Peptides Neutralizing Lipopolysaccharide – Structure and Function

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Abstract: Lipopolysaccharide (LPS) induced Gram-negative sepsis and septic shock remain lethal in up to 60 % of cases, and LPS antagonists that neutralize its endotoxic action are the subject of intensive research. In the last decade peptidic antagonists have become increasingly important in providing leads for treatment of LPS-mediated diseases. In this review an overview of the sources, functions and structures of antiseptic and antibacterial peptides that interact with LPS is presented.

Dedicated to Professor Dušan Hadži on the occasion of his 80th birthday.

INTRODUCTION

A key feature of innate immunity in mammals is the ability to limit the infectious challenges rapidly [1]. Recognition of endotoxin or lipopolysaccharide (LPS) is an important function of innate immunity and may have profound consequences for the host [2]. Failure to contain the infection can result in Gram-negative sepsis as a result of the release of LPS [3]. Very low concentrations of LPS (0.01 nM) are required to incite septic shock, a multiple organ dysfunction syndrome that is associated with high mortality in intensive care unit patients; recent reports calculate that 0.1-0.3 million deaths are caused annually in the U.S. by sepsis [4]. Septic shock is initiated by mediators released from host cells stimulated by LPS, of which tumor necrosis factor α (TNF-α) appears to be the most important one [5]. Antibiotics used to treat the bacterial infection can actually be harmful because they can stimulate the release of endotoxin [6]. Therefore, there is substantial interest in identifying novel strategies to overcome endotoxic shock. Many strategies, including neutralizing antibodies, soluble cytokine receptors, various endotoxin binding factors and antibacterial agents that inhibit lipid A biosynthesis, have been tested with mixed results [7-11]. Tetracyclines were found to prevent the patho-physiological changes associated with LPS *in vivo*; however, recent results suggest that tetracycline activity *in vivo* is based on the induction of acute phase-like response which antagonizes the LPS-induced activity [12] rather than on the suppression of synthesis of inflammatory mediators. In this review we will shortly overview the recent developments involving LPS-neutralizing peptides that are either derived from natural antimicrobial peptides or from proteins that bind LPS. This strategy is increasingly important despite of known difficulties with rapid degradation of peptides in the organism, and provides leads to substances that may be clinically useful in the treatment of Gram-negative bacterial sepsis and shock.

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LIPOPOLYSACCHARIDE (LPS) AND THE MAIN LPS SIGNALING PATHWAY

Lipopolysaccharide (LPS) is the main constituent of the outer membrane of Gram-negative bacteria. It is an amphiphile, consisting of the lipid A, a core oligosaccharide and an O-specific chain. The lipid A moiety is the most highly conserved part of the structure, typically with two glucosamines, two phosphate esters and five to seven fatty acids (Fig. **1**). The core region contains several sugars, among them 3-deoxy-D-manno-2-octulosonate (KDO), unique to LPS [13]. Studies of synthetic LPS derivatives reveal that lipid A is responsible for the endotoxic activity. LPS responsive cell types are (i) monocytes / macrophages that release TNF- α and consequently activate the cytokine cascade that involves interleukin-1 (IL-1) and IL-6, (ii) polymorphonuclear leukocytes (PMNs) and (iii) endothelial and epithelial cells [14]. The biological analysis of synthetic lipid A partial structures proved that the expression of endotoxic activity depends on a unique primary structure and a peculiar endotoxic conformation [15]. Other findings confirmed that the effect of LPS was dependent on both the LPS polysaccharide chain length and the hydrophilic portion of LPS [16].

