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COMMENTARY

The Three-Dimensional Structure of Human Bactericidal/Permeability-Increasing Protein

IMPLICATIONS FOR UNDERSTANDING PROTEIN–LIPOPOLYSACCHARIDE INTERACTIONS

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ABSTRACT. Gram-negative bacterial infections are often complicated by the inflammatory properties of lipopolysaccharides (LPS) on or released from the bacterial outer membrane. When present in the mammalian bloodstream, LPS can trigger a series of pathological changes, sometimes resulting in septic shock. Two related mammalian proteins, bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP), are known to affect the LPS-induced inflammatory response and are, therefore, of clinical interest. The recently determined three-dimensional structure of human BPI provides information on the overall protein fold, domain organization, and conserved regions of these two proteins. In addition, the discovery of two apolar lipid binding pockets in BPI indicates a possible site of interaction with LPS. The BPI structure is a powerful tool for the design of site-directed mutants, peptide mimetics/inhibitors, and BPI/LBP chimeras. These studies should help further define the functions of BPI and LBP, and their mechanism of interaction with LPS. BIOCHEM PHARMACOL **57**;3:225–229, 1999. © 1998 Elsevier Science Inc.

KEY WORDS. LPS-binding proteins; lipopolysaccharide; bactericidal; X-ray crystallography; inflammation

LPS‖ from Gram-negative bacteria can trigger a powerful inflammatory response in mammals. LPS, also known as endotoxin, is a complex glycolipid from the bacterial outer membrane. It has no catalytic or pore-forming activities as is common for protein toxins. However, when present in the mammalian bloodstream, LPS can cause profound pathological changes including low blood pressure, fever, disseminated intravascular clotting, and multiple organ failure [1]. In some cases, these changes culminate in septic shock, an overwhelming systemic inflammatory response to LPS in the bloodstream. Although the invading bacteria can generally be killed with antibiotics, LPS on or released from dying bacteria is still capable of inducing the inflammatory response [2]. The toxic effects of LPS were noted more than a century ago by a student of Robert Koch in Germany, who described a heat-resistant component of *Vibrio cholerae* with pyrogenic activity. Despite the tremendous advances in medical science over this period, at present no effective treatment exists for LPS-related complications, such as septic shock.

The biochemical pathway by which LPS produces an inflammatory response is complex and has not been fully characterized. However, a number of critical protein–LPS and protein–protein interactions have been identified. We focus here on two proteins relevant to the initial stages of the inflammatory cascade. One of these is LBP, a protein constitutively produced by hepatocytes and secreted into the bloodstream [3]. LBP binds to LPS with high affinity, and the levels of LBP are increased in response to LPS [4]. Several years ago, it was found that LBP plays a critical role in the activation of immune cells by LPS, by acting as an LPS-transfer protein [5, 6]. After binding LPS, LBP transports it to a cell surface protein (CD14) [7] of macrophages and monocytes, increasing host sensitivity to LPS by several orders of magnitude [8] (Fig. 1). Although proteins further down the inflammation pathway, e.g. TNF, mediate many of the damaging effects of the inflammatory response [2], identification and characterization of the proteins involved in the initial activation may help identify ways to block the inflammatory cascade before TNF and other damaging proteins are released.

LBP is closely related to a predominantly intracellular protein found in the primary granules of polymorphonuclear neutrophils, BPI. Discovered and characterized by Elsbach and Weiss, BPI also binds to LPS with high affinity [9, 10]. In addition, it is potently bactericidal for Gram-negative bacteria, acting in the phagocytic compartment of the neutrophil to kill engulfed bacteria [11]. Normally found in the bloodstream at low levels [12], BPI retains its high affinity-binding to LPS in sera. However, in contrast

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‖*Abbreviations:* BPI, bactericidal/permeability-increasing protein; CETP, cholesteryl ester transfer protein; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide(s); PLTP, phospholipid transfer protein; and TNF, tumor necrosis factor.

