Phosphatidylinositol bind to Plasma Membrane CD14 and Can Prevent Monocyte Activation by Bacterial Lipopolysaccharide*

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Although bacterial lipopolysaccharides (LPS) and several other microbial agonists can bind to mCD14 (membrane CD14), a cell-surface receptor found principally on monocytes and neutrophils, host-derived mCD14 ligands are poorly defined. We report here that phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate, and other phosphatidylinositides can bind to mCD14. Phosphatidylserine (PS), another anionic glycoprophospholipid, binds to mCD14 with lower apparent affinity than does PtdIns. LPS-binding protein, a lipid transfer protein found in serum, facilitates both PS- and PtdIns-mCD14 binding. PtdIns binding to mCD14 can be blocked by anti-CD14 monoclonal antibodies that inhibit LPS-mCD14 binding, and PtdIns can inhibit both LPS-mCD14 binding and LPS-induced responses in monocytes. Serum-equilibrated PtdIns also binds to mCD14-expressing cells, raising the possibility that endogenous PtdIns may modulate cellular responses to LPS and other mCD14 ligands in vivo.

Responses of monocytes and neutrophils to low concentrations of lipopolysaccharide (LPS),1 a bacterial glycoprophospholipid, occur after LPS molecules bind to mCD14, a glycosylphosphatidylinositol (GPI)-anchored plasma membrane protein. mCD14 can bind exogenous molecules as diverse in structure as peptidoglycan (1), LPS (2, 3), lipoolarabinomannan (4), Borrelia lipopeptides (5), and several polysaccharides (6). Although endogenous (host-derived) mCD14 ligands are not so well understood, it is known that soluble CD14 (sCD14), the anchorless form of CD14, can function as a lipid transfer protein, moving both LPS and phospholipids to lipoproteins and other acceptors (7). A plasma protein, LPS-binding protein (LBP), greatly facilitates ligand binding to both soluble and membrane CD14 (8). LBP can also catalyze the transfer of various lipids to sCD14 (7) and to phospholipid-cholesterol artificial membranes (9, 10). Although its phospholipid-binding properties have not been detailed, mCD14 has been implicated in macrophage recognition of apoptotic cells (11) and an ability to bind anionic phospholipids on these cells has been inferred (11, 12).

Phosphatidylinositol (PtdIns), an anionic glycerophospholipid, is a precursor for several prominent intracellular signaling molecules. It is present in low (50–100 μM) concentrations in plasma (13–16), where it seems to be largely bound to lipoproteins (high density lipoprotein > low density lipoprotein) (17). The ability of macrophages to take up phospholipid-rich liposomes has been appreciated for many years (18, 19), and recent reports indicate that both PtdIns and PS can bind to members of the class B scavenger receptor family (SR-BI/CLA-1, CD36) (20, 21), which are found on monocytes and macrophages. Certain lymphocyte subsets are also known to bind both PtdIns- and PS-containing liposomes with high affinity (22), and there is evidence that extracellular PtdIns can modulate macrophage functions such as phagocytosis (23). A role for lipid transfer proteins in these phenomena has not been described.

We report here that PtdIns binds to mCD14 with specificity and saturability, that LBP is necessary for this interaction, and that PtdIns can both prevent LPS-mCD14 binding and inhibit LPS-induced cellular responses. The results suggest that PtdIns and LPS bind to the same or nearby sites on mCD14 and raise the possibility that PtdIns modulates mCD14-dependent responses to LPS and other mCD14 ligands in vivo.

