Mechanisms of Platelet-activating Factor–induced Lipid Body Formation: Requisite Roles for 5-Lipoxygenase and De Novo Protein Synthesis in the Compartmentalization of Neutrophil Lipids

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Summary

Lipid bodies, lipid-rich cytoplasmic inclusions, are characteristically abundant in vivo in leukocytes associated with inflammation. Because lipid bodies are potential reservoirs of esterified arachidonate and sites at which eicosanoid-forming enzymes may localize, we evaluated mechanisms of lipid body formation in neutrophils (PMN). Among receptor-mediated agonists, platelet-activating factor (PAF), but not C5a, formyl-methyl-phenylalanine, interleukin 8, or leukotriene (LT) B_4, induced the rapid formation of lipid bodies in PMN. This action of PAF was receptor mediated, as it was dose dependently inhibited by the PAF receptor antagonist WEB 2086 and blocked by pertussis toxin. Lipid body induction by PAF required 5-lipoxygenase (LO) activity and was inhibited by the 5-lipoxygenase-activating protein antagonist MK 886 and the 5-LO inhibitor zileuton, but not by cyclooxygenase inhibitors. Corroborating the dependency of PAF-induced lipid body formation on 5-LO, PMN and macrophages from wild-type mice, but not from 5-LO genetically deficient mice, formed lipid bodies on exposure to PAF both in vitro and in vivo within the pleural cavity. The 5-LO product inducing lipid body formation was not LTB_4 but was 5(*S*)-hydroxyeicosatetraenoic acid [5(*S*)-HETE], which was active at 10-fold lower concentrations than PAF and was also inhibited by pertussis toxin but not by zileuton or WEB 2086. Furthermore, 5-HETE was equally effective in inducing lipid body formation in both wild-type and 5-LO genetically deficient mice. Both PAF- and 5(*S*)-HETE–induced lipid body formation were inhibited by the protein kinase C (PKC) inhibitors staurosporine and chelerythrine, the phospholipase C (PLC) inhibitors D609 and U-73122, and by actinomycin D and cycloheximide. Prior stimulation of human PMN with PAF to form lipid bodies enhanced eicosanoid production in response to submaximal stimulation with the calcium ionophore A23187; and the levels of both prostaglandin (PG) E_2 and LTB_4 correlated with the number of lipid bodies. Furthermore, pretreatment of cells with actinomycin D or cycloheximide inhibited not only the induction of lipid body formation by PAF, but also the PAF-induced "priming" for enhanced PGE_2 and LTB_4 in PMN. Thus, the compartmentalization of lipids to form lipid bodies in PMN is dependent on specific cellular responses that can be PAF receptor mediated, involves signaling through 5-LO to form 5-HETE and then through PKC and PLC, and requires new protein synthesis. Since increases in lipid body numbers correlated with priming for enhanced PGE_2 and LTB_4 production in PMN, the induction of lipid bodies may have a role in the formation of eicosanoid mediators by leukocytes involved in inflammation.

Eicosanoids, including PG, thromboxanes, leukotrienes (LT)[1], hydroxyeicosatetraenoic acids (HETE), and lipoxins, are enzymatically formed oxidative derivatives of arachidonic acid that have a wide range of biological activities, including roles as paracrine mediators of inflammation (1, 2). Arachidonate is released by the actions of phospholipases from arachidonyl phospholipids, which may reside in various membranes within cells. In addition to membranes, another lipid-bearing domain in cells can be lipid bodies, which are lipid-rich cytoplasmic inclusions. Although lipid

[1] *Abbreviations used in this paper:* FLAP, 5-lipoxygenase-activating protein; 5-oxo-ETE, 5-oxo-eicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LO, lipoxygenase; LT, leukotriene; PAF, platelet-activating factor; PKC, protein kinase C; PLC, phospholipase C.

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bodies are solubilized with alcohol-based hematologic stains, with appropriate fixation and staining, lipid bodies have been recognized to be prominent in leukocytes at sites of natural and experimental inflammation. For instance, increased lipid body numbers have been noted in PMN from inflammatory arthritis (3), from bronchoalveolar lavage of patients with acute respiratory distress syndrome (4), and from elicited rabbit peritoneal exudates (5). Lipid bodies can be sites of esterified arachidonate localization in cells including PMN and eosinophils (6, 7). In addition, PG endoperoxide synthase has been localized to lipid bodies in several types of leukocytes and other cells (8–10). Although lipid bodies are prominent in cells associated with inflammation and might have roles in the oxidative metabolism of arachidonate to form eicosanoids, little is known about how these cytoplasmic structures form.

We have evaluated the capacity of several agonists, known to stimulate PMN through specific cognate G protein–linked plasma membrane receptors, to elicit lipid body formation. Of these PMN agonists, platelet-activating factor (PAF) specifically induces lipid body formation; and the actions of PAF are receptor mediated, obligately dependent on 5-lipoxygenase (LO) activation, as well as dependent on subsequent stimulation of protein kinase C (PKC), phospholipase C (PLC), and new protein synthesis. The PAF-elicited compartmentalization of lipids to form new lipid bodies is associated with enhanced capacity for eicosanoid generation, suggesting that the cellular responses leading to lipid body formation may be important in the formation of eicosanoid mediators of inflammation.

Materials and Methods

LTB_4, rhC5a, FMLP, aspirin, indomethacin, ibuprofen, piroxicam, salicylic acid (sodium salt), nordihydroguaiaretic acid, and cycloheximide were obtained from Sigma Chemical Co. (St. Louis, MO). PAF (1-*O*-hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), lyso-PAF (1-*O*-alkyl-*sn*-glyceryl-3-phosphorylcholine), staurosporine, chelerythrine, actinomycin D, pertussis toxin, and A23187 were from Calbiochem Novabiochem Corp. (La Jolla, CA). 5(*S*)-HETE [5(*S*)-hydroxy-6,8,11,14-eicosatetraenoic acid] and 5(*R*)-HETE [5(*R*)-hydroxy-6,8,11,14-eicosatetraenoic acid] were from Cayman Chemical Co. Inc. (Ann Arbor, MI). The PLC inhibitors D609 and U-73122, and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) were from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA). The following were generous gifts: WEB 2086 from Boehringer-Ingelheim (Ingelheim, Germany), zileuton from Abbott Laboratories (North Chicago, IL), CP-105,696 from Dr. Henry Showell (Pfizer Central Research, Groton, CT), and MK886 from Dr. Jilly Evans (Merck Frosst, Point Claire-Dorval, Quebec, Canada).

Human Neutrophil Purification. Fresh human blood was obtained by venipuncture from healthy adult volunteers and collected into acidified citrate. After addition of 6% dextran 70 (McGaw, Irvine, CA), RBC were allowed to sediment for 1 h at room temperature. The leukocyte-rich supernatant was overlaid onto an equal volume of Ficoll–Paque (Pharmacia Biotechnology Inc., Piscataway, NJ), and centrifuged at 400 *g* for 20 min. PMN (>95% pure, rest being eosinophils) were recovered from the pellet and washed in Ca^{2+}/Mg^{2+}–free HBSS. Residual RBC were lysed with hypotonic saline.

Investigations with Mouse Leukocytes. 5-LO gene-targeted mice ($5\text{-}LO^{-/-}$; Dr. Beverly Koller, University of North Carolina, Chapel Hill, NC) and control wild-type mice ($5\text{-}LO^{+/+}$) were generated as described (11). All experiments were carried out with male 8–12-wk-old mice. Bone marrow, from both femurs and humeri, was obtained by lavaging the marrow cavity with 3 ml of RPMI containing heparin (20 IU/ml). After dissociating the bone marrow with a transfer pipette, the cell suspension was overlaid onto an equal volume of Ficoll–Paque and centrifuged at 400 *g* for 20 min. The supernatant was aspirated, and the pellet, rich in granulocytes (>90% pure), was resuspended in Ca^{2+}/Mg^{2+}–free HBSS.