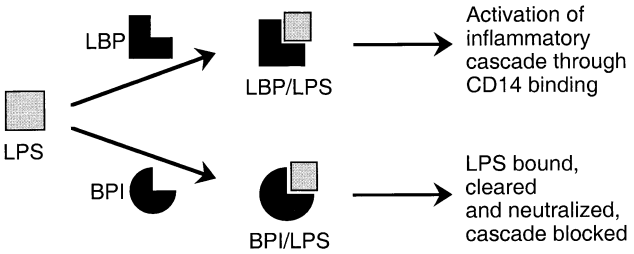

FIG. 1. Schematic showing the different effects of LBP and BPI on the LPS-induced inflammatory cascade.

to LBP, BPI neutralizes the inflammatory properties of LPS *in vitro* [13], in whole blood *ex vivo*, and in animal and human studies [14]. These observations suggested that by adding BPI to the bloodstream, it should be possible to reduce the amount of LPS available to bind to LBP, thereby diminishing the inflammatory response. A number of clinical applications for human BPI are currently under investigation [15, 16].

Because of their roles in the LPS-induced inflammatory cascade and their ability to mediate the biological activity of LPS, BPI and LBP have been the subject of extensive biochemical characterization [17, 18]. However, until recently, these studies proceeded without the benefit of detailed structural information for either protein. The recent determination of the three-dimensional structure of BPI has opened a new chapter in understanding the mechanism of action of these two proteins and their interaction with LPS. Because of the close relationship (45% amino acid identity) between BPI and LBP, the structure of BPI provides a good model for LBP. In the following sections, we describe the structure of BPI with emphasis on its relevance for LBP and protein–LPS interactions.

CRYSTAL STRUCTURE OF BPI

The structure of full-length (456 amino acids), human BPI was determined by X-ray crystallography and has been refined to 2.4 Å resolution [19]. It has several remarkable features (Fig. 2A). One of these is the overall shape of the protein. Unlike most soluble proteins, which are approximately globular, BPI has an unusual "boomerang" shape. Its longest dimension (135 Å) is nearly four times the other two. BPI is organized into two domains (N- and C-terminal), each with approximately 200 amino acids (Fig. 2B). The two domains are connected by a proline-rich linker of 21 amino acids, which runs along the surface of the protein. Each domain contains a "barrel" formed by two α-helices and a highly twisted antiparallel β-sheet. In the center of the protein, the two barrels are connected by a smaller antiparallel β-sheet formed by residues from the beginning and end of each domain. The overall topology of BPI is distinct from other proteins of known structure, and defines a novel protein fold.

The crystal structure revealed that the two domains of BPI have remarkably similar structures, a feature not predicted from its amino acid sequence. In fact, the sequence similarity between the N- and C-terminal domains (<20% identity) is considerably less than that between the BPI and LBP protein sequences. Nevertheless, the two domains share all major secondary and tertiary structural

