We report that membrane CD14 (mCD14), a cell surface receptor found principally on leukocytes, can mediate the uptake and metabolism of extracellular phosphatidylinositol (PtdIns). mCD14 facilitates PtdIns internalization, targeting it to intracellular sites where, following stimulation with a calcium ionophore, it can be acted upon by cytosolic phospholipase A<sub>2</sub>. The [1<sup>4</sup>C]arachidonate released from mCD14-acquired [1<sup>4</sup>C]arachidonyl-PtdIns is either esterified to triacylglycerol and retained in the cell or secreted as free[14C]arachidonate. Although less than 10% of the arachidonate-derived lipids secreted from endogenous cellular stores are 5-lipoxygenase metabolites, over one-half of the secreted 14C-lipids derived from mCD14-acquired PtdIns are hydroxyeicosatetraenoic acids or leukotriene B<sub>4</sub>. mCD14 may allow these highly active blood cells to acquire and use extracellular PtdIns as a source of arachidonate for leukotriene synthesis.

Monocytes and neutrophils are highly differentiated, specialized cells that figure prominently in antimicrobial host defense. In addition to phagocytosing and killing bacterial and fungal cells, they play key roles in the innate immune process by which animals recognize invading microbes. They react to conserved microbial molecules, such as bacterial lipopolysaccharide, by releasing mediators that amplify and diversify the host inflammatory response. They are also major cellular sources of the leukotrienes, potent agonists for chemotaxis, smooth muscle contraction, and cell cycle stimulation (proliferation).

Microbial recognition is mediated by a multistep process in which mCD14, a 55-kDa glycosphosphatidylinositol (GPI)-anchored membrane protein, is thought to play an important role in binding bacterial lipopolysaccharide and other ligands to the plasma membrane. In addition to its key role in antimicrobial host defense, CD14 may be an important lipid transfer protein. Soluble CD14, which lacks the GPI anchor, can transfer several lipids to plasma lipoproteins or artificial lipid membranes (1, 2), and mCD14 is a receptor for phosphatidylinositol (PtdIns) and phosphatidylserine (3). We recently reported that binding PtdIns and phosphatidylserine to mCD14 is facilitated by bacterial lipopolysaccharide-binding protein (LBP), a molecule that, like PtdIns, is found in plasma (3). PtdIns is an important precursor of numerous signaling molecules (diacylglycerol, inositol 1,4,5-trisphosphate, and various phosphatidylinositides) and a potential source of arachidonic acid (the precursor of prostaglandins and leukotrienes). It is also incorporated into the glycosylphosphatidylinositol anchors used by many membrane proteins. We therefore asked whether extracellular, mCD14-bound PtdIns is utilized by the cells.

We show here that mCD14-bound extracellular PtdIns is rapidly internalized to an intracellular compartment where, upon cell activation, it can be acted upon by cytosolic phospholipase A<sub>2</sub>. The arachidonate released from PtdIns then has several fates, including conversion to HETEs and leukotriene B<sub>4</sub> (LTB<sub>4</sub>), but not to prostaglandins E<sub>2</sub> or F<sub>2</sub>alpha and secretion from the cell.

**EXPERIMENTAL PROCEDURES**

**Reagents**—1-Stearoyl-2-[14C]arachidonoyl-phosphatidylinositol (26.7 or 48 μCi/mmol), [1H]arachidonic acid (212 Ci/mmol), and 1-A-([3H]-inositol-2-[3H]phosphatidylcholine (6.6 Ci/mmol) were from NEN Life Science Products (Boston, MA). Arachidonic acid, prostaglandin E<sub>2</sub>, prostaglandin F<sub>2alpha</sub>, 5-hydroperoxyeicosatetraenoic acid, 5-hydroxy-6,8,11,14-eicosatetraenoic acid, diacylglycerol, triarachidonate, phorbol 12-myristate 13-acetate (PMA), and cholesteroyl oleate were purchased from Sigma. 1,2-Dihydroxyvitamin D<sub>3</sub> (VD<sub>3</sub>), leukotriene B<sub>4</sub>, MK-886, ibuprofen, and MAFP were from Biomol Research Laboratories (Plymouth Meeting, PA). Phosphatidylinositol-specific phospholipase C (Bacillus cereus) was from Roche Molecular Biochemicals. Newborn calf serum (Sigma) was delipidated by ultracentrifugation at r = 1.3 g/ml (KBr) and then dialyzed against normal saline. After delipidation, it contained 38 mg/liter cholesterol, 27 mg/liter triglyceride, and 5.2 g/liter protein.

