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Review

Lipopolysaccharide-binding molecules: transporters, blockers and sensors

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Abstract. Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, can be beneficial to the host by activating the innate immune system, or harmful, by inducing inflammation, disseminated intravascular coagulation, multiple organ failure, shock and often death. On the bacteria, and in host biological fluids and cells, LPS is never free but constantly attached to cognate-binding proteins. Understanding how LPS is transported and further recognized by sensors able to deliver a signal, or by inactivating molecules able to neutralize its biological effects, is an important goal. This review describes the large panel of peptides and proteins reported to associate with LPS, and provides information on their origin, their structure and the location of amino acid residues involved in their interaction with LPS. A better understanding of the mode of recognition of LPS by cognate proteins prompted many laboratories to design on a rational basis synthetic molecules which can be used to detect low amounts of endotoxin, or to act as efficient blockers of in vitro and in vivo responses to LPS.

Key words. Lipopolysaccharides; endotoxin; binding; antimicrobial; defense.

Introduction

Lipopolysaccharides (LPSs) are amphiphilic molecules present on the outer leaflet of Gram-negative bacteria. Despite a great compositional variation depending on their particular bacterial origin, they all consist of a hydrophilic polysaccharide part covalently bound to a hydrophobic lipid component termed lipid A. The polysaccharide is composed of a very variable O-specific chain defining the serologic specificity of the LPS, and of a core oligosaccharide. The core is linked to lipid A via the acidic deoxysugar 3-deoxy-2-octulosonic acid (Kdo). One of the shortest LPS (Re-LPS) consists of lipid A and two Kdo units. The lipid A component is often composed of a 1,4′-bisphosphorylated glucosamine disaccharide that carries several amide- and ester-linked fatty acids [1]. Lipid A has been shown to constitute the bioactive centre of LPS, since free natural or chemically synthesized lipid A can reproduce the biological effects of LPS.

LPS is certainly one of the most potent microbial inducers of inflammation, and of a cascade of physiological events which may lead to toxic shock and death [2]. However, low amounts of LPS permanently access to body fluids and organs (via the airways or by translocation from gut bacteria) and prove to be beneficial for the host by stimulating its resistance to infection and malignancy. Exposure to low doses of LPS can also result in a transient state of refractoriness to LPS itself, termed endotoxin tolerance [3].

At the molecular level, a prerequisite for the induction of harmful as well as beneficial host responses to LPS is its interaction with LPS-binding molecules. The association of LPS with these molecules likely dictates whether LPS will lead to beneficial or harmful effects. It should be noted that the very first LPS-binding molecule is LPS itself. Indeed, early after the development of techniques of extraction and purification, it has been recognized that because of their amphiphilic nature, LPS molecules asso-

ciate together in aqueous media to form aggregates [4], and this explains why in the past the LPS mass was estimated in millions, whereas it is now established that LPS monomers are actually in the range of 2–20 kDa. Later, other molecules able to interact with LPS have been found, and this still growing family has now reached a considerable size.

A first group of molecules that recognize LPSs are lectins directed against the O-specific polysaccharide chain or the core region of particular LPSs. This is, for example, the case of short-tail fibre proteins of bacteriophages [5], of the cystic fibrosis transmembrane conductance regulator (CFTR) of lung epithelial cells [6], of horseshoe crab tachylectins [7], of galectin-3 (Mac-2 antigen), a β-galactoside-binding lectin of mammalian cell [8], of the mannose receptor [9] and of the mannose-binding lectin (MBL), a component of the lectin pathway of complement activation [10]. All these proteins, as well as antibodies directed against LPS O-chains, cannot be classified sensu stricto as LPS-binding molecules inasmuch as they interact only with a restricted group of LPSs: those carrying an appropriate carbohydrate determinant.

A second group of molecules that interact with LPS are enzymes involved in its degradation, such as the lysosomal phosphatase involved in LPS catabolism [11] and the granule acyloxyacyl hydrolase which removes the secondary acyl chains of LPS [12]. It should be noted that acyloxyacyl hydrolase belongs to a family of saposin-like proteins (SAPLIP) involved in a variety of functions associated with the binding or interaction of lipids [13].

However, the most important LPS-binding molecules, on which we will particularly focus in this review, are those belonging to a third group of proteins that interact with the biologically active lipid A region of LPS, but do not directly degrade it.

Bacterial LPS-binding molecules (FhuA, OmpT, MsbA, Polymyxin B)

Bacterial proteins able to interact with LPS are either constitutive proteins of Gram-negative bacteria, required for their survival, or molecules produced by other (Gram-positive) microorganisms and aiming to destroy Gram-negative bacteria. Among the first group of molecules, we can mention components which are often associated to LPS preparations as contaminants, such as lipoproteins [14], or the 'lipid A-associated protein' described by Sultzer and consisting of a lipid-containing protein resistant to various proteases [15].

Other more extensively studied constitutive proteins of Gram-negative bacteria which interact with the lipid A region of LPS are FhuA, OmpT and MsbA. FhuA is found on the surface of *Escherichia coli* and belongs to a family of proteins that mediates the active transport of siderophores, such as ferrichrome, into Gram-negative bacteria. It consists of a C-terminal β barrel and an N-terminal cork that fills the barrel interior. FhuA has been shown to bind LPS by both electrostatic interactions via eight positively charged residues, and numerous van der Waals contacts between hydrophobic side chains of FhuA and acyl chains of LPS [16]. Like FhuA, OmpT is found on the surface of *E. coli*. It is an outer-membrane protease with a 10-stranded antiparallel β-barrel structure. It contains an LPS-binding site on the exterior of the barrel (homologous to that found on FhuA), and ligation of LPS is strictly required for the enzymatic activity of OmpT [17]. This proteolytic activity is probably involved in the cleavage of antimicrobial peptides, and thus in the virulence of the bacteria [18].

A third important component, essential for bacterial viability, is MsbA, a 'lipid flippase' which facilitates the export of lipid A [19]. Its amino acid sequence is remarkably similar to those of several mammalian P-glycoproteins involved in multidrug resistance, so that it can be considered as a member of the superfamily of transporters that contain an ATP binding cassette (ABC), also called a nucleotide binding domain (NBD). In bacteria, ABC transporters considerably limit antibiotic effectiveness by exporting them as fast as they enter the cells, and in humans, ABC transporters export chemotherapy drugs and thus increase mortality rates in cancer patients. A possible mechanism of action of bacterial MsbA has been proposed by Chang [20] (fig. 1). MsbA consists of two monomers forming a chamber. The inner membrane part of the chamber contains a cluster of positively charged residues allowing the binding of lipid A. This binding induces tertiary arrangements of transmembrane α helices, triggering ATP hydrolysis. Then, the lipid A molecule flips to the outer membrane leaflet side of the chamber where it forms hydrophobic interactions, and becomes properly oriented for insertion into this outer membrane. The expulsion of lipid A to the outer membrane is the final step of the transport mechanism, and occurs when structural rearrangements triggered by the substrate flipping reposition MsbA in its original conformation. Therefore, MsbA represents a good example of a complex interaction involving entropically driven binding, flipping, and expulsion of lipid A.

It has also been reported that the exotoxin of the Gram-negative bacteria *Bordetella pertussis* (pertussis toxin) can bind LPS. This interaction is mediated through the disaccharide backbone of the lipid A region of LPS, and involves the S2 subunit of the pertussis toxin, which has a high degree of amino acid sequence similarity with lysozyme [21].

The second type of bacterial LPS-binding molecules are antibiotic-type molecules produced by some Gram-positive bacteria. These microorganisms with only one external membrane appeared before Gram-negative bacteria,

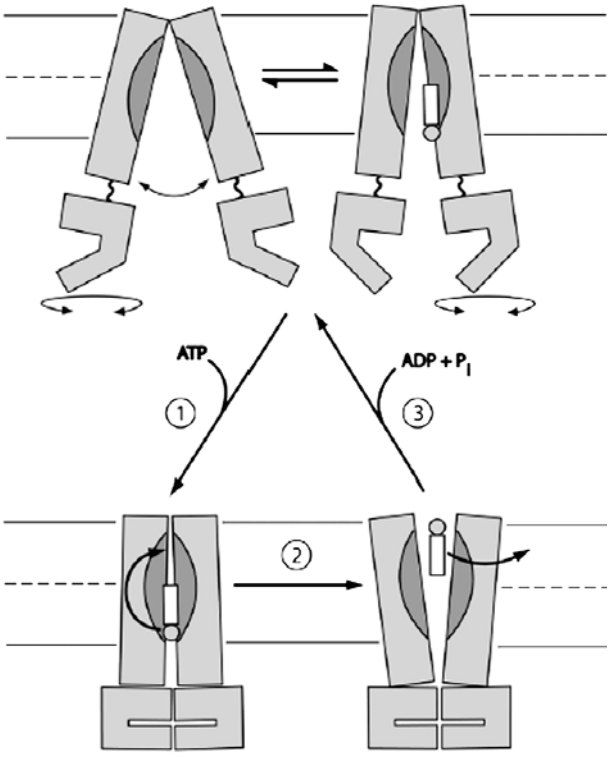

Figure 1. Model for lipid A transport by MsbA. From [20], with permission of Elsevier Science Ltd, copyright 2003. Lipid A binds to a cluster of positively-charged residues in the chamber formed by the two monomers of MsbA (top). This triggers ATP hydrolysis and closure of the chamber (1). Then, lipid A flips inside the chamber and translocates to the outer membrane level (2). After nucleotide exchange and chamber opening, lipid A is then expulsed into the outer bilayer leaflet (3).

which have two membranes and LPS on the outermost layer of their external membrane. Probably as soon as the first Gram-positive bacteria had to compete with the first Gram-negative bacteria, some 3.5 billion years ago, they selected the first weapons against them, and these weapons were of course directed against the newly emerged molecule LPS. An example of one of these weapons is polymyxin B (PMB). This compound is produced by *Bacillus polymyxa*, a Gram-positive organism living in soil. PMB is an antibiotic consisting of a cyclic and cationic decapeptide with five positive charges, and an N-linked fatty acid tail (fig. 2A). PMB binds to the LPS of Gram-negative bacteria, changes the packing order of LPS and increases the permeability of the outer membrane to a variety of molecules, including PMB itself (self-promoted uptake of PMB) [22, 23]. PMB binds LPS by a two-step process involving electrostatic attraction between charged parts of the molecules, followed by hydrophobic interactions between their nonpolar regions [24, 25] (fig. 2B). Another bacterium-derived cyclic antimicrobial peptide, gramicidin S, binds LPS with a four-times lower affinity than PMB.

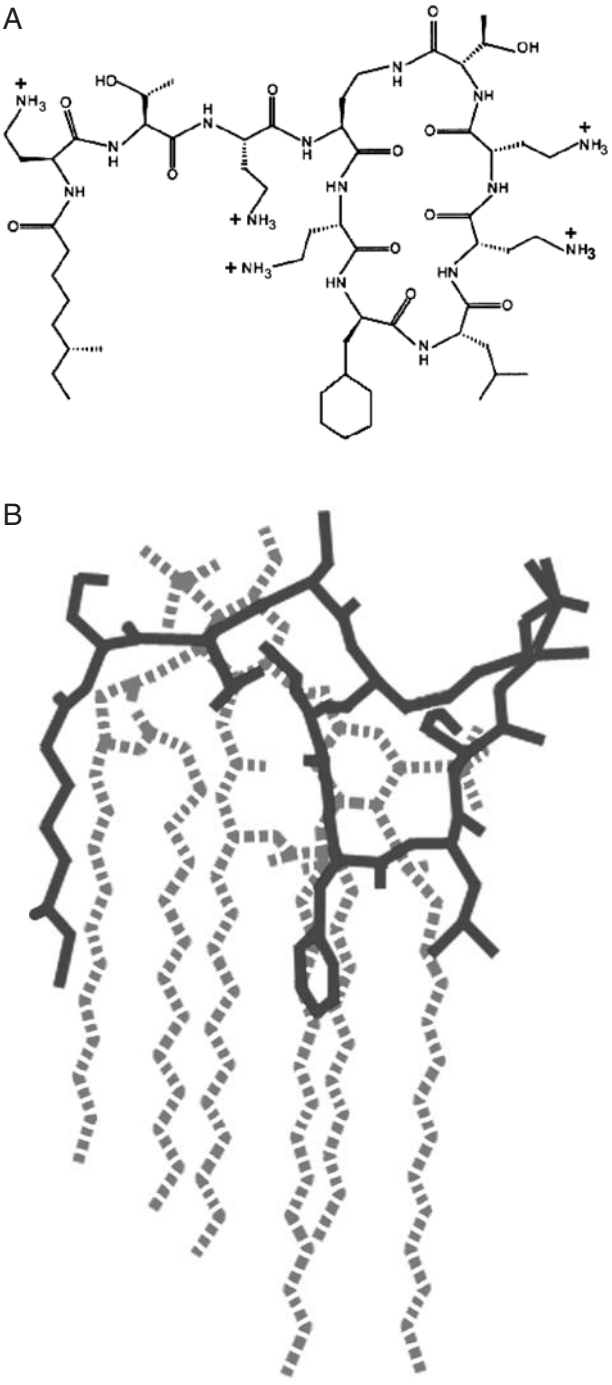

Figure 2. Polymyxin B alone (*A*) and docked to lipid A (*B*). Lipid A is represented in dotted gray line. Modified from [25], with permission of the American Chemical Society, copyright 1999.

