Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation

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Su, Grace L. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. Am J Physiol Gastrointest Liver Physiol 283: G256–G265, 2002; 10.1152/ajpgi.00550.2001.—Endogenous gut-derived bacterial lipopolysaccharides have been implicated as important cofactors in the pathogenesis of liver injury. However, the molecular mechanisms by which lipopolysaccharides exert their effect are not entirely clear. Recent studies have pointed to proinflammatory cytokines such as tumor necrosis factor-α as mediators of hepatocyte injury. Within the liver, Kupffer cells are major sources of proinflammatory cytokines that are produced in response to lipopolysaccharides. This review will focus on three important molecular components of the pathway by which lipopolysaccharides activate Kupffer cells: CD14, Toll-like receptor 4, and lipopolysaccharide binding protein. Within the liver, lipopolysaccharides bind to lipopolysaccharide binding protein, which then facilitates its transfer to membrane CD14 on the surface of Kupffer cells. Signaling of lipopolysaccharide through CD14 is mediated by the downstream receptor Toll-like receptor 4 and results in activation of Kupffer cells. The role played by these molecules in liver injury will be examined.

endotoxins; tumor necrosis factor; macrophages; CD14

LIPOPOLYSACCHARIDES (LPS) are glycolipids found in abundance on the outer membrane of all gram-negative bacteria and have the ability to incite a vigorous inflammatory response. In humans, nanograms of LPS injected into the bloodstream can result in all the physiological manifestations of septic shock (70, 118). Given that gram-negative bacteria normally colonize the colon, the body has developed strong defensive mechanisms that tightly regulate the entry and processing of LPS. The liver plays a central role in this process by virtue of its dual ability not only to clear LPS, but to respond energetically to LPS. The vast majority of LPS that enters the host in normal and pathological states does so through the gastrointestinal (GI) tract. Strategically and uniquely located at the gateway of the portal blood flow draining the GI tract, the liver is the final barrier to prevent GI bacteria and bacterial products, such as LPS, from entering the systemic blood stream (74). In experimental studies on healthy animals, LPS is cleared from the circulation within a few minutes of intravenous injection, and the majority is traced to the liver (65, 128). The liver’s primary role in clearing LPS can be demonstrated in patients with liver failure. Endotoxemia is frequently found in patients with cirrhosis, and the degree of endotoxemia is correlated with the degree of liver failure (12, 58).

In addition to its ability to clear LPS, the liver also responds to LPS with production of cytokines (63) and reactive oxygen intermediates (7, 8). Both ex vivo and in vitro studies with isolated liver perfusion and liver slice models have demonstrated that tumor necrosis factor (TNF)-α and interleukin (IL)-1 are released in response to LPS, primarily by Kupffer cells (63). Most of the toxicities of LPS, both in the liver and in the systemic circulation, have been related to the release of these inflammatory cytokines and mediators (35, 111). In baboons, passive immunization with murine monoclonal anti-TNF antibodies protects against multisystem organ failure caused by lethal doses of live gram-negative bacteria (111).
ROLE OF LPS IN LIVER INJURY

Multiple lines of evidence point to LPS as a cofactor in liver injury (80, 81). In animal models of liver injury, including carbon tetrachloride toxicity, choline deficiency, and alcohol-induced liver disease, injury to the liver is mitigated or prevented by the addition of oral nonabsorbable antibiotics, colectomy, or germ-free conditions (2, 54, 62, 90). In contrast, the addition of LPS augments liver injury caused by these hepatotoxins (11, 14). Furthermore, in carbon tetrachloride- and galactosamine-induced liver injury, the addition of anti-LPS antibody E-5 significantly reduces hepatotoxicity caused by these agents (19). These studies strongly support a role for endogenous LPS in toxicity caused by these agents (19). These studies suggest that LPS plays a role in the development of liver injury.

