Lysophosphatidylcholine Modulates Neutrophil Oxidant Production through Elevation of Cyclic AMP

Phoebe Lin, Emily J. Welch, Xiao-Pei Gao, Asrar B. Malik and Richard D. Ye

J Immunol 2005; 174:2981-2989; doi: 10.4049/jimmunol.174.5.2981
http://www.jimmunol.org/content/174/5/2981
Lysophosphatidylcholine Modulates Neutrophil Oxidant Production through Elevation of Cyclic AMP

Phoebe Lin, Emily J. Welch, Xiao-Pei Gao, Asrar B. Malik, and Richard D. Ye

Lysophosphatidylcholine (LPC) is an oxidized phospholipid present in micromolar concentrations in blood and inflamed tissues. The effects of LPC on neutrophil functions remain incompletely understood, because conflicting reports exist for its stimulatory and inhibitory roles. We report in this study that LPC inhibits superoxide generation in fMLP- and PMA-stimulated neutrophils without affecting fMLP-induced Ca^{2+} mobilization and cell viability. This effect was observed with LPC dissolved in ethanol, but not with LPC stock solutions prepared in water or in BSA-containing aqueous solution with sonication. Under the same experimental conditions, platelet-activating factor primed neutrophils for superoxide generation. The inhibitory effect of LPC was observed within 30 s after its application and was maximal at LPC concentrations between 0.1 and 1 μM. Inhibition of superoxide generation was accompanied by a 2.5-fold increase in the intracellular cAMP concentration. In addition, LPC reduced fMLP-stimulated phosphorylation of ERK and Akt and membrane translocation of p67^{phox} and p47^{phox}. The protein kinase A inhibitors H-89 and adenosine 3’5’-cyclic monophosphorothioate Rp-isomer (Rp-cAMP) partially restored superoxide production in LPC-treated neutrophils, indicating involvement of protein kinase A in LPC-mediated inhibition. Using an ex vivo mouse lung perfusion model that measures lung weight change and capillary filtration coefficient, we found that LPC prevented lung vascular injury mediated by fMLP-activated neutrophils. Taken together, these results suggest that LPC-induced elevation of intracellular cAMP is partially responsible for its inhibition of neutrophil NADPH oxidase activation. A similar mechanism of inhibition may be used for the control of neutrophil-mediated tissue injury. The Journal of Immunology, 2005, 174: 2981–2989.
We have previously shown that G2A, a potential LPC receptor, is coupled constitutively to the Gs, Gi, and G12/13 family of G proteins (16). In transfected cells, LPC stimulation of G2A results in additional activation of Gs and its downstream effector adenylyl cyclase, leading to elevation of intracellular cAMP. Because cAMP is known to inhibit neutrophil activation, including inhibition of superoxide generation (17–20), and neutrophils express G2A, we examined whether LPC had an effect on neutrophil NADPH oxidase activity via elevation of cAMP. Our results provide direct evidence showing that LPC increases the intracellular cAMP concentration in neutrophils. We have also found that LPC treatment inhibits fMLP- and PMA-induced superoxide generation, and blocking cAMP-dependent protein kinase (protein kinase A (PKA)) partially reversed the inhibition by LPC. These observations suggest a link between LPC-induced cAMP elevation and inhibition of oxidative production. Finally, we found that LPC-mediated inhibition of neutrophil superoxide generation parallels a protective effect against neutrophil-mediated lung injury.

Materials and Methods

Materials

Individual LPCs (16:0, 18:0, and 18:1 LPC) were obtained from Avanti Polar Lipids. PAF was purchased from BIOMOL. The lipids were dissolved in 50% (v/v) ethanol/H2O unless otherwise stated. Anti-phospho-ERK (p44/p42), phospho-Akt, Akt, and ERK Abs were purchased from Cell Signaling. We obtained the anti-p76 phospho Ab from Upstate Cell Signaling Solutions. The anti-p67 phospho Ab was purchased from BD Biosciences. The PKA inhibitors H-89 and adenosine 3′,5′-cyclic monophosphorothioate Rp-isomer (Rp-cAMP) were purchased from Calbiochem. Isoluminol, PMA, and fMLP were obtained from Sigma-Aldrich. HRP was purchased from Roche.

