Interleukin-1 Rapidly Stimulates Lysophosphatidate Aciyltransferase and Phosphatidate Phosphohydrolase Activities in Human Mesangial Cells*

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Phosphatidic acid (PA) is a cytokine in a variety of cell types, and an intermediary in cell activation. It is produced from membrane phospholipids by either lysophosphatidate acyl-CoA:acyltransferase (lyso-PA AT) or phospholipase D. Interleukin-1 (IL-1) stimulation of human mesangial cells (HMC) induced activation of lyso-PA AT, and synthesis of new PA species with significant increase in PA mass. These PA species were enriched in long-chain unsaturated acyl side chains (C18:1, C18:2, C20:5, and C22:6) in both the sn-2 and sn-1 positions, and stimulated the action of the lyso-PA AT as a positive feedback mechanism. Gas-liquid chromatography and mass spectrometry demonstrated that the acyl composition of phosphatidic acid does not resemble that of the major phospholipid fractions of this preparation and therefore is not the product of phospholipase D. The PA species were rapidly converted to 1,2-sn-diacylglycerols by phosphatidate phosphohydrolase, which was also activated by IL-1 via a separate mechanism involving a pertussis-sensitive G-protein. The activities of lyso-PA AT and phosphatidate phosphohydrolase were associated with plasma membrane enriched and refined microsomal fractions. IL-1 stimulation of a murine T cell (thymoma) line, EL-4, also caused stimulation of lyso-PA AT, resulting in PA formation. EL-4 mutants with defective IL-1 receptors did not demonstrate stimulation of lyso-PA AT, showing the necessity of intact IL-1 receptors for activation of this enzyme. We conclude that PA is a significant signaling intermediary for IL-1 via activation of lyso-PA AT and a G-protein, which activates phosphatidate phosphohydrolase. This system suggests a novel mechanism whereby a low intensity signal may be translated into cellular activation.

The cytokines interleukin-1α and -1β (IL-1α and IL-1β) are central mediators of inflammation (Dinarello, 1985; Mizel, 1989). The cellular responses to IL-1 molecules are protein, and include activation of T and B lymphocytes, maturation of precursor thymocyte forms, and induction of lymphokine synthesis. IL-1 binding induces proliferation of T and B cells, fibroblasts, smooth muscle cells, and glomerular mesangial cells (Lovett and Ryan, 1983; Mizel, 1988). In addition to glomerular mesangial cell (MC) proliferation, IL-1 induces production of prostaglandin E2 and a type IV collagenase in rat and human MC (Lovett et al., 1986, 1986, 1987). IL-1 may act in the glomerulus to amplify local tissue injury processes, such as those seen in acute immune complex-mediated glomerulonephritis (Werber et al., 1987).

The sequence in cellular signaling initiated by IL-1 has not been completely elucidated. In murine 70Z/3 B lymphocytes, binding of IL-1 to a high affinity receptor results in enhanced GTP binding to a pertussis toxin-sensitive G-protein (Gp), which then activates an adenyl cyclase with resulting increases in cAMP concentration (Shirakawa et al., 1988; Chedid et al., 1989). However, this pathway does not appear to play a role in all cell lines affected by IL-1 (Abraham et al., 1987; Mizel, 1989). Binding of IL-1 to its receptor complex in T lymphocytes and fibroblasts causes little to no change in cAMP levels, suggesting functional differences between IL-1 receptors on these cells and those found on B lymphocytes (Bomsztyk et al., 1989; Ballou et al., 1991). IL-1-signal transduction in some T lymphocytes appears to involve conversion of phosphatidylcholine to 1,2-diacylglycerols rather than derivation of DAG from the polyphosphoinositide cycle (Rosoff et al., 1988). Similarly, fibroblast stimulation by IL-1 does not seem to involve the polyphosphoinositide pathway, but rather may involve production of DAG from another phospholipid (Ballou et al., 1991). However, activation of protein kinase C has been reported only in one lymphoid cell type, T sub 2 (Munoz et al., 1990), which might indicate a variant function for

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The abbreviations used are: IL-1, interleukin-1α; AT, acyltransferase; BSA, bovine serum albumin; cis-PI, cis-parinaric acid (9,11,13,15-all-cis-octadecatetraenoic acid); DAG or 1,2-DAG, 1,2-sn-diacylglycerol; diilioloeoyl-PC, 1,2-sn-dilinoleoyl phosphatidylcholine; FAB/MS, fast-atom bombardment mass spectrometry; GLC, gas-liquid chromatography; Gp, G-protein; HMC, human mesangial cells; HPLC, high performance liquid chromatography; lyso-PA AT, lysophosphatidic acid acyltransferase; mAb, monoclonal antibody; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PBS, phosphate-buffered sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GTPγS, guanosine 5'-O-(thiotriphosphate); ANOVA, analysis of variance.
diacylglycerols in T cell/fibroblast systems.

Phosphatidic acid (PA) has been implicated as a cytokine in 3T3 fibroblasts (Moolenaar et al., 1986; Yu et al., 1987). This simple phospholipid interacts with arachidonate and glucagon to stimulate calcine influx in hepatocytes (Alton and Bygrave, 1987). Lyso-PA, a precursor of PA, is also a cytokine and Gp activator in 3T3 fibroblasts (VanCorven et al., 1989). PA can be synthesized by three distinct pathways: 1) DAG kinase phosphorylation of 1,2-sn-DAG, 2) lyso-PA acyl-CoA:acyltransferase (lyso-PA AT)-catalyzed acylation of lysophosphatidic acid, and 3) phospholipase D hydrolysis of phospholipids, particularly phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Farese et al., 1987; Bocckino et al., 1987; Martin, 1988; Martin and Michaelis, 1989). The PA generated in cells can be dephosphorylated via phosphatidate phosphohydrolyase to 1,2-DAG (Farese et al., 1987; Martin, 1988; Qian and Drewes, 1990), or can be metabolized to CDP-DAG. Lyso-PA AT may be regulated by phosphorylation related to calmodulin-associated kinase, whereas activation of phospholipase D is associated with Gp activation (Soeling et al., 1988; Bocckino et al., 1987).

We have recently suggested that PA is an important cellular activator of MC (Bursten et al., 1989) and that lipid A may derive its biological effect from its mimicry of PA structure. In this paper we show that IL-1 acts to stimulate rapid formation of PA by a pathway involving lyso-PA AT acylation of lyso-PA. IL-1 initially activates lyso-PA AT to produce transiently a subspecies of PA enriched in palmitate and C18:1, C18:2, C20:5, C22:6 unsaturated fatty acids. The newly synthesized PA is converted in 15-30 s into 1,2-DAG by phosphatidate phosphohydrolyase. Lyso-PA AT activity is not regulated via a G-protein, whereas phosphatidate phosphohydrolyase is dependent upon the initial activation of a pertussis-sensitive Gp. These pathways interact independently of phosphoinositide metabolism, which is quiescent throughout the first 5 min after IL-1 stimulation. These data indicate the presence of a unique signal pathway activated by IL-1.

