Neutralization and Transfer of Lipopolysaccharide by Phospholipid Transfer Protein*

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Phospholipid transfer protein (PLTP) and lipopolysaccharide-binding protein (LBP) are lipid transfer proteins found in human plasma. PLTP shares 24% sequence similarity with LBP. PLTP mediates the transfer and exchange of phospholipids between lipoprotein particles, whereas LBP transfers bacterial lipopolysaccharide (LPS) either to lipoprotein particles or to CD14, a soluble and cell-surface receptor for LPS. We asked whether PLTP could interact with LPS and mediate the transfer of LPS to lipoproteins or to CD14. PLTP was able to bind and neutralize LPS; incubation of LPS with purified recombinant PLTP (rPLTP) resulted in the inhibition of the ability of LPS to stimulate adhesion responses of neutrophils, and addition of rPLTP to blood inhibited cytokine production in response to LPS. Transfer of LPS by rPLTP was examined using fluorescence quenching experiments and native gel electrophoresis. The results suggested that rPLTP was able to mediate the exchange of LPS between micelles and the transfer of LPS to reconstituted HDL particles, but it did not transfer LPS to CD14. Consonant with these findings, rPLTP did not mediate CD14-dependent adhesion responses of neutrophils to LPS. These results suggest that while PLTP and LBP both bind and transfer LPS, PLTP is unable to transfer LPS to CD14 and thus does not mediate responses of cells to LPS.

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1 The abbreviations used are: LPS, lipopolysaccharide; BPI, bacterial/cidal/permeability-increasing protein; BODIPY, boron dipyrromethene difluoride; CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; LBP, lipopolysaccharide-binding protein; mCD14, membrane-bound CD14; PBS, phosphate-buffered saline; PD-EDTA, PBS without divalent cations, with 1 mM EDTA; PLTP, phospholipid transfer protein; apo, apolipoprotein; HDL, high density lipoprotein; R-HDL, reconstituted HDL; sCD14, soluble CD14; PAGE, polyacrylamide gel electrophoresis; IL, interleukin.
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Purification of Plasma-derived PLTP—PLTP was purified to homogeneity from human plasma using dextran sulfate/CaCl₂ precipitation and a combination of phenyl-Sepharose, CM-cellulose, DEAE-cellulose, heparin-Sepharose, and hydroxyapatite chromatography as described (10).

Preparation of Anti-PLTP Immunoglobulin—Polyclonal antibody was raised in a goat by injecting approximately 300 μg of rPLTP with an equal volume of Freund's adjuvant. The goat was immunized once after 3 weeks with the same dose of rPLTP and bled at 3–4-week intervals. Anti-rPLTP IgG was prepared following the procedure of McKinnny and Parkinson (12). A control goat IgG (goat anti-rabbit IgG) was from Incstar (Stillwater, MN).

Reconstitution of HDL Particles—R-HDL was prepared by the sodium cholate dialysis method as described previously (13). Briefly, purified apolipoprotein A-I (apoA-I), was mixed with egg phosphatidylcholine and cholate at a molar ratio of 80:1:80 (phosphatidylcholine:apoA-I:cholate), and cholate was removed with extensive dialysis against PD-EDTA (Dulbecco's PBS lacking Ca²⁺ and Mg²⁺ with 1 mM EDTA) containing 0.01% sodium azide. For gel electrophoresis, a homogeneous population of particles was further purified by gel filtration over two Superose 6 columns (Pharmacia, Uppsala, Sweden) in series. Final preparations were stored in PD-EDTA with 0.01% azide at 4 °C. All concentration values for R-HDL particles are expressed as the equivalent concentration of apoA-I in μg/ml.

Stimulation of Neutrophils by LPS—To assess the biologic activity of LPS, a measured adhesion of human neutrophils to fibrinogen-coated surfaces as described (2, 4). In this assay, stimulation of neutrophil adhesion by LPS is dependent on the presence of LBP or plasma. Briefly, mixtures containing LPS were diluted in APBS (Dulbecco's PBS with Ca²⁺ and Mg²⁺, 0.5% human serum albumin) to the concentrations indicated, yielding a final volume of 50 μl. 10 μl of freshly isolated neutrophils (2 × 10⁶ cells/ml) in HAP (Dulbecco's PBS with 0.5 unit/ml aprotinin, 0.05% human serum albumin, 3 mM glucose) fluorescently labeled with 5-(and 6-)carboxyfluorescein diacetate, succinimidyl ester as described (14) were added and incubated for 10 min at 37 °C to stimulate the cells. Neutrophils were then washed into HAP and added to a 72-well Terasaki plate precoated with fibrinogen. After 15 min at 37 °C, adherence of neutrophils to the plate was quantitated. Fluorescence in each well was measured using a Cytofluor 2300 (Millipore Corp.) as a way of quantitating the total number of cells per well. The plate was then washed and fluorescence was measured again. Binding is expressed as the percentage of cells remaining in the well after the washing step (adhesion (%)). Donor to donor variation in maximal responses prohibited averaging results of separate experiments, but the pattern of responses was highly reproducible.