In alcoholic liver disease, the importance of LPS has also been demonstrated. Rats fed the Leiber-DeCarli liquid ethanol diet develop only fatty liver unless subsequent injections of LPS are given. Only the combination of LPS and chronic ethanol will produce a histological picture of hepatic necrosis and inflammation consistent with alcoholic hepatitis (11, 34). In contrast, with the Tsukamoto and French model of rat alcoholic hepatitis, in which rats are fed ethanol intragastrically in conjunction with a high-fat diet, the pathological picture of alcoholic hepatitis can be produced without further LPS challenge (114). Part of the discrepancy may be due to the presence of endogenous endotoxemia after the addition of alcohol to the diet. The level of endotoxemia present correlates with pathological injury (76, 78). Furthermore, pathological injury can be ameliorated by decreasing the level of endogenous endotoxemia with intraluminal lactobacillus or antibiotics (2, 75).

Endotoxin or LPS itself is not hepatotoxic at low concentrations. However, its ability to stimulate an inflammatory response may account for its pathogenicity in the liver. Although many parameters of the inflammatory response contribute to liver injury (72), one well-studied pathway is the production of TNF-α. In many models of liver injury, elevated TNF-α levels are present and correlate with injury (17, 48, 68). Inhibition of TNF-α activity can decrease liver injury. The addition of soluble TNF receptors that diminish the biological effect of TNF-α will significantly decrease liver enzymes, improve liver histology, and decrease mortality acutely after acute carbon tetrachloride administration (18). Similar results are seen in chronic alcohol-induced liver disease (44) in rats. In humans, elevated levels of TNF-α are seen in alcoholic hepatitis and are associated with increased mortality (24). As potent producers of inflammatory cytokines such as TNF-α, Kupffer cells have been implicated in the pathway leading to liver injury (106). Accordingly, inhibition of Kupffer cells with gadolinium decreases injury in alcohol-induced liver disease (1). Although the greatest attention has been focused on the role of TNF-α in liver injury, other cytokines have been shown to play important pathogenic roles, including proinflammatory cytokines IL-6 and IL-18 (52, 115) as well as anti-inflammatory cytokines such as IL-10 (13). In alcoholic liver disease, it is not just a marked elevation of one proinflammatory cytokine that results in liver injury, but more likely an alteration in the balance of pro- and anti-inflammatory factors that result in disease (77).

CD14

Given the critical importance of LPS and Kupffer cells in the pathogenesis of many forms of liver injury, increasing attention has focused on the mechanisms by which LPS activates Kupffer cells. In peripheral blood monocytes, LPS activation is mediated through LPS binding protein (LBP), CD14, and Toll-like receptor (TLR)4. LPS binds to LBP, a 60-kDa acute-phase protein produced predominantly by the liver and secreted into the circulation (93, 121). Although LBP can be expressed extrahepatically, the majority of LBP is produced by the liver (102). Present in normal and acute-phase serum, LBP binds with high affinity to the lipid A portion of LPS and catalyzes the transfer of LPS to cell surface receptors such as membrane CD14 (31). In the presence of LBP, markedly less LPS is needed to activate peripheral blood monocytes. Although LBP is not required for interactions of LPS with CD14, its presence significantly decreases the concentration of LPS sufficient for cellular activation (92). Thus the LBP-CD14 pathway is critical at the low concentrations of LPS found under physiological condition (109).

Because of its location in the liver sinusoids, which drain blood from the GI tract, Kupffer cells are chronically exposed to higher concentrations of LPS than circulating peripheral blood monocytes. Thus it is likely that Kupffer cells have evolved specialized different mechanisms to interact with LPS. Unlike peripheral blood monocytes, Kupffer cells have relatively low baseline expression of CD14 (6, 103, 127). However, expression of CD14 on Kupffer cells can be upregulated with multiple stimuli including LPS (67). By immunohistochemical staining, low levels of CD14 are detected in livers of unstimulated mice. The liver CD14 levels rapidly increase and peak 6 h after intraperitoneal injection with LPS (67). CD14 expression in the liver is also increased in many types of liver disease, including alcoholic and cholestatic liver injury in rodents (20, 98, 112). In the intragastric model of alcohol-induced liver injury in rats, the level of hepatic inflammation varies with alterations in the fat composition of the diet (76). Animals fed more unsaturated fats have greater liver injury; the degree of injury correlates with the level of endotoxemia and with the level of Kupffer cell CD14 expression (100). In human disease, CD14 expression on Kupffer cells is low in normal human liver but increases with different inflammatory liver diseases (110). Kupffer cell CD14 expression also varies with different stages of the same disease. In biliary atresia, Kupffer cell CD14 increases in early stages but declines in the later stages (4).