EXPERIMENTAL PROCEDURES Lipids and Other Reagents—Nonradioactive lipids and related compounds were purchased from Serdary Laboratories (London, Ontario) (egg phosphatidylcholine), Avanti Polar Lipids (Alabaster, AL) (liver phosphatidylinositol, egg phosphatidylglycerol, brain phosphatidylserine), or Sigma (nonradioabeled inositides, bovine brain PtdIns(4)P). Synthetic dipalmitoylated phosphatidylinositides were purchased from Echelon Laboratories (Salt Lake City, UT), 1-Stearo-yl-2-[14C]arachidonoylphosphatidylinositol (28.7 μCi/mmol), 1-palmitoyl-2-[9,10-3H]palmitolipophosphatidylcholine (32.9 μCi/mmol), 1-palmitoyl-2-[1-14C]arachidonoylphosphatidylethanolamine (55 μCi/mmol), 1-palmitoyl-2-[2-14C]arachidonoylphosphatidylethanolamine (54.6 μCi/mmol), dipalmitoyl [glycerol-U-14C]phosphatic acid (144 μCi/mmol), and [9,10-3H]palmitic acid (35.9 μCi/mmol) were from NEN Life Science Products, and 1,2-dioleoylphosphatidyl-1[14C]serine (50 μCi/mmol) was from Amersham Pharmacia Biotech. Lipids were dried underargon and resuspended by sonication in 10 mM potassium phosphate, 140 mM NaCl, or SEBDAF (see below) containing 1 mg/ml bovine serum albumin (Sigma). Murine TNA-α was from Life Technologies, Inc.

Cells—Human monocyte THP-1 cells were cultured in RPMI 1640 with 10% fetal bovine serum (JRH Biosciences, Lenexa, KS), and where indicated, the cells were differentiated to express mCD14 by treatment with calcitriol (50 μM) for 3 days (24). Undifferentiated mCD14-expressing cells were produced by transfection (pRc/RSV-CD14-GPI) and selected for G418 resistance and CD14 expression by fluorescence-activated cell sorting (FacStar®PLUS, Becton Dickinson Immunocytometry, San Joe, CA). Control cells were transfected with the empty vector (pRc/RSV) and selected for G418 resistance (25). To minimize ligand internalization during binding studies, cells were incubated in SEBDAF buffer (20 mM HEPES, pH 7.4, 140 mM NaCl, 1 mM EDTA, 2 mM NaF, 300 μg/ml BSA, 10 mM Na2HPO4, 5 mM deoxyglucose) for 30 min at 37 °C (26, 27) before ligands were added. Incubation was then continued for 15 min before the cells were chilled, washed twice with PBS by centrifugation at 750 × g for 5 min at 4 °C, and resuspended in 100 μl

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‡ The abbreviations used are: LPS, lipopolysaccharide; mCD14, membrane CD14; sCD14, soluble CD14; LBP, LPS-binding protein; GPI, glycosylphosphatidylinositol; PtdIns, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TNF, tumor necrosis factor; BSA, bovine serum albumin; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; mAb, monoclonal antibody.
Anionic Glycerophospholipids Bind to Monocyte CD14

RESULTS AND DISCUSSION

Extracellular PtdIns and PS Bind to mCD14—We first used sonicated suspensions of several radiolabeled lipids to study mCD14-phospholipid binding. Radiolabeled lipids (1 μM) were added to metabolically inhibited THP-1 cells that expressed either recombinant mCD14 or no recombinant protein (vector-only control). LBP-containing medium or control medium was also added. As shown in Fig. 1, approximately 5-fold more PtdIns bound to the cells when both mCD14 and LBP were present (5.6 ± 1.8-fold increase above control, n = 12). LBP did not increase PtdIns binding to cells that lacked mCD14. Of the other phospholipids tested, only PS was bound by the cells in a mCD14- and LBP-dependent fashion (2.3 ± 0.5-fold increase above control, n = 4). PC and PE that had different acyl chain compositions (dipalmitoyl versus 1-palmitoyl, 2-arachidonoyl) gave virtually identical results (not shown).

PtdIns Binding to mCD14 Is Saturable and Specific—Since the greatest mCD14-dependent augmentation in phospholipid binding was found using PtdIns, we chose to study this interaction in detail. To estimate the apparent affinity with which mCD14 binds PtdIns, mCD14-expressing THP-1 cells were again incubated in SEBDAF buffer to inhibit ligand internalization. Increasing concentrations of [14C]PtdIns were added for 10 min in the presence or absence of LBP (0.07 μg/ml or ~1 μM). As shown in Fig. 2A, LBP-dependent PtdIns binding was saturable, Half-maximal binding occurred at approximately 0.5–1.0 μM PtdIns.

