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Distinct Responses of Lung and Liver Macrophages to Acute Endotoxemia. Role of Toll-Like Receptor 4

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Abstract

Exposure to excessive quantities of bacterial-derived lipopolysaccharide (LPS) is associated with injury to the lung and the liver. Macrophages are thought to play a key role in the pathogenic response to LPS by releasing proinflammatory/cytotoxic mediators. Macrophage responses to LPS are mediated in large part by toll-like receptor 4 (TLR4). In the present studies we used C3H/HeJ mice, which possess a mutated nonfunctional TLR4, to examine its role in lung and liver macrophage responses to acute endotoxemia induced by LPS administration. Treatment of control C3H/HeOuJ mice with LPS (3 mg/ml) was associated with a significant increase in the number of macrophages in both the lung and the liver. This was most prominent after 48 h, and was preceded by expression of proliferating cell nuclear antigen (PCNA), suggesting that macrophage proliferation contributes to the response. In liver, but not lung macrophages, LPS administration resulted in a rapid (within 3 h) increase in mRNA expression of Mn superoxide dismutase (SOD) and heme oxygenase-1 (HO-1), key enzymes in antioxidant defense. In contrast, HO-1 protein expression decreased 3 h after LPS administration in liver macrophages, while in lung macrophages it increased. mRNA expression of enzymes mediating the biosynthesis of eicosanoids, including cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1), but not 12/15-lipoxygenase (LOX), was upregulated in liver macrophages 3–24 h after LPS, with no effect on lung macrophages. However, COX-2 protein expression increased in both cell types. Loss of functional TLR4 significantly blunted the effects of LPS. Thus, no major changes were observed after LPS administration in the number of lung and liver macrophages recovered from TLR4 mutant mice, or on expression of PCNA. Increases in HO-1, MnSOD, COX-2 and PGES-1 mRNA expression in liver macrophages were also reduced in these mice. Conversely, in lung macrophages, loss of functional TLR4 resulted in increased expression of COX-2 protein and 12/15-LOX mRNA. These results demonstrate distinct lung and liver macrophage responses to acute endotoxemia mediated in part by functional TLR4.

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Conflict of interest statement

The authors declare no conflict of interest.

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Keywords

LPS; TLR4; macrophages; inflammation; liver; lung

Introduction

Lipopolysaccharide (LPS), typically found in the gastrointestinal tract, is a major component of the cell wall of Gram-negative bacteria. It is a glycolipid predominantly composed of oligo- and polysaccharides, and lipid A endotoxin (Raetz and Whitfield, 2002). LPS is primarily cleared from the body by Kupffer cells in the liver (Protzer et al., 2012; Vazquez-Torres et al., 2004). However, excessive levels of LPS can readily overwhelm this clearance mechanism resulting in acute endotoxemia. This is associated with systemic inflammation which can lead to septic shock, multiple organ failure and death (Annane et al., 2005). The lung is particularly sensitive to endotoxin and is usually the first organ to fail, followed closely by the liver (Ciesla et al., 2005; Fry, 2012). A characteristic feature of acute endotoxemia is an accumulation of macrophages in target tissues (Ahmad et al., 2002; Chen et al., 2007; McCloskey et al., 1992; Pilaro and Laskin, 1986; Wizemann and Laskin, 1994). These cells are activated by LPS to release reactive oxygen and nitrogen species, proinflammatory cytokines, proteases, and bioactive lipids, which are thought to contribute to tissue injury and the pathogenesis of organ failure (Laskin et al., 2011; Murray and Wynn, 2011).

