The EMBO Journal vol.11 no.13 pp.4917-4922, 1992

Macrophage colony stimulating factor activates phosphatidylcholine hydrolysis by cytoplasmic phospholipase A_2

Takashi Nakamura, Lih-Ling Lin[1], Surender Kharbanda, John Knopf[1] and Donald Kufe[2]

Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, and [1]Genetics Institute, Inc., Cambridge, MA 02140, USA

[2]Corresponding author

Communicated by R.Kamen

The macrophage colony stimulating factor (M-CSF) is required for the proliferation and differentiation of monocytes. Previous studies have demonstrated that M-CSF stimulation is associated with phosphatidylcholine (PC) hydrolysis and increased formation of both diacylglycerol (DAG) and phosphorylcholine. The present work extends those results by demonstrating that treatment of human monocytes with M-CSF is associated with increases in a cytoplasmic Ca^{2+}-dependent activity which hydrolyzes 1-palmitoyl,2-arachidonoyl PC to arachidonic acid. The finding that this hydrolysis of PC is associated with increases in production of lysophosphatidylcholine indicates that M-CSF stimulates a cytoplasmic phospholipase A_2 ($cPLA_2$) activity. These results are supported by the demonstration that M-CSF induces $cPLA_2$ gene expression. M-CSF-induced increases in $cPLA_2$ mRNA levels were biphasic and corresponded with rapid (30-60 min) and delayed (24-72 h) increases in $cPLA_2$ activity. The results demonstrate that this effect of M-CSF on $cPLA_2$ expression is controlled at least in part by post-transcriptional stabilization of $cPLA_2$ transcripts. The finding that M-CSF treatment is also associated with phosphorylation of the $cPLA_2$ protein further suggests that expression of this enzyme is regulated at multiple levels. Finally, the stimulation of $cPLA_2$ activity and arachidonate release is supported by increases in prostaglandin (PG) synthesis. In this regard, levels of both PGE_2 and $PGF_{2\alpha}$ were increased in response to M-CSF. Taken together, these results indicate that M-CSF stimulates PC hydrolysis in human monocytes by inducing $cPLA_2$ activity and thereby formation of eicosanoids.

Key words: arachidonate/$cPLA_2$ expression/lysophosphatidylcholine/monocytes/prostaglandins

Introduction

Colony-stimulating factor 1 (M-CSF) is required for the growth and differentiation of monocytes (Stanley *et al.*, 1983). These effects are elicited by M-CSF binding to a single class of high affinity cell surface receptors (Stanley *et al.*, 1983). The M-CSF receptor is identical to the product of the c-*fms* proto-oncogene (Sherr *et al.*, 1985) and undergoes autophosphorylation on tyrosine following ligand binding (Guilbert and Stanley, 1986). Multiple other substrates for tyrosine phosphorylation have been identified following activation of the M-CSF receptor (Downing *et al.*, 1988; Morrison *et al.*, 1988; Sengupta *et al.*, 1988). However, with the exception of phosphatidylinositol-3 kinase (Varticovski *et al.*, 1989), GTPase activating protein (GAP) and GAP-associated protein p62 (Reedijk *et al.*, 1990), the identity of these other substrates is unknown. M-CSF also stimulates protein synthesis (Tushinski and Stanley, 1983), glucose uptake (Hamilton *et al.*, 1988) and Na^+, K^+-ATPase activity (Vairo and Hamilton, 1988) during induction of macrophage DNA synthesis. Other studies have indicated that M-CSF signaling involves a GTP binding protein (G protein) (Imamura and Kufe, 1988) and that increases in GTPase activity, Na^+/H^+ exchange and DNA synthesis are in part sensitive to pertussis toxin (Imamura and Kufe, 1988; Cheung and Hamilton, 1992; Hartmann *et al.*, 1990).

While certain insights are thus available regarding a variety of cellular responses to M-CSF stimulation, less is known about the activation of effector enzymes that catalyze phospholipid hydrolysis. Indeed, previous studies have demonstrated that M-CSF-induced proliferation of mouse bone marrow-derived macrophages is not associated with hydrolysis of inositol lipids (Whetton *et al.*, 1986). Similar findings have been obtained in c-*fms*-transfected hamster fibroblasts (Hartmann *et al.*, 1990) and human monocytes (Imamura *et al.*, 1990). In contrast, other studies have demonstrated that activation of the M-CSF receptor is associated with accumulation of polyphosphoinositides phosphorylated at the D-3 position of the inositol ring (Varticovski *et al.*, 1989). However, the role of these intermediates in M-CSF-induced signaling remains unknown. More recent work has demonstrated that M-CSF-stimulated human monocytes exhibit increased hydrolysis of phosphatidylcholine (PC) and enhanced formation of diacylglycerol (DAG) (Imamura *et al.*, 1990). Similar findings have been obtained during M-CSF treatment of mouse NIH 3T3 fibroblasts transfected with a human c-*fms* cDNA (Choudhury *et al.*, 1991a). Moreover, murine bone marrow-derived macrophages respond to M-CSF with stimulation of DAG production (Veis and Hamilton, 1991).

The hydrolysis of PC to DAG can occur through the activation of a PC-specific phospholipase C (PLC) and/or through the combined activities of phospholipase D (PLD) and phosphatidate phosphohydrolase. PC can also serve as a substrate for phospholipase A_2 (PLA_2) and thereby the formation of eicosanoids. The present studies demonstrate that M-CSF stimulates a cytoplasmic PLA_2 ($cPLA_2$) activity in human monocytes.