**EXPERIMENTAL PROCEDURES**

*Materials—* Proliferating human MC were maintained in RPMI 1640 containing 20% fetal bovine serum (Irvine Scientific, Irvine, CA) and supplemented with 30 units/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml vancomycin (Sigma), 5 µg/ml transferrin, 10 µM insulin, and 5 ng/ml selenous acid (ITS Pre-mix, Collaborative Research, Waltham, MA). All media components were screened for the presence of exogenous endotoxin using a Limulus amoebocyte lysate assay sensitive to 10-100 pg of endotoxin/ml (E-toxate, Sigma). Human recombinant interleukin-1α was purchased from Endogen (Boston, MA) and treated with pertussis toxin, 0.1-100 ng/ml, for 4 h prior to harvesting and microsomal preparation. EL-4 wild-type cells and EL-4 mutants were maintained as previously described (Bomskyt et al., 1989).

Microsomes (crude microsomal fraction containing plasma membranes) were prepared from HMC and EL-4 cells as reported (Lovett et al., 1988). Confluent human MC layers were washed in PBS (4 °C), scraped, and pelleted by centrifugation at 400 X g for 10 min. The pellets were suspended in 20 mM sodium borate, pH 10.2, 10 mM EDTA, 1 µg/ml pepstatin, and 2 mM phenylmethylsulfonyl fluoride, and homogenized by disruption with a French press (50 strokes) with tight-fitting Dounce homogenizers. The homogenate was centrifuged at 500 X g for 10 min and the supernatant recentrifuged at 15,200 X g for 25 min. The resultant crude microsomal preparation was washed in 20 mM HEPES, pH 7.4, followed by centrifugation immediately or stored in liquid nitrogen at a concentration of 2-6 µg protein/µl. Microsomal protein was quantitated using the Bradford (1976) method. Plasma membrane-enriched and refined microsomal fractions were prepared by layering crude microsomal fractions on a 35% (w/w) sucrose cushion in 20 mM HEPES buffer, pH 7.4, followed by centrifugation at 75,000 X g for 30 min, and removing the plasma membrane-enriched fraction. The plasma membrane-enriched fraction was verified in three ways: 1) a 30-fold increase in Na+/K+-ATPase activity as compared to crude microsomes, 2) transmission electron microscopy after staining with 2% aqueous uranyl acetate and lead citrate demonstrated >90% open mesangial cells and >80% open capillary loops of bilayers, and 3) the presence of IL-1 receptors as determined by 125I-IL-1 photolabeling (described in detail in Lovett et al., 1988).

**Whole Cell-labeling Studies—* Human MC were grown to near-confluence in 75-cm² culture flasks in complete growth medium. The medium was removed, the cell layers washed three times in 37 °C PBS, and fresh serum-free medium containing 0.5% BSA added. To label the glycerol moiety of cellular lipids, the cultures were incubated for 4 h at 37 °C with 5 µCi/ml [3H]glycerol (diluted with unlabeled glycerol to a final specific activity of 400 mCi/mmol). The labeling medium was removed and the cell layers washed twice with 37 °C PBS. Fresh medium containing 0.5% BSA with or without 10-10 M IL-1 or -10 was added. Flasks were harvested at sequential times by washing twice with 4 °C PBS, followed by two washes with methanol. Precipitated cells were scraped from the dishes and the lipids extracted in 2:1 chloroform:methanol (Folch et al., 1957). For [3H]glycerol labeling, washed cell layers were incubated for 3 h with 0.2 µCi/ml [3H]glycerol (3.5 Ci/mmol), and [14C]arachidonate-labeled cells were incubated with 5 µCi/ml (51 mCi/mmol). Lipids were extracted with chloroform:methanol:water in a 2:1:0.1 or 3:1:1 ratio and fractionated on thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) as described below. Gas-liquid chromatography (GLC) of methyl esters was used to confirm acyl chain composition. The identity of dilinoleoyl-PA was confirmed by TLC and mass spectrometry. Acyl chain characterization by GLC demonstrated that >99.5% of acyl ester side chains were linoleate.

**Dilinoleoyl-PA Acylation—*Stock solutions of phospholipids were prepared by dissolving the compounds above in phosphate-buffered saline without Ca²⁺ containing 0.5% (w/v) BSA, such that final concentrations of BSA in reaction volumes ranged from 0.002-0.04% (w/v). Lipids were dispersed in 500 mM stock solutions by active vortexing alternated with 15-s sonicating bursts, and then sonicated in 4 °C suspensions of 12000 S. Reactions were performed in suspension for all lipid solutions with BSA in the range described. All phospholipids were characterized for purity via thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) as described below. Gas-liquid chromatography (GLC) of methyl esters was used to confirm acyl chain composition. The identity of dilinoleoyl-PA was confirmed by TLC and mass spectrometry. Acyl chain characterization by GLC demonstrated that >99.5% of acyl ester side chains were linoleate.
IL-1 Stimulates Lyso-PA Acyltransferase

RESULTS AND DISCUSSION

Evidence for Rapid IL-1-stimulated PA Formation from 1-Acylglycerol Phosphate Pools—Human MC in culture were labeled with [3H]glycerol to study interconversion between lipid species in vitro. Lipid species were separated by HPLC and quantitated using UV absorption at 206 nm, a wavelength measuring unsaturated acyl chains. By comparing the distribution of radioactivity with the distribution of UV-absorbing phospholipid mass, it was possible to follow alterations in lipid species mass by [3H]glycerol content and also detect changes in relative mass of unsaturated acyl side chains due to lypo phospholipid acylation or lipase activity. Data shown was obtained in the absence of NaF, and are identical to those obtained in the presence of 25 mM NaF, used to inhibit phospholipase C activity. PA and PE were single out as the lipids whose acyltransferase solution, no Ca2+ was present in the final solution; a small amount of Mg2+ was present. PA concentration was calculated by using phospholipid phosphorous determinations and a stock solution of parinyl PA made at a concentration of 500 nm. 2-sn-Parinyl-PA was then added to HMC microsomes or plasma membranes in an amount (5-10%) of the concentration in the presence and absence of 10^-14 to 10^-16 M IL-1, and 100 µM GTPpS. The conversion rate to 1,2-sn-diaclylglycerol was calculated by using the extinction coefficient of cis-parinaric acid. These methods of determining 1,2-sn-DAG formation were then compared to rates determined in conventional fashion using [3-3H]oleyl PA (Jamal et al., 1991; Cascaes al., 1984), and were found to correspond closely in measuring rates of phosphate phosphodiesterase activity (also cf. Bursten and Harris, 1991).