RT-PCR

To confirm the expression of G2A in human polymorphonuclear cells by RT-PCR, first-strand cDNA was synthesized using 2 μg of total RNA isolated using TRIzol reagent (Invitrogen Life Technologies), and reverse transcriptase was purified from Escherichia coli containing the pol gene of Moloney murine leukemia virus (Invitrogen Life Technologies). Fifteen percent of the first-strand cDNA synthesis product was then used for PCR with the primers XGR3 (5′-CTCGTGGGGAT CTGTCACTAC-3′) and HG2AC1 as described previously (16) to amplify human G2A (hg2A). Primers Fmid GPR4 (5′-CGGGGACCTCTGGGGG-3′) and RevGPR4 (5′-GTCTGGGCAGCAGATC-3′) were used to amplify GPR4. Primers for G3PDH were used as a housekeeping gene control.

cAMP assay

cAMP was measured using a competitive ELISA (BIOMOL). For the cAMP dose response, neutrophils were incubated at 37°C with 1 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich) and different concentrations of cAMP just before addition of LPC (1 μM). Samples were taken at different time points for up to 10 min. Neutrophils were collected by centrifugation at 14,000 rpm for 10 min at 4°C, and supernatant was harvested as Triton-soluble fraction. The intracellular cAMP concentration was measured according to the instructions provided by the manufacturer. The kit uses a polyclonal Ab against cAMP (0.001% cross-reactivity with cGMP). Absorbance was measured at 405 nm in a SpectraMax 340 microplate reader (Molecular Devices).

Preparation of neutrophils

Blood was taken from healthy human donors by venipuncture, using a protocol approved by the institutional review board at University of Illinois at Chicago. Acid citrate dextran was used as anticoagulant. RBC were then sedimented by adding 0.5 vol of 6% Hetastarch (Abbott Laboratories) and incubating for 1 h. The white blood cell-rich buffy coat was layered on top of a double-layer discontinuous Percoll gradient (12 ml each of 55 and 74%, in 0.9% NaCl), which was then centrifuged at 1500 rpm (~450 × g) at 12°C for 60 min with slow acceleration. Cells at the plasma/55% Percoll interface were carefully discarded as the PBMC, and polymorphonuclear cells were taken from the 74%/55% Percoll interface. Cells were washed twice and resuspended in 1% BSA/RPMI 1640 until use. Approximately 98% of the cells were viable. By flow cytometric analysis, 95–98% of the prepared cells were identified as neutrophils. Flow cytometry was conducted on a Coulter ELITE ESP flow cytometer (Beckman Coulter).

Superoxide generation

Neutrophils were resuspended in BSA buffer (0.5% BSA in HBSS with Ca2+ and Mg2+, and 10 mM HEPES) at 5 × 106 cells/ml. The superoxide anion release was measured as described previously (21). Briefly, isolated minol was added to the cell suspension to a final concentration of 50 μM, and HRP was added to a final concentration of 40 U/ml. Cells were then seeded into a white, 96-well, flat-bottom tissue culture dish (E&K Scientific). Chemiluminescence was measured every minute using a Wallace multilabel counter plate reader (PerkinElmer) starting from 5 min before and continuing to 30 min after stimulation with iMLP or PMA. Unstimulated controls were recorded simultaneously. Alternatively, some samples were preincubated with LPC or other inhibitors before addition of iMLP or PMA.

