ASBMB
JOURNAL OF LIPID RESEARCH

1-*O*-Alkyl-linked glycerophospholipids of human neutrophils: distribution of arachidonate and other acyl residues in the ether-linked and diacyl species

Howard W. Mueller,* Joseph T. O'Flaherty, Dianne G. Greene,* Michael P. Samuel,* and Robert L. Wykle***

Departments of Biochemistry* and Medicine,** Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

Abstract This study was done to determine whether human neutrophils contain sufficient 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine to support the synthesis of platelet activating factor by a deacylation-reacylation mechanism, and to examine the relative distribution of arachidonate among the 1,2-diacyl, 1-*O*-alkyl-2-acyl, and the 1-*O*-alk-1'-enyl-2-acyl classes of choline- and ethanolamine-containing phospholipids. The predominant phospholipid species of human neutrophils were choline-containing glycerophospholipids (41%), ethanolamine-containing glycerophospholipids (39%), and sphingomyelin (14%), with smaller quantities of phosphatidylserine (4%) and phosphatidylinositol (1%). The choline-linked fraction contained high amounts of 1-*O*-alkyl-2-acyl-X (44%) and 1,2-diacyl-*sn*-glycero-3-phosphocholine (47%), and a lesser amount of 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphocholine (9%). In contrast, the ethanolamine-linked fraction contained a large amount of 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (66%), and lower levels of the 1,2-diacyl (24%) and 1-*O*-alkyl-2-acyl (10%) species. The major 1-*O*-alkyl and 1-*O*-alk-1'-enyl ether chains found in the choline and ethanolamine phospholipid pools were 16:0, 18:0, 18:1, and 20:0. The predominant fatty acyl residues found in the 1,2-diacyl and the *sn*-2 position of the 1-*O*-alkyl-2-acyl and 1-*O*-alk-1'-enyl-2-acyl choline and ethanolamine glycerophospholipids were 16:0, 18:0, 18:1, 18:2, and 20:4. Although arachidonate comprised only 7% of all fatty acids associated with choline-linked glycerophospholipids, 63% of this amount was found in the *sn*-2 position of 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine. On the other hand, while arachidonate constituted 27% of all fatty acids associated with ethanolamine-containing glycerophospholipids, 80% of this fraction was found in the 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine pool. This work not only demonstrates that human neutrophils contain sufficient precursor for the synthesis of platelet activating factor by a deacylation-reacylation pathway, it also suggests a close relationship between arachidonate metabolism and these ether-linked species.—**Mueller, H. W., J. T. O'Flaherty, D. G. Greene, M. P. Samuel, and R. L. Wykle.** 1-*O*-Alkyl-linked glycerophospholipids of human neutrophils: distribution of arachidonate and other acyl residues in the ether-linked and diacyl species. *J. Lipid Res.* 1984. **25:** 383–388.

Supplementary key words platelet activating factor • neutrophils • arachidonate • plasmalogen • phospholipid • 1-*O*-alkyl-linked glycerophospholipids

Platelet activating factor (PAF) is a very potent inducer of rabbit platelet aggregation (1) and the release of serotonin, adenine nucleotides, and granular constituents from platelets (2). It has now been identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-*O*-alkyl-2-acetyl-GPC) (3, 4). Originally PAF was found to be secreted from antigen-stimulated IgE-sensitized basophils and has since been shown to be produced by a number of other cells including polymorphonuclear neutrophils (PMN) (5–7).

One plausible mechanism for the synthesis of 1-*O*-alkyl-2-acetyl-GPC, the deacylation-reacylation pathway, has been demonstrated in microsomal preparations of rat spleen and several other tissues. This mode of synthesis involves an acetyltransferase, which transfers acetate from acetyl-CoA to 1-*O*-alkyl-2-lyso-GPC (8–10). This synthetic scheme is thought to utilize preexisting 1-*O*-alkyl-2-acyl-GPC containing a long-chain residue in the *sn*-2 position. Upon stimulation of the cell, a phospholipase A_2 reaction (11, 12) would make available the necessary substrate for acetylation. There is now evidence in the literature that this pathway is operative in PMN (7, 10, 13), platelets (12, 14), and macrophages (9, 15).

