The Journal of Immunology

RESEARCH ARTICLE | MAY 15 2003

Calcium-Independent Phospholipase A_2 Is Required for Lysozyme Secretion in U937 Promonocytes[1] FREE

María A. Balboa; ... et. al

J Immunol (2003) 170 (10): 5276–5280.
https://doi.org/10.4049/jimmunol.170.10.5276

Calcium-Independent Phospholipase A_2 Is Required for Lysozyme Secretion in U937 Promonocytes[1]

María A. Balboa, Yolanda Sáez, and Jesús Balsinde[2]

As a part of their surveillance functions in the immune system, monocytes/macrophages secrete large amounts of the bactericidal enzyme lysozyme to the extracellular medium. We report here that lysozyme secretion in activated U937 promonocytes depends on a functional calcium-independent phospholipase A_2 (iPLA$_2$). Inhibition of the enzyme by bromoenol lactone or by treatment with a specific antisense oligonucleotide results in a diminished capacity of the cells to secrete lysozyme to the extracellular medium. Calcium-independent PLA$_2$ is largely responsible for the maintenance of the steady state of lysophosphatidylcholine (lysoPC) levels within the cells, as manifested by the marked decrease in the levels of this metabolite in cells deficient in iPLA$_2$ activity. Reconstitution experiments reveal that lysoPC efficiently restores lysozyme secretion in iPLA$_2$-deficient cells, whereas other lysophospholipids, including lysophosphatidic acid, lysophosphatidylserine, and lysophosphatidylethanolamine, are without effect. Arachidonic acid mobilization in activated U937 cells is under control of cytosolic phospholipase A_2 (cPLA$_2$). Selective inhibition of cPLA$_2$ results in a complete abrogation of the arachidonate mobilization response, but has no effect on lysozyme secretion. These results identify iPLA$_2$-mediated lysoPC production as a necessary component of the molecular machinery leading to lysozyme secretion in U937 cells and rule out a role for cPLA$_2$ in the response. Collectively, the results demonstrate distinct roles in inflammatory cell signaling for these two intracellular phospholipases. ***The Journal of Immunology*, 2003, 170: 5276–5280.**

Lysozyme degrades bacterial cell walls of Gram-positive bacteria and the chitinous components of fungal cell walls. The enzyme occurs in many body fluids, such as tears, saliva, and mucus, and is produced and secreted by phagocytic cells and a variety of cells of epithelial origin (1). Stimuli that induce lysozyme secretion from phagocytic cells also induce the phospholipase A_2 (PLA$_2$)[3]-mediated mobilization of free arachidonic acid (AA). Whether these two responses are causally related has been the subject of recent research (2–4).

PLA$_2$ enzymes are frequently classified into three main classes on the basis of whether the enzyme is secreted (sPLA$_2$), cytosolic Ca^{2+}-dependent (cPLA$_2$), or cytosolic Ca^{2+}-independent (iPLA$_2$) (5, 6). The sPLA$_2$s are low molecular mass, secreted enzymes that require millimolar concentrations of calcium for their catalytic activity and do not show fatty acid selectivity (7). The cPLA$_2$ is an 85-kDa protein that requires nanomolar to micromolar concentrations of calcium, is specific for AA-containing phospholipids, and appears to play a crucial role in agonist-induced AA mobilization (8). The iPLA$_2$ has been shown to play in important role in regulating basal phospholipid deacylation/reacylation reactions in phagocytic cells (9).

U937 promonocytic cells are derived from a human histiocytic lymphoma and can be activated by a variety of agonists, such as phorbol myristate acetate (PMA) (10, 11). U937 cells express cPLA$_2$ (12) and iPLA$_2$ (13), but not sPLA$_2$ (14). The objective of the current study was to determine the involvement of cPLA$_2$ and iPLA$_2$ in lysozyme release and AA mobilization in activated U937 cells. We report that U937 cells deficient in iPLA$_2$ activity show a decreased capacity to secrete lysozyme. On the contrary, cPLA$_2$ inhibition has no effect on lysozyme secretion, but abrogates AA release. Together, the results suggest that iPLA$_2$ and cPLA$_2$ play distinct roles in regulating the proinflammatory responses of activated U937 promonocytes.

Materials and Methods

Reagents

[5,6,8,9,11,12,14,15-^{3}H]AA (sp. act., 100 Ci/mmol) and [*methyl*-^{3}H]choline chloride (sp. act., 79 Ci/mmol) were obtained from NEN (Boston, MA). [2-^{14}C]Ethanolamine (sp. act., 57 mCi/mmol) was purchased from Amersham Pharmacia Biotech (Arlington Heights, IL). Bromoenol lactone, (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one (BEL), and methyl arachidonyl fluorophosphonate (MAFP) were from purchased Cayman Chemicals (Ann Arbor, MI). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO). The specific cPLA$_2$ inhibitor pyrrophenone was provided by Dr. K. Seno (Shionogi, Osaka, Japan) (15).