Perfusion of mouse lung with neutrophils and assessment of lung injury

Isolated and perfused mouse heart/lung preparations were obtained from anesthetized wild-type C57BL/6 mice, using a protocol approved by the institutional animal care committee. The isolated heart/lung was perfused with human neutrophils as described previously (22). Briefly, the trachea was cannulated for constant positive pressure ventilation at a rate of 186 breaths/min. The pulmonary artery was then cannulated via the right atrium, and an incision was made in the left atrium to allow for drainage of venous effluent. Lungs were immediately perfused through the pulmonary artery with Krebs buffer supplemented with 5 g/100 ml BSA at a constant flow of 2 ml/min and a temperature of 37°C, then transferred en bloc onto the perfusion apparatus where changes in lung weight were measured using a force displacement transducer. TNF-α (1000 U/ml) was added to the perfusate and allowed to recirculate for 1 h through the lung before inflating the lung with 2 × 106 freshly isolated human neutrophils without or with fMLP. Alternatively, neutrophils were preincubated with LPC, which was then washed to remove excess LPC before perfusion of the cells through the lung. The capillary filtration coefficient (Kc) was measured by rapidly elevating outflow pressure by 10 cm of H2O for 2 min after neutrophil challenge. The Kc was calculated as the slope of the lung weight change normalized against the acute change in pressure and the dry lung weight and is shown as milliliters per minute per centimeter of H2O per dry lung weight in grams (23).

Up-regulation of CD11b

Neutrophils were preincubated with or without LPC for 10 min, followed by stimulation with iMLP for 10 min, all at 37°C. Cells were then placed on ice and incubated with an anti-CD11b Ab conjugated to PE (BD Biosciences). Cells were pelleted and resuspended in 0.5% paraformaldehyde in 1× PBS. Flow cytometry was conducted on a Coulter ELITE ESP flow cytometer, with gating on neutrophils as determined by forward vs side scatter. Quadrant analysis was performed using WinMDI 2.8 software (http://facs.scripps.edu/software).

Calcium mobilization

Increases in intracellular calcium were detected using Indo-1/AM labeling of human neutrophils kept in a 0.5% BSA/HBSS buffer as described previously (24).

Membrane translocation

Neutrophils (20 × 106/sample) were resuspended in BSA buffer and preincubated without or with LPC at 37°C for 10 min before addition of iMLP for 3 min. Cells were then placed on ice, lysed with cold hypotonic buffer A (20 mM Tris–Cl (pH 7.4), 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 1 mM PMSF, and a 1/50 dilution of Calbiochem protease inhibitor mixture set I), then subjected to freeze/thaw in a liquid nitrogen/37°C water bath three times. Samples were spun at 14,000 rpm for 10 min at 4°C, and supernatant was collected as the cytosolic fraction. Pellets were washed twice in buffer A, spun down, and resuspended in buffer B (buffer A plus 1% Triton X-100). Samples were incubated for 30 min with agitation at 4°C to release membrane proteins. Samples were then spun again at 14,000 rpm for 10 min at 4°C, and supernatant was harvested as Triton-soluble membrane fraction. Aliquots of all samples were set aside to determine the relative protein concentration using the Bio-Rad protein assay. Sample buffer (5×) was added to all samples, which were then boiled and run on SDS-PAGE at equivalent protein concentrations.
Results

LPC inhibits fMLP-induced neutrophil superoxide production

To determine whether LPC affects neutrophil NADPH oxidase activation, neutrophils from peripheral blood were incubated with LPC (16:0, 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine) before being challenged with fMLP. 

\[ \text{O}_2^\cdot \] production was determined by means of isoluminol-ECL and expressed as a function of time. As shown in Fig. 1, LPC prepared by dissolving in ethanol/water (1/1), as described in Materials and Methods, significantly reduced fMLP-induced \[ \text{O}_2^\cdot \] production. Integrated chemiluminescence (area under the curve), based on experimental data derived from multiple blood donors (n = 3), is shown in Fig. 1B. Preincubation of neutrophils for 10 min with 0.1–1 \( \mu \)M LPC resulted in a 75–82% reduction in fMLP-induced superoxide production. LPC treatment alone did not significantly increase or decrease \[ \text{O}_2^\cdot \] production.

To determine whether LPC-mediated inhibition of superoxide production was due to nonspecific effects, such as disruption of fMLP integrity or interference with fMLP binding to its receptor, we examined the effect of LPC on fMLP-induced \( \text{Ca}^{2+} \) mobilization. This response requires fMLP to activate G proteins as well as the downstream effector phospholipase C\( \beta \) (PLC\( \beta \)). As shown in Fig. 2, LPC did not stimulate \( \text{Ca}^{2+} \) mobilization in neutrophils, nor did it disrupt the fMLP-induced \( \text{Ca}^{2+} \) response. These results suggest that cellular integrity and responsiveness to fMLP were not affected by LPC treatment under the experimental conditions.

