Cytokine 64 (2013) 71–78

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Liver transplantation and inflammation: Is lipopolysaccharide binding protein the link?

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ARTICLE INFO

Article history:
Received 5 March 2013
Received in revised form 19 June 2013
Accepted 22 July 2013
Available online 16 August 2013

Keywords:
LPS
LBP
Warm ischemia reperfusion
Cold ischemia
Liver transplantation

ABSTRACT

Background: Lipopolysaccharide (LPS) binding protein (LBP) is an acute phase protein, which upregulated in response to surgical interventions. LBP plays an important role in modulating LPS-induced inflammatory response. We investigated the expression of LBP and the translocation of LPS in rat models of hepatic ischemia reperfusion injury and liver transplantation (LTx). We also elucidated the effect of LBP on the inflammatory response.

Methods: In this study, cold ischemia (CI), warm ischemia/reperfusion (WI/R), and LTx models were used to model relevant physiologic situations during LTx. Serum and effluent protein levels as well as hepatic-mRNA and protein levels of LBP were examined. LBP released into the effluent during CI was used in a macrophage-LPS-stimulation assay to investigate the role of LBP in modulating the LPS-induced inflammatory response. Blocking experiments using an LBP-inhibitory peptide were performed to confirm the relevance of LPS/LBP for the induction of the inflammatory response. Impairment of the intestinal barrier and translocation of LPS into the liver was visualized by immunohistochemistry. Induction of tumor necrosis factor-alpha (TNF-α) mRNA expression in the liver was taken as indicator of the inflammatory response.

Results: Upregulation of LBP in serum and/or liver tissue was observed after WI/R, CI and LTx, respectively. The LBP released during CI enhanced the LPS induced inflammatory response in vitro as indicated by an induction of TNF-α. On the other hand, blocking LBP using LBP inhibitory peptide, suppressed the induction of TNF-α in vitro markedly. After LTx, elevated serum LBP levels were associated with post-operative LPS translocation and production of inflammatory cytokines.

Conclusions: Our findings suggest that translocation of LPS occurs after LTx and that LBP is mediating the LPS-induced inflammatory response after LTx. Blocking LBP using LBP-inhibitory peptide might represent a novel strategy to reduce the I/R-induced inflammatory response.

1. Introduction

Ischemia/reperfusion (I/R) injury is the main cause of initial graft dysfunction and primary failure in liver transplantation (LTx) [5,18,22]. Cold ischemia (CI) injury is an inevitable consequence of liver explantation and organ preservation prior to transplantation. Warm ischemia/reperfusion (WI/R) occurs during the implantation procedure. In LTx, both CI and WI/R lead to liver damage and cause a systemic inflammatory response.

I/R induces an inflammatory response and cytokine release from non-parenchymal cells and passenger leucocytes in the liver [5,18,22]. The inflammatory response following I/R which leads to organ damage have been studied extensively [25]. In the LTx setting, the initial inflammatory is caused by a sterile organ injury inflicted by the surgical trauma and the I/R injury. However the

Abbreviations: LPS, lipopolysaccharide; LBP, lipopolysaccharide binding protein; CI, cold ischemia; WI/R, warm ischemia/reperfusion; LTx, liver transplantation; I/R, ischemia/reperfusion; CD14, cluster of differentiation 14; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; HPRT, hypoxanthine guanine phosphoribosyltransferase; cDNA, complementary DNA.

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http://dx.doi.org/10.1016/j.cyto.2013.07.025

impact of a non-sterile, bacterial damage on the inflammatory reaction has not been elucidated so far.

Lipopolysaccharid (LPS) and bacterial translocation is observed in case of an impaired intestinal barrier function [6]. LPS is the major component of the outer membrane of Gram-negative bacteria, and functions as major pathophysiological factor in initiating the inflammatory response. The barrier function of the intestine is impaired when the portal pressure rises subsequent to clamping the hepatoduodenal ligament as done during the anhepatic phase of liver transplantation. Portal hypertension as well as hepatic I/R injury are associated with bacterial translocation [6,7,27]. Bacterial translocation leads to increased levels of circulating LPS, a main component of the cell wall of gram negative bacteria [1].

The inflammatory response to LPS is mediated via binding to LPS binding protein (LBP). LBP recognizes LPS molecules in systemic circulation, and then transfers LPS to cluster of differentiation 14 (CD14), which in turn initiates the toll-like receptor 4 (TLR4) signaling cascade via forming the CD14-TLR4 complex [19,20]. These events result in the activation of the nuclear factor κB (NF-κB) pathways, and cause the production of cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 [16].

LBP is an acute phase protein which plays an important role in modulating the inflammatory response. It is reported that LBP is upregulated in infectious diseases, as well as in surgical stress situations [10]. We previously demonstrated that LBP was upregulated after liver resection in rats, and the systemic LBP levels did correlate to the remnant functional liver mass [13]. Furthermore, increased LBP levels were associated with an upregulation of the downstream inflammatory cytokines after LPS challenge.

