Membrane-Anchored Forms of Lipopolysaccharide (LPS)-Binding Protein Do Not Mediate Cellular Responses to LPS Independently of CD14

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Membrane-Anchored Forms of Lipopolysaccharide (LPS)-Binding Protein Do Not Mediate Cellular Responses to LPS Independently of CD14

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Inflammatory responses of myeloid cells to LPS are mediated through CD14, a glycosyolphosphatidylinositol-anchored receptor that binds LPS. Since CD14 does not traverse the plasma membrane and alternatively anchored forms of CD14 still enable LPS-induced cellular activation, the precise role of CD14 in mediating these responses remains unknown. To address this, we created a transmembrane and a glycosylphosphatidylinositol-anchored form of LPS-binding protein (LBP), a component of serum that binds and transfers LPS to other molecules. Stably transfected Chinese hamster ovary (CHO) fibroblast and U373 astrocytoma cell lines expressing membrane-anchored LBP (mLBP), as well as separate CHO and U373 cell lines expressing membrane CD14 (mCD14), were subsequently generated. Under serum-free conditions, CHO and U373 cells expressing mLBP responded to as little as 0.1 ng/ml of LPS, as measured by NF-κB activation as well as ICAM and IL-6 production. Conversely, the vector control and mLBP-expressing cell lines did not respond under serum-free conditions even in the presence of more than 100 ng/ml of LPS. All the cell lines exhibited responses to less than 1 ng/ml of LPS in the presence of the soluble form of CD14, demonstrating that they are still capable of LPS-induced activation. Taken together, these results demonstrate that mLBP, a protein that brings LPS to the cell surface, does not mediate cellular responses to LPS independently of CD14. These findings suggest that CD14 performs a more specific role in mediating responses to LPS than that of simply bringing LPS to the cell surface. The Journal of Immunology, 1999, 162: 5483–5489.

Lipopolysaccharide, or endotoxin, a molecule that comprises the outer membrane of Gram-negative bacteria, provides a highly potent stimulus to cells of the immune system. LPS-induced cellular activation is mediated through two host proteins: LPS-binding protein (LBP) and the leukocyte receptor CD14. LBP is a 60-kDa serum glycoprotein that interacts with LPS aggregates and delivers LPS to other host proteins, most notably CD14. CD14 is a 55-kDa receptor expressed on the surface of neutrophils and macrophages that appears to function in a variety of important biological processes. In addition to mediating inflammatory responses of cells to LPS, CD14 has been shown to mediate responses to many pathogen-derived ligands, including Gram-positive bacterial peptidoglycan (3), mycobacterial lipoarabinomannan (4), fungal products (5, 6), and spirochetal lipoproteins (7, 8).

Paradoxically, in addition to their roles as proinflammatory effector molecules, LBP and CD14 also act in the cellular clearance of endotoxin from body fluid. In this role, LBP is an opsonin for whole Gram-negative bacteria, and CD14 is a receptor for both bacteria opsonized with LBP (9, 10) and for LBP-LPS aggregates (11). Binding of these particles leads to cellular internalization, followed by degradation and inactivation of LPS (12). Several lines of evidence have shown that this clearance function is separate from those events leading to cellular activation (11, 13–15).

In this regard, CD14 is also one of many receptors involved in the recognition and clearance of apoptotic cells, a process that also does not lead to cellular activation (16).

Most notable of the functions of CD14 is that it is the critical receptor that allows cells of myeloid lineage to respond to low concentrations of LPS (2). In fact, the maturation of the LPS response in monocytes has been shown to be dependent on the expression of CD14 on the cell surface (17). In addition, expression of CD14 confers LPS responsiveness to a variety of nonmyeloid cells. This ability was first demonstrated using 70Z/3 cells, a pre-B cell line whose sensitivity to LPS is increased over 1000-fold upon transfection with CD14 (18). Subsequent studies have shown that low ng/ml concentrations of LPS can activate NF-κB in both CHO and HT1080 fibroblast cell lines only after these cell lines are transfected with CD14 (19, 20). The disaggregation and delivery of LPS to CD14 that are mediated by LBP act to enhance the sensitivity of CD14-expressing cells to LPS.

