–5 min. Furthermore, PD98059, an inhibitor of MEK1, a kinase upstream of p44/42 kinase, attenuated effects of *Ps. aeruginosa* (Figure 9B) and $sPLA_2$ IB (Figure 9D) on PtdCho efflux in lung epithelium.

Transfection of cells with constitutively active MEK1 plasmid induced PPARα, RXR and ABCA1 expression without altering β-actin levels (Figure 9E). These data suggest that activation of p44/p42 kinase stimulates ABCA1 expression via PPARα–RXR heterodimerization.

Ps. aeruginosa and $sPLA_2$ IB transcriptionally activate the *ABCA1* gene in lung epithelia

MLE-12 cells were transfected with various *ABCA1* promoter constructs followed by exposure to *Ps. aeruginosa* or $sPLA_2$ IB (Figure 10A). *Ps. aeruginosa* and $sPLA_2$ IB each produced a modest stimulatory effect on *ABCA1* promoter activity using various ABCA1 constructs relative to an empty reporter construct (Figures 10B and 10C). The *ABCA1* gene is activated by an RXR–LXR-dependent pathway via binding of this heterodimer to a specific DR-4 element located within the proximal 5′-flanking region of the gene. Furthermore, PPARα–RXR heterodimers bind to a DR-1 (direct repeat 1) element. $ABCA1_{-949/+274}$ harbours only a partial DR-1 sequence and an intact DR-4 locus. Deletion of DR-1 half sites in $ABCA1_{-949/+274}$, $ABCA1_{-205/+274}$ and $ABCA1_{-77/+274}$ did not attenuate promoter activation by *Ps. aeruginosa* and $sPLA_2$ IB (Figures 10B and 10C). In contrast, deletion or mutation of the DR-4 elemen ($ABCA1_{-49/+274,-77mut/+274}$) totally abrogated *Ps. aeruginosa*- and $sPLA_2$ IB-mediated ABCA1 promoter activation (Figures 10B and 10C). These observations suggest that *Ps. aeruginosa* and $sPLA_2$ IB trigger receptor heterodimerization that transactivates the *ABCA1* gene via DR-4 binding.