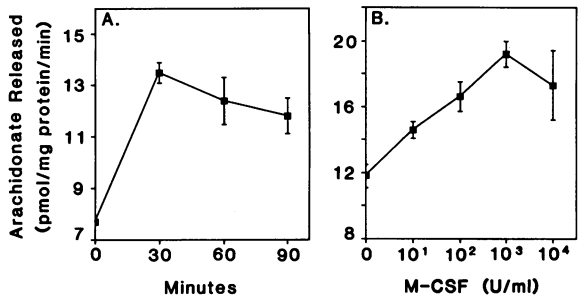

Fig. 1. M-CSF stimulates arachidonate release in human monocytes. (**A**) Monocytes were treated with M-CSF (10^3 U/ml) for the indicated times. (**B**) Monocytes were treated with the indicated concentrations of M-CSF for 30 min. The soluble fractions were obtained by sonication in buffer containing 50 mM HEPES, 0.25 M sucrose, 1 mM PMSF, 1 mM EDTA and 1 mM EGTA (pH 7.5), followed by low (1000 *g*) and then high (200 000 *g*) speed centrifugation. PLA_2 activity was assessed in the presence of 4 mM Ca^{2+} by measuring labeled arachidonate release from the *sn*-2 position of 1-palmitoyl,2-arachidonoyl PC. Results were expressed as pmol/mg protein/min. Baseline levels of arachidonate release varied from 7.5 to 12 pmol/mg protein/min depending on the donor.

Table I. Effects of M-CSF on lysophospholipid formation

Lipid	M-CSF treatment (min)	Percentage of total labeled lipids	
		[^{3}H]Glycerol	[^{14}C]Arachidonate
LPC	0	0.4 ± 0.1	0.9 ± 0.1
	15	0.5 ± 0.1	1.1 ± 0.2
	30	1.2 ± 0.2*	3.4 ± 0.7*
LPE	0	2.3 ± 0.6	7.1 ± 2.1
	15	2.0 ± 0.3	5.9 ± 0.9
	30	3.0 ± 0.3	8.9 ± 0.9

The results were expressed as the mean ± SD of three replicates. The asterisk denotes a significant difference from control as determined by the students' *t*-test. Similar results were obtained in three separate experiments.

Results

Previous work has demonstrated that M-CSF treatment is associated with increased PC, but not phosphatidylinositol (PI), turnover (Whetton *et al.*, 1986; Hartmann *et al.*, 1990; Imamura *et al.*, 1990). The finding that M-CSF induces production of both phosphorylcholine and DAG supports the role of a PC-specific PLC (Imamura *et al.*, 1990; Choudhury *et al.*, 1991a). However, PC can also serve as a substrate for PLA_2 with the formation of fatty acids and lysophosphatidylcholine (LPC). PLA_2 activity was therefore studied in monocytes using soluble extracts prepared by homogenization in the presence of 1 mM EDTA and 1 mM EGTA (Gronich *et al.*, 1988). This activity was then measured in the presence of excess Ca^{2+} and 1-palmitoyl,2-arachidonoyl PC. An increase in the hydrolysis of 1-palmitoyl,2-arachidonoyl PC to arachidonate was detectable by 30 min and reached maximal levels at 30–60 min after addition of 10^3 U/ml M-CSF (Figure 1A). Longer exposures to M-CSF were associated with a progressive decline in arachidonate release which returned to near baseline by 180 min. The effect of M-CSF on the hydrolysis of 1-palmitoyl,2-arachidonoyl PC was dose dependent with maximal increases observed at 10^3 U/ml (Figure 1B). These findings indicate that M-CSF stimulates PLA_2 activity in human monocytes.

Additional studies were performed to determine if M-CSF

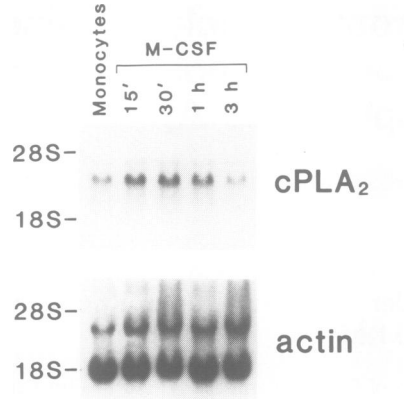

Fig. 2. M-CSF rapidly and transiently increases $cPLA_2$ mRNA levels. Monocytes were treated with 10^3 U/ml M-CSF for the indicated times. Total cellular RNA (20 μg) was hybridized to ^{32}P-labeled $cPLA_2$ and actin probes.

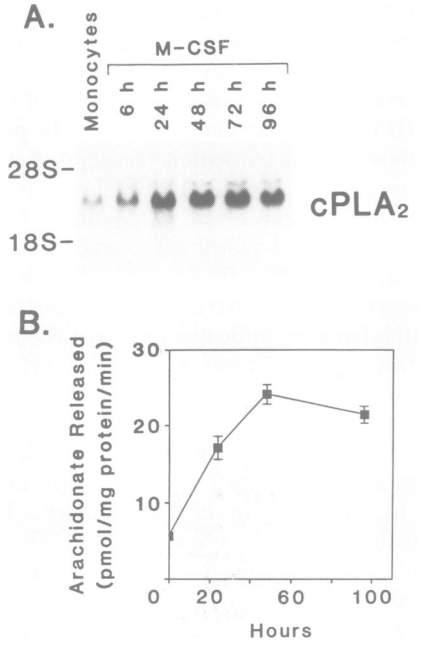

Fig. 3. Long term effects of M-CSF on $cPLA_2$ mRNA levels and arachidonate release. Monocytes were treated with 10^3 U/ml M-CSF for the indicated times. (**A**) Total cellular RNA (20 μg) was hybridized to the $cPLA_2$ probe. Actin signals demonstrated equal loading of the lanes. (**B**) PLA_2 activity (assayed in 4 mM Ca^{2+}) was determined by measuring arachidonate release from labeled 1-palmitoyl,2-arachidonoyl PC. Results are expressed as pmol/mg protein/min.