**Preparation of Phospholipids**—Phospholipids were dried under argon and resuspended by sonication (400 watts, three pulses of 30 s each; Braunsonic 1510 sonicator; B. Braun, Melsungen AG) in serum-free RPMI 1640 medium. For most of the experiments, the resuspended lipids were then added to RPMI 1640 medium that contained 10% lipoprotein-poor newborn calf serum.

**Cells**—Human monocytic THP-1 cells (4) were obtained, cultured in RPMI 1640 medium with 10% fetal calf serum, and transfected to express wild-type human CD14 (CD14-GPI) as described previously (5). The human myeloid HL-60 cell line was obtained from the American Type Culture Collection and cultured in the same medium. Before the experiments, HL-60 cells were differentiated into monocyte-like cells by adding 100 nM VD<sub>3</sub> for 5 days. Human monocytes were kindly provided by David Wilkinson (The University of Texas Southwestern Medical Center, Dallas, TX). To label cellular lipids, [1H]arachidonic acid was added to the culture medium (0.5 μCi/ml) for 24 h before each experiment. HL-60 cells were labeled on the fourth day of VD<sub>3</sub> treatment.

**Lipid Analysis**—1 volume of cell suspension was added to 6 volumes of methanol/chloroform/acetonic acid (2:1:0.01, v/v). After incubating with frequent mixing for 1 h at room temperature, 3 volumes of 0.05 M KCl were added. After vortexing, 2 volumes of chloroform were added. The sample was vortexed again and then centrifugated for 5 min at ~7000 × g.
The organic phase was collected, and the sample was re-extracted with 3 volumes of chloroform. The combined organic phases were dried under argon, and the lipids were resuspended in chloroform/methanol (1:1).

The lipids were separated on Silica Gel G plates (J.T. Baker, Inc., Phillipsburg, NJ or Whatman, Inc., Clifton, NJ) using solvent A (chloroform/acetic acid/water, 10:4:3:2:1) or, for better separation of eicosanoids, solvent B (the upper (organic) phase of ethyl acetate/isooctane/acetic acid/water (55:25:10:50)) (6, 7). Bands were identified by fluorography or phosphorimager, and those that co-migrated with lipid standards were scraped and added to scintillation fluid for radioactivity counting. Typical Rf values of compounds separated in solvent B were as follows: (a) triglyceride, 0.83; (b) diacylglycerol, 0.73; (c) arachidonate, 0.66; (d) 5-hydroxyeicosatetraenoic acid, 0.59; (e) 5-hydroxy-6,8,11,14-eicosatetraenoic acid, 0.48; (f) LTB4, 0.39; (g) prostaglandin E2, 0.20; and (h) prostaglandin F2α, 0.10. Phospholipids remained at the origin. Lipids that migrated in a band with Rf = 0.45–0.49 were considered “HETE-like.”

Detection of Surface-accessible PtdIns—[3H]PtdIns, labeled to high specific activity in the inositol moiety, was used to measure PtdIns internalization by CD14-expressing THP-1 cells (8). A trace amount of [3H]PtdIns was mixed with [14C]PtdIns, dried under argon, and then resuspended in RPMI 1640 medium. The PtdIns concentration was estimated by counting the [14C]PtdIns because the [3H]PtdIns mass was negligible.