The physiological significance of these observed differences in Kupffer cell CD14 expression is not entirely clear.
clear. However, it is tempting to hypothesize that the changes in CD14 expression could be the underlying mechanism that determines the liver’s sensitivity to LPS toxicity. This line of reasoning is supported indirectly by studies showing that CD14 expression is linked with LPS responsiveness in the monocytic cell line THP-1 (64). Immature THP-1 cells respond poorly to LPS in the presence and absence of serum. Treatment with the maturational agent calcitriol causes dose- and time-dependent increases in CD14 mRNA and surface CD14 expression. This increased expression is associated with enhanced responsiveness of these THP-1 cells to LPS (64). Similarly, transfection of CD14-negative 70Z/3 cells, a murine pre-B cell line, results in heightened sensitivity to LPS (55). In vivo, CD14 transgenic mice that overexpress CD14 on monocytes have increased sensitivity to LPS (25). In contrast, genetically engineered CD14-deficient mice are insensitive to LPS (37). It should be noted that although CD14-deficient mice are insensitive to LPS, their responses to whole bacteria remain intact (71). Isolated peritoneal macrophages from CD14-deficient animals have deficient responses to LPS, but their response to whole bacteria remains intact (71) and appears to be mediated, in part, by other LPS receptors such as CD11b/CD18.

Because “resting” Kupffer cells have low basal expression of CD14, one may hypothesize that Kupffer cells, unlike peripheral blood monocytes, react to LPS in a CD14-independent manner (10, 57). Although this is an attractive hypothesis, the data support a more complicated paradigm. Although Kupffer cells express little CD14, CD14 is still critical for LPS activation (98). Isolated human Kupffer cells can respond to low concentrations of LPS (<10 ng/ml) with the production of TNF-α. Preincubation of the Kupffer cells with a monoclonal mouse anti-human CD14 antibody MY4 inhibits production of TNF-α and supports an essential role for the CD14 receptor in Kupffer cell activation by LPS under physiological conditions. In addition, Kupffer cells isolated from CD14-deficient mice are significantly less sensitive to LPS than wild-type animals (98).

Discovered as a myeloid differentiation marker, CD14 was previously thought to be expressed exclusively by myeloid cells (127). Both a membrane and soluble form of CD14 exist in serum. The soluble form is identical to the membrane, with the exception of the glycosylphosphatidylinositol (GPI) anchor, which is not present in the soluble form of the protein. sCD14 also binds LPS and can facilitate interactions with CD14-negative cells such as endothelial cells (26, 39, 87). The source of sCD14 was previously attributed to shedding from monocyctic cells bearing CD14 or from direct secretion by monocyctic cells (9, 15). However, it has become clear that other cells can also secrete soluble CD14. In situ hybridization and immunohistochemistry for CD14 show induction of CD14 mRNA and protein in mouse hepatocytes after LPS injection in vivo (22). In the intragastric ethanol model of rat alcoholic liver disease, isolated hepatocytes from dextrose-fed animals do not have significant expression of CD14 mRNA. However, with the addition of ethanol in the diet, the level of CD14 mRNA increases and is correlated with the degree of liver injury observed (100). Isolated hepatocytes from in vivo LPS-treated rats have upregulation of both cell-associated and soluble CD14 protein production when plated in vitro (59). Basal expression of hepatocyte CD14 is present, but upregulation after inflammatory stimuli appears to be mediated, in part, by IL-1 and TNF-α (23, 59, 61).

The finding of hepatocellular sCD14 production is also supported by studies of transgenic mice generated using an 80-kb human CD14 genomic DNA fragment (40). These mice have high expressions of CD14 in both monocyctic cells and hepatocytes. Further analysis of these transgenic mice demonstrated that monocyte and hepatocellular expression of CD14 are differentially regulated. An upstream regulatory element beyond a 24-kb region of the genomic DNA but within the 33-kb region of human CD14 was critical for hepatocellular CD14 expression but not monocyctic expression (84). Furthermore, the 33-kb transgenic mice produced a soluble form of human CD14 in serum, confirming that hepatocytes are a source of sCD14 in vivo.