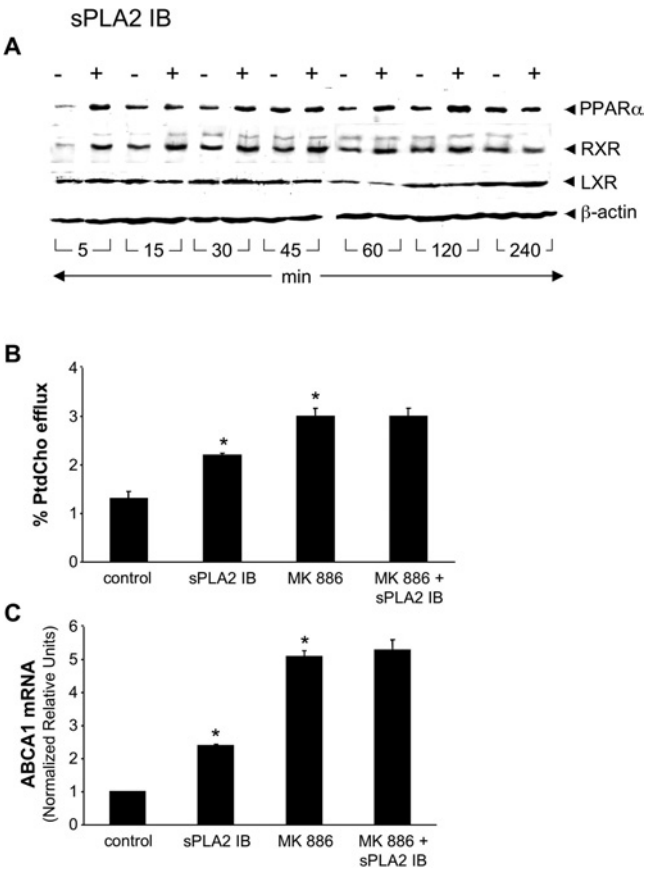

Figure 8 $sPLA_2$ IB up-regulates PPARα and RXR in MLE-12 cells

(**A**) MLE-12 cells were incubated with (+) or without (−) $sPLA_2$IB (100 μg/ml) for various times. Cell lysates were harvested, separated by SDS/10 % PAGE, and probed with anti-ABCA1, anti-PPARα, anti-RXR, anti-LXR and anti-β-actin antibodies. Relative protein levels in each experiment were detected by stripping one nitrocellulose membrane and reprobing with various antibodies. (**B**) Basolateral PtdCho efflux. MLE-12 cells were plated on transwells, labelled with [*methyl*-^{3}H]choline for 20 h, incubated with MK 886 (5 μM) for 1 h and then exposed to $sPLA_2$ IB (100 μg/ml) for 2 h using HDL (20 μg/ml) as a PtdCho acceptor. Basolateral medium and cells were harvested for PtdCho analysis. (**C**) MLE-12 cells were incubated with MK 886 and $sPLA_2$ IB as described above, and total RNA was harvested for *ABCA1* mRNA analysis by real-time PCR using GAPDH transcripts as an internal control. $^*P < 0.05$ compared with control. Results in histograms are means ± S.E.M. for three independent experiments.

DISCUSSION

Bacterial infection has been shown to accelerate phospholipid hydrolysis within various systems, but little is known regarding the effects of these pathogens on lipid transport. The new data from the present study indicate that (i) *Ps. aeruginosa* triggers basolateral export of the major eukaryotic phospholipid, PtdCho, from lung epithelia, (ii) this effect is mediated, in part, by the ATP-dependent pump, ABCA1, (iii) *Ps. aeruginosa* increases ABCA1 expression by increasing gene transcription, and (iv) *Ps. aeruginosa* up-regulates expression of a distinct array of signalling molecules known to be involved in ABCA1 expression. These effects of *Ps. aeruginosa* are also observed with $sPLA_2$ IB, a key inflammatory mediator involved in phospholipid catabolism and

