Endotoxin-neutralizing Properties of the 25 kD N-Terminal Fragment and a Newly Isolated 30 kD C-Terminal Fragment of the 55–60 kD Bactericidal/Permeability-increasing Protein of Human Neutrophils

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Summary

The bactericidal/permeability-increasing protein (BPI) of polymorphonuclear leukocytes (PMN) is a potent cytotoxin, specific for Gram-negative bacteria, that also inhibits endotoxin activity by neutralizing isolated bacterial lipopolysaccharides (LPS). We have previously shown that an isolated 25 kD N-terminal fragment of human BPI carries all the antibacterial activities of the parent 55–60 kD molecule. In this study, we have compared the LPS-neutralizing activities of human holo-BPI, the N-terminal fragment and a 30 kD C-terminal fragment that we have now isolated. We show that the N-terminal fragment also has LPS-neutralizing activity as detected by inhibition (up to 95%) of (a) activation by LPS of procoagulant proteases in Limulus amebocyte lysates, (b) LPS “priming” of PMN, and (c) LPS-mediated production of tumor necrosis factor in whole human blood. Holo-BPI and the 25 kD fragment have similar neutralizing potency (in nanomolar range) in all assays toward “smooth” LPS from Escherichia coli O111:B4 and O55:B5 (possessing long chain polysaccharide or O-antigen), and “deep rough” LPS from Salmonella minnesota R6595 mutant (possessing no O-antigen). The C-terminal fragment of BPI is devoid of antibacterial activity when tested against BPI-sensitive E. coli J5, but does have endotoxin-neutralizing activity. This activity is weak relative to holo-BPI and the 25 kD N-terminal fragment in the Limulus and PMN-priming assay, but is comparable for inhibition of TNF production in whole blood. We conclude that the principal determinants for LPS recognition and neutralization, like those for antibacterial action, reside in the N-terminal half of the BPI molecule, but that sites within the C-terminal half can also contribute to BPI-LPS interaction once LPS is detached from the bacterial envelope.

Endotoxins, complex LPS that are major components of the outer envelope of Gram-negative bacteria, can account for the most serious clinical consequences of Gram-negative bacterial infections (1). At concentrations as low as subnanogram-to-ng/ml these agents elicit in the host many cellular and extracellular responses that include the production and release of a diverse network of mediators such as activated Complement components, cytokines (e.g., tumor necrosis factor, interleukins) and arachidonate metabolites (2). While these reactions must have evolved as part of essential host defenses, they may also become excessive and self-destructive (1–3), prompting many attempts to attenuate host responses to LPS. Recent reports indicate life-saving effects of mAbs, directed against the biologically active lipid A portion of the LPS molecule, in patients with Gram-negative bacteremia and sepsis (4–6) in accord with a primary role of endotoxin in the pathogenesis of Gram-negative sepsis and supporting the potential clinical usefulness of anti-LPS directed therapies.

Progress has also been made in the identification and characterization of endogenous LPS-binding proteins, produced by various cells and tissues (7–11), that may mediate and regulate the response of the host to LPS. Among these proteins is an LPS-binding protein (LBP) that is produced and secreted by the liver and that accumulates in plasma at >100 times higher than baseline concentrations during the acute phase response that is triggered by LPS and other irritants (10).
In vitro LBP enhances the delivery of LPS and LPS-containing cells to macrophages and magnifies responses of macrophages and PMN to LPS (12-14), suggesting that the function of LBP is to heighten the host’s response to LPS. In contrast, a related LPS-binding protein, the bactericidal/permeability-increasing protein (BPI) of PMN (14, 15), suppresses endotoxin-mediated activation of procoagulant protease(s) in Limulus amebocyte lysates and upregulation of CR1 and CR3 receptors on the surface of PMN (16). Together with its potent antibacterial properties (17, 18), these findings suggest that BPI may be able to downregulate responses to LPS both by halting the proliferation of bacteria producing LPS and by directly inhibiting LPS itself.

