Plasma Lipopolysaccharide Binding Protein and Bactericidal/Permeability Increasing Factor in CRF and HD Patients

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ABSTRACT

The recent characterization of lipopolysaccharide binding protein (LBP) and bactericidal/permeability increasing factor (BPI) have provided the opportunity to examine the natural factors that regulate cytokine production in response to endotoxin in patients on hemodialysis (HD). Whole blood was collected in EDTA from 28 undialyzed patients with chronic renal failure (undialyzed CRF), 36 patients on chronic HD (HD) and 15 healthy controls, and plasma levels of LBP and BPI were measured by a sandwich ELISA. Plasma LBP levels in undialyzed patients with CRF (P = 0.04) and patients on HD (P = 0.01) were significantly higher than those in healthy controls, but not significantly different from each other. Plasma BPI levels in undialyzed patients with CRF and patients on HD were not significantly different from those in healthy controls. There was no correlation between serum creatinine and plasma levels of either LBP or BPI. Peripheral blood mononuclear cells (PBMC) were harvested from healthy volunteers by Ficoll-Hypaque separation, and 0.125 ml of 10 × 10^6/ml suspensions were incubated with 0.125 ml of test plasma (containing different LBP/BPI ratios) and 0.25 ml of RPMI, containing 1 ng/ml of endotoxin, for 24 h at 37° C. Samples were subjected to three freeze-thaw cycles, and total interleukin-1 receptor antagonist (IL-1Ra) or interleukin-1α (IL-1α) production was measured by a specific non-crossreactive RIA. The results of this study showed: (1) IL-1Ra production by endotoxin-stimulated PBMC incubated with pooled plasma from HD patients with LBP/BPI ratios of 11 × 10^2, 167 × 10^2, 379 × 10^2, and 778 × 10^2, respectively, was 1466 ± 195 pg, 3105 ± 462 pg, 8179 ± 1020 pg, and 4770 ± 1185 pg (P < 0.001); (2) Paired plasma collected before dialysis (predialysis) and at 15 min after the start of dialysis (15 minute) with cellulose membranes showed a negligible change in plasma LBP levels (−3 ± 5%), but a 6681 ± 1788% increase in plasma BPI levels. Consequently, compared with predialysis plasma, there was a 35 ± 6% decrease in endotoxin-stimulated IL-1α production by PBMC incubated with plasma drawn at 15 min (P = 0.001); (3) Compared with the PBMC incubated with predialysis plasma from HD patients, there was a 39 ± 5%, 53 ± 5%, and 60 ± 5% decrease in endotoxin-stimulated IL-1α production in the presence of 1 ng/ml, 10 ng/ml, or 1 μg/ml of recombinant BPI, respectively (P < 0.003). These results suggest that the plasma LBP:BPI ratio could influence cytokine production in response to bacterial endotoxin; the high LBP:BPI ratios observed in patients with chronic renal failure probably imparts an increased susceptibility to endotoxin-stimulated cytokine production; and natural or pharmacological increases in plasma BPI levels and the consequent decrease in LBP:BPI ratios could attenuate this susceptibility to endotoxin-stimulated cytokine production.

Key Words: Lipopolysaccharide binding protein, bactericidal permeability increasing factor, cytokines, hemodialysis

Patients on hemodialysis are afflicted by several defects in cellular and humoral mechanisms, including abnormal chemotaxis, adherence, phagocytosis, and release of mediators by granulocytes (1), impaired function of macrophage Fc receptors (2), and defective T-lymphocyte function (3). Consequently, gram-positive and gram-negative infections are an important cause of morbidity and mortality among patients on dialysis, and account for 12% to 38% of deaths in this population (1,4,5). Gram-negative sepsis is often complicated by hypotension, disseminated intravascular coagulation, and multisystem organ failure, and proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) play a pivotal role in the pathophysiology of this lethal syndrome (6–14). A better understanding of the factors that regulate the production of these cytokines in response to bacterial toxins could potentially lead to interventions that would improve outcomes in these patients.