Much attention has also been given recently to the metabolism of arachidonic acid by PMN. Numerous investigators have reported that PMN can convert arachidonate to mono- and dihydroxylated lipoxygenase products including 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) (16) and 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE) (17, 18), and to cyclooxy-

Abbreviations: PAF, platelet activating factor; GPC, *sn*-glycero-3-phosphocholine; PMN, polymorphonuclear neutrophils; TLC, thin-layer chromatography; PC, choline-containing glycerophospolipids; PE, ethanolamine-containing glycerophospholipids; PS, phosphatidylserine; PI, phosphatidylinositol; HPLC, high performance liquid chromatography; GPE, *sn*-glycero-3-phosphoethanolamine; GLC, gas–liquid chromatography.

Downloaded from www.jlr.org by guest, on June 19, 2012

genase products including prostaglandins and thromboxanes (19, 20).

Since stimulated PMN synthesize both PAF and arachidonate metabolites, this study was carried out with a twofold purpose in mind. First, we wanted to assess the levels of 1-*O*-alkyl-2-acyl-GPC, which would serve as a precursor for PAF synthesis in a deacylation-reacylation scheme. Second, we wanted to determine the degree of association of arachidonate with ether-lipid species in the cell, since this information might yield a clue to the interrelationship of PAF and arachidonate metabolism.

EXPERIMENTAL PROCEDURES

Materials

All chemicals were reagent grade or better. Dulbecco's phosphate-buffered saline was purchased from Gibco, Grand Island, NY. Plasma gel was prepared by the method of DeChatelet and Shirley (21). Isolymph was obtained from Gallard-Schlesinger Chemical Manufacturing Corporation, Carle Place, NY. Phospholipase C (*Bacillus cereus*), primulin dye, and silica gel H were purchased from Sigma Chemical Company, St. Louis, MO. Silica gel G thin-layer chromatography (TLC) plates were obtained from Analtech, Inc., Newark, DE. Vitride ($NaAlH_2[OCH_2CH_2OCH_3]_2$) was purchased from Alfa Products, Danvers, MA. Phospholipid standards were purchased from Supelco, Inc., Bellefonte, PA, 1-*O*-alkylglycerol standards were from Serdary Research Laboratories, London, Ontario, Canada, and fatty alcohol acetate standards were from Nu-Chek Prep, Elysian, MN. Fatty acid methyl ester standards were obtained also from Supelco, Inc., and Nu-Chek Prep.

Preparation of cells

Human PMN were purified by the method of Shirley et al. (22). Briefly, venous blood was drawn into 500-ml collection bags containing 3000 units of heparin. Erythrocytes were removed by sedimentation in plasma gel (blood–plasma gel 4:1 (v/v) for 45–60 min at 1 *g* and room temperature. The supernatant was removed and centrifuged at 200 *g* and 4°C for 8 min. The pellet was washed once in phosphate-buffered saline and the monocytes were eliminated by centrifugation on Isolymph at 400 *g* for 30 min at room temperature. After washing again in buffer, the remaining erythrocytes were removed by hypotonic lysis. The leukocyte population contained better than 95% PMN and there were less than 10 platelets/100 PMN. One unit of blood yielded 6–8 $\times 10^8$ cells.

TLC solvent systems

Several solvent systems were used in the purification of the cellular lipids and their derivatives: system I, chloroform–methanol–glacial acetic acid–water 50:25:8:4 (v/v); system II, hexane–ethyl ether 70:30 (v/v); system III, ethyl ether–hexane–conc. ammonium hydroxide 60:40:1 (v/v); and system IV, ethyl ether–hexane 60:40 (v/v).

Extraction and fractionation of lipids

Cellular lipids were extracted by the method of Bligh and Dyer immediately after cell isolation (23). The choline-linked (PC) and ethanolamine-linked (PE) glycerolphospholipids were purified by TLC on silica gel H using solvent system I. After visualization with a primulin spray reagent (24), the purified PC and PE fractions were eluted from the gel by the extraction method of Bligh and Dyer (23).