treatment also results in changes in lysophospholipid formation. Monocytes were labeled with both [^{3}H]glycerol and [^{14}C]arachidonate, and lysophospholipids were analyzed by two-dimensional TLC (Margolis *et al.*, 1988). M-CSF treatment was associated with a significant increase in LPC production at 30 min (Table I). This effect was detectable for both [^{3}H]glycerol and [^{14}C]arachidonate labeled phospholipids. Moreover, the extent of increased LPC formation was similar to that for M-CSF-induced fatty acid production. In contrast, there was no significant increase in LPE formation following M-CSF stimulation (Table I). Taken together with the hydrolysis of PC to fatty acids, the demonstration that M-CSF increases LPC production further supports the activation of a $cPLA_2$ that appears to be selective for PC over phosphatidylethanol (PE).

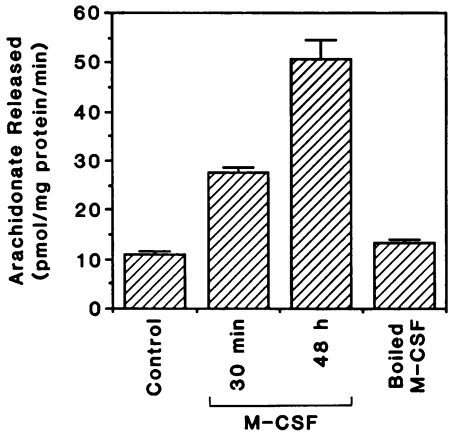

Fig. 4. M-CSF stimulates cPLA$_2$ activity assayed in the presence of 1 μM Ca^{2+}. Monocytes were treated with 10^3 U/ml M-CSF for the indicated times or with denatured M-CSF (boiled for 30 min) for 48 h. cPLA$_2$ activity was assessed in the presence of 1 μM Ca^{2+} by measuring arachidonate release from 1-stearoyl-2-arachidonyl PC. The results (pmol/mg protein/min) are expressed as the mean ± SD for three determinations.

Recent studies have described a Ca^{2+}-dependent cytosolic PLA$_2$ (cPLA$_2$) which mediates the hormonally stimulated release of arachidonate (Clark *et al.*, 1991). The effects of M-CSF on expression of this cPLA$_2$ were first determined by Northern analysis. There were low levels of 3.4 kb cPLA$_2$ transcripts in resting monocytes (Figure 2). In contrast, treatment with 10^3 U/ml M-CSF was associated with an increase in cPLA$_2$ expression which was detectable at 15 min (Figure 2). This stimulation of cPLA$_2$ mRNA levels was maximal at 30 min (3-fold as determined by laser densitometry) and then returned to pretreatment levels at 3 h (Figure 2). The finding that M-CSF has little if any effect on actin mRNA levels indicated that the changes in cPLA$_2$ expression were selective for the cPLA$_2$ gene (Figure 2). Additional studies over a longer time course revealed a biphasic pattern. Treatment with M-CSF was associated with increasing cPLA$_2$ mRNA levels which were maximal at 48 h and remained elevated for at least 96 h (Figure 3A). This later induction of cPLA$_2$ expression was associated with the hydrolysis of 1-palmitoyl,2-arachidonoyl PC. In this regard, arachidonate release was increased at 24 h of M-CSF stimulation, reached a second peak at 48 h and remained above control levels at 96 h (Figure 3B). In order to confirm these findings, we used another cPLA$_2$ assay that employs a more physiologic (1 μM) Ca^{2+} concentration (Lin *et al.*, 1992). Under these experimental conditions, treatment with M-CSF for 30 min was associated with a 2.5-fold increase in cPLA$_2$ activity (Figure 4). This activity was also increased (4.6-fold) at 48 h of M-CSF treatment (Figure 4). In contrast, boiling the M-CSF abolished the stimulation of this activity. Taken together, these findings indicated that M-CSF-induced increases in soluble PLA$_2$ activity correspond with biphasic changes in cPLA$_2$ expression at the mRNA level.

While these findings indicated that M-CSF induces cPLA$_2$ expression, further studies were performed to determine whether these effects are controlled at the transcriptional or post-transcriptional levels. Using nuclear run-on assays, we were unable to detect sufficient signals to determine transcriptional rates of the cPLA$_2$ gene in either resting or M-CSF-treated monocytes (data not shown).

