Lipopolysaccharide-Binding Protein (LBP) as an Indicator of Disease States in Multiple Species

Amanda Flies, Ph.D. and Thomas H. Ermak, Ph.D.
Cell Sciences, Inc., Canton, Massachusetts, USA. tech@cellsciences.com

Abstract

Lipopolysaccharide binding protein (LBP) is a 58-kD glycoprotein involved in the acute-phase immunologic response to Gram-negative bacterial infections. It binds with high affinity to the lipid A portion of lipopolysaccharide (LPS), which Gram-negative bacteria express on their outer membrane. The presence of LBP in blood and bodily fluids is an indicator of bacterial infection, and as such is a useful marker of a number of disease states in humans, cows, mice and other animals. As a method of detecting this important acute-phase protein, ELISA (enzyme-linked immunosorbent assay) offers specificity and reliable quantitation of the protein levels in samples such as blood, serum, lavage fluid, or milk. The Cell Sciences® Multispecies LBP ELISA kit maintains specificity for human LBP, while also providing flexibility to assess a broad range of LBP homologs in other species.

LPS/LBP/CD14 pathway

LPS-binding protein (LBP) is mainly produced by the liver as part of the innate immune response to infection or damage involving exposure to Gram-negative bacteria (1). LBP binds lipopolysaccharide (LPS), which is a component of the outer membrane of Gram-negative bacteria (Fig. 1). LPS is highly inflammatory and is found in the blood or other bodily fluids when shed as a result of damage to the bacterial cells. It is composed of carbohydrate "O-antigens," core oligosaccharides, and Lipid A, which is both highly immunogenic and hydrophobic (2, 3). LBP binds via the lipid A portion of the molecule with high affinity, and can bind multimers of LPS, which aggregate due to the hydrophobic nature of lipid A (4). LBP can then transfer monomers of LPS to CD14 (3, 4; Fig. 2a). The CD14 molecule exists either as a glycosylphosphatidylinositol (GPI)-anchored membrane protein on monocytes, macrophages and dendritic cells, or as a soluble protein which, when bound to LPS, can activate CD14-deficient cells (4, 5; Fig. 2b). Once LPS has been transferred to CD14, the immune cell detects LPS via the Toll-like receptor 4 (TLR4):MD2 complex, resulting in signaling through a number of possible pathways (3, 6).

The biological activity of LBP is complicated. Low concentrations of LBP can bind LPS and mediate the recognition of this bacterial product by way of the CD14:TLR4:MD2 pathway on immune cells (2, 3, 6). Depending on the concentration of LPS presented to TLR4, this could result in either a pro- or anti-inflammatory response (6). High concentrations of LBP can inhibit that same CD14:TLR:MD2 recognition (7) by dissociating LPS from CD14 (8). Furthermore, LBP has been shown to be involved in transferring LPS to lipoprotein molecules, resulting in clearance of LPS from the bloodstream (9–11). Activation of innate immune cells allows for a swift, but controlled, immune response to Gram-negative bacteria, while inhibition of that signal and clearance of LPS from the system may aid in preventing septic shock (7, 12).

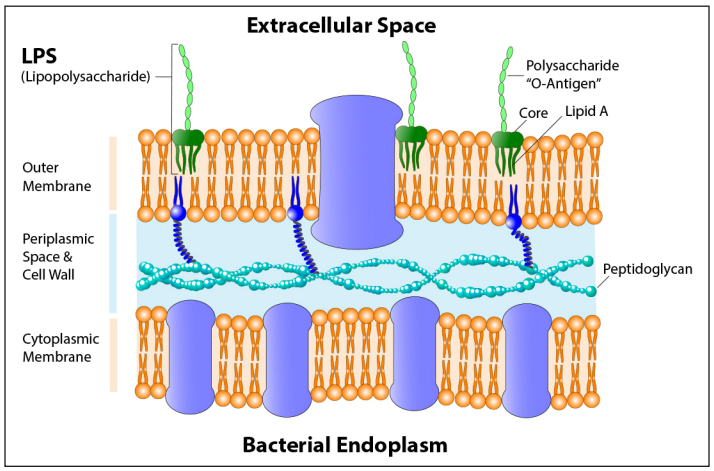

Fig. 1: *Cross-section of the envelope of a typical Gram-negative bacterium. LPS is a component of the outer membrane, and is itself composed of the hydrophobic Lipid A domain, a core oligosaccharide, and the polysaccharide chain(s) that make up the "O-antigen" extending from the surface of the bacterium into the extracellular space.*

LBP as a marker of disease in humans

Quantification of LBP (and by proxy, LPS) levels has been useful in detection of various disease states, including many in which Gram-negative bacteria have been implicated. Traditionally, the presence of LPS in the bloodstream was an indicator of sepsis, and if the amount of LPS was large enough, the cause of septic shock. However, recent research has utilized LBP as an indicator of bacteria or bacterial products that may not be classified as sepsis. Microbial/ bacterial translocation (MT/ BT), or movement of bacteria from the lumen of the intestine through gut mucosal barriers to the bloodstream (13), is implicated in complications for various diseases. Indeed, it is currently thought that the presence of low levels of LPS, which are found in many patients with chronic diseases, are in fact contributing to a constant state of low-grade inflammation that prevents the normal healing