Quantitation of phospholipid species

The quantitation of the individual phospholipid species was carried out in two ways. First, an aliquot (200–300 μg) of the total lipid extract was analyzed by TLC on silica gel H using solvent system I. The various phospholipids were visualized and assayed for lipid phosphorus using the method of Rouser, Siakotos, and Fleischer (25). This TLC method did not resolve phosphatidylserine (PS) from phosphatidylinositol (PI). In the second method high performance liquid chromatography (HPLC) (26) was utilized to separate the individual species. An aliquot (200–400 μg) of the total lipid extract was injected into a μPorasil column (3.9 mm × 30 cm, Waters) with a flow rate of 2.2 ml/min at 50°C. The eluant solvent system consisted of isopropanol–hexane 8:6 (v/v) containing 1.5% water. After 5 min, the water was increased to 9% over a period of 20 min. The column was then eluted isocratically for an additional 10 min. The resolved lipid species were detected by absorbance at 206 nm, and the peaks were collected and assayed for phosphorus (25). Recovery of lipid phosphorus was greater than 90% by TLC and HPLC.

Separation and quantitation of 1,2-diacyl-, 1-*O*-alkyl-2-acyl-, and 1-*O*-alk-1′-enyl-2-acyl-GPC and -GPE

The various lipid classes were analyzed as described earlier (27). Briefly, the purified choline- and ethanolamine-linked fractions (1–3 mg) were treated with HCl gas for 5 min to hydrolyze the 1-*O*-alk-1′-enyl groups. The cleaved aldehyde, the lyso-phospholipid, and the unreacted phospholipid were purified by preparative TLC in solvent system I. The amount of lyso-phospholipid determined by lipid phosphorus analysis (25) was taken as the 1-*O*-alk-1′-enyl content.

Downloaded from www.jlr.org by guest, on June 19, 2012

The unhydrolyzed phospholipid was treated with phospholipase C and the products were acetylated (28) to yield 1,2-diacyl-3-acetylglycerol and 1-*O*-alkyl-2-acyl-3-acetylglycerol. These two species were separated by preparative TLC on silica gel G using solvent system II. To monitor for possible acyl migration, an aliquot of the phospholipase C-treated lipid (containing 1,2-diacylglycerol and 1-*O*-alkyl-2-acylglycerol) was analyzed by TLC on silica gel G using solvent system III. In this solvent system 1,2-diacyl- and 1,3-diacylglycerols co-migrate and are resolved from 1-*O*-alkyl-2-acyl- and 1-*O*-alkyl-3-acylglycerols, which also co-migrate (29). Analyses of the diglycerides in system III and the acetylated derivatives in system II showed similar relative intensities of the 1,2-diacyl and 1-*O*-alkyl-2-acyl bands. This confirmed that the product identified as 1-*O*-alkyl-2-acyl-*sn*-glycerol was not 1,3-diacyl-*sn*-glycerol formed by acyl migration. The purified acetylglycerides and the lyso-phospholipid from acid treatment were methylated (27), and the fatty acid methyl esters were analyzed by gas–liquid chromatography (GLC) using 15:0 methyl ester as an internal standard. Each mole of methyl ester was taken to represent 1 mole of 1-*O*-alkyl-2-acyl-, 1 mole of 1-*O*-alk-1′-enyl-2-acyl-, or 0.5 mole of 1,2-diacyl-GPC or -GPE.

Determination of 1-*O*-alkyl chain distribution

An aliquot (1–2 mg) of the unreacted phospholipid from acid treatment was reduced with Vitride as described earlier (30). The 1-*O*-alkylglycerol product was purified by preparative TLC on silica gel G using solvent system IV, and the isopropylidene derivative was prepared as described earlier (27). The 1-*O*-alkyl chain distribution, which was determined by GLC–mass spectroscopy, was based on peak area percentages.

Determination of 1-*O*-alk-1′-enyl distribution

Aldehydes formed from acid treatment of plasmalogen were reduced with Vitride to the corresponding alcohol (30). The fatty alcohols were then acetylated in the same manner as the diglycerides (28) and analyzed by GLC–mass spectroscopy as described below. The 1-*O*-alk-1′-enyl chain distribution was based on peak area percentages.

GLC and GLC–mass spectroscopy analyses

Analysis of fatty acid methyl esters was performed on a Varian 3700 gas chromatograph with a CDS-111 data processor. The methyl esters were separated on a 60-m column coated with OV-351. Column temperature ranged from 175 to 245°C with a programmed rate of 10°C/min for the first 5 min, 0°C/min for 20 min, and 2°C/min for another 10 min. The injector and detector temperatures were both 250°C, and the helium flow rate was 1 ml/min.