Neutralization of LPS—Neutralization of LPS was measured as the loss of its ability to stimulate adhesion of neutrophils to fibrinogen, as described previously (2). Briefly, LPS was incubated alone or with PLTP in 50 μl of APBS. After incubation for the indicated intervals at 37 °C, the amount of biologically active LPS remaining in the tube was assessed. Neutrophils were resuspended in 50 μl of APBS and 50 μl of the suspension was applied to each well of a 96-well plate. After incubation of LPS alone or with medium from untransfected cells for 30 min, the plate was washed to remove unbound LPS, and fluorescence was measured. The amount of fluorescence was determined by comparing the amount of fluorescence measured after incubation with LPS alone with the amount of fluorescence measured after incubation with LPS and neutralizing agent. Neutralization was expressed as the percentage of cells remaining in the well after the washing step (adhesion (%)). Donor to donor variation in maximal responses prevented averaging results of separate experiments, but the pattern of responses was highly reproducible.

Stimulation of Interleukin 6 (IL-6) Production in Whole Blood—Heparinized blood was obtained by venipuncture from healthy human volunteers. Samples were incubated for 5 h at 37 °C in a 5% CO₂ environment, then centrifuged for 3 min at 1500 × g. Plasma was collected and stored at −20 °C until assayed for IL-6 by enzyme-linked immunosorbent assay. IL-6 levels were measured using a commercially available human IL-6 ELISA kit (Central Laboratories of the Netherlands Red Cross Transfusion Service, Amsterdam, The Netherlands), modified for use with smaller volumes of reagents and for detection of IL-6 with a fluorescent alkaline phosphatase substrate as described (15).

Electrophoresis—[³¹]P LPS was sonicated for 1 min and incubated at 37 °C for various times with R-HDL and other proteins as indicated in the figure legends. J ust before electrophoresis, 1/2 volume of a loading buffer containing 0.005% bromphenol blue and 20% glycerol in PBS was added to each sample. The samples were run in continuous buffer (pH 8.6) with 0.05% Triton X-100, 0.1% Triton X-100, 3% N,N,N',N'-tetramethyl-ethylene diamine, 0.025% sodium dodecylsulfate (Novex, San Diego, CA) at 100–150 V for 2–3 h in a running buffer containing 192 mM glycine, 24 mM Tris, pH 8.3. After electrophoresis, the gels were soaked in ENHANCE (DuPont NEN) for 45 min, washed three times for 15 min in ddH₂O, dried, and exposed to Kodak XAR film for 5–22 days.

Labeling of LPS with BODIPY and Measurement of Fluorescence—LPS from 5, 8-methyl-desoxypodophyllotoxin (Camptothecin) was labeled with the fluorophore BODIPY FL C₃ as described (16), using a kit from Molecular Probes, Inc. (Eugene, OR). LPS self-associates in aqueous solution, forming high molecular weight complexes (17) which we call micelles, although other physical structures have been suggested by physical data (18). The high local concentration of BODIPY in BODIPY-LPS micelles results in self-quenching of the fluorophore (16). The addition of detergent to BODIPY-LPS, or the binding of BODIPY-LPS to a protein such as CD14 physically separates the BODIPY molecules, resulting in a rise in fluorescence (16), a process referred to as dequenching. Thus, fluorescently labeled LPS can be used to study binding and transfer of LPS in real time (16, 19).

Fluorescence of BODIPY-LPS was measured as described (16) with an SLM-SF500C spectrofluorimeter (SLM Instruments, Urbana, IL). Time-dependent changes in fluorescence were measured by diluting BODIPY-LPS and other reagents in PD-EDTA or in PBS (with Ca²⁺ and Mg²⁺) and quickly mixing in a submicrocuvette (Starna Cells Inc., Atascadero, CA). Fluorescence emission at 518 nm was digitally recorded over time, with excitation at 485 nm.

