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Arachidonic acid mobilization in P388D$_1$ macrophages is controlled by two distinct Ca^{2+}-dependent phospholipase A$_2$ enzymes

(platelet-activating factor/prostaglandin)

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ABSTRACT **Macrophage-like P388D$_1$ cells mobilize arachidonic acid (AA) and produce prostaglandin E$_2$ upon stimulation with bacterial lipopolysaccharide and platelet-activating factor. We have now demonstrated that AA mobilization in these cells is composed of two distinct events: a transient phase in which AA accumulates in the cell and a sustained phase in which the fatty acid accumulates in the incubation medium. Both phases are markedly dependent on the presence of Ca^{2+} in the extracellular medium. Treatment with an antisense oligonucleotide to group II phospholipase A$_2$ inhibits the accumulation of AA in the incubation medium, but has no effect on the accumulation of this fatty acid in the cell. In addition, treatment with antisense oligonucleotide to group II phospholipase A$_2$ has no effect on the uptake or the esterification of AA. Collectively, these results indicate that, in addition to the previously demonstrated role of group II phospholipase A$_2$ in AA mobilization in activated P388D$_1$ cells, another phospholipase A$_2$, distinct from the group II enzyme, is implicated in raising the levels of intracellular AA during the early steps of P388D$_1$ cell activation and in modulating deacylation/reacylation reactions involving AA. The data suggest that each of the different phospholipase A$_2$ enzymes present in P388D$_1$ cells serves a distinct role in cell function.**

The phospholipase A$_2$ enzymes (PLA$_2$; phosphatidylcholine 2-acylhydrolase, EC 3.1.1.4) have attracted considerable attention because of their role in a number of biological phenomena. In addition to their well-established functions as digestive enzymes (1) and in the degradation of invading microorganisms (2), PLA$_2$ enzymes are now recognized to play a central role in cellular signaling mechanisms (3) by regulating the synthesis of lipid mediators [e.g., the eicosanoids and platelet-activating factor (PAF)] as well as the remodeling of phospholipid fatty acyl chains (4–6).

The macrophage-like P388D$_1$ cell line contains several PLA$_2$ enzymes (7), including the 14-kDa group II and the 85-kDa group IV Ca^{2+}-dependent PLA$_2$s (4, 8). This fact makes this cell system an especially attractive model in which to study signaling mechanisms involving PLA$_2$ activation and the generation of prostaglandins. The presence of multiple PLA$_2$ enzymes within cells has raised the question of whether each of these enzymes serves different roles in cell function. The 85-kDa group IV PLA$_2$ has been suggested to play a crucial role in stimulus-coupled arachidonic acid (AA) release in a number of cell types (9–14). However, compelling evidence suggests that the 14-kDa secretory group II PLA$_2$, an enzyme associated with the membrane fraction in some mammalian cells (15–17), also participates in AA release and prostaglandin production in certain cell types (8, 17, 18–21). In previous work from our laboratory, antisense RNA technology has been used to investigate the role of group II PLA$_2$ in AA release and prostaglandin E$_2$ (PGE$_2$) production by activated P388D$_1$ cells (8). This approach has allowed us to establish that phospholipid hydrolysis by group II PLA$_2$ provides at least part of the free AA used for PGE$_2$ synthesis and that a cell-surface-associated pool of enzyme is involved in this process (8). However, this does not account for all of the AA released or of the PGE$_2$ produced (8).

We now report that AA mobilization in PAF-stimulated P388D$_1$ cells is temporally composed of two phases. In the first phase, AA accumulates in the cell, whereas, in the second phase, AA is released into the extracellular medium. This appears to involve at least two different PLA$_2$ enzymes.

MATERIALS AND METHODS

Materials. P388D$_1$ cells were obtained from the American Type Culture Collection. Bacterial lipopolysaccharide (LPS) Re595 was the kind gift of R. J. Ulevitch (Scripps Clinic and Research Foundation, La Jolla, CA). Iscove's modified Dulbecco's medium (endotoxin < 0.05 ng/ml) was from Whittaker Bioproducts. Fetal bovine serum was from HyClone. Nonessential amino acids were from Irvine Scientific. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid ([^{3}H]AA, 100 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear. PAF, bovine serum albumin (fatty acid free), and lipid standards were from Sigma. Lipofectin was from Life Technologies (Grand Island, NY). Silicagel G-60 TLC plates were from Avanti Polar Lipids. Organic solvents (analytical grade) were from Baker or Fisher. Antisense oligonucleotide specific for group II PLA$_2$ (ASGII) was synthesized exactly as previously described (7).