A number of receptors have been identified on macrophages that are involved in LPS responsiveness. These include CD14 and toll-like receptor 4 (TLR4) (Kawai and Akira, 2010; Schumann et al., 1990). Following its release from dividing or damaged bacteria, LPS is sequestered by an LPS-binding protein in serum, which transports it to CD14 on the surface of macrophages (Akira and Takeda, 2004; Wright et al., 1990). Subsequently LPS is transferred to MD2, a soluble protein which associates with the extracellular domain of TLR4. Activation of TLR4 initiates a cell signaling cascade leading to translocation of NF- κ B into the nucleus and transcription of proinflammatory genes including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumor necrosis factor alpha (TNF α), proteins implicated in lung and liver injury (Kawai and Akira, 2010; Laskin et al., 2011). Previous studies have shown protection against LPS-induced inflammation and tissue injury in animals with TLR4 deficiency (Guo et al., 2009; Roger et al., 2009; Wittebole et al., 2010). Similar protective effects of loss of TLR4 have been described in sterile inflammatory responses to tissue injury induced by various target-organ specific toxicants (Connor et al., 2012; Lin et al., 2011; Matzinger, 2002). The present studies demonstrate that macrophage accumulation and responsiveness in the lung and the liver following acute endotoxemia depend on functional TLR4. These data provide additional evidence for an essential contribution of TLR signaling to inflammatory pathologies (Jiang et al., 2005; Matzinger and Kamala, 2011; Ostuni and Natoli, 2011).

Materials and methods

Animals and treatments

Male TLR4 mutant C3H/HeJ and control C3H/HeOJ mice (11–12 weeks) were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in sterile microisolation cages and provided autoclaved food and water *ad libitum*. Animal care was in compliance with Rutgers University guidelines as outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences. Acute endotoxemia was induced by i.p. injection of mice with 3 mg/kg *Escherichia coli* LPS (serotype 0128:B12

Sigma L4255, Sigma Chemical Co., St. Louis, MO), repurified as described previously (Chen et al., 2007). PBS was used as a control.

Macrophage isolation

Macrophages were isolated from the liver as described previously (Chen et al., 2007). Briefly, the liver was perfused through the portal vein with 10 ml of $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free Hank's balanced salt solution (HBSS) containing 25 mM HEPES, 0.5 mM EGTA and 4.4 mM NaHCO_3 (pH 7.3) at 37 °C for 2 min at rate of 10 ml/min. This was followed by perfusion for 1 min with Leibovitz L-15 media containing 100 U/ml collagenase type IV. The liver was then removed, gently combed, and filtered through 220 μm nylon mesh. Hepatocytes were separated from nonparenchymal cells by centrifugation at $50 \times g$ for 5 min. Nonparenchymal cells were recovered by centrifugation of the supernatant at $330 \times g$ for 7 min. Macrophages were then purified according to their size and density on a Beckman J-6 elutriator (Beckman Instruments Inc., Fullerton, CA) equipped with centrifugal elutriation rotor speed set at 2500 rpm. The pump speed was set at 12 ml/min to load the cells and at 33 ml/min to collect macrophages, which were further purified using Nycodenz® (osmolality 265 mOsm, density 1.077 g/ml). Cells were identified morphologically by Giemsa staining and electron microscopy, and were >85% macrophages (Chen et al., 2007). The major contaminating cell population was endothelial cells. We have previously demonstrated that the inflammatory activity of hepatic endothelial cells is significantly reduced, relative to liver macrophages (McCloskey et al., 1992; Chen et al., 2007). Thus, their contribution to the observed responses in these studies is unlikely to be significant.

Lung macrophages were isolated by bronchoalveolar lavage (BAL) as described previously with some modifications (Connor et al., 2012). After liver perfusion, the trachea was cannulated and the lung removed from the chest cavity. BAL was collected by slowly instilling and withdrawing 1 ml of HBSS 7–10 times through the cannula. BAL fluid was centrifuged ($300 \times g$ for 8 min). Cell pellets were washed 4 times with HBSS containing 2% FBS and then enumerated using a hemocytometer. Viability was 98% as determined by trypan blue dye exclusion, and cell purity >97% macrophages as assessed morphologically after Giemsa staining.

Immunohistochemistry

Tissues were fixed in 10% formalin buffer overnight at room temperature, followed by 50% ethanol. Sections (6 μm) were deparaffinized, then incubated overnight at 4 °C with rabbit antibody to heme oxygenase-1 (HO-1, 1:1000; Stressgen/Assay Designs, Ann Arbor, MI), proliferating cell nuclear antigen (PCNA, 1:250, Abcam, Cambridge, MA), COX-2, (1:400, Abcam) or normal rabbit serum followed by a 30 min incubation with biotinylated secondary antibody (Vector Labs, Burlington, CA). Binding was visualized using a VECTASTAIN® Elite ABC kit (Vector Labs).