RESULTS

rPLTP Neutralizes LPS—To investigate whether PLTP could modulate the biologic activity of LPS, we measured the effect of PLTP on the LPS-induced adhesion of neutrophils to fibrinogen-coated surfaces. Incubation of LPS with conditioned medium from cells transfected with the cDNA for human PLTP (rPLTP medium) resulted in the rapid neutralization of the activity of LPS (Fig. 1A). Neutralization was not seen upon incubation of LPS alone or with medium from untransfected cells (control medium) (Fig. 1A). Control medium failed to neutralize LPS when added at up to 20 times the concentration of rPLTP medium needed to neutralize LPS (not shown). To confirm that the neutralizing activity in rPLTP medium was due to rPLTP, we examined the ability of anti-rPLTP IgG to inhibit neutralization of LPS by rPLTP medium. Anti-PLTP IgG caused a dose-dependent inhibition of neutralization of LPS by rPLTP medium, whereas a control IgG had no effect (Fig. 1B).

To further confirm that PLTP neutralizes LPS and to determine the amount of LPS transferred by PLTP (rPLTP), we measured the effect of purified recombinant PLTP (rPLTP-His) on LPS-induced responses. In a 30-min incubation of rPLTP-His with LPS, complete neutralization of 10 ng/ml LPS was seen with 3 μg/ml rPLTP-His (Fig. 2), and 1 μg/ml rPLTP-His neutralized >90% of the activity of LPS (determined by comparing Fig. 2 with a dose-response curve for LPS from the same experiment; not shown). PLTP purified from human plasma also neutralized LPS, with a time course and dose dependence similar to that seen with purified rPLTP-His (Fig. 3).

rPLTP-His Causes Dequenching of BODIPY-LPS—The neutralization of LPS upon incubation with PLTP suggested a physical interaction of PLTP with LPS. To detect such an interaction, we measured the effect of rPLTP-His on the fluorescence of LPS labeled with the fluorophore BODIPY (BODIPY-LPS). In previous studies, incubating LBP with fluorescently labeled LPS resulted in fluorescence dequenching (an increase in fluorescence) (16, 19). Consistent with these published results, we found that the incubation of equimolar rLBP with BODIPY-LPS caused an increase in fluorescence, reaching three times the fluorescence of BODIPY-LPS alone in 200 s (Fig. 3A, curves 1 and 4). The same concentration of rPLTP-His also caused dequenching of BODIPY-LPS, to at least twice background levels (Fig. 3A, curve 3). With rLBP, there was a rapid phase of dequenching of BODIPY-LPS for the first ~20 s which was not seen with rPLTP-His, but, after this time, the two proteins had similar effects on fluorescence. Dequenching of BODIPY-LPS was not a general property of proteins, since human serum albumin caused only a slight increase in the fluorescence of BODIPY-LPS (Fig. 3A, curve 2). Because rPLTP-His was added in a low-pH buffer, we incubated BODIPY-LPS with the same amount of this buffer as was added with rPLTP-His, as a control. The rise of fluorescence...
caused by rPLTP-His was not seen with low-pH buffer alone (not shown), demonstrating that this effect of rPLTP-His was not due to changes in pH.

The change in fluorescence of BODIPY-LPS caused by rPLTP-His strongly suggests that there is a physical interaction of PLTP with LPS. LBP is known to bind LPS (19-21), and LBP and PLTP have similar abilities to cause dequenching of BODIPY-LPS (Fig. 3A). Additionally, several proteins have been identified which neutralize LPS by binding it (see "Discussion"), and PLTP shares this ability to neutralize LPS (Figs. 1 and 2). Taken together, these results suggest that PLTP binds LPS, and neutralization of LPS occurs as a result of this binding.