Pleurisy was induced in methoxyfluorane anesthetized mice by intrathoracic injection of PAF (1 μg/cavity) in a final volume of 100 μl. Each experiment included an equivalent number of control animals receiving the same volume of sterile Ca^{2+}/Mg^{2+}–free HBSS. The animals were killed in CO_2 4 h after the intrathoracic injection of PAF or buffer. The thoracic cavity was washed with 1 ml of heparinized Ca^{2+}/Mg^{2+}–free HBSS (10 IU/ml).

Lipid Body Induction and Treatments. PMN (10^6 cells/ml) were incubated with varying concentrations of PAF, lyso-PAF, 5-HETE, 5-oxo-ETE, C5a, LTB_4, FMLP, IL-8, or vehicle at 37°C in a 5% CO_2 95% O_2 atmosphere; and after incubation periods (5 min–4 h), PMN (10^5/slide) were cytocentrifuged (550 rpm, 5 min) onto glass slides. During inhibitory studies, PMN were pretreated for 1 h with varying concentrations of receptor antagonist, enzyme inhibitors, protein synthesis inhibitors, or vehicle as indicated. When PKC inhibitors were used, the preincubation time was reduced to 30 min to avoid toxic effect to the cells. The cell viability, determined by trypan blue dye exclusion at the end of each experiment, was always >90%. Stock solutions for A23187, WEB 2086, D609, U-73122, zileuton, indomethacin, CP-105,696, actinomycin D, and MK886 were prepared in DMSO and stored at −20°C. Aliquots were diluted in Ca^{2+}/Mg^{2+}–free HBSS to the indicated concentration immediately before use. The final DMSO concentration was always <0.1% and had no effect on lipid body numbers. Ibuprofen, aspirin, piroxicam, salicylic acid, staurosporine, chelerythrine, cycloheximide, and pertussis toxin were diluted in Ca^{2+}/Mg^{2+}–free HBSS.

Leukocyte Counts. Blood or pleural samples were diluted in Turk fluid (2% acetic acid) for total leukocyte counts in a hemocytometer. Differential analyses were performed on cytocentrifuged samples or blood smears stained with Diff-Quik (Baxter Healthcare Corp., Miami, FL).

Lipid Body Staining and Enumeration. Slides, while still moist, were fixed in 3.7% formaldehyde in Ca^{2+}/Mg^{2+}–free HBSS, pH 7.4, rinsed in 0.1 M cacodylate buffer, pH 7.4, stained in 1.5% OsO_4 (30 min), rinsed in dH_2O, immersed in 1.0% thiocarbohydrazide (5 min), rinsed in 0.1 M cacodylate buffer, restained in 1.5% OsO_4 (3 min), rinsed in dH_2O, and then dried and mounted. The morphology of fixed cells was observed, and lipid bodies were enumerated by phase contrast microscopy with an objective lens at a magnification of 100 in 50 consecutively scanned PMN.

PGE_2 and LTB_4 Measurement. Human PMN (5×10^6 cells/5 ml) were stimulated with PAF (10^{-8}–10^{-6} M) or vehicle at 37°C for 5 or 60 min for lipid body formation. After incubations, samples were taken for lipid body enumeration and PMN were washed in Ca^{2+}/Mg^{2+}–free HBSS. PMN were resuspended in 1 ml of HBSS containing Ca^{2+}/Mg^{2+} and then stimulated with A23187 (0.5 μM) for 15 min. Reactions were stopped on ice, and the

samples were centrifuged at 500 *g* for 10 min at 4°C. PGE_2 and LTB_4 in the supernatants were assayed by ELISA according to the manufacturer's instructions (Cayman Chemical Co., Inc.).

Statistical Analysis. Results were expressed as mean ± SEM and were analyzed statistically by means of analysis of variance followed by the Newman-Keuls-Student test with the level of significance set at $P < 0.05$. Correlation coefficients were determined by linear regression, and correlation analysis was performed by Fisher's *r* to *z* transformation with the level of significance set at $P < 0.05$.

Results

Agonist Induction of Lipid Body Formation in Human PMN. Several PMN agonists, known to act via specific G protein–linked receptors, were evaluated for their ability to stimulate lipid body formation. LTB_4 (10^{-9}–10^{-7} M), C5a (10^{-9}–10^{-7} M), IL-8 (1–25 ng/ml), and FMLP (10^{-8}–10^{-6} M), in concentrations that significantly induce chemotaxis in human PMN (12), failed to cause lipid bodies to form (Table 1). In contrast, PAF stimulated a dose-dependent induction of lipid bodies in PMN in vitro (Fig. 1 *A*). The PAF induction of lipid body formation was significant within 15 min, maximal at 1–2 h, and decreased thereafter (Fig. 1 *B*). Several findings indicated the actions of PAF were receptor mediated. First, lyso-PAF failed to induce lipid body formation (Fig. 1 *A*). Second, the PAF receptor antagonist, WEB 2086, dose dependently inhibited PAF-induced lipid body formation (Fig. 2), with an approximate IC_{50} of 0.4 μM. Moreover, prior treatment of cells with pertussis toxin (100 ng/ml, 1 h before PAF) completely prevented PAF-induced lipid body formation (Fig. 2).

Requirement for 5-LO Activation in PAF-induced Lipid Body Formation. The possibility that eicosanoids might be involved in mediating PAF-initiated signaling to form lipid bodies was evaluated with a variety of inhibitors. Four cyclooxygenase inhibitors (mean ± SEM lipid bodies/PMN: 13.2 ± 0.6 with 1 μg/ml of indomethacin; 13.5 ± 0.5 with 10 μg/ml of aspirin; 12.3 ± 0.4 with 10 μg/ml of ibuprofen; 13.2 ± 0.5 with 10 μg/ml of piroxicam vs. 12.2 ± 0.5 for vehicle-treatment alone, NS) and salicylic acid (13.5 ± 0.4 with 10 μg/ml of salicylic acid vs. 12.2 ± 0.5 for vehicle-

Table 1. *Effect of Agonists Acting on G Protein–linked Receptors on PMN Lipid Body Formation*

Stimuli	Dose	Lipid bodies
		(mean ± SEM)/PMN
C5a	0	3.9 ± 0.3
	10^{-9} M	3.9 ± 0.2
	10^{-8} M	4.0 ± 0.2
	10^{-7} M	3.7 ± 0.2
LTB_4	0	3.9 ± 0.3
	10^{-9} M	3.9 ± 0.2
	10^{-8} M	4.0 ± 0.2
	10^{-7} M	4.0 ± 0.3
FMLP	0	3.9 ± 0.3
	10^{-8} M	4.0 ± 0.3
	10^{-7} M	4.0 ± 0.3
	10^{-6} M	4.0 ± 0.3
IL-8	0	1.4 ± 0.2
	1 ng/ml	1.8 ± 0.2
	5 ng/ml	1.7 ± 0.2
	25 ng/ml	2.0 ± 0.2

PMN (10^6 cells/ml) were treated with each agonist for 1 h at 37°C. Results are means ± SEM from 50 consecutively counted PMN. Data from one individual donor are representative of three experiments with similar results.