The viability of cells was examined by trypan blue exclusion, and no significant difference was detected between LPC-treated and vehicle (0.25% ethanol)-treated cells in the course of the experiments. Less than 4% of the cells were trypan blue-positive when treated with either vehicle or LPC for up to 30 min (data not shown).

PMA-induced superoxide production is susceptible to LPC inhibition

We examined whether the inhibitory effect of LPC was selective for fMLP-induced production or was also applicable to PMA-stimulated neutrophils. PMA activates neutrophil NADPH oxidase by mimicking diacylglycerol that stimulates conventional and novel protein kinase C (25). Neutrophils were treated for 10 min with 1 \( \mu \)M PMA, then stimulated with PMA at various concentrations (100, 200, and 400 ng/ml). PMA at these concentrations induced superoxide production to similar levels (Fig. 3). LPC-treated neutrophils exhibited reduced responsiveness to PMA compared with untreated cells, as determined by integration of isoluminol-ECL over a period of 30 min. The extent of the reduction ranged from 26% (with 400 ng/ml PMA) to 45% (with 100 ng/ml PMA), indicating that the inhibitory effect of LPC could be reversed by higher concentrations of PMA.

Protective effect of LPC in neutrophil-induced lung injury

To determine the functional significance of LPC inhibition of neutrophil oxidant production, we used a model of lung injury in which human neutrophils are perfused through an isolated mouse lung preparation (22, 23). Freshly prepared human neutrophils were then added to the perfusate and challenged with or without fMLP (1 \( \mu \)M). In some samples, neutrophils were first incubated with LPC, washed with Krebs/BSA buffer to remove excess LPC, added to the perfusate, and then challenged with fMLP. Our results demonstrate that challenging neutrophils with fMLP led to an 11-fold increase in lung wet weight (indicative of lung injury) over that in unchallenged control neutrophils (Fig. 4A). Treating neutrophils with LPC reversed this increase in lung wet weight.

Data were analyzed by paired Student’s t test using PRISM (version 3.0) software (GraphPad).

Statistical analysis

FIGURE 1. LPC inhibition of fMLP-induced oxidant production in neutrophils. A, Superoxide generation was determined in real-time based on isoluminol-ECL. cps, Counts per second of light emitted. Approximately \( 0.5 \times 10^8 \) cells/sample were preincubated with either LPC or vehicle (0.25% ethanol) for 10 min before being stimulated with fMLP (1 \( \mu \)M). Shown are representative curves from one of the three experiments that produced similar results. B, Bar graph depicting the integrated total area under the chemiluminescence curves. C, Time course of LPC inhibition, based on integrated total area under the chemiluminescence curves as described above. Data shown are the mean \( \pm \) SEM from three separate experiments. **, \( p < 0.01 \).
with LPC (1 μM) for 10 min before challenge with fMLP markedly reduced the fMLP-stimulated, neutrophil-mediated lung wet weight change (Fig. 4, A and B) and $K_{c, f}$ (Fig. 4C).

**LPC prevents up-regulation of CD11b by fMLP**

In addition to stimulating oxidant production, fMLP up-regulates β2 integrin expression and thereby facilitates neutrophil adhesion to activated endothelial cells, an event closely related to neutrophil-mediated injury of the endothelium (4, 26). We examined whether LPC could affect fMLP-induced β2 integrin up-regulation. Fig. 5 shows that LPC treatment prevented up-regulation of CD11b by fMLP, whereas in untreated neutrophils, fMLP induced an ~2-fold increase in CD11b expression. This inhibitory function of LPC may contribute to the reduced lung injury, as observed in our ex vivo lung perfusion model.

