Relative Concentrations of Endotoxin-binding Proteins in Body Fluids in Children During Infection
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Abstract
The serum protein lipopolysaccharide-binding protein (LBP) binds to the lipid A component of bacterial endotoxin and facilitates its delivery to the CD 14 antigen on the macrophages, where proinflammatory cytokines are released and a cascade of host mediators is initiated. The neutrophil granular protein bactericidal/permeability-increasing protein (BPI) competes with LBP for endotoxin binding and functions as a molecular antagonist of LBP-endotoxin interactions. We have measured concentrations of BPI and LBP in abscess cavities, enclosed infected body fluids, and non-infected body fluids from 36 children whose age ranged between 2 to 12 years (21 males and 15 females). The mean values ± SD of BPI/LBP in different body fluids were 12.12 ± 5.11 in abscess cavities, 0.778 ± 0.104 in infected body fluids, and 0.022 ± 0.0624 in non-infected body fluids. The differences in BPI/LBP ratio between the three types of body fluids were highly significant (P<0.0001). The mean BPI concentrations was higher in the 8 abscess cavities that contained gram negative organisms than in the 8 with gram positive or no organisms (P<0.005). BPI concentrations were directly correlated with the quantity of neutrophils within abscess fluids (r = 0.844, P< 0.001) and in infected body fluids (r = 0.484, P<0.05). In conclusion, BPI is available in sufficient quantities within abscess cavities for effective competition with LBP. BPI may attenuate the local inflammatory response and the systemic toxicity of endotoxin release during gram-negative infections.

Introduction
Bacterial endotoxin is the principle mediator of septic shock caused by gram-negative bacteria.1 The host response to the presence of endotoxin is complex and may vary substantially between patients. Many of the pathophysiological effects of endotoxin are the result of activation of mononuclear cells and the subsequent generation of proinflammatory cytokines. Endogenous serum proteins facilitate the delivery of endotoxin to the macrophage cell surface. This interaction stimulates the production of proinflammatory mediators.2,3

Two closely related endotoxin-binding proteins may ultimately determine the host response to endotoxin during gram negative bacterial infection. The serum protein lipopolysaccharide-binding protein (LBP, synthesized in the liver) and the neutrophil-granule-derived protein bactericidal/permeability-increasing protein (BPI) share up to 44% sequence homology and contain a high-affinity binding domain for the lipid A component of bacterial endotoxin.4,5

Despite their structural similarities, LBP and BPI differ in their ability to deliver endotoxin to the macrophage. LBP binds to endotoxin and the complex then binds readily to the CD14 antigen on the macrophage cell surface, where endotoxin trigger an additional signal transduction pathway that activates the cell.6,7 By contrast, BPI interaction with endotoxin blocks endotoxin delivery to the CD14 antigen.8 BPI binding to endotoxin attenuates cytokine release by mononuclear cells and inhibits endotoxin-mediated activation of neutrophils.3,5 The physiological role of BPI in modulatory endotoxin interactions with LBP in vivo has been questioned, because there is a large molar excess of LBP compared with BPI in the systemic circulation.9,10 Moreover, the solubility and extracellular availability of this protein during active clinical infection are unknown.

The aim of this study was to measure bactericidal permeability increasing protein (BPI) and lipopolysaccharide-binding protein (LPB) concentrations in abscesses and enclosed body infected fluids in children.
Subjects and Methods

The study was conducted on 36 children whose age ranged between 2 to 12 years (21 males and 15 females). Samples of biological fluids (16 from abscesses, 10 from infected fluid, and 10 from non-infected fluid) were obtained in the operating room or invasive radiology department in HAI AL-JAMEA HOSPITAL, and KING KHALED HOSPITAL, JEDDAH, K.S.A. Within 2 hour of collection, samples were transferred to sterile, endotoxin-free tubes and centrifuged at 13600 g for 15 minute at 4°C. After filtration (0.45 μm filter, Gelman Sciences, Ann Arbor, MI), supernatants were stored at -70°C. From medical and clinical laboratory records we collected clinical data and cell count, gram-stain, and culture results for each sample of biological fluid. BPI and LBP concentrations were measured by sandwich enzyme-linked immunosorbent assays (ELISA) with rabbit polyclonal antibodies against human BPI and LBP as primary and secondary antibodies. Binding was visualized by addition of polyclonal rabbit anti-BPI or ant-LBP IgG coupled to biotin, followed by streptavidin-alkaline phosphatase with p-nitrophenyl disodium phosphate as substrate. Optical density at 405 nm was measured on a kinetic microtire reader (Molecular Devices, Menlo Park, CA). The sensitivity of assays for both BPI and LBP was 2.5 ng/mL.11

Results

Table I: Mean values ± SD of BPI/LBP ratios in abscess fluids, infected body fluids, and non-infected body fluids

<table>
<thead>
<tr>
<th>BPI/LBP</th>
<th>Abscess fluids (16)</th>
<th>Infected body fluids (10)</th>
<th>Non-infected fluids (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>12.21±5.119</td>
<td>0.778±0.1045</td>
<td>0.029±0.0197</td>
</tr>
<tr>
<td>F test</td>
<td>7.036, P&lt;0.0001</td>
<td></td>
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</tr>
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</table>

The mean age of 21 male and 15 female children was 6.5±4.3. The samples studied were from closed-space abscesses (n = 16), infected body fluids (n = 10) [empyema (n=7), secondary peritonitis (n=3)], and non-infected body fluids (n=10: pleural 4, joint 4, peritoneal 2). No patient was bacteremic at the time of tissue analysis. Escherichia coli was the microorganism most commonly isolated (9), followed by Bacteroids fragilis (5), Staphylococcus aureus (5), Streptococci (5), and enterococci (2). 5 abscesses contained more than one organism.