The primary pathway whereby the non-immune host recognizes LPS and mounts inflammatory responses involves LPS complexed with LPS-binding protein (LBP), a plasma protein, that binds to CD14 leading to cell activation at pico/nanomolar LPS concentration. LBP is a 60 kDa serum glycoprotein synthesized in hepatocytes that binds with high affinity to lipid A [17]. It lowers the stimulatory dose of LPS and increases the rate of cellular responses to LPS; however, acute phase LBP in mice has a protective effect against LPS and bacterial infection [18]. LPS is transferred to sCD14 by LBP via an ordered ternary complex reaction model [19]. LBP probably has both an LPS binding domain and another domain which is required for transfer of LPS to CD14 [20]. Recently evidence was accumulated that Toll-like receptor 4 (TLR 4) is a signal transducing receptor that is activated by LPS in a response that depends on LBP and is enhanced by CD14 (for recent reviews see [21-23]).

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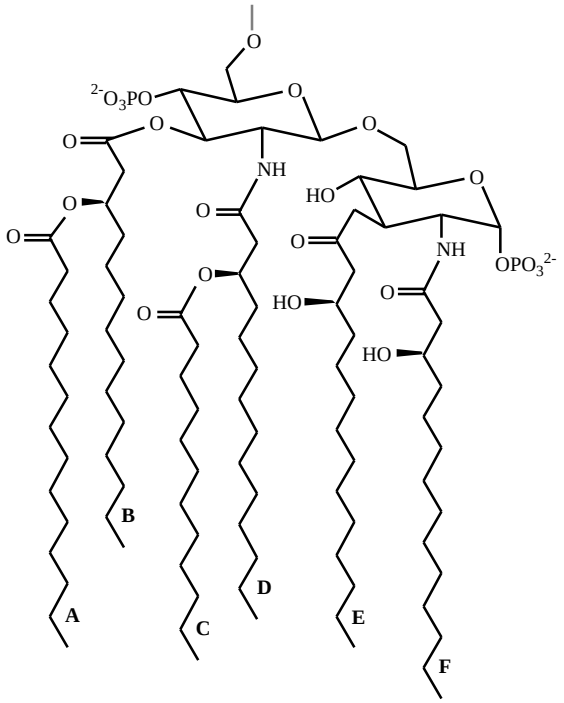

Fig. (1). Chemical structure of lipid A as found in *E. coli* strains.

CD14 is a 55 kDa protein that is found either anchored to the membrane (mCD14) of myeloid cells by its glycosylphosphatidyl inositol (GPI) moiety, or in free form in the serum lacking the GPI anchor (sCD14). The apparent dissociation constant for LPS binding to CD14 is $4\text{-}5x10^{-8}$ M^{-1}; mCD14 mediates rapid uptake of isolated LPS into isolated human monocytes [24]. CD14 plays a key role in initiating cell activation by a group of bacterial envelope components of Gram-negative and Gram-positive microorganisms, as well as mycobacteria, making it a cantidate for a pattern recognition receptor where common cellular recognition pathways might be involved in responses to molecules with similar structural features from a variety of pathogens [25]. According to the far UV circular dichroic (CD) spectrum, CD14 contains a substantial share of β-sheet secondary structure [26]. The molecular basis for therapeutic concepts utilizing CD14 were extensively studied [27]. The smallest fully functional fragment of CD14 generated up to now consists of 152 residues, comprising the amino terminal domain and 3 LRR fragments [28]. It was successfully expressed and refolded in *E. coli* and *P. pastoris* [29].

PROTEINS AND PEPTIDES THAT INTERACT WITH LPS

A large number of proteins binds to LPS [30], i.e. intracellular, cell membrane and plasma proteins, among them lysozyme that actually detoxifies LPS [31-32]. In this review we will concentrate on proteins that have been the source of anti-endotoxic peptides.