GPI-CD14 THP-1 cells were washed twice in serum-free RPMI 1640 medium, resuspended in either serum-free RPMI 1640 medium containing 0.3 mg/ml bovine serum albumin and 10 mM HEPES, pH 7.4, or SEBDAF buffer (20 mM HEPES, pH 7.4, 140 mM NaCl, 1 mM EDTA, 2 mM NaF, 300 μg/ml bovine serum albumin) to provide LBP, and 30 μM A23187 (secretory PLA2 inhibitor) (10) or MAFP (cytosolic PLA2 inhibitor) (11, 12) was added before the cells were primed with 100 nM LPS for 5–30 min at 37°C. After stimulation, the cells were chilled to provide LBP, and 30 μg/ml anti-CD14 mAb (60bca or 1H3) (3) or isotype-matched IgG was incubated with the cells for 10–20 min on ice before adding [3H]PtdIns. The cells were incubated with 3–5 μM [3H]PtdIns for 5–20 min at 37°C, primed with TNF-α (100 ng/ml) or PMA (100 nM) for 15 min at 37°C, and finally stimulated with 1–2 μg/ml A23187 for 5–30 min at 37°C. After stimulation, the cells were chilled and pelleted by centrifugation. Both the supernatant and cell pellet were immediately subjected to lipid extraction as described above.

To compare the fate of arachidonate derived from exogenous [14C]arachidonyl-PtdIns with that of endogenous cellular arachidonate, the cells were labeled overnight with [14C]arachidonate. PLA2 inhibitor LY311727 (secretory PLA2 inhibitor) (10) or MAFP (cytosolic PLA2 inhibitor) (11, 12) was added before the cells were primed with 100 nM PMA. After TLC analysis, the 3H and 14C in the arachidonate bands were counted to determine the arachidonate derived from exogenous [3H]PtdIns or endogenous [14C]PtdIns.

# FIG. 1. Effect of serum on PtdIns binding to mCD14. 1-Stearoyl-2-[14C]arachidonyl-PtdIns (2 μM) was incubated for 5 min at 37°C with either CD14-expressing or vector-transfected THP-1 cells (3 × 10⁵) in 50 μl of RPMI 1640 medium (with or without 10% (v/v) fetal bovine serum or lipoprotein-poor newborn calf serum). To pre-incubate the PtdIns with serum, 5 μM [14C]PtdIns was incubated with 25% serum in RPMI 1640 medium for 30 min at 37°C. 20 μl were then added to 30 μl of RPMI 1640 medium containing 3 × 10⁵ cells, and incubation was continued for 5 min. The cells were washed, and the cell-associated [14C]PtdIns was counted. Bars, the means ± S.E. of triplicates. The experiment was repeated with closely similar results.

incubated cells to obtain an estimate of the internalized [3H]PtdIns at each time point. Cell viability, as assessed by trypan blue exclusion, was >85% for both RPMI 1640 medium- and SEBDAF-incubated cells.

CD14/LBP-dependent Metabolism of PtdIns—GPI-CD14-transfected THP-1 cells or VD₃-differentiated HL-60 cells (3–5 × 10⁶) were suspended in RPMI 1640 medium containing 0.3 mg/ml bovine serum albumin and 10 mM HEPES, pH 7.4. When indicated, recombinant LBP-containing medium or control medium (10% of the total volume) (3) was used (final LBP concentration, approximately 0.07 μg/ml). To examine the role of GPI-CD14 in mediating arachidonate release, 5–10% lipoprotein-poor newborn calf serum was added to the medium to provide LBP, and 30 μg/ml anti-CD14 mAb (60bca or 1H3) (3) or isotype-matched IgG was incubated with the cells for 10–20 min on ice before adding [14C]PtdIns. The cells were incubated with 3–5 μM [14C]PtdIns for 5–20 min at 37°C, primed with TNF-α (100 ng/ml) or PMA (100 nM) for 15 min at 37°C, and finally stimulated with 1–2 μg/ml A23187 for 5–30 min at 37°C. After stimulation, the cells were chilled and pelleted by centrifugation. Both the supernatant and cell pellet were immediately subjected to lipid extraction as described above.

