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LIPID REQUIREMENTS FOR LEUKOCYTE CHEMOTAXIS AND PHAGOCYTOSIS: EFFECTS OF INHIBITORS OF PHOSPHOLIPID AND CHOLESTEROL SYNTHESIS

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Leukocyte chemotaxis and phagocytosis are accompanied by alterations in the morphology of the cellular plasma membrane, suggesting that modification of membrane composition or synthesis might be required for these biologic activities. We therefore determined whether the synthesis or utilization of the major lipid components of membranes, cholesterol, and/or phospholipids was required for leukocyte chemotaxis and phagocytosis. The antilipidemic drugs, clofibrate and triparanol, were tested for their effects on the chemotaxis and phagocytosis of different types of leukocytes. Clofibrate, an inhibitor of phospholipid and cholesterol synthesis, inhibited the chemotaxis of human monocytes and guinea pig peritoneal macrophages by >95%. The dose that produced a 50% inhibition (ID_{50}) of macrophage chemotaxis correlated well with the ID_{50} for inhibition of phospholipid synthesis. Administration of clofibrate to mice depressed macrophage accumulation *in vivo* in response to an i.p. injection of an inflammmatory stimulus. The incubation of monocytes or macrophages with the inhibitor of cholesterol synthesis, triparanol, also inhibited the chemotaxis of these cells by >95%. The ID_{50} for inhibition of chemotaxis was identical to the ID_{50} for inhibition of cholesterol synthesis. The inhibition of chemotaxis by triparanol but not by clofibrate could be reversed by incubation of cells with the low-density lipoprotein fraction from human plasma, indicating that triparanol inhibited chemotaxis by curtailing cholesterol synthesis. The chemotaxis of human polymorphonuclear leukocytes was also markedly decreased by triparanol but was much less sensitive to treatment with clofibrate when compared to monocyte and macrophage chemotaxis. Phagocytosis by human monocytes and guinea pig macrophages was also less sensitive to treatment with clofibrate unless a large quantity of material was to be ingested. These findings suggest that chemotaxis and, under some conditions, phagocytosis require new membrane synthesis and/or modification of the cholesterol: phospholipid ratios in cellular membrane.

The chemotaxis of leukocytes requires such metabolic processes as transmembrane fluxes of ions (1–3), glucose utilization (4, 5), reorganization of cytoskeletal elements (6, 7), and methylation mediated by *S*-adenosyl-L-methionine (8, 9). Alternatively, it has been established that neither DNA, RNA, nor protein synthesis is required for the chemotactic response (10). Since directed cellular migration is accompanied by dynamic alterations in the morphology and topography of the plasma membrane, we sought to investigate whether the metabolism of the major lipid components of membranes, phospholipids, and cholesterol (11) is required for the chemotactic and phagocytic responses of leukocytes. To this end, we studied the effects of the antilipidemic drugs, clofibrate (chlorophenoxyisobutyrate, Atromid-S) (12–14) and triparanol [1-*p*-(2-diethylaminoethoxy) phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl) ethanol (15, 16) on the chemotaxis and phagocytosis of leukocytes. These drugs have been shown to reduce triglycerides, phospholipids, and/or cholesterol synthesis when administered *in vivo* (12, 15).

MATERIALS AND METHODS

Chemicals. The sodium salt of clofibrate was obtained from Ayerst Laboratories, New York, N. Y. Triparanol was provided by Merrell-National Laboratories, Cincinnati, Ohio. Carrier-free $^{32}P_i$ as orthophosphoric acid, $Na^{51}CrO_4$ (200 to 300 Ci/g) and [^{14}C] sodium acetate (57 mCi/mmole) were obtained from New England Nuclear, Boston, Mass. [^{3}H] leucine (62 Ci/mmole) was purchased from Schwarz-Mann, Orangeburg, N. Y. Cycloheximide was obtained from Sigma Chemical Co., St. Louis, Mo.

Cell preparations. Human mononuclear leukocytes (MNL)[2] containing 20 to 25% monocytes and 75 to 80% lymphocytes were isolated from heparinized (10 units/ml) blood by sedimentation in Ficoll-Hypaque gradients (17, 18) and were washed twice in Gey's balanced salt solution containing 2% bovine serum albumin and 0.01 M HEPES buffer (GBSS), pH 7.0. The resulting MNL preparations were then suspended in the appropriate media for chemotaxis and phagocytosis.