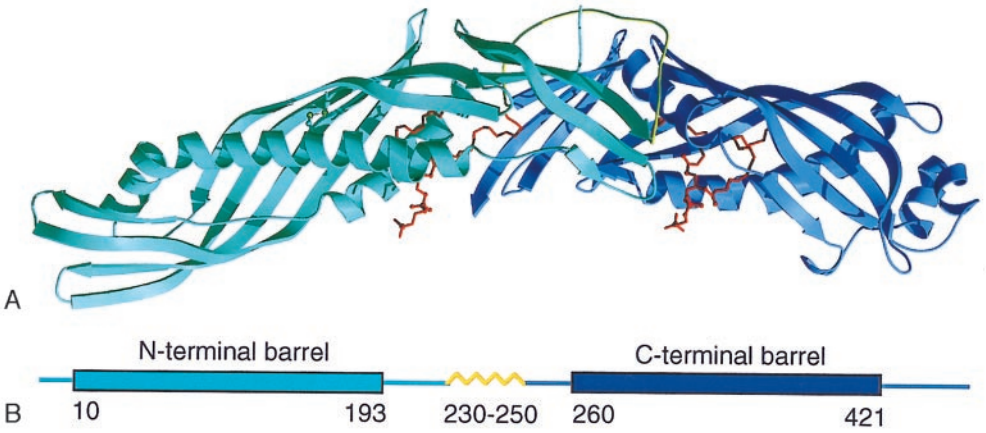

FIG. 2. (A) A ribbon diagram of human BPI. The N-terminal domain (on the left) is cyan, the C-terminal domain is blue, and the proline-rich linker is in light green. The two bound phospholipids are shown as ball-and-stick in red. Note the elongated shape of the protein and approximate 2-fold symmetry. Reprinted with permission from Ref. 19 (Beamer LJ, Carroll SF and Eisenberg D, Crystal structure of human BPI and two bound phospholipids at 2.4 angstrom resolution. *Science* 276: 1861–1864, 1997). Copyright 1997 American Association for the Advancement of Science. (B) Schematic showing the arrangement of structural elements relative to the amino acid sequence of BPI. Residues in the N-terminal domain are indicated in cyan; residues in the C-terminal domain are in blue. The two barrels are shown as boxes, and the proline-rich linker as a zig-zag line. Remaining residues (shown as a line) form the central β-sheet, which connects the two barrels. Amino acid numbers for each structural unit are indicated below.**

elements, giving the protein pseudo 2-fold symmetry. This two-domain organization is apparently conserved in all known BPI and LBP sequences [20], and it seems likely that these two domains arose from a gene duplication event of a primitive monomeric ancestor [21]. Consistent with the two-domain organization, both BPI and LBP show distinct functional differences for their two domains. In the case of BPI, the N-terminal domain retains the bactericidal, LPS-binding, and LPS-neutralization activities of the intact protein [10, 13, 22]. The C-terminal domain shows some LPS-neutralization activity [10, 23] and has been implicated in opsonization of Gram-negative bacteria [24]. The N-terminal domain of LBP also carries the LPS-binding activity [25, 26], while its C-terminal domain is necessary for transferring LPS to CD14 [25].

LIPID BINDING POCKETS

Perhaps the most unexpected result of the determination of the structure of BPI was the discovery of two lipid binding sites [19]. During model building, two extended regions of electron density were discovered in deep, apolar pockets of the protein. Electrospray mass spectrometry determined the bound ligands to be phosphatidylcholine. The lipid-binding pockets are located on the concave surface of the boomerang, one in each domain near the interface of the barrels and central β-sheet. Although each pocket is found primarily in one domain of the protein, residues from both domains make contacts with each ligand. The phospholipid molecule binds with its acyl carbon chains buried in the core of the protein. Extensive van der Waals contacts are formed between the carbon tails and apolar side chains of the protein, whereas the zwitterionic headgroup lies at the opening of the pocket and is exposed to solvent.

The presence of apolar binding pockets in the BPI structure suggests that several related proteins may share similar modes of ligand binding. In the BPI–phosphatidylcholine complex, the most extensive protein–lipid contacts are made with the carbon tails of the lipid. This "tails-in, head-out" orientation of the bound phosphatidylcholine shields the apolar regions of the ligand from solvent, and would be consistent with lipid transport activity such as that shown by LBP [5, 6]. It is also consistent with the known relationship between BPI and LBP and two mammalian lipid transport proteins, CETP and PLTP. These two proteins regulate the size and composition of lipoprotein particles in the blood [27]. Although less closely related to BPI (~25% amino acid identity) than LBP, amino acid sequence comparisons indicate that CETP and PLTP share the major structural features of the family, including the two domain architecture, overall protein fold, and, presumably, the lipid binding pockets. The ligands transported by CETP and PLTP include fatty acids, cholesteryl esters, retinyl esters, and phospholipids. It seems likely that these ligands bind in apolar pockets similar to those seen in the BPI structure, allowing these poorly soluble compounds to be transported through the aqueous environment of the bloodstream.

An analysis of the amino acid sequences of BPI and LBP in light of the BPI crystal structure suggests that the pockets are of functional importance to both proteins. Multiple sequence alignments indicate that the most highly conserved regions of BPI and LBP cluster in the center of the protein, near the lipid binding pockets [20]. This evolutionary conservation implies that the pockets have functional significance. However, the known functions of BPI do not require phospholipid binding. In contrast, it has been shown that LBP can transfer LPS into certain phospholipid membranes [28, 29], and can also transfer phospholipids from membranes to high density lipoprotein particles [30]. In addition, several lines of evidence suggest that LPS and phospholipid may be exchanged in a reciprocal fashion by LBP. This highlights the intriguing possibility, already implied by the crystal structure, that the apolar pockets are involved in LPS-binding and/or transport, in addition to phospholipid binding.