Cell culture

U937 cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) FCS, 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml). For experiments, the cells were incubated at 37°C in a humidified atmosphere of CO_2/O_2 (1/19) at a cell density of 0.5–1 $\times$ 10^6 cells/ml in 12-well plastic culture dishes (Costar, Cambridge, MA).

Treatment of the cells with antisense oligonucleotides

The antisense oligonucleotides used in these studies were derived from prior publications reporting their effects (16–18). The iPLA$_2$ antisense sequence corresponded to nt 59–78 in the murine group VI iPLA$_2$ sequence, which is conserved in human group VI iPLA$_2$ (19, 20). The antisense

Institute of Molecular Biology and Genetics, University of Valladolid School of Medicine, Valladolid, Spain

Received for publication October 31, 2002. Accepted for publication March 17, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the Ramón y Cajal Program, Spanish Research Council (to M.A.B.), Grant BMC2001-2244 from the Spanish Ministry of Science and Technology, Grant CSI-4/02 from the Education Department of the Autonomous Government of Castile and León, and Grant 011232 from Fundació La Marató de TV3.

[2] Address correspondence and reprint requests to Dr. Jesús Balsinde, Instituto de Biología y Genética Molecular, Facultad de Medicina, Universidad de Valladolid, Avenida Ramón y Cajal 7, E-47005 Valladolid, Spain. E-mail address: jbalsinde@ibgm.uva.es

[3] Abbreviations used in this paper: PLA$_2$, phospholipase A_2; AA, arachidonic acid; BEL, bromoenol lactone; cPLA$_2$, group IV cytosolic phospholipase A_2; iPLA$_2$, calcium-independent phospholipase A_2; lysoPAF, lysoplatelet-activating factor; lysoPC, lysophosphatidylcholine; lysoPE, lysophosphatidylethanolamine; MAFP, methyl arachidonyl fluorophosphonate; PAF, platelet-activating factor; PMA, phorbol 12-myristate 13-acetate; sPLA2, secreted phospholipase A_2.

0022-1767/03/$02.00

sequence was 5′-CTC CTT CAC CCG GAA TGG GT-3′. As a control, the $iPLA_2$ sense sequence was 5′-ACC CAT TCC GGG TGA AGG AG-3′. Phosphorotioate-modified oligonucleotides were used to limit degradation. The antisense and sense oligonucleotides were mixed with Lipofectamine (Invitrogen, Carlsbad, CA), and complexes were allowed to form at room temperature for 15–30 min. The complexes were then added to the cells, and the incubations were allowed to proceed under standard cell culture conditions. The final concentrations of oligonucleotide and Lipofectamine were 1 μM and 10 μg/ml, respectively. Oligonucleotide treatment and culture conditions were not toxic for the cells as assessed by the trypan blue dye exclusion assay and by quantitating adherent cell protein.

Lysozyme release assay

The cells were stimulated with PMA, Con A, or platelet-activating factor (PAF) for the indicated times. After centrifugation, the supernatants were removed, and the cell pellets were overlaid with 1 ml of PBS and homogenized. Lysozyme in the supernatants and the cell pellets was measured spectrophotometrically as follows. Briefly, 1 ml of sample was mixed with 1 ml of a *Micrococcus lysodeikticus* suspension (0.3 mg/ml in 0.1 M sodium phosphate buffer, pH 7.0). The decrease in absorbance at 450 nm was measured at room temperature. A calibration curve was constructed using chicken egg white lysozyme as a standard. Lysozyme release is expressed according to the formula: $(S/S + P) \times 100$, where S is the amount of lysozyme measured in the supernatant, and P is the amount of lysozyme measured in the cell pellets.

Measurement of AA release

The cells were labeled with 0.5 μCi/ml [^{3}H]AA for 18 h. After this period, the cells were washed an placed in serum-free medium for 1 h before the addition of 100 nM PMA in the presence of 0.5 mg/ml BSA. The supernatants were removed, cleared of cells by centrifugation, and assayed for radioactivity by liquid scintillation counting.

Lysophospholipid determination

Cells labeled with 0.5 μCi/ml [^{3}H]choline or [^{14}C]ethanolamine for 2 days were used. After the incubations, lipids were extracted with ice-cold *n*-butanol and separated by TLC with chloroform/methanol/acetic acid/water (50/40/6/0.6) as a solvent system. Spots corresponding to lysophosphatidylcholine (lysoPC) or lysophosphatidylethanolamine (lysoPE) were scraped into scintillation vials, and the amount of radioactivity was estimated by liquid scintillation counting (16).

Data presentation

Assays were conducted in duplicate or triplicate. Each set of experiments was repeated at least three times with similar results. Unless otherwise indicated, the data presented are from representative experiments.

