Relationship between Plasma Levels of Lipopolysaccharide (LPS) and LPS-Binding Protein in Patients with Severe Sepsis and Septic Shock

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Plasma endotoxin and lipopolysaccharide-binding protein (LBP) levels were measured in a group of 253 patients at the onset of severe sepsis and/or septic shock. Endotoxin levels were significantly greater than control levels (n = 33; mean ± SD, 5.1 ± 7.3 pg/mL) in 78.3% of patients. Median endotoxin levels in patients with sepsis were 300 pg/mL (25%–75% interquartile range, 110–726 pg/mL). LBP levels were elevated in 97% of patients compared with normal control values of 4.1 ± 1.65 µg/mL. Median LBP levels in patients with sepsis were 31.2 µg/mL (interquartile range, 22.5–47.7 µg/mL). Median endotoxin levels at study entry were more highly elevated (515 vs. 230 pg/mL; P < .01), and LBP levels were less highly elevated (28.0 vs. 33.2 µg/mL; P < .05) in nonsurvivors than survivors over the 28-day study period. No correlation was found between endotoxin and LBP levels. The quantitative level of both endotoxin and LBP may have prognostic significance in patients with severe sepsis.

Bacterial endotoxin, a component of the outer cell membrane of gram-negative bacteria, is a principal mediator in the pathophysiology of gram-negative bacterial sepsis [1]. This complex macromolecule induces its injurious effects by a noncytotoxic interaction with CD14-bearing inflammatory cells, which include the macrophage-monocyte lineage and circulating neutrophils. These effector cells appear to be activated through a family of Toll-like receptors [2–6] and subsequently release a network of proinflammatory mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, IL-12, cyclic endoperoxides, platelet-activating factor, complement tissue factor, and other inflammatory products. These host-derived mediators function in concert to induce the systemic inflammatory response syndrome [7, 8].

Administration of endotoxin to humans in minute quantities (2–4 ng/kg) precipitates typical signs and symptoms of clinical sepsis, activates the complement and coagulation systems, and triggers release of the proinflammatory cytokines [9]. Injection of larger doses of endotoxin (~1 mg) into humans has been followed by severe hypotension and multiorgan dysfunction within 2 h of intravenous administration [10]. The measurement of endotoxin in the systemic circulation has been complicated by the fact that humans are exquisitely susceptible to endotoxin, and accurate measurement of endotoxin in plasma is technically difficult. Endotoxin levels are often measured by using a Limulus amebocyte lysate reactivity assay. Although this detection system is highly sensitive, it is susceptible to a number of interfering substances found in human plasma, which may activate or inhibit the Limulus reaction independent of endotoxin itself [11, 12]. A large number of previous studies have attempted to measure endotoxin in the circulation of patients with sepsis and relate these findings to other clinical, microbiologic, and pathophysiologic findings [13]. Although some studies have shown that endotoxin measurements may be of value in predicting outcome and presence of gram-negative bacteremia [14–17], other studies have been inconclusive, and the clinical value of endotoxin measurement in patients with sepsis remains unclear [13, 18–21].

The host response to endotoxin is dependent on the physicochemical characteristics of the endotoxin molecule [13], the relative concentrations of endotoxin-binding proteins [22], and the degree of cellular responsiveness to endotoxin [1]. The principal plasma protein responsible for transporting endotoxin to immune effector cells is lipopolysaccharide-binding protein (LBP) [23]. LBP is a hepatically synthesized, acute-phase protein that shuttles endotoxin molecules to effector cells bearing CD14 on their cell surfaces. It has recently been shown that LBP-knockout mice have markedly diminished responses to endotoxin [24] yet are rendered susceptible to systemic Salmonella infection [25]. Therapeutic administration of high doses of LBP may actually protect normal mice from endotoxin challenge [26, 27]. These seemingly paradoxical findings are attributable to other activ-

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All patients provided written informed consent to participate in this institutional review board-approved clinical trial.

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ities, including transfer of endotoxin to soluble CD14 (sCD14) [28]; transfer of endotoxin to high-density lipoprotein (which neutralizes endotoxin activity) [23]; and the formation of endotoxin-LBP-sCD14 complexes, which bind and activate non-myeloid cell lines such as endothelial cells [29]. These activities shunt endotoxin away from immune cells bearing membrane-bound CD14, and this limits endotoxin-mediated toxicity [23].