It should be noted that in specific growth conditions, some strains of enterobacteria become resistant to PMB. This was shown to be due to the addition of L-4-amino-4-deoxy-arabinopyranose (L-Ara*p*4N) to the phosphate at position 4′ of their lipid A. Therefore, this additional positive charge prevents the interaction between PMB and lipid A [26].

Molecules produced by insects and amphibians

Of course, later during evolution, all other species improved the faculty to produce molecules able to interact with LPS. In insects, we can mention cecropin in *Drosophila* hemolymph [27, 28] (fig. 3A), sarcotoxin IA from flesh fly [29], melittin in bee venom [30] and attacin in silkmoth [31], which all bind LPS. Two synthetic α-helical peptides (MBI-27 and MBI-28) derived from parts of cecropin and melittin bind LPS with an affinity equivalent to that of polymyxin B [32]. Anti-LPS peptides have also been isolated from the skin of amphibians. This is the case of magainin 2 [33], which contains, like cecropin, a basic and amphipathic α-helix motif (GKWKAQKRFLKM) with LPS-binding capacity.

The coagulation system, from arthropod hemolymph to mammalian plasma

Early in species evolution, invertebrates have developed an innate immune system that responds to surface components of potential pathogens. A very ancient arthropod on the evolutionary scale is the horseshoe crab *Limulus polyphemus*. Large granules of its hemocytes contain a factor called *Limulus* factor C, which is released in response to invading microbes via exocytosis. Factor C interacts with minute amounts of LPS, and this feature is used in gelation or colorimetric assays for the detection of LPS. It is a large (109 kDa) protein with an N-terminal LPS binding domain, and a C-terminal serine protease domain. The N-terminal domain contains actually three short 3.5–4 kDa LPS-binding consensus repeat sites, which work in cooperativity for ligation of LPS [34]. In contrast, another LPS-binding protein, *Limulus* anti-LPS factor (LALF), is found in *Limulus* hemolymph. This is a small (11.8 kDa) basic protein which inhibits the LPS-mediated coagulation cascade [35]. Its structure consists of three α helices packed against a four-stranded β sheet, with an LPS binding site in an extended amphipathic loop (residues 31–52) [36] (fig. 3B). A Japanese horseshoe crab, *Tachypleus tridentatus,* has also a similar anti-LPS factor, TALF [37].

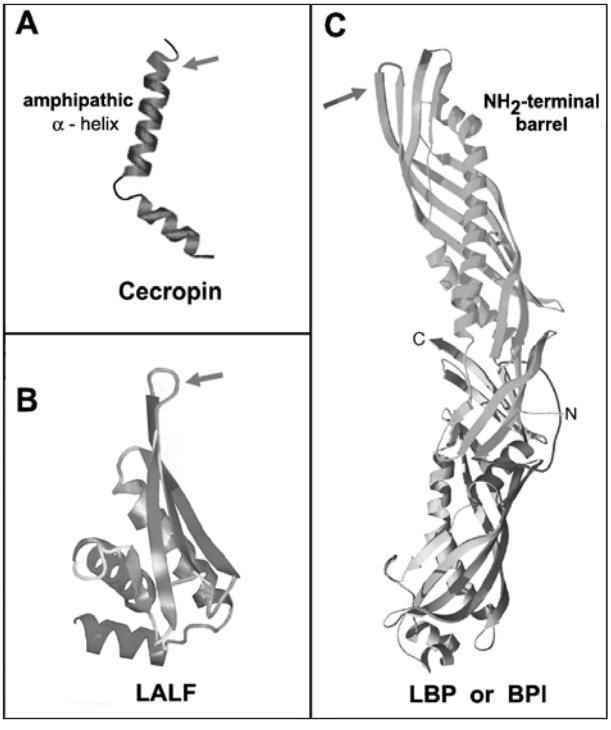

Figure 3. Ribbon diagrams of three soluble LPS binding molecules. LPS-binding sites are indicated by arrows. (*A*) Cecropin A, with a basic motif (KWKLFKK) followed by an amphipathic α helix. (*B*) *Limulus* anti-LPS factor (LALF). From [36], with permission of Nature Publishing Group, copyright 1993. (*C*) bactericidal permeability-increasing protein (BPI). Figure modified from [119] with permission of the Protein Society, copyright 1998. The structure of LBP is very similar to that of BPI.

In mammals, one of the pathological effects of LPS is the induction of disseminated intravascular coagulation (DIC). The observation that the lipid A region of LPS interacts directly with plasma factor XII (Hageman factor) [38] suggests that this factor contains an LPS binding site. This interaction with LPS activates factor XII, and triggers the intrinsic coagulation pathway. On the other hand, tissue factor (TF), expressed by macrophages and endothelial cells, activates the extrinsic coagulation pathway. This second mechanism can be regulated by a tissue factor pathway inhibitor (TFPI), which contains a heparin binding and LPS binding site, located on the third Kunitz-type domain (residues 212–243) in the carboxy terminus [39]. Therefore, in mammals as in arthropods, LPS can bind to both activators and inhibitors of the coagulation system.

Lipid transport proteins (PLTP, CETP, LBP, sCD14)

Phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) are lipid transport proteins found in plasma in association with apo A1. Their role is to transfer or exchange lipids (phospholipids, cholesteryl esters, triglycerides) between plasma lipoprotein particles [40, 41]. PLTP has been reported to bind LPS, and to transfer it from Gram-negative bacterial membranes [42] or LPS aggregates [43] to high-density lipoprotein (HDL) particles. After its transfer to HDLs, LPS is no more recognized by the 'LPS signaling complex' of responsive cells [44], but rather is cleared by phagocytic cells bearing HDL receptors [45]. Therefore, phospholipid transfer protein and HDLs collaborate for a scavenging role that may be completed by cell surface scavenger receptors [46].

Another plasma protein in association with apo A1 is lipopolysaccharide-binding protein (LBP), which shows

24% sequence identity to PLTP [47] and 23% to CETP [48]. LBP is one of the most extensively studied soluble proteins with LPS-binding capacity. It is mainly produced by hepatocytes, where it is synthesized intracellularly as a 50-kDa precursor and secreted as the mature 60-kDa glycoprotein found in plasma [49]. Its serum concentration varies from 0.5–10 μg ml^{-1} in normal serum, to more than 200 μg ml^{-1} during the acute phase of the inflammatory response. It interacts with the lipid A region of LPS with an affinity of 10^{-9} M [50]. The LPS-binding activity is contained within the N-terminal half of LBP (fig. 3C), and synthetic peptides such as CRWKVRKSFFKLQCG that mimic that region also present LPS-binding capacities [51]. It was recently shown that LBP has a concentration-dependent dual role: at low concentrations LBP intercalates into cell membranes as a transmembrane protein which binds LPS aggregates and enhances LPS-induced responses, whereas at high concentrations (in acute phase) soluble LBP intercalates into LPS aggregates and inhibits LPS-induced stimulation [52]. In this context, it is noteworthy that LPS aggregates, and not monomers, are the biologically active units [53]. A second function of LBP is to increase the interaction of LPS with soluble CD14 (sCD14) by forming a stable trimolecular complex [54]. This requires an interaction between LBP and CD14 which is mediated by the C-terminal half of LBP [55]. The complex can be transported to lipoprotein particles [56] or to cells [57] which can then respond to picomolar concentrations of LPS. A third function of LBP has been identified by Yu et al. [58] as the capacity to transport mammalian phospholipids (PI, PC, PE). This phospholipid transfer activity of LBP was overlooked for a long time because unlike PLTP which can act directly, LBP requires an additional partner, sCD14, which acts as a carrier or shuttle [58]. Therefore, sCD14 is both a phospholipid and an LPS transporter.

At least two forms of sCD14 have been described: a 48-kDa form produced by shedding the cell surface form, and a 56-kDa form released from cells before addition of the glycosylphosphatidylinositol (GPI) anchor [59]. In whole blood, the amount of soluble CD14 is 100–1000 times higher than that of membrane-bound CD14. sCD14 is a single-chain protein containing intrachain disulfide bonds and 10 leucine repeats in its carboxyl-terminal region (residues 67–367) (fig. 4). Unlike the membrane form, which requires LBP, sCD14 can directly bind LPS with a dissociation constant of 74 nM [60]. Lipoteichoic acid and phosphatidyl inositol also bind to sCD14 [57]. The amino terminus of sCD14 contains four motifs (fig. 4) involved in ligation of LPS (residues 9–12, 22–25, 35–39 and 59–63) [61]. The region (residues 53–63) which contains the fourth LPS binding motif is also the region of highest amphipathicity in sCD14, and may represent a domain analogous to the amphiphatic loops in LALF (residues 31–52), LBP (residues 86–104) and BPI

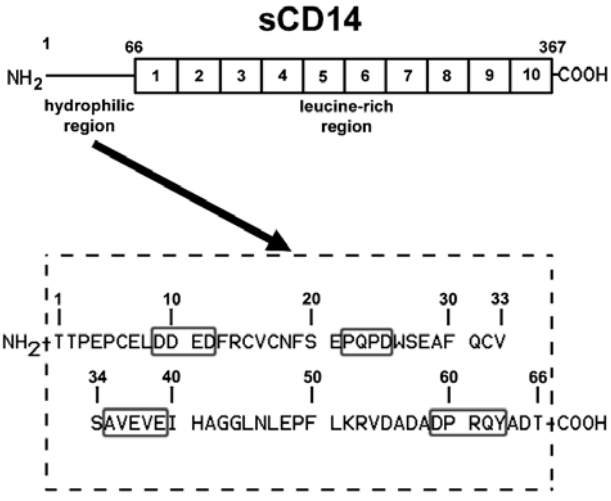

Figure 4. Schematic representation of soluble human CD14. The dashed box is an enlarged representation of residues 1–66. Residues in the four filled boxes represent proposed LPS binding sites. Figure modified from [61] with permission of the American Society for Microbiology, copyright 1997.

(residues 86–104) that bind lipid A [62]. It has been suggested that sCD14-LPS complexes can activate cells that are normally deficient in membrane CD14, such as endothelial cells [63].

Other circulating proteins (complement constituents, HDL, serum amyloid P, hemoglobin)

LPS-binding molecules are involved at different levels in the three pathways that make up the complement system (classical, lectin and alternative pathways). The polysaccharide portion of LPSs is recognized by components of the alternative pathway [64] and, for some mannose-containing O-chains, by the mannan-binding protein of the lectin pathway [65]. In addition, the lipid A portion of LPSs activates the classical pathway [66] via interaction with C1q [67, 68] and C3 [69, 70]. In C1q, the LPS-binding site is located in a highly cationic region (residues 14–26) of the collagen-like domain, and is also a major binding site of polyanionic molecules (heparin, chondroitin sulfate, DNA). Another related component, the C1 inhibitor (a serine inhibitor of the classical complement pathway and of factor XII of the coagulation system), also interacts with LPS via its N-terminal mucin domain [71]. LPS also binds to all of the major plasma lipoproteins: HDLs, low-density lipoproteins (LDLs), very low density lipoproteins (VLDLs) and chylomicrons [72]. The structural elements of LPSs interacting with HDLs are the phosphates and the diglucosamine backbone of lipid A [73]. At least one type of protein constituent of lipoproteins, the apolipoprotein apoE, contains a heparin-binding sequence [74] and can directly bind LPS, possibly by

its exposed hydrophilic domain involving arginine residues [75]. This binding is required for the redirection of LPS from Kupffer cells to parenchymal liver cells, and subsequent secretion of LPS into the bile, where it is inactivated [76].