cDNA synthesis and real time-PCR

Total RNA was extracted from cells using QIAshredder and RNeasy Mini kit with on-column DNase digestion (Qiagen, Valencia, CA) according to the manufacturer's protocol. RNA concentration was determined by absorbance at 260 nm. For cDNA synthesis, 200 ng of RNA was reverse transcribed using the High-capacity cDNA reverse Transcription Kit (Applied Biosystems, Foster City, CA). Standard curves were generated using serial dilutions from pooled randomly selected cDNA samples. Real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on a 7900HT Thermal cycler using 96-well optical reaction plates. Samples from three to four animals per treatment group were analyzed and presented relative to hypoxanthine phosphoribosyltransferase (HPRT) mRNA expression. All PCR primer sequences were

generated using Primer Express 2.0 (Applied Biosystems) and primers were synthesized by Integrated DNA Technologies (Coralville, IA). Forward and reverse primer sequences were as follows: 12/15-lipoxygenase (LOX), TCGGAGGCAGAATTCAAGGT and CAGCAGTGGCCCAAGGTATT; COX-2, CATTCTTTGCCAGCACTTCAC and GACCAGGCACCAGACCAAAGAC; microsomal prostaglandin E synthase (mPGES)-1, GGCCTTTCTGCTCTGCAGC and GCCACCGCGTACATCTTGAT; CuZn superoxide dismutase (SOD), ACCAGTGCAGGACCTCATTTTAA and TCTCCAACATGCCTCTCTTCATC; cyclin D1, AAAATGTTCTCCCTGCCCCT and CATCCTGAACGGATCCATGC; HPRT, GTTGGATACAGGCCAGACTTTGTG and GAAGGGTAGGCTCTATA-GGCT; HO-1, CCTCACTGGCAGGAA-ATCATC and CCTCGTGGAGACGCTTTACATA; MnSOD, CACATTAA-CGCGCAGATCATG and CCAGAGCCTCGTGGTACTTCTC; p21, AAGGCCTGAAGACTCCACCA and TGTGACCAATGA-AGGGCAAG.

Statistics

Data were analyzed by one-way ANOVA (Holm-Sidak method). A p-value of 0.05 was considered significant.

Results

Response of lung and liver macrophages to acute endotoxemia

Initially we analyzed the effects of acute endotoxemia on macrophage accumulation in the lung and the liver. Approximately 0.6×10^6 lung macrophages and 2×10^6 liver macrophages were recovered from control C3H/HeOuJ mice (Fig. 1). Induction of acute endotoxemia resulted in a two-fold increase in the number of macrophages in both tissues, a response observed after 48 h. Evidence suggests that local proliferation of macrophages at sites of injury contributes to increased numbers of these cells in tissues (Davies et al., 2011; Isbel et al., 2001; Nakata et al., 1999; Wohl et al., 2010). To assess this, we measured markers of proliferation in lung and liver macrophages. Following LPS administration, expression of PCNA increased significantly in both lung and liver macrophages (Figs. 2 and 3). This response was noted within 12 h and was maintained for at least 48 h. We also observed a trend toward increased cyclin D1 mRNA expression in liver macrophages 3–24 h after LPS, with no effect on the lung macrophages (Fig. 4). Interestingly, the cell cycle regulatory protein p21 also increased in liver, but not lung macrophages, within 3 h of LPS administration, remaining elevated for at least 24 h (Fig. 4).

To determine if acute endotoxemia was associated with oxidative stress in the macrophages, we analyzed expression of the antioxidants SOD and HO-1. In liver macrophages, but not lung macrophages, a rapid (within 3 h) increase in MnSOD mRNA expression was observed after LPS administration (Fig. 5). This persisted for 24 h, although at reduced levels. LPS had no effect on expression of CuZnSOD in either macrophage type. HO-1 mRNA also increased in liver macrophages after LPS treatment of the mice at 3–24 h. In contrast, LPS caused a transient decrease in constitutive expression of HO-1 protein in these cells at 3 h (Fig. 6). Whereas in lung macrophages LPS administration had no effect on HO-1 mRNA expression, HO-1 protein increased after 3 h persisting for at least 24 h (Figs. 5 and 7). We also observed increased expression of HO-1 in pulmonary epithelium and interstitium, which was most prominent 24 h after LPS.