Plasma samples taken at the onset of severe sepsis and/or septic shock in a large clinical trial [30] afforded us an opportunity to examine the interactions between endotoxin and LBP measurements in human sepsis. The primary objective of this study was to determine the association between plasma endotoxin and LBP levels with outcome in a large group of patients with sepsis. Secondary objectives were to assess the relationship between endotoxin and LBP levels and hemodynamic, clinical, microbiologic, and prognostic parameters in sepsis.

Materials and Methods

Patient selection and sample collection. Plasma endotoxin samples were obtained from 253 patients randomized to the placebo control group in a phase III multicenter sepsis trial [30]. Samples were obtained at study entry within 24 h of onset of severe sepsis. Patients were classified into severe sepsis and/or septic shock groups according to recent consensus definitions [26]. A clinical evaluation committee reviewed the clinical and laboratory findings in each patient enrolled in this clinical trial, and the committee then applied a consistent prespecified and standardized classification system to define the source of infection, organ dysfunction, and probable causative organism(s) responsible for sepsis in each patient [31].

Plasma samples were obtained by using endotoxin-free heparinized blood samples (Chromogenix, Franklin, OH) [32]. Blood samples were placed on ice and then centrifuged at 4°C for 15 min at 1500 g within 30 min. Tubes were then frozen at −70°C and shipped on dry ice to a single research laboratory. Plasma samples were obtained with endotoxin-free needles, glassware, and pipettes.

Endotoxin assays. Samples were diluted 1:10 in endotoxin-free water and then heated at 75°C for 5 min to remove interfering plasma components [11]. Endotoxin assays were done by using a quantitative turbidimetric Limulus amebocyte lysate assay (LAL5000; Associates of Cape Cod, Woods Hole, MA) [33]. Pooled human plasma with known quantities of Escherichia coli 0:113 LPS was used as the endotoxin standard. The functional limit of detection of this endotoxin assay was 2 pg/mL.

Thirty-three ambulatory adult subjects aged 22–55 years had blood samples obtained as a control group for this endotoxin assay by use of the identical methods as described earlier. All endotoxin measurements were performed at the same time period with the same lot of reagents to minimize assay variability. The median endotoxin level in these subjects was 2 pg/mL; the mean (± SD) value was 5.1 ± 7.3 pg/mL. A baseline value of ≥20 pg/mL (mean + 2 SD) was considered to be evidence for elevated endotoxin levels in the study population of patients with sepsis. LBP assays. Plasma LBP determinations were done by means of ELISA methods by using plasma from the same sample tubes as the endotoxin assays. Fifty-nine healthy adults served as controls. In this control group, the mean plasma level of LBP was 4.1 ± 1.65 μg/mL, and the mean ± 2 SD was 7.4 μg/mL. Plasma levels of LBP that were >7.4 μg/mL were considered elevated in the patient samples in this study. Endotoxin and LBP measurements were determined by laboratory personnel who were blinded to the clinical parameters, treatment assignments, and ultimate outcome of patients enrolled in this clinical trial.

Statistical methods. Data were entered into a computer database (Epi Info, version 6, Centers for Disease Control and Prevention, Atlanta). Numeric data were analyzed by using the Mann-Whitney U test or the Kruskal-Wallis 1-way analysis of variance test for multiple groups. Categorical values were compared by using a Mantel-Haenszel χ² test. The results are presented as median values with 25–75 interquartile ranges unless otherwise stated. A 2-sided P value of <.05 was considered statistically significant.

Results

Clinical characteristics and endotoxin levels in severe sepsis patients. A summary of patient demographics and clinical parameters of the study population is found in table 1. The source of infection responsible for the septic process is shown in figure 1. All patients met consensus definitions of severe sepsis, and 80% were found to be in septic shock [30]. The average APACHE II score was 26 ± 13.6, and the 28-day all-cause mortality rate for the entire study group (n = 253) was 32.4%.

Elevated endotoxin levels were found in 198 (78.3%) of the 253 patient samples analyzed. Plasma endotoxin levels ranged from undetectable (<2 pg/mL) to 5.1 ng/mL. The median value was 300 pg/mL for the 25%–75% interquartile range (110–726 pg/mL), whereas the mean (± SD) value was 581 ± 49 pg/mL.