To probe the basis for PtdIns-mCD14 binding in more detail, we tested the ability of a series of nonradioactive structures to inhibit this interaction. In 10-fold molar excess, liver-derived PtdIns inhibited [14C]PtdIns-mCD14 binding by ≥90%, whereas egg PC and brain PS were not inhibitory (Fig. 2B and data not shown). When we then studied the inhibitory potency of several soluble headgroup analogs, we found that [14C]PtdIns-mCD14 binding could be blocked (≥80%) by a 100-fold excess of 1,2-glycerophospho-di-my-o-inositol-4-monophosphate, but not by di-my-o-inositol, dib-my-o-inositol-1-monophosphate, di-my-o-inositol-1,4-bisphosphate, 1,2-glycerophospho-di-my-o-inositol, or glycerophosphoinositol-4,5-phosphate (<40% inhibition, data not shown). Since glycerophosphoinositol 4-phosphate inhibited while inositol-1,4-phosphate did not, these results implicate the glycerol moiety as one determinant of binding specificity. To test the role of headgroup structure in the context of uniform acylation of the glycerol backbone, we then compared a series of dipalmitoylated, synthetic PtdIns derivatives as inhibitors (Fig. 2B). We found that PtdIns(3)P, PtdIns(4)P, PtdIns(3,4)P₂, and PtdIns(4,5)P₂ were potent inhibitors of [14C]PtdIns-mCD14 binding (>90% inhibition by a 10-fold molar excess of inhibitor), whereas PtdIns(4)P, PtdIns(3,5)P₂, and PtdIns(3,4,5)P₃ were less inhibitory. Taken together, these results suggest that binding specificity is conferred by the glycerophosphoinositol headgroup and that phosphorylation at 3-OH or 4-OH favors binding. The acylated structures were more potent inhibitors than the nonacylated ones, in keeping...
with a role for hydrophobic interactions in either presentation of the headgroup or in binding to mCD14.

PtdIns Binds to mCD14 in the Presence of Normal Serum; LBP Is the Major Serum PtdIns Transfer Protein—Monocytes and neutrophils, the principal mCD14-expressing cells, normally are found in blood. Normal human serum contains approximately 50–100 μM PtdIns (13–15) and LBP (~7 μg/ml (31, 32)). Accordingly, we asked whether PtdIns can bind to mCD14 in the presence of serum. As shown in Fig. 3A, when [3H]PtdIns was added to cells in the presence of 10% human serum, it bound rapidly to CD14-expressing cells but not to control cells; after incubation for 5 min at 37 °C, mCD14-expressing cells bound 5.3 ± 1.6-fold (n = 3 experiments) more PtdIns than control cells. We next allowed trace amounts (approximately 1–2 pmol of [3H]PtdIns) to equilibrate with normal human serum (50–100 μM), and incubated for 5 min at 37 °C, mCD14-expressing cells bound 50–100-fold more than control cells. Normal human serum was the source of the transfer protein (Fig. 4A). Therefore, a monoclonal antibody to human LBP greatly inhibited PtdIns-mCD14 binding when 10% human serum was the source of the transfer protein (Fig. 4B).

These findings strongly suggest that LBP is the major transfer protein in serum that facilitates PtdIns-mCD14 binding.

PtdIns Inhibits LPS Binding to mCD14 and Blocks LPS Signaling.—We next asked whether PtdIns could prevent LPS binding to mCD14. [3H]LPS (5 ng/ml) was added to CD14-expressing THP-1 cells in the presence of a constant amount of LBP (0.07 μg/ml) and graded concentrations of nonradioactive PtdIns, PtdIns(4)P, or PC. As shown in Fig. 5A, PtdIns and PtdIns(4)P inhibited [3H]LPS uptake during incubation at 37 °C for 2 h. (Inhibition was incomplete since there was substantial CD14-independent uptake of LPS and phosphatidylinositides by live cells during the 2 h period). In the same experiment, we tested the ability of PtdIns to block LPS-induced IL-8 production. Fig. 5B shows that PtdIns and PtdIns(4)P inhibited IL-8 release, whereas PC did not. Similar experiments were also performed using freshly prepared human peripheral blood mononuclear cells. PtdIns and PtdIns(4)P blocked [3H]LPS cell binding (not shown) and a 5 μM concentration of either inhibitor reduced LPS-induced cell responses by 10–100-fold (shift in dose-response curve, Fig. 5C), whereas PC had no effect. In keeping with the above findings, PtdIns also inhibited LPS-induced translocation of NF-κB to the nucleus (Fig. 6). The inhibitory effect of PtdIns was specific for LPS, since PtdIns did not inhibit TNF-α-induced IL-8 production or NF-κB translocation in THP-1 cells.
IL-8 release from human mononuclear cells (Fig. 6 and data not shown). At least as measured in these assays, neither PtdIns nor PtdIns(4)P was stimulatory.