**LPC causes cAMP elevation in human neutrophils**

We recently characterized G2A, one of the potential LPC receptors identified to date, for its G protein-coupling profile (16). G2A responds to LPC stimulation with activation of G_s and elevation of intracellular cAMP concentration in transfected HeLa cells and primary T lymphocytes (16). Based on RT-PCR analysis, the transcript for G2A was detected in abundance, but the transcript for GPR4, a low affinity receptor for LPC, was barely detectable (Fig. 6A).

To determine whether LPC could affect fMLP-induced β2 integrin up-regulation. Fig. 5 shows that LPC treatment prevented up-regulation of CD11b by fMLP, whereas in untreated neutrophils, fMLP induced an ~2-fold increase in CD11b expression. This inhibitory function of LPC may contribute to the reduced lung injury, as observed in our ex vivo lung perfusion model.

**Neutrophils were treated with LPC for various times before fMLP stimulation. As shown in Fig. 6C, there was a progressive increase in the inhibitory effect for the first 2 min, after which LPC produced slightly more inhibition at 5 and 10 min. These results suggest a temporal correlation between LPC-induced elevation of intracellular cAMP concentration and inhibition of fMLP-induced superoxide generation.** The LPC-induced increase in cAMP concentration and decrease in superoxide production were consistently seen in neutrophils derived from different blood donors ($n \geq 6$).

**Inhibition of superoxide generation by different species of LPC, but priming by PAF**

Because other, less prevalent LPC species (e.g., 18:0 and 18:1) are normally present along with 16:0 LPC in plasma and tissues, we examined the effects of these phospholipids on oxidant production. As shown in Fig. 7A, both 18:0 and 18:1 LPCs exhibited inhibitory effects similar to that of 16:0 LPC. In contrast, PAF (C16), prepared in the same ethanol/water (1/1) solution, markedly primed...
neutrophil, resulting in a 7.5-fold enhancement of fMLP-induced superoxide production.

A recent report indicated that LPC stock prepared by dissolving in water or in BSA-containing aqueous solution by sonication positively regulates neutrophil oxidant production (14). To determine whether different solvents could affect the functional properties of LPC, we compared three methods of LPC preparation. As shown in Fig. 7B, an LPC stock solution made in water no longer exhibited any inhibitory effect on fMLP-induced superoxide generation. At 1 μM, the LPC thus prepared slightly enhanced fMLP-stimulated superoxide production, although at this concentration the enhancement was statistically insignificant. Likewise, the 18:0 and 18:1 species of LPC, prepared in water vs ethanol/water (1/1), exhibited similar functional differences (data not shown). Similarly, LPC prepared by sonication in water containing 1.25% essentially fatty-acid free BSA (14) did not inhibit oxidant production at 1 μM (Fig. 7B). We also tried higher concentrations of LPC dissolved in the BSA-containing aqueous solution (4.5, 10, and 30 μM) and found no significant effect on oxidant production (data not shown). Because lipids dissolved in aqueous solutions tend to form micelles, it is possible that LPC in this form loses its inhibitory effect. Interestingly, we found that the LPC preparation that inhibited superoxide generation (made by dissolving in 50% ethanol) also induced cAMP elevation, whereas the LPC preparations that did not have inhibitory effects (dissolved either in H2O or in BSA-containing aqueous solution by sonication) failed to elevate cAMP (Fig. 7C).
We have shown that fMLP-induced Ca\textsuperscript{2+} mobilization was not affected by LPC (Fig. 2), suggesting that LPC does not inhibit PLC\(\beta\) activation, because this isozyme of PLC is required for Ca\textsuperscript{2+} mobilization by the activated formyl peptide receptor. To test whether other fMLP-mediated downstream signals were affected by LPC, we examined ERK and Akt activation, because these kinases are involved in fMLP-induced NADPH oxidase activation (27–29). Phosphorylation of both ERK and Akt activation was partially inhibited by LPC preincubation in fMLP-stimulated cells (Fig. 8A). In addition, membrane translocation of the essential NADPH oxidase components p47\textsuperscript{phox} and p67\textsuperscript{phox} was inhibited by LPC preincubation in fMLP-stimulated cells (Fig. 8B). These results indicate that inhibition of fMLP-induced signaling pathways by LPC is partially responsible for the observed suppression of oxidant production.

