A Family of Lipopolysaccharide Binding Proteins Involved in Responses to Gram-negative Sepsis*

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The lipopolysaccharides (LPS) of Gram-negative bacteria initiate potentially fatal processes in many host organisms. Recently published amino acid sequence data suggest that there is a family of LPS binding proteins that may participate in the host response to Gram-negative bacteremia. The first two members of the family to be identified are an LPS binding protein present in serum after an acute phase response in humans, mice, rabbits, and rats and a bactericidal/permeability increasing protein present in the primary granules of human and rabbit neutrophils. LPS binding protein and bactericidal/permeability increasing protein share an ability to bind to LPS, have homologous NH2-terminal amino acid sequences, and are immunologically cross-reactive. Nevertheless, these two molecules differ in their effects on LPS and Gram-negative bacteria, in their sites of biosynthesis, and localization in vivo.

The lipopolysaccharides (LPS) of Gram-negative bacteria initiate injurious and potentially fatal processes in animals and man. The observation that organisms as venerable as the horseshoe crab (1) possess protective mechanisms against Gram-negative bacteremia suggests that there has been considerable time for anti-LPS mechanisms to evolve. Thus it would not be surprising to discover a family of proteins possessing LPS, and more specifically lipid A, binding sites, but with different protective functions. We report here that lipopolysaccharide binding protein (LBP) (2) and bactericidal/permeability increasing protein (BPI) (3) are the first two members of such a protein family to be recognized. The two proteins share the ability to bind to LPS, have homologous NH2-terminal amino acid sequences, and are immunologically cross-reactive. Nevertheless, these two molecules differ in their effects on LPS and Gram-negative bacteria, in their sites of biosynthesis, and localization in vivo.

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Both function in the complex host response to Gram-negative septicemia and endotoxemia.

** MATERIALS AND METHODS **

Purified LBP (2) and rabbit neutrophil granules (4, 5) were prepared by published procedures; BPI was partially purified by extraction from granules with sodium acetate (3). Electroblotting (6), the bactericidal assay using BPI (3), the protein-LPS binding assay in serum (7), and amino acid sequence similarity searches (8) were performed as described previously. Immunofluorescence studies of rabbit neutrophils were as described (9) using a goat anti-rabbit LBP to stain the elicited rabbit neutrophils.

** RESULTS AND DISCUSSION**

We have recently described the isolation and characterization of LBP from acute phase rabbit serum (2). The 60-kDa glycoprotein is estimated to be present in normal rabbit serum at ≤100 ng/ml, while the concentration of LBP in acute phase serum rises to 10–50 μg/ml 24 h after the induction of an acute phase response (2). LBP is also present in the acute phase sera of humans, rats, and mice (2). Although LBP was discovered as a result of its ability to bind to LPS from the Re595 rough mutant of Salmonella minnesota, subsequent work (10) has established that LBP binds to many types of rough or smooth forms of LPS as well as to lipid A with association constants of at least \(10^9\) M\(^{-1}\). The work of Elsbach and co-workers (11) has also resulted in the recognition of another LPS-binding protein known as BPI. This is a protein of either 50 kDa (11, rabbit) or 60 kDa (3, human) and has been isolated from rabbit and human neutrophils where it is tightly bound in the membranes of myeloperoxidase containing primary granules (9). Isolated BPI binds avidly to LPS in the outer membrane of Gram-negative bacteria and rapidly kills these organisms (12).

With the publication of NH2-terminal sequences for both LBP (2) and BPI (3), the relationship between the two proteins became apparent. The 20 amino-terminal residues of human BPI (3) and the first 20 residues of rabbit LBP (2) are shown in Fig. 1. Eleven of the 20 residues are identical, with five others being chemically conservative substitutions that could be accomplished by single base changes in the DNA. Given this degree of homology, we asked whether the two proteins might be immunologically cross-reactive. To answer this question, sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of whole rabbit neutrophil granules (4,5) and purified LBP (2) were electroblotted to nitrocellulose membranes and probed with an anti-LBP antiserum (6). The results in Fig. 2 show that the granules have two components reactive with anti-LBP serum. The electrophoretic mobility of one is near that expected for rabbit BPI (50 kDa, 11); this component is most likely BPI. Ooi et al. (3) have also described a 25–30-kDa amino-terminal fragment of rabbit BPI that retains BPI activity. The second component of rabbit neutrophil granules reactive with anti-LBP serum has the electrophoretic mobility expected for this fragment. Applying immuno-
A Family of LPS Binding Proteins

FIG. 2. Immunologic cross-reactivity of LBP and whole rabbit neutrophil granules. Lane a, LBP (25 ng, anti-LBP); lane b, granules (50 μg, anti-LBP); lane c, LBP (25 ng, preimmune serum); lane d, granules (50 μg, preimmune serum); lane e, granules (50 μg, Coomassie Blue); lane f, LBP (1 μg, Coomassie Blue); lane g, molecular mass standards, 94, 67, 43, 30, and 20 kDa.

FIG. 3. Immunofluorescence of rabbit peritoneal exudate neutrophils using polyclonal anti-(rabbit-LBP) antiserum. A, Goat anti-(rabbit-LBP) antiserum; B, nonimmune goat serum; C, LBP blocked goat anti-(rabbit-LBP) antiserum.

Immunofluorescence techniques with permeabilized whole rabbit neutrophils and anti-LBP the cross-reactive material appeared to be intracellular and punctate (Fig. 3) as reported for human BPI (9). Despite the sequence and immunologic similarities, the two proteins originate in different cells. BPI is a specific product of the neutrophil that is synthesized at or around the promyelocyte stage of differentiation (9). On the other hand, LBP synthesis can be detected in rabbit liver and explanted rabbit hepatocytes after an acute phase stimulus.

Given the structural and immunologic similarity of LBP and BPI, we next compared the functional properties of the two molecules. BPI has two activities, killing and permeabilizing Gram-negative bacteria (12). We tested the ability of LBP to kill two strains of Gram-negative bacteria as described by Elsbach et al. (11). As shown in Table I, purified LBP had no effect on the viability of these organisms at concentrations where purified BPI is very effective. In other experiments with as much as 240 μg/ml LBP, no bactericidal effects were seen. It may be that the carbohydrate present on LBP prevents expression of antibacterial activity. Experiments to prepare deglycosylated LBP for testing the role of the carbohydrate moiety are underway.

A well characterized activity of LBP is to bind to LPS in acute phase serum and, with the LPS isolated from S. minnesota Re595, to slow the binding of LPS to high density lipoprotein (7). To determine if this property of LBP is shared with BPI, an extract prepared from rabbit neutrophil granules was added to normal rabbit serum, and the kinetics of binding to high density lipoprotein of added 3H-Re595 LPS were tested as described previously (7). As seen in Fig. 4, the addition of granule extract to normal rabbit serum altered the buoyant density of Re595 LPS from 1.33 (7) to 1.29. However,

TABLE I

| Protein       | Concentration | Surviving bacteria |
|---------------|---------------|--------------------|
|               | μg/ml         | E. coli J5         | E. coli 0111:B4 |
| LBP           | 4             | 110                | 194             |
| LBP           | 15            | 98                 | 245             |
| Granule extract | 100           | 0.5                | 2.8             |
| None          |               | 100                | 100             |

and explanted rabbit hepatocytes after an acute phase stimulus.²

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binding site may provide new insights into how bacterial control transcription, and the determination of the DNA sequence for these proteins will provide new insights into how bacterial lipopolysaccharides interact with mammalian cells and proteins to initiate changes that lead to fatal endotoxic shock.

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