Cell Culture. P388D$_1$ cells were maintained at 37°C in a humidified atmosphere of 90% air/10% CO_2 in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin at 100 units/ml, streptomycin at 100 μg/ml, and nonessential amino acids. Adherent cells were continually selected by passage of only adherent cells. The cells used in the experiments reported below had been passaged between 15 and 30 times. Cells were plated at a density of 10^6 cells per well in six-well plates, allowed to adhere overnight, and used for experiments the following day. All experiments were conducted in serum-free

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Abbreviations: AA, arachidonic acid; ASGII, antisense oligonucleotide specific for group II PLA$_2$; LPS, bacterial lipopolysaccharide; PAF, platelet-activating factor; PC, choline-containing glycerophospholipids (phosphatidylcholine); PE, ethanolamine-containing glycerophospholipids (phosphatidylethanolamine); PGE$_2$, prostaglandin E$_2$; PI, inositol-containing glycerophospholipids (phosphatidylinositol); PLA$_2$, phospholipase A$_2$; PS, serine-containing glycerophospholipids (phosphatidylserine).

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Iscove's modified Dulbecco's medium. When required, radiolabeling of the cells was achieved by including [^{3}H]AA (0.5 μCi/ml) in the medium during the overnight adherence period.

Stimulation of P388D$_1$ Cells. The standard regimen for activating cells with LPS and PAF has been described previously (8). Briefly, P388D$_1$ cells were placed in serum-free medium for 30–60 min before the addition of LPS (200 ng/ml) for 1 h. After the LPS incubation, cells were challenged with 20 nM PAF for the time indicated.

Antisense Inhibition of Group II PLA$_2$ Expression in P388D$_1$ Cells. Transient transfection of P388D$_1$ cells with ASGII (5′-GATCCTCTGCCACCCACACC-3′) or sense strand oligonucleotide to group II PLA$_2$ (5′-GGTGTGGGTGGCAGAGGATC-3′) was carried out as described (8). Briefly, P388D$_1$ cells were transfected with 250 nM ASGII in the presence of 5 μg/ml Lipofectin under serum-free conditions for 6 h prior to cell activation. When cells labeled with [^{3}H]AA were used, the [^{3}H]AA was added at the beginning of the transfection (8). The incorporation and distribution of [^{3}H]AA among phospholipids at 6 h was the same whether or not serum was included in the incubation medium. In addition, ASGII-transfected cells incorporated [^{3}H]AA to the same extent as control sense-strand transfected cells or untransfected cells.

Measurement of [^{3}H]AA Release and Cell-Associated Free [^{3}H]AA. LPS-treated, [^{3}H]AA-labeled cells were stimulated with 20 nM PAF for the times indicated. For [^{3}H]AA release experiments, the prelabeled cells were stimulated in serum-free medium containing 5 mg/ml bovine serum albumin (fatty acid free) as a trap for liberated [^{3}H]AA. After the indicated times, supernatants were removed, cleared of detached cells by centrifugation, and assayed for radioactivity by liquid scintillation counting. For analysis of cell-associated, free [^{3}H]AA, the cell monolayers were scraped in 1 ml of 0.05% Triton X-100. After detergent extraction (22), lipids were separated by thin-layer chromatography using *n*-hexane/diethyl ether/acetic acid (70:30:1, vol/vol) as the solvent system. Authentic AA was cochromatographed and visualized by exposing the plates to iodine vapors. Areas containing AA were scraped and radioactivity was measured by liquid scintillation counting. Extracellular [^{3}H]AA release is expressed as the percentage of radioactivity released to the incubation medium compared with the total cellular radioactivity. Cell-associated, free [^{3}H]AA is expressed as the percentage of radioactivity in free [^{3}H]AA in cells compared with total cellular radioactivity.