Results

Lysozyme release in U937 cells

Lysozyme is one of the proteases involved in nonspecific immune defense against bacterial infection. Fig. 1 shows that the U937 cells released large quantities of lysozyme to the incubation medium when activated with the phorbol ester PMA. More than 60% of total enzyme was found in the supernatant after 2-h incubation with PMA. Fig. 1 also shows that lysozyme secretion was strongly blunted by the PLA_2 inhibitors BEL and MAFP, suggesting the possible involvement of $iPLA_2$ and/or $cPLA_2$ in this response.

To test the above suggestion more rigorously, the effect of an $iPLA_2$ antisense oligonucleotide on lysozyme secretion was evaluated. The antisense oligonucleotide used is the human counterpart of the murine one that we and others have successfully employed previously (16–18). In these experiments this antisense produced a 70–80% decrease in both immunoreactive $iPLA_2$ protein and cellular $iPLA_2$ activity (data not shown, but see Ref. 21). Under these conditions, lysozyme release in PMA-treated cells was strongly inhibited (Fig. 2), thus providing strong evidence for the involvement of $iPLA_2$ in this process.

The $cPLA_2$ inhibition was achieved by the use of pyrrophenone, a highly selective inhibitor of $cPLA_2$ vs $iPLA_2$ in cells (15, 22). Pyrrophenone exerted no significant effect on the release of lysozyme (Fig. 1).

Studies were conducted next to evaluate the effect of $iPLA_2$ inhibition on lysozyme release in response to the receptor-mediated agonists Con A and PAF. The significant release of lysozyme induced by both agonists was strongly abrogated by BEL (Fig. 3). These data indicate that $iPLA_2$ inhibition also leads to modulation of receptor-mediated lysozyme release in U937 promonocytes.

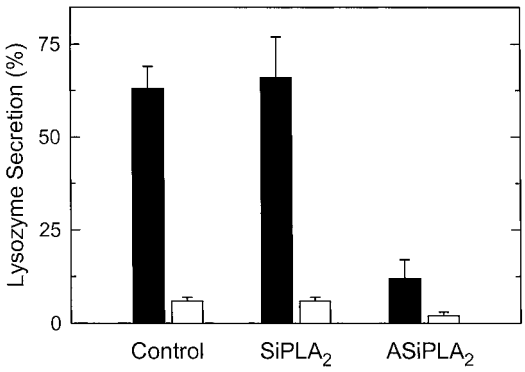

FIGURE 2. The $iPLA_2$ antisense oligonucleotide inhibits lysozyme secretion in U937 cells. The cells were either untreated (control) or treated with sense ($SiPLA_2$) or antisense ($ASiPLA_2$) oligonucleotides as indicated. Lysozyme secretion was measured in the supernatants of cells treated (■) or untreated (□) with 100 nM PMA for 2 h.

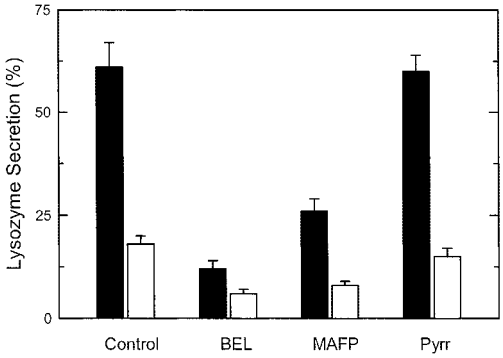

FIGURE 1. Lysozyme secretion from activated U937 cells. The cells were treated with (■) or without (□) 100 nM PMA for 2 h in the absence or the presence of 25 μM BEL, 25 μM MAFP, or 10 μM pyrrophenone (Pyrr), as indicated. Lysozyme secretion was quantitated as described in *Materials and Methods*.

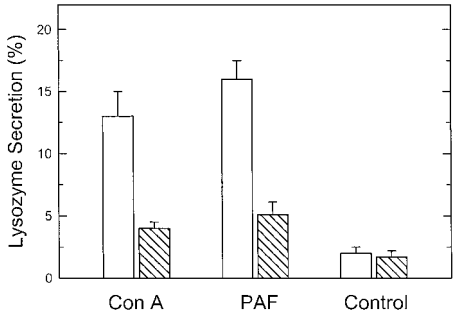

FIGURE 3. The $iPLA_2$ inhibition blocks lysozyme secretion by receptor agonists. The cells were treated with 100 μg/ml Con A, 1 μM PAF, or neither (control) as indicated, in the absence (□) or the presence (■) of 25 μM BEL. Lysozyme secretion was determined as described in *Materials and Methods*.