In this Kaplan-Meier survival plot over 28 days for patients with sepsis grouped by plasma endotoxin levels is presented in figure 2. Severe sepsis patients with elevated endotoxin levels (n = 198) had significantly greater mortality rates than those patients without measurable endotoxin (n = 55) at study entry. The median endotoxin level at study entry in survivors was 230 pg/mL; the median level in nonsurvivors was 515 pg/mL (P < .01). The 28-day all-cause mortality was 35% in endotoxemic

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<th>Variable</th>
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<tr>
<td>Study size</td>
<td>n = 253</td>
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<tr>
<td>Age (years)</td>
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<tr>
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<td>Mean ± SD 59.7 ± 13.1%</td>
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<td>Clinical findings</td>
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<tr>
<td>Acute respiratory distress syndrome</td>
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<td>Disseminated intravascular coagulation</td>
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<td>Acute tubular necrosis</td>
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<td>Hepatic dysfunction</td>
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<td>Bacteremia/fungemia</td>
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The quantitative level of circulating endotoxin present at the onset of severe sepsis was inversely proportional to the length of survival in patients with fatal sepsis. The greater quartiles of plasma endotoxin levels were associated with shorter survival times \((P < .05)\). Detectable levels of endotoxin in the plasma were more common in patients with shock than in nonshock patients with sepsis (86.7% vs. 74.5%; \(P < .05\)).

Association between plasma LBP levels and outcome. LBP levels were significantly elevated in 97% of these patients with severe sepsis. Measured values ranged between 4.1 and 162 \(\mu g/mL\), with a median level of 31.2 \(\mu g/mL\) (interquartile range, 22.5–47.7 \(\mu g/mL\)). The mean LBP value was 36.6 \(\pm 1.4 \mu g/mL\). As shown in figure 3, LBP levels at study entry were significantly greater in patients surviving at 24 h than in nonsurvivors (33.2 vs. 28.0 \(\mu g/mL\); \(P < .05\)). This difference in outcome noted within 24 h of the onset of sepsis was also observed up to 28 days.

LBP levels were highest in younger patients with sepsis; LBP levels were \(>8 \mu g/mL\) higher in patients aged \(<63\) years than in older patients \((P < .01)\), despite similar acute physiology scores (APACHE II, 26.5 vs 25.5; \(P\) NS) and frequency of septic shock (79% vs. 81%; \(P\) NS).

Discussion

This study shows that endotoxin is frequently found in the systemic circulation in the presence of sepsis, regardless of the type of microorganism that caused the septic process and either plasma endotoxin or LBP levels. In general, patients with documented gram-negative bacterial sepsis had similar levels of endotoxin and LBP as did patients with gram-positive bacterial sepsis or fungal sepsis (figure 5). Patients without a defined microbial cause of sepsis \((n = 47)\) had LBP and endotoxin levels that were similar to those of other patients with a culture-documented cause of sepsis (figure 5). However, in patients with documented gram-negative bacteremia \((n = 48)\), LBP levels (36.1 vs. 30.4 \(\mu g/mL\); \(P < .05\)) and endotoxin levels (697 vs. 567 \(\mu g/mL\); \(P < .01\)) were both significantly higher than levels measured in patients with gram-positive and/or fungal bloodstream infections \((n = 68)\).

Association between endotoxin, LBP, and infection. No correlation was observed between the endotoxin levels and LBP levels found in the systemic circulation of these patients (figure 4). There was also no significant association between the type of microorganism that caused the septic process and either plasma endotoxin or LBP levels. In general, patients with documented gram-negative bacterial sepsis had similar levels of endotoxin and LBP as did patients with gram-positive bacterial sepsis or fungal sepsis (figure 5). Patients without a defined microbial cause of sepsis \((n = 47)\) had LBP and endotoxin levels that were similar to those of other patients with a culture-documented cause of sepsis (figure 5). However, in patients with documented gram-negative bacteremia \((n = 48)\), LBP levels (36.1 vs. 30.4 \(\mu g/mL\); \(P < .05\)) and endotoxin levels (697 vs. 567 \(\mu g/mL\); \(P < .01\)) were both significantly higher than levels measured in patients with gram-positive and/or fungal bloodstream infections \((n = 68)\).

Association between endotoxin and LBP levels and clinical and laboratory findings of sepsis. No association was found between endotoxin and LBP levels and the presence or absence of acute respiratory distress syndrome, acute renal failure, or other sepsis-related organ dysfunctions (table 1). Moreover, no relationship was observed between endotoxin and/or LBP and plasma levels of IL-6, IL-1\(\beta\), TNF-\(\alpha\), or IL-1 receptor antagonist (data not shown). LBP levels were significantly lower in patients with lactic acidosis \((n = 18); 22.4 vs. 47.6 \mu g/mL\) and in those with low mixed venous oxygen saturation \((n = 21); 31.1 vs. 44.2 \mu g/mL; P < .05\). These differences were not detected with plasma endotoxin levels.
inferring microorganism. Similar findings have been reported by other investigators [13, 15, 21]. Endotoxemia may have originated from unrecognized gram-negative infections in some patients or from enteric bacteria within the gastrointestinal tract [13]. Regional hypoperfusion and mucosal ischemia are thought to promote the translocation of endotoxin from the intestinal lumen to the systemic circulation [13, 34].

