Alterations of New Methylated Phospholipid Synthesis in the Plasma Membranes of Macrophages Exposed to Chemoattractants

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ABSTRACT Chemotactic factors have been shown to inhibit the methylation of phosphatidylethanolamine in macrophages without affecting total phospholipid synthesis. It would thus be anticipated that newly synthesized membranes of macrophages exposed to chemoattractants would have an increased ratio of phosphatidylethanolamine to its methylated derivatives. These ratios were measured directly in newly synthesized phospholipids of plasma membranes isolated from guinea pig peritoneal macrophages. The phosphatidylethanolamine:methylated phospholipid ratio in such plasma membranes was increased by 53 to 111% upon exposure of the cells to chemotactic factors. This increase was due to decreased synthesis of methylated phospholipids and not to altered formation of phosphatidylethanolamine or activation of phospholipases. Methylated phospholipid ratios were also studied in the leading front lamellipodia isolated from macrophages migrating under chemotactic and nonchemotactic conditions. The phosphatidylethanolamine:methylated phospholipid ratios were increased up to fourfold in lamellipodia of macrophages migrating towards chemotactic agents when compared to those from cells migrating randomly. Biophysical changes in the plasma membrane produced by an increase in the ratio of phosphatidylethanolamine:methylated phospholipids as a result of exposure of cells to chemoattractants may be required for sustained directed migration.

The chemotaxis of mononuclear phagocytes requires methylation reactions mediated by *S*-adenosyl-L-methionine (17, 24). Interestingly, chemotactic factors, when incubated with macrophages, produce a marked depression of one type of transmethylation reaction, the formation of methylated derivatives of phosphatidylethanolamine (PE) (18). Chemotactic factors do not, however, alter total phospholipid synthesis in these cells. On the basis of these observations, we have hypothesized that, when chemotactic factors bind to their cell surface receptors, phospholipid methylation is inhibited locally in the area of receptor occupancy (24). Because total phospholipid synthesis is unchanged in macrophages exposed to chemoattractants, one would expect to increase the ratio of PE to methylated phospholipids in the newly synthesized membrane lipids of such cells. Changes in the ratio of PE to methylated phospholipids could alter the biophysical characteristics in focal areas of the membranes of chemotactically responsive cells. Such changes might well be asymmetric and most pronounced at the leading front membrane where the greatest degree of chemotactic factor receptor occupancy would be expected to occur. To test this hypothesis, we have examined the effects of chemoattractants on the ratio of newly synthesized PE to total methylated phospholipids in plasma membranes isolated from guinea pig macrophages. In addition, the effects of exposure to a chemotactic gradient on these phospholipid ratios in the leading front portions (lamellipodia) of migrating macrophages were studied.

MATERIALS AND METHODS

Chemicals

N-formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe) and fNle-Leu-Phe were obtained from Peninsula Laboratories (San Carlos, Calif.). PE, phosphatidyl-*N'*-dimethylethanolamine, lysophosphatidylcholine, and phosphatidylcholine (PC) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Phosphatidyl-*N'*-monomethylethanolamine was purchased from Gibco Laboratories (Grand Island Biological Co., Grand Island, N. Y.). *L*-[methyl-^{3}H]methionine (10 Ci/mmol) and [1,2-^{14}C]ethanolamine (95 mCi/mmol) were obtained from ICN (Irvine, Calif.). The chemotactic stimulants dialyzed activated guinea pig serum

(AS) and the chemotactic fragment derived from the fifth component of guinea pig complement (C5a) were prepared as described (23).

Cell Preparations

Mononuclear leukocytes containing ~80% macrophages and 20% lymphocytes were obtained from the peritoneal cavities of male Hartley guinea pigs (500–600 g) 3 d after an intraperitoneal injection of 25 ml of 0.5% shellfish glycogen (Sigma Chemical Co.). Leukocytes were removed by lavage of the peritoneal cavities with buffer consisting of 0.135 M NaCl, 4.5 mM KCl, 0.1% dextrose, 1.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$ (pH 7.0) (incubation buffer).

Preparation of [^{3}H]methyl-, [^{14}C]Ethanolamine-labeled Plasma Membranes

Intact macrophages (30–40 × 10^6/ml) suspended in incubation buffer were labeled with 30 μCi/ml [methyl-^{3}H]methionine and 1.5 μCi/ml [^{14}C]ethanolamine for 1 h at 37°C. The cells were centrifuged, washed once, resuspended in 1 ml of buffer containing 10 mM Tris, 1 mM $MgCl_2$, and 1 mM KCl (pH 7.4), and twice frozen in liquid nitrogen and thawed (26). An equal volume of 10 mM Tris, pH 8.1, was then added and the cells were frozen and thawed once more. Plasma membranes were isolated by centrifugation at 95,000 *g* for 45 min in a SW41 rotor in a Beckman L575 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) on discontinuous gradients (10). These gradients consisted of 3 ml each of 40, 36, and 26% sucrose (wt/wt) in 10 mM HEPES buffer containing 1 mM sodium azide (pH 7.6). Plasma membranes at the 0/26 and 26/36% sucrose layers were pooled, diluted with the HEPES buffer, and collected by centrifugation in a type 30 rotor (Beckman Instruments, Inc.) for 25 min at 22,000 *g*. These membrane fractions were enriched fivefold for the plasma membrane ectoenzyme, 5′ nucleotidase, assayed as previously described (3).