Serum amyloid P (SAP) is a serum glycoprotein which may play a role in clearance of cell debris at sites of acute inflammation [77]. It is a multispecific protein which binds heparin, various 3-sulfated carbohydrates, mannose-6-phosphate and LPS [78]. Three LPS binding sites (27–39, 61–75 and 186–200) have been identified in SAP, but only the two latter are accessible on the intact SAP molecule. The corresponding synthetic peptides exhibit LPS-neutralizing activity [79].

Hemoglobin (Hb) is an oxygen-carrying globular protein located in erythrocytes. Hb is an LPS binding protein [80] that enhances LPS's biological activities [81]. The binding of Hb does not involve ionic interactions with the phosphate groups of lipid A, but rather hydrophobic and/or hydrogen interactions, which causes a slight rigidification of the lipid A acyl chains [82]. Hb is also a source of biologically active peptides: a C-terminal fragment of its β subunit, isolated from human placenta, was reported to exhibit LPS-binding and antibacterial activities [83].

Proteins produced in neutrophil granules (BPI, lactoferrin, heparin-binding protein, lysozyme)

Bactericidal permeability-increasing protein (BPI) is a 57-kDa cationic antimicrobial protein that is present principally in the azurophilic granules of polymorphonuclear leukocytes, but is also expressed on the surface of human mucosal epithelia, where it is markedly upregulated by exposure to endogenous antiinflammatory eicosanoids [84]. BPI is toxic only toward Gram-negative bacteria. It has both heparin- and LPS-binding capacity, and shares ~44% sequence homology with LBP (fig. 3C). Three regions (residues 17–45, 65–99 and 142–169) have been described to cooperate for the total binding to LPS [85].

Lactoferrin (Lf) is a multispecific protein which binds iron, heparin, proteoglycan, DNA, oligodeoxyncleotides and LPS [86, 87]. It is found at mucosal surfaces and in biological fluids, and is released from neutrophil granules and mucosal epithelial cells in response to inflammatory stimuli [88]. Proteolytic digestion of human lactoferrin in vitro yields a peptide fragment called lactoferricin H, which corresponds to the helix portion of lactoferrin, in the region of LPS binding [89]. Actually, two LPS binding sites have been identified in Lf: the 28–34 loop region [89] and an N-terminal stretch of only four arginines (residues 2–5) [86]. Addition of a 12-carbon acyl chain to a lactoferricin fragment enhances ligation of LPS by up to two orders of magnitude [90].

Heparin-binding protein (HBP) is a cationic antimicrobial protein of 37 kDa (also known as CAP37 or azurocidin) produced by human neutrophil granules [91]. Structurally, HBP belongs to the serprocidin subgroup of the chymotrypsin-like protease superfamily comprising elastase and cathepsin G, but unlike these enzymes, HBP lacks proteolytic activity. Once released from neutrophils, HBP binds to endothelial cell surface proteoglycans, such as syndecans and glypican, and contributes to the progression of inflammation. This multifunctional protein has, adjacent to its proteoglycan/heparin-binding site, a high-affinity binding site for lipid A [92], consisting of an ionic and hydrophilic pocket (Asn^{20}, Gln^{21} and Arg^{23}) suited for binding a phosphate group, and a hydrophobic pocket (Phe^{25}, Cys^{26}, Cys^{42} and Phe^{43}) suitable for binding the fatty acid chains of lipid A [92]. A synthetic peptide corresponding to residues 20–44 has been shown to possess the capacity to bind lipid A [93]. Like LBP, HBP enhances LPS-induced tumor necrosis factor-alpha (TNF-α) release by monocytes. However, HBP cannot bind to CD14, and its capacity to increase the responses of monocytes to LPS is likely mediated by its internalization [94].

Lysozyme, a major cationic protein of leukocyte polymorphonuclear granules, was also found to bind LPS. Like polymyxin B, lysozyme binds first electrostatically to the phosphate groups of lipid A, whereas the carboxylates of the Kdo units do not play any role [95]. This electrostatic interaction is followed by a hydrophobic interaction.

Another group of neutrophil cationic proteins able to bind LPS involves proline-rich peptides. Indolicidin, Bac5 and prophenin represent three examples of such peptides. Indolicidin is a 13-amino acid (ILPWKWPWWPWRR) proline-rich peptide present in bovine neutrophil granules, which binds LPS efficiently (K_d = 45.2 µM) [96]. This is also the case of another proline-rich peptide found in ruminants, Bac5 [97]. The third peptide belonging to this group is prophenin. This peptide has been isolated from leukocytes but is also detectable in preparations of lung surfactant [98]. It consists of a 79-residue peptide rich in proline (53%) and phenylalanine (19%), and contains repeating decameric elements. Its affinity for LPS is 60 times greater than that of polymyxin B [99].

Proteins produced by epithelial cells, T lymphocytes and NK cells (SLPI, HRP, NKL)

In addition to TFPI, another enzyme inhibitor is the 11.7-kDa secretory leukocyte protease inhibitor (SLPI), which is essentially produced by epithelial cells and inhibits leukocyte serine proteases such as elastase and cathepsin G. SLPI can also suppress the production of inflammatory mediators by macrophages, and exhibits de-

fensin-like antibacterial activity. In addition, SLPI was found to interact directly with different sulfated polysaccharides (dermatan, heparan and dextran sulfates) [100], and with LPS [101].

In saliva, histatins are small histidin-rich peptides (HRPs) with antimicrobial activity [102], secreted by epithelial cells of parotid and submandibular glands. They can bind LPS and neutralize its effects on monocytes. This is particularly the case of HRP-2, and of synthetic polyhistidine [103]. It is noteworthy that the chemokine CCL28 (mucosae-associated epithelial chemokine) shares with histatin-5 a similar molecular arrangement of hydrophobic and polarized regions [104].

Although neutrophils and epithelial cells are the main producers of soluble LPS-binding proteins, other cell types can also do so. For example, porcine cytolytic T lymphocytes and natural killer (NK) cells produce a 9-kDa cationic polypeptide, NK-lysin (NKL), which exhibits LPS-binding and -neutralizing activity [105]. Its human counterpart is granulysin [106].

Mammalian antimicrobial peptides that ligate LPS: defensins and cathelicidins

Humans neutrophils contain two structurally distinct types of antimicrobial peptides, defensins (β-sheet peptides with three disulfide bridges) and cathelicidins (α-helical peptides).

Human α-defensins (HNP-1 to HNP-4) have been shown to interact with LPS, although less efficiently than BPI [107]. The binding capacity may be due to exposed cationic and nonpolar residues oriented at different faces of the molecule. For example, HNP-3 is an amphiphilic basket-shaped molecule with hydrophilic residues at the top and hydrophobic residues at the bottom [108].

Another group of antimicrobial proteins is the cathelicidins, which are stored in the intracellular granules of neutrophilic granulocytes, and are liberated into the phagocytic vacuoles during phagocytosis. SMAP-29, rCAP18 and hCAP18 are antimicrobial peptides found in sheep, rabbit and human leukocytes, respectively. They are classified as 'cathelicidins' because they are synthesized from a precursor that contains a highly conserved 11-kDa N-terminal cathelin domain. The human cathelicidin hCAP18 carries a 37-residue α-helical peptide termed LL-37, with antimicrobial and LPS-binding activity [109]. Structural studies of the sheep homolog SMAP-29 indicated that residues 8–17 are helical, residues 18–19 form a central hinge and residues 20–28 form a hydrophobic segment. SMAP-29 contains two LPS binding sites: the highest-affinity site (RGLRRLGR) is located in the N-terminal region, and the lowest-affinity site (VLRIIRIA) is in the C-terminal region. These two sites bind LPS with intramolecular positive cooperativity [110]. Concerning the mechanism of their antimicrobial action, it has been shown that after accumulation of CAP18 at the membrane, its orientation changes under the influence of an LPS-specific transmembrane potential from a nonconductive to a transmembranous conductive state [111]. This change in membrane intercalation is governed by the type of LPS. For example, addition of L-Ara*p*4N to the first Kdo of the LPS is the structural feature responsible for the resistance of *Proteus mirabilis* to CAP18 lethality [111].

LPS-binding proteins in the lungs

Because of inhalation of airborne particles containing bacteria and LPS, the respiratory system is continuously exposed to this potent proinflammatory compound. Acute inhalation of LPS induces airway inflammation. Lung injury is also a common clinical complication of sepsis, with frequent development of an acute respiratory distress syndrome. However, under normal exposure conditions, the lung has efficient defense mechanisms against LPS. When LPS reaches the alveolar spaces, it encounters a surfactant layer that covers the epithelium and consists of lipids and surfactant-associated proteins. Surfactant proteins are produced by lung type II epithelial cells. Two of these proteins (SP-A and SP-D) are hydrophilic, whereas the other two (SP-B and SP-C) are hydrophobic. SP-A and SP-D belong to a family of proteins named collectins because they contain collagen-like and lectin domains. It has been established that the two hydrophilic surfactant proteins, SP-A and SP-D, bind phospholipids and LPS. However, fine specificities are different: SP-D, binds to phosphatidylinositol and to the core carbohydrates of LPS [112], whereas SP-A recognizes dipalmitoylphosphatidycholine and the lipid A region of LPS [113].

Structural analysis of SP-A established that the C-terminal region of the collagen-like domain of three monomers are linked together by a coiled-coil neck, and that six of these trimers are assembled by the N-terminal region of their collagen-like domain. The binding region for lipid A (fig. 5A) may require a cluster of basic or amine side chains (Arg^{216}, Arg^{222}, Gln^{220}), and a hydrophobic patch of aromatic and aliphatic rings on the surface of the carbohydrate recognition domain (Tyr^{161}, Asn^{162}, Asn^{163}, Tyr^{164}, Tyr^{192}, Tyr^{208}) [114]. These structural requirements are very similar to those involved in electrostatic (Arg^{382}, Arg^{384}, Gln^{353}) and hydrophobic (four phenylalanine residues) interactions between LPS and the FhuA surface.

Concerning the hydrophobic surfactant proteins SP-B and SP-C, recent studies of the present author's group did not detect any LPS-binding activity with SP-B [115], in spite of some structural analogy with NK-lysin [116], suggesting that their common amphipathic helical motif does not contain the LPS-binding motif of NK-lysin. In

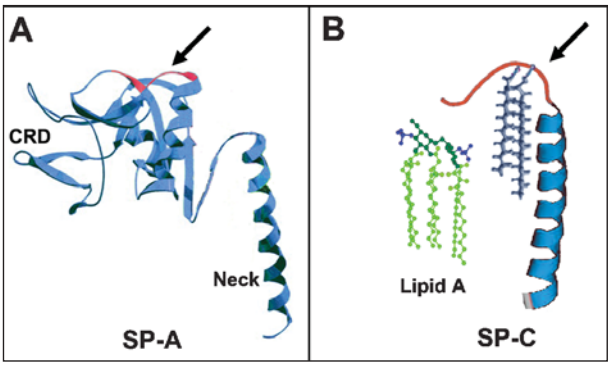

Figure 5. Structures of two LPS binding proteins of the lungs. LPS-binding sites (in red) are indicated by arrows. (*A*) CRD and neck regions of an isolated monomer of lung surfactant protein A (SP-A). Figure modified from [114] with permission of the American Society for Biochemistry and Molecular Biology, copyright 2003. (*B*) Surfactant protein C (SP-C) with its two palmitoyl residues, and lipid A in the vicinity of the binding site.

contrast, the authors reported that SP-C binds efficiently to LPS [115]. A more detailed analysis indicated that the N-terminal region of SP-C, which contains polar and basic residues, interacts with the lipid A region of LPS, whereas the C-terminal hydrophobic domain of SP-C may be involved for an optimal conformation of the protein [117] (fig. 5B). The α configuration of the terminal phosphate group at the reducing end of the lipid A disaccharide plays a determinant role in LPS recognition. We also demonstrated that CD14 shares with LPS the same binding region on SP-C, and that the interaction with SP-C modifies the conformation of CD14, allowing it to bind LPS more efficiently [118].