The effect of rPLTP-His or rLBP on the fluorescence of BODIPY-LPS was less pronounced in the presence of divalent cations and in the absence of EDTA (Fig. 3B) than in EDTA-containing buffer (Fig. 3A). In divalent cation-containing buffer (PBS), rPLTP-His and rLBP consistently caused a rise in fluorescence of BODIPY-LPS, although only to ~1.5 times the

FIG. 1. Neutralization of LPS by rPLTP medium. A, time course of neutralization. LPS (10 ng/ml) was incubated at 37 °C for the indicated times alone (●-●), with medium from control (nontransfected) cells (0.5×) (○-○), or with medium from cells expressing rPLTP (0.5×) (□-□). After the incubation, neutrophils were then added and adhesion to fibrinogen-coated plates was measured as described under "Materials and Methods." Adhesion in the absence of LPS was measured as a control (□-□). B, neutralization of LPS by rPLTP medium is prevented by anti-rPLTP IgG. LPS (10 ng/ml) was incubated for 30 min at 37 °C alone (●-●), with rPLTP medium (0.5×) (□-□), or with rPLTP medium (0.5×) and the indicated concentrations of anti-rPLTP IgG (△-△) or control IgG (○-○). Neutrophils were then added and adhesion was measured as in A. Adhesion in the absence of LPS was measured as a control (□-□).

FIG. 2. Neutralization of LPS by purified rPLTP-His. LPS (10 ng/ml) was incubated at 37 °C for 30 min alone (●-●) or in the presence of the indicated concentrations of purified rPLTP-His (○-○). Neutrophils were then added and adhesion to fibrinogen-coated plates was measured as described under "Materials and Methods." Adhesion in the absence of LPS was measured as a control (□-□).

FIG. 3. Dequenching of BODIPY-LPS by LBP or rPLTP-His. BODIPY-LPS (40 nm) was incubated at 37 °C in PD-EDTA (A) or PBS (B) alone (curve 1), with human serum albumin (HSA) (40 nm, curve 2), with rPLTP-His (40 nm) (curve 3), or with rLBP (40 nm, curve 4), and the fluorescence emission at 518 nm was measured over time.
shown in Fig. 3A was performed with EDTA to emphasize the effects of rPLTP-His and rLBP on BODIPY-LPS. Subsequent experiments, using BODIPY-LPS to assess the transfer activities of PLTP and LBP, were performed in PBS, taking advantage of the limited effect of PLTP or LBP alone on the fluorescence of BODIPY-LPS under these conditions.

rPLTP-His and rLBP Transfer BODIPY-LPS to Unlabeled LPS Micelles—Because PLTP appears to bind LPS and acts as a transfer protein for phospholipids (6), we reasoned that PLTP may have the ability to transfer LPS. We assayed transfer activity using BODIPY-LPS donor micelles and unlabeled LPS acceptor micelles. Transfer of BODIPY-LPS to acceptor micelles should result in dequenching due to dilution of BODIPY-LPS monomers in micelles of unlabeled LPS. Incubation of BODIPY-LPS micelles alone, with a 100-fold excess of unlabeled LPS micelles, with rPLTP-His or with rLBP caused little change in fluorescence (Fig. 4, curves 1–4). However, rapid dequenching was seen when BODIPY-LPS was incubated with unlabeled LPS micelles and rPLTP-His together (Fig. 4, curves 5 and 6) or unlabeled LPS micelles and rLBP together (Fig. 4, curves 7 and 8), suggesting that both rLBP and rPLTP-His can transfer BODIPY-LPS between micelles of LPS. rPLTP-His appeared to be somewhat less efficient than rLBP in mediating this transfer event, as there was more rapid dequenching with LBP than with rPLTP-His at the two concentrations tested.

rPLTP-His Mediates the Transfer of $^{3}H$LPS to R-HDL—We have shown recently that rLBP transfers LPS to R-HDL particles: rLBP facilitates transfer of $^{3}H$LPS aggregates to R-HDL particles separated with nondenaturing polyacrylamide gel electrophoresis (native PAGE) (22), and rLBP enables R-HDL to functionally neutralize LPS (2, 22). Since PLTP neutralizes LPS without added R-HDL, we could not use functional assays to determine if PLTP transfers LPS to R-HDL. Instead, we examined transfer of $^{3}H$LPS to R-HDL using native PAGE.