From a structural standpoint, there are similarities between LPS and phosphatidylcholine (Fig. 3). Both BPI and LBP show high affinity binding to the lipid A region of LPS, its biologically active component. Lipid A is composed of a disaccharide headgroup substituted with a variable number of carbon tails, usually six [31]. It is conceivable that some of the carbon tails of lipid A could fit in the apolar pockets in a manner similar to the observed phosphatidylcholine. Therefore, we have proposed that the pockets might be sites of interaction between LPS and BPI. Biochemical data indicate other potential sites of interaction with LPS. LPS-binding peptides derived from the sequence of both BPI and LBP have been identified [32, 33]. In general, they are positively charged and map to areas at the tip of the N-terminal domain (left side of Fig. 2A). Mutants of several conserved, positively charged amino acids from this area of LBP show a decrease in LPS-binding and transfer activities [34]. The relative importance of the apolar pockets and positively charged tip remains to be determined, but it appears likely that both play some role in the function and action of these proteins

FUTURE DIRECTIONS

While the three-dimensional structure of BPI has provided important new insights, many questions remain about the mechanisms of action of BPI and LBP. For example, does lipid A bind in the apolar pockets? How does BPI neutralize LPS? Why is BPI bactericidal, and, conversely, why isn't LBP? Does BPI have lipid transfer activity like the other members of the family? Perhaps the most important contribution of the crystal structure of BPI will be as a platform for designing future studies. The detailed structural information provided allows the rational design of site-directed mutants, deletion mutants, BPI/LBP chimeras, and bioactive peptides, to name a few. Along with additional structural data, these studies should provide insights into

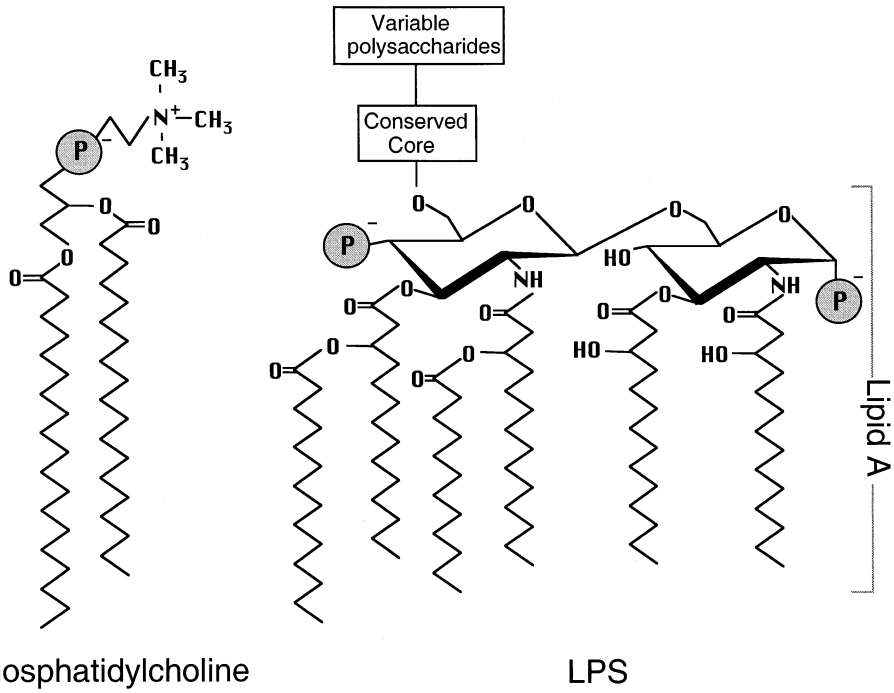

FIG. 3. Covalent structures of LPS and phosphatidylcholine. BPI and LBP bind with high affinity to lipid A, the highly conserved, bioactive fragment of LPS. Lipid A and phosphatidylcholine share some structural features including negatively charged phosphate groups (P), and acyl carbon chains. Reprinted with permission from Ref. 19 (Beamer LJ, Carroll SF and Eisenberg D, Crystal structure of human BPI and two bound phospholipids at 2.4 angstrom resolution. *Science* 276: 1861–1864, 1997). Copyright 1997 American Association for the Advancement of Science.

protein–LPS interactions and the mechanism of action of this expanding protein family. At the same time, they will provide clues about how proteins mediate the toxic effects of LPS and may help develop new therapies for sepsis and other complications of Gram-negative bacterial infections.