Since CD14 is a glycosylphosphatidylinositol (GPI)-anchored molecule that does not traverse the cell membrane, the mechanism by which it confers LPS responsiveness to cells has remained a long-standing question in the field. This question is not restricted to CD14 since it remains unknown how other GPI-anchored receptors mediate cellular signaling (21). Clearly, the GPI anchor itself is not required for the ability of CD14 to transduce signals since expression of various transmembrane-anchored forms of this receptor still confer LPS responsiveness to cells (22, 23). Analogous to CD14, the leukocyte integrins CD11/CD18 also bind whole bacteria and LPS (24), and expression of these heterodimeric transmembrane proteins imparts LPS responsiveness to...
CHO cells (25). As with CD14, it is not known how these integrins trigger cellular activation signals across the cell membrane in response to LPS. This ability is not a function of any cytoplasmic protein sequences since expression of mutant receptors of CD11/CD18 that lack their cytoplasmic tails still elicit LPS-induced activation of NF-κB in CHO cells (26).

The fact that different LPS-binding receptors lacking traditional intracellular signaling domains still enable cellular responses to LPS has led to the theory that only the role of CD14 is to bring LPS in close proximity to the cell membrane where other events subsequently lead to cellular activation. To address this, and to better understand the mechanism of CD14-mediated activation, we engineered cell lines expressing membrane-anchored forms of the LPS delivery molecule, LBP. Here, we show that, although membrane-anchored LBP (mLBP) binds LPS, it does not confer LPS responsiveness to either CHO or U373 cells even at high ng/ml concentrations of LPS. Conversely, expression of CD14 in CHO or U373 cells confers LPS responsiveness to these cells in the presence of pg/ml concentrations of LPS. These results suggest that CD14 performs a more specific function in mediating LPS-induced activation of cells than that of merely bringing LPS to the cell surface.

Materials and Methods

Generation of CHO and U373 cell lines

A vector-expressing LBP with a carboxyl-terminal GPI-anchoring sequence was generated by first modifying full-length LBP by PCR. The oligonucleotides 5′-GTAAGCTTGTACACTGACGTGGGAATCTAGG-3′ and 5′-GCACACGTCTAGAAACTCTCATGTACCGTTGG-3′ were used as primers to the amino terminus and carboxyl terminus of LBP, respectively, in a PCR reaction that amplified LBP from a vector containing a cDNA encoding full-length human LBP (27). The ends of the amplified human LBP DNA were cleaved at the newly introduced NotI overhang. The amplified and cleaved human LBP and the GPI-anchoring sequence of CD55, decay accelerating factor (28), was made synthetically by annealing the complementary oligonucleotides 5′-CTAGAAGCTTGGTACCACTGCACTGGGAATCTAGG-3′ and 5′-GGCCGCGCTAAGTCAGCAAGCCCATGGTTAAGCCCTGAGCTCAGTG-3′. The ends of the amplified GPI-anchored LBP vector encode full-length human LBP with the addition of 35 amino acids SRRSITGSLRTLSCHTFTLTLGLTLWTMGLLT at the carboxyl terminus, which is the minimal GPI-anchoring sequence of CD55 (29). This construct was confirmed by sequencing at the Core Facility of The Scripps Research Institute.

CHO and U373 cell lines expressing mLCD14, mLBP, and vector alone were made by transfecting U373 cells with the vectors pRSV-CD14 (18), pCDNA-LBP, and pCDNA3.1 empty vector, respectively. Transfections were performed by electroporating 2 × 106 cells in 0.5 ml of electroporation buffer (20 mM HEPES, 150 mM NaCl, 2 mM EDTA, and 0.5% BSA, 10 mM NaF, 5 mM deoxyglucose, 2 mM NaI (pH 7.4)) containing 800 ng/ml [3H]LPS from Escherichia coli strain K12 (List Biologicals, Campbell, CA) for 5 min at 37°C. The cell suspension was then layered on 200 μl of 1 N sodium hydroxide. Cell-bound counts were determined after adding EDTA scintillation fluid to the vial and resuspended in 200 μl of 1 N sodium hydroxide. Cell-bound counts were determined after adding EDTA scintillation fluid to the vial. Well plates containing mLBP and CD14 (28C5) were a gift from A. Moriarty and D. Leturcq (R. W. Johnson Pharmaceutical Research Institute, La Jolla, CA).