**Role of cAMP/PKA in LPC-mediated inhibition of oxidant production**

Having determined that 16:0 LPC could inhibit neutrophil oxidant production and at the same time increase intracellular cAMP concentration, we addressed the potential signaling mechanisms activated by LPC. The cAMP-dependent protein kinase (PKA) is a serine/threonine kinase activated by cAMP that mediates important physiological functions of this second messenger (30). To determine the role of PKA in LPC-mediated inhibition of neutrophil oxidant production.
oxidant production, we used two different PKA inhibitors: H-89, a general PKA inhibitor, and Rp-cAMP, a cAMP analog that specifically inhibits cAMP-dependent PKA activation. When used for pretreatment of neutrophils, H-89 and Rp-cAMP abrogated the inhibitory effects of LPC on ERK and Akt activation as well as membrane translocation of p47^phox and p67^phox (Fig. 9, A and B). LPC-mediated inhibition of Akt activation was more effectively rescued with the general PKA inhibitor H-89 than with Rp-cAMP. The latter result suggests that a cAMP-independent PKA activation mechanism may exist and contribute to LPC-mediated inhibition of Akt activation.

We next determined whether the PKA inhibitors could also rescue LPC-mediated inhibition of superoxide generation. Neutrophils were first incubated with one of the PKA inhibitors, then treated with LPC. Although neither inhibitor alone affected basal or fMLP-induced superoxide generation, both inhibitors could rescue the inhibitory effect of LPC by ~60% in the superoxide assay (Fig. 9C). These results suggest that cAMP-mediated PKA activation is partially responsible for LPC-induced inhibition of neutrophil oxidant production. A higher dose of PKA inhibitor (10 μM H-89) provided no further correction of the inhibitory effect of LPC. The lack of a complete rescue by the PKA inhibitors suggests that a PKA-independent component may also contribute to LPC signaling.

**Discussion**

The results presented in this paper demonstrate that LPC, prepared in 50% ethanol, inhibits neutrophil NADPH oxidase activation. This inhibition is more prominent in fMLP-stimulated neutrophils than in PMA-stimulated cells, probably because PMA is a more potent and direct activator of protein kinase C. The inhibitory response is observed at relatively low concentrations of LPC (0.1–1
2988 LPC INHIBITS SUPEROXIDE GENERATION THROUGH cAMP

Because LPC does not contain the susceptible fatty acid chain length, the saturation level, and the solvent used for the preparation of stock solutions. In addition, the source of LPC and method of preparation (purification vs synthesis) can contribute to the variability in outcome due to the presence of contaminants in certain preparations. These discrepancies may be responsible for the different results obtained by several laboratories. Several published studies have shown that LPC activates calcium signaling and induces proinflammatory cytokine expression, functions that require the PAF receptor (31–33). Because LPC has not been found to bind to the PAF receptor (34), these effects may be caused by contaminants in certain LPC preparations. A study conducted by McIntyre and colleagues (35) showed that several commercially available LPC preparations were able to induce calcium mobilization in a PAF receptor-expressing cell line and to stimulate inflammation in a murine model of pleurisy. An important finding resulting from this study was that the LPC preparations used in these studies exhibited PAF and PAF-like activity that could be abolished by treatment with PAF acetylhydrolase and/or saponification (35). Because LPC does not contain the susceptible sn-2 residue, the study convincingly demonstrated that the LPC used in that work were contaminated with other biologically active phospholipids that possess proinflammatory activity.

The results obtained from this study are different from those published recently by Silliman et al. (14). In their work, LPC was dissolved in water or in aqueous solution containing essentially fatty acid-free human albumin by sonication. The lysophospholipids thus prepared were shown to prime neutrophils for oxidant production by either lysis or apoptosis. Recently, it has been shown that 16:0 LPC is produced by apoptotic cells (12). It is possible to measure the portion of endothelial cell-produced LPC is available to bind its receptor. In contrast, dissolving LPC in an aqueous solution facilitates formation of micelles, because the hydrophobic fatty acyl chain of the lipid tends to be concealed from the aqueous environment. Furthermore, LPC bound to albumin (BSA-LPC) is unable to exhibit the cAMP-elevating and NADPH oxidase inhibitory property that is displayed by the free monomeric LPC. These results reinforce the idea that the method of lysophospholipid preparation can drastically affect the functional properties of these phospholipids.