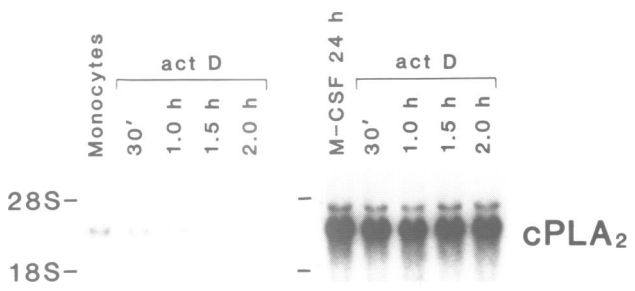

Fig. 5. M-CSF stabilizes the cPLA$_2$ transcript. Monocytes were incubated in the presence and absence of 10^3 U/ml M-CSF for 24 h. Actinomycin D (act D; 5 μg/ml) was then added for the indicated times. Total cellular RNA (20 μg) was hybridized to the cPLA$_2$ probe. Actin hybridization demonstrated equal loading of the lanes.

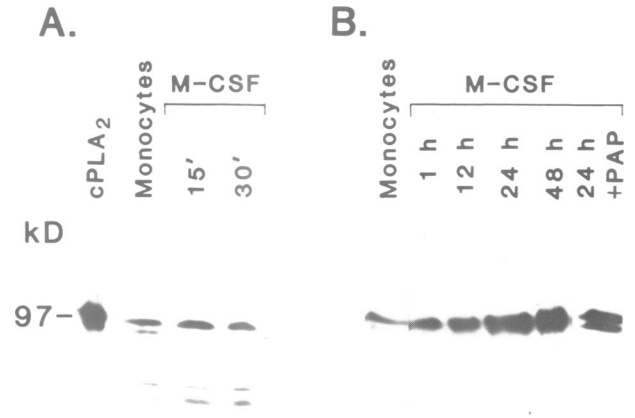

Fig. 6. Effects of M-CSF on electrophoretic mobility of cPLA$_2$. Monocytes were treated with 10^3 U/ml M-CSF. Cell lysates were prepared at the indicated times and analyzed by immunoblotting with a cPLA$_2$-specific rabbit antiserum. Migration of the bands corresponded with recombinant cPLA$_2$. PAP refers to the sample treated with potato acid phosphatase.

However, mRNA stability studies were feasible and demonstrated control at the post-transcriptional level. For example, the half-life of cPLA$_2$ transcripts in resting monocytes treated with actinomycin D was ~30 min (Figure 5). In contrast, treatment with M-CSF for 24 h was associated with an increase in half-life to over 2 h (Figure 5). These results indicated that M-CSF increases cPLA$_2$ mRNA levels at least in part by post-transcriptional mechanisms.

Recent studies have demonstrated that cPLA$_2$ protein from CHO cells migrates as a doublet and that a decrease in electrophoretic mobility following phosphorylation is associated with increased activity (Lin *et al.*, 1992). Western blot analysis of cPLA$_2$ in resting monocytes demonstrated a similar doublet, although the ratio of the two species varied from preparation to preparation (Figure 6). Interestingly, M-CSF stimulation was associated with a loss of the rapidly migrating species and an increase in intensity of the slower migrating species (Figure 6A). This effect was detectable at 15 min (Figure 6A). The intensity of the slower migrating species was increased to an even greater extent at 12–48 h (Figure 6B). Moreover, treatment of this species with potato acid phosphatase to decrease the extent of phosphorylation resulted in reappearance of the doublet (Figure 6B). These findings indicated that both phosphorylation of cPLA$_2$ and

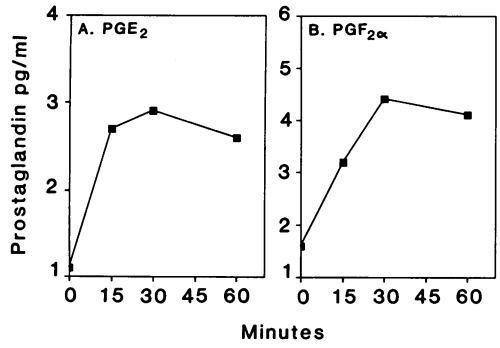

Fig. 7. M-CSF stimulates PGE_2 and $PGF_{2\alpha}$ production in human monocytes (2×10^7) were suspended in 5 ml of RPMI 1640 supplemented with 10% FBS and then treated with M-CSF (10^3 U/ml) for the indicated times. PGE_2 and $PGF_{2\alpha}$ levels in culture supernatants were measured by radioimmunoassay. The results represent the mean of duplicate determinations with a standard deviation of $< 10\%$. Similar results were obtained in three separate experiments.

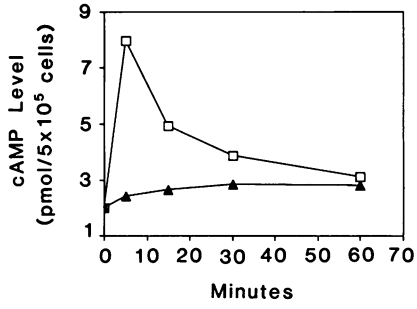

Fig. 8. Effects of M-CSF and PGE_2 on monocyte cAMP levels. Monocytes were treated with M-CSF (10^3 U/ml) (▲) or PGE_2 (1 μM) (□), for the indicated times. The results represent the mean of three determinations with a standard deviation of $< 10\%$. Similar results were obtained in four separate experiments.

increases in levels of this enzyme occur in association with M-CSF-induced arachidonate release.

PLA_2 hydrolyzes the *sn-2* ester bonds of phospholipids and thereby releases arachidonic acid for the synthesis of eicosanoids. The demonstration that M-CSF stimulates PLA_2 activity therefore prompted further studies on the production of prostaglandins and leukotrienes. Prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels were studied in the supernatants of M-CSF-stimulated monocytes. PGE_2 levels were increased at 10 min of M-CSF treatment and were maximal at 30 min (Figure 7A). Similar findings were obtained for $PGF_{2\alpha}$ (Figure 7B). Moreover, levels of both prostaglandins remained elevated at 60 min of M-CSF stimulation (Figure 7). The demonstration that M-CSF increases the production of prostaglandins is thus in concert with the stimulation of $cPLA_2$ activity and the metabolism of arachidonic acid by cyclooxygenase.