Over 500 peptides have been reported to participate in innate immunity in all multicellular organisms that were investigated, including plants, insects and humans [2],[33]. These potent broad spectra peptides are included as immediate effector molecules in innate immunity. In mammals, antimicrobial peptides are represented by two families, the defensins and the cathelicidins [34]. The defensins are a group of compact (3-5 kD) protease-resistant molecules with three or four disulphide bridges linking β-sheet secondary structure, but may contain an α-helical segment near the N-terminus [35]. Other antimicrobial peptides encompass a wide variety of structural motifs [36]. Many peptides have alpha-helical structures. The majority of these peptides are cationic and amphipathic but there are also hydrophobic alpha-helical peptides which possess antimicrobial activity. In spite of the structural diversity, a common feature of the cationic antimicrobial peptides is that they all have an amphipathic structure which allows them to bind to the membrane interface. Antimicrobial peptides are generally not cytotoxic at concentrations where they kill microorganisms (see [2] and references therein). The detailed killing mechanism for these effectors is partly known, but nearly all of them have membrane affinity, and permeate bacterial membranes, resulting in lysis of the bacteria [37], [34], [38].

Two α-helical cationic peptides, MBI-27 and MBI-28 (Table **1**), derived from parts of the silk moth cecropin and bee mellitin peptides, have displayed antiendotoxic as well as antimicrobial activity *in vitro* and *in vivo* in animal models [39]. A series of synthetic α-helical cationic antimicrobial peptides displayed considerable variations in their ability to bind LPS, which correlated significantly with their microbicidal activity [40].

Vasoactive intestinal peptide (VIP), a neuropeptide synthesized by immune cells, and the structurally related neuropeptide pituary adenylate cyclase-activating polypeptide (PACAP38), were found to rapidly and specifically inhibit the LPS-stimulated production of TNF-α [41]. The latter peptide also reduced the LPS-induced neurotoxicity in mixed cortical neuron/glia cultures [42]. A synthetic LPS-binding peptide based on amino acids 27-39 of serum amyloid P component (SAP, Table **1**) inhibited LPS-induced response in human blood, although the parent protein does not neutralize LPS and its role in the pathophysiology of Gram-negative infections has yet to be elucidated [43].

A variety of polycationic compounds including gentamicin and streptomycin was shown to bind LPS [44]. The interaction of melitin, an amphipathic and basic 26-residue polypeptide isolated from bee venom, was characterized by fluorescence methods [45]. Several classes of cationic amphiphilic drugs including phenothiazines, aminoquinolines, biguanides and aromatic diamidines interact with lipid A; the sequestration of endotoxin by pentamidine reduces its propensity to bind to cells, and the complex exhibits attenuated toxicity in biological assays [46]. Compounds in a series of amine- and guanidine-functionalized cholic acid derivatives, designed to mimic the activities of PmB, act both as potent antibiotics and effective permeabilizers of the outer membranes of Gram-negative bacteria, some even rivaling PmB in antibacterial activity [47]. Lipopolyamines were demonstrated to bind LPS; DOSPER, a commercially available transfection agens, was

Table 1. Primary Sequences of Peptides That Bind and Neutralize Lps. Underlined Letters Denote Cycle Closure

$BPI_{82\text{-}108}$ [83]	NANCKISGKWKAQKRFLKMSGNFDCSI
BU3* [80]	*HIKELQV*KWKAQKRFLKMS*IIVKLNDGRELSLD*
$CAP18_{106\text{-}137}$ [84]	GLRKRLRKFRNKIKEKLKKIGQKIQGLLPKLA
$CAP37_{20\text{-}44}$ [70]	NQGRHFCGGALIHARFVMTAASCFQ
$LALF_{28\text{-}54}$ [83]	DHECHYRIKPTFRRLKWKYKGKFWCPS
$LBP_{82\text{-}108}$ [83]	DSSIRVQGRWKVRKSFFKLQGSFDVSV
$LBP_{91\text{-}105}$ [77]	WKVRKSFFKLQGSFD
$LBP_{91\text{-}108}$ [77]	WKVRKSFFKLQGSFDVSV
LF-33 [96]	GRRRRSVQWCAVSQPEATKCFQWQRNMRKVRGP
MBI-27[39]	KWKLFKKIGIGAVLKVLTTGLPALIS
MBI-28[39]	KWKLFKKIGIGAVLKVLTTGLPALKLTK
PmB** [53]	mo-K'TK'K'K'fLK'K'T
SAP [43]	EKPLQNFTLCFRA

* the italicised sequences are derived from the β-turn initiation sequences from IL-8 [102].
** mo: methyl octanoate; K': diaminobutyric acid; f: D-Phe.

confirmed as a novel antiendotoxin compound that reduced mortality in experimental sepsis caused by Gram-negative bacteria [48].