The recent characterization of lipopolysaccharide binding protein (LBP) and bactericidal/permeability increasing factor (BPI) have provided the opportunity to examine some of the natural factors that regulate endotoxin-stimulated cytokine production. LBP is a 60-kd protein that is synthesized by the liver. It is an
acute-phase reactant that binds to the lipid A fraction of lipopolysaccharide (LPS) (15,16). The LBP-LPS complex binds to the CD14 receptor on monocytes and enhances the production of IL-1 and TNF (17). In contrast, bactericidal/permeability increasing factor, a 55-kd cationic protein found in the primary granules of polymorphonuclear leukocytes (PMN), binds to LPS and “neutralizes” its biological effects (18–20). The aminoterminal of BPI is homologous to LBP (21–23). However, BPI has a tenfold-higher binding affinity for endotoxin, compared with LBP (24,25). The relative production/release of these proteins could play an important role in determining the susceptibility of individuals to endotoxin-stimulated cytokine production.

This study examines the role of LBP and BPI as modulators of endotoxin-stimulated cytokine production among patients with chronic renal failure. We measured plasma levels of LBP and BPI in undialyzed patients with chronic renal failure, patients on hemodialysis, and healthy controls. Further, we investigated the effect of plasma from hemodialysis patients with different LBP/BPI ratios on cytokine production by endotoxin-stimulated peripheral blood mononuclear cells (PBMC).

PATIENTS AND METHODS

Patients and Controls

The study was approved by the Human Investigation Review Committees of The New England Medical Center and St. Elizabeth's Hospital. Informed consent was obtained from each participant. Sixty-four patients with chronic renal failure (CRF), between 20 and 80 years of age, were included in the study. Of these, 28 patients were undialyzed (serum creatinine levels greater than 2.0 mg/dL) and 36 patients were on chronic hemodialysis (HD). The HD patients were dialyzed three times a week with bicarbonate dialysate and reused (average 10 reuses) cellulose membranes (Cirrasa CO81; Terumo Corporation, Tokyo, Japan). The reuse procedure included cleansing with reverse-osmosis water, processing with sodium hypochlorite (<1%), and disinfection with glutaraldehyde (0.8%) by using an automated system (DRS 4®; Seratronics Inc., Concord, CA). All dialysis patients received human recombinant erythropoetin alpha (Epogen; Amgen, Thousand Oaks, CA) to maintain hematocrit levels above 35%. Fifteen healthy volunteers, 20 to 50 years of age, who were taking no medications, served as controls. Patients with acute infection or blood transfusion in the past month, chronic infections (hepatitis B, hepatitis C, human immunodeficiency virus, etc.), active immunological disease (systemic lupus erythematosus, rheumatoid arthritis), immunosuppressive therapy, or malignancy were excluded from participation in the study.

Laboratory Methods

Blood Collection. In patients on hemodialysis, blood was drawn from the fistula needle immediately after venipuncture before a routine hemodialysis session. In undialyzed patients with chronic renal failure, blood was drawn from a peripheral vein at the time of routine laboratory investigations. Blood was drawn from a peripheral vein in healthy controls. Blood was collected in sterile standard vacuum blood-collection tubes containing EDTA (1.5 mg/mL of blood) (Terumo Medical, Elkton, MD). Centrifuges were available in the dialysis clinic or laboratory to ensure that the tubes were immediately centrifuged (400 g, 10 min). Plasma was removed without disturbing the buffy coat, aliquots of 500 μL were spun at 10,000 g for 1 min at 4°C and then stored at −70°C.

Assays for LBP and BPI. Plasma levels of LBP and BPI were measured by a sandwich ELISA using rabbit polyclonal antibodies against human LBP and BPI as primary and secondary antibodies (a gift from Dr. Marian Marra, Incyte Pharmaceuticals, Palo Alto, CA) (20). Microtiter plates were developed with polyclonal rabbit anti-LBP/BPI immunoglobulin G (IgG) coupled with biotin, followed by streptavidin-alkaline phosphatase conjugate (Gibco BRL Life Technologies Inc., Grand Island, NY), with P-nitrophenyl disodium phosphate as a substrate (Sigma). Absorbances were determined at 405 nm on a Vmax kinetic microtiter reader (Molecular Devices, Menlo Park, CA). The lower limit of detection for the assays was 160 ng/mL for LBP and 160 pg/mL for BPI.