$^{3}H$LPS incubated alone, with rLBP, with rPLTP-His, or with R-HDL did not comigrate with the main band of R-HDL seen with Coomassie staining (Fig. 5, lanes 1–5), indicating that $^{3}H$LPS does not spontaneously bind to R-HDL under these conditions, as we have shown previously (22). However, upon incubation of $^{3}H$LPS with rLBP and R-HDL together, a portion of the $^{3}H$LPS migrated at the position of the main Coomassie-stained band of R-HDL incubated with rLBP (Fig. 5, lanes 6–9). Similarly, incubation of $^{3}H$LPS with rPLTP-His and R-HDL together yielded a radioactive band at the position of the main Coomassie-stained band of R-HDL incubated with rPLTP-His (Fig. 5, lanes 10–13). The dramatic change in mobility of R-HDL caused by rPLTP-His (compare lanes 5 and 13 of Fig. 5) was time-dependent (not shown) and may reflect the ability of rPLTP-His to mediate HDL conversion, a modification of HDL resulting in populations of larger and smaller particles (8–10). Very similar electrophoresis patterns were seen upon incubation of $^{3}H$LPS and R-HDL with rPLTP-His, with plasma-derived PLTP, or with rPLTP medium, but not with control medium (not shown). These results suggest that PLTP can transfer $^{3}H$LPS to R-HDL.

rPLTP-His Transfers BODIPY-LPS to R-HDL—We used BODIPY-LPS donor micelles and R-HDL as acceptor particles to confirm the ability of rPLTP-His to mediate transfer of LPS to R-HDL. Incubation of BODIPY-LPS with rLBP, rPLTP-His, or R-HDL resulted in a modest increase in fluorescence (Fig. 6, curves 1–4), whereas incubation with rLBP and R-HDL or rPLTP-His and R-HDL resulted in more rapid dequenching of BODIPY-LPS (Fig. 6, curves 5 and 6), a result consistent with transfer of BODIPY-LPS to R-HDL particles. Dequenching of BODIPY-LPS might also result from transfer of phospholipid molecules from R-HDL particles to BODIPY-LPS micelles. If this were the case, however, one would expect fluorescence to rise only after a time lag: BODIPY-LPS molecules in a micelle would only become dequenched after transfer of many phospholipid molecules into the aggregate. If dequenching were due to transfer of BODIPY-LPS molecules to R-HDL particles, however, fluorescence dequenching would not have a time lag, since the first BODIPY-LPS molecules transferred to an HDL particle would be dequenched. Since rPLTP-His caused an increase in BODIPY fluorescence without a time lag (Fig. 6, curve 6), we believe that the rapid dequenching observed represents the transfer of BODIPY-LPS to R-HDL.

PLTP Does Not Mediate the Transfer of LPS to CD14—CD14...
is a 55-kDa glycoprotein found as a membrane-bound, glycosylphosphatidylinositol-linked protein on the surfaces of monocytes, macrophages, and neutrophils (mCD14) and as a soluble protein in blood (sCD14). Both of these forms of CD14 can bind LPS and mediate responses of cells to LPS. LBP enhances responses of CD14-bearing cells to LPS (3), an activity which reflects its ability to transfer LPS to CD14 (4). Because LBP can transfer LPS either to lipoproteins or to CD14, we asked whether PLTP could also transfer LPS to CD14.

A 15-min incubation of [3H]LPS with sCD14 did not yield [3H]LPS-sCD14 complexes (Fig. 7, lane 2). However, when the incubation was done in the presence of at least 0.1 μg/ml rLBP, most of the [3H]LPS comigrated with sCD14, suggesting complete transfer of [3H]LPS to sCD14 by rLBP (Fig. 7, lanes 3–6), consistent with our previous findings (4). When [3H]LPS and sCD14 were incubated with increasing concentrations of rPLTP-His (0.13–13 μg/ml), there was no apparent formation of [3H]LPS-sCD14 complexes (Fig. 7, lanes 7–9). Similar concentrations of rPLTP-His to those used here were able to transfer [3H]LPS to R-HDL (Fig. 5), suggesting that PLTP is able to transfer LPS, but unable to transfer LPS to CD14.

PLTP also failed to transfer BODIPY-LPS to sCD14. Little change of fluorescence was seen when BODIPY-LPS was incubated alone or with rLBP, rPLTP-His, or sCD14 alone (Fig. 8, lanes 1–4). However, incubation of BODIPY-LPS with sCD14 and as little as 0.4 nm rLBP resulted in rapid dequenching of BODIPY-LPS (Fig. 8A, curves 5 and 6), reflecting rLBP-mediated binding of BODIPY-LPS to CD14 (16). In contrast, the change in fluorescence seen when BODIPY-LPS was incubated with rPLTP-His and CD14 together (Fig. 8B, curves 5 and 6) was the same as when BODIPY-LPS was incubated with the same concentrations of rPLTP-His alone (Fig. 8B, curves 3 and 4). Thus, concentrations of rPLTP which appear to transfer BODIPY-LPS to LPS acceptor micelles (Fig. 4B) or to R-HDL particles (Fig. 6) are unable to transfer LPS to CD14.