Common structural motif of soluble LPS-binding proteins

Several investigators searched for common structural motifs in soluble LPS-binding proteins. The first studies focused on LBP and BPI because of their high (44%) sequence homology. The interaction of these proteins with LPS seems to be a complex multistep process involving, on the one hand, the lipid-binding pockets of the molecule (which can accommodate the apolar moiety of LPS, but with little specificity), and, on the other hand, positively charged residues in a region of the N-terminal domain [85], leading to electrostatic interactions with phosphorylated carbohydrate groups of LPS [119]. On the other hand, LALF does not display any degree of sequence homology with LBP and BPI, and therefore no obvious region responsible for LPS ligation could be postulated until the three-dimensional structure analysis of LALF revealed the presence of a solvent-exposed, positively charged, amphipathic loop that interacts specifically with lipid A [120], and that could also be identified in LBP and BPI [121].

In a very nice study in which they compared the sequences of a number of LPS-binding proteins and peptides, Frecer et al. [122] suggested that the more favorable amino acid sequence for specific binding of lipid A is the amphipathic cationic binding pattern BHPHB (B, basic; H, hydrophobic; P, polar residue). A sequence of that type is found in LBP (sequence RVQGR, residues 111–115), BPI (sequence KISGK, residues 113–117), LALF (sequence RLKWK, residues 39–43) and TLR4 (sequence KLTLR, residues 190–194). The best minimum binding sequence was found to be KFSFK.

However, this type of analysis was a pure molecular modeling study, a method which was poorly predictive with LPS and lipid A in the past. In addition, the continuous sequence of BHPHB residues does not always account for LPS ligation: in some proteins, the basic residues that interact with the phosphate groups of lipid A likely belong to discontinuous but spatially proximal β sheets or α helices. A second model based on this hypothesis has been proposed by Ferguson et al. [16]. The authors searched in protein data bases structurally characterized proteins containing residues in a three-dimensional arrangement similar to that of the LPS-binding residues of FhuA. They identified four residues (K-K-R-K) common to several known LPS-binding proteins (BPI, lactoferrin, lysozyme, LALF). Two of the lysine residues form hydrogen bonds with the 4′-phosphate of lipid A, the third lysine residue interacts with the 1-phosphate and the arginine residue interacts with the carbonyl of the fatty acid residue at position 3′. It should be noted, however, that cocrystallized LPS and protein were analyzed in this study, which might differ from the physiological situation at high water content.

Membrane molecules involved in signaling and internalization

CD14 does not exist only as a soluble protein, but also as a membrane-bound glycoprotein (mCD14) expressed on the surface of monocytes, macrophages and neutrophils. It plays an important role in the LPS-induced activation of these cells, after recognition of LPS-LBP complexes [123]. mCD14 is anchored to cell plasma membranes by a glycosylphosphatidylinositol (GPI) moiety. Because it lacks a traditional transmembrane domain, it cannot alone provide direct cell signaling. Furthermore, cell activation by high doses of LPS cannot be blocked by anti-CD14 monoclonal antibodies, and species specificity of lipid A analogs was found to be independent of CD14 [124]. This means that additional membrane molecules are required for cellular activation. The most important of these molecules is a member of the Toll-like receptor (TLR) family: TLR4. It has been identified by the group of Beutler as

the product of the *lps* gene, which is deficient in LPS hyporesponsive C3H/HeJ mice [125]. Although TLR4 is the main signaling receptor of LPSs isolated from enterobacteria, other LPSs containing structurally different lipid A regions, such as those of *Porphyromonas gingivalis*, *Leptospira interrogans*, *Rhizobium species* Sin-1 and *Legionella pneumophila*, require TLR2 rather than TLR4 [126-128]. Using a radiolabeled LPS coupled to a photoactivatable crosslinking probe, the group of Ulevitch [129] observed labeling of both TLR2 and TLR4 in the presence of CD14. However, this shows only a spatial proximity between these molecules and the probe, but not necessarily a direct interaction with LPS. Another study [130] demonstrated direct binding of ^{3}H-labeled LPS to TLR2, with low affinity (K_d = 500–700 nM).

Overall, there is presently no clear evidence that lipid A interacts directly with TLR4. Its role in LPS signaling was better explained after the demonstration that TLR4 needs the coexpression of an adaptor protein called MD-2. The MD-2 polypeptide monomer (18.4 kDa) contains two N-linked glycosylation sites, and seven Cys residues that participate in the formation of large, disulfide-linked oligomers of dimeric subunits [131]. It has been shown that MD-2 can directly bind LPS (K_d = 65 nM) [60]. MD-2 belongs to a large family of proteins (ML proteins) containing a recently identified lipid-recognition domain which does not show any homology to the hydrophobic cluster of nonspecific lipid transfer proteins such as those of the PLTP/LBP family [132]. On the other hand, a region of human MD-2 (residues 119–132) rich in basic and aromatic residues contains several features common to other LPS-binding proteins, and a synthetic peptide derived from this sequence was shown to possess both LPS-binding and antimicrobial activities [133]. Therefore, MD-2 may bind LPS both by its lipid-recognition domain and its positively charged region. Point mutations of two residues in this region (lysines 128 and 138) greatly blunted the cellular LPS response [134]. In a more exhaustive study of mouse MD-2, Kawasaki et al. [135] found that some residues located outside the 119–132 region, such as Tyr^{34}, Ile^{85} and Ile^{153}, are also responsible for LPS responsiveness. The same residues also confer responsiveness to Taxol, a plant-derived stabilizer of microtubules which exhibits LPS-mimetic activity in mice. In contrast, cysteine residues such as Cys^{37}, Cys^{95}, Cys^{105} and Cys^{148} are important for forming a cell surface TLR4-MD-2 complex, in which MD-2 is docked to the extracellular leucine-rich repeats of TLR4. The affinity of this complex for LPS (K_d about 3 nM) is 20 times higher than that of free soluble MD-2 [136], suggesting a contribution of TLR4 to LPS ligation.

It should be noted that in insects, the *Toll* pathway is not involved in the response to LPS. In contrast, it has been reported recently that two isoforms of the peptidoglycan recognition protein (PGRP) family of transmembrane proteins, PGRP-LCx and PGRP-LCa are both required for activation of *Drosophila* cells by LPS via the *Toll*-independent *imd-Relish* pathway [137]. PGRP-LCx and PGRP-LCa share an identical intracellular region, but differ in their extracellular domain, suggesting that at least one of the two extracellular domains may recognize LPS. This LPS-binding capacity could be due to PGRP-LCa, because PGRP-LCx is required for activation by both peptidoglycan and LPS, whereas PGRP-LCa is required only for activation by LPS. The simultaneous requirement of the two isoforms for the response to LPS suggests that they may act as heterodimers or multimers.

In mammals, in addition to mCD14, TLR4 and MD-2, several other membrane-bound molecules have been shown to participate in cellular recognition of LPS: in a study using affinity chromatography, peptide mass fingerprinting and fluorescence resonance energy transfer, Triantafilou et al. identified heat shock proteins 70 and 90 (HSP70 and HSP90), chemokine receptor 4 (CXCR4) and growth differentiation factor 5 (GDF-5) as four new molecules on the cell surface that bind LPS and are involved in LPS-induced signaling [138]. A specific LPS binding motif was also shown in the CD11b chain of one of the three adhesion molecules (CR3) belonging to the β-integrin family [139]. CR3 can mediate LPS-induced activation of NF-κB in transfected CHO cells, although its cytosolic domain is not required for signaling [140]. Another membrane molecule involved in LPS recognition and signaling is the decay accelerating factor (DAF, CD55). Complexes of this GPI-anchored membrane molecule with LPS have been isolated [141]. A transmembrane form of LBP and a mechano-sensitive K^+ channel (MaxiK) are also involved in LPS-induced signaling [53]. However, although MaxiK modulation by LPS clearly results from early-mediated events, it also seems indirect since it involves the membrane-bound receptor mCD14. Therefore, MaxiK should not be considered as an LPS-binding molecule, but rather as a component of the LPS-signaling cluster. Several of the LPS receptors, including mCD14, CD55, CD11b and TLR4, coassemble in special membrane lipid domains called 'rafts', as shown by fluorescence resonance energy transfer (FRET) analysis [142].

All LPS receptors mentioned above (fig. 6, upper part) were characterized in macrophages. On other cell types, different molecules can participate to LPS recognition and signaling. For example, it has been reported that in human neutrophils, L-selectin (CD62L) can bind LPS [143] and trigger LPS signaling [144]. In human platelets, LPS ligation occurs via another selectin, P-selectin [145]. The LPS-binding site of the two selectins is distinct from their lectin domain, and ligation of LPS is independent of Ca^{2+} [143]. Concerning B lymphocytes, in addition to TLR4-MD-2, another cell surface complex, RP105-MD-1, is also involved in the recognition of LPS

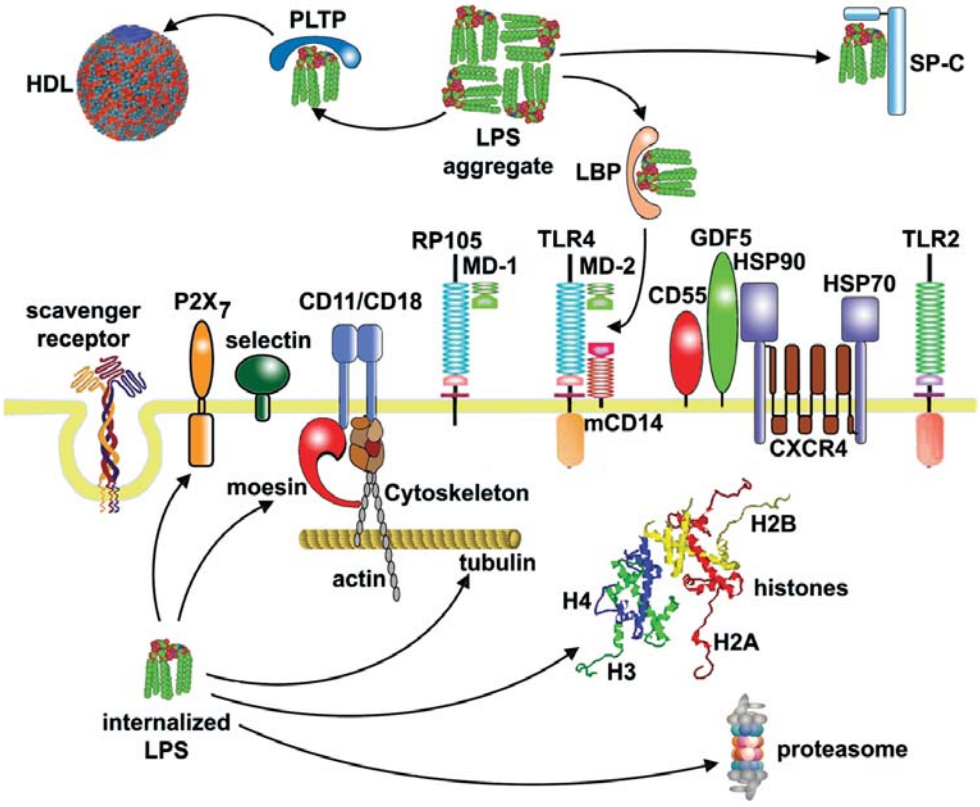

Figure 6. Overview of intracellular, membrane and some extracellular LPS-binding proteins. The presence of a particular molecule depends on the cell type (RP105/MD-1 exclusively on B lymphocytes) and the tissue environment (SP-C exclusively in lung alveoli).