The isoproylidene derivatives of alkyl glycerol and the fatty alcohol acetates were both analyzed on a Ribermag R10-10 quadrupole mass analyzer using a PDP-8A minicomputer for data acquisition and reduction. Software for this system is from R. D. S. Nermag. The isopropylidene derivatives were separated on a DB-1 WCOT fused-silica column with a helium velocity of 35 cm/sec. Column temperature ranged from 170 to 270°C at a programmed rate of 5°C/min. The injector, source, and interface temperatures were 250°C, 200°C, and 280°C, respectively. Filament current was 0.3 mA and the electron voltage was 70 eV. The fatty acetates were separated on a SE-52 WCOT fused-silica column with a helium velocity of 40 cm/sec. Column temperature ranged from 125 to 225°C, beginning with a 5-min hold and followed by a programmed rate of 3°C/min. The injector, source, and interface temperatures were 200°C, 170°C, and 225°C, respectively. Filament current for this analysis was also 0.3 mA and the electron voltage was 30 eV.

RESULTS AND DISCUSSION

The phospholipids of human PMN were analyzed by TLC and HPLC as described in Experimental Procedures. As shown in **Table 1,** the major phospholipid species were PC (41%), PE (39%), and sphingomyelin (14%), with smaller amounts of PS (4%) and PI (1%). These findings are in general agreement with previously published data, which reported circulating human PMN to contain similar high amounts of PC (44%) and PE (33%), a slightly lower content of sphingomyelin (5%), and higher amounts of PS (8%) and PI (5%) (31). Rabbit peritoneal (27) and guinea pig peritoneal (32) PMN have also been shown to have comparable phospholipid distributions. In this study platelet lipid contamination was calculated to

TABLE 1. Phospholipid composition of human polymorphonuclear leukocytes

	mol % Phosphorus (N = 14)[a]
Sphingomyelin	14.4 ± 2.7
Choline-containing glycerophospholipids	40.8 ± 1.8
Phosphatidylinositol	1.1 ± 0.5
Phosphatidylserine	4.3 ± 1.9
Ethanolamine-containing glycerophospholipids	38.6 ± 2.7

[a] Each determination is based on a separate population of 6–8 × 10^8 PMN. The data are presented as the mean ± standard deviation. There were 17.2 ± 3.7 mg of total lipid/10^9 PMN (N = 9) and 8290 ± 117 nmol of phosphorus/10^9 PMN.

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 2. Ether class composition of choline- and ethanolamine-containing glycerophospholipids

	Choline-containing Glycerophospholipids (N = 5)[a]	Ethanolamine-containing Glycerophospholipids (N = 5)[a]
	mol %	
1,2-Diacyl	47.3 ± 8.5	24.1 ± 6.2
1-*O*-Alkyl-2-acyl	44.1 ± 8.9	10.4 ± 4.4
1-*O*-Alk-1′-enyl-2-acyl	9.4 ± 1.6	65.5 ± 3.3

[a] Each determination is based on a separate population of cells. Data are presented as the mean ± standard deviation.

be less than 1% based on the phospholipid content of human platelets (33).

As shown in **Table 2,** the PC fraction of human PMN contained 47% 1,2-diacyl-, 44% 1-*O*-alkyl-2-acyl-, and 9% 1-*O*-alk-1′-enyl-2-acyl-GPC. Recent work has shown that several other cell types have elevated levels of 1-*O*-alkyl-2-acyl-GPC including rabbit peritoneal PMN (46%) (27), rabbit alveolar macrophages (33%) (34), rat alveolar macrophages (35%) (15), and guinea pig PMN (16%), macrophages (14%), and lymphocytes (19%) (35). The PE fraction of human PMN, however, contained high levels of 1-*O*-alk-1′-enyl-2-acyl-GPE (66%), and lower levels of 1,2-diacyl- (24%) and 1-*O*-alkyl-2-acyl-GPE (10%). The elevated level of plasmalogen in the PE pool agrees with earlier work done with human (36) and rabbit peritoneal PMN (27).