Previous studies have demonstrated that PGE_2 increases cAMP levels in different cell types and that cAMP acts as a negative regulatory signal in monocytes (Jackowski *et al.*, 1990; Vairo *et al.*, 1990). In view of the M-CSF-induced increases in PGE_2, further studies were performed to determine if this effect is associated with changes in cAMP levels. Exposure of monocytes to 1 μM PGE_2 was associated with a transient increase in cAMP levels that was maximal at 5 min and returned to baseline at 60 min (Figure 7). While these findings indicated that monocytes respond to PGE_2 with an increase in cAMP, there was no detectable effect of M-CSF on levels of this cyclic nucleotide (Figure 7). These findings may be related to desensitization of the target receptor/signaling pathway or insufficient production of PGE_2 to stimulate a detectable enhancement in cAMP levels.

Discussion

PC is hydrolyzed by phospholipases of the C and D types to yield DAG and PA, respectively (Exton, 1990). Stimulation of PC hydrolysis has been detected in certain cells exposed to growth factors, such as interleukin-1 (Rosoff *et al.*, 1988), platelet-derived growth factor (PDGF) (Besterman *et al.*, 1986; Larrodera *et al.*, 1990) and epidermal growth factor (EGF) (Wright *et al.*, 1988; Farese *et al.*, 1989). Moreover, recent studies have demonstrated that M-CSF increases PC hydrolysis, as well as DAG and phosphorylcholine production, in monocytes (Imamura *et al.*, 1990; Choudhury *et al.*, 1991a; Veis and Hamilton, 1991). While turnover of PC in the presence of increased DAG and phosphorylcholine formation supports the involvement of a PC-specific PLC, these findings could also be related to stimulation of PLD. However, measurement of PLD activity in monocytes labeled with oleic acid (Liscovitch and Amsterdam, 1989; Sandmann, 1991) has failed to demonstrate activation of this pathway by M-CSF (unpublished data). More recent studies have indicated that M-CSF activates a PC-specific PLC in rat peritoneal macrophages and that this event involves tyrosine phosphorylation and a pertussis toxin-sensitive G protein (Choudhury *et al.*, 1991b). Thus, the available information suggests that M-CSF-induced increases in PC turnover is at least in part due to the activation of PLC. The significance of these findings in M-CSF-induced signaling is unclear, although recent studies have demonstrated that this step is important in PDGF-stimulated DNA synthesis (Larrodera *et al.*, 1990).

The present findings demonstrate that M-CSF stimulates a soluble PLA_2 activity in human monocytes. A similar approach has been used to characterize a hormonally regulated form of soluble PLA_2 in rat renal mesangial cells (Gronich *et al.*, 1988). Treatment of these cells with EGF is associated with enhanced PLA_2 activity as evidenced by the ability of soluble extracts to cleave PC to arachidonate (Bonventre *et al.*, 1990). A $cPLA_2$ has recently been identified in U-937 myeloid leukemia cells which hydrolyzes phospholipids containing arachidonoyl at the 2-position (Clark *et al.*, 1990; Kramer *et al.*, 1991). Cloning of the $cPLA_2$ gene has identified a 45 amino acid Ca^{2+}-dependent phospholipid binding domain with homology to protein kinase C (PKC), GAP, the synaptic vesicle p65 and PLC (Clark *et al.*, 1991; Sharp *et al.*, 1991). Using a $cPLA_2$ cDNA clone, the present results demonstrate that M-CSF induces expression of this gene. The M-CSF-induced increases in $cPLA_2$ expression were detectable in a biphasic pattern and corresponded with stimulation of arachidonate release. The precise mechanisms responsible for this increase in $cPLA_2$ transcripts are unclear, although the results indicate that this effect is in part related to an increase in mRNA stability. Moreover, it is not yet clear whether the latter increase in $cPLA_2$ activity is directly related to M-CSF-stimulated signaling events or through the effects of another cytokine induced by M-CSF.

The signaling events responsible for the stimulation of

cPLA$_2$ activity are not yet known. Previous work has indicated that cPLA$_2$ in certain cell types is stimulated by the activation of PKC (Parker *et al.*, 1987; Bonventre and Swindler, 1988; Halenda *et al.*, 1989). Treatment of human monocytes with M-CSF is associated with increases in both DAG production and PKC activity (Imamura *et al.*, 1990). Thus, activation of PKC, and thereby potential phosphorylation of cPLA$_2$, could contribute to increases in cPLA$_2$ activity in these cells. Other studies have indicated that G proteins may be involved in the regulation of cPLA$_2$ (Burch and Axelrod, 1987; Gupta *et al.*, 1990; Lowndes *et al.*, 1991). The treatment of human monocytes with M-CSF is associated with increases in GTPase activity (Imamura and Kufe, 1988). However, with the exception of ras, the role of G proteins in M-CSF-induced signaling remains unclear. Moreover, while certain M-CSF-induced signaling events are sensitive to pertussis toxin (He *et al.*, 1988; Imamura and Kufe, 1988; Hartmann *et al.*, 1990; Choudhury *et al.*, 1991b; Cheung and Hamilton, 1992), it is not known whether these effects are related to inhibition of G protein function or to indirect effects of this toxin such as transient increases in cAMP (Burch *et al.*, 1988). Further studies are needed to determine whether this enzyme is phosphorylated on tryosine and/or serine/threonine residues in M-CSF-treated cells. The finding in fibroblasts that EGF and PDGF activate cPLA$_2$ by phosphorylation on serine residues indicates that cPLA$_2$ is not a direct substrate for certain receptor tyrosine kinases (Lin *et al.*, 1992).