[146]. MD-1 is structurally similar to MD-2, and RP105 (assigned as CD180) is structurally similar to TLR4, except that it does not have an interleukin-1 (IL-1) receptor-like (TIR) signaling domain in the cytoplasmic portion.

In addition to the membrane LPS-binding molecules mentioned above, which are involved in LPS signaling, other membrane LPS-binding structures (fig. 6) are involved in LPS clearance. This is particularly the case of the scavenger receptor, a family of cell surface glycoproteins able to bind acetylated low-density lipoproteins (AcLDLs). Scavenger receptors belonging to class A (SR-A) are found in macrophages and Kupffer cells. Two major isoforms have been identified, type I (SR-AI) and type II (SR-AII). They have an identical trimeric structure consisting of a transmembrane domain linked to an α-helical coiled coil domain and a collagenous region, except that type I receptors have an additional extracellular C-terminal cysteine-rich domain not present in the type II receptors. Both SR-AI and SR-AII recognize a wide range of anionic ligands, including LPS [147]. As in C1q and SP-A, the positively charged C-terminal portion of the collagenous domain of SR-A is involved in LPS binding [148]. Interaction of LPS with SR-A induces its internalization, followed by the dephosphorylation of its lipid A moiety.

Intracellular LPS-binding molecules

After binding to the cell surface, LPS was found to be internalized and detectable in the cytoplasm of macrophages within seconds [149]. This internalization occurs in many cell types, and appears to be necessary for certain cellular responses to LPS, such as the integrin-mediated adhesion of neutrophils [150], or the production of the chemokine MIP-1 in intestinal epithelial cells [151]. This suggests that LPS can also be recognized in the cytoplasm. In epithelial cells, the intracellular sensor of LPS is the TLR4-MD-2 complex present in the Golgi apparatus. LPS internalization and delivery to the Golgi may thus be an important requirement for cell activation in different cell types [152].

Once in the cytoplasm, LPS can also interact with other intracellular LPS binding motifs (fig. 6, lower part). Such motifs can belong to the cytoplasmic domain of integral membrane proteins. This is, for example, the case of $P2X_7$, a nucleotide receptor which potentiates the LPS-induced activation of macrophages [153]. It has been shown by Denlinger et al. [154] that the C-terminal region of $P2X_7$, which is located inside the cell after the second transmembrane domain, contains a motif (residues 573–590) which shares strong amino acid homol-

ogy with the LPS binding sites of LBP and BPI. Furthermore, peptides derived from this sequence were shown to bind LPS in vitro, and to neutralize some LPS responses in macrophages. Moesin, a cytoskeletal linker actin-binding protein associated with the cytoplasmic side of plasma membranes, can also bind LPS [155], and at the cytoskeleton level, tubulin can do so as well [156]. It has also been suggested that LPS has the capacity to bind to selected subunits (C2 and N3) of the macrophage proteasome, with further activation of the proteasome chymotrypsin-like activity [157].

Another new class of LPS-binding molecules recently discovered by the present author's group is that of histones [158]. The authors found that histones H1, H2A, H2B, H3 and H4 can all bind LPS. For example, H2A binds to the rough-type LPS Re-595 of *Salmonella minnesota* with an affinity, determined by isothermal titration calorimetry, higher than that of PMB. Polynucleotides inhibit this association, thus indicating that they share with LPS a common binding site. Furthermore, H2A inhibits the ligation of LPS to macrophages, and the LPS-induced production of TNF-α and nitric oxide. Analysis of interactions with synthetic peptides representing partial structures indicated that the LPS-binding motif is located in the C-terminal moiety (residues 71–115) of H2A (fig. 7). However, in this region, the putative LPS-binding sequence of 'basic-hydrophobic-polar-hydrophobic-basic' amino acids mentioned above is not detectable, suggesting that another arrangement of amino acids can also participate in LPS ligation. The physiological importance of the histone-LPS interaction remains unknown, but may take place at different levels. Of course, the classical localization of histones is the cell nucleus, but they have also been found in the cytosol, the plasma membrane and the extracellular milieu. Emlen et al. detected histones on the surface of monocytes, probably bound via a particular receptor [159], and Bolton and Perry found that the major LPS-associated protein of the brain is an isoform of histone H1, predominantly located at the surface of neuronal cells [160]. Histones were also found in placenta, where they exhibit LPS-neutralizing and antibacterial activities [161]. Finally, during the apoptotic process, nuclear material including histones is released in the extracellular milieu, and some of their fragments produced by enzymatic cleavage may have conserved their LPS-binding property and can thus act as antibacterial peptides. This is actually the case of buforin I and parasin I, which are fragments of histone H2A [162, 163], and histogranin, which is similar to a C-terminal sequence in histone H4 [164]. However, the location where histones are the most abundant is the cytosol, because after their biosynthesis only a small number of these molecules are transported by karyopherins into the nucleus [165]. Therefore, an exciting hypothesis could be that another function of histones is to act as an intracellular receptor or transporter for LPS.

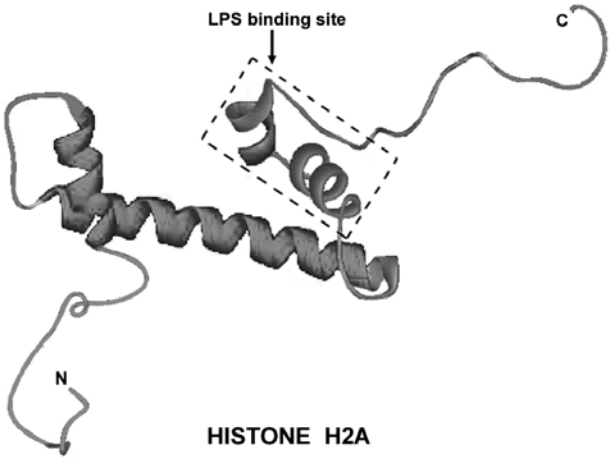

Figure 7. Ribbon diagram of histone H2A.

It should be mentioned in this connection that until recently, another candidate molecule for intracellular recognition of LPS was Nod1, a molecule consisting of an N-terminal caspase-recruitment domain linked to a nucleotide-binding domain and a C-terminal LRR domain. Although it has been claimed that Nod1 confers responsiveness to LPS [166], it is now clearly established that Nod1 detects actually a peptidoglycan motif [167].

Conclusions and perspectives

One of the main features which emerges from the above overview is that LPS is constantly transferred from one associated state to another, but is never free as a soluble monomer in biological fluids. These frequent transfers require the existence of various LPS transporters. The transport of lipid A from the inner to the outer membrane of Gram-negative bacteria by MsbA, or the internalization of LPS by the scavenger receptor of macrophages, is an example of transmembrane transport, whereas the transfer of LPS from bacteria or aggregates to mammalian cell membranes by LBP or to lipoprotein particles by PLTP can be considered as intermembrane transports, and the presentation of LPS to the TLR4/MD-2 complex by mCD14 is an example of intramembrane transport.

During transport, LPS can also encounter two other types of binding molecules: blockers (inactivators) and sensors (activators). The existence of blockers and sensors accounts for the different effects of LPS-binding proteins on endotoxicity, varying from reduction (polymyxin B, BPI, lysozyme, lactoferrin) to enhancement (hemoglobin, LBP, HBP). Blockers can be very short peptides. This is the case of indolicidin, a 13-amino acid peptide. In contrast, sensors must have a more complex structure since they must also deliver a recognition signal. The coagulation system is a good example, because it contains both

blockers (LALF in *Limulus*, TFPI in humans) and sensors (factor C in *Limulus*, factor XII in humans). The soluble sensors of the coagulation system are proteolytic enzymes activated upon LPS recognition. At the cell membrane level, TLR4 is of course the best example of a sensor which delivers a transmembrane signal. It is noteworthy that the majority of LPS transporters and sensors contain multiple LPS binding sites that often work cooperatively. For example, three LPS-binding regions have been found in *Limulus* factor C, two in lactoferrin, three in cathelicidins (SMAP-29 and CAP-18), three in BPI and four in CD14. These individual LPS binding motifs are usually very short (about five amino acids). This shows that the association of several sites of moderate affinity is an efficient way to confer to a molecule the ability to recognize LPS more specifically. This may also be necessary for induction of tertiary rearrangements of the molecule, required for the sensor function.

It should also be noted that almost all LPS binding molecules can also recognize other ligands. This is particularly the case of CD14, which is a pattern-recognizing receptor (PRR) [168]. Cross reactivities with other ligands have also been described for MD-2 (recognition of taxol and phosphatidylinositol) and TLR4 (recognition of lipoteichoic acid, flavolipin and extradomain A of fibronectin). Other LPS-binding molecules frequently cross-react with heparin, or other anionic molecules such as phosphatidylinositol and DNA.

The beneficial and, above all, the harmful host responses to the lipid A region of LPS prompted many laboratories to design synthetic molecules with lipid A binding sites [32, 51, 120], and some of them were efficient blockers of in vitro and in vivo responses to LPS. Another important goal was the design of endotoxin detection reagents. A green fluorescent protein mutant bearing an LPS binding motif was recently created [169], and can now be used to detect low amounts of endotoxin for the evaluation on the safety of water and pharmaceutical preparations.

The exciting developments in the field of LPS-binding molecules have thus led to a much better understanding of how the host processes an LPS molecule and respond to it. Future research will tell us whether the promising molecules designed and produced as a result of these studies will give rise to pharmaceutical applications.

1 Imoto M., Kusumoto S., Shiba T., Rietschel E. T., Galanos C. and Lüderitz O. (1985) Chemical structure of *Escherichia coli* lipid A. Tetrahedron Lett. **26:** 907–908

2 Parrillo J. E. (1993) Pathogenetic mechanisms of septic shock. N. Engl. J. Med. **328:** 1471–1477

3 Medvedev A. E., Kopydlowski K. M. and Vogel S. N. (2000) Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J. Immunol. **164:** 5564–5574

4 Peterson A. A., Haug A. and McGroarty E. J. (1986) Physical properties of short- and long-O-antigen-containing fractions of lipopolysaccharide from *Escherichia coli* 0111:B4. J. Bacteriol. **165:** 116–122

5 Thomassen E., Gielen G., Schutz M., Schoehn G., Abrahams J. P., Miller S. et al. (2003) The structure of the receptor-binding domain of the bacteriophage T4 short tail fibre reveals a knitted trimeric metal-binding fold. J. Mol. Biol. **331:** 361–373

6 Schroeder T. H., Lee M. M., Yacono P. W., Cannon C. L., Gerceker A. A., Golan D. E. et al. (2002) CFTR is a pattern recognition molecule that extracts *Pseudomonas aeruginosa* LPS from the outer membrane into epithelial cells and activates NF-kappa B translocation. Proc. Natl. Acad. Sci. USA **99:** 6907–6912

7 Beisel H. G., Kawabata S., Iwanaga S., Huber R. and Bode W. (1999) Tachylectin-2: crystal structure of a specific GlcNAc/GalNAc-binding lectin involved in the innate immunity host defense of the Japanese horseshoe crab *Tachypleus tridentatus*. EMBO J. **18:** 2313–2322

8 Mey A., Leffler H., Hmama Z., Normier G. and Revillard J. P. (1996) The animal lectin galectin-3 interacts with bacterial lipopolysaccharides via two independent sites. J. Immunol. **156:** 1572–1577

9 Stahl P. D. (1992) The mannose receptor and other macrophage lectins. Curr. Opin. Immunol. **4:** 49–52

10 Zhang Y., Suankratay C., Zhang X. H., Lint T. F. and Gewurz H. (1999) Lysis via the lectin pathway of complement activation: minireview and lectin pathway enhancement of endotoxin-initiated hemolysis. Immunopharmacology **42:** 81–90

11 Hampton R. Y. and Raetz C. R. (1991) Macrophage catabolism of lipid A is regulated by endotoxin stimulation. J. Biol. Chem. **266:** 19499–19509

12 Munford R. S. and Hall C. L. (1989) Purification of acyloxyacyl hydrolase, a leukocyte enzyme that removes secondary acyl chains from bacterial lipopolysaccharides. J. Biol. Chem. **264:** 15613–15619