Isolated human hepatocytes from normal livers express CD14 mRNA and protein in culture, predominantly the soluble form (97). This sCD14 is biologically active and able to facilitate LPS responses in the CD14-negative epithelial cell line SW620, resulting in activation and production of IL-8 in response to low concentrations of LPS. It is important to note, however, that sCD14 can exert widely varying effects on cellular responses to LPS. The balance between activation vs. inhibition of LPS responses by sCD14 is dependent on its concentration. At what are thought to be supraphysiological concentrations, sCD14 can compete with mCD14 and inhibit LPS activation of CD14-positive cells (29, 38, 96, 113). The exact concentrations of mCD14 and sCD14 present in the microenvironment of the liver are not known. Whether local hepatocyte expression of sCD14 can lead to high enough concentrations to suppress Kupffer cell reactivity to LPS is not known. However, interpretations of studies in liver injury should take into account the relative contributions of Kupffer cell and hepatocyte CD14.

In CD14 knockout mice that are deficient in both membrane and soluble CD14 expression, the predominant phenotype is that of LPS insensitivity (37). Yin et al. (126) demonstrated that after 4 wk of continuous enteral ethanol delivery, CD14-deficient animals had less liver injury than wild-type BALB-c controls. These studies in the rodent model of ethanol-induced liver disease suggest that increases in CD14 expression contribute to the pathogenesis of liver injury. In human alcoholic disease, a promoter polymorphism that is associated with increased CD14 expression was found to be more common in men with alcoholic hepatitis and cirrhosis (47). A polymorphism at −159 (cytosine to thymidine) of the human CD14 gene, which lies within the Sp1 transcriptional binding site known to affect CD14 expression in monocytes, was examined in au-
TLPs

Because membrane CD14 is a GPI-anchored protein without a transmembrane component, downstream partners for this receptor have been long sought. Recent studies in TLRs have been instrumental in elucidating the pathway by which ligand binding to membrane CD14 results in cellular activation. The Toll receptors are evolutionarily well conserved and were first described in Drosophila with biological functions in development and immunity (89). A series of TLRs, which are human homologs of the Toll receptors in Drosophila, have been cloned (69). To date, at least 10 TLRs have been found to have LPS resistance (42) similar to the TLR4 knockout mice have been produced and have confirmed the importance of TLR4 in mediating Kupffer cell responses to LPS (99). A role for TLR4 in liver injury is suggested by studies with intragastric ethanol administration in TLR4 mutant C3H/HeJ mice. After 4 wk of intragastric ethanol, these mice have less liver injury histologically, lower alanine amino transferase levels, and lower TNF-α mRNA expression than wild-type controls (116). Given the narrow specificity of TLR4 for LPS and not for other immunogenic bacterial products such as bacterial lipopolysaccharide and bacterial CpG-DNA, this study suggests that in mouse ethanol model of liver injury, LPS rather than other bacterial components is the specific pathophysiologic agent. Whether this will be true in other models of liver injury remains to be seen. In addition to TLR4, TLR2 mRNA is expressed by rodent hepatocytes (60, 66) and can be upregulated by LPS in vivo. Regulation of TLR2 mRNA appears to be mediated, in part, by IL-1 and TNF-α (60). The significance of TLR2 expression on hepatocytes and its role in liver injury is, as yet, unknown.