A striking property of BPI is its selective cytotoxicity for Gram-negative bacteria (17-19), which reflects the avid and specific affinity of BPI for the Gram-negative bacterial surface, and is best explained by a strong attraction of the positively charged protein for the negatively charged LPS in the bacterial outer membrane (20-22). We have shown before (23) that the antibacterial activities and therefore the extraordinary affinity of BPI for Gram-negative bacteria reside entirely within a highly cationic 25 kD N-terminal fragment (23, 24). However, in the case of interactions with cell-free forms of LPS in which the lipid A region is not buried in an intact outer membrane, additional interactions with hydrophobic sites in the C-terminal portion (23, 24) of BPI may also be involved. It should be noted that the homology between BPI and LBP (approx. 45% overall sequence identity) extends throughout the whole 55-60 kD molecules (14), further suggesting that the determinants of protein-LPS interaction may not be entirely limited to the N-terminal region. We now report the isolation of a ca. 30 kD C-terminal BPI fragment and describe further the functional properties of holo-BPI and its N- and C-terminal fragments. Our findings demonstrate that the potent endotoxin-neutralizing activity of BPI is conferred mainly by the N-terminal fragment. This activity is manifest both in physiological salt solution and in whole blood indicating that BPI or fragments of BPI may suppress endotoxin activity in vivo.

Materials and Methods

Materials. Purified LPS from E. coli O55:B5 (S chemotype) was obtained from Calbiochem Corp. (La Jolla, CA) and resuspended in pyrogen-free water with vigorous vortexing according to the instructions of Whittaker Bioproducts, Inc. (Walkersville, MD). Serial dilutions of LPS were prepared in the same way and stock solutions of LPS (10 ng/ml) were stored at 4°C. LPS from Salmonella minnesota mutant R, 595 (R, chemotype) was obtained from List Biologicals (Campbell, CA). Solutions of this LPS were prepared by sonication as previously described (25). The chromogenic Limulus amebocyte lysate assay kit was from Whittaker Bioproducts, Inc., and zymosan was from Sigma Chemical Co. (St. Louis, MO). Leukotriene B4 (LTB4) standards were from Calbiochem Corp. (La Jolla, CA). Tumor necrosis factor (TNF-α) was the kind gift of Drs. J. Le and J. Vilecek (New York University Medical Center, New York, NY). Hanks’ buffered salt solution (minus Ca2+ and Mg2+) (HBSS−) was from Gibco Laboratories (Grand Island, NY). Acetonitrile was from J. T. Baker, Inc. (Phillipsburg, NJ), trifluoroacetic acid from Pierce Biochemicals (Rockford, IL), and bovine serum albumin from United States Biochemical Corp. (Cleveland, OH). The Bio-Rad protein assay kit was from Bio-Rad Laboratories (Richmond, CA), and nitrocellulose was from Schleicher & Schuell, Inc. (Keene, NH). Nutrient broth and Bile agar were from Difco Laboratories (Detroit, MI).

Polymorphonuclear Leukocytes. Polymorphonuclear leukocytes were isolated from the venous blood of normal volunteers using standard procedures of dextran sedimentation and centrifugation on Ficoll-Hypaque as previously described (26).

Purification of Human BPI and its 25 kDa NH2-Terminal Fragment. Human BPI was purified from crude extracts of PMN-rich populations using E. coli as an affinity matrix, as previously described (22). The 25 kD NH2-terminal fragment of human BPI was isolated after limited proteolysis of purified human BPI as described before (23).

Reversed-Phase High Performance Liquid Chromatography (HPLC). Reversed-phase HPLC was carried out on a reversed-phase Vydac C18 column (The Separations Group, Hesperia, CA) using a Beckman HPLC system model 332 (Beckman Instruments, Inc., Someset, NJ). A linear gradient of acetonitrile in 0.1% (v/v) trifluoroacetic acid was developed at a flow rate of 1 ml/min (see legend, Fig. 1). Elution of protein was monitored by absorbance at 214 nm. Eluted proteins were immediately dialyzed against 50 volumes of 10-50 mM sodium acetate, pH 4.0. All protein samples were stored at 4°C.

Western Blot. SDS-PAGE of proteins (12% polyacrylamide gel system of Laemmli), transfer of proteins to nitrocellulose, and immunodetection of proteins with rabbit anti-BPI/125I-Protein G was carried out as previously described (27-29).

Determination of Protein Mass. Protein mass was estimated by the Bio-Rad protein assay kit and by absorbance at 280 nm, with bovine serum albumin as the standard. Protein estimates were confirmed by Coomassie blue staining after SDS-PAGE.

NH2-Terminal Amino Acid Sequencing. NH2-terminal amino acid sequence analyses were performed by sequential Edman degradation on an Applied Biosystems 470A (Applied Biosystems, San Francisco, CA).