Methyl Esterification of Lipids and GLC—The acyl content of free fatty acid fractions, neutral lipids, or phospholipids was determined by GLC after esterification with BF3/methanol (Johnson et al., 1990). GLC was performed on a Hewlett-Packard model 5790 A GLC using a 6-ft x 1/8-inch column packed with GP 3%, SP-2385% on 100-120 Chromasorb WAW using nitrogen as a carrier gas. Oven temperature was programmed for initial 2 min at 190 °C, followed by a gradient to 255 °C at the rate of 2 °C/min.

Fast-Atom Bombardment Mass Spectrometry (FAB/MS) of HPLC Isolated Fractions—FAB/MS spectra were acquired using a VG 70 SEQ tandem hybrid instrument of EBGQ geometry (VG Analytical, Altrincham, U.K.). The instrument was equipped with a standard unheated VG FAB ion source and a standard sapphire-field gun (Ion Tech Ltd., Middlesex, U.K.) producing a beam of xenon atoms at 8 keV and 1 mA. The mass spectrometer was adjusted to a resolving power of 1000 and spectra were obtained at 8 kV and a scan speed of 10 s/decade. In this study, all samples were applied to the FAB target as solutions of known concentrations.

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Change in [3H]glycerol content of PA and PE in HMC stimulated with 10^-11 M IL-1 Sn for 5 min is shown in Fig. IA. Stimulation of cells with IL-1 resulted in labeled glyceroe in PE decreasing between 2 and 4 min, with little change in the first minute of stimulation (p > 0.05, ANOVA). [3H]Glycerol concentrations by GLC (cf. below). Due to formation of PA in acyltransferase solution, no Ca2+ was present in the final solution; a small amount of Mg2+ was present. PA concentration was calculated by using phospholipid phosphorous determinations and a stock solution of parinyl PA made at a concentration of 500 nm. 2-sn-Parinyl-PA was then added to HMC microsomes or plasma membranes in an amount (5-10%) of the concentration in the presence and absence of 10^-14 to 10^-16 M IL-1, and 100 µM GTPpS. The conversion rate to 1,2-sn-diaclylglycerol was calculated by using the extinction coefficient of cis-parinaric acid. These methods of determining 1,2-sn-DAG formation were then compared to rates determined in conventional fashion using [3-3H]oleyl PA (Jamal et al., 1991; Cascaes al., 1984), and were found to correspond closely in measuring rates of phosphate phosphodiesterase activity (also cf. Bursten and Harris, 1991).

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Statistics—Representative experiments performed independently two to three times are given for the whole cell labeling studies. In each case, the phospholipid analyses were performed in groups of two to four, and the data are presented as the means ± S.E. For analysis of lipid samples at 5 min, replication of experiments two to six times showed that control for a given lipid fraction varied by no more than 1-2% of total unsaturated acyl mass. Other data were analyzed by analysis of variance and multiple comparison techniques, with p values of <0.05 considered significant (Tukey, 1949; Dunnnett, 1964). For polarization of fluorescence studies, samples from 5-10 individual batches of HMC were run three to five times each in the acyltransferase assay system and the data are given as the means of these determinations (S.E. <10% in all cases). Linear regression analysis of polarization of fluorescence data was performed using STATview II (Abacus Concepts, Berkeley, CA).

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content of PA fractions did not change over the first min of stimulation (p > 0.05), succeeded by a steady increase. This later increase in PA was reciprocal to the decrease in PE. In contrast, examination of HMC lipid unsaturated acyl chains by A_200 nm demonstrated a 33% decrease in unsaturated PE (Fig. 1B; p < 0.01) and a 200% increase in unsaturated PA between 5 and 15 s (Fig. 1B; p < 0.001) followed by slower changes in the same directions for both lipid species between 2 and 4 min. Unsatuated acyl content was unchanged in PE thereafter, while a decrease in PA was observed. Increases in relative PA mass in IL-1-stimulated HMC observed within 5 s to 1 min (Fig. 1B) therefore appeared to be correlated with incorporation of unsaturated acyl chains into PA rather than shift of phospholipid mass. Subsequent increases in PA mass (2 min to 4 min) reflect parallel changes in the glycerol backbone and acyl chains (Fig. 1) and are the inverse of PE changes.

Whole HMC were then studied for changes in absolute phospholipid species mass during the first min of IL-1 stimulation (Table I). During this time, corresponding to the period when unsaturated acyl absorption in PA was increasing (Fig. 1B), there was a parallel 3–4-fold increase in PA mass in stimulated cells compared to control cells (p < 0.01 by ANOVA). The mass in other phospholipid species, including PE, did not change during this time. We hypothesized from these data that IL-1 stimulated immediate increases in PA mass in HMC (5–60 s), resulting from rapid incorporation of unsaturated acyl groups into both preexisting stable pools of 1-acylglycerol phosphate (lyso phosphatidic acid), and pools formed from saturated PA. These latter pools did not appear to originate from other phospholipid species, as there was no increase in glycerol incorporation in PA, but rather from possible phospholipase A_2 action on saturated PA species (Land cycle). [3H]Glycerol levels did not decrease in other phospholipid species during the first min of stimulation (cf. Fig. 1A and legend) in either the presence or absence of 25 mM NaF, which suggests that phospholipase C does not play a role in these lipid changes. The stability in phospholipid glycerol pools and phospholipid mass over 60 min may be due to the presence of phosphatidic acid, which is known to inhibit phospholipase C (Mandell et al., 1985).

**TABLE I**

| Phospholipid, condition | 15 s | 45 s | 60 s |
|-------------------------|------|------|------|
| PA, control             | 13.4 ± 2.7 | 12.8 ± 3.6 | 14.2 ± 2.7 |
| PA, +IL-1               | 38.6 ± 3.4^a | 49.5 ± 2.9^a | 57.3 ± 5.9^a |
| PE, control             | 157.4 ± 8.9 | 168.7 ± 11.3 | 162.1 ± 9.4 |
| PE, +IL-1               | 159.0 ± 7.3 | 147.3 ± 13.2 | 151.5 ± 8.6 |
| PC, control             | 224 ± 11.8 | 239 ± 13.6 | 244 ± 9.5 |
| PC, +IL-1              | 238 ± 9.6 | 229 ± 17.1 | 247 ± 7.5 |
| PI, control             | 17.3 ± 3.4 | 15.8 ± 2.6 | 16.9 ± 3.1 |
| PI, +IL-1               | 16.4 ± 2.7 | 17.5 ± 5.8 | 15.4 ± 1.9 |