Macrophages activated by LPS are known to release proinflammatory eicosanoids implicated in endotoxin-induced tissue injury (Funk, 2001; Laskin et al., 2011). We next analyzed the effects of acute endotoxemia on lung and liver macrophage expression of enzymes mediating the generation of eicosanoids. Low constitutive levels of COX-2,

mPGES-1, and 12/15-LOX mRNA were detected in lung and liver macrophages from control C3H/HeOuJ mice (Fig. 8). While induction of acute endotoxemia had no major effect on mRNA expression of these enzymes in lung macrophages, in liver macrophages a rapid (within 3 h) and persistent increase in COX-2 and mPGES-1 mRNA was observed, with no significant effect on 12/15-LOX. COX-2 protein expression also increased at 3 h following LPS administration in liver, as well as lung macrophages (Figs. 9 and 10). COX-2 protein expression also increased in alveolar epithelial cells (Fig. 10).

Effects of loss of functional TLR4 on macrophage responses to acute endotoxemia

In our next series of studies, we analyzed the role of TLR4 in lung and liver macrophage responsiveness to acute endotoxemia using C3H/HeJ mice, which possess a mutated nonfunctional TLR4 (Poltorak et al., 1998). In contrast to control C3H/HeOuJ mice, in TLR4 mutant C3H/HeJ mice, LPS administration had no effect on the number of macrophages recovered from the lung or the liver (Fig. 1). LPS-induced lung and liver macrophage proliferation, as measured by PCNA expression, was also reduced (Figs. 2 and 3), along with increases in cyclin D1 and p21 expression in liver macrophages (Fig. 4). Additionally, in TLR4 mutant C3H/HeJ mice, the effects of LPS on liver macrophage expression of HO-1 mRNA and protein, and MnSOD mRNA, were diminished (Figs. 5 and 6), with no major effect on HO-1 protein in lung macrophages (Fig. 7). Loss of functional TLR4 also resulted in an attenuated response of liver macrophages to LPS-induced increases in expression of COX-2 mRNA and protein, and of mPGES-1 mRNA (Figs. 8 and 9). In contrast, COX-2 protein expression increased in lung macrophages, as well as type II cells in TLR4 mutant C3H/HeJ mice after LPS administration, a response which was most prominent after 24 h (Fig. 10). In TLR4 mutant C3H/HeJ mice, 12/15-LOX mRNA also increased in lung macrophages 3–24 h after LPS administration, with no effects on mPGES-1 or COX-2 mRNA (Fig. 8).

Discussion

Exposure to excessive levels of circulating endotoxin is associated with both acute and chronic inflammatory diseases (Miller et al., 2005; Qin et al., 2007; Rojas et al., 2005; Xu et al., 2010). Macrophages and cytotoxic/proinflammatory mediators they release play a key role in the development of these pathologies (Jiang et al., 2005; Laskin et al., 2011; Murray and Wynn, 2011). Of particular importance are macrophages located in the lung and liver which are highly responsive to endotoxin (Ahmad et al., 2002; Chen et al., 2007; Protzer et al., 2012; Sunil et al., 2002; Wizemann and Laskin, 1994). Evidence suggests that binding of LPS to TLR4 is a major triggering event in the signaling pathway leading to endotoxin-induced tissue injury (Akira and Takeda, 2004; Kawai and Akira, 2010). In the present studies we characterized the response of lung and liver macrophages to acute endotoxemia, with the goal of assessing the role of functional TLR4 in their response.

Acute endotoxemia is associated with an accumulation of macrophages in the lung and the liver, key target organs (Ahmad et al., 2002; Chen et al., 2007; McCloskey et al., 1992; Pilaro and Laskin, 1986; Sunil et al., 2002). Consistent with these reports, the present studies demonstrate increased numbers of macrophages in both the lung and the liver following LPS administration to mice, a condition which causes acute endotoxemia; this was most prominent 48 h after exposure. Inflammatory macrophages accumulating in tissues in response to injury or infection are thought to be derived mainly from blood and bone marrow precursors (Gordon and Taylor, 2005). Our observations that macrophages in lung and liver of LPS treated mice stained positively for PCNA, and that cyclin D1 expression increased in liver macrophages, suggest that local proliferation may also contribute to increases in the numbers of these cells in the tissues. These findings are in accord with previous studies showing local proliferation of macrophages following injury or infection in