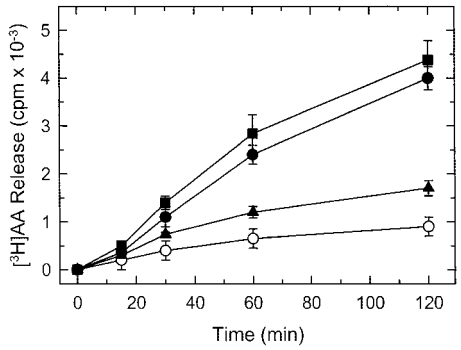

FIGURE 4. Effects of BEL and MAFP on PMA-induced AA release. The cells were treated with 25 μM BEL (■), 25 μM MAFP (▲), or neither (●) for 30 min before the addition of 100 nM PMA. ○, Control incubations, i.e., those that received neither PMA nor inhibitors. The inhibitors alone did not significantly affect the control release.

AA mobilization in activated U937 cells is not involved in lysozyme release

[^{3}H]AA-labeled U937 cells were stimulated with the phorbol ester PMA (100 nM) for different periods of time, and the release of radiolabel in the supernatant was measured. After a time lag of ~15 min, PMA-treated cells showed a modest, but significant, release of radiolabel (Fig. 4). This sustained release was completely blocked by MAFP, but was unaffected by BEL (Fig. 4), suggesting that, in agreement with previous data (13, 21), cPLA$_2$, not iPLA$_2$, mediates AA release in activated U937 promonocytic cells.

The cPLA$_2$-mediated AA release was sensitive to the unspecific kinase inhibitor quercetin (23). Fifty percent inhibition was observed at a quercetin concentration of 5 μM, while higher concentrations of the inhibitor were required to inhibit lysozyme secretion (Fig. 5). The lack of correspondence between the concentration-response effects of quercetin on AA release and lysozyme secretion suggests that both responses are unrelated.

LysoPC levels are decreased in iPLA$_2$-deficient cells

The iPLA$_2$ plays a major role in a number of cells in the regulation of basal phospholipid deacylation reactions by providing the bulk of lysoPC acceptors used in these reactions (9, 24). Fig. 6*A* shows that U937 cells made deficient in iPLA$_2$ by antisense treatment

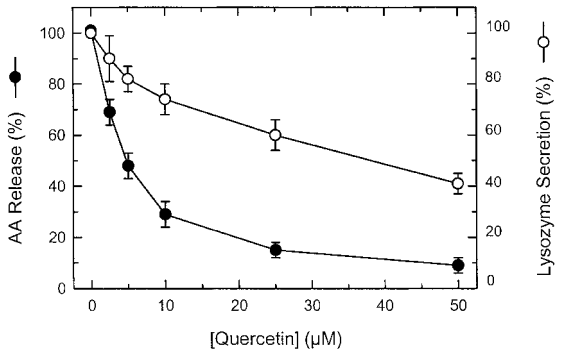

FIGURE 5. Effect of quercetin on AA release and lysozyme secretion in activated U937 cells. The cells were incubated with the indicated concentrations of quercetin for 30 min before the addition of 100 nM PMA. AA release (●) and lysozyme secretion (○) were measured as described in *Materials and Methods*. To allow for direct comparison, the data are presented as a percentage of the responses obtained in the absence of the inhibitor.