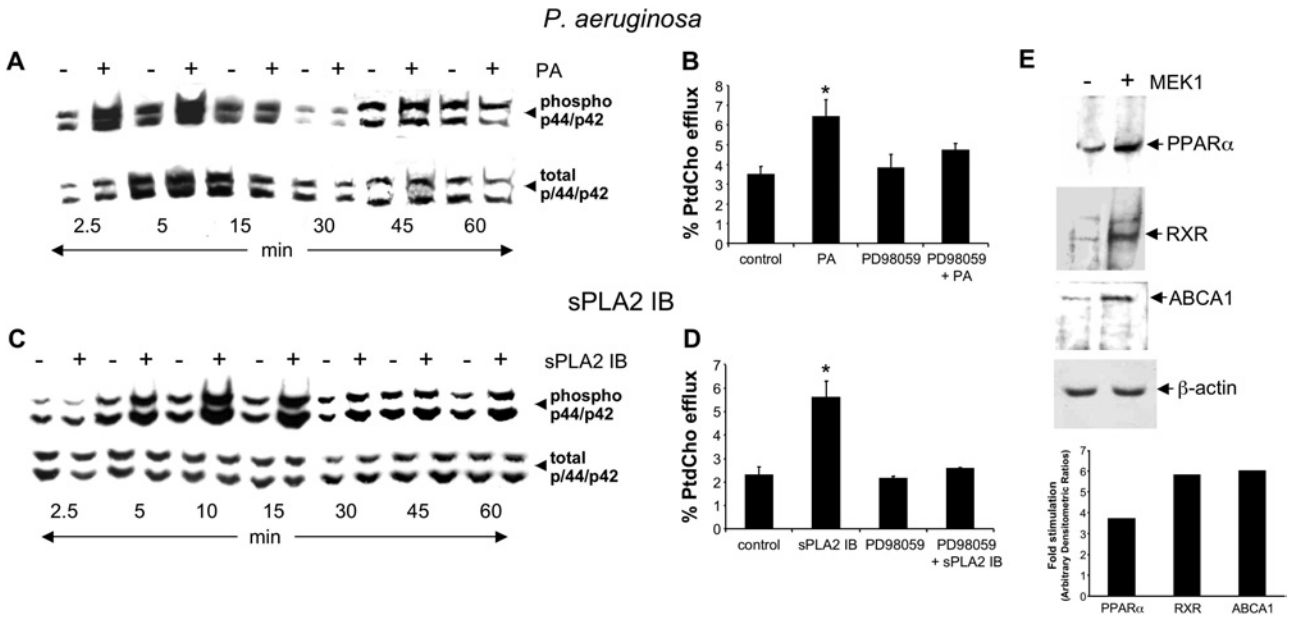

Figure 9 ***Ps. aeruginosa*** **and sPLA$_2$ IB activate p44/p42 kinase**

MLE-12 cells were exposed to *Ps. aeruginosa* (MOI = 5) (PA) (**A**) or sPLA$_2$ IB (100 μg/ml) (**C**) for various times, and cell lysates were harvested and processed for immunoblotting. Nitrocellulose membranes were probed with antibodies against phosphorylated (active) and total p44/p42 isoforms. (**B**) Basolateral PtdCho export. MLE-12 cells were labelled for 20 h with [*methyl*-^{3}H]choline, incubated for 1 h with PD98059 (10 μM) and subsequently exposed for 2 h to *Ps. aeruginosa* (**B**) or sPLA$_2$ IB (**D**). Medium was harvested and processed for PtdCho analysis. (**E**) MLE-12 cells were transfected with a MEK1 plasmid (4 μg) overnight, and the next day harvested and processed for immunoblot analysis using anti-PPARα, anti-RXR, anti-ABCA1 and anti-β-actin antibodies. $^*P < 0.05$ compared with control. Results in histograms are means $\pm$ S.E.M. for three independent experiments.

the innate host response [43]. Indeed, sPLA$_2$ IB is released from MLE-12 cell in response to *Ps. aeruginosa* infection (Figure 6). The data as a whole suggest that *Ps. aeruginosa* infection exploits a phospholipid-trafficking mechanism, catalysed by ABCA1, as a means to deplete the intracellular pool of PtdCho. Because alveolar type II epithelial cells utilize PtdCho for new cell membrane formation and surfactant biosynthesis, these results expand on the pathobiology of bacterial infections as it relates to properties that intrinsically link to cellular viability. The secretion of sPLA$_2$s by alveolar epithelia after infection as shown in the present study is intriguing, and may initially represent an attempt by host cells to degrade bacterial phospholipids [43,44]. However, sPLA$_2$ IB secretion by epithelia after *Ps. aeruginosa* infection may also facilitate hydrolysis of extracellular surfactant PtdCho or trigger signalling events that eventually lead to the demise of the type II cell by altering phospholipid homoeostasis.

Previous studies have identified ABCA1 as a lipid sensor that might serve to help maintain the delicate balance of phospholipids within lung epithelium [28–30]. By using two different pro-inflammatory agonists, *Ps. aeruginosa* and exogenous sPLA$_2$ IB each increased PtdCho export via a basolateral route in epithelial cells. These physiological changes in response to pro-inflammatory agonist stimulation were associated with increases in ABCA1 protein and mRNA levels and transactivation of the *ABCA1* promoter via a DR-4 locus-dependent mechanism. These effects of *Ps. aeruginosa* resemble those of LPS that either increases or decreases *ABCA1* mRNAs in other systems, suggesting that effects of the bacterium may be due partly to LPS components. However, LPS did not trigger ABCA1-mediated efflux in our studies (Figure 2D). Phospholipid efflux after bacterial infection was abolished further after pharmacological inhibition of ABCA1 and not observed in Tangier disease cells, in support of a causal role for ABCA1 in mediating effects of *Ps. aeruginosa*. Furthermore, *Ps. aeruginosa* increases in PtdCho efflux were not observed in H441 and CaCo-2 cells, recapitulated with related Gram-negative pathogens, nor did *Ps. aeruginosa* increase ABCG1 and ABCG5 expression or stimulate lyso-PtdCho or SM export. The lack of export of SM by the bacterium may be an attempt to conserve cellular levels of this lipid, as *Ps. aeruginosa* triggers SM hydrolysis within membrane rafts, an event that appears to be essential for the cytokine and apoptotic response in infected cells [45]. Taken together, these results indicate that *Ps. aeruginosa* effects on lipid trafficking in lung epithelia appear to be fairly specific with regard to the nature of lipid exported in cells.

A new observation from this study is that *Ps. aeruginosa* stimulates secretion of immunoreactive sPLA$_2$ IB and sPLA$_2$ IIA, rather than sPLA$_2$ V and sPLA$_2$ X, from distal lung epithelia. The mRNAs for these phospholipases are all detected in the lung, but it appears that sPLA$_2$ IIA serves as a major effector in acute lung injury [9]. However, sPLA$_2$ IIA and sPLA$_2$ IB, unlike sPLA$_2$ V and sPLA$_2$ X, exhibit different substrate requirements, in that they have higher activities against phosphatidylglycerol with little preference for PtdCho [11]. Phosphatidylglycerol does provide some surface-tension-lowering activity and, with PtdCho, may be important in maintaining lung stability; however, this phospholipid is a minor component of surfactant [11,46]. Thus sPLA$_2$ IB and sPLA$_2$ IIA release after *Ps. aeruginosa* infection might reduce surfactant by inciting other inflammatory pathways distinct from their enzymatic properties on surfactant phospholipids. As shown in the present study, sPLA$_2$ IB stimulation of basal PtdCho export in combination with sPLA$_2$ IIA activity might be one mechanism whereby these lipases accentuate pulmonary injury; this could occur by reducing phospholipids destined for secretion as part of surfactant or ultimately directed into alveolar membranes. Both lipases were released rapidly (within 30 min) from epithelia (Figure 6) after bacterial infection, but the kinetics of *Ps. aeruginosa* compared with sPLA$_2$ IB differed somewhat with regard to effects on ABCA1 protein. Maximal effects of