Bacterial Cultures. E. coli J5, a "rough" (short-chain LPS) UDP-4-galactose-epimeraseless mutant of the "smooth" (long-chain LPS) parent E. coli O111:B4, was grown overnight, and then in subculture at 37°C in triethanolamine-buffered medium (20). Bacterial subcultures were harvested at mid-logarithmic phase and resuspended to the desired concentration in sterile physiological saline.

Bioassays

Bacterial Growth Inhibition. Bacteria were incubated with protein to be tested for 15 min at 37°C in the standard incubation mixture containing 0.8% (w/v) nutrient broth in saline buffered with 20 mM sodium phosphate pH 6.0. After the incubation, bacteria were serially diluted with sterile physiological saline to 4 x 103 organisms/ml, then plated on nutrient agar (0.8% (w/v) nutrient broth, 0.5% (w/v) NaCl, 1.5% (w/v) Bile agar) by pouring 5 ml molten nutrient agar (48°C) into a sterile petri dish containing 30 μl of the diluted bacteria. Bacterial growth was measured as the number of colony forming units enumerated after overnight incubation at 37°C.

Bacterial Outer-Membrane Permeability-Increasing Activity. Permeability of the E. coli outer membrane to the normally impermeant actinomycin D was assessed by measuring sensitivity of bacteria to actinomycin D added to a final concentration of 50 μg/ml in the standard incubation mixture. After incubation for 15 min at 37°C, bacterial growth was measured as described above (30).
**Endotoxin-Neutralizing Activities of Human BPI and its N- and C-Terminal Fragments**

Comparison of the antibacterial activities of BPI and its two fragments confirms our previous findings showing that the antibacterial activities of BPI can be accounted for entirely by a region within the 25 kD N-terminal fragment. To determine which portion(s) of BPI is (are) required for endotoxin neutralization, we measured the effects on endotoxic activity of holo-BPI and the N- and C-terminal fragments in three different settings of LPS-mediated responses: (a) Activation of proteases in Limulus amebocyte lysates; (b) Priming of arachidonate metabolism by PMN; (c) Induction of TNF production in whole blood ex vivo.

**Limonus Amebocyte Lysate Assay**  Pre-incubation of “smooth” LPS (from *E. coli* O111:B4 or O55:B5, S chemotype) with holo-BPI produced a dose-dependent inhibition of the antibacterial activity of BPI to a small extent, whereas the N-terminal fragment(s) completely inhibited the activity of the 25 kD N-terminal fragment (Fig. 2).

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**Figure 1**. Purification of a ca. 30 kD fragment of human BPI by reversed-phase HPLC. Chromatography was carried out as described under Materials and Methods. The (broken line) indicates the gradient of acetonitrile; the (solid line) indicates elution of material absorbing at 214 nm. (Left panel) shows chromatography of the protein (~100 μg) from the peak containing both holo-BPI and the ca. 30 kD species (20); (right panel) shows chromatography of purified ca. 30 kD fragment(s) (~25-μg). Inset in (right panel) shows Western blot of the purified 30 kD fragment(s) (see Materials and Methods), with numbers indicating migration of protein mol wt standards.

**Results**

**Purification of ca. 30 kD C-Terminal Fragment(s) of Human BPI**

To permit a more complete assessment of the structural and functional properties of human BPI, we sought to isolate the C-terminal portion of the protein. During the limited proteolysis that generates the active 25 kD N-terminal fragment, similar amounts of a ca. 30 kD species accumulated (23). Whereas the 25 kD fragment was readily isolated from the digest by reversed-phase HPLC, the ca. 30 kD species coeluted with the holo-protein (23). However, rechromatography using a shallower acetonitrile gradient allowed separation of the 60 kD and ca. 30 kD protein species (Fig. 1). The peak eluting earlier conformed with the 60 kD species upon SDS-PAGE (not shown), and the later peak migrated as a slightly heterogeneous ca. 30 kD species (Fig. 1). N-terminal amino acid sequence analysis of the latter protein fraction revealed the presence of two overlapping species: (a) Asp-Ser-Val-Gly-Ile-Asn-Tyr-Gly-Leu-Val, and (b) Ala-Gly-Ile-Tyr-Gly-Leu-Val-Ala-Pro, corresponding to the sequence of BPI starting respectively from residues 200 and 204 (24). The size and location of the 30 kD fragments indicate therefore that they represent the C-terminal half of the BPI molecule. The greater hydrophobicity of this portion of BPI explains the later elution of the 30 kD fragments during reversed-phase HPLC.