Preparation of PBMC Suspensions. The water and tissue-culture media used in this study were subjected to ultrafiltration with a polyamide hollow-fibre ultrafilter (U2000; Gambro AB, Hechingen, Germany) to remove cytokine-inducing agents (26). PBMC were harvested as previously described (27,28). In brief, each 10 mL sample of blood was drawn into a heparinized (10 U/mL) polypropylene syringe. The blood was then diluted with 10 mL of sterile pyrogen-free normal saline (Abbott Laboratories, Rockford, IL) and ultracentrifuged with 10 mL of Ficoll-Hypaque. The tube was centrifuged at 450 g for 45 min at room temperature. The PBMC layer was harvested, washed in saline, and centrifuged at 400 g for 10 min. The cells were washed in saline two additional times, and resuspended in ultrafiltered tissue culture medium (RPMI 1640, pH 7.4; Sigma Chemical Co., St. Louis, MO), containing 10 mmol/L L-glutamine, 24 mmol/L NaHCO3 (Mallinkrodt, Paris, KY), 10 mmol/L N-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES; Sigma, Chemical Co., St. Louis, Mo), 100 U/mL penicillin and 100 μg/mL streptomycin (Irvine Scientific, Santa Ana, CA). Cells were counted by using a standard hemocytometer and adjusted to 10 × 10⁶/mL, and 0.125 mL of cell suspensions were aliquoted into 12 × 75 mm polypropylene tubes.

Effect of Plasma LBP/BPI Ratios on In Vitro Cytokine Production by Endotoxin-Stimulated PBMC. The aim of this experiment was to study the effect of plasma from HD patients with different LBP/BPI ratios on cytokine production by endotoxin-stimulated PBMC. Plasma from three hemodialysis patients with low (6 × 10⁶), medium (244 × 10⁶), and high (494 × 10⁶) LBP/BPI ratios were incubated with PBMC from a healthy volunteer and varying concentrations of endotoxin. PBMC were harvested from a single donor by Ficoll-Hypaque separation, and 0.125 mL of 10 × 10⁶/mL suspensions (1.25 × 10⁵ PBMC) were aliquoted into polystyrene tubes. To each tube was added one of the three different LBP/BPI ratios, and 0.25 mL of RPMI containing either endotoxin-free RPMI, or 10 pg/mL, 100 pg/mL, or 1 ng/mL of endotoxin (Escherichia coli, serotype 055:B5; Sigma). The tubes were then incubated upright at 37°C and 5% CO2. At the end of 24 h, the three tubes were frozen at −70°C. The cell suspensions were subjected to two additional freeze-thaw cycles and total (intracellular and extracellular) interleukin-1 receptor antagonist (IL-1Ra) production was measured by a specific noncross-reactive radioimmunoassay (RIA) (27). The IL-1Ra was used as an index of endotoxin-stimulated cytokine production.
production by PBMC was made on the basis of our previous observations that IL-1Ra is a very sensitive indicator of dialysis-related cytokine production (28–30).

On the basis of the results of the above experiment, the role of plasma LBP:BPI ratios was examined further by using pooled plasma from hemodialysis patients achieving LBP/ BPI ratios of 11 × 10^4, 167 × 10^4, 379 × 10^4, and 778 × 10^4, respectively. PBMC were harvested from eight healthy volunteers by Ficoll-Hypaque separation, and 0.125 mL of 10 × 10^6/mL suspensions were aliquoted into polypropylene tubes. To each tube was added 0.125 mL of plasma containing one of the four different LBP:BPI ratios, and 0.25 mL of RPMI containing 1 ng/mL of endotoxin. The tubes were then incubated upright at 37°C for 24 h and total IL-1Ra production was measured.