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# FIG. 2. mCD14 (LBP)-dependent PtdIns internalization. Vesicles containing phosphatidylinositol-[3H]inositol were incubated with mCD14-expressing THP-1 cells in RPMI 1640 medium for the indicated times at 37°C in the presence (●) or absence (○) of recombinant LBP. After washing to remove unbound PtdIns, the cells were treated with PI-PLC (0.2 unit/ml) on ice in 50 μl of RPMI 1640 medium for 120 min and then pelleted by centrifugation. The 3H in the supernatant and cells was counted. A, PI-PLC-resistant, cell-associated 3H. Solid line, live cells; dotted line, SEBDAF-treated cells. Each point is the average of duplicates. Bars, minimal and maximal values. B, internalized (surface inaccessible) PtdIns, expressed as a percentage of the total cell-bound 3H. Phosphatidyl-[3H]inositol became inaccessible to extracellular PI-PLC only when it was bound to live cells in the presence of LBP, suggesting energy-dependent, mCD14-mediated internalization. The experiment was repeated three times with similar results.

[CD14-dependent Phosphatidylinositol Metabolism]
Vesicles containing phosphatidyl-[3H]inositol were then added in the cytochalasin D or SEBDAF buffer for 30 min at 37 °C as indicated. mCD14-expressing THP-1 cells were incubated with metabolism of arachidonate derived from extracellular [14C]PtdIns. The cellular lipids were labeled with [3H]arachidonic acid as described above. When desired, inhibitors of 5-lipoxygenase activating protein (inhibitor MK-886) or cyclooxygenase (ibuprofen) were added to the cells for 10 min at 37 °C before adding [14C]PtdIns. After priming with 100 nM PMA for 15 min at 37 °C, calcium ionophore A23187 (1 μM) was added for 5 min. The lipids in the cell suspension were extracted, and the [14C]arachidonate was isolated by TLC and quantitated by scintillation counting. Each point represents the average of triplicates (± 1 S.E.). Control, vehicle only. Asterisks indicate groups that were different from all others at p < 0.05 (analysis of variance). The experiment was repeated with similar results. Both PMA and TNF-α primed the cells to release [14C]arachidonate from exogenous PtdIns.

FIG. 3. Cytochalasin D inhibits mCD14-dependent PtdIns internalization. mCD14-expressing THP-1 cells were incubated with cytochalasin D or SEBDAF buffer for 30 min at 37 °C as indicated. Vesicles containing phosphatidyl-[3H]inositol were then added in the presence or absence of recombinant LBP, and incubation was continued for 10 min at 37 °C. The cells were then chilled, washed, treated with PI-PLC, and pelleted by centrifugation. A, the PI-PLC-resistant, cell-associated [3H] dpm are shown. Cytochalasin D inhibited only LBP-dependent (mCD14-mediated) PtdIns internalization, reducing it almost to background (SEBDAF buffer) levels. The points are the average (range) of duplicates; the experiment was repeated with almost identical results. B, energy- and LBP-dependent PtdIns internalization, expressed as a percentage of the total cell-associated PtdIns (the values for SEBDAF-treated cells were subtracted from each point).

VD₃-differentiated HL-60 cells were used to study the specific metabolism of arachidonate derived from extracellular [14C]PtdIns. The cellular lipids were labeled with [3H]arachidonic acid as described above. When desired, inhibitors of 5-lipoxygenase activating protein (inhibitor MK-886) or cyclooxygenase (ibuprofen) were added to the cells for 10 min at 37 °C before adding [14C]PtdIns. After priming with 100 nM PMA for 15 min at 37 °C and stimulating with 2 μM A23187 for 30 min, the cells were pelleted, and lipids were extracted from the medium and cells. The lipids were separated by TLC using solvent B, and the bands that co-migrated with each standard were scraped and counted.

RESULTS

Specific binding of extracellular PtdIns to mCD14 was accomplished by adding PtdIns to mCD14-expressing cells in the presence of recombinant LBP or serum (3). Alternatively, in some experiments, PtdIns-mCD14 binding was inhibited by pre-incubating the cells with an anti-mCD14 mAb. When desired, PtdIns internalization was blocked by incubating the cells in SEBDAF buffer to deplete ATP stores; cell-associated PtdIns under these conditions was assumed to be bound to the plasma membrane so that its headgroup is no longer surface-exposed.