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References

1. Bone RC, The pathogenesis of sepsis. *Ann Intern Med* **115:** 457–469, 1991.
2. Wiessner W, Casey L and Zbilut J, Treatment of sepsis and septic shock: A review. *Heart Lung* **24:** 380–392, 1995.
3. Tobias PS, Soldau K and Ulevitch RJ, Isolation of a lipopolysaccharide binding acute phase reactant from rabbit serum. *J Exp Med* **164:** 777–793, 1983.
4. Schumann R, Kirschning C, Unbehaun A, Aberle H, Knopf H, Lamping N, Ulevitch R and Herrmann F, The lipopolysaccharide-binding protein is a secretory class 1 acute-phase protein whose gene is transcriptionally activated by APRF/STAT-3 and other cytokine-inducible nuclear proteins. *Mol Cell Biol* **16:** 3490–3503, 1996.
5. Tobias PS, Soldau K, Gegner JA, Mintz D and Ulevitch RJ, Lipopolysaccharide binding protein-mediated complexation of lipopolysaccharide with soluble CD14. *J Biol Chem* **270:** 10482–10488, 1995.
6. Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelly M, Busse LA, Zukowski MM and Wright SD, Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med* **179:** 269–377, 1994.
7. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ and Mathison JC, CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* **249:** 1431–1433, 1990.
8. Mathison JC, Tobias PS, Wolfson E and Ulevitch RJ, Plasma lipopolysaccharide (LPS)-binding protein: A key component in macrophage recognition of gram-negative LPS. *J Immunol* **149:** 200–206, 1992.
9. Appelmelk BJ, An Y-Q, Thijs BG, MacLaren DM and deGraaff J, Recombinant human bactericidal/permeability-increasing protein ($rBPI_{23}$) is a universal lipopolysaccharide-binding ligand. *Infect Immun* **62:** 3564–3567, 1994.
10. Ooi CE, Weiss J, Doerfler ME and Elsbach P, 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. *J Exp Med* **174:** 649–655, 1991.
11. Weiss J, Elsbach P, Olsson I and Odeberg H, Purification and characterization of a potent bacterial membrane active protein from the granules of human polymorphonuclear leukocytes. *J Biol Chem* **253:** 2664–2672, 1978.
12. White ML, Ma JK, Birr CA, Trown PW and Carroll SF, Measurement of bactericidal/permeability-increasing protein in human body fluids by sandwich ELISA. *J Immunol Methods* **167:** 227–236, 1994.
13. Horwitz AH, Leigh SD, Abrahamson S, Gazzano-Santoro H, Liu P-S, William RE, Carroll SF and Theofan G, Expression and characterization of cysteine-modified variants of an amino-terminal fragment of bactericidal/permeability-increasing protein. *Protein Exp Purif* **8:** 28–40, 1996.
14. von der Mohlen MAN, Kimmings AN, Wedel NJ, Mevissen MLCM, Jansen J, Friedmann N, Lorenz TJ, Nelson BJ, White ML, Bauer R, Hack CE, Eerenber AJM and van Deventer SJH, Inhibition of endotoxin-induced cytokine release and neutrophil activation in humans by use of recombinant bactericidal/permeability-increasing protein. *J Infect Dis* **172:** 144–151, 1995.
15. Elsbach P and Weiss J, Prospects for use of recombinant BPI in the treatment of Gram-negative bacterial infections. *Infect Agents Dis* **4:** 102–109, 1995.
16. Giroir BP, Quint PA, Barton P, Kirsch EA, Kitchen L, Goldstein B, Nelson BJ, Wedel NI, Carroll SF and Scannon PJ, Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeability-increasing pro-