Human polymorphonuclear leukocytes (PMN) (ca. 70% PMN, 30% MNL) were obtained by gravity sedimentation of whole blood in 3% dextran T500 (Pharmacia Fine Chemicals, Piscataway, N. J.) contained in normal saline (19). Guinea pig peritoneal macrophage preparations containing ca. 80% macrophages and 20% lymphocytes were obtained by lavage of the peritoneal cavities of animals that had been injected i.p. with 0.5% shellfish glycogen (Sigma Chemical Co.) 3 days earlier (20). The resulting cell suspensions were contained in the

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[2] Abbreviations used in this paper: MNL, mononuclear leukocytes; ^{51}CrShea, $Na^{51}CrO_4$-labeled antibody-coated sheep erythrocytes; fMet-Leu-Phe, *N*-Formyl-methionyl-leucyl-phenylalanine; GBSS, Gey's balanced salt solution; LDL, low density lipoprotein; s.c., subcutaneous; HEPES, *N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

appropriate buffers for the different functional and biochemical assays. Determination of monocytes and macrophages was made by nonspecific esterase stain and by differential counting on Wright-stained preparations.

Chemotaxis assays. The chemotaxis of human monocytes and guinea pig macrophages was assayed in modified Boyden chambers with 5 μ polycarbonate filters (Nuclepore) as previously described (18, 21). Chemoattractants used were 10^{-8} M formyl-Methionyl-Leucyl-Phenylalanine (fMet-Leu-Phe) (22, 23) for human monocytes and 3% (v/v) zymosan-activated guinea pig serum for guinea pig macrophages (24). Monocytes and macrophages were suspended in GBSS, pH 7.0, to a concentration of 1.5×10^6 monocytes/ml or 2.0×10^6 macrophages/ml, respectively. In experiments where the effects of clofibrate and triparanol on chemotaxis were tested, equimolar amounts of the drugs were placed on both sides of the chemotaxis chambers to eliminate artifacts due to concentration gradients. Chemotaxis was scored as the average number of migrating cells per oil immersion microscopic field (X1540) and in some instances is expressed as the percentage of normal chemotaxis $\frac{E}{C} \times 100$ where E is the chemotactic response of cells treated with drugs and C is the chemotactic response of cells incubated in buffer alone. In some experiments, blind well chemotaxis chambers were used, and the number of cells migrating through the filter into the bottom compartment of the chamber were counted with a hemacytometer. The chemotaxis of human PMN suspended to a concentration of 3.3×10^6 cells/ml in GBSS, pH 7.2, in response to 3% (v/v) zymosan activated human serum was quantified in modified Boyden chambers by using 5 μ nitrocellular filters as described previously (25).

Phagocytosis assay. The phagocytosis of $Na^{51}CrO_4$-labeled opsonized sheep erythrocytes (^{51}CrShEA) by human monocytes and guinea pig macrophages was measured as previously described (26).

Assay of in vivo macrophage accumulation. C3HeB/FeJ male mice aged 7 to 9 weeks (Jackson Laboratories, Bar Harbor, Maine) were injected i.p. with 2 ml of sterile saline containing 35 μg of purified phytohemagglutinin (PHA, Wellcome Research Laboratories, Research Triangle Park, N. C.). Forty-eight hours later, the mice were killed, the peritoneal cavities were lavaged with GBSS, and the total differential cell counts for the individual mice were performed as described (27). The effect of clofibrate on macrophage accumulation was tested by injecting various concentrations of the drug contained in 0.2 ml normal saline subcutaneously (s.c.) into the thighs of groups of four mice 14 hr before PHA administration.

Assay of cellular phospholipid synthesis. Total phospholipid synthesis in macrophages and PMN was assayed by measuring the incorporation of $^{32}P_i$ into organic solvent extractable radioactivity as previously described (21). Briefly, cells were incubated for 60 min at 37°C in a phosphate-free buffer containing 0.135 M NaCl, 4.5 mM KCL, 0.1% dextrose (w/v), 1.5 mM $MgCl_2$, 0.15 mM $CaCl_2$ and 0.01 M HEPES buffer, pH 7.0 (macrophages) or pH 7.2 (PMN) and 5 μCi of $^{32}P_i$. The cells were then pelleted, resuspended in 0.1 ml of 0.01 M sodium phosphate-buffered isotonic saline, pH 7.1 (PBS) and phospholipids were extracted with 0.5 ml of chloroform/methanol (2/1). This extraction procedure gives a phospholipid yield of 93% when using [^{14}C]-phosphatidylcholine as a marker. The organic phase was washed once with PBS after which 0.1 ml was transferred to a plastic scintillation vial and the radioacitvity was quantified by liquid scintillation spectrophotometry after the addition of 10 ml of Aquasol (New England Nuclear, Boston, Mass.).