Measurement of [^{3}H]AA Uptake and Incorporation into Cellular Phospholipids. Unlabeled cells were exposed to exogenous [^{3}H]AA (0.5 μCi/ml). At the indicated times, supernatants were removed and the cell monolayers were washed with medium containing 5 mg/ml albumin. Supernatants and wash media were combined and assayed for radioactivity by liquid scintillation counting. Fatty acid uptake is expressed as a percentage of the total radioactivity originally present in the medium (23). The cell monolayers were scraped with 0.05% Triton X-100, and total lipids were extracted according to Bligh and Dyer (22). Phospholipids were separated by thin-layer chromatography with chloroform/methanol/ammonia (65:25:5, vol/vol). Authentic phospholipid standards were run in parallel and visualized by exposing the plates to iodine vapors. Areas of the TLC plates containing the specific cellular phospholipids were scraped and the percentage of each phospholipid class as a function of the total was estimated by liquid scintillation counting.

Measurement of PGE$_2$ Production. PGE$_2$ was measured in the supernatant of unlabeled cells by using a PGE$_2$-specific radioimmunoassay (Advanced Magnetics, Cambridge, MA).

RESULTS

[^{3}H]AA Incorporation into Membrane Phospholipids. Experiments were performed to determine the extent of incorporation of exogenous, radiolabeled AA into P388D$_1$ cell phospholipids. As shown in Fig. 1, [^{3}H]AA was quickly taken up by the cells. Approximately 60% of the total [^{3}H]AA added to the medium became cell associated within the first 5 min of incubation. Remarkably, the same pattern of uptake occurred at concentrations of [^{3}H]AA in the culture medium of 5 nM or 1 μM (Fig. 1*A*). Approximately 25% of the total [^{3}H]AA added to the medium was found to be esterified in phospholipids at 15 min (Fig. 1*B*), whereas neutral lipids contained less than 5% (data not shown) and 30% remained as free fatty acid. At very early incubation times (up to 5 min), PC contained the bulk of the [^{3}H]AA recovered in phospholipids, representing approximately 60% of the total radioactive phospholipids. However, shortly thereafter the distribution of radioactivity in phospholipids changed, such that PE became the predominant [^{3}H]AA-containing class, accounting for approximately 60% of total radioactivity in phospholipids (Fig. 1*C*).

After 5 h, the incorporation of radioactivity into cell phospholipids ceased, and no further changes in the distribution of radioactivity in phospholipids occurred. Accordingly, all subsequent [^{3}H]AA release experiments were conducted with cells prelabeled for at least 5 h. It is interesting to note that the distribution of radiolabeled AA into phospholipids at 5 h is similar to that of endogenous, preexisting AA, as measured by gas-liquid chromatography (R. Asmis and E.A.D., unpublished data).

[^{3}H]AA Release and PGE$_2$ Production in PAF-Stimulated P388D$_1$ Cells. Given the high capacity of P388D$_1$ cells to

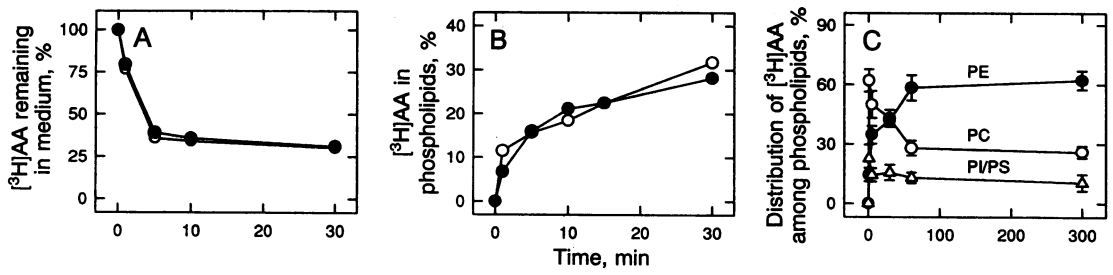

FIG. 1. AA incorporation into P388D$_1$ cell phospholipids. Cells were incubated for the indicated periods of time in the presence of 5 nM (●) or 1 μM (○) [^{3}H]AA (0.5 μCi) and the amount of AA remaining in the medium (*A*) or incorporated into phospholipid (*B*), expressed as a percentage of total radioactivity added to the medium, is indicated. These data are means ± SEM from triplicates in a representative experiment. (*C*) Cells were exposed to 0.5 μCi of [^{3}H]AA (5 nM) for the indicated periods of time. Phospholipids were separated into classes by thin-layer chromatography. The radioactivity incorporated into each phospholipid class is expressed as a percentage of the total radioactivity found in all phospholipid classes. These data are presented as means ± SEM of four experiments. PC, choline-containing glycerophospholipids; PE, ethanolamine-containing glycerophospholipids; PI, inositol-containing glycerophospholipids; PS, serine-containing glycerophospholipids.