Table I, shows that, the ratio of BPI and LBP concentrations in body fluids varied greatly according to whether infection was present. BPI concentrations exceeded those of LBP (ratio > 1.0) in all samples of fluid from closed-space infections, whereas BPI and LBP were present in similar concentrations in infected body fluids. The BPI/LBP ratio was lower than 0.1 in 9 of 10 non-infected body fluids. The differences in BPI/LBP ratio between the three types of body fluids [closed-space infections mean value 12.12 (6.05-25), infected body fluids 0.778 (0.587-0.91), and non-infected fluids 0.029 (.009-0.12)] were highly significant (P < 0.0001). Figure 1, shows the mean BPI concentrations was higher in the 8 abscess cavities that contained gram negative organisms than in the 8 with gram positive or no organisms (5600 ± 1562 vs 2512.97± 941.34 ), (P< 0.005).

Figure 2, shows, BPI concentrations were significantly higher in enclosed abscess fluids than in infected body fluids (P<0.005), and non-infected fluids (P<0.001). LBP concentrations were significantly lower in abscess cavities than in infected body fluids (P < 0.001) and non-infected fluids (P<0.0005).

Figures 3 and 4, shows that the concentrations of BPI was directly proportional to the quantity of neutrophils within the enclosed abscess cavities (r= 0.844, P<0.001), and neutrophils within the infected body fluids (r=0.484, P<0.05). However, no relation was identified between LBP concentrations and neutrophils count in different body fluids.
Discussion

BPI is a highly cationic 55 KDa protein found within the azurophilic granules of human neutrophils. This molecule was first recognized for its antibacterial properties against certain gram-negative bacteria, but its endotoxin-neutralizing properties have lately been recognized as a potential treatment strategy for gram-negative sepsis. The molecule has a high-affinity endotoxin-binding domain, which competes effectively with LBP in vivo and prevents death in animals with endotoxic shock. The endotoxin-binding domain is within the amino-terminal domain of the protein, which also contains short sequences that are homologous with other endotoxin-binding molecules such as LBP, endotoxin-neutralizing protein of Limulus Polyphemus, and polymyxin B.

Our findings indicate, however, that BPI functions as an important host defense mechanism against endotoxin in extravascular fluids. Within abscess cavities BPI is present in sufficient quantities (mean BPI/LBP=12.12) for effective competition with LBP. Heumann et al calculated that a three-fold or greater molar excess of BPI is sufficient to inhibit LBP-endotoxin interactions in plasma. These conditions were regularly met in abscess fluids measured in our study. BPI is readily solubilized without detergent from neutrophil membranes and is stable and functionally active in aqueous solution. The large molar excess of BPI over LBP and its greater binding avidity for endotoxin mean that the host response in local abscesses would favor BPI-endotoxin interactions. Such binding would diminish endotoxin delivery to macrophages and reduce endotoxin-mediated cytokine release.

In the present work, BPI/LBP ratios in sterile, non-infected body fluids were similar to those found in plasma samples. BPI concentrations were 40 times higher in infected body fluids than in non-infected body fluids.
(P < 0.05), but LBP was also increased in infected body fluid samples and the net ratio (mean BPI/LBP = 0.7787) continued to favor LBP. By contrast, in abscess cavities BPI was the predominant endotoxin-binding protein. These results were consistent with data reported by others.\(^{15,16,17,18,19}\) In contrast, others reported that the physiological importance of BPI as an endogenous inhibitor of endotoxin action has been questioned. First, they had found that measured BPI concentrations in normal human plasma were low (8 ng/mL) compared with LBP, which is produced constitutively and is measurable in μg/mL. Despite the evidence that BPI has ten or more times the binding affinity for endotoxin that LBP has, the large molar excess of LBP in the plasma would favor initial endotoxin-binding with LBP in preference to BPI. Second, BPI has a short plasma half-life of about 42 min, which limits the duration of its effects in the systemic circulation. Third, BPI is primarily found in the azurophilic granule and remains cell-associated within granules until the neutrophil is activated.\(^{20,21,22}\)

Our study demonstrates that not only were BPI concentrations greatly increased, but also abscess-fluid concentrations of LBP were lower than those in infected body fluids, or non-infected body fluids. These results were in agreement with the findings of Opal et al,\(^{15}\) Weiss et al,\(^{16}\) Hoess et al,\(^{18}\) and Gazzano et al.\(^{19}\) Low amounts of LBP may be related to limited penetration of the plasma protein or its proteolysis within abscess fluids. As expected, BPI concentrations were directly related to neutrophil numbers within body fluids, whereas LBP showed no such relation.

In conclusion, BPI predominates in local abscess, whereas LBP predominates in the plasma and in non-infected body fluids. There is a complex and dynamic relation between an invasive gram-negative bacterium and the host. BPI may have an essential physiological role during local infection with gram-negative bacteria. It could neutralize endotoxin within abscess fluids and limit the systemic toxicity of CD 14-bearing effector cells. Therapeutic interventions that change the balance between BPI and LBP in the systemic circulation may prove efficacious in the prevention and treatment of endotoxemic states. Further work will be necessary to assess the usefulness of BPI as treatment in gram-negative sepsis.

References

14. Heumann D, Galley P, Betz-Corradin S, Barras C, Baumgartner JD, Glauser MP. Competition between bactericidal/permeability increasing and lipopolysaccharide-binding protein for...