Whole cell binding assays were performed as described previously (30). Briefly, 5 × 105 dissociated U373 cells were incubated in 0.1 ml of binding buffer (20 mM HEPES, 150 mM NaCl, 2 mM EDTA, and 0.5% BSA, 10 mM NaF, 5 mM deoxyglucose, 2 mM NaI (pH 7.4)) containing 800 ng/ml [3H]LPS. After 6-h incubation with LPS, U373 cell supernatants were collected and assayed for IL-6 production. The IL-6 ELISA was performed using 96-well Immulon plates (Dynatech Laboratories, Chantilly, VA), which were coated using 2 μg/ml goat anti-human IL-6 polyclonal Ab (R&D Systems, Minneapolis, MN). The standard curve was generated using recombined human IL-6 CHO cells (Genzyme, Cambridge, MA). Bound IL-6 was detected using 2 μg/ml rabbit anti-human IL-6 polyclonal Ab (Endogen, Woburn, MA) followed by a 1:1000 dilution of goat anti-rabbit IgG-conjugated HRP (Biosource, Camarillo, CA). Plates were developed using o-phenylenediamine as a substrate, and optical density was determined at a wavelength of 490 nm using a Spectramax plate reader and software (Molecular Devices, Sunnyvale, CA). All values were interpolated from a four-parameter fit of the standard IL-6 curve.

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After a 6-h incubation with LPS, cell surface expression of ICAM was determined by removal of the U373 cells with cell dissociation buffer (Life Technologies) and staining the cells in FACs buffer containing PE-conjugated mouse anti-human ICAM (PharMingen) followed by FACs analysis.

LPS binding assays

Adherent U373 cells were washed with PBS and removed from flasks using Life dissolution buffer (Life Technologies, Gaithersburg, MD). Cell suspensions were washed with FACS buffer (20 mM HEPES, 150 mM NaCl, 0.2% BSA, 10 mM NaF, (pH 7.4)), and 1 × 105 cells were resuspended in 0.1 ml of FACS buffer on ice. FITC-labeled Re595 LPS was added to a final concentration of 500 ng/ml. Cell suspensions were shifted to 37°C for 5 min and put back on ice before analysis by flow cytometry. When used, Abs were added to the cell suspension for 30 min on ice at a final concentration of 20 μg/ml before the addition of LPS. Abs against LBP (8F5, 1E8, and 2B5) and CD14 (28C5) were a gift from A. Moriarty and D. Leturcq (R. W. Johnson Pharmaceutical Research Institute, La Jolla, CA).

After a 1-h incubation with LPS, nuclear extracts were prepared by a standard method (31) with modifications as described (32). Briefly, six-well plates of adherent CHO or U373 cells were washed with ice cold PBS. The cells were scraped off of the plates, centrifuged, and resuspended in 0.4 ml of ice cold buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF (pH 7.9)). After 10 min on ice, 25 μl of NP-40 detergent was added. The mixture was quickly mixed, and nuclei were immediately pelleted by centrifuging at 14,000 × g for 10 s. The nuclear pellet was resuspended in 50 μl of buffer B (20 mM HEPES (pH 7.9), 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1 mM PMSF), and, after 30 min on ice, nuclear lysates were cleared by centrifuging at 14,000 × g for 30 s. The supernatant containing nuclear proteins was recovered and mixed with 2 μg of protein to determine the protein concentration. The activity of NF-κB in the nuclear extracts was determined by a standard EMSA. Briefly, 3 μg of nuclear extract was preincubated with 10 μl of