the lung, liver, kidney, eye, and peritonium (Davies et al., 2011; Isbel et al., 2001; Nakata et al., 1999; Vadiveloo, 1999; Wohl et al., 2010). We also observed LPS-induced increases in p21 in liver macrophages. Previous studies have shown that deficiency in p21 renders mice more susceptible to septic shock (Scatizzi et al., 2009; Trakala et al., 2009), potentially due to loss of p21-induced suppression of macrophage NF- κ B (Coqueret, 2003; Lloberas and Celada, 2009; Vadiveloo, 1999). Increases in p21 in the liver may represent a compensatory response to limit excessive inflammatory responses and minimize tissue injury. The fact that this was not evident in lung macrophages suggests distinct regulation of macrophage proliferation and inflammatory activity in the lung and the liver following acute endotoxemia. This is supported by our observation of increased cyclin D1 in liver, but not lung macrophages, in LPS treated mice.

We have previously demonstrated that acute endotoxemia leads to increased production of reactive oxygen and nitrogen species by macrophages in the lung and the liver (Ahmad et al., 2002; Feder and Laskin, 1994; McCloskey et al., 1992; Wizemann and Laskin, 1994). These cytotoxic mediators cause oxidative stress which is thought to contribute to tissue injury and multiple organ failure (Ciesla et al., 2005; Fry, 2012). Markers of oxidative stress including F2-isoprostanes, isofurans, peroxiredoxin 4, and lipocalin 24p3 have been identified in blood from septic patients (Kumpers et al., 2010; Schulte et al., 2011; Ware et al., 2011). Expression levels of 24p3 are also increased in lung and liver macrophages following induction of acute endotoxemia in rodents (Sunil et al., 2007). In response to oxidative stress, cells upregulate antioxidants including SOD and HO-1 (Kinnula and Crapo, 2003; Paine et al., 2010). Consistent with an oxidative stress response, we observed a rapid induction of MnSOD and HO-1 mRNA in liver macrophages following LPS administration, and increased HO-1 protein expression in lung macrophages. In contrast, HO-1 protein, which is constitutively expressed at relatively high levels in liver macrophages, decreased. Similar decreases in constitutive expression of antioxidants have been described in liver macrophages in response to other hepatotoxicants known to induce oxidative stress (Gardner et al., 2010). High levels of constitutive HO-1 in liver, but not lung macrophages, are likely due to continuous exposure of these cells to LPS in the portal circulation (Protzer et al., 2012). Previous studies have demonstrated that in addition to its antioxidant activity, HO-1 has anti-inflammatory functions suppressing macrophage NF- κ B and promoting an alternatively activated wound repair phenotype in these cells (Paine et al., 2010; Weis et al., 2009). Upregulation of HO-1 in lung macrophages may reflect an attempt to reduce their proinflammatory activity.

Prostaglandins, synthesized from membrane derived arachidonic acid via COX-2 and mPGES-1, are known to be involved in inflammation and tissue injury (Funk, 2001). The present studies show that acute endotoxemia leads to a rapid upregulation of COX-2 and mPGES-1 mRNA in liver macrophages. Deficiency in mPGES-1 has been reported to result in a 95% decrease in the synthesis of prostaglandin E₂ (PGE₂) (Trebino et al., 2005). These data suggest that liver macrophages are major producers of PGE₂ during acute endotoxemia. This is supported by previous studies in endotoxemic rats demonstrating increased COX-2 expression and PGE₂ production by liver macrophages (Ahmad et al., 2002). Interestingly, while COX-2 mRNA expression persisted at lower levels, for at least 24 hr in liver macrophages, protein expression was only noted at 3 hr post LPS. It may be that LPS also stimulates turnover of COX-2 protein and this is currently under investigation. In contrast to liver macrophages, LPS had no effect on mRNA expression of COX-2 or mPGES-1 in lung macrophages. However, COX-2 protein expression increased, suggesting distinct mechanisms regulating expression of COX-2 and PGE₂ production in lung and liver macrophages in response to endotoxin. LPS-induced COX-2 protein expression was also noted in alveolar epithelial cells, providing support for the idea that these cells play a role in pulmonary inflammatory responses to tissue injury (Fehrenbach, 2001; Punjabi et al., 1994;

Sunil et al., 2002). Eicosanoids generated via 12/15-LOX are thought to promote the resolution of inflammation (Levy et al., 2001; Serhan et al., 2008). Our findings that expression of 12/15-LOX mRNA was not altered in either lung or liver macrophages after LPS administration to the mice are in accord with prolonged inflammation in these tissues during acute endotoxemia (Chen et al., 2007; Rojas et al., 2005).