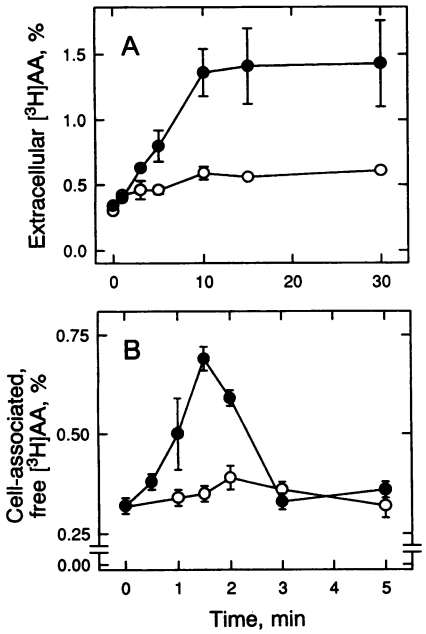

FIG. 2. AA release in activated P388D$_1$ cells. (*A*) Time course of the extracellular release of [^{3}H]AA. (*B*) Time course of the accumulation of cell-associated, free [^{3}H]AA. LPS-primed cells, prelabeled with [^{3}H]AA for 20 h, were incubated with (●) or without (○) PAF (20 nM). Radioactivity released into the medium (*A*) or cell-associated [^{3}H]AA (*B*) was measured at the indicated time points. Data in *A* represent the means ± SEM of three different experiments and are expressed as the percentage of radioactivity released into the incubation medium compared to the total cellular radioactivity. Data in *B* are presented as means ± SEM from one experiment with triplicate determinations and are expressed as the percentage of radioactivity in free [^{3}H]AA in cells compared to the total cellular radioactivity.

incorporate [^{3}H]AA into phospholipids, we examined the question of whether PAF inhibited incorporation of [^{3}H]AA into phospholipids as a mechanism for increasing free [^{3}H]AA levels. For this purpose, LPS-primed P388D$_1$ cells were treated with or without stimulus (20 nM PAF) in the presence of varied amounts of exogenous [^{3}H]AA. We found no differences in [^{3}H]AA incorporation between PAF-stimulated and control cells under any condition tested (data not shown). Therefore, these data suggest that inhibiting esterification of AA is not a mechanism for enhancing free AA levels in PAF-stimulated P388D$_1$ cells.

Fig. 2*A* shows the time course of PAF-induced AA release from prelabeled P388D$_1$ macrophage-like cells. AA release proceeded linearly from 1 to 10 min, after which it began to plateau. AA release was measured in the presence of 5 mg albumin per ml of incubation medium. The use of albumin allows one to measure the amounts of AA cleaved from phospholipids, since trapping of AA by albumin decreases cellular AA utilization. For this reason, unmetabolized AA accumulates predominantly in the supernatants under these experimental conditions. In PAF-stimulated P388D$_1$ cells, more than 99% of released radioactive material remains as AA in the presence of albumin (24). AA release into the incubation medium was preceded by a rapid and transient accumulation of free [^{3}H]AA in the cell (Fig. 2*B*). Maximal accumulation of cellular, free [^{3}H]AA was observed at 1.5 min, after which a sharp decrease was detected that was likely due to reacylation (Fig. 1) or release into the extracellular medium (Fig. 2*A*).

In parallel with both phases of AA release, LPS-primed, PAF-stimulated P388D$_1$ cells synthesized and released PGE$_2$ into the incubation medium. In the absence of albumin, PGE$_2$ production was detected by 30 sec after PAF addition to the cells and was maximal after 3–5 min (Fig. 3). PAF-treated P388D$_1$ cells secreted all of the PGE$_2$ they produced into the incubation medium; none was detected in the cells (data not shown).

The incubation medium in which the above experiments were performed contained 1.3 mM Ca^{2+}. To determine whether PAF-stimulated [^{3}H]AA mobilization required the presence of extracellular calcium, the effect of removing calcium from the incubation medium on extracellular AA release and on accumulation of intracellular, free AA was examined. As shown in Table 1, both processes were inhibited by the absence of extracellular calcium.