In Vitro Cytokine Production by Endotoxin-Stimulated PBMC Cultured with Plasma Before and After Exposure to Cellulose Membranes. The aim of this experiment was to examine the effect of exposure to cellulose membranes on endogenous BPI release, the consequent changes in plasma LBP:BPI ratios, and its effect on in vitro cytokine production by endotoxin-stimulated PBMC. Paired plasma samples were collected before dialysis (predialysis) and at 15 min after the start of dialysis (15 minute) in three patients on chronic hemodialysis with cellulose membranes, and levels of LBP and BPI were measured in each sample. PBMC were harvested from three healthy volunteers, and 0.125 mL of 10 × 10^6/mL suspensions from each donor were aliquoted into six polypropylene tubes. Either a predialysis or 15-minute plasma sample (0.125 mL) from each of the three HD patients was added to the PBMC suspensions from each healthy donor, along with 0.25 mL of RPMI containing 1 ng/mL of endotoxin. The tubes were then incubated upright at 37°C for 24 h, and total interleukin-1α (IL-1α) production was measured by RIA (31).

Effect of Addition of Recombinant BPI on In Vitro Cytokine Production by Endotoxin-Stimulated PBMC Incubated with Plasma from Hemodialysis Patients. The aim of this experiment was to examine the effect of adding increasing concentrations of recombinant human BPI to plasma from HD patients, the consequent decrease in plasma LBP:BPI ratios, and its effect on in vitro cytokine production by endotoxin-stimulated PBMC. Human recombinant BPI was produced by transfected Chinese hamster ovary cells and was purified sequentially by ion-exchange column and size-exclusion columns (25). Plasma was collected before dialysis from three patients on chronic HD and levels of LBP and BPI were measured in each sample. PBMC were harvested from three healthy volunteers, and 0.125 mL of 10 × 10^6/mL suspensions from each donor were aliquoted into polypropylene tubes. Plasma (0.125 mL) from each of the three hemodialysis patients was added to the PBMC suspensions from each healthy donor, along with 0.25 mL of RPMI containing 1 ng/mL of endotoxin. In addition, either RPMI (control), or 1 ng/mL, 10 ng/mL, or 1 μg/mL of recombinant BPI was added. The tubes were then incubated upright at 37°C for 24 h and total IL-1α production was measured by RIA (31).

Radioimmunoassay for IL-1Ra and IL-1α. Total IL-1Ra or IL-1α production was measured by a specific non-crossreactive RIA as previously described (27,31). In brief, undiluted or diluted samples were added to RIA buffer (0.01 mol/L phosphate-buffered saline, pH 7.4), 0.25% bovine serum albumin, and 0.05% sodium azide and total cytokine synthesis (cell-associated and secreted) were measured by specific non-crossreactive RIA. The cytokine concentrations were then read from a logit plot of percent specific binding versus the log concentration of a serially diluted cytokine from the linear portion of the curve (usually between 35% and 85% specific binding). The lower limit of detection for both IL-1Ra and IL-1α was 160 pg/mL.

Statistical Analysis

The statistical analysis was performed with the SYSTAT software package (SYSTAT Inc., Evanston, IL). Analysis of variance using Fisher's least significant difference and the Tukey test for simultaneous pair-wise comparisons were utilized to detect differences between groups. All data are expressed as mean ± SE of mean. Cytokine production by PBMC is expressed as pg/2.5 million PBMC. Differences were considered significant if \( P < 0.05 \).

RESULTS

Plasma Levels of LBP

Among healthy controls, undialyzed patients with CRF, and HD patients, plasma levels of LBP were 5424 ± 749 ng/mL (\( N = 15 \)), 8513 ± 797 ng/mL (\( N = 28 \)), and 8972 ± 652 ng/mL (\( N = 36 \)), respectively (Figure 1). Plasma LBP levels in undialyzed patients with CRF (\( P = 0.04 \)) and patients on HD (\( P = 0.01 \)) were significantly higher than those in healthy controls. However, the levels in patients on HD were not significantly different from those in undialyzed patients with CRF.

Plasma Levels of BPI

Among healthy controls, undialyzed patients with CRF, and HD patients, plasma levels of BPI were 2582 ± 921 pg/mL (\( N = 15 \)), 3785 ± 2842 pg/mL (\( N = 28 \)), and 3765 ± 1651 (\( N = 36 \)) pg/mL, respectively.
Plasma BPI levels in undialyzed patients with CRF and patients on HD were not significantly different from those in healthy controls.