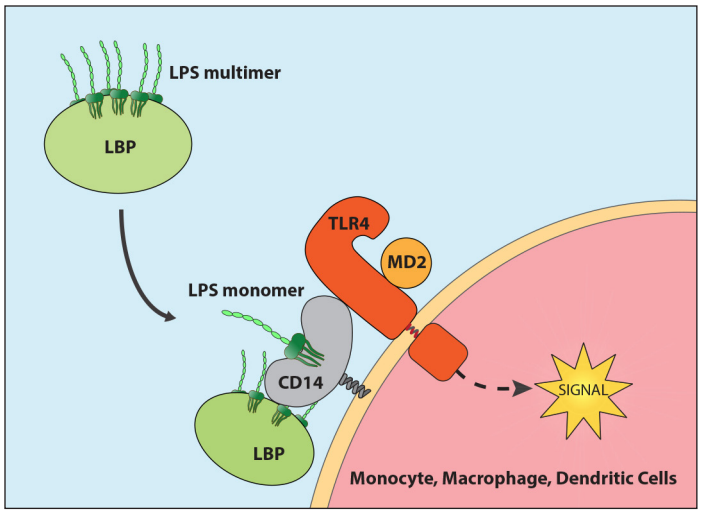

Fig. 2a: *LBP binds multimers of LPS in the blood or other bodily fluids and transfers LPS monomers to CD14 on the surface of an innate immune cell. LPS is detected at the cell's surface by a protein complex involving CD14, MD2 and TLR4, which signals through its intracellular domain. Recognition of bacterial products such as LPS may activate innate immune cells to produce inflammatory mediators.*

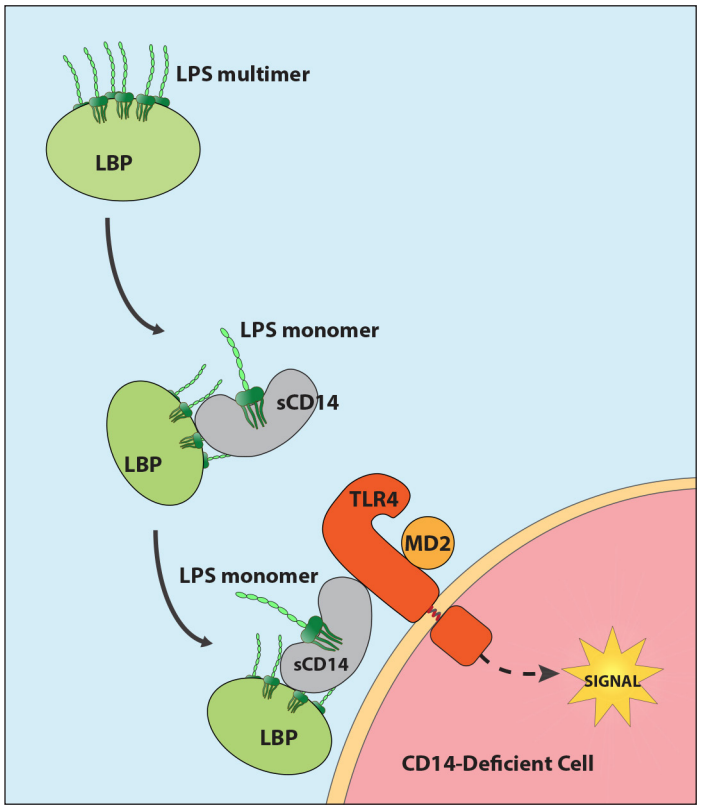

Fig. 2b: *LBP binds multimers of LPS and transfers monomers to soluble CD14 (sCD14), which may then potentiate the recognition of LPS by cells which do not themselves express CD14 on their cell membranes.*

process (6). Research on various chronic inflammatory conditions, such as Type 1 diabetes mellitus (T1DM) (14), obesity (15), and fatty-liver disease (16), have posited or positively correlated LBP levels with worsening disease states. Studies in HIV-infected patients have used LBP levels as a measure of gut damage, as well as implicating it in the ongoing activation of monocytes in that disease (17). LBP has also been used to study Parkinson's Disease, wherein exposure to bacterial products from the gut may be the cause of neurotoxic inflammation (18). Additionally, LBP has been studied as a marker of bacterial infections in patients with liver disease, which are associated with higher mortality rates (19). Furthermore, LBP levels have been studied in connection with predicting the outcome of treatment for lung cancer (20) and HCV (21). As a protein associated with many disease states and complications, LBP is a useful tool for clinical and research studies.