The fatty acyl distribution of PC and PE is shown in **Table 3** and **Table 4,** respectively. The major fatty acids in both the PC and PE fractions were 16:0, 18:0, 18:1, 18:2, and 20:4. These findings agree with recent analyses of lipids of rabbit (27) and guinea pig (35) peritoneal PMN, although considerably more 18:2 and less 20:4 was found in the peritoneal cells. The high content of 16:0 in the *sn*-2 position of the PC fraction has also been seen in rabbit peritoneal PMN (27), although it is not known whether this is linked to a disaturated species. Of all fatty acids associated with the PC fraction in human PMN, 7% were arachidonate. However, if one considers the ether class composition of the PC pool and the percentage of 20:4 in each class, it can be calculated that 63% of all 20:4 in PC is associated with the 1-*O*-alkyl class. The PE fraction, on the other hand, contained an overall higher percentage of 20:4; 27% of all fatty acids associated with PE were arachidonate. However, considering the relative amounts of ether-linked species in PE and the 20:4 content in each of these, it can be determined that 80% of all 20:4 in the ethanolamine-containing phospholipids is associated with the 1-*O*-alk-1′-enyl fraction. Sugiura et al. (34) have recently found similar arachidonyl enrichment of 1-*O*-alkyl-2-acyl-GPC and 1-*O*-alk-1′-enyl-2-acyl-GPE in rabbit alveolar macrophages.

To determine the 1-*O*-alkyl chain distribution, 1-*O*-alkyl-2-acyl-GPC and -GPE were analyzed as the isopropylidene derivatives of alkylglycerol as described in Experimental Procedures. Analysis of the derivatives by

TABLE 3. Fatty chain distribution of choline-containing glycerophospholipids

	1,2-Diacyl	1-*O*-Alkyl-2-acyl		1-*O*-Alk-1′-enyl-2-acyl	
	Position 1 + 2 (N = 5)[a]	Position 1 (n = 3)[a,b]	Position 2 (N = 4)[a]	Position 1 (N = 1)[a,b]	Position 2 (N = 5)[a]
			mol %		
16:0	33.7 ± 5.4	39.8 ± 7.6	23.8 ± 3.2	42.4	21.3 ± 2.2
16:1	0.7 ± 0.3		1.7 ± 0.6		1.5 ± 1.3
18:0	13.9 ± 0.9	22.5 ± 0.7	1.1 ± 0.6	39.1	7.9 ± 2.5
18:1	35.8 ± 3.2	25.6 ± 1.8	33.4 ± 7.0	18.5	35.9 ± 3.6
18:2	10.0 ± 2.9		22.9 ± 6.2		18.0 ± 2.6
20:0	0.1 ± 0.1	3.3 ± 1.1			0.1 ± 0.1
20:1	1.3 ± 0.1	2.4 ± 0.7	0.2 ± 0.1		2.3 ± 3.3
20:2	0.8 ± 0.2	0.4 ± 0.6	0.8 ± 0.2		1.9 ± 1.5
20:3	0.4 ± 0.3		1.9 ± 0.1		1.2 ± 0.4
20:4	2.2 ± 2.9		10.4 ± 5.6		6.4 ± 4.6
22:1		2.4 ± 1.6	0.3 ± 0.3		0.4 ± 0.4
22:4	0.3 ± 0.3		1.9 ± 1.1		0.7 ± 0.6
24:1		1.5 ± 0.6	0.1 ± 0.1		0.1 ± 0.3
Other[c]	0.8	1.8	1.9		2.5

[a] Each determination is based on a separate population of cells. Data are presented as mol % ± standard deviation.
[b] Based on area % rather than mol %.
[c] Other fatty acids that were detected but constituted less than 1% each were 14:0, 14:1, 17:0, 18:3, 20:5, 22:0, 22:5, 22:6, and 24:0. Additional minor components of the 1-*O*-alkyl chain distribution included 15:0, 17:0, 22:0, 22:2, and 24:2.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 4. Fatty acid chain distribution of ethanolamine-containing glycerophospholipids