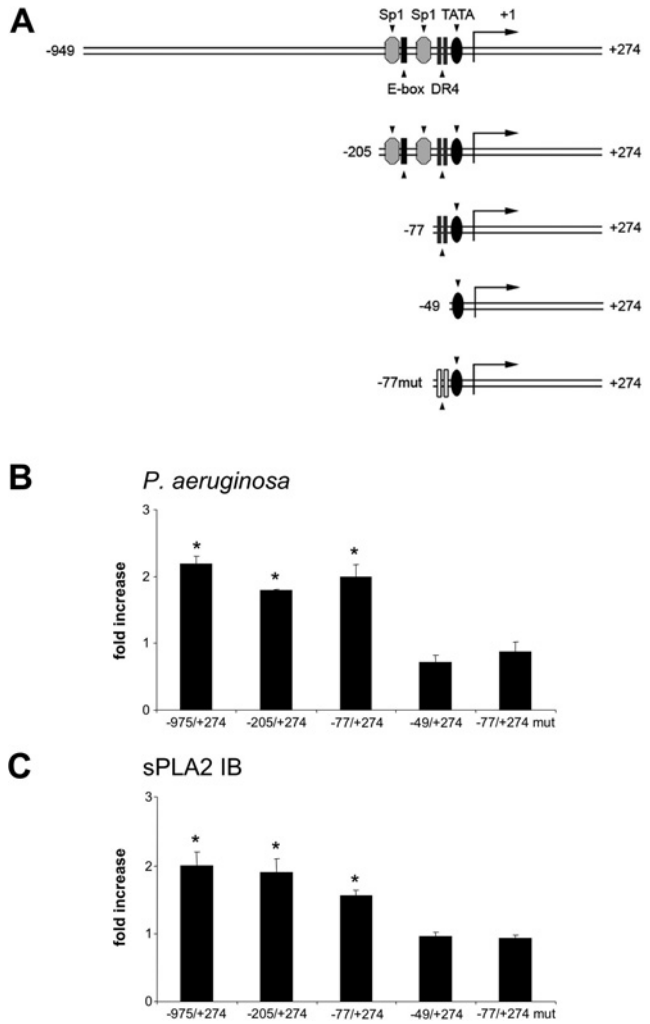

Figure 10 *Ps. aeruginosa* and sPLA$_2$ IB increase *ABCA1* promoter activity

(**A**) Schematic illustration of *ABCA1* promoter constructs. Three constructs contain a DR-4 element (−949/+274, −205/+274 and −77/+274), one lacks this element (−49/+274), and one contains a mutation within DR4 (−77/+274 mut). (**B**, **C**) Cells were co-transfected with these *ABCA1* promoter reporters and pSV-β-galactosidase for 2–4 h before exposure to *Ps. aeruginosa* (MOI = 5) (**B**) or PLA$_2$ IB (100 μg/ml) (**C**) for 2 h. Lysates were assayed for luciferase and β-galactosidase activities. Results are means ± S.E.M. fold increases of luciferase/β-galactosidase activities in the cells exposed to *Ps. aeruginosa* and sPLA$_2$ IB compared with control for three independent experiments. *$P < 0.05$ compared with control.

Ps. aeruginosa on induction of ABCA1 levels were observed within 1 h, whereas sPLA$_2$ IB optimally increased the transporter after 4 h (Figure 3). These data suggest that other yet unidentified factors associated with intact bacteria may synergize to more rapidly stimulate *ABCA1* gene transcription.

Ps. aeruginosa and sPLA$_2$ IB each triggered PPARα and RXR activation without affecting LXR expression in lung epithelia. PPARα, RXR and LXR serve as key transcription factors that regulate ABCA1 expression, resulting in enhanced cholesterol efflux [47]. sPLA$_2$ IB also triggers sPLA$_2$ IIA release via an autocrine loop involving PPARα and induces PPARα–RXR complex formation in mesanglial cells [18]. Of note, MJ33 and pyrrolidin-1, inhibitors of sPLA$_2$ IB and cPLA$_2$ activities respectively, did not attenuate the effect of *Ps. aeruginosa* or sPLA$_2$ IB on PtdCho efflux in our experiments (results not shown). Thus it is possible that sPLA$_2$ IB exerts its signalling effects on this ABCA1 pathway via transmembrane signalling through binding to membrane receptors independently of its catalytic activity, as has been suggested previously [18].