Role of Group II PLA$_2$ in AA Metabolism in P388D$_1$ Cells. Using antisense RNA technology, we have previously demonstrated that treatment of P388D$_1$ cells with a phosphorothioate antisense oligonucleotide specific for ASGII blocks the expression of the enzyme (8). In the current work, we have used this reagent to further investigate the role of group II PLA$_2$ in the AA metabolism of LPS-primed, PAF-stimulated P388D$_1$ cells. Group II PLA$_2$ depletion by ASGII had no effect on PAF-induced accumulation of cell-associated, free [^{3}H]AA (Fig. 3*A*). In addition to using nontransfected cells as a control for this experiment, as an additional control, other cells were transfected with the sense complement of oligonucleotide ASGII as shown in Table 2. Within experimental error, the results are consistent with a lack of effect of ASGII on cell-associated AA release. Compared with the controls, transfection of the cells with ASGII decreased both PAF-stimulated [^{3}H]AA release into the extracellular medium (Fig. 3*B*) and PGE$_2$ production (Fig. 3*C*). These results are comparable to our previous data showing a 60% decrease in PGE$_2$ production in ASGII-treated cells (once the data are corrected for the nonspecific effects of oligonucleotide treatment, indicated by the effects of the sense oligonucleotide) (8).

We also studied the effect of group II PLA$_2$ depletion by ASGII on the incorporation of AA into phospholipids. For

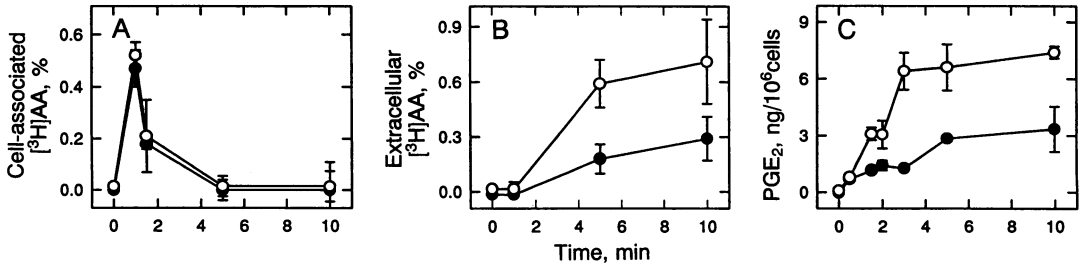

FIG. 3. Effect of antisense inhibition of group II PLA$_2$ on AA metabolism. LPS-primed cells, either treated (●) or untreated (○) with ASGII (8), were stimulated with 20 nM PAF for the indicated periods of time. Cell-associated [^{3}H]AA accumulation (*A*), [^{3}H]AA release into the extracellular medium (*B*), and PGE$_2$ production (*C*) were determined as described under *Materials and Methods*. Background values at each time point have been subtracted to allow direct comparison. Data are presented as means ± SEM from one experiment with triplicate determinations.

Table 1. Effect of extracellular Ca^{2+} on cell-associated, free [^{3}H]AA

Cells	Cell-associated, free [^{3}H]AA, %		Extracellular [^{3}H]AA, %	
	Without Ca^{2+}	With Ca^{2+}	Without Ca^{2+}	With Ca^{2+}
Unstimulated	0.23 ± 0.01	0.24 ± 0.01	0.55 ± 0.10	0.77 ± 0.11
PAF-stimulated	0.37 ± 0.01	0.61 ± 0.02	0.77 ± 0.06	1.63 ± 0.07

Cell-associated [^{3}H]AA was measured by thin-layer chromatography in prelabeled, LPS-primed cells incubated for 1.5 min with or without PAF (20 nM) in the absence or presence of 1.3 mM $CaCl_2$ in the incubation medium. The Ca^{2+}-free medium contained, in addition, 1 mM EGTA. Data are presented as means ± SEM from one experiment with triplicate determinations. Extracellular release of [^{3}H]AA was measured in prelabeled LPS-primed cells incubated for 15 min with or without PAF (20 nM) in the absence or presence of 1.3 mM $CaCl_2$ in the incubation medium. The Ca^{2+}-free medium contained, in addition, 1 mM EGTA. These data are expressed as the means ± SEM of two experiments with triplicate determinations.