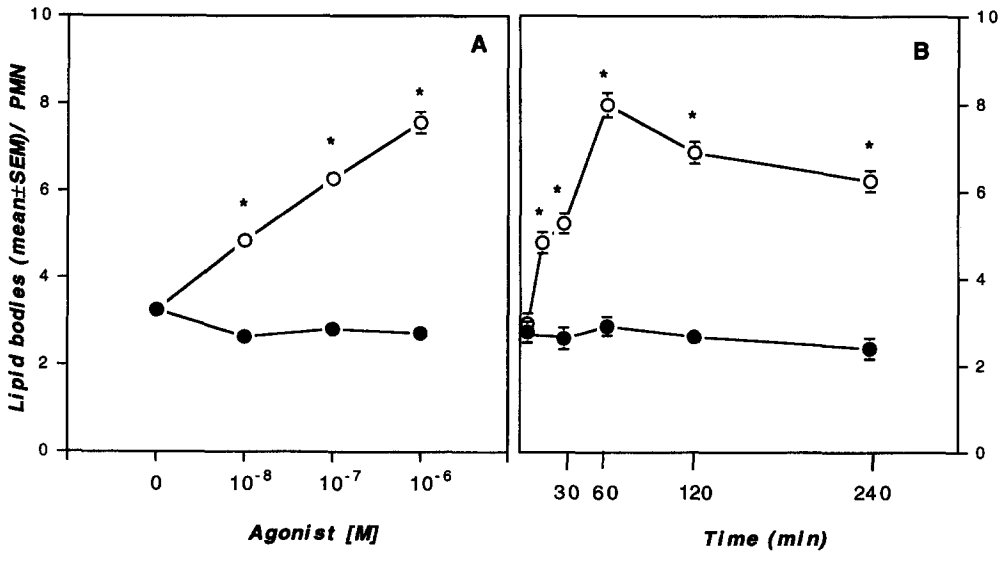

Figure 1. Dose response (*A*) and time course (*B*) for PAF-induced lipid body formation in human PMN. (*A*) PMN (10^6 cells/ml) were treated with PAF (10^{-8}–10^{-6} M) (*open circle*) or lyso-PAF (10^{-8}–10^{-6} M) (*solid circle*) for 1 h at 37°C. (*B*) PMN were treated with PAF (1 μM) at 37°C. Samples were taken at various time intervals (5–240 min). The open and solid circles represent PAF and vehicle, respectively. Lipid bodies were enumerated using light microscopy after osmium staining. Each point represents the mean ± SEM from 50 consecutively counted PMN. Data from one individual donor are representative of four to six experiments with similar results. Statistically significant differences are indicated by asterisks.

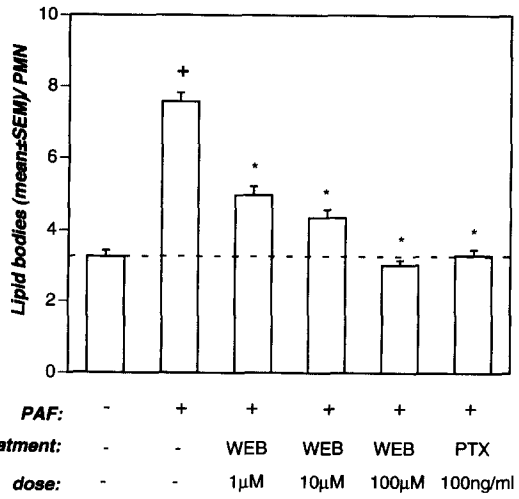

Figure 2. Effect of the PAF receptor antagonist WEB 2086 (*WEB*) and pertussis toxin (*PTX*) on PAF-induced lipid body formation in human PMN. PMN (10^6 cells/ml) were pretreated with WEB 2086 (1–100 μM), pertussis toxin (100 ng/ml) or vehicle for 1 h at 37°C. Cells were then treated with PAF (1 μM) or vehicle for 1 h at 37°C. Lipid bodies were enumerated using light microscopy after osmium staining. Each point represents the mean ± SEM from 50 consecutively counted PMN. Data from one individual donor are representative of four experiments with similar results. The statistically significant difference between PAF and the vehicle is marked by a cross, whereas differences due to the pretreatment with WEB 2086 or pertussis toxin are indicated by asterisks.

Table 2. *Effect of LT Biosynthesis Inhibitors on PAF-induced Lipid Body Formation in Human PMN*

Treatment	Dose	Lipid bodies	Percent inhibition
		(mean ± SEM)/PMN	
MK 886	0	8.0 ± 0.1	—
	2 μM	5.4 ± 0.3*	61
	10 μM	4.1 ± 0.3*	93
Zileuton	0	12.3 ± 0.4	—
	0.1 μM	7.6 ± 0.2*	73
	1 μM	5.2 ± 0.2*	100
NDGA	0	8.9 ± 0.4	—
	10 μM	4.4 ± 0.3*	61
	20 μM	3.9 ± 0.3*	67
DEC	0	8.9 ± 0.4	—
	5 mM	2.2 ± 0.3*	90

PMN (10^6 cells/ml) were pretreated with MK886, zileuton, nordihydroguaiaretic acid (*NDGA*), diethylcarbamazine (*DEC*), or vehicle for 1 h at 37°C. PAF (1 μM) or vehicle was added, and another incubation at 37° C for 1 h followed. Lipid bodies were enumerated using light microscopy after osmium staining. Each point represents the mean ± SEM from 50 consecutively counted PMN. Data from one individual donor are representative of two to five experiments with similar results. Percentage of inhibition was calculated using the following formula: Percent inhibition = 100 − (net no. lipid bodies in treatment group) (100)/(net no. lipid bodies PAF alone). The statistically significant differences caused by pretreatment with MK886, zileuton, NDGA, and DEC are indicated by asterisks.

treatment alone, NS) failed to inhibit the increase in lipid body numbers induced by PAF in human PMN. In contrast, several mechanistically different inhibitors of 5-LO blocked PAF-induced lipid body formation (Table 2). The 5-lipoxygenase-activating protein (FLAP) antagonist MK886 (13), the 5-LO inhibitor zileuton (14), and two other lipoxygenase inhibitors, nordihydroguaiaretic acid and diethylcarbamazine, inhibited lipid body formation elicited by PAF.

To confirm the 5-LO dependence of PAF-induced lipid body formation, leukocytes from wild-type and 5-LO knockout mice were studied. As with human cells, wild-type mouse PMN and macrophages contain only a few lipid bodies under normal conditions and could be stimulated in vitro to form lipid bodies with PAF (10^{-8}–10^{-6} M), but not with lyso-PAF (10^{-6} M) (Fig. 3 *A*). In agreement with the results obtained using human PMN, MK886 (5 μM) drastically inhibited PAF-induced lipid body formation in wild-type mice, whereas aspirin (10 μg/ml) had no effect (data not shown). As shown in Fig. 3 *A*, PAF failed to induce lipid body formation in both PMN and macrophages from 5-LO knockout animals in all the concentrations analyzed. That PAF induction of lipid body formation in leukocytes occurred in vivo as well as in vitro and was also requisitely dependent on 5-LO activity was established in mice. Intrathoracic injection of PAF into wild-type mice induced a drastic increase in the number of lipid bodies in resident macrophages after 4 h in comparison to the animals that received buffer only (Fig. 3 *B*). In accord with results obtained in vitro, PAF failed to induce lipid body formation in pleural resident macrophages from 5-LO knockout mice (Fig. 3 *B*). As shown in Fig. 3 *C*, PAF induced a significant increase in PMN migration to the pleural cavity of wild-type mice but failed to attract PMN to the pleural cavity of 5-LO knockout mice.