NF-κB activation assay

After a 1-h incubation with LPS, nuclear extracts were prepared by a standard method (31) with modifications as described (32). Briefly, six-well plates of adherent CHO or U373 cells were washed with ice cold PBS. The cells were scraped off of the plates, centrifuged, and resuspended in 0.4 ml of ice cold buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF (pH 7.9)). After 10 min on ice, 25 μl of NP-40 detergent was added. The mixture was quickly mixed, and nuclei were immediately pelleted by centrifuging at 14,000 × g for 10 s. The nuclear pellet was resuspended in 50 μl of buffer B (20 mM HEPES (pH 7.9), 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1 mM PMSF), and, after 30 min on ice, nuclear lysates were cleared by centrifuging at 14,000 × g for 30 s. The supernatant containing nuclear proteins was recovered and mixed with 2 μg of protein to determine the protein concentration. The activity of NF-κB in the nuclear extracts was determined by a standard EMSA. Briefly, 3 μg of nuclear extract was preincubated with 10 μl of
binding buffer (5 mM HEPES, 5 mM MgCl₂, 50 mM KCl, 0.5 mM DTT, 10% glycerol containing 0.4 mg/ml poly(dI-dC), and 0.1 mg/ml sonicated salmon sperm DNA (pH 7.9)) for 10 min at room temperature. Then 25 fmol (50,000 cpm) of 32P end-labeled double stranded oligonucleotide encoding the consensus NF-κB site 5\textsuperscript{9}-AGTTGAGGGGACTTTC CCAGGC-3\textsuperscript{9} (Promega, Madison, WI) was added, and the reaction was incubated for another 10 min at room temperature. Samples were analyzed on 5% polyacrylamide gels prepared in 50 mM Tris borate buffer containing 1 mM EDTA. Electrophoresis was performed at 4°C for 2 h at 12 V/cm, after which gels were dried on Whatman paper followed by phoshoimaging (Molecular Dynamics, Sunnyvale, CA) or autoradiography.

**Results**

Cell surface expression of CD14, an LPS-binding protein, confers LPS responsiveness to a variety of non-CD14-expressing cell types. Since LBP is another well-characterized LPS binding and transfer protein, we were interested in comparing the effect of LPS on cells expressing a membrane-anchored form of LBP. To this end, we constructed a vector encoding full-length human LBP with the addition, at the carboxyl terminus, of the 35-aa sequence required for the GPI anchoring of CD55 (decay-accelerating factor). This mLBP expression vector, along with an expression vector encoding normal GPI-anchored CD14, was separately transfected into CHO and U373 cells. These fibroblast and epithelial-like astrocytoma cells are not known to endogenously express CD14.

Stably transfected CHO and U373 cell lines expressing mCD14, mLBP, or vector alone were generated under gentamicin selection followed by FACS using anti-CD14 or anti-LBP Abs (see Materials and Methods). A typical FACS analysis of these sorted cell lines using mAbs to CD14 and LBP reveals that CD14-transfected and mLBP-transfected cells specifically express cell surface CD14 and LBP, respectively (Fig. 1). Expression of either CD14 or mLBP is undetectable in the vector control cells.

The ability of the different cell lines to bind LPS was determined under serum-free conditions. When incubated with 500 ng/ml of FITC-labeled LPS on ice, all the U373 cell lines exhibited the same low level of background fluorescence as determined by FACS (Fig. 2A). When the whole cell binding reaction was shifted to 37°C, the vector control and mCD14-U373 cells exhibited a small increase in median fluorescence; however, the fluorescence of the mLBP-U373 cells dramatically increased, indicating that these cells specifically bind FITC-labeled LPS. The apparent binding of FITC-labeled LPS to mLBP-U373 cells was almost completely blocked by preincubation of the cells with the anti-LBP Abs 1E8 and 2B5, but was unaffected by the control Abs IB4 and 8F5, which recognize CD18 and denatured LBP, respectively. Similarly, the binding was inhibited by Fab fragments of the anti-LBP Ab 2B5 but not by Fab fragments of the anti-CD14 Ab 28C5. These results demonstrate that LBP expressed on the surface of cells as a GPI-anchored protein is capable of binding LPS. To confirm these results, whole cell-binding assays were performed using biosynthetically radiolabeled LPS (Fig. 2B). The results obtained with radiolabeled LPS were essentially identical to those obtained with FITC-labeled LPS, confirming that at 37°C membrane-anchored LBP is capable of binding LPS. A time course of the binding of LPS by mLBP revealed that it is rapid, saturating within 2 min (data not shown). Cells expressing mCD14 did not exhibit any significant measurable binding of LPS compared with vector control cells, even after incubating the binding reaction for several hours at 37°C. In this regard, mCD14 has been shown to be a receptor for LBP-LPS complexes (2, 11), and, as expected, addition of soluble LBP caused a dramatic increase in the association of FITC-LPS to the mCD14-expressing cells (Fig. 2A).
To address whether expression of mLBP enables LPS-induced cellular activation, we compared the responses of the different cell lines following exposure to LPS. To this end, we incubated the cells with various concentrations of LPS under serum-free conditions and measured activation of NF-κB in nuclear extracts by a standard EMSA. As expected, NF-κB was activated by as little as 1 ng/ml of LPS in both mCD14-CHO and CD14-U373 cells (Fig. 3). This activation was dependent on the expression of CD14 since the vector control cells were not activated even after exposure to as much as 100 ng/ml of LPS. Expression of mLBP did not alter the LPS responses of either the CHO or U373 cells, which, like the vector control cells, remained unresponsive even after exposure to as much as 100 ng/ml of LPS. Since the soluble form of CD14 has been shown to enable epithelial and endothelial cells to respond to LPS (33, 34), we added recombinant soluble CD14 (sCD14) to our assay to ensure that our cell lines are still capable of LPS-induced cellular activation. Addition of sCD14 enabled all the cell lines to respond to LPS (Fig. 3). These results demonstrate that, even though the CHO and U373 cells are capable of LPS-induced activation, expression of a membrane-anchored form of LBP on the cell surface does not confer LPS-induced activation of NF-κB.