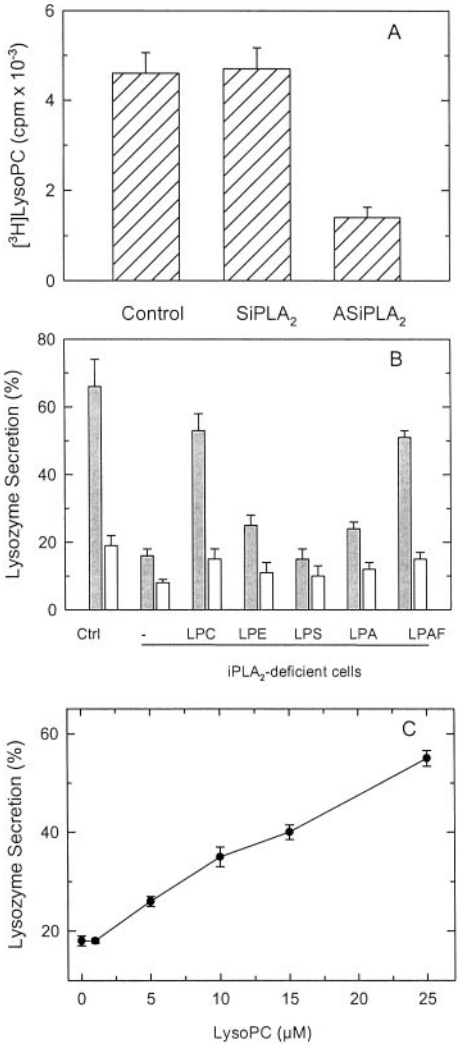

FIGURE 6. LysoPC effects on lysozyme secretion in U937 cells. *A*, Effect of iPLA$_2$ antisense oligonucleotide on lysoPC levels. The cells were labeled with [^{3}H]choline at the time they were treated with oligonucleotides. The cellular amount of lysoPC in control, sense-treated (SiPLA$_2$), or antisense-treated (ASiPLA$_2$) cells was determined as described in *Materials and Methods*. *B*, LysoPC restores lysozyme secretion in BEL-treated cells. Cells deficient in iPLA$_2$ activity by treatment with 25 μM BEL were incubated without (□) or with (■) 100 nM PMA in the presence of lysoPC (LPC), lysoPE (LPE), lysophosphatidylserine (LPS), lysophosphatidic acid (LPA), or lysoPAF (LPAF) for 2 h, as indicated. Afterward lysozyme secretion was measured as described in *Materials and Methods*. *C*, Dose response of the effect of lysoPC on the restoration of lysozyme secretion by iPLA$_2$-deficient cells stimulated with 100 nM PMA.

exhibited considerably lesser amounts of lysoPC than control cells, and this was readily observable in control unstimulated cells as well as in PMA-stimulated U937 cells. Changes in cellular lysoPC due to activation with PMA were too small to be detected (not shown). No significant effect of the iPLA$_2$ antisense on cellular levels of lysoPE in [^{14}C]ethanolamine-labeled cells was detected.

Importantly, addition of lysoPC (25 μM) restored lysozyme release in the iPLA$_2$-deficient cells (Fig. 6*B*). Other lysophospholipids tested, i.e., lysoPE, lysophosphatidylserine, and lysophosphatidic acid were ineffective. LysoPAF was as effective as lysoPC in restoring lysozyme release (Fig. 6*B*). Together, these results suggest that it is the choline headgroup of the lysophospholipid that is necessary for biological activity under these settings. The dose response of the effect of lysoPC on the restoration of lysozyme

release in activated cells is shown in Fig. 6*C*. Significant effects of lysoPC were already observed at concentrations between 5–10 μM. Lysophospholipid concentrations >25 μM induced significant lysozyme release on their own and thus were not suitable for these reconstitution experiments.

Addition of free fatty acids such as AA, palmitic acid, or linoleic acid (up to 10 μM) failed to restore lysozyme release in iPLA$_2$-deficient U937 cells (not shown). Collectively, the results suggest that lysoPC, and not other putative PLA$_2$-derived metabolites such as a free fatty acid or lysoPE, is required for U937 cells to release lysozyme to the incubation medium.

Studies of the regulation of iPLA$_2$ sctivity

Homogenates of U937 cells, either untreated or treated with PMA, were prepared, and assays were conducted to assess iPLA$_2$ activity using a vesicle substrate assay. Under these conditions we failed to detect any change in the iPLA$_2$ specific activity of homogenates from PMA-treated cells vs untreated cells. As an alternative approach, iPLA$_2$ was measured using the mammalian membrane assay system described by Diez and colleagues (25). We have previously used this system to detect iPLA$_2$ activity changes in homogenates from H_2O_2-treated U937 cells (21). In this system, purified [^{3}H]AA-labeled mammalian membranes are used as a substrate. Using this assay, again no differences in the iPLA$_2$ activity of untreated cells vs that of PMA-treated cells could be demonstrated.

Discussion

The current study addresses the possible involvement of two intracellular PLA$_2$s in lysozyme secretion in activated U937 cells. In particular, the data identify iPLA$_2$ as an important player in the secretion process and rule out a significant role for cPLA$_2$. These conclusions are based on two different approaches that yield cells deficient in either iPLA$_2$ activity or cPLA$_2$ activity, namely the use of chemical inhibitors and antisense oligonucleotides. Thus, experimental approaches leading to the blockade of cellular iPLA$_2$ result in abrogation of the cell's capacity to secrete lysozyme. On the contrary, strategies that lead to the blockade of cPLA$_2$ activity do not significantly affect lysozyme release.

The iPLA$_2$ appears to play an important role in regulating phospholipid fatty acid recycling in a variety of cell types by providing the lysophospholid acceptors used in the acylation reaction (9). Thus, inhibition of either iPLA$_2$ activity by chemical inhibitors or iPLA$_2$ expression by antisense oligonucleotides results in a marked decrease in the steady state levels of lysoPC, the primary acceptor of free AA for its incorporation into membrane phospholipids (24, 26). The contribution of iPLA$_2$ to the steady state level of cellular lysoPC appears to largely depend on cell type. Thus, it is estimated that the iPLA$_2$ contribution to cellular lysoPC levels ranges from as much as 80–90% in rat submandibular ductal cells (27) to 50–60% in phagocytic cells (17, 28, 29) and to 20–25% in rat uterine stromal cells and rat pancreatic islets (30, 31). We confirmed that in common with the aforementioned cell types, U937 cells deficient in iPLA$_2$ also exhibit significantly lower lysoPC levels.