Assay of cellular cholesterol synthesis. Cholesterol synthesis was measured by using modifications of previously described techniques (28, 29). Guinea pig macrophages were suspended to a concentration of 6×10^6 cells/ml in GBSS, to which were added 10 μCi/ml of [^{14}C]-acetate. After incubation for 1 hr at 37°C, the cells were pelleted and then dissolved in 0.5 ml of 50% KOH for 30 min at 37°C. After the addition of 2.5 ml of ethanol, the cellular lipids were saponified for 2 hr at 60°C. The nonsaponifiable lipids were extracted with 4 ml of hexane, which was then evaporated under N_2 to dryness. The lipids were then resuspended in 0.1 ml hexane and applied to a silica gel G plate along with 5 μg of unlabeled cholesterol (Analtech, Inc., Newark, Del.) and developed in a solvent system consisting of petroleum ether: diethyl ether: acetic acid (80:20:1). Cholesterol spots were visualized with iodine vapor, scraped into scintillation vials, and the radioactivity was counted by liquid scintillation spectroscopy after the addition of 10 ml Aquasol.

Protein synthesis. Protein synthesis was determined by measuring the incorporation of [^{3}H]-leucine into acid precipitable radioactivity (8). Guinea pig macrophages (1 ml) were incubated in GBSS, pH 7.0, at a concentration of 2×10^6 cells/ml with 5 μCi of [^{3}H] leucine for 30 min at 37°C. The cells were then pelleted, and proteins were precipitated with 1 ml of 1 N perchloric acid. The precipitates were washed one time with 70% ethanol and vacuum filtered onto Whatman GFC3 glass fiber filters. The filters were washed four times with ethanol, dried, and placed into plastic scintillation vials for quantification of radioactivity after the addition of 10 ml of Aquasol.

Preparation of low density lipoprotein (LDL). LDL was isolated from human plasma as previously described (30). Briefly, plasma was placed into Beckman cellulose nitrate tubes, overlayed with normal saline, and centrifuged at 105,000 × G for 24 hr. The very low density lipoprotein fraction at the top of the tube was removed with the aid of a tube cutter, and the density of the remaining plasma fraction was adjusted to 1.066 g/ml with sodium bromide. The plasma was then centrifuged at 105,000 × G for 24 hr after which time the LDL was recovered by using the tube cutter. The LDL was then dialyzed overnight against 0.15 M NaCl to remove contaminating sodium bromide and then stored in an 0°C water bath until used. LDL preparations were used within 5 days of isolation.

RESULTS

Effect of clofibrate on leukocyte chemotaxis and phospholipid synthesis. The antilipidemic drug, clofibrate, inhibits the synthesis of monoacylglycerols from sn-glycerol-3-phosphate and acetyl CoA, the formation of which is necessary for subsequent synthesis of diacylglycerols and of phospholipids (14). In addition, the drug had been shown to inhibit competitively the regulatory enzyme of cholesterol biosynthesis, hydroxymethylglutaryl CoA reductase (13, 31). To determine if the synthesis of these compounds is required for the chemotactic response of leukocytes, monocytes, macrophages, and PMN were incubated with various concentrations of clofibrate for 15 min at 37°C and tested for their chemotactic response. Human monocyte and guinea pig macrophage chemotaxis was markedly inhibited by incubation with clofibrate; complete inhibition of the response was obtained at a concentration of 10 mM for monocytes and of 4 mM for macrophages (Fig. 1). The inhibition of chemotaxis by clofibrate in monocytes and macrophages could not be explained by increased numbers of cells falling off of the underside of the chemotaxis filters since clofibrate did not increase the quantity of cells recovered from the lower wells of blind well chemotaxis chambers (data not shown). In addition, the viability of the cells as measured by trypan blue dye exlusion

was not altered in the presence of clofibrate during the time period in which chemotaxis was measured. The random migration of human monocytes responding to Gey's BSS alone was not affected by incubation with doses of clofibrate ranging from 1.0 mM to 10.0 mM. In contrast to the mononuclear phagocytes, the chemotaxis of human PMN was inhibited by only 30% at a concentration of 10 mM clofibrate (Fig. 1). The effect of the drug on total phospholipid synthesis in guinea pig macrophages and human PMN was also measured (Table I). Good correlation was observed for the doses of clofibrate that inhibited both chemotaxis and phospholipid synthesis in macrophages. The dose of clofibrate that inhibited 50% (ID_{50}) of the chemotactic response for macrophages was 2.0 mM and the ID_{50} concentration for inhibition of phospholiid synthesis was 2.5 mM. Furthermore, the doses of clofigrate that inhibited chemotaxis and phospholipid synthesis in macrophages were in the range of the published values for the ID_{50} for inhibition of monoacyglycerol formation (ID_{50} = 2.5 to 5.0 mM) (14) in rat liver. Human PMN had a lower baseline synthesis and no significant inhibition was observed until clofibrate concentrations of 5.0 mM or greater were used. There was no clearcut quantitative relationship between inhibition of phospholipid synthesis and chemotaxis in human neutrophils. Phospholipid synthesis was inhibited by 34% at a concentration of 5.0 mM clofibrate, whereas chemotaxis was unaffected. The effect of clofibrate on phospholipid synthesis in human monocytes was not attempted because of the heterogeneity of the MNL preparations.