It is known that cAMP-elevating agents such as adenosine have anti-inflammatory properties and can act as negative regulators of neutrophil activation (17–20, 36). However, it is not as well known that LPC can elevate cAMP and suppress neutrophil activation. Yuan et al. (37) reported previously that LPC could elevate cAMP in platelets and inhibit platelet aggregation, agonist-induced protein kinase C activation, and thromboxane A2 generation. Additionally, Engelmann et al. (38) reported that LPC suppressed the expression of tissue factor in human monocytes, an effect related to LPC-induced elevation of cAMP levels in these cells. The latter study also used 16:0 LPC dissolved in ethanol. These findings combined with our recent observation that G2A-expressing cells respond to LPC with G2-mediated cAMP elevation (16) suggest that the LPC-mediated increase in intracellular cAMP is an inhibitory mechanism in several types of cells. The current study provides the first direct evidence for LPC-induced cAMP elevation in neutrophils.

Our investigation also shows that LPC pretreatment prevents lung injury caused by FMLP-induced neutrophil activation, presumably through inhibition of superoxide production and up-regulation of β2 integrins. These results implicate a greater pathophysiological context for LPC action. Resolution of a microbial infection occurs to the detriment of neutrophils, which die in the process by either lysis or apoptosis. Recently, it has been shown that 16:0 LPC is produced by apoptotic cells (12). It is possible to conjecture that a local elevation of LPC in extravascular tissues, perhaps released from apoptotic neutrophils and synthesized through PLA2 activation, contributes to the down-regulation of neutrophil activity once the infection is resolved. Apoptotic cells are cleared without inflicting tissue damage, and this may be attributed in part to the inhibitory effect of LPC. A published report indicates that septic patients, in whom an uncontrolled inflammatory response yields extensive tissue damage, have lower levels of plasma LPC (39). However, it is unclear at present whether LPC has a systemic effect on neutrophil activation.

Although the concentration of monomeric LPC in the circulation remains to be determined, it is reasonable to speculate that a portion of endothelial cell-produced LPC is available to bind its cell surface receptors, whereas the remaining portion complexes with albumin. Because the concentration of LPC required for its inhibitory effect is relatively low (0.1–1 μM), it is possible that LPC can suppress neutrophil oxidant production in the blood circulation and during transendothelial migration. However, other mechanisms may also exist that can effectively prevent oxidant production by circulating neutrophils. Along these lines, it becomes interesting to consider LPC as a therapeutic agent. Recently, Yan et al. (40) showed that injection of 18:0 LPC into mice protected against sepsis-induced lethality, although they attributed mouse survival to activation rather than inhibition of neutrophils. A possible explanation for the improved survival is that the protective role of LPC may be derived from its ability to inhibit, rather
than stimulate, neutrophil activation, which would serve to prevent the hyperinflammatory response associated with sepsis. In fact, other lipid mediators, such as certain lipoxins, have been shown to have anti-inflammatory properties (reviewed in Ref. 41). Despite these beneficial effects on the immune system, the use of LPC as a therapeutic agent poses considerable difficulty, because it is a lipid with varying effects on different cell types. Therefore, a potential application for this and other studies is to explore the mechanisms of cell surface receptor-mediated activation by LPC and to eventually generate pharmacological inhibitors that mimic the inhibitory effect of LPC. Future studies toward this goal will require detailed characterization of G2A and GPR14 (16, 42–44) as well as other potential receptors for LPC.

Acknowledgments

We thank Drs. Papasani Subbaiah, Mark Quinn, Oswald Quehenberger, and members of the laboratory for helpful discussions. We also thank Dr. Stephen Vogel for his help with the lung perfusion experiment, and the flow cytometry facility at the Research Resources Center at University of Illinois-Chicago for technical assistance.

Disclosures

The authors have no financial conflict of interest.

References