To confirm that the NF-κB results reflect LPS-induced cellular activation events, we also measured the expression of two markers of inflammation, ICAM and IL-6, in our U373 cells. Under serum-free conditions, mCD14-U373 cells exhibited increased ICAM expression after treatment with as little as 100 pg/ml of Re595 LPS (Fig. 4). In contrast, mLBP-U373 cells and vector control cells showed no measurable ICAM response even after treatment with as much as 100 ng/ml LPS. To confirm this finding, we performed similar experiments using IL-6 production as a measure of cell activation. Activation of IL-6 production was measurable after the exposure of mCD14-U373 cells to as little as 100 pg/ml LPS and was maximal using 10 ng/ml LPS (Fig. 5). In contrast, mLBP-U373 cells and vector control U373 cells showed no measurable response to LPS even at concentrations as high as 100 ng/ml. These results demonstrate that, under serum-free conditions, expression of CD14 enables U373 cells to respond to low pg/ml concentrations of LPS while expression of LBP on the surface of these cells does not confer LPS responsiveness. In support of this

FIGURE 2. Membrane LBP binds LPS. A, Different U373 cell lines were incubated at 37°C for 5 min in FACS buffer containing 500 ng/ml FITC-labeled LPS. Cells were put back on ice, washed with FACS buffer, and analyzed by flow cytometry. Median channel fluorescence was determined using Becton Dickinson (Mountain View, CA) software. B, Different U373 cell lines were incubated at 37°C for 5 min in binding buffer containing 800 ng/ml of [3H]LPS from E. coli strain K12. Cell-bound counts were determined as described in Materials and Methods. Error bars represent the range of duplicate values. When used, Abs were preincubated with cells for 30 min on ice before addition of LPS.

FIGURE 3. LPS activates NF-κB in cells expressing mCD14 but not mLBP. CHO and U373 cell lines were incubated for 1 h under serum-free conditions with various concentrations of Re595 LPS as indicated, and cell extracts were analyzed for NF-κB activity by EMSA as described in Materials and Methods. Where indicated, 1 μg/ml of sCD14 was added with LPS. The data shown are from a representative experiment that was repeated with similar results.

FIGURE 4. LPS induces activation of ICAM in U373 cells expressing CD14 but not mLBP. U373 cell lines were incubated for 6 h under serum-free conditions with various concentrations of Re595 LPS as indicated and subsequently analyzed for ICAM expression by FACS as described in Materials and Methods. The data shown are from a representative experiment that was repeated with similar results.
conclusion, U373 cells expressing LBP as a transmembrane-anchored fusion protein, using sequences encoding the transmembrane domain of tissue factor, were also unresponsive to LPS under serum-free conditions (data not shown).