tein in children with severe meningococcal sepsis. *Lancet* **350:** 1439–1443, 1997.
17. Weiss J, Purification and assay of bactericidal/permeability-increasing protein. *Methods Enzymol* **236:** 173–196, 1994.
18. Schumann R, Rietschel E and Loppnow H, The role of CD14 and lipopolysaccharide-binding protein (LBP) in the activation of different cell types by endotoxin. *Med Microbiol Immunol* **183:** 279–297, 1994.
19. Beamer LJ, Carroll SF and Eisenberg D, Crystal structure of human BPI and two bound phospholipids at 2.4 angstrom resolution. *Science* **276:** 1861–1864, 1997.
20. Beamer LJ, Carroll SF and Eisenberg D, The BPI/LBP family of proteins: A structural analysis of conserved regions. *Protein Sci* **7:** 906–914, 1998.
21. Beamer LJ, Fischer D and Eisenberg D, Detecting distant relatives of mammalian LPS-binding and lipid transport proteins. *Protein Sci* **7:** 1643–1646, 1998.
22. Ooi CE, Weiss J, Elsbach P, Frangione B and Mannion B, A 25-kDa NH-2 terminal fragment carries all the antibacterial activities of the human neutrophil 60-kDa bactericidal/permeability-increasing protein. *J Biol Chem* **262:** 14891–14894, 1987.
23. Abrahamson SL, Wu H-M, Williams RE, Der K, Ottah N, Little R, Gazzano-Santoro H, Theofan G, Bauer R, Leigh S, Orme A, Horwitz AH, Carroll SF and Dedrick RL, Biochemical characterization of recombinant fusions of lipopolysaccharide binding protein and bactericidal/permeability-increasing protein: Implications in biological activity. *J Biol Chem* **272:** 2149–2155, 1997.
24. Iovine NM, Elsbach P and Weiss J, An opsonic function of the neutrophil bactericidal/permeability-increasing protein depends on both its N- and C-terminal domains. *Proc Natl Acad Sci USA* **94:** 10973–10978, 1997.
25. Han J, Mathison JC, Ulevitch RJ and Tobias PS, Lipopolysaccharide (LPS)-binding protein, truncated at IIe-197 binds LPS but does not transfer LPS to CD14. *J Biol Chem* **269:** 8172–8175, 1994.
26. Theofan G, Horwitz AH, Williams RE, Liu P-S, Chan I, Birr C, Carroll SF, Meszaros K, Parent JB, Kasler H, Aberle S, Trown PW and Gazzano-Santoro H, An amino-terminal fragment of human lipopolysaccharide-binding protein retains lipid A binding but not CD14-stimulatory activity. *J Immunol* **152:** 3623–3629, 1994.
27. Tall A, Plasma lipid transfer proteins. *Annu Rev Biochem* **64:** 235–257, 1995.
28. Wurfel MM, Kunitake ST, Lichenstein HS, Kane JP and Wright SD, Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J Exp Med* **180:** 1025–1035, 1994.
29. Schromm AB, Brandenburg K, Rietschel ET, Flad H-D, Carroll SF and Seydel U, Lipopolysaccharide-binding protein mediates CD14-independent intercalation of lipopolysaccharide into phospholipid membranes. *FEBS Lett* **399:** 267–271, 1996.
30. Yu B, Hilman E and Wright SD, Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. *J Clin Invest* **99:** 315–324, 1997.
31. Raetz CRH, Biochemistry of endotoxins. *Annu Rev Biochem* **59:** 129–170, 1990.
32. Little RG, Kelner DN, Lim E, Burke DJ and Conlon PJ, Functional domains of recombinant bactericidal/permeability increasing protein ($rBPI_{23}$). *J Biol Chem* **269:** 1865–1872, 1994.
33. Taylor AH, Heavner G, Nedelman M, Sherris D, Brunt E, Knight D and Ghrayeb J, Lipopolysaccharide (LPS) neutralizing peptides reveal a lipid A binding site of LPS binding protein. *J Biol Chem* **270:** 17934–17938, 1995.
34. Lamping N, Hoess A, Yu B, Park TC, Kirschning C-J, Pfeil D, Reuter D, Wright SD, Herrmann F and Schumann RR, Effects of site-directed mutagenesis of basic residues (Arg 94, Lys 95, Lys 99) of lipopolysaccharide (LPS)-binding protein on binding and transfer of LPS and subsequent immune cell activation. *J Immunol* **157:** 4648–4656, 1996.