Lipopeptides - Polymyxin

Various microorganisms produce lipopeptides [49] that are powerful antibiotics with antifungal activities [50]. Their biosurfactant properties [51] result from a combination of polar (sugars, amino acids, phosphates or alcohol and esther groups) and apolar (frequently a hydrocarbon chain) structural elements in the molecule. Synthetic lipopeptides based on bacterial lipoprotein are efficient activators for monocytes/macrophages [52].

The polymyxins, a class of lipopeptides produced by *Bacillus polymyxa*, have important anti-endotoxic properties. Investigations of the interactions between LPS (or lipid A) and oligopeptides were motivated by the finding that polymyxin B (PmB), a cyclic, cationic peptide antibiotic, binds to lipid A [53] with an apparent dissociation constant in the μM range [54] and neutralises its pathogenicity. In an early review discussing LPS antagonists PmB was the only peptide mentioned [55]. From there on the number of peptidic antagonists has vastly increased. The antibiotic action of PmB could be accounted for by its ability to direct intermembrane exchange [56]. Polymyxin E differs in only one amino acid residue at position 6 and displays similar biological functions; however, PmB but not PmE inhibits insulin mediated hypoglycemia [57]. Unfortunately, PmB is toxic and can not be used for therapy. However, experiments with rats show that PmB administered intramuscularly in low doses may improve the mortality of sepsis [58], and PmB-dextran 70 has shown potential for treatment of horses with endotoxemia when used in combination with a cyclooxygenase-inhibiting drug [59]. A series of peptides designed to mimic the primary and secondary structure of PmB was synthesised and tested for binding and detoxification of LPS / lipid A [60], [61]. Multiple factors were responsible for optimal binding of peptide structures to lipid A, including the amphipatic and cationic features of the primary structure, the size of the structure and the peptide conformation. Binding affinity, *per se*, is an unsatisfactory predictor of endotoxin-neutralising activity, since ligands may either opsonize or sequester the toxin. Strict structural or stereochemical requisites are probably not critical in LPS-binding ligands. Basicity and hydrophobicity of peptides are important structural features in determining their endotoxin-binding properties. The binding of the peptides to lipid A appears to be relatively independent of their amino acid sequence suggesting that the interactions are not sensitive to the conformations of the non-bound peptides.

Another study using linear polycationic amphiphilic peptides suggested that antibacterial and LPS neutralising activities are dissociable, which might be of value in designing LPS-sequestering agents of low toxicity [62]. A polycationic, amphiphilic, terminally branched oligopeptide $K(K)KLVFLYG\text{-}NH_2$ closely resembled the effects of PmB with regard to its interaction with lipid A/LPS and attenuated LPS activity in cytokine release, but was devoid of microbicidal activity against Gram-negative bacteria.

Little structural information obtained by spectroscopic methods exists for the peptides and the peptide – LPS / lipid A complexes. Initial NMR studies were focused on free PmB in water solution [63],[64]. Conformations of PmB analogues in dimethylsulphoxide from NMR spectra and molecular modelling have been proposed [65]. Recently, an NMR and molecular dynamics (MD) study of polymyxin B-nonapeptide (PmBN), a PmB analogue lacking the mo-Dab 1 residue, has been published [66] and a model of the PmB-lipid A complex proposed that was based on the LPS bound PmBN conformation derived from transferred NOE [67]

data, and on electrostatic contacts of the γ-amino and the phosphate groups. However, a refined knowledge of interactions is desirable since the determination of the thermodynamic properties of LPS - PmB complexes using calorimetric titration indicates that complexation is entropically driven [68], thus confirming previous assumptions of its hydrophobic nature [53]. The study was repeated using PmB and PmE that also showed the transferred NOE effect in mixture with LPS. The structure of the bound peptide could be determined with NOE refinement. It turned out to be amphiphilic, clearly separating the two hydrophobic residues in the seven-membered cycle from the positively charged Dab side-chains by an envelope-like fold of the cycle [69]. A model of the peptides bound to lipid A was proposed that decreases the total hydrophobic area of both molecules and thus explains the entropy-driven binding of the polymyxins to LPS.