Importantly, the low lysoPC level found in iPLA$_2$-deficient cells appears to be related to the diminished capacity of these cells to secrete lysozyme after activation. This conclusion is based on the finding that exogenous supplementation with lysoPC fully restores the capacity of the cells to release lysozyme to the extracellular medium. Interestingly, iPLA$_2$ depletion does not substantially change the steady state level of lysoPE, and exogenous lysoPE lacks the ability to restore lysozyme secretion in iPLA$_2$-deficient cells, indicating that the lysoPC effect is specific. In support of this contention, structure-function relationship studies revealed that the choline headgroup appears to be important for the biological activity of lysoPC, since lysoPAF was the only lysophospholipid tested that was able to restore lysozyme release. In turn, these studies indicate that the type of linkage present at the sn-1 position of the lysophospholipid is unimportant.

LysoPC is a natural amphiphile; it incorporates into membranes and affects membrane fluidity and permeability (32–34). For instance, lysoPC, but not lysoPE, enhances the exocytosis of ram spermatozoa treated with Ca^{2+} (35), which is in accord with the results of this study. Thus, we hypothesize that the continuing iPLA$_2$-mediated phospholipid fatty acid recycling of membranes and concomitant generation of choline-containing lysophospholipid are important for secretion to take place.

It is interesting to note that surface receptors for lysoPC that mediate some of the biological actions of this phospholipid have recently been described (36). Signaling through lysoPC receptors involves rapid activation of the mitogen-activated protein kinase cascade as well as increased intracellular Ca^{2+} levels (36). However, exogenous lysoPC neither activates the mitogen-activated protein kinase cascade nor increases the intracellular Ca^{2+} levels in U937 cells (M. Balboa and J. Balsinde, unpublished observations), thus ruling out a role for lysoPC receptor signaling in the current study.

It was somewhat unexpected that exogenous AA and other fatty acids failed to restore lysozyme secretion in iPLA$_2$-deficient cells. This suggests that stimulus-triggered increases in free AA levels have no role in regulating lysozyme secretion. In keeping with this observation the data have shown that both responses can be dissociated by the use of the unspecific kinase inhibitor quercetin. Moreover, AA mobilization in the activated U937 cells appears to be under the control of cPLA$_2$, an enzyme that has no role in lysozyme secretion. In turn, iPLA$_2$ plays no role in stimulus-induced AA mobilization in these cells. It is interesting to note that cPLA$_2$ activation transiently elevates cellular lysoPC levels in activated cells (37). Since cPLA$_2$ plays no discernible role in lysozyme release, it is the steady state level of lysoPC (iPLA$_2$-mediated), not the transient increases in this metabolite that occur as a consequence of cellular activation (cPLA$_2$-mediated), that are important for lysozyme secretion. In agreement with this observation, no changes in the specific activity of iPLA$_2$ were detected after activation of the cells with PMA.

An interesting aspect of these results, showing the importance of lysoPC in lysozyme secretion, is the possibility of functional redundancy with exogenous secreted PLA$_2$s that might act on the lysozyme-secreting cells in a paracrine fashion. Certain PLA$_2$ forms that are secreted by immunoinflammatory cells are able to attack the outer membrane phosphatidylcholine very well. These include group X PLA$_2$ (38, 39) and also group V PLA$_2$ (40). The temporal accumulation of lysoPC at discrete sites on the plasma membrane induced by these enzymes might facilitate the exocytotic process

Recently, two important cellular functions that, like enzyme secretion, require profound membrane rearrangement, have been suggested to involve the participation of the iPLA$_2$. These are chemotaxis (16) and cell spreading (41). Coincident with the results of this study, it was the constitutive activity of the iPLA$_2$ that was found to be necessary to sustain both these functions, and in addition, the contribution of iPLA$_2$ was dissociated from cPLA$_2$ activation (16, 41). Collectively, these studies underscore the importance of iPLA$_2$ in regulating processes that require changes in membrane phospholipid homeostasis and support the growing idea that the iPLA$_2$ and the cPLA$_2$ play separate and often unique roles in inflammatory cell signaling.