13 Munford R. S., Sheppard P. O. and O'Hara P. J. (1995) Saposin-like proteins (SAPLIP) carry out diverse functions on a common backbone structure. J. Lipid. Res. **36:** 1653–1663

14 Hirschfeld M., Ma Y., Weis J. H., Vogel S. N. and Weis J. J. (2000) Repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. J. Immunol. **165:** 618–622

15 Goodman G. W. and Sultzer B. M. (1979) Characterization of the chemical and physical properties of a novel B-lymphocyte activator, endotoxin protein. Infect. Immun. **24:** 685–696

16 Ferguson A. D., Welte W., Hofmann E., Lindner B., Holst O., Coulton J. W. et al. (2000) A conserved structural motif for lipopolysaccharide recognition by procaryotic and eucaryotic proteins. Structure Fold Des. **8:** 585–592

17 Vandeputte-Rutten L., Kramer R. A., Kroon J., Dekker N., Egmond M. R. and Gros P. (2001) Crystal structure of the outer membrane protease OmpT from *Escherichia coli* suggests a novel catalytic site. EMBO J. **20:** 5033–5039

18 Stumpe S., Schmid R., Stephens D. L., Georgiou G. and Bakker E. P. (1998) Identification of OmpT as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*. J. Bacteriol. **180:** 4002–4006

19 Zhou Z., White K. A., Polissi A., Georgopoulos C. and Raetz C. R. (1998) Function of *Escherichia coli* MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis. J. Biol. Chem. **273:** 12466–12475

20 Chang G. (2003) Structure of MsbA from *Vibrio cholera*: a multidrug resistance ABC transporter homolog in a closed conformation. J. Mol. Biol. **330:** 419–430

21 Lei M. G. and Morrison D. C. (1993) Lipopolysaccharide interaction with S2 subunit of pertussis toxin. J. Biol. Chem. **268:** 1488–1493

22 Daugelavicius R., Bakiene E. and Bamford D. H. (2000) Stages of Polymyxin B interaction with the *Escherichia coli* cell envelope. Antimicrob. Agents Chemother. **44:** 2969–2978
23 Vaara M. (1992) Agents that increase the permeability of the outer membrane. Microbiol. Rev. **56:** 395–411
24 Koch P. J., Frank J., Schuler J., Kahle C. and Bradaczek H. (1999) Thermodynamics and structural studies of the interaction of Polymyxin B with deep rough mutant lipopolysaccharides. J. Colloid Interface Sci. **213:** 557–564
25 Pristovsek P. and Kidric J. (1999) Solution structure of polymyxins B and E and effect of binding to lipopolysaccharide: an NMR and molecular modeling study. J. Med. Chem. **42:** 4604–4613
26 Helander I. M., Kilpelainen I. and Vaara M. (1994) Increased substitution of phosphate groups in lipopolysaccharides and lipid A of the polymyxin-resistant pmrA mutants of Salmonella typhimurium: a 31P-NMR study. Mol. Microbiol. **11:** 481–487
27 De Lucca A. J., Jacks T. J. and Brogden K. A. (1995) Binding between lipopolysaccharide and cecropin A. Mol. Cell. Biochem. **151:** 141–148
28 Bland J. M., De Lucca A. J., Jacks T. J. and Vigo C. B. (2001) All-D-cecropin B: synthesis, conformation, lipopolysaccharide binding and antibacterial activity. Mol. Cell. Biochem. **218:** 105–111
29 Okemoto K., Nakajima Y., Fujioka T. and Natori S. (2002) Participation of two N-terminal residues in LPS-neutralizing activity of sarcotoxin IA. J. Biochem. (Tokyo) **131:** 277–281
30 David S. A., Mathan V. I. and Balaram P. (1992) Interaction of melittin with endotoxic lipid A. Biochim. Biophys. Acta **1123:** 269–274
31 Carlsson A., Nystrom T., de Cock H. and Bennich H. (1998) Attacin – an insect immune protein – binds LPS and triggers the specific inhibition of bacterial outer-membrane protein synthesis. Microbiology **144:** 2179–2188
32 Gough M., Hancock R. E. and Kelly N. M. (1996) Antiendotoxin activity of cationic peptide antimicrobial agents. Infect. Immun. **64:** 4922–4927
33 Matsuzaki K., Sugishita K. and Miyajima K. (1999) Interactions of an antimicrobial peptide, magainin 2, with lipopolysaccharide-containing liposomes as a model for outer membranes of Gram-negative bacteria. FEBS Lett. **449:** 221–224
34 Tan N. S., Ng M. L., Yau Y. H., Chong P. K., Ho B. and Ding J. L. (2000) Definition of endotoxin binding sites in horseshoe crab factor C recombinant sushi proteins and neutralization of endotoxin by sushi peptides. FASEB J. **14:** 1801–1813
35 Aketagawa J., Miyata T., Ohtsubo S., Nakamura T., Morita T., Hayashida H. et al. (1986) Primary structure of limulus anticoagulant anti-lipopolysaccharide factor. J. Biol. Chem. **261:** 7357–7365
36 Hoess A., Watson S., Siber G. R. and Liddington R. (1993) Crystal structure of an endotoxin-neutralizing protein from the horseshoe crab, Limulus anti-LPS factor, at 1.5 A resolution. EMBO J. **12:** 3351–3356
37 Kloczewiak M., Black K. M., Loiselle P., Cavaillon J. M., Wainwright N. and Warren H. S. (1994) Synthetic peptides that mimic the binding site of horseshoe crab antilipopolysaccharide factor. J. Infect. Dis. **170:** 1490–1497
38 Morrison D. C. and Cochrane C. G. (1974) Direct evidence for Hageman factor (factor XII) activation by bacterial lipopolysaccharides (endotoxins). J. Exp. Med. **140:** 797–811
39 Park C. T., Creasey A. A. and Wright S. D. (1997) Tissue factor pathway inhibitor blocks cellular effects of endotoxin by binding to endotoxin and interfering with transfer to CD14. Blood **89:** 4268–4274
40 Tollefson J. H., Ravnik S. and Albers J. J. (1988) Isolation and characterization of a phospholipid transfer protein (LTP-II) from human plasma. J. Lipid Res. **29:** 1593–1602
41 Tall A. R. (1993) Plasma cholesteryl ester transfer protein. J. Lipid Res. **34:** 1255–1274
42 Vesy C. J., Kitchens R. L., Wolfbauer G., Albers J. J. and Munford R. S. (2000) Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from gram-negative bacterial membranes. Infect. Immun. **68:** 2410–2417
43 Hailman E., Albers J. J., Wolfbauer G., Tu A. Y. and Wright S. D. (1996) Neutralization and transfer of lipopolysaccharide by phospholipid transfer protein. J. Biol. Chem. **271:** 12172–12178
44 Grunfeld C., Marshall M., Shigenaga J. K., Moser A. H., Tobias P. and Feingold K. R. (1999) Lipoproteins inhibit macrophage activation by lipoteichoic acid. J. Lipid Res. **40:** 245–252
45 Maier R. V., Mathison J. C. and Ulevitch R. J. (1981) Interactions of bacterial lipopolysaccharides with tissue macrophages and plasma lipoproteins. Prog. Clin. Biol. Res. **62:** 133–155
46 van Oosten M., van de Bilt E., van Berkel T. J. and Kuiper J. (1998) New scavenger receptor-like receptors for the binding of lipopolysaccharide to liver endothelial and Kupffer cells. Infect. Immun. **66:** 5107–5112
47 Day J. R., Albers J. J., Lofton-Day C. E., Gilbert T. L., Ching A. F., Grant F. J. et al. (1994) Complete cDNA encoding human phospholipid transfer protein from human endothelial cells. J. Biol. Chem. **269:** 9388–9391
48 Schumann R. R., Leong S. R., Flaggs G. W., Gray P. W., Wright S. D., Mathison J. C. et al. (1990) Structure and function of lipopolysaccharide binding protein. Science **249:** 1429–1431
49 Ramadori G., Meyer zum Buschenfelde K. H., Tobias P. S., Mathison J. C. and Ulevitch R. J. (1990) Biosynthesis of lipopolysaccharide-binding protein in rabbit hepatocytes. Pathobiology **58:** 89–94
50 Mathison J. C., Tobias P. S., Wolfson E. and Ulevitch R. J. (1992) Plasma lipopolysaccharide (LPS)-binding protein. A key component in macrophage recognition of gram-negative LPS. J. Immunol. **149:** 200–206
51 Dankesreiter S., Hoess A., Schneider-Mergener J., Wagner H. and Miethke T. (2000) Synthetic endotoxin-binding peptides block endotoxin-triggered TNF-alpha production by macrophages in vitro and in vivo and prevent endotoxin-mediated toxic shock. J. Immunol. **164:** 4804–4811
52 Gutsmann T., Müller M., Carroll S. F., MacKenzie R. C., Wiese A. and Seydel U. (2001) Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. Infect. Immun. **69:** 6942–6950
53 Müller M., Scheel O., Lindner B., Gutsmann T. and Seydel U. (2003) The role of membrane-bound LBP, endotoxin aggregates, and the MaxiK channel in LPS-induced cell activation. J. Endotoxin Res. **9:** 181–186
54 Thomas C. J., Kapoor M., Sharma S., Bausinger H., Zyilan U., Lipsker D. et al. (2002) Evidence of a trimolecular complex involving LPS, LPS binding protein and soluble CD14 as an effector of LPS response. FEBS Lett. **531:** 184–188
55 Han J., Mathison J. C., Ulevitch R. J. and Tobias P. S. (1994) Lipopolysaccharide (LPS) binding protein, truncated at Ile-197, binds LPS but does not transfer LPS to CD14. J. Biol. Chem. **269:** 8172–8175
56 Wurfel M. M., Hailman E. and Wright S. D. (1995) Soluble CD14 acts as a shuttle in the neutralization of lipopolysaccharide (LPS) by LPS-binding protein and reconstituted high density lipoprotein. J. Exp. Med. **181:** 1743–1754
57 Gegner J. A., Ulevitch R. J. and Tobias P. S. (1995) Lipopolysaccharide (LPS) signal transduction and clearance. Dual roles for LPS binding protein and membrane CD14. J. Biol. Chem. **270:** 5320–5325
58 Yu B., Hailman E. and Wright S. D. (1997) Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. J. Clin. Invest. **99:** 315–324
59 Labeta M. O., Durieux J. J., Fernandez N., Herrmann R. and Ferrara P. (1993) Release from a human monocyte-like cell