CAP37

The oxygen-independent bactericidal action of human neutrophil polymorphonuclear leukocytes is due to a number of potent antimicrobial cationic proteins [70], (i) serine proteases (24-29 kDa) such as cathepsin G, elastase and p29b, (ii) cationic antimicrobial protein CAP37 (HBP, azurocidin, BP30) of 37 kDa, and (iii) bactericidal/permeability increasing protein (BPI) or CAP57 (55-57 kDa).

CAP37 is a multifunctional, LPS binding protein of 37 kDa with antimicrobial activity that acts as mediator of the second wave of inflammation [71]. A synthetic bactericidal peptide based on CAP37 was shown to mimic its antibiotic and LPS binding action [70]. It also significantly inhibited the endotoxin-induced TNF-α release in sensitized rats [72]. It comprises residues 20-44 (Table **1**) that are 56% hydrophobic and possess a net charge +2. LPS/lipid A preparations inhibited antibiotic action strongly suggesting that antibiotic and lipid A binding domains are the same. The disulfide bridge between C26 and C42 turned out to be necessary for biological activity. Maximum antibacterial activity was observed at pH 5.0-5.5; at pH 7.0 the activity was attenuated. The structure of CAP37 was solved by X-ray crystallography; the 20-44 fragment consists of two anti-parallel β-strands in the full length protein [73].

An *S. typhimurium* strain, SH7426, resistant to PmB was also resistant to CAP37 and $CAP37_{20-44}$. This bacterial phenotype may be due to substitution of 4-aminopentose on the acidic phosphate groups of lipid A, reducing the negative charges in the outer membrane and leaving fewer putative binding sites for CAP37. Studies of $CAP37_{20-44}$ with dipalmitoyl-phosphatidylcholine membranes may suggest that the antibiotic action of the molecule is effected through its interaction with the lipid components of the Gram-negative bacterial membrane [74]; however, phosphatidylcholines are only present in traces in the latter.

LBP

LPS binding proteins play a pivotal role in physiology as well as pathophysiology of Gram-negative infection by mediating the binding of minute amounts of LPS to membrane-bound CD14 triggering a proinflammatory response of macrophages [75] (see also description in »LPS signaling pathway«); additionally, it plays a critical role in clearance of bacteria by phagocytosis by alveolar macrophages and is essential for survival after bacterial challenge [76]. In the intact protein, LBP residues 91-108 play a critical role in the formation of specificity to LPS, forming at least part of the LPS binding site. Two overlapping 15-mer peptides were identified, LBP_{91-108} and LBP_{91-105}, that specifically bound lipid A with high afinity and blocked release of TNF α following LPS challenge both *in vivo* and *in vitro* [77].

BPI

Bactericidal/permeability increasing protein (BPI) is a 55 kDa protein causing bacterial killing and endotoxin neutralisation. It binds to LPS and Gram-negative bacteria via LPS. Contrary to LBP it is directly bactericidal. The crystal structure was solved at 2.4 Å resolution showing two apolar pockets on the concave surface that each bind a molecule of phosphatidylcholine primarily by interacting with their acyl chains, suggesting that the pockets might also bind the acyl chains of LPS [78]. Overall differences in charge and electrostatic potential between BPI and LBP suggest that BPI's bactericidal activity is related to the high positive charge of its N-terminal domain [79].