Fig. 1. Effect of IL-1 on HMC phospholipid metabolism. Confluent HMC layers were prelabeled with [3H]glycerol in serum-free media (see “Experimental Procedures”). Following removal of the labeling media, the washed cell layers were incubated for 0–5 min with serum-free media containing 10−8 M IL-1. Lipids from unstimulated cultures were analyzed in parallel to stimulated cultures. At the indicated times, cells were washed with 4 ml of 4 °C methanol, then extracted into 2:1 chloroform:methanol and lipids analyzed via HPLC. Lipid species were detected and relative mass determined by UV absorption spectroscopy at 206 nm (A_200 for C16-C22 unsaturated acyl side chains). Fractions of eluent were collected, and analyzed by β-scintillation counting. [3H]Glycerol labeling of phosphatidylycerine (1,2-di[3H]-diacylglycerol/100 μg protein), and absorption at 206 nm (A_200, 103,000 dpm/100 μg protein), and absorption at 206 nm for PI, PS, and PC did not vary significantly during the incubation. For clarity, only data at 0 and 300 s are shown for PA and PE controls. *Panel A*: [3H]glycerol labeling of HMC PA and PE, A, PA, control, 0 s; C, PA, control, 300 s; A, PA, + IL-1, C, PE, control, 0 s; C, PE, control, 300 s; A, PA, + IL-1, C, PE, + IL-1. *Panel B*: Absorption at 206 nm of HMC PA and PE (relative unsaturated acyl mass). A, PA, + IL-1; C, PE + IL-1. Control values at zero time are the same as those found at 5 s.
Fig. 2. Effect of IL-1 on fatty acid incorporation into HMC PA and HMC microsomal PA. Panel A, incorporation of [3H]linoleic acid into PA in HMC. Confluent HMC layers were labeled with [3H]linoleic acid (see "Experimental Procedures"). Following removal of the labeling media, the washed cell layers were incubated for 0-5 min with serum-free medium with or without 10^{-10} M IL-1. At the indicated times, washed cell layers were fixed and scraped into 4 ml of methanol and the lipids extracted with 2:1 chloroform/methanol. The lipids were separated by HPLC, monitoring UV absorption spectroscopy and 3H-scintillation counting. During the 5-min time period analyzed, [3H]linoleate in the following phospholipid fractions did not change after stimulation with IL-1: PC (92,500 ± 9,600 dpm/100 μg protein), PS (1145 ± 150 dpm/100 μg protein), and PI (700 ± 125 dpm/100 μg protein). During the first 2 min, the radioactivity in PE did not vary significantly (33,000 ± 1,400 dpm/100 μg protein), but began to decrease over the next 3 min (to 16-17,000 dpm/100 μg protein). Free fatty acid fractions (FFA) were labeled to 29,000 ± 1,075 dpm/100 μg protein at zero time, but decreased to ≈16,350 within 2 min after IL-1 stimulation. Radioactivity in PA of control cells (□), PA in cells stimulated with IL-1 (■). Panel B, uptake of [3H]linoleoyl-CoA into HMC microsomes used as a lyso-PA acyltransferase assay. [3H]Linoleoyl CoA (4 μM) and 1-palmitoyl-lyso-PA (50 nm) were added to 100-300 μg HMC microsomes in the presence of absence of 10^{-10} M IL-1. At indicated times, chloroform/methanol (2:1) was added to stop the acylation reaction, and the lipids extracted, separated by HPLC, and the radioactivity in individual fractions determined. >85% of radioactivity was recovered within the PA fractions at all time points examined for controls. The remaining radioactivity was either in the solvent front (10-12%) or PE (3-5%). In HMC microsomes treated with IL-1, 15-20% of radioactivity was incorporated into 1,2-sn-DAG fractions after the first 5 s. Recalculating enzyme activity as nanomoles of fatty acid incorporated/mg protein/min gave a range of 17-75 nmol/mg min for control microsomes, and a range of 36-168 nmol/mg/min for IL-1-stimulated microsomes. This is comparable to measured lyso-PA acyl-CoA acyltransferase activity (Soelting et al., 1989). Radioactivity of PA fraction in control microsomes (□), radioactivity of PA fraction in IL-1-stimulated microsomes (■). Panel C, incorporation of cis-parinaric acid into phospholipids of HMC microsomes. The incorporation process is followed by changes in polarization of fluorescence of cis-PnA, as described under "Experimental Procedures" (cf. Harris and Stahl, 1983). Upper curve (□), IL-1-mediated increases in polarization of fluorescence indicating stimulated incorporation of cis-PnA relative to unstimulated microsomes. Lower curve (■), changes in polarization of unstimulated HMC microsomes; lower curve (■), acyltransferase cofactor requirement of IL-1-mediated increases. Removal of AT cofactors in medium resulted in loss of cis-PnA incorporation. HMC microsomes sonicated for 30 min or incubated at 55 °C did not incorporate cis-PnA. HMC microsomes incubated at 95 °C for less than 15 min retained significant activity, consistent with former descriptions of lyso-PA acyl-CoA acyltransferase (Yamashita et al., 1975).
**TABLE II**

Relative initial kinetics of acyl-CoA:acyltransferase in human MC microsomes

| Additions         | -IL-1 | +IL-1 |
|-------------------|-------|-------|
| Control (no additions) | 1.0 ± 0.06 | 1.66 ± 0.045* |
| 1-Palmitoyl-lyso-PA, 100 nM | 1.71 ± 0.003* | 2.32 ± 0.14** |
| 1-Palmitoyl-lyso-PC, 100 nM | 1.07 ± 0.03 | 1.63 ± 0.05* |
| Dlinoleoyl-PA, 1 μM | 1.53 ± 0.007* | 2.16 ± 0.09** |
| Dlinoleoyl-PA, 10 μM | 1.89 ± 0.07* | 2.23 ± 0.13** |
| Dlinoleoyl-PC, 10 μM | 0.98 ± 0.06 | 1.58 ± 0.10 |
| CaCl₂, 5 mM | 0.72 ± 0.06 | 1.21 ± 0.10 |
| NaF, 5 mM | 1.18 ± 0.12 | 1.83 ± 0.06* |
| GTP+PS, 50 μM | 1.21 ± 0.09* | 1.96 ± 0.12** |
| Pertussis toxin, 0.1 mg/ml (preincubated) | 1.03 ± 0.01 | 1.62 ± 0.12* |
| Pertussis toxin, 100 mg/ml (preincubated) | 1.01 ± 0.01 | 1.55 ± 0.06* |

*Significant change, p < 0.05, comparing conditions to the initial control curve, using the chi-squared test.