	1,2-Diacyl	1-*O*-Alkyl-2-acyl		1-*O*-Alk-1'-enyl-2-acyl	
	Position 1 + 2 (N = 5)[a]	Position 1 (N = 3)[a,b]	Position 2 (N = 5)[a]	Position 1 (N = 1)[a,b]	Position 2 (N = 5)[a]
			mol %		
16:0	4.7 ± 0.2	33.7 ± 1.7	13.6 ± 6.3	20.9	1.6 ± 0.2
16:1	0.6 ± 0.3		1.3 ± 0.8		0.7 ± 0.1
17:0	0.2 ± 0.2	1.2 ± 1.2	0.1 ± 0.2		
18:0	34.2 ± 1.3	56.2 ± 7.3	5.7 ± 3.1	59.9	0.6 ± 0.2
18:1	40.4 ± 5.7	4.5 ± 6.1	32.3 ± 7.7	14.2	29.9 ± 3.6
18:2	6.3 ± 1.4		13.1 ± 3.8		21.1 ± 5.6
18:3	0.1 ± 0.2		1.4 ± 2.0		
20:0	0.2 ± 0.1	4.0 ± 2.0	0.3 ± 0.4	5.0	
20:1	1.7 ± 0.4		1.6 ± 1.6		0.3 ± 0.1
20:2	1.0 ± 0.5		0.5 ± 0.7		0.8 ± 0.3
20:3	1.2 ± 0.3		1.4 ± 0.9		3.1 ± 0.6
20:4	6.6 ± 6.3		22.3 ± 8.4		32.7 ± 6.2
22:4	1.5 ± 0.4		3.5 ± 1.6		4.9 ± 1.8
22:5	0.5 ± 0.4		0.9 ± 0.7		2.4 ± 1.2
22:6	0.2 ± 0.3		0.5 ± 0.4		1.1 ± 0.5
Other[c]	0.7	0.4	1.6		0.9

[a] Each determination is based on a separate population of cells. Data are presented as the mean ± standard deviation.
[b] Based on area % rather than mol %.
[c] Other fatty acids that were detected but constituted less than 1% each were 14:0, 20:5, 22:0, 22:1, 24:0, and 24:1. A minor component of the 1-*O*-alkyl chain distribution was 22:0.

GLC–mass spectroscopy yielded characteristic peaks of m/z = 101 and m/z = M − 15, indicative of a glyceryl ether structure (4). The predominant alkyl chains of 1-*O*-alkyl-2-acyl-GPC and -GPE were 16:0 and 18:0, with lesser amounts of 18:1 and 20:0. Traces of other alkyl chains are listed in the tables. The alkyl chain distributions of PC and PE from guinea pig PMN and macrophages (35) and from rabbit alveolar macrophages (34) have been reported to consist primarily of 16:0, 18:0, and 18:1, which is in general agreement with our findings. However, the distribution of alkyl chains from PC and PE of rabbit peritoneal PMN contains significantly higher amounts of 20:0 (PC, 16%; PE, 25%) and 22:0 (PC, 9%; PE, 10%) (27).

The 1-*O*-alk-1'-enyl chain distribution, which was also determined by GLC–mass spectroscopy as described in Experimental Procedures, showed that the predominant vinyl ether chains in the PC and PE plasmalogen pools were 16:0, 18:0, and 18:1. The PE fraction also contained a small amount (5%) of 20:0. These findings concur with the 1-*O*-alk-1'-enyl chain distribution of guinea pig peritoneal PMN (35). Rabbit peritoneal PMN, however, contain significant quantities of 14:0 (13%) and 18:2 (3%) in the *sn*-1 position of PE plasmalogen (27).

While this work provides no direct proof for mechanisms of PAF synthesis and arachidonate release, we have shown that human PMN contain sufficient 1-*O*-alkyl-2-acyl-GPC for the synthesis of PAF by a deacylation-reacylation pathway, and that this pool of proposed precursor is enriched with arachidonate. Rabbit macrophages, which also synthesize PAF, have also been shown to contain elevated amounts of 1-*O*-alkyl-2-arachidonyl-GPC (34). It is noteworthy that 1-*O*-alkyl-2-arachidonyl-GPC might serve as a precursor of both PAF and active arachidonate metabolites through the action of phospholipase A_2, thereby coupling the synthesis of the two inflammatory agents. Whether PAF synthesis and arachidonate mobilization are tightly linked or not remains to be determined, but the presence of high amounts of 1-*O*-alkyl-2-arachidonyl-GPC certainly makes it appear feasible.