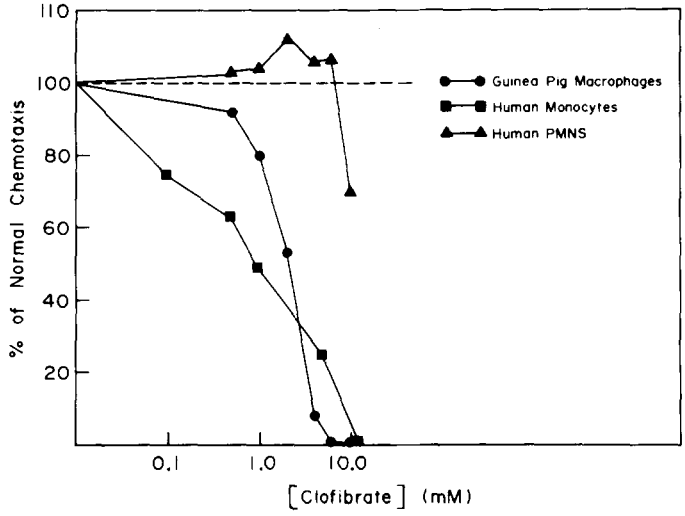

Figure 1. Effects of clofibrate on the chemotactic responsiveness of guinea pig macrophages, human monocytes and human polymorphonuclear leukocytes. The cell preparations were preincubated with buffer alone or containing clofibrate for 15 min at 37°C before deposition into the chemotaxis chamber. The incubation times at 37°C in the chemotaxis chambers were 90 min for human monocytes; 2 hr for guinea pig macrophages and 3 hr for human PMN. The chemotactic agents used were 3% (v/v) guinea pig or human zymosan activated serum, respectively, for guinea pig macrophages and human PMN and 10^{-8} M fMet-Leu-Phe for human monocytes. % of normal = $\frac{E}{C} \times 100$ where E is the response of cells incubated in the presence of drugs and C is the response of cells incubated in buffer alone.

TABLE I

Effects of clofibrate on phospholipid synthesis in guinea pig macrophages and human polymorphonuclear leukocytes (PMN)

Clofibrate Concentration[a]	$^{32}P_i$ Incorporated into Phospholipids	
	Macrophages	PMN
mM	*(fmol/10⁶ cells/hr)*[b]	
10.0	16.0 (75)[c]	20.3 (48)
6.0	21.6 (66)	
5.0		25.6 (34)
4.0	29.3 (54)	
2.5		31.3 (19)
2.0	40.1 (37)	
1.0	54.4 (14)	38.4 (1)
0.5	63.5 (0)	44.4 (−15)
0	63.4	38.7

[a] Cells were incubated with the indicated concentration of clofibrate for 15 min at 37°C before addition of 5 μCi of $^{32}P_i$.

[b] Cells were incubated for 1 hr at 37°C in the presence of 5 μCi $^{32}P_i$ and the total amount of $^{32}P_i$ incorporation into phospholipids was then determined.

[c] Numbers in parentheses indicate percent inhibition of $^{32}P_i$ incorporation in the presence of the indicated concentration of clofibrate.

Effects of triparanol on monocyte and macrophage chemotaxis and cholesterol synthesis. Triparanol has been shown to inhibit the formation of cholesterol (15, 16), but does not inhibit phospholipid synthesis in macrophages at concentrations ranging from 5 to 50 μM (data not shown). To determine if cholesterol is required for chemotaxis, guinea pig macrophages were preincubated for 15 min at 37°C with various concentrations of triparanol and then tested for *in vitro* chemotactic responsiveness and for the ability to incorporate [^{14}C] acetate into cholesterol. Figure 2 indicates that doses of triparanol ranging from 5 μM to 50 μM produced a dose-dependent inhibition of macrophage chemotaxis; as much as 98% inhibition of the response was noted at a concentration of 50 μM. Cholesterol synthesis in the macrophages was similarly depressed by incubation with the drug. There was good agreement between the ID_{50} for inhibition of chemotaxis (17.5 μM) and cholesterol synthesis (17.5 μM).