TLR4 mutant C3H/HeJ mice have been reported to be hyporesponsive to LPS (Chen et al., 2007; Hoshino et al., 1999; Poltorak et al., 1998). In agreement with these findings, the present studies demonstrate that loss of functional TLR4 leads to reduced responsiveness of lung and liver macrophages to acute endotoxemia. Thus, in TLR4 mutant C3H/HeJ mice, LPS had no effect on macrophage accumulation in the lung or the liver. LPS-induced increases in macrophage proliferation, as measured by PCNA and cyclin D1 expression, were also decreased. Reduced numbers of inflammatory cells have also been described in lungs of TLR4 deficient mice after exposure to bleomycin, *Mycobacterium tuberculosis*, or ozone (Abel et al., 2002; Connor et al., 2012; Hollingsworth et al., 2005). Whether this is due to decreased recruitment of inflammatory cells from blood and bone marrow precursors or reduced proliferation of these cells remains to be determined.

In liver macrophages, LPS-induced upregulation of MnSOD mRNA and HO-1 mRNA and protein was also attenuated in TLR4 mutant mice relative to control mice. Our findings that in lung macrophages, LPS-induced HO-1 protein expression was unaltered by loss of functional TLR4 suggest that TLR4 is not a major regulator of antioxidant responses in these cells which is in accord with previous reports (Mandal et al., 2010). Interestingly, HO-1 protein expression in pulmonary epithelium and interstitium was significantly reduced in TLR4 mutant mice, relative to control C3H/HeOuJ mice. These data indicate that antioxidant defense is regulated by distinct pathways in different lung cell populations.

We also found that LPS-induced effects on COX-2 mRNA and COX-2 protein expression, and mPGES-1 mRNA were significantly reduced in liver macrophages in TLR4 mutant mice, relative to control mice. Conversely, loss of functional TLR4 resulted in an increase in COX-2 protein in lung macrophages, as well as type II cells; however this was delayed for 12–24 h. We also found that lung macrophages from TLR4 mutant mice express increased levels of 12/15-LOX mRNA following LPS administration. This may contribute to reduced inflammation and oxidative stress in the lung in these mice. In this regard, recent studies have demonstrated that 12/15-LOX plays a key role in macrophage clearance of apoptotic cells from tissues and in the maintenance of immunological tolerance, which may contribute to its anti-inflammatory activity (Uderhardt et al., 2012).

Macrophages exhibit a diverse spectrum of activation states in response to pathogens and microenvironmental signals they encounter in the lung and the liver (Laskin et al., 2011; Mosser and Edwards, 2008). The present studies demonstrate that while TLR4 signaling plays a role in promoting inflammatory responses of macrophages to LPS, this activity is tissue specific, which may be due, at least in part, to different expression levels of TLR4 on these cells. This is supported by findings that TLR4 expression is reduced on lung macrophages relative to liver macrophages, which may account for their attenuated response to LPS (Fan et al., 2002; Maris et al., 2006). Understanding pathways regulating the proinflammatory responses of lung and liver macrophages to LPS maybe important in the design of novel therapies to treat multiple organ failure.

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Abbreviations

BAL	bronchoalveolar lavage
COX-2	cyclooxygenases-2
HBSS	Hank's balanced salt solution
HO-1	heme oxygenase-1
iNOS	inducible nitric oxide synthase
LOX	lipoxygenase
LPS	lipopolysaccharide
mPGES-1	microsomal prostaglandin E synthase-1
NF-κB	nuclear factor-kappa B
PCNA	proliferating cell nuclear antigen
SOD	superoxide dismutase
TLR4	toll-like receptor 4
TNFα	tumor necrosis factor alpha

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Highlights

- Acute endotoxemia results in an accumulation of activated macrophages in the lung and the liver.
- Liver macrophages are generally more sensitive to acute endotoxemia than lung macrophages.
- The effects of acute endotoxemia are significantly blunted in mice with a mutated nonfunctional TLR4.
- TLR4 is key to LPS-induced oxidative stress, inflammation and macrophage activation.