Molar Ratios of Plasma Levels LBP/BPI

Among healthy controls, undialyzed patients with CRF, and hemodialysis patients, molar ratios of LBP/BPI in plasma showed a wide variation in all three groups, and were 6892 ± 1408 (N = 15), 22,122 ± 3341 (N = 28), and 24,668 ± 3962 (N = 36), respectively. As in the case with LBP, plasma LBP/BPI ratios in undialyzed patients with CRF (P = 0.04) and patients on HD (P = 0.01) were significantly higher than those in healthy controls. However, the ratios in patients on HD were not significantly different from those in undialyzed patients with CRF.

Correlation between Plasma Levels of LBP, BPI, and Serum Creatinine

Among healthy controls and undialyzed patients with CRF, there was no correlation between serum creatinine and plasma levels of either LBP (r = 0.277, P = not significant [NS]) or BPI (r = 0.161, P = NS). Similarly, among all groups studied, there was no correlation between plasma levels of LBP and BPI (r = 0.064, P = NS).

Effect of Plasma LBP/BPI Ratios on the In Vitro Production of IL-1Ra by Endotoxin-Stimulated PBMC

In the first set of experiments (Figure 2), IL-1Ra production by $1.25 \times 10^6$ PBMC incubated with RPMI (control) or plasma with LBP/BPI ratios of 6 × $10^2$, 244 × $10^2$ or 494 × $10^2$ was: (1) 160 pg, 404 pg, 637 pg, and 896 pg, respectively, without endotoxin; (2) 160 pg, 613 pg, 657 pg, and 1071 pg, respectively, with an endotoxin concentration of 10 pg/mL; (3) 227 pg, 796 pg, 552 pg, and 1535 pg, respectively, with an endotoxin concentration of 100 pg/mL; and (4) 971, 1470, 1723, and 2418 pg, respectively, with an endotoxin concentration of 1 ng/mL. For each concentration of endotoxin, the production of IL-1Ra by PBMC increased with increasing LBP/BPI ratios (P = 0.005).

On the basis of the results of the above experiment, the study was extended to IL-1Ra production by PBMC stimulated with 1 ng/mL of endotoxin in the presence of pooled plasma from hemodialysis patients, with LBP/BPI ratios of $1 \times 10^2$, $167 \times 10^2$, $379 \times 10^2$, or $778 \times 10^2$, respectively. The IL-1Ra production by endotoxin-stimulated PBMC incubated with plasma with LBP/BPI ratios of $1 \times 10^2$, $167 \times 10^2$, $379 \times 10^2$, and $778 \times 10^2$, respectively, was 1466 ± 195 pg, 3105 ± 462 pg, 8179 ± 1,020 pg, and 4770 ± 1,185 pg (Figure 3). Higher plasma ratios of LBP/BPI were associated with higher levels of IL-1Ra production by endotoxin-stimulated PBMC (P < 0.001). Among different LBP/BPI ratios, IL-1Ra production by endotoxin-stimulated PBMC incubated with plasma with a LBP/BPI ratio of $167 \times 10^2$ (3105 ± 462 pg) was significantly higher than that with plasma with a LBP/BPI ratio of $11 \times 10^2$ (1466 ±
195 pg, \( P = 0.01 \). Likewise, IL-1Ra production by endotoxin-stimulated PBMC incubated with plasma with a LBP/BPI ratio of \( 379 \times 10^2 \) \((8179 \pm 1020 \text{ pg})\) was significantly higher than that with plasma with a LBP/BPI ratio of \( 167 \times 10^2 \) \((3105 \pm 462 \text{ pg}, P = 0.002)\). However, IL-1Ra production by endotoxin-stimulated PBMC incubated with plasma with a LBP/BPI ratio \( 778 \times 10^2 \) \((4770 \pm 1185 \text{ pg})\) was not significantly different from that with plasma with a LBP/BPI ratio of \( 379 \times 10^2 \). The IL-1Ra production by endotoxin-stimulated PBMC incubated with plasma with the two higher LBP/BPI ratios \( (379 \times 10^2 \text{ and } 778 \times 10^2)\) was significantly higher than that with plasma with the two lower LBP/BPI ratios \( (11 \times 10^2 \text{ and } 167 \times 10^2, P = 0.001)\).