In this study, we wanted to investigate whether LBP was upregulated and released after hepatic I/R and LTx in rats. We also wanted to observe whether LBP, released during ischemic damage of liver, was associated with the LPS-induced inflammatory response after LTx. As LBP is an important host protein for interaction with LPS, the modulation of LBP may be of potential interest for elucidating mechanisms of post-operative inflammation.

2. Materials and methods

2.1. Experimental design

In this study, three animal models were used to cover different situations – WI/R, CI, and LTx – modeling the different pathophysiological aspects of the surgical procedure during LTx.

Selective warm I/R injury was induced by clamping the vascular blood supply to the median and left lateral lobe of the liver for 90 min followed by 0.5 h, 6 h and 24 h reperfusion (n = 6/group). As a CI model, livers were explanted and stored in saline at 4 °C. Effluents were collected every hour during cold ischemia and liver tissue was obtained at 0 h, 4 h, 8 h, and 12 h. Six rats were subjected to LTx after cold-preservation of the graft for 6 h and sacrificed 24 h postoperatively. Another set of 6 rats was included as normal control group. Rats subjected to 2 mg/kg LPS injection (*Escherichia coli* serotype O55:B05 type, Sigma Aldrich, St. Louis, CO, US) and an observation time of 1 h, 6 h, 24 h were used as control for LBP elevation (n = 6/group). Serum and effluent protein levels as well as hepatic-mRNA and protein levels of LBP were examined after warm I/R, CI and LTx. LPS translocation and hepatic mRNA expression of inflammatory cytokines was observed after LTx.

In in vitro experiment, the effluent was used to co-stimulate rat peritoneal macrophages with LPS. Effluent was collected at defined time-points of cold liver storage. The LBP in effluent was measured by western blot. The time point with highest LBP concentration was chosen for macrophage stimulation. The macrophages were co-cultured with effluent (50 μl) and 0.33 ng/ml LPS. The same volume 0.9% NaCl and 0.33 ng/ml LPS was added in control group. In the LBP blockade experiments, macrophages were stimulated with effluent and LPS in combination with LBP inhibitory peptide (80 μg/ml). The culture suspensions were taken after 4 h stimulation and the TNF-α levels was detected by ELISA.

2.2. Animals

Male inbred Lewis rats, purchased from the Central Animal Facility of the University Hospital Essen, and weighing 300–350 g, were employed in this study. All animals were housed under standard animal care conditions and had free to access to water and rat chow ad libitum. All procedures were carried out according to the German Animal Welfare Legislation. Animal experiments were approved by the Bezirksregierung Düsseldorf. All injection and operative procedures were performed under inhalation anesthesia with 3 % isoflurane (Sigma Delta, London, UK).

2.3. Surgical models: selective in vivo liver WI/R, ex vivo liver CI, and LTx model

For the warm ischemia–reperfusion model, the procedure was performed as reported before [12]. The left hepatoduodenal ligament containing the hepatic artery, portal vein and bile duct of the left lateral and median liver lobes was clamped using a micro vascular clamp. For the cold ischemia model, livers were explanted and subjected to cold ischemia as described previously in detail [14]. Liver grafts were subjected to 6 h cold ischemia prior to performing the transplantation procedure according to the cuff technique described by Kamada [9]. Postoperative analgesia was achieved by subcutaneous injection of buprenorphine (0.01 mg/kg) (TemgesicTM, Essex Pharma, Munich, Germany).

2.4. Enzyme-linked immunosorbent assay (ELISA)

For analysis of hepatic IL-6 and TNF-α level, commercially available ELISA kits were used (R&D Systems, Minneapolis, US). All procedures were performed according to the instructions of the manufacturers.

For measurement of serum LBP levels, a recently established, novel LPS-LBP ELISA system was used as described before (Manuscript submitted, Fang, et al., 2011 Oct). Standard 96 well ELISA plates were coated with 1 μg LPS (Sigma Aldrich, St. Louis, USA) in PBS. The plates were subsequently washed three times with PBST (0.01% Tween-20, pH 7.4) and blocked with 1% BSA in PBST for 2 h. 100 μl of each dilution of the calibrator and samples were loaded and incubated for 2 h. The plates were incubated with monoclonal mouse anti-LBP antibody (1:10,000, cell science, Canton, MA) for 2 h. After the plates were washed for three times, rabbit anti-mouse IgG-H&L antibody (1:5000, Abcam, Cambridge, UK) was loaded and incubated for 1 h. And then, 100 μl 1:1 mixed of H_2O_2 and Tetramethylbenzidine (BD, Franklin Lakes, US) was added to each well and incubated for 12–15 min. The color reaction was stopped by adding 50 μl of sulfuric acid (AppliChem, Darmstadt, Germany) and the plates were measured with the ELx 808 ELISA plate reader (Bio-Tek Instruments Inc., Winooski, VT, US) at 450 nm. Quantification was based on a non-linear regression standard curve using Sigma Plot 10.0 (Systat-Software, Erkrath, Germany).

2.5. Quantitative polymerase chain reaction (PCR)

Total RNA was isolated from liver tissue using the RNeasy kit (Qiagen, Hilden, Germany). cDNA was synthesized by using the