Studying LBP as a marker of disease in animals

Over the last decade, the multispecies LBP immunoassay has facilitated study of the acute phase response to LPS in bovine models (22–25). In initial studies, investigators examined whether intra-mammary challenge with LPS could influence bovine blood and milk levels of LBP (22, 23). Increased levels of LBP were observed in circulation and milk within 12 hours after LPS challenge and maximized within 24 hours. Elevated levels of blood and milk concentrations of LBP were also detected in cows with naturally occurring mastitis (24). Recently, studies were performed to assess acute phase protein levels in a setting of naturally occurring pneumonia in a calf feedlot (25). The study showed that measurement of LBP is associated with clinically diagnosed BRD (Bovine Respiratory Disease) under field conditions, and the levels correlate with those previously described in challenged experimental studies.

LBP ELISA

Much of the current understanding of the acute-phase immunologic response comes from measurement of LBP in plasma, serum, or bodily fluids in humans, mice, and veterinary models. The concentration of LBP is determined using a "multispecies" human ELISA kit that cross-reacts with bovine, porcine, sheep, goat, and rabbit LBP. A monoclonal antibody specific for human LBP is used for coating modular plates. The assay measures LBP in the 1.5 to 50 ng/ml range (Fig. 3).

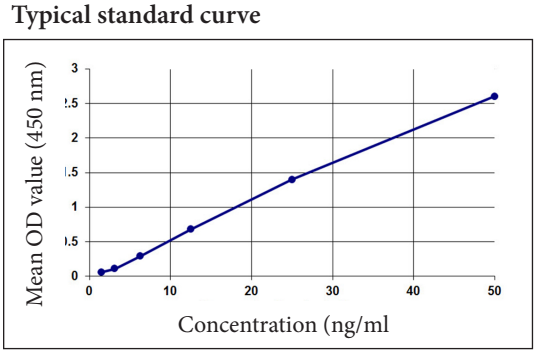

Fig. 3: *The standard curve from a typical Cell Sciences® "Multispecies" ELISA kit showing a concentration range from 1.5 to 50 ng/ml.*

Levels of LBP in humans range from 5-15 µg/ml and the reference serum provided in the kit is approximately 10 µg/ml. Accordingly, samples must be diluted to bring concentrations into the range of the assay (Table 1). For example, a dilution of 1:800 is recommended for humans, whereas a dilution of 1:10 to 1:100 is recommended for bovine samples where the levels of LBP typically range from 0.05 – 2.5 µg/ml.

Table 1: Normal LBP Range using the Human LBP Standard

Human	5 - 15 µg/ml
Bovine	0.05 - 2.5 µg/ml
Sheep & Goat	10 - 30 ng/ml
Porcine & Rabbit	4 - 10 µg/ml

LBP and related reagents

In addition to multispecies and mouse LBP ELISAs, Cell Sciences® offers LBP monoclonal antibodies that do or do not inhibit binding of LPS to membrane-bound CD14 (Table 2). These antibodies may be paired to act as controls in assays measuring LPS activation of CD14 expressing cells. Furthermore, Cell Sciences® offers recombinant human or mouse LBP proteins that have been shown to mediate binding of LPS to membrane-bound CD14.

ELISAs are also available to measure human and mouse soluble CD14, and several CD14 monoclonal antibody clones and recombinant proteins may be used study binding of LPS to CD14. Whatever your goals regarding the study of LBP, Cell Sciences® can provide you with high-quality reagents which meet your needs.

Table 2: LBP Product Family

LBP	
Catalog No:	**Description**
CKH113	Human LBP Multispecies Reactive ELISA Kit
CKM043	Mouse LBP ELISA Kit
CML002	Mouse Anti-Human LBP Clone biG 42 mAb†
CML003	Mouse Anti-Human LBP Clone biG 48 mAb†
CML004	Mouse Anti-Human LBP Clone biG 412 mAb*
CML007	Mouse Anti-Human LBP Clone biG 43 mAb†
CPC402	Rabbit Anti-Human LBP pAb
CML001	Mouse Anti-Mouse LBP Clone biG 33 mAb*
CML005	Mouse Anti-Mouse LBP Clone biG 35 mAb†
CPL105	Rabbit Anti-Mouse LBP pAb
CRL701	Recombinant Human LBP
CRL700	Recombinant Mouse LBP

* Inhibits binding to CD14 † Does not inhibit binding to CD14

CD14	
Catalog No:	**Description**
CKH114	Human sCD14 ELISA Kit
CKM034	Mouse sCD14 ELISA Kit
CDM150	Mouse Anti-Human CD14 Clone B-A8 Azide Free mAb
CMC000	Mouse Anti-Human CD14 Clone biG 10 mAb
CMC001	Mouse Anti-Human CD14 Clone biG 13 multi-species mAb
CMC005	Mouse Anti-Mouse CD14 Clone biG 53 mAb
CRCC01	Recombinant Human CD14
CRCC03	Recombinant Mouse CD14

Recent Publications Citing Cell Sciences® LBP ELISA kit:

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