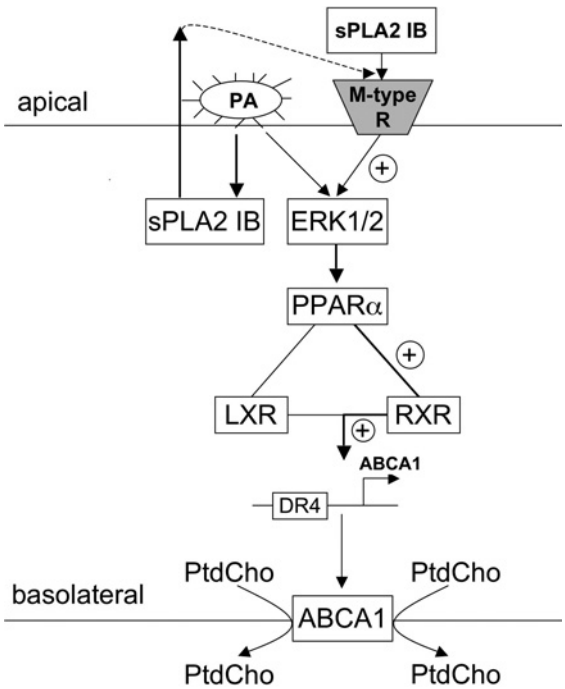

Figure 11 Mechanisms of ABCA1 activation by *Ps. aeruginosa* and exogenous sPLA$_2$ IB

The solid lines indicate known pathways, the broken lines indicate putative mechanisms, and the thick solid lines indicate mechanisms discussed in the present paper.

Although *Ps. aeruginosa* or sPLA$_2$ IB increased PPARα and RXR expression, it is also notable that MK 886, a PPARα inhibitor, increases ABCA1 expression and phospholipid efflux when added to cells alone (Figures 7C, 7D, 8B and 8C). This apparent inconsistency can be explained by competition for heterodimeric association by individual receptors. For example, LXR–PPARα heterodimerization leads to reduction of PPARα–RXR formation, and PPARα activation reduces LXR–RXR heterodimerization [38,39]. Such overlapping and competitive transcriptional programs involving PPARα, RXR and LXR were also demonstrated in mouse liver [40]. Our data are consistent with these results, where, depending on the relative abundance of each heterodimeric pair, PPARα could serve as an endogenous repressor of LXR–RXR heterodimer formation. Thus inhibition of PPARα in our experiments by MK 886 may induce heterodimerization of LXR–RXR, the most potent activator of *ABCA1* gene transcription [24,41].

Finally, *Ps. aeruginosa* or sPLA$_2$ IB rapidly activated p44/42 MAPK, MEK1 induced PPARα–RXR and ABCA1 expression, and effects of physiological agonists were blocked using PD98059. These results strongly implicate ERKs as upstream regulators of the ABCA1 efflux pathway. Indeed, *Ps. aeruginosa* activates p44/p42 kinase in airway epithelia, and PPARα is activated by p44/42 MAPK in response to insulin via phosphorylation of its N-terminus [42,48]. Moreover, sPLA$_2$ IB, via binding to its receptor, dramatically induces p44/42 MAPK activation in human neutrophils [49,50]. As a whole, these observations suggest a *Ps. aeruginosa* → sPLA$_2$ IB → p44/p42 kinases → PPARα/RXR → ABCA1 pathway (Figure 11). According to this scheme, *Ps. aeruginosa* induces sPLA$_2$ IB secretion from alveolar epithelium. sPLA$_2$ IB via binding to its membrane receptor

activates p44/p42 kinases that phosphorylate and activate PPARα. PPARα heterodimerizes with RXR and activates ABCA1 via binding of this heterodimer to a DR-4 element within the *ABCA1* promoter. Thus *Ps. aeruginosa* may impair lung function via induction of basolateral ABCA1-mediated surfactant PtdCho export in murine alveolar epithelium. The results do not exclude effects of *Ps. aeruginosa* that are independent of $sPLA_2$. Future studies may involve investigating this pathway using animal models of bacterial infection where secretory phospholipases might emerge as physiologically relevant molecular targets for therapeutic intervention in inflammatory lung injury.

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