Human monocyte chemotaxis was also inhibited by triparanol when the cells were incubated in RPMI 1640 (Fig. 3*A*). Likewise, the chemotaxis of human PMN was inhibited by triparanol; the ID_{50} for inhibition was 15 μM (data not shown). It is well known that leukocytes derive the majority of their cholesterol for membrane synthesis *in vivo* from extracellular

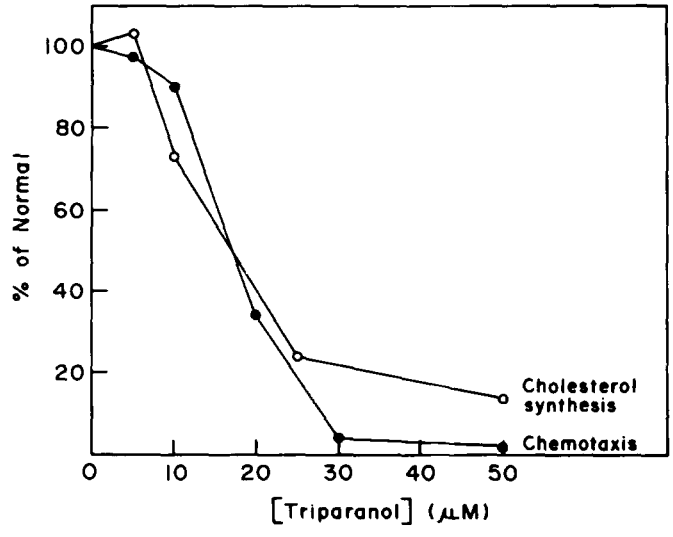

Figure 2. Effects of triparanol on guinea pig macrophage chemotaxis and cholesterol synthesis. Cells were preincubated with buffer alone or containing triparanol and then assayed for chemotactic responsiveness and the ability to incorporate [^{14}C] acetate into cholesterol. % of normal was calculated as indicated in the legend to Figure 1. The incorporation of [^{14}C] acetate into untreated macrophages was 8.6 pmol/10^6 cells/hr, assuming that one molecule of radioactive acetate is incorporated per cholesterol molecule synthesized.

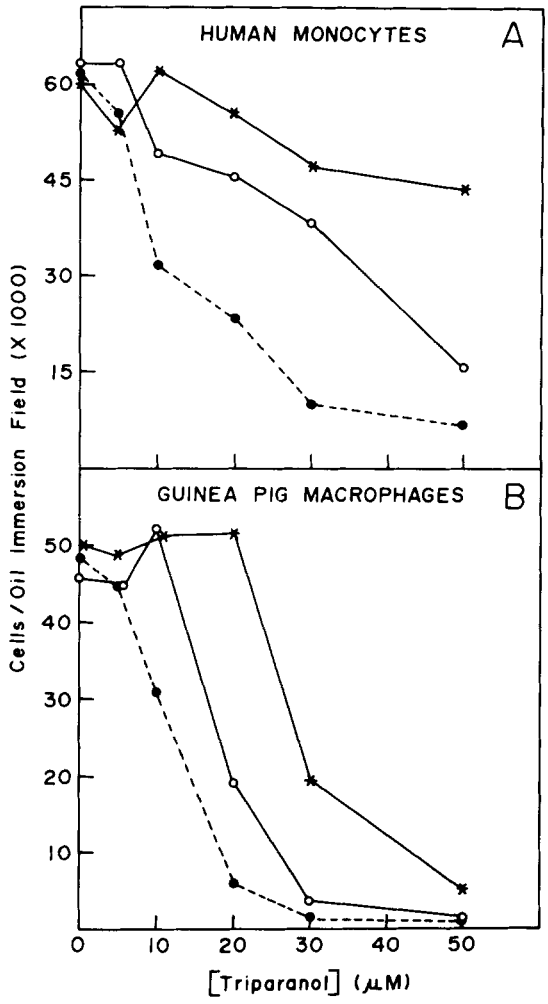

Figure 3. Effect of low-density lipoproteins on triparanol-induced inhibition of (*A*) human monocyte and (*B*) guinea pig macrophage chemotaxis. Cells were preincubated with various doses of triparanol for 15 min at 37°C in the presence or absence of 0.45 or 0.09 mg LDL protein/ml before quantification of chemotaxis. ●----●, triparanol alone; ○——○, triparanol plus 0.09 mg LDL protein/ml; *——*, triparanol plus 0.45 mg LDL protein/ml.