In preliminary experiments, we found that [14C]PtdIns binding to mCD14-expressing cells occurs in 10% fetal bovine serum and that binding is substantially higher when the serum has been depleted of lipoproteins (Fig. 1). Pre-incubating labeled PtdIns in normal serum greatly inhibited PtdIns binding to both mCD14-expressing and mock-transfected THP-1 cells; however, specific binding was still observed (in three independent experiments, 6- to 8-fold more PtdIns bound to mCD14-expressing cells than to mock-transfected cells). Pre-incubating [14C]PtdIns in lipoprotein-poor serum was less inhibitory, suggesting that much of the PtdIns added to normal serum binds to lipoproteins. For many experiments we used 10% lipoprotein-poor newborn calf serum to provide both a source of LBP and a more physiological medium for the studies. The serum proteins also bound secreted radiolabeled lipids and prevented their re-uptake by the cells.

**PtdIns Internalization**—We found that phosphatidyl-[3H]inositol that binds to mCD14 quickly becomes inaccessible to cleavage by extracellular PI-PLC. Loss of surface accessibility did not occur in metabolically poisoned cells, and PtdIns that bound to cell surface molecules other than mCD14 remained largely on the cell surface (Fig. 2). Loss of surface accessibility thus required both the expenditure of energy and binding to mCD14. Cytochalasin D inhibited mCD14-mediated PtdIns disappearance from the cell surface by approximately 80% (Fig. 3), suggesting that the actin cytoskeleton is involved in PtdIns internalization and arguing that the loss of surface accessibility is not simply due to the movement of PtdIns within the plasma membrane.

**Stimulus-dependent Release of Arachidonate from PtdIns**—When mCD14-expressing THP-1 cells that had bound 1-stearoyl-2-[14C]arachidonoyl-PtdIns for 20 min were treated with the calcium ionophore A23187 for 5 min, [14C]arachidonate was released into the medium (Fig. 4). Priming the cells for 15 min with PMA or TNF-α increased [14C]arachidonate release above that observed after A23187 treatment alone. When labeled PtdIns bound mCD14, arachidonate release occurred more promptly and to a greater extent than when it bound other
We next tested the ability of two selective phospholipase A2 inhibitors to block [14C]arachidonate release from mCD14-acquired exogenous PtdIns. MAFP, a selective inhibitor of cytosolic phospholipase A2 (12), effectively blocked [14C]arachidonate release from mCD14-acquired PtdIns (Fig. 6). In contrast, the highly selective inhibitor of secretory PLA2 (10), LY311727, had little effect. The ID50 for MAFP on arachidonate release from mCD14-acquired PtdIns (<2.5 μM) was slightly lower than its ID50 for the release of arachidonate from endogenous cellular stores (Fig. 6). Similar findings were obtained using another human leukocyte cell line, HL-60 (Fig. 7), and normal human peripheral blood mononuclear cells (data not shown). The data suggest that arachidonate release from mCD14-acquired exogenous PtdIns is mediated principally by cytosolic PLA2.

Metabolic Fate of PtdIns-derived Arachidonate—We then compared the fate of mCD14-acquired [14C]arachidonate (from extracellular PtdIns) with that of endogenously labeled [3H]arachidonate. Essentially no free [3H]arachidonate was found in unstimulated HL-60 cells (data not shown), and the free [3H]arachidonate released from [3H]arachidonate-labeled cellular lipids was almost entirely (>90%) secreted into the culture medium. Of the other [3H]-lipsids recovered in the medium, [3H]triglyceride was the most abundant (Fig. 7A), although it represented less than 10% of the total cellular [3H]triglyceride (data not shown). The ability of the 5-lipoxygenase activating protein inhibitor MK-886 to diminish the release of [3H]arachidonate, HETEs, or LTB4 (Fig. 7B, C, and D) was significantly inhibited by the selective inhibitor of secretory PLA2 (10), LY311727, had little effect. The ID50 for MAFP on arachidonate release from mCD14-acquired PtdIns (<2.5 μM) was slightly lower than its ID50 for the release of arachidonate from endogenous cellular stores (Fig. 6). Similar findings were obtained using another human leukocyte cell line, HL-60 (Fig. 7), and normal human peripheral blood mononuclear cells (data not shown). The data suggest that arachidonate release from mCD14-acquired exogenous PtdIns is mediated principally by cytosolic PLA2.

