LPS in Liver Disease
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A role for lipopolysaccharide has been suggested in various types of liver diseases:

- Alcoholic steatohepatitis
- Non-alcoholic steatohepatitis
- Primary biliary cirrhosis
- Cholestasis - sepsis associated
- Cholestasis of pregnancy
- Ischemic liver injury
- Liver regeneration
- Chronic HCV progression
- Cirrhosis

- SBP
- Hepatic encephalopathy
Lipopolysaccharide (LPS)
LPS is recognized by the Toll-like receptor 4 (TLR4) complex

**TLR4 complex:**
- TLR4 (Toll-like receptor 4)
- CD14
- MD2 (myeloid differentiation factor 2)
- LBP (lipopolysaccharide binding protein)
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**TLR4 adapters:**
- TIRAP (TIR domain-containing adaptor protein)
- MyD88 (myeloid differentiation response gene 88)
- TRAM (TRIF-related adaptor molecule)
- TRIF (TIR domain containing adaptor inducing TGFβ)

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LPS is sensed by multiple cell types in the liver

- Bile duct epithelium: TLR4
- Hepatocyte: TLR4, CD14
- Stellate cell: TLR4
- Endothelial cell: TLR4, CD14
- Kupffer cell: TLR4, CD14, MD2
- Myeloid dendritic cells: TLR4, CD14, MD2
- Hepatic sinusoid
Gut - Liver Access
Homeostasis

Detoxification
Liver
Gut

Portal circulation
LPS

Detoxification
Barrier function
Hepatocyte function
Permeability
Kupffer cells
Bacterial overgrowth
LPS tolerance
Microbial translocation

LIVER
GUT
Gut - Liver Access Microbial Translocation

**Systemic circulation**

- TNFα, pro-inflammatory cytokines
- LPS

**Portal circulation**

- Detoxification
- Hepatocyte function
- Kupffer cell activation
- LPS sensitization

**Gut - Liver Access**

- Barrier dysfunction
- Permeability
- Bacterial overgrowth
- Microbial translocation

**Liver**

- TLR4
- LPS

**Kupffer Cell**
Mechanisms of Bacterial Translocation

**Intestinal Bacterial Overgrowth**
- Dysmotility
- Delayed transit time
- Nutrition?

**Intestinal Permeability**
- Mucosal Hypoxia, Acidosis
- ATP depletion, NO, LPS, TNF

**Impaired Immunity**
- Impaired chemotaxis, migration, phagocytic function, complement deficiency, etc.

Anaerobic bacteria
- Entero-cytes

Aerobic bacteria
- Lamina propria
Evidence for increased intestinal permeability or serum LPS levels in liver disease

- **Cholestasis of pregnancy**
  - Hepatology. 2006;43:715-722

- **Chronic hepatitis C infection in HIV co-infected patients**

- **Chronic HCV patients (no cirrhosis)**

- **Alcoholic liver disease**

- **Cirrhosis**
In Humans, Bacterial Translocation Increases with Severity of Cirrhosis

Cirera et al., J Hepatol 2001: 34:32
MECHANISMS OF LPS DETOXIFICATION

- **Mechanisms that bind LPS**
  - Anti-endotoxin antibodies
  - Collectins: mannose-binding lectin, surfactants
  - LPS binding neutrophil granule proteins
  - Lipoproteins: HDL, LDL, VLDL, chylomicrons, apolipoproteins
  - Soluble CD14, soluble MD2
- **Enzymes that degrade Lipid A**
  - Deacetylation
  - Dephosphorylation
- **Inactivation by cellular uptake**
  - Liver – Kupffer cells, sinusoidal endothelial cells, hepatocytes
  - Spleen
- **Adaptation that modifies cell responses**
  - LPS/TLR4 tolerance
  - LPS/TLR “cross-tolerance”
Acute LPS administration

- Intravenous bolus of 3-4ng/kg LPS into healthy volunteers (approx 60 pg/ml blood concentration)
  - Acute increase in serum pro-inflammatory cytokines: TNFα, IL-1
  - Delayed induction of anti-inflammatory mediators: IL-10, IL-1ra
- Administration of anti-CD14 antibody prior to LPS can prevent the pro-inflammatory cytokine induction

Roth et al. Inf Immun 1993;61:3209-3215
Spek et al. JCI 2003;23:132-140
Repeated LPS stimulation results in TLR tolerance

TLR (endotoxin)-tolerance

1st
24 hrs
LPS

2nd
LPS

CD14
TLR4

NF-κB
Inflammatory mediators

NF-α, IL-1, etc
Chronic hepatitis C virus infection

Loss of TLR4 tolerance in blood monocytes

Increased serum endotoxin in the absence of portal hypertension

The effects of LPS on HCV clinical course

Jirillo et al
J Endotoxin Res 2002;8:319-327

Endotoxemic patients with HCV

Treatment for 6 mo with IFNα/Rib

Responders
No LPS detected

Non-responders
48% LPS detected

Balagopal et al
Gastroenterology 2008:125:226-233

HIV/HCV coinfected patients

Association of microbial translocation markers and cirrhosis:
LPS >42pg/ml
LBP
soluble CD14
EndoCab IgM
CD14 <350/mm²