Preparation of [^{3}H]-Methyl-, [^{14}C]Ethanolamine-labeled Macrophage Lamellipodia

Lamellipodia containing [^{3}H]methyl- and [^{14}C]ethanolamine-labeled phospholipids from macrophages migrating randomly or to chemotactic agents were isolated as follows. Macrophages (3 × 10^6) suspended in incubation buffer containing 10 μCi/ml [methyl-^{3}H]methionine and 1.0 μCi/ml [^{14}C]ethanolamine were placed in the top compartment of modified Boyden chambers and were separated from control solutions or chemotactic agents in the bottom compartment by 1.0 μm polycarbonate filters (Nuclepore Corp., Pleasanton, Calif.). The cells cannot migrate through the 1-μm pores but do extend membrane processes through them. After a 2-h incubation at 37°C, the chambers were emptied and the top of the filters were wiped free of cells by the use of cotton tipped swabs. The filters containing labeled lamellipodia membranes were removed and extracted in chloroform:methanol (2:1 vol/vol) for analysis of labeled phospholipids.

Analysis of [^{3}H]Methyl-, [^{14}C]Ethanolamine-labeled Phospholipids

Polycarbonate filters (duplicate samples of 15 filters for each determination) or isolated plasma membranes were extracted in 1 vol of incubation buffer and 6 vol of chloroform:methanol (2:1 vol/vol). The resulting organic phase was removed and evaporated to dryness under N_2 gas, then resuspended in 0.15 ml of chloroform:methanol and applied to a silica gel G plate (Analtech Inc., Newark, Del.) along with 25 μg of the phospholipid standards, PE, PC, phosphatidyl-*N*′-monomethylethanolamine, phosphatidyl-*N*′-*N*′-dimethylethanolamine and lysophosphatidylcholine. Plates were developed in chloroform:propionic acid:n-propanol:water (2:2:3:1) (8), and phospholipid spots were visualized with iodine and marked. After vaporization of the iodine, the spots were either scraped directly into scintillation vials containing 10 ml of Aquasol (New England Nuclear, Boston, Mass.) or, for determination of specific activity, scraped into test tubes and the phospholipids were eluted with 1 ml of chloroform:methanol. Portions of the eluate were assayed for radioactivity and inorganic phosphorus content. Tritium and ^{14}C were counted at efficiencies of 31 and 79%, respectively. Greater than 90% of the ^{14}C label was incorporated into PE, with <10% incorporated into the methylated derivatives of PE. Where indicated, results are expressed as the ratio of newly synthesized PE to total methylated phospholipids (excluding lysophosphatidylcholine) or to PC alone. Lysophosphatidylcholine was not included in the calculations because the levels of this derivative are low in extracts of macrophage membranes, and no changes in the amount of this compound are found in extracts of macrophages treated with chemoattractants (18). Ratios are obtained by dividing [^{14}C]cpm incorporated into PE by [^{3}H]cpm incorporated into methylated phospholipids.

Assay of Inorganic Phosphate

Portions of chloroform:methanol extracts of macrophage membranes or of individual phospholipids isolated by thin-layer chromatography (TLC) were washed using 10% $Mg(NO_3)_2$ in absolute ethanol, and the resulting pyrophosphate was hydrolyzed in the presence of 0.5 N HCl. The resulting inorganic phosphate was assayed colorimetrically (1).

Preparation of Filters for Electron Microscopy

Polycarbonate filters containing macrophage lamellipodia were fixed for 2 d at 37°C in 2% glutaraldehyde contained in 0.05 M sodium cacodylate buffer (pH 7.4). After staining for 1 h in 1% osmium tetroxide, the filters were washed in sucrose buffer and dehydrated in ethanol. The filters were critical point dried, coated with carbon followed by platinum, and examined in a Philips 501 scanning electron microscope.