Small synthetic peptides based on the amino acid sequence of the LPS binding domain of BPI neutralize LPS, albeit inefficiently; BU3 (Table **1**), a hybrid peptide on the basis of a portion of the LPS binding domain from BPI and amino acids known to initiate a β-turn, displayed enhanced LPS neutralization [80]. This data demonstrated that the biological activity of peptides may be optimised via manipulation of its structure.

LALF

Another source of potential peptide ligands to lipid A was the limulus anti-LPS factor (LALF), a small (101 amino acids) basic protein which binds and neutralizes LPS; its crystal structure has been determined at 1.5 Å [81]. It has a single domain consisting of three α-helices packed against a four-stranded β-sheet. The binding site for LPS (residues 32-50) that involves an extended amphipathic loop was also proposed for LBP and BPI (residues 86-104) based on sequence alignment. The loop of LALF is distinguished by an alternating series of positively charged and hydrophobic residues that, by virtue of the extended β-conformation, point in opposite directions and maintain the amphipathicity.

The proposed LPS-binding domains of LBP, BPI and LALF are interchangeable in the context of a whole protein model, demonstrating their structual similarity [82]. Although discrete point mutations within the LPS-binding domain of LBP disrupted its specfic functions, the hybrid proteins were still able to bind LPS and, in addition, retained the wild type LBP activity of e.g. transferring LPS aggregates to CD14.

Three peptide sequences of the proposed LPS-binding motif from BPI, LALF and LBP, each 27 amino acids in length, were synthesized, namely BPI_{82-108}, $LALF_{28-54}$ and LBP_{82-108} (Table **1**); all three peptides significantly inhibited LPS-induced TNF α secretion by macrophages [83].

CAP18

CAP18 (18 kDa cationic antimicrobial protein) is an LPS binding protein of 142 residues first isolated from rabbit granulocytes. It possesses no homology to BPI. Its C-terminus of 37 amino acids (residues 106-142, Table **1**) has LPS-binding activity; an even more potent region was identified between residues 106 and 137. Recently a 21-mer peptide (residues 106-126) with potent activity against Gram-positive and -negative bacteria was synthesized (Table **1**). The CD spectra indicated an unordered state in absence of lipid A; with addition of lipid A the helical content increased. In NMR severe line broadening was observed that made structure determination impossible. In presence of 30% TFE the model structure of the peptide was found to be a complete and very rigid helix [84] which is probably also true in contact with lipid A [85]. The major impetus for forming the helical structure is to form charged or hydrophobic patches and stripes. The human CAP18 C-terminal fragments $CAP18_{104-140}$ and the more truncated $CAP18_{104-135}$ were shown to inhibit LPS-induced release of nitric oxide from macrophages and protect mice from LPS lethality [86]. The synthetic fragment $CAP18_{109-135}$ was capable of preventing antibiotic-induced endotoxic shock in mice with septicemia due to its LPS-neutralizing activity rather than to its antibacterial properties [87].