Conclusion:
Microbial translocation may be a mechanism through which HIV accelerates progression of chronic liver disease.
Evidence for increased sensitivity to endotoxin-induced liver injury

- **Human disease:**
  - Alcoholic fatty liver and alcoholic steatohepatitis
  - Non-alcoholic fatty liver and steatohepatitis
  - Primary biliary cirrhosis
  - Cholestasis

- **Animal models:**
  - Alcoholic liver disease
  - Non-alcoholic liver disease
  - Ischemia-reperfusion liver injury
  - Priming with Propionibacterium acnes
  - D-galactosamine
Fatty liver has increased sensitivity to LPS
Increased LPS sensitivity in mouse models of fatty liver disease

LPS hyper-responsiveness: increased TNFα production

* p<0.05

* p<0.03

p<0.03
Mechanisms of alcoholic liver injury

Hypothesis:
MyD88-dependent and -independent signaling pathways may have different roles in alcoholic liver disease.
Chronic alcohol feeding in mice

**Animals:**

WT, TLR4-, MyD88- and IRF3-deficient (KO) male mice aged 6-8 weeks received Lieber-DeCarli diet with 4.5 V/V% ethanol or isocaloric liquid control diet for 5 weeks (4-8 mice/group).

**Measurements:**

- Serum ALT levels
- Liver steatosis and triglyceride levels
- TNFα, ISG56 mRNA expression in the liver
- Liver mRNA expression of the NADPH-oxidase complex subunits
- mRNA expression of IRF7 and TNFα in isolated Kupffer cells
TLR4 and IRF3 but not MyD88 deficiency protect against alcohol-induced steatosis

Wild type | TLR4⁻/⁻ | MyD88⁻/⁻ | IRF3⁻/⁻
---|---|---|---
Pair fed
EtOH
Liver triglyceride

Liver TG (mg/g)

<table>
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<tr>
<th></th>
<th>WT</th>
<th>TLR4⁻/⁻</th>
<th>MyD88⁻/⁻</th>
<th>IRF3⁻/⁻</th>
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<tr>
<td>p-value</td>
<td>0.03</td>
<td>0.02</td>
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The graph shows the liver triglyceride content in different genotypes under control (Pair fed) and alcohol (EtOH) conditions. The deficiency of TLR4 and IRF3 significantly protects against steatosis compared to the wild type and MyD88⁻/⁻, as indicated by lower liver triglyceride content.
TLR4 and IRF3 but not MyD88 deficiency protects from alcoholic liver damage

TLR4 and IRF3 but not MyD88 deficiency protects from alcoholic liver damage

Schematic model of TLR4 activation in ALD

MyD88-dependent

MyD88-independent

LPS

TLR4

IL-1β

NF-κB

Promoter

Inflammatory cytokines

Type I IFNs

NF-κB responsive genes

IFN promoter
Metabolic Syndrome and NAFLD Associated with Adiposity

- **Visceral fat**
- **Peripheral fat**
- **Kupffer Cell function**
- **Adiponectin**
- **NAFL/NASH**

**References:**
2. Day et al FALK Symposium, Innsbruck 2005
HYPOTHESIS

Activation of the TLR4 receptor complex and TLR4-induced downstream signaling plays a role in the development of NASH.
Methods

Animal model: 10-12 week-old female mice.

Strains (C57BL/6):

- WT
- MyD88-/-
- TLR4-/-

MCS diet (Methionine-Choline-Supplemented)

MCD diet (Methionine-Choline-Deficient)

8 wks

Statistical Analysis:

- mean+SD.
- Kruskal-Wallis & Mann-Whitney U tests.
Deficiency in TLR4 and MyD88 attenuates liver injury and steatosis

Serum ALT

Liver TG

* = p<0.04 vs. corresponding MCS group

Velayudham DDW 2006
Deficiency in TLR4 and MyD88 results in decreased induction of the proinflammatory cytokine, TNF$_{\alpha}$

Serum TNF$_{\alpha}$

* = p<0.04 vs. corresponding MCS group*

MCS MCD

WT MyD88-/- TLR4-/-
Differential involvement of downstream TLR4 signaling pathways in ASH and NASH

**NASH**
- MyD88-dependent
- IRAK1/4
- TRAF6
- IKKα, IKKβ
- p65, p50
- IκB
- NF-κB responsive genes
- Pro-inflammatory cytokines: TNFα, IL-1, IL-6
- Chemokines

**ASH**
- MyD88-independent
- TLR4
- TRAF3
- TRIF
- TBK1/IKKε
- IRF3
- Type I IFNs
- IFN promoter

**Pro-inflammatory cytokines**

**Chemokines**
Future perspectives:
Therapies to target the LPS pathway

• Anti-lipopolysaccharide antibodies
  - anti-endotoxin monoclonal antibody, E5,
    binds lipid A
  - antibodies to the O-polysaccharide chain

• Anti-CD14 blocking antibodies

• Small molecule inhibitors
  - lipidA antagonist: E5564

• TLR4 antagonists
Thank you
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Intestinal Bacteria
Role in NAFL / NASH

Gut flora promote
- absorption of dietary lipids
- hepatic fatty acid synthesis

Gut-derived bacterial products
- escape steatotic livers
- stimulate cytokine production by peripheral fat

↑ Hepatic Fatty Acids
↑ TNF