**In vitro Cytokine Production by Endotoxin-Stimulated PBMC Cultured with Plasma Before and After Exposure to Cellulose Membranes**

The plasma levels of LBP and BPI before the start of dialysis and at 15 min after the start of dialysis in the three chronic hemodialysis patients are provided in the table that accompanies Figure 4. At 15 min after the start of hemodialysis with cellulose membranes, there was a negligible change in plasma LBP levels \((-3 \pm 5\%)\), but a \( 6681 \pm 1788\% \) increase in plasma BPI levels. As a result, the plasma LBP/BPI ratios decreased from \( 62,123 \pm 9662 \) before dialysis to \( 961 \pm 100 \) after 15 min of dialysis with cellulose membranes. The IL-1α production by endotoxin-stimulated PBMC incubated with plasma before dialysis and at 15 min into dialysis was \( 3285 \pm 212 \text{ and } 1473 \pm 145; 2275 \pm 105 \text{ and } 1523 \pm 202; 1967 \pm 87 \text{ and } 1653 \pm 142; \text{ and } 2509 \pm 206 \text{ pg/mL and } 1,550 \pm 99 \text{ pg/mL, respectively, for Patient 1, Patient 2, Patient 3, and overall. Compared with predialysis plasma, the } 35 \pm 6\% \text{ overall decrease in endotoxin-stimulated cytokine production by PBMC incubated with plasma drawn at 15 min was highly significant (} P = 0.001\).**

**Effect of Addition of Recombinant BPI on In vitro Cytokine Production by Endotoxin-Stimulated PBMC Incubated with Plasma from Hemodialysis Patients**

Predialysis plasma levels of LBP and BPI from three patients on chronic hemodialysis patients are provided in the table that accompanies Figure 5. The overall IL-1α production by endotoxin-stimulated PBMC incubated with plasma from hemodialysis patients without the addition of recombinant BPI (control) or with the addition of 1 ng/mL, 10 ng/mL, or 1 \( \mu \)g/mL BPI decreases in IL-1α production was observed with the addition of 1 ng/mL, 10 ng/mL, or 1 \( \mu \)g/mL of recombinant BPI, respectively \( (P < 0.003)\). Data are from three donors for each experiment.
Among BPI human normal in state could attenuate in IL-1α production was observed with the addition of 1 ng/mL, 10 ng/mL, or 1 μg/mL of recombinant BPI, respectively (P < 0.003).

DISCUSSION

The results of this study provide new insights into the susceptibility of patients with chronic renal failure to the consequences of infections. We observed that: (1) among undialyzed patients with CRF and patients on HD, plasma levels of LBP but not BPI were significantly higher than those among healthy controls; (2) plasma LBP:BPI ratios varied widely in healthy controls as well as in both groups of patients with CRF; (3) plasma LBP:BPI ratios among undialyzed patients with CRF and patients on HD were significantly higher than those in healthy controls; (4) plasma levels of LBP and BPI did not correlate with each other or with serum creatinine levels; (5) endotoxin-stimulated PBMC incubated with plasma from hemodialysis patients containing higher LBP:BPI ratios produced significantly higher levels of IL-1Ra compared with PBMC incubated with plasma containing lower LBP:BPI ratios; (6) the release of BPI, as observed during hemodialysis with cellulose membranes, and the consequent decrease in the endogenous LBP:BPI ratios attenuates endotoxin-stimulated cytokine production; and (7) addition of recombinant human BPI to plasma attenuates endotoxin-stimulated cytokine production.

These results suggest that: (1) plasma LBP:BPI ratio could influence cytokine production in response to bacterial endotoxin; (2) the high baseline LBP:BPI ratios observed among patients with chronic renal failure could impart an increased susceptibility to endotoxin-stimulated cytokine production; and (3) natural or pharmacological increases in plasma BPI levels and the consequent decrease in LBP:BPI ratios could attenuate this susceptibility to endotoxin-stimulated cytokine production.