RESULTS

Effects of Chemotactic Factors on the Ratio of Newly Synthesized PE to Methylated Phospholipids in Macrophage Plasma Membranes

Guinea pig macrophages were incubated for 1 h at 37°C with [methyl-^{3}H]methionine and [^{14}C]ethanolamine in buffer alone or in buffer containing the chemotactic factors fMet-Leu-Phe, fNle-Leu-Phe, or C5a. The doses used were maximally active for producing a chemotactic response in vitro. After incubation, the plasma membranes were isolated and the amounts of newly synthesized methylated phospholipids and PE were determined. Treatment of the macrophages with the three chemotactic agents increased the ratio of newly synthe-

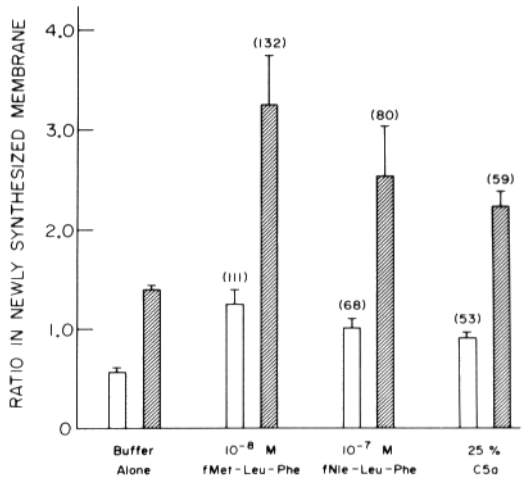

FIGURE 1 Effects of chemotactic factors on newly synthesized phosphatidylethanolamine:methylated phospholipid ratios in macrophage plasma membranes. Duplicate samples of intact macrophages were incubated for 1 h at 37°C in the presence or absence of chemotactic factors in medium containing [^{3}H-methyl]methionine and [^{14}C]ethanolamine. The plasma membranes were then isolated, extracted with chloroform:methanol, and the labeled phospholipids were analyzed by thin-layer chromatography. Ratios (±SD) were calculated by dividing the number of [^{14}C]cpm incorporated into PE by the number of [^{3}H]cpm incorporated into the total methylated phospholipids (□) or into PC alone (▨). The actual cpm used to calculate the data were as follows: *Buffer*, 853, 1450, 609; *fMet-Leu-Phe*, 769, 629, 244; *fNle-Leu-Phe*, 867, 777, 304; and *C5a*, 958, 1068, 531 for PE, total methylated phospholipids and PC, respectively. Numbers in parentheses indicate the percentage of increase calculated as indicated in the legend to Fig. 2. Similar results were obtained in three other experiments.

sized PE:total methylated phospholipids in the plasma membrane by 53 to 111% (Fig. 1). The PE:PC ratio was similarly increased in the plasma membranes by 59 to 132%. FMet-Leu-Phe increased the ratio of PE:methylated phospholipids in a dose-response fashion that paralleled the ability of this peptide to produce a chemotactic response (Fig. 2). The effective concentration (EC_{50}) of fMet-Leu-Phe that gave 50% of a maximal chemotactic response was 2×10^{-9} M whereas the EC_{50} for increasing the plasma membrane PE:methylated phospholipids ratio was 2.5×10^{-9} M.

The increase in the ratio of PE:methylated phospholipids produced by chemotactic factors was most pronounced in the plasma membrane-rich fraction of the cells, as compared to lysosomal membrane fractions (obtained from 36/40% sucrose interface) or pelleted material containing organelles and non-disrupted cells. For example, the increase in the PE:PC ratio produced by 10^{-7} M fMet-Met-Met-Met was 79% in the plasma membrane fraction, 50% in the pelleted cellular material, and only 28% in lysosomal membranes.

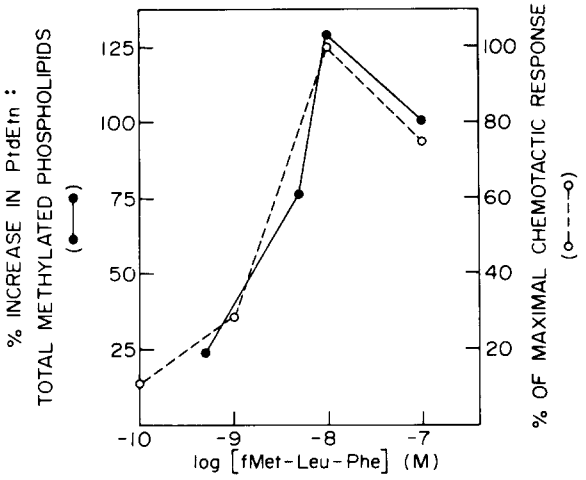

FIGURE 2 Chemotaxis and change in the PE:methylated phospholipid ratio induced by various concentrations of fMet-Leu-Phe. Chemotaxis of guinea pig macrophages suspended in incubation buffer was performed in modified Boyden chambers using 5.0 μM polycarbonate filters and a 2-h incubation at 37°C. *The percentage of maximal response* is equal to (S/M) × 100 where *M* is the maximal chemotactic response (12.5 cells per oil immersion field) induced by *fMet-Leu-Phe* and *S* in the response induced at a submaximal concentration. Phospholipids were analyzed in plasma membranes from guinea pig macrophages that had been incubated with various concentrations of fMet-Leu-Phe under conditions indicated in the legend to Fig. 1. The percentage of increase in the PE:total methylated phospholipid ratio is equal to [(E/C) − 1] × 100 where *E* is the ratio obtained in the presence of *fMet-Leu-Phe* and *C* is the ratio obtained when cells were incubated with buffer alone. Similar results were noted in two additional experiments.