In contrast to the 25 kD N-terminal fragment, the purified C-terminal fragment(s) displayed no antibacterial activity toward BPI-sensitive *E. coli*. The C-terminal fragment(s) produced neither growth inhibitory nor outer membrane permeability-increasing effects on *E. coli* J5, even when tested at doses thirty times higher than an effective dose of the 25 kD fragment (Fig. 2).
Inhibition was nearly complete at 10 nM BPI in accord with the report of Marra et al. (16). Maximum inhibition required pre-incubation of BPI with LPS for 20-30 min (data not shown). The endotoxin-neutralizing activity of the 25 kD N-terminal fragment was nearly the same as that of holo-BPI. The 30 kD C-terminal fragment also inhibited endotoxin, but at 5-10-fold higher molar protein concentrations (Fig. 3 a).

**Figure 2.** Comparison of antibacterial activities of the 25 kD N-terminal and the ca. 30 k D C-terminal fragments. E. coli J5 (5 × 10⁹) were incubated in the standard incubation mixture (see Materials and Methods) (125 μl) with increasing amounts of the 25 kD fragment (–○–) or the ca. 30 kD fragment in the absence (–△–) or presence (–□–) of actinomycin D (50 μg/ml). Growth inhibitory effects (closed symbols) were measured by assessing colony formation on nutrient agar. Since the ca. 30 kD fragment did not show any growth inhibitory effect, outer membrane permeability-increasing effects (open symbols) could be assessed by measuring bacterial viability in the presence of actinomycin D. The data are presented as percent of values obtained for bacteria incubated alone, and are the mean of two similar experiments.

Both holo-BPI and the 25 kD fragment also inhibited activation of proteolysis in the *Limulus* assay by “rough” LPS from *S. minnesota* (R595 LPS). The 30 kD fragment, however, showed only weak LPS-neutralizing activity (Fig. 3 b).

### Priming of PMN

Pretreatment of PMN with LPS causes a dose-dependent increase in release of leukotriene B₄ (LTB₄) and its metabolites during subsequent incubation of the primed PMN with opsonized zymosan (reference 25; Fig. 4 a). Pretreatment for 15 min of R595 LPS with either holo-BPI or the 25 kD fragment suppressed, in dose-dependent fashion, this primed PMN response (Fig. 4 b). The two protein species caused suppression of endotoxicity activity by 50 percent at respectively 1.0 and 0.3 nM and nearly complete inhibition at 10 nM concentrations of each protein. In contrast, the 30 kD C-terminal fragment(s) did not inhibit priming. Neither holo-BPI nor its fragments reduced the basal response of PMN to opsonized zymosan (data not shown). The inhibition of priming by BPI and the N-terminal fragment was apparently attributable to a direct effect on endotoxic because: (a) Pretreatment of PMN with BPI and washing before addition of LPS did not inhibit priming; and (b) priming of PMN by tumor necrosis factor (1 ng/ml) was unaffected by BPI (reference 16; data not shown).

**Figure 4.** Effect of holo-BPI and its N- and C-terminal fragments on priming of PMN by LPS. Priming of PMN by R595 LPS was determined by measuring the release of LTB₄ (and metabolites) in response to opsonized zymosan after pre-incubation of PMN with LPS, as described in Materials and Methods. The amount of LTB₄ (and metabolites) released by unprimed cells (i.e., preincubation with buffer alone) was subtracted from the actual amount of LTB₄ (and metabolites) released, to yield the amount of LTB₄ (and metabolites) released as a result of LPS priming. (a) Release of LTB₄ (and metabolites) was measured after preincubation of PMN with varying amounts of R595 LPS. Priming of 5 × 10⁶ PMN by 1.0 ng/ml LPS resulted in release of 32.0 ± 4.5 ng LTB₄ and metabolites (n = 4), representing an enhancement of 5-10-fold over unprimed cells. Values are expressed as % of maximum release, which is defined as amount of LTB₄ released in response to 1 ng/ml LPS. (b) LPS (0.6 or 2 ng/ml) was pre-incubated alone or with the indicated concentrations of holo-BPI and the N- and C-terminal fragments at 37°C for 15 min before adding LPS to the PMN suspension (final LPS concentration 0.3 or 1.0 ng/ml). The effect of added protein was the same at both LPS doses, and therefore these data are pooled. Values are expressed as % LPS activity, as calculated using the LPS standard curve shown in (a). Each value shown represents the mean ± SEM of eight more determinations.