The 5-LO pathway product active in eliciting PMN lipid body formation was not LTB_4. Exogenous LTB_4 was not active in stimulating lipid body formation (Table 1). To confirm this and to establish that endogenously formed LTB_4 was not involved, an LTB4 receptor agonist, CP-105,696 (which inhibits LTB_4-induced PMN chemotaxis with an IC_{50} of 5.2 nM [15]), at 0.1–1 μM failed to inhibit PAF-induced lipid body formation (mean ± SEM lipid bodies/PMN 7.4 ± 0.2 with 1 μM of CP-105,696 vs. 8.2 ± 0.2 for vehicle treatment alone, NS). In contrast, 5-HETE (10^{-9}–10^{-7} M) significantly induced lipid body formation in a dose-dependent way (Fig. 4 *A*). Similar to PAF-induced lipid body formation, 5-HETE–induced increases in lipid body numbers were significant within 15 min, maximal within 1–2 h, and decreased thereafter (Fig. 4 *B*). Both 5-HETE enantiomers induced lipid body formation; however, 5(*S*)-HETE was significantly more potent than 5(*R*)-HETE in all concentrations tested (Fig. 4). The effect of the 5(*S*)-

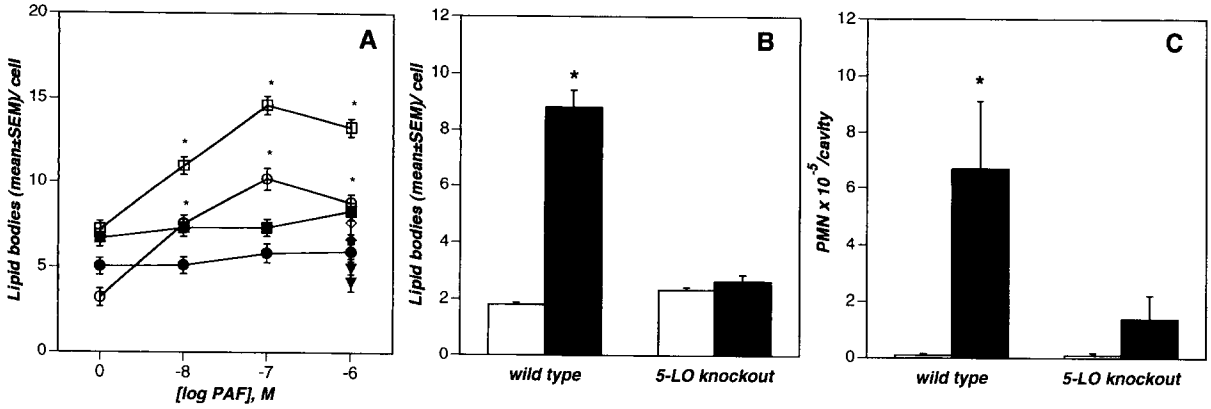

Figure 3. In vitro and in vivo effects of PAF in wild type and 5-LO knockout mice. Effect of PAF on lipid body formation in peritoneal macrophages or bone marrow PMN from wild-type or 5-LO knockout mice (*A*). Macrophages (*circle*) or PMN (*square*) (10^6 cells/ml) were treated with PAF (10^{-8}–10^{-6} M) for 1 h at 37°C. Lyso-PAF (10^{-6} M) is represented by triangles and diamonds in macrophages and PMN, respectively. The open and solid symbols represent wild-type and 5-LO knockout mice, respectively. Effect of intrathoracic injection of PAF (1 μg/cavity) (*solid columns*) or vehicle (*open columns*) on lipid body formation in resident macrophages (*B*) and pleural PMN accumulation (*C*) in wild-type or 5-LO knockout mice. Lipid bodies were enumerated in 50 consecutive cells using light microscopy after osmium staining. Each point represents the mean ± SEM from four to seven animals. Statistically significant differences are indicated by asterisks.

HETE metabolite, 5-oxo-ETE, on lipid body formation was also analyzed. 5-oxo-ETE significantly increased the number of lipid bodies in PMN, with a similar time course observed for 5(*S*)-HETE, but with a lesser potency, comparable to 5(*R*)-HETE. Pretreatment of PMN with the PAF receptor antagonist WEB 2086 (mean ± SEM lipid bodies/PMN 8.1 ± 0.2 with 50 μM of WEB 2086 vs. 7.9 ± 0.2 for vehicle treatment alone, NS) and the 5-LO inhibitor zileuton (mean ± SEM lipid bodies/PMN 8.3 ± 0.3 with 0.5 μM of zileuton vs. 7.9 ± 0.2 for vehicle treatment alone, NS) failed to inhibit the lipid body formation induced by 5(*S*)-HETE. Induction of lipid body formation by 5(*S*)-HETE, however, was significantly inhibited by pertussis toxin (mean ± SEM lipid bodies/PMN 4.5 ± 0.2 with 100 ng/ml of pertussis toxin vs. 7.9 ± 0.2 for vehicle treatment alone, $P < 0.05$). As shown in Fig. 4 *C*, 5(*S*)-HETE and 5(*R*)-HETE dose-dependently stimulated lipid body formation in bone marrow PMN from both wild-type and 5-LO–deficient mice, and no differences in the pattern or intensity of 5-HETE–induced lipid body formation between wild-type and 5-LO–deficient PMN were noted. Collectively, these results indicate that 5-HETE, but

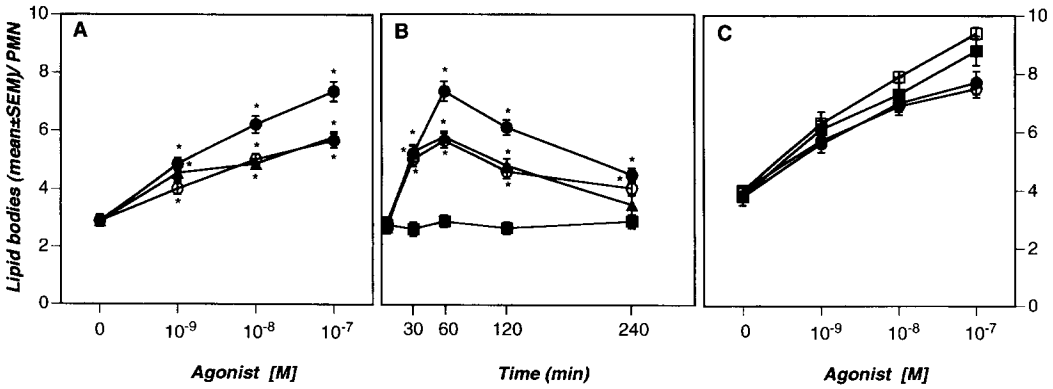

Figure 4. Stimulation of lipid body formation by 5(*S*)-HETE, 5(*R*)-HETE, and 5-oxo-ETE in human and murine PMN. (*A*) Dose-dependent lipid body formation induced by 5(*S*)-HETE, 5(*R*)-HETE, and 5-oxo-ETE in human PMN. PMN (10^6 cells/ml) were treated with 5(*S*)-HETE (10^{-9}–10^{-7} M) (*solid circles*), 5(*R*)-HETE (10^{-9}–10^{-7} M) (*open circles*), or 5-oxo-ETE (10^{-9}–10^{-7} M) (*solid triangles*) for 1 h at 37°C. (*B*) Time-dependent lipid body formation induced by 5(*S*)-HETE, 5(*R*)-HETE, and 5-oxo-ETE in human PMN. PMN were treated with 5(*S*)-HETE (10^{-7} M) (*solid circles*), 5(*R*)-HETE (10^{-7} M) (*open circles*), 5-oxo-ETE (10^{-7} M) (*closed triangles*) or vehicle (*solid squares*) at 37°C. Samples were taken at various time intervals (5-240 min). Each point represents the mean ± SEM from 50 consecutively counted PMN. Data from one individual donor are representative of two to four experiments with similar results. (*C*) Effect of 5-HETE on lipid body formation in bone marrow PMN from wild-type and 5-LO knockout mice. PMN (10^6 cells/ml) were treated with 5(*S*)-HETE (10^{-9}–10^{-7} M) (*squares*) or 5(*R*)-HETE (10^{-9}–10^{-7} M) (*circles*) for 1 h at 37°C. The open and solid symbols represent wild-type and 5-LO knockout mice, respectively. Each point represents the mean ± SEM from three animals. Lipid bodies were enumerated using light microscopy after osmium staining. Statistically significant differences are indicated by asterisks.

Table 3. *Effect of PKC, PLC, and Protein Synthesis Inhibitors on PAF- and 5(S)-HETE–induced Lipid Body Formation*

Treatment	Dose	PAF		5 (S)-HETE	
		Lipid bodies	Percent inhibition	Lipid bodies	Percent inhibition
	μM	(mean ± SEM)/PMN		(mean ± SEM)/PMN	
Staurosproine	0	8.5 ± 0.3	–	7.8 ± 0.3	–
	0.1	6.3 ± 0.3*	47	6.1 ± 0.2*	63
	1	5.7 ± 0.2*	60	5.6 ± 0.2*	83
Chelerythrine	0	7.5 ± 0.2	–	7.8 ± 0.3	–
	0.5	6.7 ± 0.2*	22	6.2 ± 0.3*	61
	1	6.2 ± 0.2*	46	5.6 ± 0.3*	84
U-73122	0	8.5 ± 0.3	–	7.8 ± 0.3	–
	1	6.6 ± 0.3*	57	6.1 ± 0.2*	63
	5	5.8 ± 0.2*	80	5.6 ± 0.2*	81
D609	0	8.1 ± 0.3	–	7.8 ± 0.3	–
	0.1	6.0 ± 0.2*	59	6.0 ± 0.2*	66
	1	5.3 ± 0.2*	78	5.3 ± 0.2*	94
Actinomycin D	0	8.0 ± 0.2	–	7.6 ± 0.2	–
	1	4.9 ± 0.3*	67	4.4 ± 0.2*	75
	10	4.3 ± 0.3*	79	3.8 ± 0.3*	88
Cycloheximide	0	7.9 ± 0.2	–	7.8 ± 0.3	–
	1	5.8 ± 0.2*	76	6.6 ± 0.2*	43
	10	5.3 ± 0.2*	96	5.4 ± 0.2*	89

PMN (10^6 ml) were pretreated with PKC inhibitors (staurosporine and chelerythrine) or the protein synthesis inhibitor cycloheximide for 30 min and with PLC inhibitors (D609 and U-73122) or the RNA synthesis inhibitor actinomycin D for 1 h and then stimulated with PAF (1 μM), 5(S)-HETE (0.1 μM), or vehicle for 1 h. Results are mean ± SEM from 50 consecutively counted PMN. Data from one individual donor are representative of two to four experiments with similar results. Percentage of inhibition was calculated using the following formula: Percent inhibition = 100 − (net no. lipid bodies in treatment group) (100)/net no. lipid bodies in agonist alone). Statistically significant differences between agonists alone and treated groups are indicated by asterisks.

not LTB_4, mediates PAF-induced lipid body formation in leukocytes.

Effect of PKC, PLC, and Protein Synthesis Inhibition on PAF- and 5(S)-HETE–induced Lipid Body Formation. The mechanisms involved in PAF-induced lipid body formation were analyzed. As shown in Table 3, staurosporine (0.1–1 μM) significantly inhibited the increase in lipid body numbers induced by PAF and 5(S)-HETE. In addition, the selective PKC inhibitor chelerythrine (0.5–1 μM) also inhibited PAF- and 5(S)-HETE–induced lipid body formation by 46 and 84%, respectively, at the highest concentration used, thus implicating PKC activation in lipid body formation. It has been previously described that PAF-induced PMN activation, including chemotaxis and superoxide anion release, depends on PLC activation (16, 17). To determine if PAF-stimulated induction of PMN lipid bodies was mediated by PLC activation, two PLC inhibitors were used: U-73122, an inhibitor of G protein–mediated PLC activation (18), and D609, a phosphatidylcholine–PLC inhibitor (19). As shown in Table 3, PAF-induced lipid body formation was significantly inhibited by 78 and 80% when the cells were pretreated with D609 (1 μM) and U-73122 (5 μM), respectively. Similarly, lipid body induction by 5(S)-HETE was also inhibited by D609 and U-73122 (Table 3). Inhibitors of mRNA and protein synthesis, actinomycin D and cycloheximide, respectively, almost completely blocked both PAF- and 5(S)-HETE–induced lipid body formation. These inhibitors did not alter numbers of preformed lipid bodies in PMN, suggesting that the induction of lipid body formation by PAF and 5(S)-HETE was dependent on gene expression and de novo protein synthesis.

Involvement of Lipid Bodies in PMN Priming for PGE_2 and LTB_4 Production. We analyzed the effect of increased numbers of lipid bodies on PGE_2 and LTB_4 production by human PMN. PMN were incubated with increasing concentrations of PAF (10^{-8}–10^{-6} M) for 5 min and 1 h. After the incubation time, lipid bodies were enumerated, and replicate leukocytes were stimulated with a submaximal dose of calcium ionophore A23187 (0.5 μM). As shown in Fig. 5, PAF induced a dose-dependent increase in the lipid body numbers in PMN after 1 h, but not as early as 5 min after stimulation. The incubation of PMN with PAF for only 5 min failed

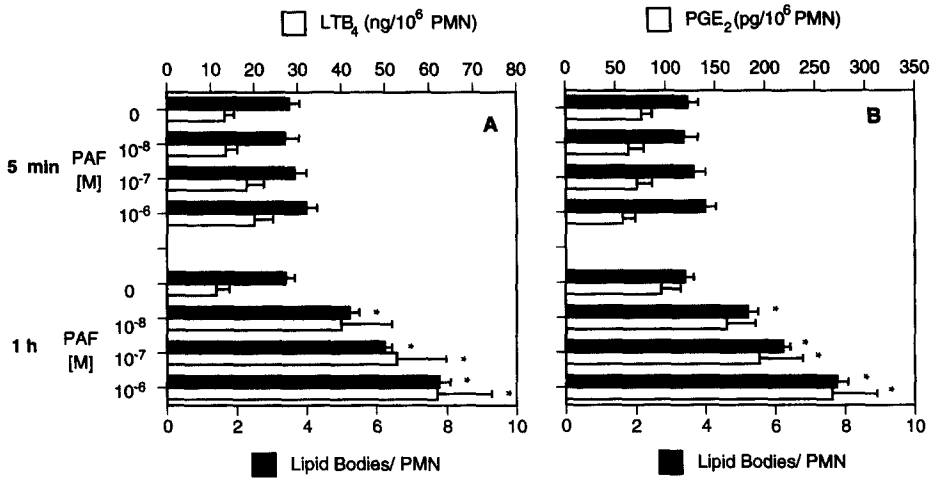

Figure 5. Effect of PAF on lipid body formation and priming for LTB_4 (*A*) and PGE_2 (*B*) production by human PMN. PMN (10^6 cells/ml in 5 ml) were stimulated by PAF (10^{-8}–10^{-6} M) for 5 min or 1 h at 37°C for the induction of lipid bodies. After this period the cells were incubated with A23187 (0.5 μM) for 15 min at 37°C for LTB_4 and PGE_2 production. Concentrations of LTB_4 and PGE_2 in the supernatant were assayed by ELISA. Results are mean ± SEM from four to six independent assays. Statistically significant differences are indicated by asterisks.

to prime PMN for LTB_4 (Fig. 5 *A*) or PGE_2 (Fig. 5 *B*) production induced by A23187. However, after 1 h of PAF stimulation, a significant and dose-dependent priming for both LTB_4 (Fig. 5 *A*) and PGE_2 (Fig. 5 *B*) production was noted. PAF induced a 5.5- and 2.8-fold increase in the production of LTB_4 or PGE_2, respectively, at the highest PAF concentration (10^{-6} M). Furthermore, statistically significant positive correlations between the content of lipid bodies in PMN and the priming for LTB_4 ($r = 0.986$, $P < 0.001$) and PGE_2 ($r = 0.971$, $P < 0.001$) release were obtained, thus suggesting that lipid bodies may contribute to eicosanoid formation in PMN.

Since the protein synthesis inhibitors actinomycin D and cycloheximide were effective in blocking lipid body formation induced by PAF, and lipid body numbers correlated with enhanced eicosanoid formation, we tested whether protein synthesis inhibitors would inhibit PAF-induced priming for eicosanoid production. Pretreatment of PMN with actinomycin D (1 μM) or cycloheximide (1 μM) inhibited not only PAF-induced lipid body formation, but also "priming" for enhanced LTB_4 (Fig. 6 *A*) and PGE_2 (Fig. 6 *B*) production. Protein synthesis inhibitors were not acting to block pathways of arachidonate release or metabolism that are activated by calcium ionophore, since actinomycin D and cycloheximide failed to inhibit calcium ionophore–induced LTB_4 and PGE_2 production in cells not prestimulated with PAF (data not shown).