Lactoferrin/Lactoferricin

Lactoferrin (Lf), a multifunctional 80 kDa iron-binding glycoprotein found in exocrine secretions of mammals, is associated with host defense through its antibacterial properties [88]. The bactericidal effect is exhibited by means of reducing the amount of iron available for the microorganisms during growth, and destabilizing of the outer membranes of Gram-negative bacteria. Lf binds directly to isolated lipid A and decreases its endotoxicity [89]. The loop region of 28-34 interacts with LPS and competes with LBP in serum for its binding, therefore interfering with the interaction of LPS with CD14. The inhibition of Lf-LPS interaction by a synthetic octadecapeptide corresponding to residues 20-37 of human Lf revealed the importance of the 28-34 loop region of Lf for LPS binding [90]. Basic sequences homologous to residues 28-34 of hLf were evidenced on LPS-binding proteins such as LBP, BPI and LALF [91]. Pepsin digestion of Lf releases antibacterial peptides named lactoferricin comprising residues from the N-terminus. Synthetic peptides corresponding to the loop region of human lactoferricin (residues 20-35) exerted significant antibacterial effects against *E. coli* [92]. The solution structure of bovine lactoferricin (LfcinB), a 25-residue antimicrobial peptide that also binds LPS, was determined using NMR spectroscopy. The NMR structure revealed a somewhat distorted β-sheet, in contrast with the X-ray structure of bovine Lf in which residues 1-13 (of LfcinB) form an α-helix [93]. The structure of the antimicrobial center of LfcinB, LfcinB4-9, in sodium dodecyl sulphate (SDS) micelles has been determined by NMR and molecular dynamics refinement; the peptide adopts a well defined amphipathic structure when bound to SDS [94]. A series of peptides derived from sequences from human, bovine, murine and caprine Lf was prepared, and novel peptides with enhanced antibacterial activities were prepared with sequences designed by molecular modeling and structure activity studies [95]. LF-33, a human lactoferrin-derived 33-mer peptide (Table **1**) representing the minimal sequence for lactoferrin binding to glycosaminoglycans, dramatically reduced the lethality of LPS in the galactosamine-sensitized mouse model, demonstrating its potential use for the treatment of endotoxin-induced septic shock [96]. Recently a method for the production of recombinant peptides in *E. coli* has been described for human Lf_{21-31} that allows expression and isotope labeling of peptides that are toxic to bacteria [97].

CONCLUSIONS AND OUTLOOK

Proteins involved in LPS signaling pathway, recognition and neutralization, together with natural cationic antibiotic peptides, provide leads to natural or synthetic peptides that display similar actvity. Structural studies of antiseptic peptides in complex with LPS using NMR may lead to an understanding of LPS action at the atomic level. The experimental work, however, is difficult because of the amphiphilic nature of the peptides that tend to aggregate at concentrations necessary for measurement; additionally, conditions for the transferred NOE effect are not always attainable. New microbiological techniques allow expression and isotope labeling of bactericidal peptides in bacteria without killing them [97], thus opening avenues to NMR studies of structure and interactions in complex with LPS. The interaction of LPS with its cognate binding proteins has been structurally elucidated in the single case of the X-ray crystallographic structure of LPS in complex with the integral outer membrane protein FhuA from Escherichia coli K-12 [98]. A subset of four out of eight positively charged residues of FhuA that provide most of the important hydrogen-bonding or electrostatic interactions with LPS was identified that is common to known LPS-binding proteins. These four residues, three of which form specific interactions with lipid A, appear to provide the structural basis of pattern recognition in the innate immune response [99]. Their arrangement can serve to identify LPS-binding sites on proteins known to interact with LPS, and could serve for design of an LPS scavenger. The molecular mechanisms of biological activity of bacterial endotoxins can additionally be probed by theoretical means, as attempted by comparing molecular modeling results for two possible mechanisms with the underlying experimental data suggesting that specific binding of lipid A to a protein receptor is energetically more favorable than nonspecific intercalation into the phospholipid membrane of a host cell [100]. Identification of conserved LPS-binding regions within each protein may provide clues for the development of new immunomodulatory reagents for use as adjuvant therapy in the treatment of Gram-negative bacterial sepsis. As the understanding of the TLR 4-LBP-CD14 pathway unfolds

new targets that modify these pathways may be effective lead compounds in the treatment of septic shock, and the ability to produce both insect and mammalian peptides and the corresponding peptidomimetics with better specificity and therapeutic potential [101] may provide new classes of antibiotics.

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ABBREVIATIONS

BPI	=	Bactericidal/permeability increasing protein
CAP37	=	Cationic antimicrobial protein of 37 kDa
CD	=	Circular dichroism
LALF	=	Limulus anti-LPS factor
LBP	=	LPS-binding protein
Lf	=	Lactoferrin
LPS	=	Lipopolysaccharide
PmB	=	Polymyxin B
TLR 4	=	Toll-like receptor 4
TNF-α	=	Tumor necrosis factor α

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