Fig. 1.

Effects of loss of TLR4 on the number of macrophages in the lung and liver following LPS administration. Macrophages were isolated from the lung and liver 12–48 h after treatment of C3H/HeOuJ and TLR4 mutant C3H/HeJ mice with control or LPS. Viable cells were counted on a hemocytometer using trypan blue exclusion. *Upper panel:* Each bar represents the mean $\pm$ SE (n=3–21 mice). *Lower panel:* Each bar represents the mean $\pm$ SE (n=11–72 mice). *Significantly different (p < 0.05) from control (ANOVA).

Fig. 2. Effects of loss of TLR4 on LPS-induced PCNA expression in the lung. Sections, prepared 3–24 h following treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS, were stained with anti-PCNA antibody. Binding was visualized using a Vector DAB peroxidase substrate kit. Slides were counterstained with hematoxylin. Original magnification 600x, inserts, 1000x. One representative section from three experiments is shown.

Fig. 3.

Effects of loss of TLR4 on LPS-induced PCNA expression in the liver. Sections, prepared 3–24 h following treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS, were stained with anti-PCNA antibody. Binding was visualized using a Vector DAB peroxidase substrate kit. Slides were counterstained with hematoxylin. Original magnification 600x, inserts, 1000x. One representative section from three experiments is shown.

Fig. 4.

Effects of loss of TLR4 on LPS-induced expression of proliferation markers. Lung and liver macrophages were isolated 3–24 h after treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS. mRNA expression was quantified by real-time RT-PCR. Data were normalized to HPRT and expressed as fold change relative to controls. Each bar represents the mean $\pm$ SE (n=3–8 mice). *Significantly different (p < 0.05) from control (ANOVA).

Fig. 5.

Effects of loss of TLR4 on LPS-induced expression of antioxidant enzymes. Lung and liver macrophages were isolated 3–24 h after treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS. mRNA expression was quantified by real-time RT-PCR. Data were normalized to HPRT and expressed as fold change relative to controls. Each bar represents the mean $\pm$ SE (n=3–8 mice). *Significantly different (p < 0.05) from control (ANOVA).

Fig. 6.

Effects of loss of TLR4 on LPS-induced HO-1 expression in the liver. Sections, prepared 12–24 h following treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS, were stained with anti-HO-1 antibody. Binding was visualized using a Vector DAB peroxidase substrate kit. Slides were counterstained with hematoxylin. Original magnification 600x, inserts, 1000x. One representative section from three experiments is shown.

Fig. 7.

Effects of loss of TLR4 on LPS-induced HO-1 expression in the lung. Sections, prepared 12–24 h following treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS, were stained with anti-HO-1 antibody. Binding was visualized using a Vector DAB peroxidase substrate kit. Slides were counterstained with hematoxylin. Original magnification 600x, inserts, 1000x. One representative section from three experiments is shown.

Fig. 8.

Effects of loss of TLR4 on LPS-induced expression of enzymes mediating eicosanoid metabolism. Lung and liver macrophages were isolated 3–24 h after treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS. mRNA expression was quantified by real-time RT-PCR. Data were normalized to HPRT and expressed as fold change relative to controls. Each bar represents the mean $\pm$ SE (n=3–8 mice). *Significantly different (p < 0.05) from control (ANOVA).

Fig. 9. Effects of loss of TLR4 on LPS-induced expression of COX-2 protein in the liver. Sections, prepared 3–12 h following treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS, were stained with anti-COX-2 antibody. Binding was visualized using a Vector DAB peroxidase substrate kit. Slides were counterstained with hematoxylin. Original magnification 600x, inserts, 1000x. One representative section from three experiments is shown.

Fig. 10.

Effects of loss of TLR4 on LPS-induced expression of COX-2 protein in the lung. Sections, prepared 3–12 h following treatment of C3H/HeOuJ and C3H/HeJ mice with control or LPS, were stained with anti-COX-2 antibody. Binding was visualized using a Vector DAB peroxidase substrate kit. Slides were counterstained with hematoxylin. Original magnification 600x, inserts, 1000x. One representative section from three experiments is shown.