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mAb 60bca blocked the release of [14C]arachidonic acid from PtdIns as well as its conversion to leukotrienes, MK-886 inhibited [14C]arachidonyl-PtdIns conversion to HETEs and LTB4, and ibuprofen and LY311727 had no effect on these metabolic steps. The fates of the PtdIns-derived extracellular [14C]arachidonate and the [3H]arachidonate from the cellular pool were thus strikingly different. Whereas less than 10% of the secreted [3H] was found in 5-lipoxygenase metabolites, over half of the [14C] was in HETEs or LTB4. The findings suggest that mCD14 targets PtdIns-derived arachidonate to intracellular sites at which rapid and selective metabolism by 5-lipoxygenase can occur.

**DISCUSSION**

Although arachidonic acid is an essential fatty acid, it is highly active toward cells. Perhaps for this reason, essentially no free arachidonate is transported in the blood or found in cells. Instead, arachidonate is both carried and stored in phospholipids (14). Although alkenyl-arachidonyl phosphatidylethanolamine is the most arachidonate-enriched lipid in plasma, over 50% of the PtdIns molecules in plasma contain sn-2 arachidonate (15, 16). Normal plasma contains approximately 50–100 μM PtdIns, most of which is bound to lipoproteins (17–19). Little is known about its sources or about how it is taken up and utilized by cells.

In addition to providing a potential source of arachidonate to the cells that acquire it, PtdIns also is the precursor of several signaling phosphatidylinositides, diacylglycerol, and inositol 1,4,5-trisphosphate. Leukocytes can synthesize PtdIns from inositol and CDP-diacylglycerol, yet de novo PtdIns biosynthesis is thought to occur very slowly (20). Having a mechanism for acquiring extracellular PtdIns may thus endow mCD14-expressing cells with the ability to supplement their existing PtdIns and arachidonate stores. In keeping with this notion, incubation with liposomes containing PtdIns suppressed stimulus-induced PtdIns turnover (biosynthesis) in mouse macrophages (21).

Approximately one-fourth of the extracellular PtdIns that bound to mCD14 on THP-1 cells was rapidly internalized. Although the internalization mechanism is not known, another CD14-binding ligand, bacterial lipopolysaccharide, is largely internalized via non-coated plasma membrane invaginations and membrane-derived vesicles (5) with a time course that resembles that found here. Although we have not tried to study the fate of PtdIns that binds PtdIns receptors such as SR-BI and CD36 (22), extracellular PtdIns that bound to cell surface molecules other than MCO14 on THP-1 cells was internalized...
very slowly (Figs. 2B and 3A), and it was a relatively poor source of secreted arachidonate (Fig. 5, A, B, and D).

Platelets can also take up and metabolize extracellular phospholipids (23, 24). Although the uptake mechanism has not been defined, transfer of phosphatidylethanolamine from plasma lipoproteins to platelets increases after thrombin stimulation, and arachidonate derived from the exogenous phosphatidylethanolamine can be metabolized to HETE and thromboxane in a stimulus-dependent manner (23). There is also a precedent for cellular uptake of extracellular PtdIns. When extracellular PtdIns was delivered to Friend erythroleukemia cells via PtdIns transfer protein, it did not undergo conversion to phosphorylated metabolites, whereas spontaneously incorporated PtdIns was both phosphorylated to PtdIns-4-phosphate and deacylated (25). Finally, both free arachidonate and cholesterol-arachidonate can be taken up by cells. When resting monocytes take up low-density lipoprotein that contains cholesterol-[14]C]arachidonate, they use the arachidonate for prostaglandin synthesis (26). Upon stimulation with A23187, however, they convert some of the low-density lipoprotein-derived [14]C]arachidonate into LTB4 and leukotriene C4. Free [14]C]arachidonate can also be converted to leukotrienes after ionophore stimulation (26).