The present studies also demonstrate that the stimulation of monocytes with M-CSF is associated with increased production of prostaglandins. While we have found increased levels of PGE$_2$ and PGF$_{2\alpha}$, multiple other prostanoids may be produced in response to M-CSF. This production of prostaglandins may contribute to the role of M-CSF-activated monocytes in the inflammatory response. Other studies have shown that PGE$_2$ and cAMP inhibit M-CSF-induced growth of murine macrophages (Jackowski *et al.*, 1990; Vairo *et al.*, 1990; Cheung and Hamilton, 1992). Our findings demonstrate that while 1 μM PGE$_2$ increases cAMP in human monocytes, there was little if any effect of M-CSF-induced increases in PGE$_2$ levels on production of this cyclic nucleotide. Finally, recent studies have demonstrated the eicosanoids, including PGE$_2$ and PGF$_{2\alpha}$, inhibit GAP function in certain cells (Tsai *et al.*, 1989; Han *et al.*, 1991). Thus, PGE$_2$ may function in blocking M-CSF signaling through a ras p21−GAP pathway. In this context, M-CSF treatment is associated with phosphorylation of GAP (Reedijk *et al.*, 1990) and ras p21 is involved in signaling mediated by the activated M-CSF receptor (Bortner *et al.*, 1991). The functional interaction between ras p21 and GAP is also involved in the activation of PC-specific PLC (Dominguez *et al.*, 1991). Thus, while M-CSF may induce PC hydrolysis through ras p21−GAP signaling to PLC, the production of eicosanoids, such as PGE$_2$ and PGF$_{2\alpha}$, could play a role in the down-regulation of this event.

Materials and methods

Monocyte isolation and culture

Human monocytes were isolated from the peripheral blood of healthy volunteers by Ficoll−Paque separation followed by adherence for 1 h and removal of the non-adherent cells. The adherent cells were collected with a plastic policeman, washed three times with culture medium, readhered and washed again three times with medium. The resultant cell population consisted of >95% monocytes as judged by morphologic examination and α-naphthyl acetate esterase staining. Monocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. The monocytes were treated with 10^3 U/ml M-CSF (Genetics Institute, Cambridge, MA), 5 μg/ml actinomycin D (Sigma Chemical Co., St Louis, MO) and 1 μM prostaglandin E$_2$ (PGE$_2$; Sigma).

Soluble PLA$_2$ activity

Monocytes were washed and resuspended in homogenization buffer containing 50 mM HEPES, 0.25 M sucrose, 1 mM phenylmethylsulfonylfluoride (PMSF), 1 mM EDTA and 1 mM EGTA (pH 7.5) (Gronich *et al.*, 1988). The cells were sonicated on ice and the lysate centrifuged at 1000 *g* for 5 min to remove nuclei and unbroken cells. The supernatant was then subjected to centrifugation at 200 000 *g* for 1 h at 4°C. The resulting cytosolic fractions were stored at −70°C until use. The PLA$_2$ assay was performed at 37°C for 30 min in Eppendorf tubes containing 190 μl of cytosolic fraction (40−60 μg protein), 4 mM $CaCl_2$ and 4 μl (3×10^5 c.p.m.) L-α-1-palmitoyl-2-arachidonoyl-arachidonoyl-1-[^{14}C]phosphatidylcholine (55 μCi/mmol). The reaction was terminated by adding 100 μl ethanol containing 2% (v/v) acetic acid and 120 μg/ml free arachidonic. The reaction mixture (50 μl) was applied to Silica Gel TLC plates and developed with the organic phase of a mixture of ethyl acetate/iso-octane/acetic acid/water (55:75:8:100, by vol). The radioactivity corresponding to authentic arachidonic was scraped into 1 ml methanol/HCl (150:1, v/v) and counted after addition by Hydrofluor.

Monocytes were also sonicated to 50 mM HEPES (pH 7.4), 1 mM PMSF, 1 mM EGTA and 10 μg/ml leupeptin (Clark *et al.*, 1991). Cell lysates were centrifuged at 8500 *g* for 30 min at 4°C. The supernatant was then subjected to centrifugation at 100 000 *g* for 60 min. The resulting supernatant (60 μg protein) was assayed in 10 mM HEPES (pH 7.4) containing 1 μM free Ca^{2+} (1 mM EGTA/0.975 mM Ca^{2+}) and 2 μM 1-stearoyl-2-[^{14}C]arachidonyl phosphatidylcholine (50−60 mCi/mmol). The assay was performed at 37°C for 5 min and stopped by the addition of isopropanol, heptane and 0.5 M sulfuric acid (20:5:1). The samples were assayed on 1 ml silicon columns (Whatmann) and monitored for radioactivity. PLA$_2$ activity is expressed as pmol of arachidonate released per mg protein per min.