Lipopolysaccharide binding protein potentiates LPS-stimulated cytokine production by cells of the monococyte/macrophage lineage (32). In the presence of LBP, LPS-treated rabbit peritoneal exudate macrophages demonstrate a more rapid induction of mRNA for cytokines such as IL-1 and TNF, higher steady state mRNA levels, and increased mRNA stability (32). Among healthy individuals, plasma levels of LBP are in the 2 to 12 μg/mL range. In contrast, BPI is stored in the primary granules of polymorphs and under normal circumstances, plasma levels are very low. Indeed, among healthy individuals, we observed that plasma levels of LBP were one thousand-fold higher than those of BPI. Consequently, the addition of plasma from healthy individuals significantly enhances cytokine production by endotoxin-stimulated human monocytes (25,33) suggesting that the LBP:BPI ratio in healthy human plasma favors endotoxin-stimulated cytokine production. In experimental endotoxemia in human volunteers, there is a fourfold to fivefold increase in plasma LBP levels, a threefold increase in surface BPI on PMN, and a fourfold to fivefold increase in plasma BPI levels (34). Similarly, in patients with clinical sepsis, there is an eightfold to 15-fold increase in plasma LBP levels, and a 1.5-fold increase in surface BPI on PMN, but a nonsignificant increase in plasma BPI levels (34). Consequently, in both situations, the LBP:BPI ratio continues to favor endotoxin binding to target cells. In contrast, BPI levels in closed-space infections such as abscess cavities are consistently higher than those of LBP (35).

Indeed, the high BPI levels in abscess cavities were directly related to the PMN numbers (35). The plasma LBP:BPI balance favoring endotoxin-stimulated cytokine production is further accentuated in patients with chronic renal failure in whom LBP:BPI ratios were, on average, threefold higher than those in healthy controls. The increased ratios among patients with chronic renal failure were to large extent a result of the high plasma levels of LBP in these patients. The elevated plasma levels of LBP are not surprising because LBP is an acute-phase reactant, and plasma levels of several acute-phase reactants such as C-reactive protein (CRP) and serum amyloid A are increased in patients on hemodialysis (36–38). Further, plasma levels of acute-phase reactants such as CRP have been shown to directly correlate with the duration of hemodialysis (37) and some (36,38), but not all, studies (36,37,39) have demonstrated a rise in plasma levels of acute-phase reactants during a hemodialysis session. Although it is tempting to speculate that chronic exposure to hemodialysis membranes may increase LBP synthesis in the liver, we observed that plasma levels of LBP among patients on hemodialysis were not significantly different from those in undialyzed patients with chronic renal failure. Further, Schindler and colleagues have shown that after 4 h of dialysis with either cellulose or polysulfone membranes, there was no rise in plasma levels of LBP (39). Therefore it is more likely that renal failure per se is associated with high plasma levels of LBP. The cause for the high levels of LBP among patients with chronic renal failure is unclear. The large molecular weight of LBP, and the absence of a correlation between plasma levels of LBP and serum creatinine, suggest that this molecule is not cleared by the kidney. However, the normal kidney could have a role in the metabolism of this protein.

An increase in plasma BPI levels and the consequent reduction in plasma LBP:BPI ratios can attenuate the cytokine response to endotoxin exposure (40,41). In a model of experimental lethal E. coli bacteremia in baboons, treatment with BPI led to lower plasma levels of TNFα and IL-6 (40). Consequently, an increase in plasma BPI levels among hemodialysis patients would be expected to attenuate the cytokine response to endotoxin exposure. During hemodialysis, complement activation and neutrophil
degranulation are associated with a greater than tenfold increase in plasma BPI levels (39), although LBP levels remain unchanged. We observed that compared with plasma before a hemodialysis session, the endogenous release of BPI during hemodialysis with cellulose membranes and the subsequent decrease in the plasma LBP:BPI ratios attenuated endotoxin-stimulated cytokine production. Further, experimental reduction in the plasma LBP:BPI ratios by adding recombinant human BPI to plasma also attenuated endotoxin-stimulated cytokine production. These results confirm our hypothesis that the plasma LBP:BPI ratio could influence endotoxin-stimulated cytokine production. However, the half-life of plasma BPI is less than 1 h (25,40), and plasma BPI levels decrease rapidly after a dialysis treatment (39). Hence, the dialysis-induced release of BPI is unlikely to affect the plasma LBP:BPI ratios between dialysis sessions.