References

1. Lemansky, P., and A. Hasilik. 2001. Chondroitin sulfate is involved in lysosomal transport of lysozyme in U937 cells. *J. Cell Sci. 114:345.*
2. Hirasawa, N., F. Santini, and M. A. Beaven. 1995. Activation of the mitogen-activated protein kinase/cytosolic phospholipase A_2 pathway in a rat mast cell line: indications of different pathways for release of arachidonic acid and secretory granules. *J. Immunol. 154:5391.*
3. Hata, D., Y. Kawakami, N. Inagaki, C. S. Lantz, T. Kitamura, W. N. Khan, M. Maeda-Yamamoto, T. Miura, W. Han, S. E. Hartman, et al. 1998. Involvement of Bruton's tyrosine kinase in FcεRI-dependent mast cell degranulation and cytokine production. *J. Exp. Med. 187:1235.*
4. Nakatani, N., N. Uozumi, K. Kume, M. Murakami, I. Kudo I, and T. Shimizu. 2000. Role of cytosolic phospholipase A_2 in the production of lipid mediators and histamine release in mouse bone-marrow-derived mast cells. *Biochem. J. 352:311.*
5. Six, D. A., and E. A. Dennis. 2000. The expanding superfamily of phospholipase A_2 enzymes: classification and characterization. *Biochim. Biophys. Acta 1488:1.*
6. Balsinde, J., M. V. Winstead, and E. A. Dennis. 2002. Phospholipase A_2 regulation of arachidonic acid mobilization. *FEBS Lett. 531:2.*
7. Valentin, E., and G. Lambeau. 2000. Increasing molecular diversity of secreted phospholipases A_2 and their receptors and binding proteins. *Biochim. Biophys. Acta 1488:59.*
8. Dessen, A. 2000. Structure and mechanism of human cytosolic phospholipase A_2. *Biochim. Biophys. Acta 1488:40.*
9. Winstead, M. W., J. Balsinde, and E. A. Dennis. 2000. Calcium-independent phospholipase A_2: structure and function. *Biochim. Biophys. Acta 1488:28.*
10. Balsinde, J., and F. Mollinedo. 1988. Specific activation by concanavalin A of the superoxide anion generation capacity during U937 cell differentiation. *Biochem. Biophys. Res. Commun. 151:802.*
11. Balsinde, J., and F. Mollinedo. 1990. Induction of the oxidative response and of concanavalin A-binding capacity in maturing human U937 cells. *Biochim. Biophys. Acta 1052:90.*
12. Clark, J. D., N. Milona, and J. L. Knopf. 1990. Purification of a 110-kilodalton cytosolic phospholipase A_2 from the human monocytic cell line U937. *Proc. Natl. Acad. Sci. USA 87:7708.*
13. Hsu, F. F., Z. Ma, M. Wohltmann, A. Bohrer, W. Nowatzke, S. Ramanadham, and J. Turk. 2000. Electrospray ionization/mass spectrometric analyses of human promonocytic U937 cell glycerolipids and evidence that differentiation is associated with membrane lipid composition changes that facilitate phospholipase A_2 activation. *J. Biol. Chem. 272:16579.*
14. Burke, J. R., L. B. Davern, K. R. Gregor, G. Todderud, J. G. Alford, and K. M. Tramposch. 1997. Phosphorylation and calcium influx are not sufficient for the activation of cytosolic phospholipase A_2 in U937 cells: requirement for a G_i α-type G-protein. *Biochim. Biophys. Acta 1341:223.*
15. Ono, T., K. Yamada, Y. Chikazawa, M. Ueno, S. Nakamoto, T. Okuno, and K. Seno. 2002. Characterization of a novel inhibitor of cytosolic phospholipase A_2α. *Biochem. J. 363:727.*
16. Carnevale, K. A., and M. K. Cathcart. 2001. Calcium-independent phospholipase A_2 is required for human monocyte chemotaxis to monocyte chemoattractant protein 1. *J. Immunol. 167:3414.*
17. Balsinde, J., M. A. Balboa, and E. A. Dennis. 1997. Antisense inhibition of group VI Ca^{2+}-independent phospholipase A_2 blocks phospholipid fatty acid remodeling in murine $P388D_1$ macrophages. *J. Biol. Chem. 272:29317.*
18. Balsinde, J., M. A. Balboa, E. A. Dennis. 2000. Identification of a third pathway for arachidonic acid mobilization and prostaglandin production in activated $P388D_1$ macrophage-like cells. *J. Biol. Chem. 275:22544.*
19. Balboa, M. A., J. Balsinde, S. S. Jones, and E. A. Dennis. 1997. Identity between the Ca^{2+}-independent phospholipase A_2 enzymes from $P388D_1$ macrophages and Chinese hamster ovary cells. *J. Biol. Chem. 272:8576.*
20. Tang, J., R. W. Kriz, N. Wolfman, M. Shaffer, J. Seehra, and S. S. Jones. 1997. A novel cytosolic calcium-independent phospholipase A_2 contains eight ankyrin motifs. *J. Biol. Chem. 272:8567.*
21. Balboa, M. A., and J. Balsinde. 2002. Involvement of calcium-independent phospholipase A_2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem. 277:40384.*