Lysophospholipid analysis

As Margolis *et al.* (1988). Monocytes were prelabeled with [2-^{3}H]glycerol for 48 h and [^{14}C]arachidonate for 24 h. After stimulation with M-CSF, the lipids were extracted and analyzed by two-dimensional TLC with $CHCl_3$/methanol/NH_4OH (130:70:11, v/v/v) and $CHCl_3$/methanol/formic acid (11:5:1, v/v/v) as the first and second solvent systems, respectively. The authentic standards were cochromatographed and visualized with iodine vapor. The TLC plates were then subjected to autoradiography and radioactivities corresponding to lysophosphatidylcholine (LPC) and lysophosphatidylethanol (LPE) scraped and counted.

Preparation of RNA and Northern blot analysis

Total cellular RNA (20 μg) was isolated by the guanidine isothiocyanate/cesium chloride method, subjected to electrophoresis in a 1% agarose−0.66 M formaldehyde gel, transferred to nitrocellulose paper and hybridized to the 3.0 kb *Sal*I insert of a human cPLA$_2$ cDNA purified from the pMT-2 plasmid (Clark *et al.*, 1991) and the 2.0 kb *Pst*I insert of a chicken β-actin cDNA gene purified from the pA1 plasmid (Cleveland *et al.*, 1980). Hybridization reactions were performed for 16−18 h at 42°C in 50% (v/v) formamide, 2× SSC, 1× Denhardt's solution, 0.1% (w/v) SDS and 200 μg/ml salmon sperm DNA. Filters were washed in 2× SSC, 0.1% SDS at room temperature for 20 min, and then 0.1× SSC, 0.1% SDS at 60°C for 20 min. Filters were exposed to Kodak X-Omat XAR film using an intensifying screen. In some experiments, the autoradiograms were scanned using an LKB (Bromma, Sweden) Ultrascan XL laser densitometer and analyzed using the Gelscan XL software package.

Immunoblot analysis

Antibodies were raised in rabbits immunized with cPLA$_2$ produced in *Escherichia coli* and purified by preparative SDS−PAGE (Lin *et al.*, 1992). Cell lysates were obtained by sonicating monocytes at 4°C for 20 s in 10 mM HEPES buffer (pH 7.4) containing 1 mM EGTA and 2 mM PMSF. The lysates were centrifuged at 1000 *g* for 5 min to remove nuclei. The supernatant fraction (40 μg protein) was analyzed by SDS−7.5% PAGE, transferred to nitrocellulose paper, incubated with the cPLA$_2$ specific antiserum and then with goat anti-rabbit IgG conjugated to horseradish peroxidase (Boehringer Mannheim, Indianapolis, IN). Alternatively, the protein was first treated with 2 μg potato acid phosphatase (PAP; Type III, Sigma) for 20 min at 30°C. Detection of immunoreactive bands was carried

out using the ECL Western blotting system (Amersham, Arlington Heights, IL).

Measurement of PGE_2 and $PGF_{2\alpha}$ levels

Monocyte culture supernatants were assayed in duplicate using radioimmunoassays specific for PGE_2 (DuPont Co., Boston, MA), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Amersham Corp., Arlington Heights, IL).

Measurement of cAMP

Monocyte proteins were precipitated by addition of 10% TCA. The TCA-soluble fraction was washed four times with diethyl ether. The aqueous phase was lyophilized and then dissolved in 0.05 M Tris−HCl, pH 7.5, containing 4 mM EDTA. cAMP content was determined using a commercially available cAMP assay (Amersham).

Acknowledgements

This investigation was supported by PHS Grant #CA34183 awarded by the National Cancer Institute, DHHS and by a Burroughs Wellcome Award in Clinical Pharmacology (DK).

References

Besterman,J.M., Duronio,V. and Cuatrecasas,P. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6785−6789.

Bonventre,J.V. and Swindler,M. (1988) *J. Clin. Invest.*, **82**, 168−176.

Bonventre,J.V., Gronich,J.H. and Nemenoff,R.A. (1990) *J. Biol. Chem.*, **265**, 4934−4938.

Bortner,D.M., Ulivi,M., Roussel,M.F. and Ostrowski,M.C. (1991) *Genes Dev.*, **5**, 1777−1785.

Burch,R.M. and Axelrod,J. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 6374−6378.

Burch,R.M., Jelsema,C. and Axelrod,J. (1988) *J. Pharmacol. Exp. Ther.*, **244**, 765−773.

Cheung,D.L. and Hamilton,J.A. (1992) *Blood*, **79**, 1972−1981.

Choudhury,G.G., Sylvia,V.L., Wang,L.-M., Pierce,J. and Sakaguchi,A.Y. (1991a) *FEBS Lett.*, **282**, 351−354.

Choudhury,G.G., Sylvia,V.L. and Sakaguchi,A.Y. (1991b) *J. Biol. Chem.*, **266**, 23147−23151.

Clark,J.D., Milona,N. and Knopf,J.L. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7708−7712.

Clark,J.D., Lin,L.-L., Kriz,R.W., Ramesha,C.S., Sultzman,L.A., Lin,A.Y., Milona,N. and Knopf,J.L. (1991) *Cell*, **65**, 1043−1051.

Cleveland,D.W., Lopata,M.A., MacDonald,R.J., Cowan,N.J., Rutter,W.J. and Kirschner,M.W. (1990) *Cell*, **20**, 95−105.

Dominguez,I., Marshall,M.S., Gibbs,J.B., Garcia de Herreros,A., Cornet,M.E., Graziani,G., Diaz-Meco,M.T., Johansen,T., McCormick,F. and Moscat,J. (1991) *EMBO J.*, **10**, 3215−3220.