This work was supported by grant HL-26818 from the National Heart, Lung, and Blood Institute, and grant AI-17287 from the National Institute of Allergy and Infectious Diseases. The authors thank Marshall Ellis for help in cell preparation and Tommye Campbell for her secretarial assistance.

Manuscript received 15 August 1983.

REFERENCES

1. Benveniste, J., P. M. Henson, and C. G. Cochrane. 1972. Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophils, and a platelet-activating factor. *J. Exp. Med.* **136:** 1356–1377.
2. Henson, P. M., and R. N. Pinckard. 1977. Platelet activating factor (PAF). A possible direct mediator of anaphylaxis in

Downloaded from www.jlr.org by guest, on June 19, 2012
JOURNAL OF LIPID RESEARCH ASBMB

the rabbit and a trigger for the vascular deposition of circulating immune complexes. *Monogr. Allergy.* **12:** 13–26.
3. Demopoulos, C. A., R. N. Pinckard, and D. J. Hanahan. 1979. Platelet activating factor: evidence for 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine as the active component (a new class of lipid chemical mediators). *J. Biol. Chem.* **254:** 9355–9358.
4. Hanahan, D. J., C. A. Demopoulos, J. Liehr, and R. N. Pinckard. 1980. Identification of platelet activating factor isolated from rabbit basophils as acetyl glyceryl ether phosphorylcholine. *J. Biol. Chem.* **255:** 5514–5516.
5. Lynch, J. M., G. Z. Lotner, S. J. Betz, and P. M. Henson. 1979. The release of platelet activating factor by stimulated rabbit neutrophils. *J. Immunol.* **123:** 1219–1226.
6. Lotner, G. Z., J. M. Lynch, S. J. Betz, and P. M. Henson. 1980. Human neutrophil-derived platelet activating factor. *J. Immunol.* **124:** 676–684.
7. Mueller, H. W., J. T. O'Flaherty, and R. L. Wykle. 1983. Biosynthesis of platelet activating factor in rabbit polymorphonuclear neutrophils. *J. Biol. Chem.* **258:** 6213–6218.
8. Wykle, R. L., B. Malone, and F. Snyder. 1980. Enzymatic synthesis of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a hypotensive and platelet-aggregating lipid. *J. Biol. Chem.* **255:** 10256–10260.
9. Ninio, E., J. M. Mencia-Huerta, F. Heymans, and J. Benveniste. 1982. Biosynthesis of platelet activating factor. I. Evidence for an acetyltransferase activity in murine macrophages. *Biochim. Biophys. Acta.* **710:** 23–31.
10. Lee, T-c., B. Malone, S. I. Wasserman, V. Fitzgerald, and F. Snyder. 1982. Activities of enzymes that metabolize platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in neutrophils and eosinophils from humans and the effect of calcium ionophore. *Biochem. Biophys. Res. Commun.* **105:** 1303–1308.
11. Elsbach, P., and M. A. Rizack. 1963. Acid lipase and phospholipase activity in homogenates of rabbit polymorphonuclear leukocytes. *Am. J. Physiol.* **205:** 1154–1158.
12. Benveniste, J., M. Chignard, J. P. Le Couedic, and B. B. Vargaftig. 1982. Biosynthesis of platelet-activating factor (PAF-acether). II. Involvement of phospholipase A_2 in the formation of PAF–acether and lyso-PAF-acether from rabbit platelets. *Thromb. Res.* **25:** 375–385.
13. Alonso, F., M. G. Gil, M. Sanchez-Crespo, and J. M. Mato. 1982. Activation of 1-*O*-alkyl-2-lyso-glycero-3-phosphocholine acetyl-CoA transferase during phagocytosis in human polymorphonuclear leukocytes. *J. Biol. Chem.* **257:** 3376–3378.
14. Chap, H., G. Mauco, M. Simon, J. Benveniste, and L. Douste-Blazy. 1981. Biosynthetic labelling of platelet activating factor from radioactive acetate by stimulated platelets. *Nature.* **289:** 312–314.
15. Albert, D. H., and F. Snyder. 1983. Biosynthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine by rat alveolar macrophages. *J. Biol. Chem.* **258:** 97–102.