LDL (32, 33). In the absence of lipid-containing proteins in the external medium, however, cellular cholesterol synthesis is induced. To determine whether triparanol-induced inhibition of chemotaxis was due to curtailment of cholesterol synthesis, monocytes and macrophages were incubated with various concentrations of triparanol in the presence and absence of 0.45 or 0.09 mg LDL protein/ml. Figure 3*A* and *B* indicates that the dose-response curves for inhibition of chemotaxis by triparanol are shifted to the right in the presence of increasing concentrations of LDL in both human monocytes (Fig. 3*A*) and guinea pig macrophages (Fig. 3*B*). The ID_{50} for inhibition of monocyte chemotaxis by triparanol was 10 μM in the presence of RPMI alone and >50 μM in the presence of 0.45 mg LDL protein/ml. The ID_{50} for guinea pig macrophage chemotaxis was increased from 12.5 μM to 28 μM in the presence of 0.45 mg LDL protein/ml. The addition of 0.45 mg/ml of lipid-poor albumin did not alter the inhibitory effects of triparanol. The incubation of monocytes or macrophages with LDL in the absence of triparanol did not significantly alter their chemotactic responsiveness when compared with cells incubated in buffer alone. In addition, the presence of LDL did not alter the dose-response curves for inhibition of either monocyte or macrophage chemotaxis by clofibrate.

Macrophage chemotaxis during inhibition of protein synthesis. Since high concentrations of clofibrate (34) and triparanol (unpublished data) inhibit protein synthesis in addition to lipid synthesis, it was important to determine whether inhibition of protein synthesis alone altered the chemotactic response of guinea pig macrophages. Preparations of macrophages were incubated with various doses of the protein synthesis inhibitor, cycloheximide, for 30 min at 37°C and then tested for chemotactic responsiveness to activated serum and for the ability to incorporate (^3H) leucine into acid precipitable radioactivity (Fig. 4). The results indicate that protein synthesis can be inhibited by as much as 93% before significant inhibition of chemotaxis is observed (17%). These results are in agreement with our previously reported observation that protein synthesis appears not to be required for the chemotaxis of human monocytes (8).

Effect of clofibrate administration on in vivo macrophage accumulation in mice. To determine whether *in vivo* administration of clofibrate to mice was capable of altering the migratory response of macrophages to an inflammatory focus *in vivo*, groups of mice were injected with various doses of clofibrate and macrophage accumulation in response to an i.p. injection of PHA was measured as described in *Methods*. A dose of 14 mg/kg clofibrate, which corresponds to one-half the recommended daily dosage given to humans for curtailment of triglyceride synthesis, inhibited the accumulation of macrophages into the peritoneal cavity by 51% (Table II). Decreased inhibition of macrophage accumulation was observed when decreasing amounts of clofibrate were administered. The animals receiving the drug did not appear ill over the entire assay period.

Effect of clofibrate on human monocyte and guinea pig macrophage phagocytosis. Human monocytes and guinea pig macrophages were incubated for 15 min at 37°C with increasing doses of clofibrate and tested for their ability to phagocytosize ^{51}CrShEA. Figure 5 illustrates that in the absence of clofibrate, guinea pig macrophages phagocytosize about 3-fold more ^{51}CrShEA than do monocytes. Doses of clofibrate as high as 10 mM did not alter the amount of ^{51}CrShEA ingested by human monocytes. In contrast to monocytes, phagocytosis of opsonized erythrocytes by macrophages was inhibited by clofibrate, maximal inhibition being 52% at a dose of 10 mM. When guinea pig macrophages were incubated with lower ^{51}CrShEA:macrophage

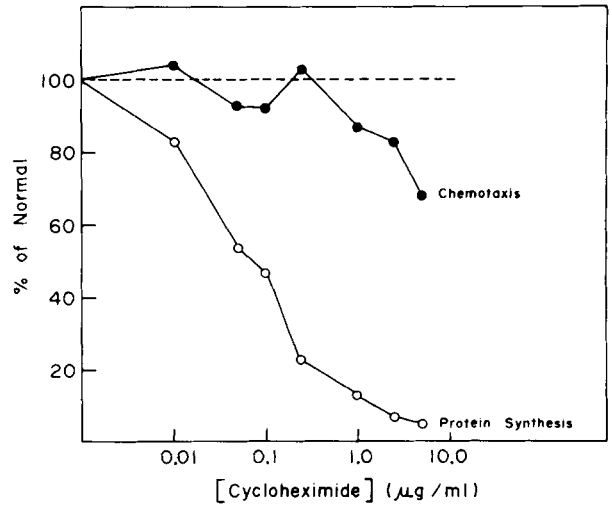

Figure 4. Effects of cycloheximide on guinea pig macrophage chemotaxis and protein synthesis. Cells were incubated with buffer alone or containing cycloheximide for 30 min at 37°C and then tested for chemotactic responsiveness and protein synthesis. % of normal was calculated as indicated in the legend to Figure 1.