Discussion

Human PMN obtained from healthy donors contain few lipid bodies. However, a dramatic increase in lipid body numbers is observed in vivo in blood or tissue PMN when these cells are involved in a variety of infectious, allergic, neoplastic, and other inflammatory diseases (6, 20). Lipid body induction in PMN can also be elicited in vitro, when cells are stimulated with *cis*-unsaturated, but not fully saturated, fatty acids (6, 21). In this study, we showed that stimulation of PMN with PAF leads to rapid (15–60 min) formation of lipid bodies. Although PAF is a lipid, mechanisms other than the simple incorporation of exogenous lipids are involved in lipid body formation. First, the PAF precursor

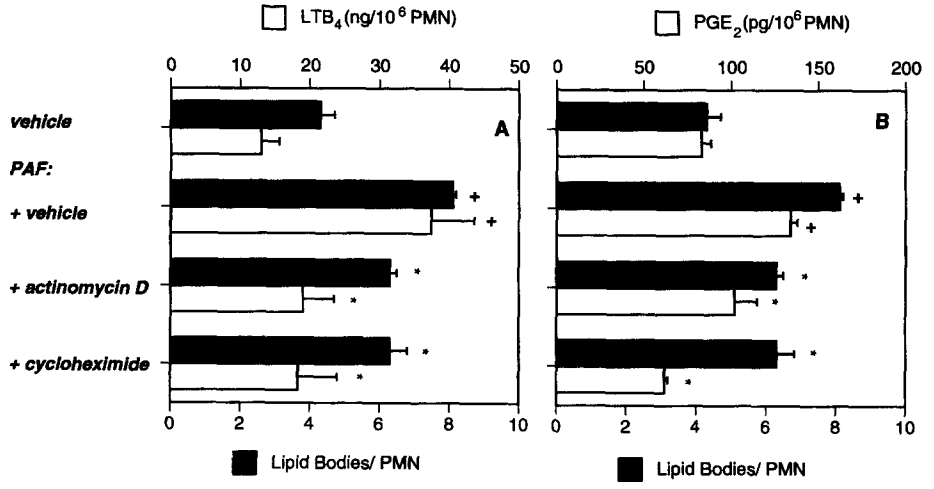

Figure 6. Inhibition of PAF-induced lipid body formation and priming for LTB_4 (*A*) and PGE_2 (*B*) production by actinomycin D and cycloheximide. PMN (10^6 cells/ml in 5 ml) were pretreated with actinomycin D (1 μM), cycloheximide (1 μM), or vehicle for 30 min at 37°C. Cells were then stimulated with PAF (1 μM) or vehicle for 1 h at 37°C for the induction of lipid bodies. After this period, the cells were incubated with A23187 (0.5 μM) for 15 min at 37°C for LTB_4 and PGE_2 production. Concentrations of LTB_4 and PGE_2 in the supernatant were assayed by ELISA. Results are mean ± SEM from four independent assays. Statistically significant differences are indicated by asterisks.

and metabolite, lyso-PAF, that shares the lipid structure of PAF but differs by the absence of an acetate group at the *sn*-2 position and has no receptor agonistic activity (for review see reference 22), lacked the capacity to induce lipid body formation. In addition, significant lipid body formation was induced by PAF at nanomolar concentrations, consistent with a receptor-dependent phenomenon. In agreement, PAF-induced lipid body formation was dose dependently inhibited by the PAF receptor antagonist WEB 2086 with an approximate IC_{50} of 0.4 μM.

The PAF receptor belongs to a family of seven-transmembrane domain rhodopsin-like G protein–coupled receptors (23). In PMN, PAF has been shown to trigger both pertussis-sensitive and -resistant responses, depending upon the reaction analyzed. For instance, the chemotactic effect of PAF on PMN is sensitive to pertussis toxin inhibition, whereas the calcium influx in response to PAF is resistant to pertussis toxin (23). The effect of pertussis toxin on PAF-induced lipid body formation was analyzed. Pretreatment of PMN with pertussis toxin completely inhibited PAF-induced lipid body formation, thus suggesting that PAF is acting through a pertussis toxin–sensitive G protein to induce the formation of lipid bodies. Interestingly, other PMN agonists, including LTB_4, C5a, FMLP, and IL-8, which also signal through seven-transmembrane spanning G protein–coupled receptors, failed to induce lipid body formation in PMN. Thus, the receptor-mediated signaling initiated by PAF that leads to lipid body formation in PMN is specific and not common to all PMN agonists that signal through G protein–linked receptors.

Since increased lipid body formation within cells is associated with inflammatory responses (5, 20), we analyzed the effects of antiinflammatory drugs on lipid body formation. Four structurally unrelated cyclooxygenase inhibitors failed to inhibit lipid body induction by PAF, ruling out involvement of cyclooxygenase activation in the process of PAF-induced lipid body formation. In contrast, lipid body formation induced by *cis*-unsaturated fatty acids is inhibited by nonsteroidal antiinflammatory drugs including aspirin, indomethacin, and sodium salicylate (Bozza, P.T., and P.F. Weller, unpublished observations). These results suggest that different metabolic pathways may be involved in lipid body formation depending on the eliciting stimuli.

Although PAF may activate inflammatory cells directly, several PAF-induced effects, including edema formation, PMN migration, pulmonary hypertension, and lethality, are mediated through LT (22). We demonstrated that the specific 5-LO inhibitor zileuton (14) and the FLAP inhibitor MK 886 (13) inhibited lipid body formation induced by PAF, suggesting that both FLAP and 5-LO are involved in PAF-induced lipid body formation. Recently, the generation of LT-deficient mice in which the 5-LO pathway was inactivated by the targeted disruption of the 5-LO gene was described (11, 24). Peritoneal macrophages obtained from 5-LO knockout animals have no residual 5-LO mRNA or protein and are incapable of synthesizing LT (11, 24). Interestingly, one feature of the 5-LO knockout phenotype is an increased resistance to PAF-induced lethality (11, 24). To further evaluate the involvement of 5-LO in PAF-induced lipid body formation, we analyzed the effect of PAF on peritoneal macrophages and bone marrow PMN from wild-type and 5-LO knockout mice. Similar to the results obtained with human PMN, PMN and macrophages from wild-type mice were dose dependently stimulated by PAF to induce lipid body formation. However, PAF failed to increase lipid body numbers in cells obtained from 5-LO knockout mice. Likewise, the capacity of PAF to elicit lipid body formation in vivo was shown to be 5-LO dependent in studies with an acute model of inflammation in mice. The intrathoracic injection of PAF significantly increased the number of lipid bodies in pleural resident macrophages (Fig. 3) and in the PMN that migrated to the pleural cavity (data not shown) from wild-type mice, but failed to stimulate pleural macrophages or PMN from 5-LO knockout mice. These findings indicate the requisite role for 5-LO activity in the in vitro and in vivo induction of leukocyte lipid bodies by PAF.