line of two different soluble forms of the lipopolysaccharide receptor, CD14. Eur. J. Immunol. **23:** 2144–2151
60 Viriyakosol S., Tobias P. S., Kitchens R. L. and Kirkland T. N. (2001) MD-2 binds to bacterial lipopolysaccharide. J. Biol. Chem. **276:** 38044–38051
61 Shapiro R. A., Cunningham M. D., Ratcliffe K., Seachord C., Blake J., Bajorath J. et al. (1997) Identification of CD14 residues involved in specific lipopolysaccharide recognition. Infect. Immun. **65:** 293–297
62 Juan T. S., Hailman E., Kelley M. J., Busse L. A., Davy E., Empig C. J. et al. (1995) Identification of a lipopolysaccharide binding domain in CD14 between amino acids 57 and 64. J. Biol. Chem. **270:** 5219–5224
63 Frey E. A., Miller D. S., Jahr T. G., Sundan A., Bazil V., Espevik T. et al. (1992) Soluble CD14 participates in the response of cells to lipopolysaccharide. J. Exp. Med. **176:** 1665–1671
64 Grossman N. and Leive L. (1984) Complement activation via the alternative pathway by purified *Salmonella* lipopolysaccharide is affected by its structure but not its O-antigen length. J. Immunol. **132:** 376–385
65 Jiang G. Z., Sugiyama T., Kato Y., Koide N. and Yokochi T. (1995) Binding of mannose-binding protein to *Klebsiella* O3 lipopolysaccharide possessing the mannose homopolysaccharide as the O-specific polysaccharide and its relation to complement activation. Infect. Immun. **63:** 2537–2540
66 Vukajlovich S. W. (1986) Antibody-independent activation of the classical pathway of human serum complement by lipid A is restricted to re-chemotype lipopolysaccharide and purified lipid A. Infect. Immun. **53:** 480–485
67 Cooper N. R. and Morrison D. C. (1978) Binding and activation of the first component of human complement by the lipid A region of lipopolysaccharides. J. Immunol. **120:** 1862–1868
68 Zohair A., Chesne S., Wade R. H. and Colomb M. G. (1989) Interaction between complement subcomponent C1q and bacterial lipopolysaccharides. Biochem. J. **257:** 865–873
69 Brade V. and Kreuzpaintner G. (1980) Interaction of lipopolysaccharides and of lipid A from *Yersinia enterocolitica* with purified guinea pig C3. Immunobiology **156:** 441–453
70 Edwards J. L. and Apicella M. A. (2002) The role of lipooligosaccharide in *Neisseria gonorrhoeae* pathogenesis of cervical epithelia: lipid A serves as a C3 acceptor molecule. Cell. Microbiol. **4:** 585–598
71 Liu D., Cai S., Gu X., Scafidi J., Wu X. and Davis A. E. 3rd (2003) C1 inhibitor prevents endotoxin shock via a direct interaction with lipopolysaccharide. J. Immunol. **171:** 2594–2601
72 Parker T. S., Levine D. M., Chang J. C., Laxer J., Coffin C. C. and Rubin A. L. (1995) Reconstituted high-density lipoprotein neutralizes gram-negative bacterial lipopolysaccharides in human whole blood. Infect. Immun. **63:** 253–258
73 Brandenburg K., Jurgens G., Andra J., Lindner B., Koch M. H., Blume A. et al. (2002) Biophysical characterization of the interaction of high-density lipoprotein (HDL) with endotoxins. Eur. J. Biochem. **269:** 5972–5981
74 Cardin A. D. and Weintraub H. J. (1989) Molecular modeling of protein-glycosaminoglycan interactions. Arteriosclerosis **9:** 21–32
75 Rensen P. C., Oosten M., Bilt E., Eck M., Kuiper J. and Berkel T. J. (1997) Human recombinant apolipoprotein E redirects lipopolysaccharide from Kupffer cells to liver parenchymal cells in rats In vivo. J. Clin. Invest. **99:** 2438–2445
76 Read T. E., Harris H. W., Grunfeld C., Feingold K. R., Calhoun M. C., Kane J. P. et al. (1993) Chylomicrons enhance endotoxin excretion in bile. Infect. Immun. **61:** 3496–3502
77 Gewurz H., Zhang X. H. and Lint T. F. (1995) Structure and function of the pentraxins. Curr. Opin. Immunol. **7:** 54–64
78 Loveless R. W., Floyd-O'Sullivan G., Raynes J. G., Yuen C. T. and Feizi T. (1992) Human serum amyloid P is a multispecific adhesive protein whose ligands include 6-phosphorylated mannose and the 3-sulphated saccharides galactose, N-acetylgalactosamine and glucuronic acid. EMBO J. **11:** 813–819
79 de Haas C. J., van der Zee R., Benaissa-Trouw B., van Kessel K. P., Verhoef J. and van Strijp J. A. (1999) Lipopolysaccharide (LPS)-binding synthetic peptides derived from serum amyloid P component neutralize LPS. Infect. Immun. **67:** 2790–2796
80 Roth R. I., Kaca W. and Levin J. (1994) Hemoglobin: a newly recognized binding protein for bacterial endotoxins (LPS). Prog. Clin. Biol. Res. **388:** 161–172
81 Kaca W., Roth R. I. and Levin J. (1994) Hemoglobin, a newly recognized lipopolysaccharide (LPS)-binding protein that enhances LPS biological activity. J. Biol. Chem. **269:** 25078–25084
82 Jurgens G., Muller M., Koch M. H. and Brandenburg K. (2001) Interaction of hemoglobin with enterobacterial lipopolysaccharide and lipid A. Physicochemical characterization and biological activity. Eur. J. Biochem. **268:** 4233–4242
83 Liepke C., Baxmann S., Heine C., Breithaupt N., Standker L. and Forssmann W. G. (2003) Human hemoglobin-derived peptides exhibit antimicrobial activity: a class of host defense peptides. J. Chromatogr. B. Analyt .Technol. Biomed. Life Sci. **791:** 345–356
84 Levy O., Canny G., Serhan C. N. and Colgan S. P. (2003) Expression of BPI (bactericidal/permeability-increasing protein) in human mucosal epithelia. Biochem. Soc. Trans. **31:** 795–800
85 Little R. G., Kelner D. N., Lim E., Burke D. J. and Conlon P. J. (1994) Functional domains of recombinant bactericidal/permeability increasing protein (rBPI23). J. Biol. Chem. **269:** 1865–1872
86 van Berkel P. H., Geerts M. E., van Veen H. A., Mericskay M., de Boer H. A. and Nuijens J. H. (1997) N-terminal stretch Arg2, Arg3, Arg4 and Arg5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. Biochem. J. **328:** 145–151
87 Britigan B. E., Lewis T. S., Waldschmidt M., McCormick M. L. and Krieg A. M. (2001) Lactoferrin binds CpG-containing oligonucleotides and inhibits their immunostimulatory effects on human B cells. J. Immunol. **167:** 2921–2928
88 Masson P. L., Heremans J. F. and Schonne E. (1969) Lactoferrin, an iron-binding protein in neutrophilic leukocytes. J. Exp. Med. **130:** 643–658
89 Elass-Rochard E., Roseanu A., Legrand D., Trif M., Salmon V., Motas C. et al. (1995) Lactoferrin-lipopolysaccharide interaction: involvement of the 28–34 loop region of human lactoferrin in the high-affinity binding to *Escherichia coli* 055B5 lipopolysaccharide. Biochem. J. **312:** 839–845
90 Majerle A., Kidric J. and Jerala R. (2003) Enhancement of antibacterial and lipopolysaccharide binding activities of a human lactoferrin peptide fragment by the addition of acyl chain. J. Antimicrob. Chemother. **51:** 1159–1165
91 Pereira H. A. (1995) CAP37, a neutrophil-derived multifunctional inflammatory mediator. J. Leukoc. Biol. **57:** 805–812
92 Iversen L. F., Kastrup J. S., Bjorn S. E., Rasmussen P. B., Wiberg F. C., Flodgaard H. J. et al. (1997) Structure of HBP, a multifunctional protein with a serine proteinase fold. Nat. Struct. Biol. **4:** 265–268
93 Brackett D. J., Lerner M. R., Lacquement M. A., He R. and Pereira H. A. (1997) A synthetic lipopolysaccharide-binding peptide based on the neutrophil-derived protein CAP37 prevents endotoxin-induced responses in conscious rats. Infect. Immun. **65:** 2803–2811
94 Heinzelmann M., Mercer-Jones M. A., Flodgaard H. and Miller F. N. (1998) Heparin-binding protein (CAP37) is internalized in monocytes and increases LPS-induced monocyte activation. J. Immunol. **160:** 5530–5536

95 Brandenburg K., Koch M. H., and Seydel U. (1998) Biophysical characterisation of lysozyme binding to LPS Re and lipid A. Eur. J. Biochem. **258:** 686–695

96 Nagpal S., Kaur K. J., Jain D. and Salunke D. M. (2002) Plasticity in structure and interactions is critical for the action of indolicidin, an antibacterial peptide of innate immune origin. Protein Sci. **11:** 2158–2167

97 Shamova O., Brogden K. A., Zhao C., Nguyen T., Kokryakov V. N. and Lehrer R. I. (1999) Purification and properties of proline-rich antimicrobial peptides from sheep and goat leukocytes. Infect. Immun. **67:** 4106–4111

98 Wang Y., Griffiths W. J., Curstedt T. and Johansson J. (1999) Porcine pulmonary surfactant preparations contain the antibacterial peptide prophenin and a C-terminal 18-residue fragment thereof. FEBS Lett. **460:** 257–262

99 Harwig S. S., Kokryakov V. N., Swiderek K. M., Aleshina G. M., Zhao C. and Lehrer R. I. (1995) Prophenin-1, an exceptionally proline-rich antimicrobial peptide from porcine leukocytes. FEBS Lett. **362:** 65–69

100 Fath M. A., Wu X., Hileman R. E., Linhardt R. J., Kashem M. A., Nelson R. M. et al. (1998) Interaction of secretory leukocyte protease inhibitor with heparin inhibits proteases involved in asthma. J. Biol. Chem. **273:** 13563–13569

101 Ding A., Thieblemont N., Zhu J., Jin F., Zhang J. and Wright S. (1999) Secretory leukocyte protease inhibitor interferes with uptake of lipopolysaccharide by macrophages. Infect. Immun. **67:** 4485–4489

102 Murakami Y., Shizukuishi S., Tsunemitsu A., Nakashima K., Kato Y. and Aimoto S. (1991) Binding of a histidine-rich peptide to *Porphyromonas gingivalis*. FEMS Microbiol. Lett. **66:** 253–256

103 Bosshart H. and Heinzelmann M. (2003) Endotoxin-neutralizing effects of histidine-rich peptides. FEBS Lett. **553:** 135–140

104 Hieshima K., Ohtani H., Shibano M., Izawa D., Nakayama T., Kawasaki Y. et al. (2003) CCL28 has dual roles in mucosal immunity as a chemokine with broad-spectrum antimicrobial activity. J. Immunol. **170:** 1452–1461

105 Andersson M., Girard R. and Cazenave P. (1999) Interaction of NK lysin, a peptide produced by cytolytic lymphocytes, with endotoxin. Infect. Immun. **67:** 201–205

106 Pena S. V., Hanson D. A., Carr B. A., Goralski T. J., and Krensky A. M. (1997) Processing, subcellular localization and function of 519 (granulysin), a human late T cell activation molecule with homology to small, lytic, granule proteins. J. Immunol. **158:** 2680–2688

107 Levy O., Ooi C. E., Elsbach P., Doerfler M. E., Lehrer R. I. and Weiss J. (1995) Antibacterial proteins of granulocytes differ in interaction with endotoxin. Comparison of bactericidal/permeability-increasing protein, p15s and defensins. J. Immunol. **154:** 5403–5410

108 Hill C. P., Yee J., Selsted M. E. and Eisenberg D. (1991) Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization. Science **251:** 1481–1485

109 Turner J., Cho Y., Dinh N. N., Waring A. J. and Lehrer R. I. (1998) Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob. Agents. Chemother. **42:** 2206–2214

110 Tack B. F., Sawai M. V., Kearney W. R., Robertson A. D., Sherman M. A., Wang W. et al. (2002) SMAP-29 has two LPS-binding sites and a central hinge. Eur. J. Biochem. **269:** 1181–1189

111 Gutsmann T., Fix M., Larrick J. W. and Wiese A. (2000) Mechanisms of action of rabbit CAP18 on monolayers and liposomes made from endotoxins or phospholipids. J. Membr. Biol. **176:** 223–236

112 Kuan S. F., Rust K. and Crouch E. (1992) Interactions of surfactant protein D with bacterial lipopolysaccharides. Surfactant protein D is an *Escherichia coli*-binding protein in bronchoalveolar lavage. J. Clin. Invest. **90:** 97–106

113 Van Iwaarden J. F., Pikaar J. C., Storm J., Brouwer E., Verhoef J., Oosting R. S. et al. (1994) Binding of surfactant protein A to the lipid A moiety of bacterial lipopolysaccharides. Biochem. J. **303:** 407–411

114 Head J. F., Mealy T. R., McCormack F. X. and Seaton B. A. (2003) Crystal structure of trimeric carbohydrate recognition and neck domains of surfactant protein A. J. Biol. Chem. **278:** 43254–43260

115 Augusto L., Le Blay K., Auger G., Blanot D., and Chaby R. (2001) Interaction of bacterial lipopolysaccharide with mouse surfactant protein C inserted into lipid vesicles. Am. J. Physiol. Lung Cell. Mol. Physiol. **281:** L776–785

116 Andersson M., Curstedt T., Jornvall H. and Johansson J. (1995) An amphipathic helical motif common to tumourolytic polypeptide NK-lysin and pulmonary surfactant polypeptide SP-B. FEBS Lett. **362:** 328–332