**Significant change, p < 0.05, comparing conditions to the initial control curve generated by control HMC microsomes in the presence of IL-1. IL-1 significantly enhances acyltransferase activity. At concentrations below those known to damage biological membranes (>1 μM), 1-palmitoyl-lyso-PA had a stimulating effect on lyso-PA AT, while 1-palmitoyl-lyso-PC had no equivalent effect. 1,2-di-Dlinoleoyl-PA-stimulated enzyme activity, and was synergistic with IL-1, whereas 1,2-dn-Dilinoleoyl-PC had no effect. NaF and GTP+PS had small stimulating effects on enzyme activity. Pretreatment with pertussis toxin did not reduce baseline acyltransferase activity, nor reduce the stimulatory effect of IL-1.

studied, no incorporation of cis-PnA into phospholipids of the microsomal membrane occurred without AT cofactors ATP, CoA, and Mg2+. Sonication of microsomes for 30 min also resulted in loss of cis-PnA incorporation (data not shown). This was evidence that a specific enzyme was responsible for incorporation of cis-PnA into HMC microsomal PA.

Table II presents other properties of lyso-PA AT activity in HMC. Linear regression analysis was used to compare initial rates of cis-PnA incorporation into control microsomes versus incorporation following additions to the reaction media. Exogenous increase in the second substrate of the lyso-PA AT-mediated acyltransferase reaction, 1-palmitoyl-lyso-PA, caused an augmentation of cis-PnA incorporation, and also augments IL-1 stimulation of this reaction. Addition of 1-palmitoyl-lyso-PC, which contains a different phospholipid head group, had no stimulatory action on this enzyme. Addition of the end product congener, 1,2-sn-dilinoleoyl-PA, resulted in strong augmentation of lyso-PA AT activity. This appears to be specific to PA, as addition of dilinoleoyl-PC did not change basal or stimulated kinetics. These data suggested that initial accumulation of PA further stimulates lyso-PA AT in a positive feedback manner. This is in agreement with earlier reports of lysophospholipid acyltransferase activity including oleoyl CoA:lysleceithin acyltransferase, oleoyl CoA:lyso-PA acyltransferase, and linoleoyl-CoA:lyso-PA acyltransferase (rat MC) (Ferber and Resch, 1977; Szamel and Resch, 1980; Bursten and Harris, 1991). This provides an explanation for delay in fully enhanced uptake observed in Fig. 2B (i.e., accumulation of PA is required before full stimulation is observed; Szamel and Resch, 1981).

Calcium salts inhibit the activity of lysophospholipid AT (Lands and Hart, 1965). Results in significant diminution in initial rate of incorporation and IL-1-stimulated uptake. 5 mM NaF, which stimulates G-proteins in the presence of aluminum (Birnbaum et al., 1987), and GTP+PS, a nonhydrolyzable activator of G-proteins, caused a small enhancement in cis-PnA uptake, but did not duplicate the action of IL-1. Preincubation of HMC with pertussis toxin prior to preparation of microsomes had no effect on lyso-PA AT activity.

The specificity of uptake of unsaturated acyl groups into PA was confirmed by chemical analysis of labeled phospholipid species. Separation of lipid species by HPLC after labeling with [14C]linoleoyl-CoA demonstrated that basal uptake of labeled PA into HMC microsomes was directed into PA fractions (Fig. 3A), and that IL-1 stimulation of microsomes resulted in enhancement of PA fraction unsaturated mass and radioactive labeling (Fig. 3D). There was no labeling of or change in unsaturated mass in other phospholipid species, consistent with Table I. This specificity of uptake of unsaturated fatty acids into PA was confirmed by fluorescent labeling of microsomal PA fractions by C18:4 cis-PnA (Fig. 3C), and significant enhancement of PA unsaturated mass and fluorescence labeling after IL-1 stimulation (Fig. 3D). We also observed that, with basal (untreated) uptake of linoleoyl-CoA or cis-PnA, there was no labeling of DAG fractions. However, following stimulation with IL-1, a significant percentage of label was found in DAG fractions (cf. Figs. 3, B and D). This suggested that an IL-1-stimulated DAG fraction found at 5–60 s originated from PA, but could not completely exclude activation of monoacylglycerol AT.
(myristate increased from 1.4 to 8%; palmitate increased from 12 to 18.4%; and stearate increased from 15 to 24.6%). This is further evidence that one source of 1-acylglycerol phosphate (lyso-PA) is the Lands cycle.

It was assumed that saturated acyl chains were found in the C-1 position and unsaturated acyl chains in the C-2 position. From these GLC data, most probable configurations supported predictions from acyl chain composition were predicted and matched with mass spectrometric data.

The microsomal phospholipids were extracted and separated by HPLC as detailed under "Experimental Procedures." The upper tracing represents relative phospholipid mass as measured at 206 nm; the lower tracing represents either β-scintillation counts due to incorporation of [14C]linoleoyl-CoA (panels A and B) or fluorescence due to incorporation of cis-PnA (panels C and D). Labeled PA fractions represent HPLC separation of 1-palmitoyl 2-unsaturated PAs from 1-oleoyl-2-oleoyl and 1-oleoyl-2-linoleoyl PAs. Panel A, HPLC profile of lipids from control HMC microsomes incubated for 5 s at 37°C; panel B, HPLC profile of HMC microsomal lipids after treatment with 10^{-6} M IL-1 for 5 s at 37°C; panel C, HPLC profile of lipids from control HMC microsomes incubated for 5 s, + cis-PnA; panel D, HPLC profile of microsomal lipids after treatment with IL-1, 10^{-6} M, 5 s, + cis-PnA.

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**Fig. 3.** [14C]Linoleoyl CoA and cis-pinaric acid incorporation into PA. HPLC analysis of lipids from HMC microsomes incubated with 4 μM [14C]linoleoyl CoA or cis-pinaric acid in the presence or absence of 10^{-6} M IL-1x is shown. 50 nM 1-oleoyl-lyso-PA and 50 nM 1-palmitoyl-lyso-PA were present. The microsomal phospholipids were extracted and separated by HPLC as detailed under "Experimental Procedures." The upper tracing represents relative phospholipid mass as measured at 206 nm; the lower tracing represents either β-scintillation counts due to incorporation of [14C]linoleoyl-CoA (panels A and B) or fluorescence due to incorporation of cis-PnA (panels C and D). Labeled PA fractions represent HPLC separation of 1-palmitoyl 2-unsaturated PAs from 1-oleoyl-2-oleoyl and 1-oleoyl-2-linoleoyl PAs. Panel A, HPLC profile of lipids from control HMC microsomes incubated for 5 s at 37°C; panel B, HPLC profile of HMC microsomal lipids after treatment with 10^{-6} M IL-1 for 5 s at 37°C; panel C, HPLC profile of lipids from control HMC microsomes incubated for 5 s, + cis-PnA; panel D, HPLC profile of microsomal lipids after treatment with IL-1, 10^{-6} M, 5 s, + cis-PnA.
TABLE III