We measured adhesion responses of neutrophils to LPS to confirm the inability of rPLTP-His to transfer LPS to CD14. Neutrophils do not adhere to fibrinogen-coated plates in response to LPS alone, but do adhere in response to LPS with LBP (2, 4). This response depends on mCD14, since it can be inhibited with monoclonal antibodies to CD14 (4). We therefore tested the ability of rPLTP-His to mediate this mCD14-dependent response by incubating neutrophils with a fixed dose of LPS and increasing amounts of rLBP or rPLTP-His (Fig. 9). Addition of as little as 1 ng/ml rLBP allowed adhesion of neutrophils, whereas addition of rPLTP-His did not enhance the adhesion response at any concentration tested. This result, and the previous experiments (Figs. 7 and 8), strongly suggest that PLTP is unable to transfer LPS to CD14.

PLTP Neutralizes LPS in Whole Blood—Since rPLTP is able to neutralize LPS directly (Figs. 1 and 2) and is able to transfer LPS to lipoprotein particles (Figs. 6 and 7), a process which results in the neutralization of LPS (2), we reasoned that increasing the concentration of PLTP in whole blood might result in the rapid neutralization of LPS. We measured IL-6 production in whole blood in the presence or absence of added rPLTP. The addition of 10 μg/ml rPLTP-His to 50% whole blood reduced the sensitivity of the IL-6 response to LPS by ~10-fold (Fig. 10), and a more modest decrease in responses was seen with 3 μg/ml rPLTP-His (not shown). The concentration of PLTP in plasma has been estimated to be ~2 μg/ml (10), based on the phospholipid transfer activity of plasma compared with the activity of purified PLTP. Thus, the addition of 10 μg/ml rPLTP to 50% whole blood may represent an increase in the PLTP concentration of roughly an order of magnitude. This experiment does not distinguish whether the neutralizing effect of rPLTP in whole blood is due to neutralization by binding LPS or by the transfer of LPS to lipoproteins, but suggests that high concentrations of PLTP in the blood may diminish inflammatory responses to endotoxin.

**DISCUSSION**

Here we have shown that PLTP, a plasma protein known to transfer phospholipids (6) and mediate HDL conversion (8–10), inhibits cellular responses to LPS. The observed inhibition was due to the action of PLTP on LPS, rather than on cells, because neutralization of LPS by PLTP was dependent on the time of incubation of LPS with PLTP (Fig. 1A), and rPLTP-His had no effect on tumor necrosis factor α-induced adhesion of neutrophils (not shown). The ability of PLTP to cause dequenching of BODIPY-LPS (Fig. 3) provides additional evidence for a physical interaction of PLTP with LPS and suggests that PLTP binds LPS. The binding of BODIPY-LPS by a protein would be expected to cause dequenching, due to the physical separation...
of BODIPY molecules, and the effect of PLTP on the fluorescence of BODIPY-LPS was similar to the effect of LBP (Fig. 3), a protein known to bind LPS (19–21).

The above results suggest that PLTP may neutralize LPS by binding it. This mechanism is consistent with the previous identification of several proteins and peptides which bind and neutralize LPS. These include bactericidal/permeability-increasing protein (BPI) (28, 29), a neutrophil granule protein with sequence similarity to LBP (44%)(3) and PLTP (26%)(5); Polymixin B, an acylated cationic peptide with antimicrobial activity for Gram-negative bacteria (30,31); endotoxin neutralizing protein, a protein from Limulus which neutralizes LPS in vitro (32); and CAP-18, a cationic protein of neutrophil granules (33). Like PLTP, all these proteins appear to neutralize LPS simply by binding it and preventing its recognition by CD14 or by other cellular factors necessary for responses to LPS.

The stoichiometry and affinity of binding of PLTP to LPS are not evident from our studies and may depend on the buffer conditions. A greater rise in the fluorescence of BODIPY-LPS was caused by PLTP and LBP with EDTA present (Fig. 3A) than without EDTA and with divalent cations (Fig. 3B). EDTA does not appear to change the intrinsic fluorescence of BODIPY-LPS alone was unaffected by EDTA (Fig. 3, A and B), and the fluorescence of complexes of BODIPY-LPS and CD14 was the same in PBS and PD-EDTA (not shown). EDTA prevents the binding of divalent cations to LPS (34), thereby weakening lateral interactions between LPS molecules (35). By destabilizing interactions between LPS molecules, EDTA may increase the affinity of LBP and PLTP for LPS or it may allow the formation of BODIPY-LPS-PLTP or BODIPY-LPS-LBP complexes with low LPS:protein ratios, which would have greater fluorescence due to the lower aggregation state.