TABLE II

Effect of clofibrate administration on macrophage accumulation in vivo

Clofibrate Administered[a]	No. of Macrophages (× 10^6) ± S.E.M. Harvested from the Peritoneum[b]	% Inhibition[c]
mg/kg		
None	5.1 ± 0.3	
14.0	2.5 ± 0.9	51
7.0	2.9 ± 0.7	43
2.8	3.4 ± 0.5	34
0.7	4.2 ± 0.5	18

[a] Groups of four mice were injected s.c. with the indicated amount of clofibrate contained in 0.2 ml saline.

[b] Mice were injected with 35 μg of PHA 14 hr after clofibrate administration and 48 hr later were killed and the peritoneal cavities lavaged. Total and differential cell counts were performed on the individual exudates. The numbers shown represent net macrophage accumulation; the number of resident macrophages present in the absence of PHA stimulation (ca. 2 × 10^6) were subtracted from the total recovered.

[c] % inhibition $= \left(1 - \frac{E}{C}\right) \times 100$ where E is the number of macrophages recovered from the peritoneal cavities of mice receiving clofibrate and C is the number of macrophages obtained from the peritoneal cavities of mice receiving no clofibrate injection.

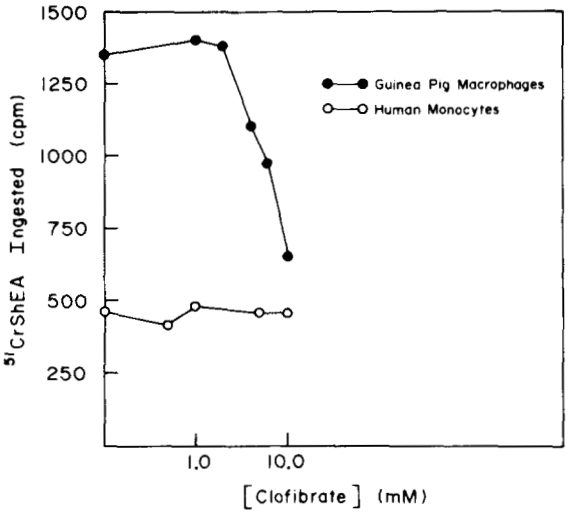

Figure 5. Effects of clofibrate in the phagocytosis of $Na^{51}CrO_4$ labeled opsonized sheep erythrocytes by human monocytes and guinea pig macrophages. Adherent cells were preincubated with buffer alone or containing clofibrate for 15 min at 37°C and then assayed for the ability to ingest ^{51}CrShEA after an additional incubation of 60 min at 37°C.

ratios, less cpm were ingested by the cells in the absence of clofibrate, and less inhibition of phagocytosis was observed in the presence of the drug (data not shown).

DISCUSSION

The chemotaxis of leukocytes is accompanied by deformation and polarization of the cellular membrane and thus might require alterations in membrane composition, microviscosity, and/or symmetry. Our previous reports have demonstrated that chemotactic agonists specifically inhibit phospholipid methylation in guinea pig macrophages, but not in chemotactically unresponsive cells (21). This finding indicated that leukocyte chemotaxis might require changes in cellular lipid composition and that indeed, the synthesis or utilization of the predominant membrane lipids, cholesterol and phospholipids, might be crucial for the chemotactic response. The present report indicates that an inhibitor of monoacylglycerol formation and of cholesterol synthesis, clofibrate (12–14), inhibits the chemotactic response of human monocytes and guinea pig macrophages. The ID_{50} for inhibition of chemotaxis (2 mM) corresponded to the ID_{50} for inhibition of phospholipid synthesis (2.5 mM) in guinea pig macrophages. The inhibition of chemotaxis caused by clofibrate could not be explained by decreased adherence of cells to the chemotaxis filter nor to altered cell viability. Another antilipidemic drug, triparanol, which curtails the formation of cholesterol (15, 16), also inhibited the chemotactic response of guinea pig macrophages. A mode of action of triparanol in other cell types is inhibition of the last step in cholesterol biosynthesis, the reduction of desmosterol (15, 16). Since the thin layer chromatography step used to assay cholesterol in the present study does not separate desmosterol from cholesterol, this site of inhibition of cholesterol synthesis by triparanol in macrophages must occur at an earlier step in the pathway. It is unlikely that this inhibition by triparanol is due to a toxic effect of the drug on the cells, e.g., via inhibition of energy production, since, at the concentrations of the drug used in these studies it had no effect on phospholipid synthesis. Furthermore, the ID_{50} concentration for inhibition of chemotaxis (17.5 μM) was the same as the ID_{50} for inhibition of cholesterol synthesis (17.5 μM). The chemotaxis of human monocytes and PMN was also inhibited by triparanol in the presence of buffers or medium that lacked lipid-containing proteins. It has been demonstrated that the synthesis of hydroxymethylglutaryl CoA reductase, the regulatory enzyme in the cholesterol biosynthetic pathway, is subject to control by extracellular LDL-cholesterol (32). In the presence of LDL-cholesterol, the cell turns off endogenous cholesterol synthesis, but in its absence, synthesis of the sterol is increased to compensate for the loss (32). Our findings indicate that a source of cholesterol is required for chemotaxis by macrophages, monocytes, and PMN. If no extracellular source of cholesterol is available, cholesterol synthesis by the cells is required. Guinea pig peritoneal macrophages appear to be more sensitive to the effects of inhibition of cholesterol synthesis in that the inhibition of chemotaxis by triparanol is not reversed by LDL to the same extent as that seen in human peripheral blood monocytes. These findings are to expected since peripheral blood cells are exposed to approximately 10 to 100 times more LDL *in vivo* than are peritoneal macrophages (32) and thus probably contain either more residual LDL on their cell surface or larger intracellular stores of cholesterol esters than do peritoneal cells. In contrast to the effects observed with triparanol, clofibrate-induced inhibition of chemotaxis could not be reversed in the presence of LDL, suggesting that the mode of action of clofibrate could not be attributed solely to inhibition of cholesterol synthesis, but must also be due to inhibition of phospholipid synthesis as well.