Acyl side chain composition of HMC phospholipid fractions ± interleukin-1

| Acyl chain | Fraction, condition (t = 15 s) |
|------------|--------------------------------|
|            | PA, Control | PA + IL-1 | PE, Control | PE + IL-1 | PC<sup>a</sup> | PS<sup>b</sup> |
| 14:0       | 14.7        | <1<sup>c</sup> | 2          | 4.4       |
| 16:0       | 33.3        | 20.9<sup>c</sup> | 2.9        | 27        | 12.4          | 2.4           |
| 18:1       | 8.0         | —          | —          | —         | 4.6           |
| 18:2       | 20.4        | 16.5<sup>c</sup> | 23.6       | 25.8      | 16.2          | 32.9          |
| 18:4       | 15.5        | 19.5<sup>c</sup> | 15.8       | 18.8      | 39.5          | 20.4          |
| 20:2       | 12.6<sup>c</sup> | 4.2       | 3.9        | 3.8       | 4.8           |
| 20:4       | 1.2         | 8.1<sup>c</sup> | 23.6       | 22.3      | 11.3          | 5.0           |
| 20:5       | 2.5         |            |            |           |               |
| 22:2       | 2.1         | 3.4        | 1.7        | 3.9       | 1.0           |
| 22:5       | 6.8<sup>c</sup> | 6.0       | 4.8        | 2.0       |               |
| 22:6       | 1.4         | 7.1<sup>c</sup> | 7.3        | 5.4       | 1.2           | 9.4           |
| 24:0       | 4.9         | —          | 6.0        | 5.2       | 2.0           | 10.3          |

<sup>a</sup> PC fractions showed no differences between controls and IL-1-stimulated HMC.

<sup>b</sup> PS fractions showed no differences between controls and IL-1-stimulated HMC.

* Significant increase or decrease (p < 0.05) in acyl chain composition.

extant lyso-PA AT. The specificity of IL-1 stimulation of the acylation reaction was shown by addition of excess α-IL-1α antibody prior to IL-1α. In the presence of α-IL-1 antibody, IL-1-stimulated uptake of cis-PnA into HMC microsomes was abolished, and original reaction rate maintained (Fig. 5A).

The dose response of cis-PnA incorporation into lyso-PA after IL-1 stimulation is shown in Fig. 5B. Addition of 10-fold IL-1-stimulated uptake of cis-PnA into HMC microsomes was abolished, and original reaction rate maintained (Fig. 5A). Considerable levels of PC were detected after stimulation with IL-1. Most probable identities of major FAB/MS m/z peaks are: 605, 1-O-tetradecanoyl-2-palmitoyl-PA; 619, 1-myristoyl-2-palmitoyl-PA; 633, 1-O-tetradecanoyl-2-oleyl-PA; 647, 1-stearoyl-2-myristoyl-PA; 661, 1-octadecanoyl-2-palmitoyl PA; 675, 1-stearoyl-2-palmitoyl (or 1-palmitoyl-2-stearoyl) PA; 689, 1-octadecanoyl-2-oleoyl PA. Panel B, PA species detected after stimulation with IL-1. Most probable identities of major FAB/MS m/z peaks are: 673, 1-palmitoyl-2-linoleoyl-PA; 695, 686, 1-palmityl-2-eicosapentaenoyl-PA; 697, 619, 1-linoleoyl-2-linoleoyl-PA; 701, 1-oleoyl-2-oleoyl-PA; 711, 1-stearoyl-2-arachidonoyl-PA; 721, 723, 1-oleoyl-2-eicosapentaenoyl-PA and 1-palmitoyl-2-docosahexaenoyl-PA; 725, 1-stearoyl-2-arachidonoyl-PA; 749, 750, 1-stearoyl-2-docasahexaenoyl-PA.

Fig. 4. Fast atom bombardment mass spectrometry of phospholipid fractions from intact HMC. HMC were stimulated with IL-1 as in preceding figures followed by fixation in cold methanol, lipid extraction in 2:1 chloroform/methanol, and fraction separation by HPLC. Samples were isolated and repurified by HPLC. An aliquot of each PA or PE fraction was treated with BF₃/methanol and resulting methyl esters of acyl side chains were analyzed by GLC (see “Experimental Procedures”). The other aliquot of each fraction was analyzed by FAB/MS (see “Experimental Procedures”). A portion of the FAB positive-ion spectra between 650 and 750 mass units of the PA control and stimulated fractions are shown. These samples were generated 5–15 s after IL-1 stimulation of HMC. FAB/MS was performed for positive-ion and negative-ion scatter. Positive-ion FAB caused cleavage of the phosphate and production of a carbonium ion in the sn-3 site; these diglyceronium ions were used to confirm the interpretation of the PA peaks (S. L. Bursten, W. E. Harris, W. Howard, and R. Talalat, manuscript in preparation). Panel A, control PA species. Note the smaller mass of the control PA compared with IL-1-stimulated PA in panel B (proportionate to findings in Table I). Most probable identities of major FAB/MS m/z peaks are: 605, 1-O-tetradecanoyl-2-palmitoyl-PA; 619, 1-myristoyl-2-palmitoyl-PA; 633, 1-O-tetradecanoyl-2-oleyl-PA; 647, 1-stearoyl-2-myristoyl-PA; 661, 1-stearoyl-2-palmitoyl PA; 675, 1-stearoyl-2-palmitoyl (or 1-palmitoyl-2-stearoyl) PA; 689, 1-octadecanoyl-2-oleoyl PA. Panel B, PA species detected after stimulation with IL-1. Most probable identities of major FAB/MS m/z peaks are: 673, 1-palmitoyl-2-linoleoyl-PA; 695, 686, 1-palmityl-2-eicosapentaenoyl-PA; 697, 619, 1-linoleoyl-2-linoleoyl-PA; 701, 1-oleoyl-2-oleoyl-PA; 711, 1-stearoyl-2-arachidonoyl-PA; 721, 723, 1-oleoyl-2-eicosapentaenoyl-PA and 1-palmitoyl-2-docosahexaenoyl-PA; 725, 1-stearoyl-2-arachidonoyl-PA; 749, 750, 1-stearoyl-2-docasahexaenoyl-PA.
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**Fig. 5.** Specificity of stimulation of acyl transferase in microsomes and whole HMC by IL-1 and lyso-PA. Change in polarization of fluorescence of cis-PnA as an index of cis-PnA incorporation by HMC microsomes is shown in Fig. 4, A and B. Panel A, effect of addition of IL-1 to microsomes preequilibrated with cis-2-palmitoyl-1-acyl-sn-glycero-3-phosphorylcholine (cis-PA) for 5 min prior to stimulation. Addition of $10^{-10}$ or $10^{-11}$ M IL-1a results in marked increase in polarization of fluorescence at this time. Preincubation of HMC microsomes with anti-IL-1a does not abolish basal uptake, but prevents the stimulation observed with addition of IL-1 after equilibration. ■, control microsomes; □, microsomes + $10^{-11}$ M IL-1a; ●, microsomes + $10^{-10}$ M IL-1 + antibody against IL-1; ●, microsomes + $10^{-9}$ M IL-1 + antibody against IL-1. Panel B, dose response of HMC microsome cis-PnA incorporation to IL-1. Microsomes were incubated with 4 pM cis-PA for 10 min in the presence of given concentrations of IL-1, and polarization of fluorescence was measured. □, control microsomes; △, microsomes + $10^{-11}$ M IL-1; ●, microsomes + $10^{-10}$ M IL-1; Δ, microsomes + 5 x $10^{-10}$ M IL-1. The effect of lyso-PA on HMC whole cell and microsomal PA unsaturated mass in the presence and absence of IL-1 is demonstrated in panels C and D. Panel C, whole HMC were incubated with 1-palmitoyl lyso-PA ranging from 1.5 to 500 nM (HMC were shown to be viable by trypan blue exclusion assay at these concentrations). Representative results at 50 nM lyso-PA are shown. Microsomes were incubated in the presence and absence of $10^{-10}$ M IL-1a. After incubation between 6 and 60 s, the reaction was stopped with 2:1 chloroform:methanol, and lipids were extracted and separated by HPLC. Absorption at 206 nm of 1,2-sn-DAG and PA fractions was determined. PA (0), addition of lyso-PA alone: DAG (0); PA (△). Panel D, HMC microsomes were incubated with 100 nM 1-palmitoyl lyso-PA in the presence and absence of $10^{-10}$ M IL-1a. After incubation between 0 and 30 s, the reaction was stopped with 2:1 chloroform:methanol, and lipids were extracted and separated by normal phase HPLC. Absorption at 206 nm of 1,2-sn-DAG and PA fractions was determined. PA (0); DAG (△). Additions of PE and PC were constant under these conditions during the time studied. Control: DAG (0); PA (0). Addition of lyso-PA alone: DAG (△); PA (△). Addition of lyso-PA + IL-1: DAG (0); PA (△).
in these microsomes was not increased compared with M-15- or pertussis toxin-treated microsomes at either 5 or 15 s. These data established that IL-1 occupation of the IL-1 receptor was necessary for stimulation of lyso-PA AT and phosphatidate phosphohydrolase. Pertussis toxin prevented activation of phosphatidate phosphohydrolase.