We also present evidence here that PLTP transfers LPS, either between micelles of LPS or to R-HDL particles. The incubation of micelles of BODIPY-LPS with unlabeled LPS micelles or with R-HDL particles resulted in rapid dequenching only in the presence of PLTP or LBP (Figs. 4 and 6), a finding consistent with transfer of BODIPY-LPS molecules. Alternatively, fluorescence dequenching might result from fusion of BODIPY-LPS micelles with LPS micelles or with R-HDL particles. The PLTP-mediated incorporation of [3H]LPS into R-HDL did not appear to change the molecular weight of R-HDL...
particles (Fig. 5, compare lanes 12 and 13), making fusion of an entire LPS micelle with a single R-HDL particle an unlikely mechanism. However, we cannot rule out the possibility that PLTP and LBP mediate the fusion of small aggregates of LPS with R-HDL particles, since this mechanism would have similar consequences as the transfer of LPS.

The ability of PLTP to transfer LPS, a membrane lipid of Gram-negative bacteria, is consistent with its ability to transfer other amphipathic molecules. PLTP has been found to transfer a variety of glycerophospholipids as well as sphingomyelin and thus appears to have little specificity for the fatty acyl composition or head group of phospholipids (36). LPS is a much larger molecule than phospholipids, having six or seven acyl chains and at least four carbohydrate groups (37). Nonetheless, the overall amphipathic nature of the molecules is similar, and the solubility of LPS in aqueous environments resembles that of glycerophospholipids (37). Our results suggest that LPS is sufficiently similar to phospholipids that it is recognized and transferred by PLTP.

PLTP and LBP share the ability to transfer LPS to R-HDL (Figs. 5 and 6). The LBP-mediated transfer of LPS to R-HDL particles has been shown to result in the neutralization of LPS (2). Because PLTP also appears to transfer LPS to R-HDL, it may also neutralize LPS by transferring it and may have a role in the transfer of LPS to lipoproteins in vivo. In our studies, PLTP neutralized LPS on its own (Figs. 1 and 2), and the rate of neutralization was not increased by adding R-HDL (not shown); therefore, we could not directly demonstrate whether PLTP could neutralize LPS by transfer to R-HDL particles. In principle, however, PLTP may neutralize LPS through two distinct mechanisms via direct interaction or by transferring LPS to lipoprotein particles. Either or both of these mechanisms may account for the finding that adding PLTP to whole blood caused a reduced sensitivity of cytokine release in response to LPS (Fig. 10).

PLTP is unable to transfer LPS to CD14. We have previously studied the transfer of LPS to CD14 by LBP using native gel electrophoresis (4) and fluorescence dequenching of BODIPY-LPS (16); using these techniques, we were unable to detect transfer of LPS to CD14 by PLTP (Figs. 7 and 8). PLTP was also unable to mediate adhesion of neutrophils to fibrinogen in response to LPS (Fig. 9). This cellular response is dependent on mCD14 (14), and the ability of LBP to mediate this response most likely reflects its ability to transfer LPS to CD14. Our results suggest that while LBP is not unique in its ability to transfer LPS, it may be unique in its ability to transfer LPS to CD14. PLTP is the first protein described which is able to transfer LPS, it may be unique in its ability to transfer LPS to CD14 and may have a role in the transfer of LPS to lipoproteins.

PLTP is part of a gene family that also includes LBP, BPI, and CETP. The abilities of LBP and BPI to interact with LPS are well established, and our results show that PLTP shares this property. CETP may also interact with LPS, insomuch as the transfer of cholesteryl esters by plasma from mice transgenic for human CETP is inhibited by LPS (40). Levels of LBP and BPI may rise during infection, as LBP is an acute phase reactant (20) and BPI is released by activated neutrophils (28). On the other hand, PLTP mRNA levels are decreased by injection of LPS into mice (41), and CETP transcription and protein levels decline upon injection of LPS in mice transgenic for human CETP (40). The role of these changes in modulating responses to LPS in different disease states and sites of infection may be a fruitful area of study.

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