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also be elicited in the complex environment of whole blood with its content of other LPS-binding proteins such as LBP and lipoproteins, we examined the effect of the proteins on the production of TNF in whole blood after incubation with LPS (32). Addition of LPS to whole blood triggers a dose-dependent synthesis and extracellular release of TNF (reference 32; Fig. 5 a). Addition of human BPI to whole human blood before adding R. 595 LPS caused a dose-dependent inhibition of TNF accumulation, over at least a 30-fold range of LPS doses (0.1–3 ng/ml). At 1 ng/ml LPS, TNF production was inhibited by 50% at a BPI concentration of 4 nM and by ca. 90% at ca. 30 nM (Fig. 5 b). As in the Limulus and the PMN-priming assays, the concentration dependence of the neutralizing activity of the 25 kD N-terminal fragment was of the same order as that of holo-BPI, i.e.: 50% inhibition at 12 nM and nearly 90% at 40 nM. However, in contrast to the weaker endotoxin-neutralizing activity in the preceding assays, the potency of the 30 kD C-terminal fragment(s) as inhibitor of TNF production falls in the same range (50% inhibition at 15 nM protein and ca. 80% at approximately 30 nM) as shown for holo-BPI and the N-terminal fragment (Fig. 5 b). Full inhibitory effects did not require preincubation of BPI (or fragments) with LPS. The proteins alone had no recognizable effect on TNF production in response to heat-killed S. aureus (Table 1).

**Figure 5.** Inhibition by holo-BPI and its N- and C-terminal fragments of LPS-triggered TNF accumulation in whole blood. (a) Effect of increasing concentrations of R. 595 LPS on TNF accumulation in whole blood. (b) Effect of increasing concentrations of holo-BPI and its 25 kD N-terminal and 30 kD C-terminal fragments on ability of R. 595 LPS (1 ng/ml) to induce accumulation of TNF in whole blood. LPS activity was calculated as described in the legend to Fig. 4. Each value shown represents the \( \bar{x} \pm SEM \) of six or more determinations.

**Table 1.** BPI (Fragments) Do Not Inhibit TNF Production in Whole Blood Triggered by Heat-killed S. aureus

| Protein added | 25 kD fragment | 30 kD fragment |
|---------------|----------------|----------------|
| None          | 10\(^7\)        | 3.8 \(\times\) 10\(^7\) | 11.5 \(\times\) 10\(^8\) |
| S. aureus added | 10\(^8\)        | 7.2             | 7.0             |
| Holo-BPI      | 25 kD fragment  | 4.1             | 10.9            |
| None          | 30 kD fragment  | 3.4             | 11.5            |
| Holo-BPI      | 30 kD fragment  | 3.9             | 10.7            |

Release of TNF in whole blood was measured as described in Methods. Protein amounts were 33 nM holo-BPI, 40 nM 25 kDa N-terminal fragment, and 33 nM 30 kDa C-terminal fragment.

* NT, not tested.

Discussion

Guided by the functional properties of an N-terminal 25 kD fragment obtained by limited proteolysis and the structural properties of the holoprotein deduced from its primary structure, we have previously proposed that human BPI is organized into two domains linked via a protease-sensitive region, with all of the determinants for the antibacterial activities of BPI residing within the N-terminal half of the protein (23, 24).

The isolation and characterization of the ca. 30 kD fragment(s) now reported provide further evidence supporting this model. The N-terminal sequence of these fragment(s) indicates that cleavages occur at peptide bonds between residues 199–200 and 203–204 during the limited proteolysis of BPI that gives rise to both these fragment(s) and the antibacterial N-terminal fragment (23). Since the 25 kD (N-terminal) and 30 kD fragments are generated in roughly equal amounts with no other fragments detected (23) these findings indicate that the principal cleavages are restricted to the predicted protease-sensitive region and that the C-terminal boundary of the active N-terminal fragment does not extend beyond residues 199 or 203. The predicted (peptide) mass of N- and C-terminal fragments comprising residues 1–199(203) and 200(204)–456 (the C-terminus of BPI; 24) is ca. 23 and 29 kDa, respectively, corresponding closely to the apparent Mr of these fragments estimated from their migration rates during SDS-PAGE. The small differences may reflect carbohydrate content (24). The absence of detectable (<5%) antibacterial activity in the purified 30 kD fragments is consistent with our previous conclusion that these activities reside entirely within the N-terminal portion of BPI.