Human IgG is a known stimulant of IL-1Ra by PBMC; this is believed to be via the Fc receptor (27). Consequently, in the first two sets of experiments (Figures 2 and 3), the use of IL-1Ra as a read-out for endotoxin-stimulated cytokine production could have potentially been confounded by the varying concentrations of IgG in the pooled plasma. Nevertheless, in subsequent experiments (Figures 4 and 5), the use of IL-1α as the read-out (which is not known to be influenced by plasma constituents) confirmed our observation that natural or pharmacological reduction in LBP:BPI ratios attenuates endotoxin-stimulated cytokine production.

The endotoxin-stimulated cytokine response is governed by the balance between plasma factors that potentiate this process, such as LBP and sepsin (15–17,42), and factors that attenuate this process, such as BPI and soluble CD14 (18–20,43,44). This study was limited to the investigation of the impact of the balance between LBP and BPI in uremic plasma, on endotoxin-stimulated cytokine production. Consequently, some of the observations in this study remain unexplained. For example, in Figure 3, increasing ratios of LBP:BPI resulted in increased endotoxin-stimulated cytokine production. However, at very high LBP:BPI ratios, endotoxin-stimulated cytokine production did not increase further and actually decreased, albeit not significantly. This could have been influenced by some of the above-mentioned factors that were not measured in this study.

The characterization of LBP and BPI also have important implications for the experimental models that have been used to study the transmembrane passage of bacterial products from dialysate challenged with bacterial filtrates (30,45–47). In some of these models, investigators circulated normal saline, tissue-culture medium, or 10% plasma rather than whole blood in the blood compartment (45–47). Samples from the blood compartment were then incubated with PBMC harvested from healthy donors, and cytokine production by these cells was used as an index of the transmembrane passage of bacterial products from dialysate. The absence of LBP in in vitro models using normal saline or tissue-culture media may have underestimated the passage of cytokine-inducing bacterial products across dialysis membranes. On the contrary, the absence of neutrophils and, consequently, low levels of BPI in in vitro models using 10% plasma may have overestimated the significance of the passage of cytokine-inducing bacterial substances across dialysis membranes. Consequently, we believe that in vitro studies should be done with whole blood to more closely mimic the balance of plasma and cellular factors that regulate the production of cytokines induced by bacterial products (30).

Finally, the high LBP:BPI ratios among hemodialysis patients add a new dimension to the cytokine production in patients on hemodialysis. Proinflammatory cytokines such as IL-1 and TNF have been incriminated in acute-phase responses such as fever and hypotension seen in patients on dialysis, as well as long-term complications such as accelerated atherosclerosis, dialysis arthropathy, hypoalbuminemia, and muscle wasting (48–50). During HD, leukocytes in circulation are exposed to the dialysis tubing, dialyzer membrane, soluble membrane constituents, complement, or other plasma products activated by the dialysis procedure, bacterial, and other contaminants in the dialysate, and the mechanical effect of pumping blood through the HD circuit (49,50). In vitro studies have shown that when human blood is circulated through a hollow-fiber cellulose membrane, transcription of mRNA for IL-1 by monocytes is apparent within 2 h. However, translation into mature IL-1 does not occur unless these cells are exposed to a second signal, such as endotoxin (51). These activated cells can either degrade this mRNA or receive a second signal from ongoing infection or contaminated dialysate (30,47), triggering translation into cytokine protein. In the presence of LPS, the high LBP:BPI ratios in patients on hemodialysis could amplify the cytokine-producing potential of these PBMC. Therefore, plasma levels of LBP and BPI could influence cytokine production in hemodialysis patients, and the ensuing cytokine-related morbidity in these patients. Potentially, plasma LBP levels and BPI release by PMN could be used to stratify the risk of infectious complications in this high-risk population.

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