OTHER LPS RECEPTORS

The focus of this review has been on the CD14 and TLR4 pathway of Kupffer cell activation by LPS, because this is the best characterized high-affinity receptor system for LPS in monocytes. However, other receptor systems may be present (56). Another receptor system well described in the liver is the scavenger receptors, also known as Ac-LDL receptors (95). This receptor system has specificity toward negatively charged ligands such as Ac-LDL, polyinosinic acid, and maleylated BSA. On macrophages such as Kupffer cells, two types of scavenger receptor A have been

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TLRs

of the −159 polymorphism promote CD14 gene expression and cause higher expression of CD14 on monocytes (43). In this study, the thymidine allele was found to be associated with alcoholic hepatitis and cirrhosis but not with fatty liver, peribiliary fibrosis, or bridging fibrosis. These findings suggest that CD14 expression may directly influence human susceptibility to alcohol-induced liver injury (47).
identified (types I and II), which are both products of a single gene. The two gene products result from alternative splicing. Both subtypes of scavenger receptor A are found in abundance on macrophages and have very broad specificity toward negatively charged ligands such as Ac-LDL, polyinosinic acid, and maleylated BSA. They also have a great affinity for LPS, lipid A, and the bioactive precursor of lipid A, lipid IV\(_A\). This receptor system has been found to participate in the binding of LPS by macrophages before its metabolism to a less active form. Binding of LPS to the scavenger receptors does not result in Kupffer cell activation or production of proinflammatory cytokines. Hepatocytes also produce sCD14, which may alter cellular reactivity to LPS.

Recently, an interesting class of intracellular receptors for LPS has been identified. Nucleotide binding domains (Nod)1 and -2 are members of a family of intracellular proteins composed of an NH\(_2\)-terminal caspase recruitment domain and a centrally located Nod (45). The COOH-terminal portions have leucine-rich repeats and serve as the sensors for intracellular ligands. LPS has been identified as a ligand for Nod1 and Nod2. Binding of LPS to Nod1 and Nod2 results in TLR4- and MYD88-independent activation of NF-\(\kappa\)B activation, as demonstrated in experiments with Nod1- and Nod2-transfected human embryonic kidney 293T cells. Not surprisingly, the leucine-rich region of the protein appears to be essential for LPS-induced NF-\(\kappa\)B activation (46). Whereas Nod1 is found in a wide range of cell types, Nod2 appears to be restricted to monocytic cells (83). A recent report of a single nucleotide polymorphism in the Nod2 gene that is associated with susceptibility to Crohn’s disease has generated much interest in this receptor. The frameshift mutation results in a truncated protein lacking part of the leucine-rich region and deficient in LPS-mediated NF-\(\kappa\)B activation (82). Whether this receptor will have any role in susceptibility to liver injury remains to be seen.

**LBP**

As noted earlier, LBP binds with a high degree of specificity and affinity (dissociation constant \(\sim 10^9\)) to the lipid A portion of bacterial LPS (107). The NH\(_2\)-terminal half of the protein is responsible for specific binding to LPS, whereas the COOH-terminal half of LBP is responsible for CD14 interactions. A truncated form of human LBP composed of the 197 amino acids in the NH\(_2\)-terminus of the parent molecule effectively binds LPS but cannot transfer the LPS to CD14 (33, 105). At low concentrations (3 ug/ml), which is present...
is normal serum, LBP can facilitate the transfer of LPS to CD14 on isolated Kupffer cells ex vivo, with accelerated binding and more TNF-α production at lower concentrations of LPS (98, 99). As an acute-phase protein, LBP is present in normal serum at a concentration of 0.5–20 μg and can increase as much as 100-fold with an acute-phase response (16, 27, 73, 93, 108). Hepatocytes are the main producers of LBP, although LBP is also produced locally in the lung and skin (50, 51, 88, 102). Within the liver, therefore, Kupffer cells can be exposed to high concentrations of LBP.

Whereas the LPS-potentiating properties of LBP are well recognized, its ability to mediate widely varying functions at different concentrations has been underappreciated. With significant sequence homology to the lipid transfer proteins, cholesterol ester transfer protein and phospholipid transfer protein, LBP can transfer LPS to lipoproteins such as high-density lipoprotein (HDL), leading to neutralization of LPS (122). In fact, the ability of lipoproteins such as HDL to bind and neutralize LPS may depend on a transfer protein such as LBP (124). Reconstituted HDL (R-HDL) prepared from purified apolipoprotein A-I combined with phospholipid and free cholesterol were not sufficient to neutralize the biological activity of LPS, but the addition of LBP enabled prompt binding and neutralization of LPS by R-HDL (124). In serum, LBP is predominantly associated with lipoproteins, and its association strongly enhances the ability of lipoproteins to bind LPS (119). Given the role that lipoproteins play in neutralizing LPS in blood, LBP is well placed to participate in the neutralization of LPS.