22. Ghomashchi, F., A. Stewart, Y. Hefner, S. Ramanadham, J. Turk, C. C. Leslie, and M. H. Gelb. 2001. A pyrrolidine-based specific inhibitor of cytosolic phospholipase A_2α blocks arachidonic acid release in a variety of mammalian cells. *Biochim. Biophys. Acta 1513:160.*
23. Middleton, E., and C. Kandaswami. 1992. Effects of flavonoids on immune and inflammatory cell functions. *Biochem. Pharmacol. 43:1167.*
24. Balsinde, J. 2002. Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J. 364:695.*
25. Diez, E., F. H. Chilton, G. Stroup, R. J. Mayer, J. D. Winkler, and A. N. Fonteh. 1994. Fatty acid and phospholipid selectivity of different phospholipase A_2 enzymes studied by using a mammalian membrane as substrate. *Biochem. J. 301:721.*
26. Chilton, F. H., A. N. Fonteh, M. E. Surette, M. Triggiani, and J. D. Winkler. 1996. Control of arachidonate levels within inflammatory cells. *Biochim. Biophys. Acta 1299:1.*
27. Alzola, E., A. Perez-Etxebarria, E. Kabre, D. J. Fogarty, M. Metioui, N. Chaib, J. M. Macarulla, C. Matute, J. P. Dehaye, and A. Marino. 1998. Activation by P2 × 7 agonists of two phospholipases A_2 (PLA_2) in ductal cells of rat submandibular gland: coupling of the calcium-independent PLA_2 with kallikrein secretion. *J. Biol. Chem. 273:30208.*
28. Balsinde, J., I. D. Bianco, E. J. Ackermann, K. Conde-Frieboes, and E. A. Dennis. 1995. Inhibition of calcium-independent phospholipase A_2 prevents arachidonic acid incorporation and phospholipid remodeling in $P388D_1$ macrophages. *Proc. Natl. Acad. Sci. USA 92:8527.*
29. Daniele, J. J., G. D. Fidelio, and I. D. Bianco. 1999. Calcium dependency of arachidonic acid incorporation into cellular phospholipids of different cell types. *Prostaglandins 57:341.*
30. Birbes, H., S. Drevet, J. F. Pageaux, M. Lagarde, and C. Laugier. 2000. Involvement of calcium-independent phospholipase A_2 in uterine stromal cell phospholipid remodelling. *Eur. J. Biochem. 267:7118.*
31. Ramanadham, S., F. F. Hsu, A. Bohrer, Z. Ma, and J. Turk. 1999. Studies of the role of group VI phospholipase A_2 in fatty acid incorporation, phospholipid remodeling, lysophosphatidylcholine generation, and secretagogue-induced arachidonic acid release in pancreatic islets and insulinoma cells. *J. Biol. Chem. 274:13915.*
32. Kitagawa, T., K. Inoue, and S. Nojima. 1976. Properties of liposomal membranes containing lysolecithin. *J. Biochem. 79:1123.*
33. Fink, K. L., and R. W. Gross. 1984. Modulation of canine myocardial sarcolemmal membrane fluidity by amphiphilic compounds. *Circ. Res. 55:585.*
34. Karli, U. O., T. Schafer, and M. M. Burger. 1990. Fusion of neurotransmitter vesicles with target membranes is calcium-independent in a cell-free system. *Proc. Natl. Acad. Sci. USA 87:5912.*
35. Roldán, E. R., and C. Fragio. 1993. Phospholipase A_2 activation and subsequent exocytosis in the Ca^{2+}/ionophore-induced acrosome reaction of ram spermatozoa. *J. Biol. Chem. 268:13962.*
36. Xu, Y. 2002. Sphingosylphosphorylcholine and lysophosphatidylcholine: G-protein-coupled receptors and receptor-mediated signal transduction. *Biochim. Biophys. Acta 1582:81.*
37. Balsinde, J., and E. A. Dennis. 1996. Distinct roles in signal transduction for each of the phospholipase A_2 enzymes present in $P388D_1$ cells. *J. Biol. Chem. 271: 6758.*
38. Hanasaki, K., T. Ono, A. Saiga, Y. Morioka, M. Ikeda, K. Kawamoto, K. Higashino, K. Nakano, K. Yamada, J. Ishizaki, et al. 1999. Purified group X secretory phospholipase A_2 induced prominent release of arachidonic acid from human myeloid leukemia cells. *J. Biol. Chem. 274:34203.*
39. Pan, Y. H., B. Z. Yu, A. G. Singer, F. Ghomashchi, G. Lambeau, M. H. Gelb, M. K. Jain, and B. J. Johnson. 2002. Crystal structure of human group X secreted phospholipase A_2: electrostatically neutral interfacial surface targets zwitterionic membranes. *J. Biol. Chem. 277:29086.*
40. Cho, W. 2000. Structure, function and regulation of group V phospholipase A_2. *Biochim. Biophys. Acta 1488:48.*
41. Teslenko, V., M. Rogers, and J. B. Lefkowith. 1997. Macrophage arachidonate release via both the cytosolic Ca^{2+}-dependent and -independent phospholipases is necessary for cell spreading. *Biochim. Biophys. Acta 1344:189.*