Compartmentalization of lipids in leukocyte membranes occurs in at least two known ways. First, lipid-rich domains exist in the plasma membrane; because mCD14 can be found in low-density fragments of the THP-1 plasma membrane (27), it is interesting to note that the conversion of PtdIns to PtdIns-4-phosphate is thought to occur in such domains (28). Whether mCD14-acquired PtdIns can be phosphorylated to PIP or PIP2 is presently unknown. Second, leukocytes can accumulate arachidonate-rich lipids into discrete intracellular organelles called lipid bodies (29, 30). However, PtdIns is only a minor component of these bodies, and we were unable to show that extracellular PtdIns or PtdIns-derived arachidonate co-localizes with lipid bodies in THP-1 cells, at least in short-term experiments.2

The data in Fig. 6 suggest that exogenous PtdIns is a substrate for cytosolic PLA2. Although this enzyme is thought to act at various sites within the cytosol, including the nuclear envelope and the inner surface of the plasma membrane, exogenous PtdIns would presumably have to flip within the membrane before it could be accessible to cytosolic PLA2 attack. Although the existence of such a "flipase" has been postulated by analogy to the aminophospholipid transporter, its presence has not been established.

Approximately 3% of the [14]C]arachidonic acid in mCD14-acquired [14]C]arachidonyl-PtdIns was cleaved after cell stimulation (data not shown). Similarly, 1–3% of the cellular [3]H]arachidonate was released from phospholipid storage after stimulation. If one assumes that 20% of the mCD14-acquired PtdIns is internalized (Fig. 2) and that it is the internalized fraction that is cleaved by PLA2, approximately 15% of the internalized PtdIns is attacked by PLA2. These calculations are obviously approximate, yet they suggest that the arachidonate in mCD14-acquired PtdIns may be more susceptible to PLA2 cleavage than the arachidonate in the endogenous cellular lipid pool.

Finding that the arachidonate contained in mCD14-acquired PtdIns undergoes stimulus-induced conversion to HETEs is consistent with previous observations on the fate of endogenous arachidonate in human neutrophils (15) and HL-60 cells (31). Differentiation of HL-60 cells with VD3 may actually enhance this conversion because VD3 treatment greatly increases 5-lipoxygenase activity in these cells (31). Because conversion of arachidonate to leukotrienes by 5-lipoxygenase and 5-lipoxygenase-activating protein is thought to occur on the nuclear membrane or in the endoplasmic reticulum (32, 33), our results suggest that extracellular PtdIns, or arachidonate derived from it, can move to these sites. Other mCD14-bound ligands have been reported to traffic within monocytes (5, 34).

Leukotriene biosynthesis occurs principally in myeloid cells, which require leukotrienes for effective phagocytosis and microbial killing (35, 36). In A23187-stimulated human neutrophils, the major source of arachidonate used for leukotriene biosynthesis is 1-ether-linked phospholipids (37), whereas endogenous PtdIns is a relatively minor source. In keeping with our results, Chilton (37) reported that a very small fraction of the arachidonate released from endogenous neutrophil lipids is converted to leukotrienes. In striking contrast, we found that over half of the 14C-lipid secreted by cells that had taken up [14]C]arachidonyl-PtdIns via mCD14 were HETE-like molecules or LTB4. Although these results do not reveal the contribution that exogenously acquired PtdIns makes to overall monocyte PtdIns content or to total signal-induced arachidonate or leukotriene release, they do indicate that the fate of the arachidonate derived from mCD14-acquired PtdIns is very different from that of the arachidonate in the bulk cellular pool. The arachidonate derived from extracellular PtdIns may be selectively targeted for leukotriene production. Therefore, it may not be coincidental that some of the major cells that produce leukotrienes (monocytes, macrophages, and neutrophils) are cells that constitutively express mCD14; mCD14 may provide these cells with a source of secreted arachidonate (Fig. 5, A, B, and 3). Monocytes, macrophages, and neutrophils are myeloid cells, and leukotriene biosynthesis in these cells occurs in the endoplasmic reticulum (32, 33), our results suggest that extracellular PtdIns is selectively targeted for leukotriene production. Therefore, leukotrienes may be biologically important for the myeloid cells that require leukotrienes for effective phagocytosis and microbial killing.

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