Blockade of lyso-PA AT activation through mAb occupation of the IL-1 receptor suggested that lyso-PA AT activity may be found in the plasma membrane. To examine this possibility further, HMC plasma membranes were isolated from crude microsomal fractions. Assay of lyso-PA AT activity indicates its presence in the plasma membrane (Table IV), that it is stimulated by IL-1, and this stimulation is blocked by M-15 mAb. We conclude that IL-1 stimulates a plasma membrane-associated lysop-PA AT, through association with its receptor.

The data in Fig. 6 also indicate that IL-1 stimulation of lyso-PA AT is not linked to a pertussis toxin-sensitive Gp. The accumulation of unsaturated mass in PA after IL-1 stimulation in pertussis toxin-treated HMC microsomes suggested that increases in DAG unsaturated mass occurring with IL-1 result from conversion of PA to DAG via phosphatidate phosphohydrolase. This latter enzyme is regulated by a pertussis-toxin sensitive Gp, and hence the blockage of phosphatidate phosphohydrolase results in PA mass accumulation. This hypothesis would also explain the stimulation of cis-PnA uptake by NaF and GTPyS (Table II) as the result of a change in equilibrium induced by shift of mass from PA to DAG due to the G-protein-induced action of phosphatidate phosphohydrolase.

To further examine Gp activation of phosphatidate phosphohydrolase, microsomes from HMC were reexamined in the presence and absence of IL-1 and GTPyS following labeling of phosphatidic acid with cis-parinaric acid or [14C]oleate allowing estimation of phosphatidate phosphohydrolase action rates. Control microsomes incubated at 37°C demonstrated formation rates of <0.1 nmol DAG/mg/min (Table V). In contrast, microsomes stimulated with 10^{-11} M IL-1 showed formation rates of DAG from cis-PnA-labeled PA of 6.2 ± 2.0 nmol DAG/mg/min within 15 s after stimulation. Microsomes stimulated with 100 μM GTPyS showed formation rates of 5.3 ± 1.5 nmol DAG/mg/min within 15 s after stimulation (Table V). NaF (5 and 10 mM) also induced moderate increases in phosphatidate phosphohydrolase activity compared to control (3.1 and 2.4 nmol DAG/mg/min), but did not increase rates to the same extent as IL-1 or GTPyS.

These data are consistent with an inhibitory effect of NaF on phosphatidate phosphohydrolase itself (Jamal et al., 1991) and with previous data concerning phosphatidate phosphohydrolase activity in rat MC (Bursten and Harris, 1991); they confirm significant activation of phosphatidate phosphohydrolase after IL-1 stimulation, via a Gp-related mechanism. Phosphatidate phosphohydrolase is present in eukaryotic cells in hormone-sensitive forms and may be tightly regulated by phosphorylation and/or translocation (Hosaka et al., 1975; Berglund et al., 1981; Cascales et al., 1984, 1988). In addition, a specific plasma membrane-associated form of phosphatidate phosphohydrolase has recently been described which may be present in association with signaling (Jamal et al., 1991) consistent with our findings (Table V). Evidence linking IL-1 to Gp activation that may mediate enzymes other than adenyl cyclase or phospholipase A2 has been reported (O’Neill et al., 1990).

**TABLE V**

| Phosphatidate phosphohydrolase activity in HMC cellular fractions |
|---------------------------------------------------------------|
| **HMC microsomes and plasma membrane enriched fractions were isolated as described under “Experimental Procedures.”** 100–300 μg of microsomes or 50–100 μg of plasma membranes were reacted with reagents as described, or following equilibration for 1 h with M-15 mAb, in the presence of 4 μM cis-PnA-labeled PA, or [14C]oleoyl-PA. The reaction was quenched by addition of 2 ml of ice-cold methanol, and lipids were then extracted in 2:1 chloroform:methanol and separated by HPLC. Formation of DAG was assayed using fluorescence intensity of DAG or scintillation counting. |
| **Activity at 15 s** |
| **Fraction + reagent** |
| Control | <0.1 |
| IL-1, 10^{-11} M | 6.2 ± 2.0 |
| GTPyS, 100 μM | 5.3 ± 1.3 |
| M-15 + IL-1, 10^{-11} M | <0.5 |
| NaF, 5 mM | 3.1 ± 1.2^a |
| NaF, 10 mM | 2.4 ± 0.7^a |
| Plasma membranes (enriched): |
| Control | <0.1 |
| IL-1, 10^{-11} M | 9.6 ± 2.6 |
| GTPyS, 100 μM | 6.1 ± 1.8 |
| M-15 + IL-1, 10^{-11} M | <0.1 |

*Activity is expressed as nmols of DAG/mg/min ± S.E.