Irrespective of its ability to transfer LPS to lipoproteins, the multiple functions of LBP can be illustrated by its dose-dependent effects on monocyte activation. Addition of low concentrations of recombinant murine LBP (up to 1 μg/ml) augments RAW 267.4 cell responses to low concentrations of LPS (0.33 ng/ml). However, at higher LBP concentrations (10 μg/ml), the previously observed enhancement of responses to LPS is lost. At higher concentrations of LPS, the previously effective concentration of LBP (10 μg/ml) is no longer sufficient to inhibit responses to LPS. In vivo, the addition of recombinant murine LBP diminishes LPS response (TNF-α levels), mortality, and ALT levels in the LPS-galactosamine model of liver injury (53). Concentration-dependent effects of LBP have also been described for priming of neutrophils by LPS (1 ng/ml) for subsequent responses to N-formyl-methionyl-leucyl-phenylalanine (113). In this system, sCD14 can activate or inhibit the priming of neutrophils. The end effect of sCD14 is dependent on the concentration of LBP. For LBP concentrations >3–10 ng/ml, the addition of sCD14 resulted in inhibition of neutrophil priming. At <3 ng/ml of LBP, the addition of sCD14 enhanced priming (113). The mechanism by which LBP can exert opposing effects is not known, but these data suggest that a complex interplay of multiple concentration-dependent factors determines the biological effect of these proteins.

The phenotype expressed by the LBP knockout mice produced by Wurfel et al. (123) supports the hypothesis that the primary role of LBP in vivo may not be to augment LPS reactivity. These LBP-deficient mice do not demonstrate any difference in TNF-α production when injected with LPS in vivo. In contrast, whole blood from these animals was 1,000-fold less responsive to LPS as assessed by TNF-α production ex vivo than that of wild-type controls. These findings suggest that in vivo sources of TNF-α production such as the liver may not require LBP for LPS responsiveness. In fact, the critical function of LBP in vivo may be to protect animals from bacterial infections. This latter supposition is supported by studies showing the exquisite sensitivity of these LBP-deficient animals to a Klebsiella pneumoniae challenge (21).

As an acute phase protein, hepatocyte LBP expression is upregulated in many models of acute-phase response and liver injury including endotoxemia, intramuscular turpentine injection, and alcoholic hepatitis (28, 100). Like other acute phase proteins, LBP production is regulated by proinflammatory cytokines such as IL-6, IL-1, and TNF-α (30, 120). The role LBP plays in liver injury when it is upregulated is not clear. Early studies with acute carbon tetrachloride-induced liver injury in LBP-deficient animals suggest that LBP-deficient animals have defective recovery from liver injury (79, 101). However, in mice given intragastric ethanol for 1 mo, a reduction in liver injury is seen in LBP-deficient animals (117). These contrasting effects of LBP deficiency suggests a more complex paradigm for liver injury that is dependent on relative concentrations of LBP and its cofactors (CD14 and TLR4) as well as the timing and type of injury.

In summary, the liver and, in particular, the Kupffer cell play an important role in the innate immune response to bacteria and bacterial products that enter the portal system. In the setting of liver injury, this function is impaired. Evidence suggests that these endogenous bacterial products, LPS in particular, can exacerbate the ongoing liver injury. Recent investigations into the molecular mechanisms by which LPS interacts with Kupffer cells and other mononuclear cells shed light on the potential role played by LBP, CD14, and TLR4 in the pathogenesis of different forms of liver injury. Although binding of LPS to LBP with transfer to CD14/TLR4 on Kupffer cells can result in activation, a complex interplay of other factors, including the relative concentrations of LBP and sCD14, may ultimately determine the Kupffer cell’s response to LPS. An understanding of these factors, which are altered in liver injury, can lead to better therapeutic options in the future.

This work was supported by National Institutes of Health Grants DK-53296 and GM-60205 and a Veteran’s Administration Merit Award.

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