this purpose, unlabeled cells were exposed to 250 nM ASGII in the presence of 5 μM Lipofectin under serum-free conditions for 6 h. After that time, both ASGII-treated and control untreated cells were exposed to [^{3}H]AA (0.5 μCi, 5 nM) for different periods of time, and the radioactive content in each phospholipid class was estimated by liquid scintillation counting. As shown in Table 3, ASGII treatment did not significantly alter the esterification or remodeling of [^{3}H]AA in P388D$_1$ cell phospholipids, indicating that group II PLA_2 does not modulate deacylation/reacylation reactions involving AA.

DISCUSSION

Role for Two PLA_2 Enzymes. PAF-stimulated AA mobilization in P388D$_1$ macrophage-like cells is composed of two different phases. In the first phase, the free fatty acid accumulates in the cell. In the second phase, AA is released into the incubation medium. An early accumulation of free AA in the cell following receptor occupancy has also been documented in bombesin-stimulated Swiss 3T3 cells (25), formyl peptide-activated, LPS-primed, human neutrophils (26), zymosan-stimulated, murine peritoneal macrophages (27), and antigen-stimulated, rat mast cells (28). Interestingly, both phases of the PAF-stimulated AA release are markedly dependent on the presence of Ca^{2+} in the incubation medium. Therefore the data highlight the necessity of extracellular Ca^{2+} for PAF to fully activate AA release in macrophages and macrophage-like cell lines (29–31). However, removal of external Ca^{2+} did not completely inhibit either the first or the second phase of the AA mobilization response. This minor AA mobilization in Ca^{2+}-free medium may be due to the PAF-induced discharge of intracellular Ca^{2+} stores (32), as suggested previously in other systems (29, 33–35).

We have previously demonstrated that group II PLA_2 is involved in PGE_2 production from PAF-stimulated P388D$_1$ cells (8). In the present work, we have analyzed the contribution of group II PLA_2 to overall AA mobilization upon PAF stimulation. The results clearly indicate that a significant portion of the AA mobilized by PAF cannot be ascribed to group II PLA_2 activity. Treatment of the cells with antisense oligonucleotide ASGII does not abolish the extracellular release of both [^{3}H]AA and PGE_2 to the external medium. Furthermore, ASGII-treated cells exhibit an intracellular AA accumulation following PAF challenge almost identical to that observed in untreated cells. Although it remains possible that the residual AA released from ASGII-treated cells is due to a stable pool of group II PLA_2, these data suggest that another PLA_2 is involved in AA mobilization as well. By analogy with data from other systems (10, 17), it would be logical for this intracellular activity to belong to the 85-kDa Ca^{2+}-dependent group IV PLA_2, an enzyme that is present in P388D$_1$ cells (8). This would be consistent with the observation that the two phases of AA mobilization in activated cells are markedly dependent on extracellular Ca^{2+} (Table 1). P388D$_1$ cells contain a third different PLA_2, a Ca^{2+}-independent cytosolic enzyme that is able to cleave AA-containing phospholipids (36). Because of the Ca^{2+} dependency of PAF-stimulated AA mobilization in LPS-primed cells, a role for such an enzyme in that process seems unlikely.

Pfeilschifter *et al.* (21) have recently reported a group II PLA_2 in rat mesangial cells that contributes in part to PGE_2 production induced by the cytokines tumor necrosis factor α and interleukin 1β. In these cells, incubation with an anti-group II PLA_2 monoclonal antibody attenuates cytokine-stimulated PGE_2 production by about 50%, and incubation with the group II PLA_2 inhibitor CGP43182 reduces the response by about 70%. Since the monoclonal antibody employed by Pfeilschifter *et al.* (21) did not cross the plasma membrane, the group II PLA_2 involved was most likely localized at the outer surface of the cell. This observation is

Table 2. Effect of sense and antisense oligonucleotides specific for group II PLA_2 on AA metabolism in PAF-stimulated cells

Cells	Relative stimulation, fold increase		
	Cell-associated, free [^{3}H]AA	Extracellular [^{3}H]AA	PGE_2
Untransfected	1.6 ± 0.3	2.2 ± 0.4	7.0 ± 1.8
ASGII-transfected	1.5 ± 0.4	1.4 ± 0.1	3.1 ± 0.9
SGII-transfected	1.6 ± 0.2	1.9 ± 0.4	7.1 ± 1.1

Cells were transfected with ASGII or the sense strand oligonucleotide to group II PLA_2 (SGII). Cell-associated, free AA, extracellular AA, and PGE_2 production were determined as described in *Materials and Methods*. Data, presented as the ratio of PAF-stimulated to unstimulated cells, are means ± SEM from one experiment with triplicate determinations.