We next determined whether the increase in the newly synthesized PE:methylated phospholipid ratio produced by chemotactic factors in macrophage plasma membranes was due to increased synthesis of PE or decreased synthesis of methylated phospholipids. Macrophages were incubated in the presence or absence of 10^{-8} M fNle-Leu-Phe, the plasma membranes were isolated, and labeled phospholipids were analyzed based on total lipid phosphate content. Table I indicates that there was no significant difference in the amount of [^{14}C]-ethanolamine incorporated into PE in the presence or absence of attractant. [^{14}C]Ethanolamine incorporation was 249 ± 21 pmol/μmol lipid P_i when cells were incubated with buffer alone and 219 ± 13 pmol/μmol lipid P_i in the presence of fNle-Leu-Phe. In contrast, the amount of [^{3}H]methyl groups incorporated into all three methylated derivatives of PE isolated from the plasma membrane was depressed by >50% in the presence of fNle-Leu-Phe (Table I). Thus, chemotactic factors do not alter PE:methylated phospholipid ratios by affecting PE synthesis. The increase in the ratio of PE:methylated phospholipids produced by chemotactic factors in the plasma membrane appears to be the result of decreased synthesis of methylated phospholipids. It would follow then that the ratio of the radioactivity incorporated into new methylated phospholipids to the sum total of methylated phospholipids (specific activity) is depressed in membranes of cells treated with chemotactic factors. We therefore determined the specific activity of PE and the methylated phospholipids in plasma membranes of cells that had been incubated in the presence and absence of chemotactic factors. The specific activity of methylated phospholipids in the presence of buffer alone was 283 cpm/mmol, which was decreased to 191 cpm/mmol in the presence of 10^{-8} M fMet-Leu-Phe. There was no significant difference in the specific activity of PE measured in the presence and absence of 10^{-8} M fMet-Leu-Phe.

Effect of fMet-Leu-Phe on Preformed Derivatives of PE in Macrophage Plasma Membranes

The observed change in PE:methylated phospholipid ratios produced by chemotactic factors could be caused by either decreased synthesis of new methylated phospholipids or enhanced degradation of preformed methylated phospholipids.

TABLE I

Effect of fNle-Leu-Phe on the Incorporation of Radioactive Precursors into Membrane Phospholipids

Membranes isolated from cells incubated with:	[^{14}C]ethanolamine incorporated into PtdEtn	[^{3}H]methyl incorporated into methylated derivatives of PtdEtn			
	pmol/μmol lipid P_i	*(pmol/μmol lipid P_i)*			
		PME*	PDE*	PtdCho*	LPC*
Buffer	249 ± 21‡	0.81 ± 0.18	1.55 ± 0.18	1.70 ± 0.11	0.50 ± 0.10
10^{-8} M fNle-Leu-Phe	219 ± 13	0.38 ± 0.02	0.76 ± 0.02	0.74 ± 0.05	0.35 ± 0.03

Macrophages were incubated for 1 h at 37°C in the presence or absence of fNle-Leu-Phe in buffer containing 30 μCi/ml of [^{3}H-methyl]methionine and 1.5 μCi/ml of [^{14}C]ethanolamine. The plasma membranes were then isolated. Labeled membranes were extracted in chloroform:methanol and the labeled phospholipids were analyzed by thin-layer chromatography. Portions of the extracts were assayed for inorganic phosphorus.

* PME: phosphatidyl-N'-monomethylethanolamine
PDE: phosphatidyl-N'-N'-dimethylethanolamine
PtdCho: phosphatidylcholine
LPC: lysophosphatidylcholine
‡ ± S.D.

To distinguish between these possibilities, macrophages were prelabeled with [methyl-^{3}H]methionine and [^{14}C]ethanolamine, washed extensively, and resuspended in buffer containing 1.0 mM unlabeled methionine and ethanolamine to prevent continued incorporation of radiolabel. The cells were then treated with 10^{-8} M fMet-Leu-Phe for 0, 15, or 60 min at 37°C, after which time the plasma membranes were isolated and the residual radioactivity associated with the phospholipids was analyzed. The treatment of cells with fMet-Leu-Phe did not alter the ratios of preformed PE:total methylated phospholipids or PE:PC, indicating that the chemotactic factor did not produce degradation of preformed methylated phospholipids through phospholipase activation.

Effects of Chemotactic Factors on the Ratio of Newly Synthesized PE:Methylated Phospholipids in the Lamellipodia of Macrophages

To determine whether similar changes in the ratio of newly synthesized PE:methylated phospholipids were produced in the lamellipodia of macrophages responding to gradients of chemoattractants, cells were placed in modified Boyden chambers containing 1-μm diameter pore size polycarbonate filters. This pore size is too small to allow for migration of total cells through the filter. Membrane processes of the macrophages do, however, protrude through the pores to the underside of the filters, as illustrated in the scanning electron micrograph shown in Fig. 3. These membrane processes constitute the leading front or lamellipodia of the migrating cells. These findings are similar to those reported by Wetzel et al. (27) showing that mononuclear leukocyte lamellipodia comprise the initial portion of the cells that migrate to the underside of polycarbonate filters (Nuclepore Corp.). In addition, Malech et al. (12) have used the technique of allowing lamellipodia to penetrate into undersized pores of millipore filters to study cytoskeletal changes that occur in this area of the cells when they are exposed to chemoattractants.

Macrophages were labeled in the chemotaxis chambers with [methyl-^{3}H]methionine and [^{14}C]ethanolamine. Buffer or various chemotactic factors were placed in the lower compartment of the chamber, and, after a 2-h incubation at 37°C, the chambers were emptied and the cells sheared from the top of the filters, leaving behind the lamellipodia that had penetrated to the underside of the filter. Chloroform:methanol extracts of these filters were analyzed for labeled phospholipids. The ratios of PE:total methylated phospholipids and PE:PC were increased two- to fourfold in lamellipodia derived from macrophages that had responded to dialyzed zymosan AS, C5a, or fMet-Met-Met when compared to those from cells responding to buffer alone (Fig. 4). The increase in these ratios produced by C5a were dose-dependent (Fig. 5) and occurred over the same concentration range that produced a chemotactic response in guinea pig macrophages (18).

DISCUSSION

The sequence of biochemical events initiated by chemoattractants in leukocytes that results in biological responses is poorly

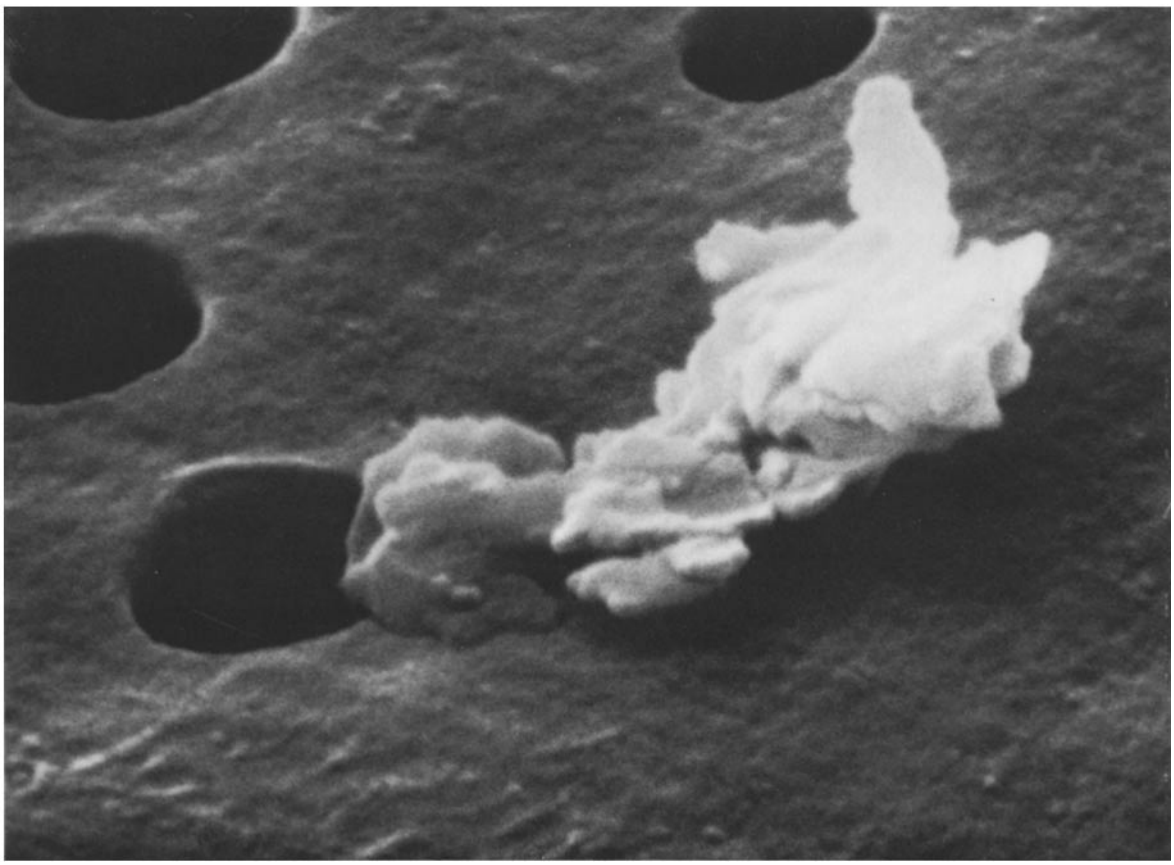

FIGURE 3 Scanning electron micrograph (× 30,000) of the underside of a 1.0-μM-pore-size polycarbonate filter through which a macrophage lamellipodium has migrated in response to buffer alone. Similar lamellipodia were observed in electron micrographs of filters in which the cells had migrated in response to C5a.

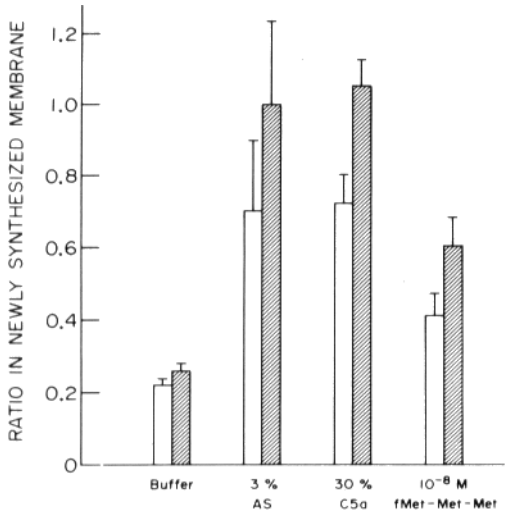

FIGURE 4 Effects of exposure to gradients of various chemotactic agents on the PE:methylated phospholipid ratios in leading front macrophage membranes. Macrophages were labeled in the chemotaxis chambers with [methyl-^{3}H]methionine and [^{14}C]ethanolamine while responding to buffer alone or the indicated chemoattractant. Duplicate samples of fifteen filters each were analyzed for labeled phospholipids as indicated in Materials and Methods, and the PE:total methylated phospholipid (□) or PE:PC (▨) ratios were calculated as described in the legend to Fig. 1. Similar results were obtained in at least three other experiments.

defined. It does appear, however, that binding of chemotactic factors to specific surface receptors (2, 6, 14, 29), ion fluxes (4, 7, 13, 18), alterations in cyclic nucleotide levels (20–22), and rearrangement of cellular cytoskeletal elements (12, 16) are involved in directed cell movement. Transmethylation reactions using *S*-adenosyl-L-methionine as a methyl donor have also been shown to be required for the chemotactic response of certain types of leukocytes because inhibitors of these reactions inhibit directed migration (15, 17, 23). In addition, we have demonstrated that chemotactic agents produce a marked depression of phospholipid methylation in intact guinea pig macrophages (18). One explanation for these seemingly paradoxical observations concerning methylation and chemotaxis is that local or asymmetric inhibition of phospholipid methylation in macrophages is required for directed migration. Phospholipid methylation in leukocytes might be a generalized-membrane property while the cell is immobile or migrating randomly. However, when exposed to a gradient of chemotactic agents, the methylation of membrane phospholipids may be altered in a nonuniform manner. The biophysical properties of the membrane would be expected to change asymmetrically under these circumstances as a consequence of inhibition of PC formation without alterations in total phospholipid synthesis. One would expect that, under these conditions and on that portion of the membrane facing the chemotactic gradient, there would be a local accumulation of PE as compared to its methylated derivatives. If this were the case, inhibitors of methylation reactions would depress chemotaxis by preventing asymmetrical changes in membrane phospholipid composition induced by chemotactic factors.

The present study shows that there is an increase in the ratio of newly synthesized PE to methylated phospholipids in the plasma membrane of guinea pig macrophages treated with biologically relevant doses of various chemotactic factors. This increased ratio resulted from inhibition of the methylation of PE in the membranes and not from alterations in the incorporation of [^{14}C]ethanolamine into PE. A decrease in the specific activity of methylated phospholipids but not of PE reflected the inhibition of phospholipid methylation produced by the chemotactic factors. Because the specific activity of PE is unaltered in the presence of chemoattractants, it seems unlikely that chemoattractants affect the decarboxylation of phosphatidylserine to form PE. In addition, it does not appear that the changes observed in methylated phospholipids in the presence of chemoattractants are due to alterations in the CDP-choline pathway, because we do not detect changes in the incorporation of [^{3}H]choline or ^{32}P (18) into PC in macrophages. The present study also examined the PE:methylated phospholipid ratios in lamellipodia of macrophages undergoing either random migration or chemotaxis in response to a variety of agents. These ratios are increased by up to fourfold in lamellipodia exposed to gradients of various chemoattractants as compared to lamellipodia isolated from cells migrating randomly.

The increase in the newly synthesized PE:methylated phospholipid ratio appears to be due to inhibition of phospholipid methylation rather than to altered degradation of preformed methylated phospholipids by phospholipase A_2. This latter mechanism has been proposed to account for the depressed levels of methylated phospholipids induced in rabbit peritoneal polymorphonuclear (PMN) leukocytes by a chemotactic peptide (9). These discrepancies with our data may reflect differences in the cell types used. It is noteworthy that the chemotaxis of PMN is less dependent upon transmethylation reactions when compared to that of macrophages (24, 28). In addition, the methylation requirements of the functional responses of leukocytes, such as phagocytosis, capping of receptors, as well as chemotaxis, also vary depending upon the species studies (4, 11, 17, 24, 25).

Activation of phospholipase *C* by chemoattractants with subsequent degradation of phosphatidylinositol has been noted in rabbit PMNs (30) and we have observed this effect in human

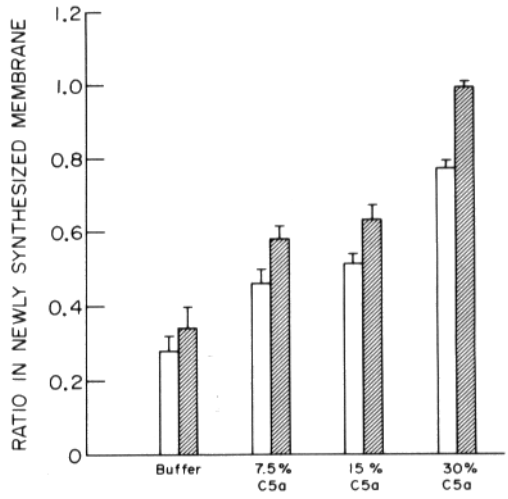

FIGURE 5 Effects of exposure to gradients of various concentrations of *C5a* on the PE:methylated phospholipid ratios in leading front macrophage membranes. Duplicate samples of 15 filters each were analyzed for labeled phospholipids as indicated in Materials and Methods, and the PE:total methylated phospholipids (□) and PE:PC (▨) ratios (± S.D.) were calculated as described in the legend to Fig. 1. Similar results were obtained in one other identical experiment.

monocytes (unpublished observations). It has not yet been determined whether activation of this pathway has any effect on phospholipid methylation.

The functional importance of the increase in newly synthesized PE:methylated phospholipid ratios produced by chemotactic factors in the plasma membranes of macrophages exposed to chemotactic gradients can only be surmised at this time. The local accumulation of PE, a molecule whose head group is both smaller than that of PC and capable of forming more hydrogen bonds, should result in local alterations in the physical state of the membrane. Although the bulk properties of the membrane may not be altered in the presence of chemoattractants, minute, local changes in the PE:PC ratio in focal areas of newly synthesized membrane exposed to chemotactic agents could be required for altering receptor mobility, for anchoring membranous structures to cellular cytoskeletal elements, or for changing the permeability of the membrane to various ions. It can be expected that further study of phospholipid methylation in chemotactically responsive cells will lead to a greater understanding of the biochemical control of cellular motility.

We thank Dr. Charles Daniels, Department of Pathology, Duke University Medical Center, for advice and for reagents for fixing and staining filters for electron micrographs, and Susan Hutchinson for taking the electron micrographs.

Received for publication 6 February 1981, and in revised form 26 May 1981.