To address the possibility that LPS was sequestered by interacting with PtdIns in the medium, we incubated THP-1 cells with LBP (0.07 μg/ml) and PtdIns, or PC (100 μM) LBP for 5 min, washed to remove unbound lipid, and added [3H]LPS (4 ng/ml) with fresh LBP for 5 min at 37 °C. Preincubation with PtdIns decreased LBP-dependent LPS binding by ≥25%, whereas preincubation with PC was ineffective. This result indicates that coinubation of PtdIns and LPS was not necessary for PtdIns to inhibit LPS-mCD14 binding. Nonetheless, since LPS can evidently insert into liposomes that contain PtdIns (9), this process may influence the outcome of longer term experiments such as those shown in Fig. 5.

Monoclonal Antibodies That Block LPS-mCD14 Binding Also Block PtdIns-mCD14 Binding—We next asked whether mAbs known to inhibit (or not to inhibit) LPS-mCD14 binding would also inhibit PtdIns-mCD14 binding. As shown in Fig. 7, inhibition of LPS-mCD14 binding by a panel of anti-CD14 mAbs.
mAbs closely paralleled inhibition of PtdIns-mCD14 binding. These data suggest that PtdIns and LPS bind to the same or nearby regions of mCD14.

As noted above, binding of mCD14 by LPS, PS, and phosphatidylsodiums, like most other lipids, is greatly facilitated by LBP. To what extent is the specificity of phospholipid-mCD14 binding actually conferred by LBP? We took several approaches to this question. Doubling the LBP concentration in the reaction mixtures did not raise the saturating concentration of PtdIns (see Fig. 2; data not shown), and a 100-fold excess of phospholipids previously shown to interact with LBP (PC, PE) (7) did not block [14C]PtdIns-mCD14 binding. We also found that inhibition of [14C]PtdIns-mCD14 binding occurred when the cells were incubated with LBP and 100 μg liver PtdIns or brain PtdIns(4)P, washed to remove unbound phosphatidylsodium, and reincubated with [14C]PtdIns and a fresh supply of LBP (25% inhibition of [14C]PtdIns-mCD14 binding by PtdIns, 60% inhibition by PtdIns(4)P, no inhibition by PC; two independent experiments, data not shown). PtdIns, PtdIns(4)P, and [14C]PtdIns thus compete for binding a cell-surface receptor, most likely mCD14. Although LBP may also contribute to the pattern of ligand-mCD14 binding observed, these observations suggest that the binding specificity observed is determined principally by mCD14.

Our experiments focused on phosphatidylsodium-mCD14 interactions, yet we also found that PS can bind to mCD14 in an LBP-dependent manner (Fig. 1). Although in our assay system mCD14 binds PS with lower apparent affinity than it binds PtdIns (a 10-fold excess of PS did not inhibit PtdIns-mCD14 binding), its ability to bind PS may account for its role in recognizing apoptotic cells (11, 33). Our results suggest that LBP should enhance this interaction and raise the possibility that mCD14 may also bind PtdIns exposed on apoptotic cells or on membrane microvesicles such as those released in response to various inflammatory stimuli (34).

We thus suggest that phosphatidylsodiums and PS are more likely than the other phospholipids tested to be natural mCD14 ligands. Its ability to compete with LPS for binding mCD14 further suggests that PtdIns, the most abundant anionic phospholipid in serum, could modulate cellular responses to low concentrations of LPS (and possibly other exogenous agonists that bind mCD14) in vivo. A great excess of PtdIns is required to inhibit LPS binding to mCD14, however, and very little is known about the movement of PtdIns between cells, lipoproteins, and other carrier molecules in blood. mCD14-bound phosphatidylinositol may also be translocated into the cell to participate in signaling reactions, to provide a source of intracellular arachidonate (35), or for other functions (23, 36). Identifying mCD14 as a phosphatidylsodium and PS receptor raises intriguing questions about the possible functions of a LBP-dependent mechanism by which myeloid cells bind these extracellular phospholipids.

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