*Significantly increased phosphatidate phosphohydrolase activity, p < 0.01, compared to control by ANOVA.

*Significantly decreased phosphatidate phosphohydrolase activity, p < 0.01, compared to IL-1-stimulated microsomes or plasma membranes.

**TABLE IV**

| Lyso-phosphatidate-CoA:Acyltransferase specific activity in HMC subcellular fractions |
|--------------------------------------------------------------------------------------------|
| **HMC microsomes and plasma membrane enriched fractions were isolated as described under “Experimental Procedures.” 100–300 μg, microsomes or 50–100 μg of plasma membranes were reacted with reagents as described, or following equilibration for 1 h with 5 μg/ml M-15 mAb, in the presence of [14C]oleoyl-CoA. The reaction was quenched by addition of 2 ml of ice-cold methanol, lipids extracted in 2:1 chloroform:methanol, and separated by HPLC. Formation of PA was assayed using scintillation counting.** |
| **Cell fraction** | **Specific activity** |
| | nmol fatty acid/ mg protein/min |
| Crude microsomes | 26 |
| Crude microsomes + IL-1 | 77^a |
| Plasma membrane-enriched | 34.7^a |
| Plasma membrane-enriched + IL-1 | 102^a |
| Plasma membrane-enriched + M-15 + IL-1 | 37.6 |
| Refined microsomes | 17.8 |

^a Significantly increased lys-PA AT activity, compared to control crude microsomes.

^b Significantly increased activity, compared to control plasma membranes.
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Fig. 7. Effect of IL-1 stimulation on PA fractions in EL-4 murine T lymphoma whole cell and microsome. Panel A, EL-4 whole cells in suspension were stimulated with 10^{-11} M IL-1 for the indicated times. Equal aliquots of cells (1 - 2 x 10^9) were then removed and the incubation halted by addition of 2 ml of cold methanol. Lipids were then extracted in 2:1 chloroform:methanol, separated by HPLC, and analyzed at A_{206 nm}. Results shown are representative of five to seven experiments. Panel B, EL-4 microsomes were isolated as described under “Experimental Procedures.” Lipids were then extracted in 2:1 chloroform:methanol, separated by HPLC, and analyzed at A_{206 nm}. Results shown are representative of two to three experiments.

Microsomes from mesangial cells (cf. Fig. 2C). Microsomes were prepared from an EL-4 mutant that contained nonfunctional IL-1 receptors. Addition of IL-1 to these microsomes had no effect on the AT activity measured by polarization of fluorescence (Fig. 7C). This suggested that a functional IL-1 receptor was required for lyso-PA AT stimulation. A dose response of EL-4 cells to IL-1 (Fig. 7D) demonstrated that the generation of unsaturated PA occurred in the same physiological conditions described under “Experimental Procedures.” Microsomes were then incubated in acylation buffer, and change in polarization of fluorescence of cis-parinaric acid was observed in the presence and absence of 10^{-11} M IL-1, added with cis-PnA at time 0. Results shown are representative of five to seven experiments. Panel C, EL-4 microsomes from wild-type cells responsive to IL-1, and microsomes from mutant EL-4 cells with greatly diminished response to IL-1 and recently characterized as having atypical IL-1 receptors were isolated as described under “Experimental Procedures.” Microsomes were incubated in acylation buffer, and change in polarization of fluorescence of cis-parinaric acid was observed in the presence and absence of 10^{-11} M IL-1. In the first series of experiments, IL-1 was added at time 0 with cis-PnA. IL-1 was also added (after equilibration of cis-PnA with microsomes) at t=10 min in the next series of experiments. Mutant EL-4 microsomes were then incubated in acylation buffer, and uptake of cis-PnA observed as previously described. Following equilibration at 10 min, mutant microsomes were stimulated with 10^{-12} M IL-1. Panel D, dose response curve of EL-4 whole cell PA unsaturated acyl group incorporation to IL-1. EL-4 whole cells in suspension were stimulated with 2 x 10^{-10} to 5 x 10^{-8} M IL-1 for 30 s or 1 min. Equal aliquots of cells (2 x 10^9) were pipetted into 2 ml of ice-cold methanol. Lipids were then extracted in 2:1 chloroform:methanol, separated by HPLC, and analyzed at A_{206 nm}. Results shown are representative of two to three experiments.
iologic range as the response of HMC to IL-1 and was maximal at 10−12 to 10−11 M.

In summary, we have demonstrated activation of two novel mechanisms produced by IL-1 stimulation of HMC (and EL-4 murine T lymphoblasts) which occurs within 5–15 s after addition of the cytokine: 1) a lyso-PA-specific acyltransferase activity that used lysophosphatidic acid as a substrate to form PA, and caused an increase in PA mass, and 2) a G-protein-dependent phosphatidate phosphohydrolase activity that hydrolyzes PA to DAG (Fig. 8). The increase in PA mass observed appears to come from incorporation of stable pools of lyso-PA (1-acylglycerol phosphate), and production from the Lands cycle, i.e. phospholipase A2-mediated conversion of PA to lyso-PA. In addition, there is evidence that lyso-PA may be produced from other sources such as lyso-PE, but this remains preliminary. Both activities are associated with the plasma membrane, and the presence of intact IL-1 receptors. Abolition of response with IL-1-receptor antibodies demonstrates that this is a specific IL-1 interaction with the cell.

The importance of PA as a cytokine and signaling intermediate has been described (Moolenaar et al., 1986; Altim and Bygrave, 1987; Murayama and Ui, 1987). Other evidence implicates lyso-PA separately as a mediator of early cell signaling pathways, and as a possible activator of G-proteins. The stimulation of lyso-PA AT activity by IL-1 provides several explanations for the effects observed with lyso-PA (Van Corven et al., 1989). In our system, lyso-PA acted synergistically with IL-1 in formation of both PA and 1,2-sn-DAG. This synergism may derive both from lyso-PA as a substrate for AT, and as an activator of phosphatidate phosphohydrolase via Gp. A second effect of lyso-PA on cell dynamics may be as the rate-limiting substrate for lyso-PA AT. Production of sn-2-un saturated PA species from 1-palmitoyl and 1-un saturated lyso-PA substrates by the acyltransferase may be sufficient as a cell activator. The importance of unsaturated acyl chains in the sn-2 position of PA in cell activation of mouse mammary epithelium has been reported (Imagawa et al., 1989).

This study provides evidence that PA is a cellular signaling intermediate; however, it also emphasizes that there are variant pathways that may be utilized to give PA and lyso-PA their relevance to cell signaling. In addition, our data elucidate the mechanisms by which IL-1 acts in early cell signaling. The complexities of rapid membrane responses to cytokines are evident, and data suggest how G-protein and non-G-protein-mediated mechanisms may interact to amplify cell responses (Fig. 8). The various interactions of both PA and lyso-PA in these mechanisms is worthy of further investigation.

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