16. Borgeat, P., M. Hamberg, and B. Samuelsson. 1976. Transformation of arachidonic acid and homo-gamma linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. *J. Biol. Chem.* **251:** 7816–7820.
17. Borgeat, P., and B. Samuelsson. 1979. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxyeicosatetraenoic acid. *J. Biol. Chem.* **254:** 2643–2646.
18. Chilton, F. H., J. T. O'Flaherty, C. E. Walsh, M. J. Thomas, R. L. Wykle, L. R. DeChatelet, and B. M. Waite. 1982. Platelet activating factor: stimulation of the lipoxygenase pathway in polymorphonuclear leukocytes by 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine. *J. Biol. Chem.* **257:** 5402–5407.
19. Knapp, H. R., O. O. Oelz, J. Roberts, B. J. Sweetman, J. A. Oates, and P. W. Reed. 1977. Ionophores stimulate prostaglandin and thromboxane synthesis. *Proc. Natl. Acad. Sci. USA.* **74:** 4251–4255.
20. Wentzell, B., and R. M. Epand. 1978. Stimulation of the release of prostaglandins from polymorphonuclear leukocytes by the calcium ionophore A23187. *FEBS Lett.* **86:** 255–258.
21. DeChatelet, L. R., and P. S. Shirley. 1982. Chemiluminescence of human neutrophils induced by soluble stimuli: effect of divalent cations. *Infect. Immun.* **35:** 206–212.
22. Shirley, P. S., T. L. Campbell, E. Cheadle, and L. R. DeChatelet. 1979. Transhydrogenase activity in human leukocytes. *J. Reticuloendothel. Soc.* **25:** 575–583.
23. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–918.
24. Wright, R. S. 1971. A reagent for the non-destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms. *J. Chromatogr.* **59:** 220–221.
25. Rouser, G., A. N. Siakotos, and S. Fleischer. 1966. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids.* **1:** 85–86.
26. Geurts Van Kessel, W. S. M., W. M. A. Hax, R. A. Demel, and J. De Gier. 1977. High performance liquid chromatographic separation and direct ultraviolet detection of phospholipids. *Biochim. Biophys. Acta.* **486:** 524–530.
27. Mueller, H. W., J. T. O'Flaherty, and R. L. Wykle. 1982. Ether lipid content and fatty acid distribution in rabbit polymorphonuclear neutrophil phospholipids. *Lipids.* **17:** 72–77.
28. Waku, K., H. Ito, T. Bito, and Y. Nakazawa. 1974. Fatty chains of acyl, alkenyl, and alkyl phosphoglycerides of rabbit sarcoplasmic reticulum. *J. Biochem.* **75:** 1307–1312.
29. Snyder, F. 1973. Thin-layer chromatographic behavior of glycerolipid analogs containing ether, ester, hydroxyl, and ketone groupings. *J. Chromatogr.* **82:** 7–14.
30. Snyder, F., M. Blank, and R. L. Wykle. 1971. The enzymic synthesis of ethanolamine plasmalogens. *J. Biol. Chem.* **246:** 3639–3645.
31. Gottfried, E. L. 1972. Lipid patterns of leukocytes in health and disease. *Sem. Hematol.* **9:** 241–250.
32. Mason, R. J., T. P. Stossel, and M. Vaughan. 1972. Lipids of alveolar macrophages, polymorphonuclear leukocytes, and their phagocytic vesicles. *J. Clin. Invest.* **51:** 2399–2407.
33. Broekman, M. J., J. W. Ward, and A. J. Marcus. 1980. Phospholipid metabolism in stimulated human platelets. *J. Clin. Invest.* **66:** 275–283.
34. Sugiura, T., M. Nakajima, N. Sekiguchi, Y. Nakagawa, and K. Waku. 1983. Different fatty chain compositions of alkenylacyl, alkylacyl, and diacyl phospholipids in rabbit alveolar macrophages: high amounts of arachidonic acid in ether phospholipids. *Lipids.* **18:** 125–129.
35. Sugiura, T., Y. Onuma, N. Sekiguchi, and K. Waku. 1982. Ether phospholipids in guinea pig polymorphonuclear leukocytes and macrophages. Occurrence of high levels of 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine. *Biochim. Biophys. Acta.* **712:** 515–522.
36. Gottfried, E. L. 1967. Lipids of human leukocytes: relation to cell type. *J. Lipid Res.* **8:** 321–327.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH