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Immunology

Phospholipid methylation in macrophages is inhibited by chemotactic factors

(*S*-adenosyl-L-methionine/carboxy-*O*-methylation/phagocytosis/phosphatidylcholine/phosphatidylethanolamine)

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ABSTRACT **Chemotaxis by human monocytes has been shown to require methylation mediated by *S*-adenosyl-L-methionine(AdoMet), but the specific transmethylation reaction necessary for this function was not elucidated. In an attempt to define the methylation requirement for chemotaxis, we examined the effect of chemotactic agonists and antagonists on protein carboxy-*O*-methylation of protein and methylation of phospholipid in guinea pig macrophages. Chemotactic agents tested over a wide dose and time range produced no alteration in carboxy-*O*-methylation. However, these agents did produce an effect on the methylation of phosphatidylethanolamine by macrophages. AdoMet-mediated phospholipid methylation was inhibited by as much as 73% by chemotactic factors, and there was excellent correlation ($r = 0.99$) between their concentrations for producing half-maximal chemotactic responses and for inhibiting phospholipid methylation. The inhibition of methylation by chemotactic factors was observed at all incubation times and could not be explained by an increased turnover of membrane phospholipid. Neither the chemotaxis antagonist fPhe-Met nor the nonchemotactic tripeptide Met-Met-Met significantly depressed phospholipid methylation. Immune phagocytosis by macrophages similarly did not alter phospholipid methylation. The chemotactic factors produced no alteration in total macrophage phospholipid synthesis or in the phospholipid methylation in a nonchemotactic cell type. The formation of newly methylated derivatives of phosphatidylethanolamine in macrophages was decreased by a biologically active dose of chemotactic factor. These findings indicate that chemotactic factors are capable of altering the methylation of phosphatidylethanolamine in chemotactically responsive cells. The inhibition of phospholipid methylation by chemotactic factors may be necessary for the translation of a chemotactic signal on the surface of the cell into directional cell movement.**

Transmethylation reactions mediated by *S*-adenosyl-L-methionine (AdoMet) are required for many functions of both eukaryotic and prokaryotic cells (1, 2). In bacteria, chemotaxis requires the methylation of certain membrane-associated proteins by protein carboxymethyltransferase (3–5). Recently, we demonstrated that transmethylation was necessary for the directed migration of at least one type of eukaryotic cell, the human monocyte (6). Incubation of monocytes with a specific inhibitor of adenosine deaminase, *erythro*-9[3-(2-hydroxynonyl)]adenine(EHNA), plus adenosine and L-homocysteine thiolactone produced marked increases in intracellular concentrations of *S*-adenosyl-L-homocysteine (AdoHcy), a competitive inhibitor of AdoMet-mediated methylation reactions (7). Concomitant with such increases in monocyte AdoHcy was a profound depression of monocyte chemotactic responsiveness and inhibition of protein carboxy-*O*-methylation (6).

Although these studies clearly demonstrated the requirement of AdoMet-mediated transmethylation for monocyte chemotaxis, they did not define the specific methylation reactions(s) involved in cell movement. Because cell division and protein synthesis appear not to be required for monocyte chemotaxis (6), we think that the methylation of DNA and RNA probably are not involved in the chemotactic response. Because of the known or suspected importance of protein carboxy-*O*-methylation (8, 9) and phospholipid methylation (10, 11) in certain specialized membrane functions, we measured the effects of chemotactic factors on these two types of methyltransferase reactions in guinea pig macrophages.

MATERIALS AND METHODS

Chemicals. *N*-Formyl-L-methionyl-L-methionyl-L-methionine (fMet-Met-Met), fPhe-Met, Met-Met-Met, and fMet-Leu were purchased from Andrulis Research (Bethesda, MD); fMet-Leu-Phe and fNorleu-Leu-Phe were obtained from Peninsula Laboratories (San Carlos, CA). Adenosine and Hepes were products of Calbiochem; L-homocysteine thiolactone, phosphatidylcholine (PtdCho), and phosphatidyl-*N*,*N*-dimethylethanolamine (PtdEtn) were obtained from Sigma, and EHNA was from Burroughs Wellcome (Research Triangle Park, NC). Phosphatidyl-*N*-monomethylethanolamine was purchased from GIBCO. L-[*methyl*-^{3}H]Methionine (80 Ci/mmol; 1 Ci = 3.7 + 10^{10} becquerels) and $^{32}P_i$ as orthophosphoric acid were purchased from New England Nuclear. The chemotactic stimulants activated guinea pig serum (AS) and the chemotactic fragment derived from the fifth component of complement (C5a) were prepared as described (12).

Cell Preparations. Mononuclear leukocytes were obtained from the peritoneal cavities of male Hartley guinea pigs (500–600 g) 3 days after an intraperitoneal injection of 25 ml of 0.5% shellfish glycogen (Sigma). Mononuclear leukocytes were removed by lavage of the peritoneal cavities with Gey's balanced salt solution containing 2% (wt/vol) bovalbumin (Flow Laboratories, Rockville, MD), 0.01 M Hepes at pH 7.0, and 10 units of heparin per ml.

Guinea pig lymphocytes were obtained by mincing resected spleens and passing the resultant cell suspension through gauze to remove large particulate matter. After centrifugation at 400 × *g* for 10 min, the cells were resuspended to 2 × 10^6 cells per ml in RPMI 1640 (GIBCO) containing 10% (wt/vol) heat-inactivated (30 min, × 37°C) fetal calf serum (GIBCO). Twelve milliliters of this suspension was placed into each of a series of 100-mm-diameter Falcon tissue culture plates. The plates were then incubated for 1 hour at 37°C in humidified air containing 5% CO_2 to allow the macrophages to adhere to the plastic. The nonadherent cells were then pooled and standardized to contain 4–6 × 10^6 cells per ml in Gey's solution.

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Abbreviations: AdoMet, *S*-adenosyl-L-methionine; EHNA, *erythro*-9[3-(2-hydroxynonyl)]adenine; AdoHcy, *S*-adenosyl-L-homocysteine; AS, activated guinea pig serum; C5a, chemotactic fragment derived from the fifth component of complement; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; TLC, thin-layer chromatography.

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Chemotaxis Assay. Chemotaxis was measured after a 2-hr incubation in modified Boyden chambers using 5.0-μm polycarbonate filters (13). Chemotactic activity of the various stimulants was determined as the mean number of migrating cells per oil-immersion field in triplicate samples.

Assay of Cellular Phospholipid Methylation. The methylation of both membrane and total intracellular PtdCho precursors was assayed in intact macrophages and lymphocytes by measuring the incorporation of the methyl group from L-[*methyl*-^{3}H]methionine into phospholipids. Aliquots (1 ml) of cell suspensions containing 4–6 $\times$ 10^6 cells per ml in Gey's solution were preincubated at 37°C for 15 min, after which 10 μCi of L-[*methyl*-^{3}H]methionine (0.125 μM) was added. Where indicated, chemotactic agonists, antagonists, or medium alone was added simultaneously with the labeled methionine. After incubation at 37°C for various times, the cells were pelleted, and washed once in isotonic saline, and the cell pellet was resuspended in 0.1 ml of saline. Then, 0.5-ml of chloroform/methanol, 2:1 (vol/vol), was added to each tube. The tubes were vortexed vigorously for 30 sec and then centrifuged for 5 min at 2000 $\times$ *g*. The aqueous phase was aspirated and 0.1 ml of the organic phase was applied to a cellulose thin-layer chromatography (TLC) plate containing a fluorescent indicator (Eastman Chromogram) along with 10 μg of PtdCho. Separation of labeled and authentic phospholipids from free L-[*methyl*-^{3}H]methionine was achieved by developing the plates in chloroform/methanol/water, 75:18:2 (vol/vol); authentic phospholipids were visualized under UV light. The spots containing labeled phospholipids were scraped into glass scintillation vials and the total amount of radioactivity in duplicate samples was determined by liquid scintillation spectrophotometry after the addition of 12 ml of Aquasol (New England Nuclear). In some instances, the specific activity of *S*-adenosyl[*methyl*-^{3}H]methionine in acid-soluble cellular extracts was determined in parallel experiments using high-performance liquid chromatography as described (6).

Characterization of Reaction Products. A chloroform/methanol extract derived from approximately 30 $\times$ 10^6 cells was spotted on cellulose TLC plates and developed as described above. The [^{3}H]methylated phospholipids contained in the TLC scrapings were eluted with 1 ml of chloroform/methanol (2:1) which was then evaporated under a stream of nitrogen gas. After the addition of 100 μl of chloroform/methanol (2:1), samples were applied to a silica gel G plate (Uniplate, Analtech, Newark, DE) and developed in each of two solvent systems: (*i*) chloroform/propionic acid/1-propanol/water, 2:2:3:1 (vol/vol); and (*ii*) chloroform/methanol/water, 65:25:4 (vol/vol) (10). Authentic PtdCho and PtdEtn and its mono- and dimethylated intermediates were chromatographed and visualized by spraying with 0.06% rhodamine 6G (Eastman).

Assay of Cellular Phospholipid Synthesis. Total phospholipid synthesis in macrophages was assayed by measuring the incorporation of $^{32}P_i$ into organic solvent-extractable material. Four to 6 $\times$ 10^6 cells contained in 1 ml of phosphate-free balanced salt solution (0.135 M NaCl/4.5 mM KCl/0.1% dextrose/1.5 mM $MgCl_2$/0.15 mM $CaCl_2$, pH 7.0) were incubated for 15 min at 37°C. $^{32}P_i$ (5 μCi) was then added, and the cell suspension was incubated for an additional 1 hr at 37°C. Phospholipids were extracted as outlined above for studies of phospholipid methylation. The aqueous phase was aspirated and the organic phase was washed once with 0.2 ml of phosphate-buffered saline. One-tenth milliliter of the organic phase was transferred to a glass scintillation vial and the radioactivity was assayed by liquid scintillation spectrophotometry after the addition of 12 ml of Aquasol.

Cellular Protein Carboxymethylase Assay. Carboxy-*O*-methylation of proteins was assayed as described (6, 9).

Preparation of Opsonized Erythrocytes for Phagocytosis. Opsonized sheep erythrocytes for use in phagocytosis were prepared as described (14).

RESULTS

A role for AdoMet-mediated transmethylation in human monocyte chemotaxis has already been established (6). A methylation requirement also exists for guinea pig macrophage chemotactic responsiveness, because incubation of these cells with EHNA, adenosine, and L-homocysteine thiolactone also inhibits their chemotaxis (data not shown). In an attempt to define the specific methyltransferase reaction(s) necessary for macrophage chemotaxis, we investigated the effects of various chemotactic agonists and antagonists on cellular phospholipid and protein methylation.

Effects of Chemotactic Factors on Macrophage Phospholipid Methylation. Guinea pig macrophage phospholipid methylation was measured during a 60-min period in the presence of a series of chemotactic formylated tripeptides and C5a (12). fMet-Met-Met, fNorleu-Leu-Phe, fMet-Leu-Phe, and C5a inhibited macrophage phospholipid methylation in a dose-dependent fashion, with maximal inhibition ranging from 50 to 73% with the various chemotactic agonists (Fig. 1*A*). The concentrations necessary to inhibit phospholipid methylation were in the same range (0.1–10 nM) that gave a chemotactic response (Fig. 1*B*). Comparison of the effective concentrations of these different substances required for 50% of a maximal response shows excellent agreement (correlation coefficient, 0.999) between their effects on chemotaxis and phospholipid methylation (Fig. 2).

fMet peptides at 10 nM and less did not significantly alter the specific activity of intracellular AdoMet when measured at 5, 30 and 60 min during the phospholipid methylation assays. However, at peptide concentrations of 1 μM or greater, AdoMet specific activities were appreciably lowered, presumably by hydrolytic release of nonradiolabeled methionine from these compounds. The data given here have been corrected for such effects.

The nonchemotactic peptide Met-Met-Met (15) produced no inhibition of phospholipid methylation at a concentration of 10 nM. The chemotactic agonist fPhe-Met (16) inhibited phospholipid methylation by only 13% at 0.1 mM.

Effect of Phagocytosis on Phospholipid Methylation. The effect of a phagocytic stimulus on phospholipid methylation was also investigated in order to determine whether the inhibition of phospholipid methylation was a general consequence of stimulated metabolic functions within the cell or more specifically related to chemotaxis. Macrophages were incubated for 15 min at 37°C with sheep erythrocytes or with sheep erythrocytes treated with anti-sheep-erythrocyte hemolysin; then L-[*methyl*-^{3}H]methionine was added and, 1 hr later, the cells were processed for phospholipid methylation. A ratio of opsonized erythrocytes to macrophages as high as 50:1 did not significantly alter the amount of macrophage phospholipid methylation (Table 1). Unopsonized erythrocytes, which are not ingested, also did not alter the methylating ability of the cells. The phospholipid methyltransferase activity of the opsonized and unopsonized erythrocytes themselves did not significantly contribute to these reactions because, at the concentrations of erythrocytes used, their phospholipid methylation was undetectable.

Kinetics of Inhibition of Phospholipid Methylation by Chemotactic Factor. Macrophages were incubated at 37°C with L-[*methyl*-^{3}H]methionine in the presence and absence of 10 nm fMet-Met-Met and harvested at various times for the measurement of phospholipid methylation. Inhibition of the rate of accumulation of radiolabel in methylated phospholipid

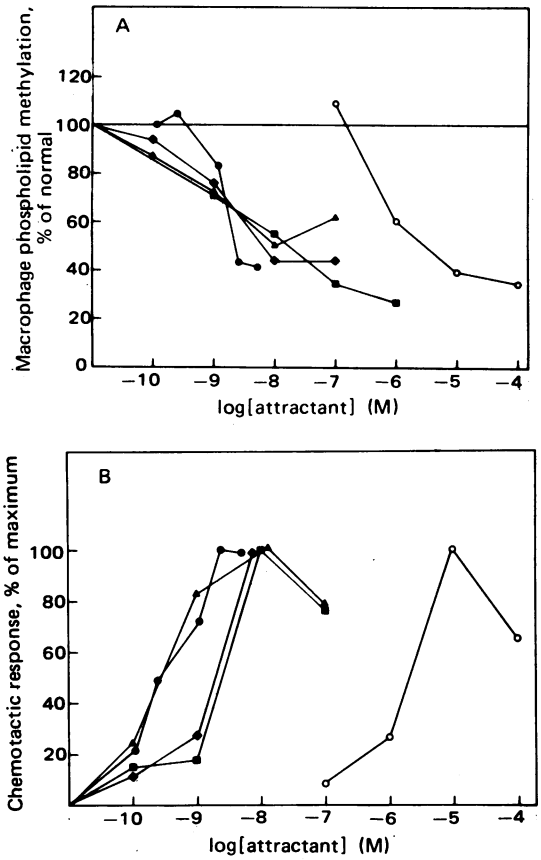

FIG. 1. (*A*) Effects of chemotactic factor on incorporation of [^{3}H]methyl groups into phospholipids in guinea pig macrophages. % of normal = $(E/C) \times 100$ in which E is incorporation in the presence of chemotactic agent and C is incorporation in the presence of buffer alone. Calculations were corrected for the alteration of specific activity of the [^{3}H]AdoMet formed in the presence of fMet-Leu. ●, C5a; ▲, fMet-Met-Met; ■, fNorleu-Leu-Phe; ◆, fMet-Leu-Phe; ○, fMet-Leu. (*B*) Chemotactic activity of chemotactic agents for guinea pig macrophages. % of maximal response = $(S/M) \times 100$ in which M is the maximal response to a chemotactic agent and S is response to the same factor at a submaximal concentration. Maximal responses for the individual chemoattractants (expressed as cells per oil-immersion field) were: C5a, 114.2; fMet-Met-Met, 29.7; fMet-Leu-Phe, 12.5; fNorleu-Leu-Phe, 28.7; fMet-Leu, 25.7. Symbols as in *A*.

by chemotactic factor was evident within 5 min of incubation and persisted for as long as 60 min (Fig. 3). At no time did phospholipid methylation appear to be stimulated by fMet-Met-Met.

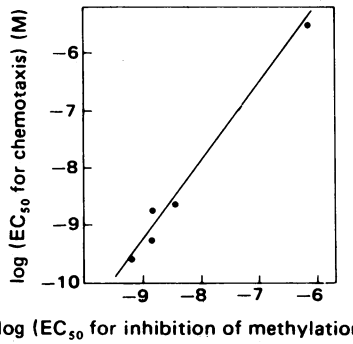

FIG. 2. Correlation of chemotactic potency of the chemotactic agonists and their ability to inhibit phospholipid methylation. The concentrations of each chemoattractant causing half maximal responses (EC_{50}) were computed from data in Fig. 1. Linear regression analysis was performed to obtain a best fit line: slope, 3.6; correlation coefficient, 0.999; standard error of estimate, ±0.23.

Table 1. Effect of immune phagocytosis on macrophage phospholipid methylation

Macrophages incubated with*	[^{3}H]Methyl incorporation, into phospholipids, pmol/10^9 cells	% of normal†
Buffer alone	51.8	100
fMet-Met-Met(10 nM)	26.6	51
ShEA (50:1)	46.7	90
ShEA (10:1)	44.9	87
ShE (50:1)	47.2	91
ShE (10:1)	48.4	93

* Macrophages (4×10^6) were incubated under the indicated conditions at 37°C for 60 min with 10 μCi of [*methyl*-^{3}H]methionine, and then total phospholipid methylation was quantified. ShEA, sheep erythrocytes treated with anti-sheep-erythrocyte hemolysin; ShE, sheep erythrocytes.

† % of normal = $(E/C) \times 100$ in which E is the [^{3}H]methyl incorporation determined in the presence of the indicated substances and C is the [^{3}H]methyl incorporation in macrophages incubated with buffer alone.

We also asked whether chemotactic factor accelerates the turnover of preformed methylated derivatives of PtdEtn in macrophages. Macrophages were labeled with L-[*methyl*-^{3}H]methionine for 1 hr at 37°C, collected by centrifugation, and resuspended in Gey's solution containing 0.1 mM unlabeled methionine. These cells were then incubated at 37°C in the presence or absence of 10 nM fMet-Met-Met and aliquots were removed at times ranging between 10 min and 5 hr for measurements of residual phospholipid radioactivity. There was no appreciable loss of newly formed methylated phospholipids in the presence or absence of chemotactic factor during the course of the 5-hour incubation. Thus, fMet-Met-Met did not accelerate the degradation of newly formed methylated phospholipid derivatives associated with the macrophages.

Specificity of Inhibition of Phospholipid Methylation by Chemotactic Factors. Because the decrease in phospholipid methylation produced by chemotactic factors could be the result of inhibiting total phospholipid synthesis, the incorporation of $^{32}P_i$ into lipid-extractable radioactivity after incubation for 1 hr at 37°C was measured in the presence and absence of various concentrations of the chemotactic factors fMet-Met-Met, fMet-Leu-Phe, and C5a. The results of these experiments (Fig. 4) indicate that the chemotactic factors produced no inhibition of phospholipid synthesis. Indeed, slight but significant

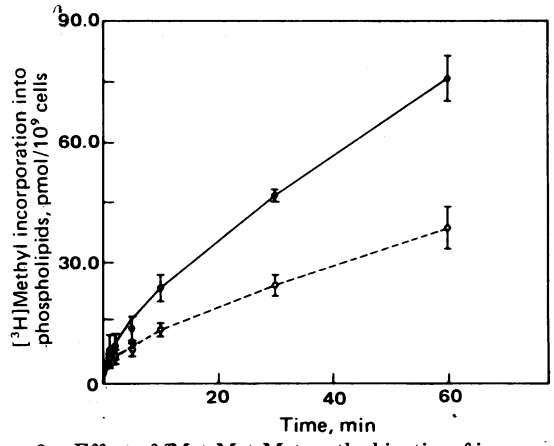

FIG. 3. Effect of fMet-Met-Met on the kinetics of incorporation of [^{3}H]methyl into guinea pig macrophage phosopholipids. Macrophages were incubated with [*methyl*-^{3}H]methionine in the presence or absence of 10 nM fMet-Met-Met, and total phospholipid methylation was determined after incubation for various times at 37°C. ●, Buffer alone; ○, fMet-Met-Met.

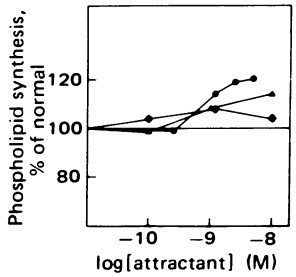

FIG. 4. Effects of chemotactic factors on macrophage total phospholipid synthesis. Macrophages were incubated with $^{32}P_i$ in the presence or absence of chemotactic factors for 60 min at 37°C; then, total phospholipid synthesis was measured. ●; C5a; ▲, fMet-Met-Met; ◆, fMet-Leu-Phe.

($P < 0.025$) enhancement of synthesis (15–20%) was consistently observed with C5a.

Effect of Chemotactic Factors on Macrophage Protein Carboxy-*O*-Methylation. To determine whether the inhibition of phospholipid methylation produced by chemotactic factors were specific or simply reflected a general inhibition of all AdoMet-mediated methylation reactions, the effects of various concentrations of fMet-Met-Met, fMet-Leu-Phe, and C5a on protein carboxy-*O*-methylation in macrophages were determined. There was no significant effect on macrophage protein carboxy-*O*-methyltransferase activity produced by incubation for 1 hr at 37°C with the three different chemotactic factors (Fig. 5*A*). Because other laboratories (17) have reported a rapid and transient (1 min) stimulation of protein carboxymethylase activity in rabbit neutrophils incubated with a fMet chemotactic peptide, we investigated the effects of 10 nM fMet-Met-Met on the kinetics of macrophage carboxy-*O*-methylation (Fig. 5*B*). No alteration in protein carboxymethylase activity was observed in the presence of the chemoattractant even as early as 1 min after introduction of labeled methionine.

Cellular Specificity of Inhibition of Phospholipid Methylation by Chemotactic Factors. The effects of three different chemotactic factors (fMet-Met-Met, fMet-Leu-Phe, and C5a) on the phosphatide methyltransferase activity of guinea pig splenic lymphocytes were examined. These cells did not respond chemotactically to the above compounds (data not shown). No inhibitory effects on lymphocyte phospholipid methylation were observed with any of the chemotactic factors tested (Fig. 6).

Characterization of Methylated Phospholipids. Radiolabeled phospholipids extracted from macrophages incubated with L-[*methyl*-^{3}H]methionine were fractionated into mono-, di-, and trimethyl(choline) derivatives by TLC using two separate solvent systems (10). Fig. 7*A* illustrates the profile of

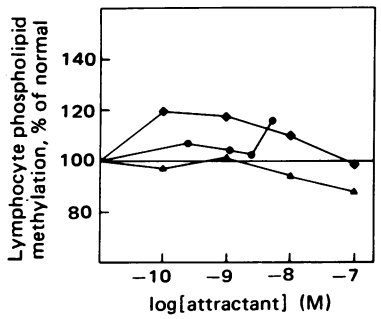

FIG. 6. Effects of chemotactic factors on [^{3}H]methyl incorporation into phospholipids of guinea pig splenic lymphocytes. Lymphocytes were incubated with or without chemotactic factors in the presence of [*methyl*-^{3}H]methionine for 60 min at 37°C. Then, total phospholipid methylation was measured; % of normal was calculated as in Fig. 1*A*. ●, C5a; ▲, fMet-Met-Met; ◆, fMet-Leu-Phe.

[^{3}H]methylated phospholipids found in macrophages incubated in the absence of chemotactic factor. Most of the radioactivity migrated with the three methylated derivatives of PtdEtn, particularly with PtdCho and phosphatidyl-*N*-*N*-dimethylethanolamine. Another peak of radioactivity, the nature of which has yet to be determined, was consistently found to migrate with the solvent front. The profile of [^{3}H]methylated phospholipids from macrophages that had been treated with 10 nM fMet-Met-Met showed a uniform depression of all three methylated derivatives compared to cells incubated in the absence of chemotactic factor (Fig. 7*B*).

DISCUSSION

The accumulation of phagocytic cells at sites of antigenic penetration or neoplastic transformation is a critical event in immunologically mediated host defense. There is substantial evidence indicating that leukocytes are capable of sensing certain chemical gradients by means of specific cellular receptors (16, 18) and of migrating directionally along such concentration gradients. Although the biochemical mechanism by which occupancy of a chemotactic receptor leads to a chemotactic response in eukaryotic cells is as yet poorly understood, we have recently shown a requirement for AdoMet-mediated methylation in this phenomenon (6). In bacteria, chemotaxis requires the binding of a chemoattractant to specific surface receptors followed by AdoMet mediated carboxy-*O*-methylation of certain membrane proteins (3–5, 19). In the studies reported here, we did not observe any significant alteration of

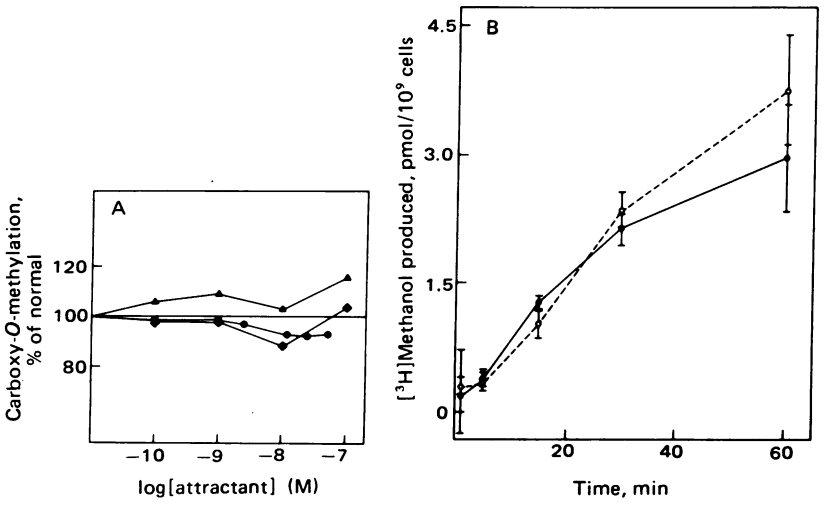

FIG. 5. Effects of chemotactic factors on macrophage protein carboxy-*O*-methylation. (*A*) Various doses of chemotactic factors were incubated with the macrophages in the presence of [*methyl*-^{3}H]methionine for 60 min at 37°C. Then, protein carboxy-*O*-methylation was determined; % of normal was calculated as in the legend to Fig. 1*A*. ●, C5a; ▲, fMet-Met-Met; ◆, fMet-Leu-Phe. (*B*) Kinetics of macrophage protein carboxy-*O*-methylation in the presence (○) and absence (●) of fMet-Met-Met. Macrophages were incubated for various times at 37°C with [*methyl*-^{3}H]-methionine in the presence or absence of fMet-Met-Met (10 nM); then, carboxy-*O*-methylation was measured.

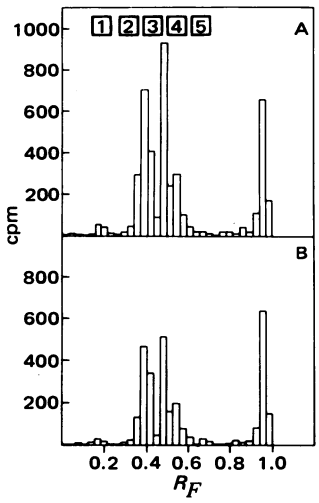

FIG. 7. Chromatographic pattern of the [^{3}H]methylated phospholipid reaction products in the presence of medium alone (*A*) and of 10 nM fMet-Met-Met. (*B*) Reaction products were isolated, applied to a silica gel G plate, and chromatographed with a solvent system of chloroform/propionic acid/n-propyl alcohol/water, 2:2:3:1 (vol/vol). Similar results were obtained with chloroform/methanol/water, 65:25:4 (vol/vol). Region identification: *1*, lysophosphatidylcholine; *2*, PtdCho; *3*, phosphatidyl-*N*,*N*-dimethylethanolamine; *4*, phosphatidyl-*N*-monomethyl-ethanolamine; *5*, PtdEtn.

carboxy-*O*-methylation over a wide dose range of chemoattractants, at any incubation time, even as early as 1.0 min after exposure of guinea pig macrophages to these agents. These findings contrast with the results of O'Dea *et al.* (17) who reported a rapid stimulation of carboxy-*O*-methyltransferase activity in rabbit peritoneal polymorphonuclear leukocytes by fMet-Leu-Phe. Whether the differences in our data reflect differences in species or cell type has not yet been determined.

In contrast to their lack of effect on protein carboxy-*O*-methylation, chemotactic factors inhibited phospholipid methylation in intact guinea pig macrophages by as much as 50–73% when measured after 5–60 min of incubation. A physiologic significance for this phenomenon is suggested by the fact that, for each compound tested, chemotactic activity and inhibition of phospholipid methylation occurred over the same concentration range. Furthermore, neither the chemotactic antagonist fPhe-Met, which binds to the formylated peptide chemotactic receptor but produces no chemotactic response (16, 18), nor the nonchemotactic peptide Met-Met-Met (15) produced any inhibition of phospholipid methylation. The lack of effect of fPhe-Met indicates that chemotactic factor receptor occupancy *per se* is not sufficient to trigger inhibition of methylation. This point is further emphasized by the finding that immune phagocytosis by macrophages did not inhibit phospholipid methylation. Phagocytosis and chemotaxis bear certain similarities in that both processes are initiated by occupancy of membrane receptors followed by cellular movement (20). However, in chemotaxis there is polarized cell movement whereas in phagocytosis there is membrane and cytoplasmic movement around the particle to be ingested but no accompanying cellular polarization. Our data thus suggest that inhibition of phospholipid methylation by chemotactic factors may be important for polarized cell movement. The failure of chemotactic agonists to inhibit phospholipid methylation in erythrocytes and lymphocytes also argues in favor of a relationship between alterations of phospholipid transmethylation reactions and chemotaxis. In the presence of chemotactic factors, macrophages failed to accumulate all three methylated derivatives of PtdEtn. Whether chemotactic factors affect these transmethylation reactions directly or indirectly remains to be determined.

The present study clearly shows that chemotactic factors inhibit a specific AdoMet-mediated methylation reaction in chemotactically responsive cells. We have previously demonstrated, however, that AdoMet-mediated reactions are indeed necessary for chemotaxis to occur, in that inhibition of methylation resulted in inhibition of chemotaxis (6). One explanation of this paradox is that methylation might be requisite for some aspect of chemotaxis, and inhibition of phospholipid methylation is necessary for another. Another possibility is that a gradient of membrane phospholipid methylation or local inhibition of this process is necessary for a directed migratory response. During nondirected migration or at rest, phospholipid methylation might be a generalized membrane property. By binding to responsive cells and by altering the methylation of membrane phospholipids in a nonuniform manner, chemotactic factors could effect local changes in the biophysical properties of the membrane. This would occur as a consequence of the inhibition of the formation of PtdCho without affecting total phospholipid synthesis. It can be expected that, under these conditions, there would be a local accumulation of PtdEtn, a molecule whose head group is both smaller than that of PtdCho and capable of forming more hydrogen bonds. Global inhibition of phospholipid methylation by EHNA, adenosine, and L-homocysteine thiolactone would depress chemotaxis by preventing a cell from establishing a gradient of the ratio of PtdEtn to PtdCho in newly synthesized membrane.

Many constructs of the possible relevance of the alteration of phospholipid composition to chemotaxis can be made. Several that deserve mention are that these changes could be accompanied by local alterations in membrane microviscosity, with the ability of the membrane to interact with external surfaces, or with the anchoring of the membrane to internal cytoskeletal elements. Determining the exact role of the inhibition of phospholipid methylation in chemotaxis will have a major impact on our understanding of cellular motility as well as the mechanism by which membrane phospholipid composition is controlled.

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