The presence of endotoxemia was not significantly greater in patients with sepsis who had major end-organ dysfunction than in those without organ dysfunction; however, endotoxemia was consistently found in patients who were in shock at study entry [15–17, 19]. Because endotoxin is a potent inducer of the proinflammatory cytokines and inducible nitric oxide synthase activity [3, 4], this may account for the frequency with which endotoxemia was associated with shock [15–19].

The correlation between elevated mortality and endotoxin in older patients is not readily explainable. Endotoxin challenge studies in aged animals show that they do not tolerate endotoxin as well as younger animals [35]. Older patients with sepsis have a larger number of comorbidities, which may adversely affect their outcome in the presence of endotoxemia. The metabolic response to endotoxin may vary with age and affect susceptibility to endotoxin-mediated systemic toxicity [13].

The finding that most (97%) of our patients with severe sepsis exhibited elevated LBP levels is compatible with the physiologic role of LBP as part of the innate host defense against systemic microbial challenge [36] and is consistent with earlier reports of LBP levels in sepsis [37]. LBP may prove to be useful as a diagnostic marker for sepsis, because elevated levels of LBP were measurable regardless of the cause of sepsis. In this study, the levels of LBP were equally elevated in patients with gram-negative sepsis and in those with gram-positive sepsis, fungal sepsis, and sepsis of unknown microbial cause.

In this study, significantly worse outcomes were observed in patients with less highly elevated levels of LBP. This finding is in contrast to other studies, in which elevated plasma levels of LBP were associated with an adverse outcome [37, 38]. It is possible that patients with rapidly progressive septic shock fail to adequately synthesize LBP. There may not be sufficient time to mount a fully expressed acute-phase response to systemic microbial challenge in such patients with rapidly fatal infection.

Alternatively, patients who are incapable of mounting a rigorous host response (secondary to preexisting hepatic dysfunction, genetic predisposition, old age, or comorbidities) may be more likely to die of overwhelming systemic infection. LBP may contribute to a physiologic microbial defense response and promote the clearance of intact microbial pathogens [25, 39] and excess endotoxin levels [23, 26, 40].

LBP has been shown to protect animals from endotoxin challenge by facilitating the removal of systemic levels of endotoxin from the circulation [26, 27]. LBP may also directly bind to intact gram-negative bacteria and participate in clearance by human immune cells [39]. LBP-knockout mice are relatively resistant to endotoxin lethality but are susceptible to invasive gram-negative bacteria [25]. It is possible that LBP serves an important functional role in sepsis and is more than a simple marker of the presence of systemic inflammation. The lack of a prompt and appropriate LBP response to infectious stimuli may portend a poor prognosis in the patient with severe sepsis. The dynamics of LBP synthesis and other components of the acute-phase hepatic protein response to sepsis is worthy of additional basic and clinical investigation.

Endotoxin and LBP levels may vary considerably over the course of a given septic episode in a patient [15]. Unfortunately, it was possible to analyze only a single sample in this study that was obtained within an hour of the initiation of the clinical study. Variations in the level of endotoxin-binding proteins [22, 41] and endogenous anti-endotoxin antibodies [42, 43] and innate susceptibility to endotoxin [44, 45] vary substantially within populations with sepsis. These factors will affect the measurement of endotoxin and alter the potential of endotoxin to induce pathophysiologic reactions. The intricate interactions...
Fig. 5. Detectable endotoxin levels at onset of sepsis by causative microorganism (P, not significant for all groups). Data are presented as median value with interquartile range and 95% confidence interval. LPS, lipopolysaccharide; gm neg, gram-negative sepsis; gm pos, gram-positive sepsis; mixed cause, polymicrobial sepsis with gram-negative or gram-positive bacteria with or without fungal pathogens.

between endotoxin, LBP, soluble and membrane-bound CD14, and the Toll-like cellular receptors for endotoxin will necessitate further research into the molecular pathogenesis of septic shock.

References