To ensure that our U373 cell lines are still capable of LPS-induced inflammatory responses, we added recombinant sCD14 to our activation assay. In the presence of 1 μg/ml of recombinant sCD14, both the vector control and mLBP-U373 cells exhibited responses to LPS even at a concentration as low as 1 ng/ml LPS (Fig. 6). These results demonstrate that these cell lines are still capable of LPS-induced activation through sCD14. Therefore, even though these cells are still capable of LPS-induced activation, expression of LBP on the cell membrane does not confer LPS responsiveness to cells under serum-free conditions. A small, but measurable, LPS response by the U373 vector control and mLBP-U373 cell lines was observed under higher LPS concentrations (Fig. 6). Interestingly, this activation was consistently higher in the mLBP-U373 cells, compared with the vector control cells (Figs. 5 and 6), even though both cell lines were treated identically. This result could suggest that cell surface expression of LBP mediates a low level of cellular activation in response to LPS. However, this activation was completely blocked by the anti-CD14 Ab 28C5, demonstrating that it is in fact due to a low level of contaminating CD14 in our assay (Fig. 6). Thus, it is likely that the higher level of residual activation observed when using the mLBP-U373 cells arises from the cellular expression of functional LBP, which is transferring the LPS to the contaminating CD14 in our assay. In support of this, the residual activation observed is also abrogated by the addition of the anti-LBP Ab 2B5 (Fig. 6).

Discussion

The basic mechanism by which CD14 mediates cellular activation has been unresolved since the discovery of this GPI-anchored molecule as a receptor for LPS-induced events (2). One theory of how CD14 signals is that it binds LPS and subsequently interacts with an as yet unidentified signal-transducing molecule. It is proposed that the interaction of CD14 and LPS with the signaling molecule would be the first step leading to cellular activation (35, 36). In support of this, monocytes pretreated with trypsin are no longer LPS responsive even though the CD14 on their surface appears intact and still binds LPS (37). The observation that certain analogues of LPS can act as potent LPS antagonists, capable of blocking cellular responses to LPS without inhibiting the apparent association of LPS with CD14, has also been cited as evidence for the existence of a separate signaling receptor (38, 39). The most compelling evidence for an accessory LPS-signaling molecule stems from the initial observation that lipid IVa, a metabolic precursor of LPS lacking two acyl chains, acts as an LPS agonist in humans and as an LPS antagonist in mice (40). Even though both of these actions are facilitated by CD14, the heterologous expression of mouse or human CD14 does not alter the species-specific cellular responses observed (20). These latter studies demonstrated that the target of the species-specific effect of lipid IVa must be a cellular-signaling component other than CD14 (20). Recently, members of a family of genes known as Toll-like receptors (41) were found to mediate cellular responses to LPS (42–44). As the Toll-like receptors signal activation of innate immune responses (45) and possess cytoplasmic signaling domains homologous to those of the IL-1 receptor, they represent interesting potential cell surface signaling partners for CD14.

Cellular responses of CD14-expressing cells to LPS are augmented by the presence of LBP. LBP is a serum protein that binds LPS and delivers it in a less aggregated state to other proteins, including CD14 (46, 47). Recent studies have shown that LBP and
sCD14 are also capable of transferring LPS to phospholipid vesicles (48). This finding has given rise to an alternate theory as to how LBP and CD14 enable cellular responses to LPS. The premise of this theory is that CD14 is an LPS carrier that merely brings LPS to the cellular membrane (48). Once at the membrane, it is proposed that the acyl chains of LPS perturb the plasma membrane, leading to activation of the cell (49). Thus, in this model, LPS and CD14 do not directly interact with a downstream signaling molecule; rather, membrane perturbations indirectly activate downstream signals. It is worth noting, however, that neither LBP nor sCD14 have been shown to transfer LPS into the membrane of an intact cell, and, in fact, it has been shown that cell-surface-associated LPS appears to be entirely removable by treatment with proteinase K (15).

In short, the mechanism by which CD14 mediates cellular responses to LPS remains undefined. To address the hypothesis that mCD14 simply functions as a molecule that brings LPS to the plasma membrane, we generated CHO and U373 cell lines expressing either mCD14 or membrane-anchored forms of LBP. Under serum-free conditions, cells expressing mCD14 responded to LPS in a manner similar to that observed with CHO cells expressing either mCD14 or sCD14, albeit at a lower degree of cellular activation (15). This finding has given rise to an alternate theory as to the role of CD14: it is not merely a lipid transporter, but rather, it has a strong influence on CD14-dependent internalization but has no measurable effect on the ability of LPS to stimulate cells (15). The ability of our mCD14-expressing cells to be directly activated by LPS, despite being able to bind LPS, supports the view that events leading to clearance of LPS vs those leading to LPS-induced cellular activation are separate. Our results also indirectly support the idea that the role of LBP in promoting cellular activation is to disaggregate LPS and deliver LPS in a more monomeric form to CD14.

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**References**


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