In support of the biologic relevance of the *in vitro* observations, the *in vivo* effect on inflammation of one of the drugs, clofibrate, was tested and was found to inhibit the accumulation of macrophages in response to an inflammatory stimulant. These findings suggested an anti-chemotactic action of the drug *in vivo*.

In contrast to its effects on mononuclear cells, clofibrate was far less effective in inhibiting chemotaxis in human PMN and there was not a clear-cut quantitative correlation between inhibition of phospholipid synthesis and chemotaxis. The reason for this observation is unknown at this time; however, it may be speculated that PMN already contain enough preformed phospholipids to execute a chemotactic response. De-

pression of cholesterol synthesis by triparanol did, however, inhibit the directed migration of human PMN, suggesting that an adequate supply of this lipid is required for chemotaxis.

Although clofibrate (34) and triparanol also have some inhibitory effects on protein synthesis, this property of the drugs is unlikely to be responsible for their inhibitory effects on chemotaxis, since almost complete inhibition of protein synthesis by cycloheximide barely affected the chemotactic response of the cells used in this study.

The phagocytic activity of human monocytes and of guinea pig macrophages was much less sensitive to inhibition by clofibrate than was chemotaxis. Phagocytosis by human monocytes was not affected by the drug, and guinea pig macrophage phagocytosis was inhibited by a maximum of 52% at 10 mM. At a concentration of 4 mM clofibrate, macrophage chemotaxis was inhibited by 92%, whereas phagocytosis was depressed by only 11%. Since human monocytes ingest approximately one-third less ^{51}CrShEA than do macrophages, and since we found that the inhibition of macrophage phagocytosis decreased as the ^{51}CrShEA:macrophage ratio was reduced, it may be that new membrane synthesis for phagocytosis is not required until a large amount of the plasma membrane of the cells is internalized along with the material that is to be ingested.

These data suggest that the directed migration of macrophages and monocytes requires the continued availability of new membrane lipids obtained either from endogenous synthesis or exogenous sources. The chemotaxis of PMN is less dependent on new phospholipid synthesis but appears to require a source of cholesterol. Chemotaxis appears to be more dependent upon optimum lipid levels than does phagocytosis unless the amount of material to be ingested is large compared to the total amount of available membrane. It is not yet clear how new lipid synthesis is needed for the chemotactic response, but studies using artificial lipid bilayers have shown that changes in the ratio of cholesterol:phosphatidylcholine greatly changes the biophysical properties of such membranes. For example, the liquid-crystalline state of phosphatidylcholine bilayers has been shown to be dependent upon hydrophobic interactions of cholesterol with the fatty acyl groups of phospholipids (11). In addition, studies have shown that the ion permeability of artificial membranes is dependent upon the cholesterol concentration, and that increased amounts of cholesterol can alter the activity of certain membrane transport proteins such as the Na^+, K^+-dependent ATPase (35). For chemotaxis to occur, appropriate cholesterol:phospholipid ratios may therefore be required for changes in leukocyte membrane viscosity, cellular ion permeability, receptor mobility, or the interaction of membrane with cytoskeletal elements within the cell.

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