We conclude, therefore, that all of the molecular determinants required for the specific and potent cytotoxicity of BPI toward Gram-negative bacteria are present within the region of the protein encompassed by residues 1–199 (203).

It is the portion of the BPI molecule that is also closely similar to holo-BPI in its activity as a neutralizing agent against isolated LPS in three different assessments of LPS-activity in vitro: (a) the limulus lysate test, (b) priming of neutrophils for arachidonate metabolism, and (c) the production of TNF in whole blood. Thus, the principal determinants of LPS recognition and neutralization also reside within this region of BPI. The strong attraction of BPI as well as of its N-terminal fragment for LPS, whether embedded in the gram-negative bacterial envelope or as isolated molecules, can be explained...
by two structural features of this portion of the BPI molecule: (a) The very high content of basic residues in BPI that are concentrated in the N-terminal region (the net charge of the region comprising residues 1-199 is +17) (20, 21, 24, 33) and (b) hydrophobic stretches in this region. The former provides a strong electrostatic attraction to the cluster of anionic sites in the core region of LPS, while the latter can account for a hydrophobic interaction with the lipid A moiety of LPS (24, 34). The finding that the C-terminal fragment that lacks the charge properties for electrostatic attraction to LPS (net charge -2 to -3), but that contains several prominently hydrophobic regions (24), does not possess antibacterial properties but does exhibit LPS-neutralizing activity, suggests that its hydrophobic properties are sufficient for LPS recognition under certain conditions and therefore that regions in the BPI molecule outside the N-terminal fragment can also contribute to interaction with LPS. However, in contrast to the activities of the holo-BPI and the N-terminal fragment that are the same or nearly the same under all conditions tested, the LPS-neutralizing activity of the C-terminal fragment varies greatly with the different endpoints examined. Thus, whereas the C-terminal fragment shows no inhibition of the priming of PMN by R.595 LPS, and very limited neutralizing activity in the limulus assay (Fig. 3 b) when the same “rough” LPS is the stimulus, with long chain LPS the C-terminal fragment is clearly inhibitory, although at molar concentrations that are much higher than of holo-BPI or the N-terminal fragment (Fig. 3 a). On the other hand, the potency of the N-terminal and the C-terminal fragments as inhibitors of the stimulation of TNF production by LPS in whole blood is similar. Apparently, the highly variable physical presentation of LPS (34-36), depending on its chemical structure and state of aggregation as well as environmental factors such as ionic composition and neighboring macromolecules, has a much more profound effect on the LPS-interactive capabilities of the C-terminal fragment than of holo-BPI and the N-terminal fragment. The extent of LPS-neutralizing activity of the hydrophobic C-terminal fragment may depend on the accessibility of the fatty acyl chains of lipid A. These are shielded when LPS forms aggregates, especially in the case of “rough” LPS or within the hydrophobic bilayer of the intact bacterial envelope (34-36), situations in which the C-terminal fragment shows little or no activity.

In whole blood the stimulation by LPS of the cellular elements has been shown to be mediated by LPS-binding plasma proteins such as LBP (14). It is possible, therefore, that the similar inhibitory effects in whole blood of the N-terminal and C-terminal fragments are by different mechanisms, involving not only the direct interaction with LPS, but also interference with the delivery of LPS by LBP (and/or other LPS-binding proteins) in plasma to TNF-producing cells. This possibility is now under investigation.

In summary, our findings together with the earlier observations of Marra et al. (16) demonstrate that BPI can inhibit a number of LPS-dependent responses elicited by a variety of LPS. Although we have not yet demonstrated this directly, it is most likely that both the endotoxin-neutralizing and the antibacterial action of BPI as well are initiated by binding of BPI to the endotoxic lipid A (and adjacent KDO) regions that are present in virtually all LPS (20, 21, 34). Plasma contains a structurally related LPS-binding protein (LBP; references 10, 14, and 15) that also appears to bind to this region of LPS (37). However, the biological properties of BPI and LBP are strikingly different: LBP has no antibacterial activity (15) and it amplifies rather than suppresses many LPS-triggered host responses (12, 13). Thus, binding of LPS per se does not necessarily lead to a particular biological effect, suggesting that the biological consequences depend on more subtle and specific aspects of protein-LPS interactions. The availability of cloned protein and the identification of the active protein regions should greatly facilitate further dissection of the determinants of LPS recognition and neutralization in BPI and homologous and divergent sites in other LPS-binding proteins such as LBP.

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