Among the metabolites of the 5-LO pathway, LTB_4 is the most potent activator of human PMN. In PMN, LTB_4 mediates different functions including adhesion, chemotaxis, and aggregation, and high levels of LTB_4 can be detected in fluids from inflammatory and allergic reactions (1, 25). Thus, LTB_4 would be a likely candidate to mediate PAF-induced lipid body formation in PMN. Nevertheless, LTB_4 itself failed to induce lipid body formation in human PMN (Table 1). Moreover, the specific LTB_4 receptor antagonist CP-105,696 (15) had no inhibitory effect on PAF-induced lipid body formation, excluding a role for both exogenous and endogenous LTB_4 as a mediator of PAF-induced lipid body formation. Another PMN activator derived from the 5-LO pathway is 5(*S*)-HETE (26, 27). Lipid body formation in human PMN was dose dependently induced by both *R* and *S* isomers of 5-HETE (Fig. 4 *A*). Dose- and time-response curves for lipid body induction stimulated by both isomers had the same shapes; however, 5(*S*)-HETE was more potent than 5(*R*)-HETE (Fig. 4, *A* and *B*). These results are in agreement with previous findings that demonstrate that 5(*S*)-HETE is more potent than 5(*R*)-HETE in mobilizing Ca^{2+} in human PMN and also in "priming" PMN to degranulate in response to PAF and diacylglycerol (28, 29). Recently, a novel pathway for the metabolization of 5(*S*)-HETE in PMN resulting in the formation of 5-oxo-ETE was described (30). 5-oxo-ETE was shown to stimulate PMN in a LTB_4-independent manner, but seems to use the same putative receptor as 5-HETE (31). In accordance, 5-oxo-ETE dose dependently stimulated human PMN to form lipid bodies (Fig. 4), but with a lesser potency than 5(*S*)-HETE. 5-HETE could bypass the 5-LO deficiency in 5-LO knockout mice and stimulated PMN from both wild-type and 5-LO knockout mice to form lipid bodies (Fig. 4 *C*). Since PAF-induced lipid body formation is 5-LO dependent and is elicited not by LTB_4 but by 5(*S*)-HETE, which is active at 10 times lower concentrations than PAF itself to induce comparable numbers of lipid bodies, endogenously formed 5(*S*)-HETE mediates PAF-induced lipid body formation.

The mechanisms by which 5-HETE stimulates PMN are not clearly understood. Although 5-HETE is able to stimulate PMN at nanomolar concentrations, in a stereospecific and G protein–dependent way, putative receptors for 5-HETE have not yet been identified (29). Receptor-independent mechanisms of action for 5-HETE have been proposed. Moreover, PMN have an enormous capacity to incorporate HETEs into their phospholipids and triglycerides (32), and such effects might contribute to lipid body formation. Nevertheless, since the induction of lipid bodies in PMN by 5(*S*)-HETE occurs at nanomolar concentrations of 5(*S*)-HETE, is inhibited by pertussis toxin, suggesting the involvement of a pertussis toxin–sensitive G protein, and is dependent on PKC and PLC activation, it is likely that a 5-HETE receptor is involved in signaling lipid body formation.

We further investigated other signaling pathways that could contribute to PAF- and 5-HETE–induced lipid body formation. It has been shown in several cells that respond to PAF, including human PMN, that PAF transmembrane signaling is related to an increased turnover of phosphoinositide due to PLC activation (23, 33). Accordingly, PAF- and 5-HETE–induced lipid body formation was inhibited by two PLC inhibitors, D609 and U-73122, thus suggesting a role for PLC in this reaction. The products generated by the action of PLC on phospholipids, inositol triphosphate and diacylglycerol, are involved in the release of Ca^{2+} from the endoplasmic reticulum and PKC activation (34). The effect of two PKC inhibitors, staurosporine and chelerythrine, on PAF-stimulated lipid body formation were observed (Table 3). The ability of the PKC inhibitor staurosporine to block lipid body formation induced by PAF and 5-HETE suggests the involvement of PKC activation in this process. However, the selectivity of staurosporine as a PKC inhibitor has been questioned (35), and at concentrations used in these experiments, staurosporine would also inhibit tyrosine protein kinase, calcium/calmodulin–dependent protein kinase, and cAMP-dependent protein kinase (35, 36). To better address the role of PKC on lipid body formation, a highly selective PKC inhibitor was used. Chelerythrine is a potent and selective PKC inhibitor that interacts with the catalytic domain of PKC and is devoid of effect on other protein kinases (36). In our experiments, a similar degree of inhibition of lipid body formation was observed for treatments with staurosporine and chelerythrine, 66 and 46%, respectively, which is consistent with involvement of PKC in PAF-induced lipid body formation. In support of the involvement of PKC in lipid body formation, it was previously demonstrated that direct PKC activators including 1-oleoyl-2-acetyl-glycerol and two active phorbol esters (PMA and phorbol 12, 13 dibutyrate) effectively induced lipid body formation in human PMN (21). The formation of lipid bodies in PMN, stimulated by PAF and 5(*S*)-HETE, requires de novo protein synthesis and was almost fully inhibited by inhibition of RNA synthesis with actinomycin D and protein synthesis with cycloheximide (Table 3). Thus, it is likely that specific early response genes are activated and participate in the metabolic processes that lead to lipid body formation.

Under normal circumstances, leukocytes stimulated with receptor-mediated stimuli, including PAF and FMLP, or with submaximal concentrations of calcium ionophore generate little or undetectable amounts of eicosanoids unless the cells are first primed (37, 38). Priming is therefore a critical step in the activation of PMN during inflammation and has been the subject of several investigations. However, the biochemical alterations and physiological site of priming remain unclear. It is interesting to note that priming agents for PMN-induced eicosanoid production, including 1-oleoyl-2-acetyl-glycerol, arachidonate, and PAF, in the concentrations and time period used (37, 39) are also active in stimulating lipid body formation (21, Fig. 1). Accordingly, in this study we observed a significant correlation between lipid body formation induced by PAF and priming for PGE_2 and LTB_4 production in PMN stimulated by submaximal concentrations of the ionophore A23187 (Fig. 5). Furthermore, pretreatment of cells with actinomycin D or cycloheximide inhibited not only the induction of lipid body formation by PAF, but also the PAF-induced priming for enhanced PGE_2 and LTB_4 in PMN, suggesting that lipid bodies are early response structures involved in the production of lipid mediators of inflammation.

Together with the findings that lipid bodies store the eicosanoid precursor arachidonate esterified in specific phospholipids (6, 7) and also may contain the key enzymes for eicosanoid metabolization (8–10), our results suggest that lipid bodies are specialized, inducible intracellular domains involved in the metabolic generation of eicosanoids during the inflammatory process.