REFERENCES

1. Amos, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. *J. Biol. Chem.* 235:769–775.
2. Aswanikumar, S., B. A. Corcoran, E. Schiffmann, A. R. Day, R. J. Freer, H. J. Showell, and E. L. Becker. 1977. Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. *Biochem. Biophys. Res. Commun.* 74:210–217.
3. Avruch, J., and D. B. Wallach. 1971. Preparation and properties of plasma membrane and endoplasmic reticulum fragments from isolated rat fat cells. *Biochem. Biophys. Acta* 233: 334–347.
4. Boucek, M. M., and R. Snyderman. 1976. Calcium influx requirement for human neutrophil chemotaxis:inhibition by lanthanum chloride. *Science (Wash. D. C.)* 193:905–907.
5. Braun, J., F. S. Rosen, and E. R. Unanue. 1980. Capping and adenosine metabolism: genetic and pharmacologic studies. *J. Exp. Med.* 151:174–183.
6. Chenoweth, D. E., and T. E. Hugli. 1978. Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* 74:3943–3947.
7. Gallin, J. I., and A. S. Rosenthal. 1974. The regulatory role of divalent cations in human granulocyte chemotaxis. *J. Cell Biol.* 62:594–609.
8. Hirata, F., O. H. Viveros, E. J. Dilberto Jr., and J. Axelrod. 1978. Identification and properties of two methyltransferases in conversion of phosphatidylethanolamine to phosphatidylcholine. *Proc. Natl. Acad. Sci. U. S. A.* 75:1718–1721.
9. Hirata, F., B. A. Corcoran, K. Venkatasubramanian, E. Schiffmann, and J. Axelrod. 1979. Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* 76:2640–2643.
10. Jakoi, E. M., G. Zampighi, and J. D. Robertson. 1976. Regular structures in unit membranes. II. Morphological and biochemical characterization of two water soluble membrane proteins isolated from the suckling rat ileum. *J. Cell Biol.* 70:97–111.
11. Leonard, E. J., A. Skeel, P. K. Chiang, and G. L. Cantoni. 1978. The action of adenosylhomocysteine hydrolase inhibitor 3-deazaadenosine, on phagocytic function of mouse macrophages and human monocytes. *Biochem. Biophys. Res. Commun.* 84:102–109.
12. Malech, H. L., R. K. Root, and J. I. Gallin. 1977. Structural analysis of human neutrophil migration:centriole, microtubule microfilament orientation and function during chemotaxis. *J. Cell Biol.* 75:666–693.
13. Naccache, P. H., H. J. Showell, E. L. Becker, and R. I. Sha'afi. 1977. Transport of sodium, potassium, and calcium across rabbit polymorphonuclear leukocyte membranes: effect of chemotactic factor. *J. Cell Biol.* 73:428–444.
14. Niedel, J., S. Wilkinson, and P. Cuatrecasas. 1979. Receptor-mediated uptake and degradation of ^{125}I-chemotactic peptide by human neutrophils. *J. Biol. Chem.* 254:10700–10706.
15. O'Dea, R. F., O. H. Viveros, S. Aswanikumar, E. Schiffmann, P. K. Chiang, G. L. Cantoni, and J. Axelrod. 1978. A protein carboxy-methylation stimulated by chemotactic peptides in leukocytes. *Fed. Proc.* 37:1656.
16. Oliver, J. M., J. A. Krawiec, and E. L. Becker. 1978. The distribution of actin during chemotaxis in rabbit neutrophils. *J. Reticuloendothel. Soc.* 24:697–704.
17. Pike, M. C., N. M. Kredich, and R. Snyderman. 1978. Requirement of S-adenosyl-L-methionine-mediated methylation for human monocyte chemotaxis. *Proc. Natl. Acad. Sci. U. S. A.* 74:3928–3932.
18. Pike, M. C., N. M. Kredich, and R. Snyderman. 1979. Phospholipid methylation in macrophages is inhibited by chemotactic factors. *Proc. Natl. Acad. Sci. U. S. A.* 76: 2922–2926.
19. Rivkin, I., J. Rosenblatt, and E. L. Becker. 1975. The role of cyclic AMP in the chemotactic responsiveness and spontaneous motility of rabbit peritoneal neutrophils. *J. Immunol.* 115: 1126–1134.
20. Sandler, J. A., J. I. Gallin, and M. Vaughan. 1975. Effects of serotonin, carbamylcholine, and ascorbic acid on leukocyte cyclic GMP and chemotaxis. *J. Cell Biol.* 67:480–484.
21. Simchowitz, L., J. P. Atkinson, and I. Spilberg. 1980. Lack of cross-deactivation of chemotactic factor-induced cyclic AMP response and superoxide generation by human neutrophils. *Fed. Proc.* 39:566.
22. Smolen, J. E., H. M. Kirchak, and G. Weissmann. 1980. Increased levels of cyclic adenosine-3′-5′-monophosphate in human polymorphonuclear leukocyte after surface stimulation. *J. Clin. Invest.* 65:1077–1085.
23. Snyderman, R., H. Gewurz, and S. E. Mergenhagen. 1968. Interactions of the complement system with endotoxic lipopolysaccharide. Generation of a factor chemotactic for polymorphonuclear leukocytes. *J. Exp. Med.* 128:259–275.
24. Snyderman, R., M. C. Pike, and N. M. Kredich. 1980. Role of transmethylation reactions in cellular motility and phagocytosis. *Molec. Immunol.* 17:209–218.
25. Sung, S. J., J. H. Raker, and S. C. Silverstein. 1980. Phagocytosis inhibition of mouse peritoneal macrophages by methylation inhibitors. *Fed. Proc.* 39:1820.
26. Turner, M. J., P. Cresswell, P. Parham, and J. L. Strominger. 1975. Purification of papain-solubilized histocompatibility antigens from a cultured human lymphoblastoid line, RPMI 4265. *J. Biol. Chem.* 250:4512–4519.
27. Wetzel, B., L. C. Altman, and S. M. Wahl. 1976. SEM of macrophage chemotaxis. *J. Cell Biol.* 70(2, Pt. 2):156*a* (Abstr.).
28. Wilkinson, P. C., and S. C. Silverstein. 1980. *In:* Proceedings of the First International Conference on Immunopharmacology. Brighton, England (New York, Pergamon Press), *In press.*
29. Williams, L. T., R. Snyderman, M. C. Pike, and R. J. Lefkowitz. 1977. Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* 74:1204–1208.
30. Wolpert, L. M., M. Volpi, P. H. Nacchache, and R. I. Sha'afi. 1980. The interrelationship of phosphatidylinositol turnover and calcium gating in the rabbit neutrophil. *Fed. Proc.* 39:878.