Downing,J.R., Rettenmier,C.W. and Sherr,C.J. (1988) *Mol. Cell. Biol.*, **8**, 1795−1799.

Exton,J.H. (1990) *J. Biol. Chem.*, **265**, 1−4.

Farese,R.V., Nair,G.P., Sierra,C.G., Standaert,M.L., Pollet,R.J. and Cooper,D.R. (1989) *Biochem. J.*, **261**, 927−934.

Gronich,L.J., Bonventre,J.V. and Nemenoff,R.A. (1988) *J. Biol. Chem.*, **263**, 16645−16651.

Guilbert,L.J. and Stanley, E. R. (1986) *J. Biol. Chem.*, **261**, 4024−4032.

Gupta,S.K., Diez,E., Heasley,L.E., Osawa,S. and Johnson,G.L. (1990) *Science*, **249**, 662−666.

Halenda,S.P., Banga,H.S., Zavoico,G.B., Lau,L. and Feinstein,M.B. (1989) *Biochemistry*, **28**, 7356−7363.

Hamilton,J.A., Vairo,G. and Lingelbach,S.R. (1988) *J. Cell. Physiol.*, **134**, 405−412.

Han,J.-W., McCormick,F. and Macara,I.G. (1991) *Science*, **252**, 576−578.

Hartmann,T., Seuwen,K., Roussel,M.F., Sherr,C.J. and Pouyssegur,J. (1990) *Growth Factors*, **2**, 289−300.

He,Y., Hewlett,E., Temples,D. and Quesenberry,P. (1988) *Blood*, **71**, 1187−1195.

Imamura,K. and Kufe,D. (1988) *J. Biol. Chem.*, **263**, 14093−14098.

Imamura,K., Dianoux,A., Nakamura,T. and Kufe,D. (1990) *EMBO J.*, **9**, 2423−2430.

Jackowski,S., Rettenmier,C.W. and Rock,C.O. (1990) *J. Biol. Chem.*, **265**, 6611−6616.

Kramer,R.M., Roberts,E.F., Manetta,J. and Putnam,J.E. (1991) *J. Biol. Chem.*, **266**, 5268−5272.

Larrodera,P., Cornet,M.E., Diaz-Meco,M.T., Lopez-Barahona,M., Diaz-Laviada,I., Guddal,P.H., Johansen,T. and Moscat,J. (1990) *Cell*, **61**, 1113−1120.

Lin,L.-L., Lin,A.Y. and Knopf,J.L. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 6147−6151.

Liscovitch,M. and Amsterdam,A. (1989) *J. Biol. Chem.*, **264**, 11762−11767.

Lowndes,J.M., Gupta,S.K., Osawa,S. and Johnson,G.L. (1991) *J. Biol. Chem.*, **266**, 14193−14197.

Margolis,B.L., Holub,B.J., Troyer,D.A. and Skorecki,K.L. (1988) *Biochem. J.*, **256**, 469−474.

Morrison,D.K., Browning,P.J., White,M.F. and Roberts,T.M. (1988) *Mol. Cell. Biol.*, **8**, 176−185.

Parker,J., Daniel,L.W. and Waite,M. (1987) *J. Biol. Chem.*, **262**, 5385−5393.

Reedijk,M., Liu,X. and Pawson,T. (1990) *Mol. Cell. Biol.*, **10**, 5601−5608.

Rosoff,P.M., Savage,N. and Dinarello,C.A. (1988) *Cell*, **54**, 73−81.

Sandmann,J., Peralta,E.G. and Wurtman,R.J. (1991) *J. Biol. Chem.*, **266**, 6031−6034.

Sengupta,A., Liu,W.-K., Yeung,Y.G., Yeung,D.C.Y., Frackelton Jr,A.R. and Stanley,E.R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8062−8066.

Sharp,J.D. *et al.* (1991) *J. Biol. Chem.*, **266**, 14850−14853.

Sherr,C.J., Rettenmier,C.W., Sacca,R., Roussel,M.F., Look,A.T. and Stanley,E.R. (1985) *Cell*, **41**, 665−676.

Stanley,E.R., Guilbert,L.J., Tushinski,R.J. and Bartelemez,S.H. (1983) *J. Cell Biochem.*, **21**, 151−159.

Tsai,M.-H., Yu,C.-L., Wei,F.-S. and Stacey,D.W. (1989) *Science*, **243**, 522−526.

Tushinski,R.J. and Stanley,T. (1983) *J. Cell. Physiol.*, **116**, 67−75.

Vairo,G. and Hamilton,J.A. (1988) *J. Cell. Physiol.*, **134**, 13−24.

Vairo,G., Argyriou,S., Bordun,A.-M., Whitty,G. and Hamilton,J.A. (1990) *J. Biol. Chem.*, **265**, 2692−2701.

Varticovski,L., Druker,B., Morrison,D., Cantley,L. and Roberts,T. (1989) *Nature*, **342**, 699−702.

Veis,N. and Hamilton,J.A. (1991) *J. Cell. Physiol.*, **147**, 298−305.

Whetton,A.D., Monk,P.N., Consalvey,S.D. and Downes,C.P. (1986) *EMBO J.*, **5**, 3281−3286.

Wright,T.M., Rangan,L.A., Shin,H.S. and Raben,M.M (1988) *J. Biol. Chem.*, **263**, 9374−9380.

Received on May 15, 1992; revised on August 24, 1992