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References

1. Samuelsson, B., S.E. Dahlen, J.A. Lindgren, C.A. Rouzer, and C.N. Serhan. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science (Wash. DC).* 237:1171–1176.
2. Serhan, C.N. 1994. Eicosanoids in leukocyte function. *Curr. Opin. Hematol.* 1:69–77.
3. Coimbra, A., and A. Lopes-Vaz. 1971. The presence of lipid droplets and the absence of stable sudanophilia in osmium-fixed human leukocytes. *J. Histochem. Cytochem.* 19:551–557.
4. Triggiani, M., A. Oriente, M.C. Seeds, D.A. Bass, G. Marone, and F.H. Chilton. 1995. Migration of human inflammatory cells into the lung results in the remodeling of arachidonic acid into a triglyceride pool. *J. Exp. Med.* 182:1181–1190.
5. Robinson, J.M., M.L. Karnovsky, and M.J. Karnovsky. 1982. Glycogen accumulation in polymorphonuclear leukocytes, and other intracellular alterations that occur during inflammation. *J. Cell Biol.* 95:933–942.
6. Weller, P.F., S.J. Ackerman, A. Nicholson-Weller, and A.M. Dvorak. 1989. Cytoplasmic lipid bodies of human neutrophilic leukocytes. *Am. J. Pathol.* 135:947–959.
7. Weller, P.F., R.A. Monahan-Earley, H.F. Dvorak, and A.M. Dvorak. 1991. Cytoplasmic lipid bodies of human eosinophils: subcellular isolation and analysis of arachidonate incorporation. *Am. J. Pathol.* 138:141–148.
8. Dvorak, A.M., E. Morgan, R.P. Schleimer, S.W. Ryeom, L.M. Lichtenstein, and P.F. Weller. 1992. Ultrastructural immunogold localization of prostaglandin endoperoxide synthase (cyclooxygenase) to nonmembrane-bound cytoplasmic lipid bodies in human lung mast cells, alveolar macrophages, type II pneumocytes and neutrophils. *J. Histochem. Cytochem.* 40:759–769.
9. Dvorak, A.M., R.P. Schleimer, A.M. Dvorak, L.M. Lichtenstein, and P.F. Weller. 1992. Human lung mast cell and alveolar macrophage cytoplasmic lipid bodies contain arachidonic acid and prostaglandin endoperoxide synthase (cyclooxygenase), the substrate and enzyme necessary for prostaglandin production. *Int. Arch. Allergy Appl. Immunol.* 99:208–217.
10. Dvorak, A.M., E. Morgan, D.M. Tzizik, and P.F. Weller. 1994. Prostaglandin endoperoxide synthase (cyclooxygenase): ultrastructural localization to nonmembrane-bound cytoplasmic lipid bodies in human eosinophils and murine 3T3 fibroblasts. *Int. Arch. Allergy Immunol.* 105:245–250.
11. Goulet, J.L., J.N. Snouwaert, A.M. Latour, T.M. Coffman, and B.H. Koller. 1994. Altered inflammatory responses in leukotriene-deficient mice. *Proc. Natl. Acad. Sci. USA.* 92: 12852–12856.
12. Kew, R.R., C.M. Grimaldi, M.B. Furie, and H.B. Fleit. 1992. Human neutrophil FcγRIIIB and formyl peptide receptors are functionally linked during formyl-methionyl-leucyl-phenylalanine-induced chemotaxis. *J. Immunol.* 149: 989–997.
13. Miller, D.K., J.W. Gillard, P.J. Vickers, S. Sadowski, C. Leveille, J.A. Mancini, P. Charleson, R.A. Dixon, A.W. Ford-Hutchinson, R. Fortin, et al. 1990. Identification and isolation of a membrane protein necessary for leukotriene production. *Nature (Lond.).* 343:278–281.
14. Carter, G.W., P.R. Young, D.H. Albert, J. Bouska, R. Dyer, R.L. Bell, J.B. Summers, and D.W. Brooks. 1991. 5-lipoxygenase inhibitory activity of zileuton. *J. Pharmacol. Exp. Ther.* 256:929–937.
15. Griffiths, R.J., E.R. Pettipher, K. Koch, C.A. Farrel, R. Breslow, M.J. Conklyn, M.A. Smith, B.C. Hackman, D.J. Wimberly, A.J. Milici, et al. 1995. Leukotriene B_4 plays a critical role in the progression of collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA.* 92:517–521.
16. Verghese, M.W., L. Charles, L. Jakoi, S.B. Dillon, and R. Snyderman. 1987. Role of a guanine nucleotide regulatory protein in the activation of phospholipase C by different chemoattractants. *J. Immunol.* 138:4374–4380.
17. Cockcroft, S. 1992. G-protein-regulated phospholipases C, D and A_2-mediated signalling in neutrophils. *Biochim. Biophys. Acta.* 1113:135–160.
18. Yule, D.I., and J.A. Williams. 1992. U73122 inhibits Ca^{2+} oscillations in response to cholecystokinin and carbachol but not to JMV-180 in rat pancreatic acinar cells. *J. Biol. Chem.* 267:13830–13835.
19. Shutze, S., K. Potthoff, T. Machleidt, D. Berkovic, K. Wiegmann, and M. Kronke. 1992. TNF activates NF-κB by phosphatidylcholine-specific phospholipase-C-induced "acidic" sphingomyelin breakdown. *Cell.* 71:765–776.
20. Galli, S.J., A.M. Dvorak, S.P. Peters, E.S. Schulman, D.W. MacGlashan, Jr., T. Isomura, K. Pyne, V.S. Harvey, I. Hammel, L.M. Lichtenstein, and H.F. Dvorak. 1985. Lipid bodies: widely distributed cytoplasmic structures that represent preferential non-membrane repositories of exogenous [^{3}H]-arachidonic acid incorporated by mast cells, macrophages and other cell types. *In* Prostaglandins, Leukotrienes, and Lipoxins. J.M. Bailey, editor. Plenum Publishing Corp., New York. 221–239.
21. Weller, P.F., S.W. Ryeom, S.T. Picard, S.J. Ackerman, and A.M. Dvorak. 1991. Cytoplasmic lipid bodies of neutrophils: formation induced by *cis*-unsaturated fatty acids and mediated by protein kinase C. *J. Cell Biol.* 113:137–146.
22. Braquet, P., L. Touqui, T. Shen, and B.B. Vargaftig. 1987. Perspectives in platelet-activating factor research. *Pharmacol. Rev.* 39:2–97.
23. Murphy, P.M. 1994. The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol.* 12:593–633.
24. Chen, X.-S., J.R. Sheller, E.N. Johnson, and C.D. Funk. 1994. Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene. *Nature (Lond.).* 372:179–182.
25. Henderson, W.R., Jr. 1994. The role of leukotrienes in inflammation. *Ann. Intern. Med.* 121:684–697.
26. Rossi, A.G., and J.T. O'Flaherty. 1991. Bioactions of 5-hydroxyicosatetraenoate and its interaction with platelet-activating factor. *Lipids.* 26:1184–1188.
27. Serhan, C.N. 1995. Leukocyte transmigration, chemotaxis, and oxygenated derivatives of arachidonic acid: when is chirality important? *Am. J. Respir. Cell Mol. Biol.* 12:251–253.
28. Rossi, A.G., M.J. Thomas, and J.T. O'Flaherty. 1988. Stereospecific bioactions of 5-hydroxyicosatetraenoate. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 240:163–166.
29. O'Flaherty, J.T., and A.G. Rossi. 1993. 5-hydroxyicosatetraenoate stimulates neutrophils by a stereospecific, G protein-linked mechanism. *J. Biol. Chem.* 268:14708–14714.
30. Powell, W.S., F. Gravelle, and S. Gravel. 1992. Metabolism of 5(S)-hydroxy-6,8,11,14-eicosatetraenoic acid and other 5(S)-hydroxyeicosanoids by a specific dehydrogenase in human polymorphonuclear leukocytes. *J. Biol. Chem.* 267: 19233–19241.
31. Powell, W.S., S. Gravel, R.J. MacLeod, E. Mills, and M. Hashefi. 1993. Stimulation of human neutrophils by 5-oxo-6,8,11,14-eicosatetraenoic acid by a mechanism independent of the leukotriene B_4 receptor. *J. Biol. Chem.* 268:9280–9286.

32. Stenson, W.F., and C.W. Parker. 1979. Metabolism of arachidonic acid in ionophore-stimulated neutrophils. *J. Clin. Invest.* 64:1457–1465.
33. Shukla, S.D. 1991. Inositol phospholipid turnover in PAF transmembrane signalling. *Lipids.* 26:1028–1033.
34. Clapham, D.E. 1995. Calcium signaling. *Cell.* 80:259–268.
35. Ruegg, U.T., and G.M. Burgess. 1989. Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinases. *Trends Pharmacol. Sci.* 10:218–220.
36. Herbert, J.M., J.M. Augereau, J. Gleye, and J.P. Maffrand. 1990. Chelerythrine is a potent and specific inhibitor of protein kinase C. *Biochem. Biophys. Res. Commun.* 172:993–999.
37. Bauldry, S.A., R.L. Wykle, and D.A. Bass. 1991. Differential actions of diacyl- and alkylacylglycerols in priming phospholipase A_2, 5-lipoxygenase and acetyltransferase activation in human neutrophils. *Biochim. Biophys. Acta.* 1991:178–184.
38. Glasser, K.B., R. Asmis, and E.A. Dennis. 1990. Bacterial lipopolyssaccharide priming of $P388D_1$ macrophage-like cells for enhanced arachidonic acid metabolism. *J. Biol. Chem.* 265:8658–8664.
39. Stewart, A.G., P.N. Dubbin, T. Harris, and G.J. Dusting. 1990. Platelet-activating factor may act as a second messenger in the release of icosanoids and superoxide anions from leukocytes and endothelial cells. *Proc. Natl. Acad. Sci. USA.* 87: 3215–3219.