Table 3. Effect of group II PLA_2 depletion on AA incorporation into phospholipids

Phospholipid	[^{3}H]AA, %*			
	10 min		90 min	
	Control	ASGII	Control	ASGII
Total	18 ± 3	15 ± 2	33 ± 5	27 ± 3
Subclass				
PE	53 ± 19	47 ± 12	66 ± 12	65 ± 14
PC	41 ± 15	41 ± 7	26 ± 7	27 ± 9
PI/PS	6 ± 1	12 ± 2	8 ± 5	8 ± 1

Cells were transfected with ASGII or the sense strand complement of ASGII (control) as described in *Materials and Methods*. After the transfection, cells were incubated with 0.5 μCi of [^{3}H]AA (5 nM) and the incorporation of radiolabeled fatty acid into each phospholipid class was measured under each experimental condition.

*The [^{3}H]AA in total phospholipids is expressed as the percentage of radioactivity incorporated in all phospholipids compared to the total radioactivity originally present in the medium. For the phospholipid subclasses, the percent values given are for the radioactivity incorporated into each phospholipid subclass expressed as a percentage of the total radioactivity found in all phospholipids.

consistent with our previous data suggesting that an extracellular pool of group II PLA_2 is involved in PGE_2 production in activated $P388D_1$ cells (8). Once bound outside the cell, group II PLA_2 may encounter the millimolar levels of Ca^{2+} that this enzyme requires for full activity. An extracellularly localized PLA_2 has also been implicated in prostaglandin production in human endothelial cells (17), rat mast cells (37), and mouse peritoneal macrophages (38).

AA Esterification in $P388D_1$ Cell Phospholipids. This work has also demonstrated that $P388D_1$ cells manifest a high capacity to import exogenous [^{3}H]AA. The AA is incorporated into PC and PI/PS first, followed by a rapid transfer to PE, mainly at the expense of PC (Fig. 1). This indicates that there is a high AA turnover in these cells. Although this process is qualitatively similar to that reported in other cell systems (39–41), what makes it unusual in the $P388D_1$ cell is that it is extremely rapid when compared with other cells (see Fig. 1 and refs. 39–41). The amount of AA present in normal macrophages represents about 25% of total cellular fatty acid (42). In $P388D_1$ cells, however, AA represents roughly only 3% (R. Asmis and E.A.D., unpublished data). Thus, it could be that the remarkably high capacity of $P388D_1$ cells to incorporate and redistribute AA in phospholipids is due to their relative "deficiency" in endogenous AA when compared with normal macrophages.

Unlike other fatty acids, AA is not incorporated during *de novo* synthesis of phospholipids, but enters at a later stage by acylation of phospholipids (43). Hence, a lysophospholipid acceptor is necessary for the esterification of exogenous [^{3}H]AA into $P388D_1$ cell phospholipids. It follows that the $P388D_1$ cell line should exhibit a basal PLA_2 activity high enough to account for its capacity to incorporate free AA. Our antisense inhibition studies have clearly shown that group II PLA_2 is not implicated in this process, indicating that another, as yet unidentified, PLA_2 modulates AA deacylation/reacylation reactions in $P388D_1$ cells.

Despite the presence of albumin in the incubation medium, AA release in LPS-primed, PAF-stimulated $P388D_1$ cells is rather modest, accounting for a 2- to 3-fold increase over constitutive background levels. The data presented in Fig. 1 suggest that in $P388D_1$ cells, the AA reacylation pathway could play an effective role in controlling cellular, free AA levels. PAF-stimulated PGE_2 production occurs very rapidly (within 30 sec) and has ceased after 3–5 min. Thus, it can be envisioned that in PAF-stimulated $P388D_1$ cells, the AA reacylating enzymes would efficiently divert a significant amount of AA away from the cyclooxygenase, and, in this manner, limit PGE_2 synthesis.

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