117 Augusto L. A., Li J., Synguelakis M., Johansson J. and Chaby R. (2002) Structural basis for interactions between lung surfactant protein C and bacterial lipopolysaccharide. J. Biol. Chem. **277:** 23484–23492

118 Augusto L. A., Synguelakis M., Johansson J., Pedron T., Girard R. and Chaby R. (2003) Interaction of pulmonary surfactant protein C with CD14 and lipopolysaccharide. Infect. Immun. **71:** 61–67

119 Beamer L. J., Carroll S. F. and Eisenberg D. (1998) The BPI/LBP family of proteins: a structural analysis of conserved regions. Protein Sci. **7:** 906–914

120 Ried C., Wahl C., Miethke T., Wellnhofer G., Landgraf C., Schneider-Mergener J. et al. (1996) High affinity endotoxin-binding and neutralizing peptides based on the crystal structure of recombinant Limulus anti-lipopolysaccharide factor. J. Biol. Chem. **271:** 28120–28127

121 Taylor A. H., Heavner G., Nedelman M., Sherris D., Brunt E., Knight D. et al. (1995) Lipopolysaccharide (LPS) neutralizing peptides reveal a lipid A binding site of LPS binding protein. J. Biol. Chem. **270:** 17934–17938

122 Frecer V., Ho B. and Ding J. L. (2000) Interpretation of biological activity data of bacterial endotoxins by simple molecular models of mechanism of action. Eur. J. Biochem. **267:** 837–852

123 Wright S. D., Ramos R. A., Tobias P. S., Ulevitch R. J. and Mathison J. C. (1990) CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science **249:** 1431–1433

124 Delude R. L., Savedra R. Jr, Zhao H., Thieringer R., Yamamoto S., Fenton M. J. et al. (1995) CD14 enhances cellular responses to endotoxin without imparting ligand-specific recognition. Proc. Natl. Acad. Sci. USA **92:** 9288–9292

125 Poltorak A., He X., Smirnova I., Liu M. Y., Van Huffel C., Du X. et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science **282:** 2085–2088

126 Hirschfeld M., Weis J. J., Toshchakov V., Salkowski C. A., Cody M. J., Ward D. C. et al. (2001) Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. Infect. Immun. **69:** 1477–1482

127 Werts C., Tapping R. I., Mathison J. C., Chuang T. H., Kravchenko V., Saint Girons I. et al. (2001) Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nat. Immunol. **2:** 346–352

128 Girard R., Pedron T., Uematsu S., Balloy V., Chignard M., Akira S. et al. (2003) Lipopolysaccharides from *Legionella* and *Rhizobium* stimulate mouse bone marrow granulocytes via Toll-like receptor 2. J. Cell Sci. **116:** 293–302

129 da Silva Correia J., Soldau K., Christen U., Tobias P. S. and Ulevitch R. J. (2001) Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. Transfer from CD14 to TLR4 and MD-2. J. Biol. Chem. **276:** 21129–21135

130 Yang R. B., Mark M. R., Gray A., Huang A., Xie M. H., Zhang M. et al. (1998) Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature **395:** 284–288
131 Mullen G. E., Kennedy M. N., Visintin A., Mazzoni A., Leifer C. A., Davies D. R et al. (2003) The role of disulfide bonds in the assembly and function of MD-2. Proc. Natl. Acad. Sci. USA **100:** 3919–3924
132 Inohara N. and Nunez G. (2002) ML – a conserved domain involved in innate immunity and lipid metabolism. Trends Biochem. Sci. **27:** 219–221
133 Mancek M., Pristovsek P. and Jerala R. (2002) Identification of LPS-binding peptide fragment of MD-2, a toll-receptor accessory protein. Biochem. Biophys. Res. Commun. **292:** 880–885
134 Visintin A., Latz E., Monks B. G., Espevik T. and Golenbock D. T. (2003) Lysines 128 and 132 enable lipopolysaccharide binding to MD-2, leading to Toll-like receptor-4 aggregation and signal transduction. J. Biol. Chem. **278:** 48313–48320
135 Kawasaki K., Nogawa H. and Nishijima M. (2003) Identification of mouse MD-2 residues important for forming the cell surface TLR4-MD-2 complex recognized by anti-TLR4-MD-2 antibodies, and for conferring LPS and taxol responsiveness on mouse TLR4 by alanine-scanning mutagenesis. J. Immunol. **170:** 413–420
136 Akashi S., Saitoh S., Wakabayashi Y., Kikuchi T., Takamura N., Nagai Y. et al. (2003) Lipopolysaccharide interaction with cell surface Toll-like receptor 4-MD-2: higher affinity than that with MD-2 or CD14. J. Exp. Med. **198:** 1035–1042
137 Werner T., Borge-Renberg K., Mellroth P., Steiner H. and Hultmark D. (2003) Functional diversity of the *Drosophila* PGRP-LC gene cluster in the response to lipopolysaccharide and peptidoglycan. J. Biol. Chem. **278:** 26319–26322
138 Triantafilou K., Triantafilou M. and Dedrick R. L. (2001) A CD14-independent LPS receptor cluster. Nat. Immunol. **2:** 338–345
139 Wright S. D., Levin S. M., Jong M. T., Chad Z. and Kabbash L. G. (1989) CR3 (CD11b/CD18) expresses one binding site for Arg-Gly-Asp-containing peptides and a second site for bacterial lipopolysaccharide. J. Exp. Med. **169:** 175–183
140 Ingalls R. R., Arnaout M. A. and Golenbock D. T. (1997) Outside-in signaling by lipopolysaccharide through a tailless integrin. J. Immunol. **159:** 433–438
141 El-Samalouti V. T., Schletter J., Brade H., Brade L., Kusumoto S., Rietschel E. T. et al. (1997) Detection of lipopolysaccharide (LPS)-binding membrane proteins by immuno-coprecipitation with LPS and anti-LPS antibodies. Eur. J. Biochem. **250:** 418–424
142 Pfeiffer A., Bottcher A., Orso E., Kapinsky M., Nagy P., Bodnar A. et al. (2001) Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts. Eur. J. Immunol. **31:** 3153–3164
143 Malhotra R., Taylor N. R. and Bird M. I. (1996) Anionic phospholipids bind to L-selectin (but not E-selectin) at a site distinct from the carbohydrate-binding site. Biochem. J. **314:** 297–303
144 Malhotra R. and Bird M. I. (1997) L-selectin: a novel receptor for lipopolysaccharide and its potential role in bacterial sepsis. Bioessays **19:** 919–923
145 Malhotra R., Priest R., Foster M. R. and Bird M. I. (1998) P-selectin binds to bacterial lipopolysaccharide. Eur. J. Immunol. **28:** 983–988
146 Miyake K., Ogata H., Nagai Y., Akashi S. and Kimoto M. (2000) Innate recognition of lipopolysaccharide by Toll-like receptor 4/MD-2 and RP105/MD-1. J. Endotoxin Res. **6:** 389–391
147 Hampton R. Y., Golenbock D. T., Penman M., Krieger M. and Raetz C. R. (1991) Recognition and plasma clearance of endotoxin by scavenger receptors. Nature **352:** 342–344
148 Krieger M., Acton S., Ashkenas J., Pearson A., Penman M. and Resnick D. (1993) Molecular flypaper, host defense and atherosclerosis. Structure, binding properties and functions of macrophage scavenger receptors. J. Biol. Chem. **268:** 4569–4572
149 Kutuzova G. D., Albrecht R. M., Erickson C. M. and Qureshi N. (2001) Diphosphoryl lipid A from *Rhodobacter sphaeroides* blocks the binding and internalization of lipopolysaccharide in RAW 264.7 cells. J. Immunol. **167:** 482–489
150 Detmers P. A., Thieblemont N., Vasselon T., Pironkova R., Miller D. S. and Wright S. D. (1996) Potential role of membrane internalization and vesicle fusion in adhesion of neutrophils in response to lipopolysaccharide and TNF. J. Immunol. **157:** 5589–5596
151 Hornef M. W., Frisan T., Vandewalle A., Normark S. and Richter-Dahlfors A. (2002) Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. J. Exp. Med. **195:** 559–570
152 Thieblemont N. and Wright S. D. (1999) Transport of bacterial lipopolysaccharide to the golgi apparatus. J. Exp. Med. **190:** 523–534
153 Hu Y., Fisette P. L., Denlinger L. C., Guadarrama A. G., Sommer J. A., Proctor R. A. et al. (1998) Purinergic receptor modulation of lipopolysaccharide signaling and inducible nitric-oxide synthase expression in RAW 264.7 macrophages. J. Biol. Chem. **273:** 27170–27175
154 Denlinger L. C., Fisette P. L., Sommer J. A., Watters J. J., Prabhu U., Dubyak G. R. et al. (2001) The nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. J. Immunol. **167:** 1871–1876
155 Amar S., Oyaisu K., Li L. and Van Dyke T. (2001) Moesin: a potential LPS receptor on human monocytes. J. Endotoxin Res. **7:** 281–286
156 Ding A., Sanchez E., Tancinco M. and Nathan C. (1992) Interactions of bacterial lipopolysaccharide with microtubule proteins. J. Immunol. **148:** 2853–2858
157 Qureshi N., Perera P. Y., Shen J., Zhang G., Lenschat A., Splitter G. et al. (2003) The proteasome as a lipopolysaccharide-binding protein in macrophages: differential effects of proteasome inhibition on lipopolysaccharide-induced signaling events. J. Immunol. **171:** 1515–1525
158 Augusto L. A., Decottignies P., Synguelakis M., Nicaise M., Le Marechal P. and Chaby R. (2003) Histones: a novel class of lipopolysaccharide-binding molecules. Biochemistry **42:** 3929–3938
159 Emlen W., Holers V. M., Arend W. P. and Kotzin B. (1992) Regulation of nuclear antigen expression on the cell surface of human monocytes. J. Immunol. **148:** 3042–3048
160 Bolton S. J. and Perry V. H. (1997) Histone H1; a neuronal protein that binds bacterial lipopolysaccharide. J. Neurocytol. **26:** 823–831
161 Kim H. S., Cho J. H., Park H. W., Yoon H., Kim M. S. and Kim S. C. (2002) Endotoxin-neutralizing antimicrobial proteins of the human placenta. J. Immunol. **168:** 2356–2364
162 Kim H. S., Yoon H., Minn I., Park C. B., Lee W. T., Zasloff M. et al. (2000) Pepsin-mediated processing of the cytoplasmic histone H2A to strong antimicrobial peptide buforin I. J. Immunol. **165:** 3268–3274
163 Park I. Y., Park C. B., Kim M. S. and Kim S. C. (1998) Parasin I, an antimicrobial peptide derived from histone H2A in the catfish, *Parasilurus asotus*. FEBS Lett. **437:** 258–262
164 Lemaire I., Yang H., Cantin M. F. and Lemaire S. (1994) Up-regulation of cytokine production in alveolar macrophages by histogranin, a novel endogenous pentadecapeptide. Immunol. Lett. **41:** 37–42
165 Chang L., Loranger S. S., Mizzen C., Ernst S. G., Allis C. D. and Annunziato A. T. (1997) Histones in transit: cytosolic histone complexes and diacetylation of H4 during nucleosome assembly in human cells. Biochemistry **36:** 469–480

166 Inohara N., Ogura Y., Chen F. F., Muto A. and Nunez G. (2001) Human Nod1 confers responsiveness to bacterial lipopolysaccharides. J. Biol. Chem. **276:** 2551–2554
167 Girardin S. E., Boneca I. G., Carneiro L. A., Antignac A., Jehanno M., Viala J. et al. (2003) Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science **300:** 1584–1587
168 Pugin J., Heumann I. D., Tomasz A., Kravchenko V. V., Akamatsu Y., Nishijima M. et al. (1994) CD14 is a pattern recognition receptor. Immunity **1:** 509–516
169 Goh Y. Y., Ho B. and Ding J. L. (2002) A novel fluorescent protein-based biosensor for gram-negative bacteria. Appl. Environ. Microbiol. **68:** 6343–6352