Kupffer Cells, Hepatic Stellate Cells, And Liver Fibrosis

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Hepatitis C Virus

Mild inflammation

Inflammation

Fibrosis

Cirrhosis
Non-alcoholic Fatty Liver Disease

Steatosis

Steatohepatitis

Fibrosis

Cirrhosis
Chronic Inflammation leads to Fibrosis

WBC recruitment

Kupffer cell activation

HSC activation

Activated Myofibroblast

INJURY
LPS
TLR4
TNF-α
IL-1
MIP-1

TGF-β
Ang II
Leptin
ROS

KO mice – TLR4, TNF-α
TLR4 induces proinflammatory responses, but not profibrogenic pathways

- **LPS** (HMGB1, Hyaluronan)
  - TLR4
    - MD2
    - CD14

MyD88-independent pathway:
- TRIF
- TRAM
- MyD88

MyD88-dependent pathway:
- IRAK1
- IRAK4
- TRAF6
- TAK1
- IKKα
- IKKβ
- JNK
- IκBα
- p50
- p65

**Nucleus**
- IRF3
- IFN-β transcription
  - RANTES, CXCL10
- NFκB
- AP-1

**Proinflammatory cytokines**
- TNF-α, IL-6, IL-12, CXCL1
Activation of Hepatic Stellate Cells

**Quiescent HSC**
- TLR4
- Proinflammatory cytokines

**Activated HSC**
- TLR4
- Proliferative cytokines (PDGF)
- Fibrogenic cytokines (TGFβ)
- Proinflammatory cytokines
- Collagen production
- α-SMA expression
- Fibrogenesis
- Proliferation

**Kupffer cell**
- Retinoid droplets
The role of TLR4 in hepatic fibrogenesis

TLR4
C3H/HeOuJ

TLR4-mutant
C3H/HeJ

Bile duct ligation
Intragastric CCl₄ administration
Thioacetamide in drinking water

Hepatic fibrosis

Analysis: Sirius red staining, IHC for αSMA, Hydroxyproline mRNA levels of profibrogenic genes by qPCR
TLR4-mutant mice display strongly reduced fibrosis after BDL

**Sirius red staining**
- TLR4<sup>WT</sup> Sham: Sirius red staining is faint.
- TLR4<sup>WT</sup> BDL: Sirius red staining is more intense.
- TLR4<sup>mutant</sup> Sham: Sirius red staining is faint.
- TLR4<sup>mutant</sup> BDL: Sirius red staining is significantly reduced.

**Hydroxyproline**
- TLR4<sup>WT</sup> Sham: Hydroxyproline levels are low.
- TLR4<sup>WT</sup> BDL: Hydroxyproline levels are high.
- TLR4<sup>mutant</sup> Sham: Hydroxyproline levels are low.
- TLR4<sup>mutant</sup> BDL: Hydroxyproline levels are significantly lower.
TLR4-mutant mice showed less fibrogenic responses 5 days after BDL

* P<0.05

TLR4 signaling is required for hepatic fibrosis after BDL
TLR4 is essential for all models of hepatic fibrosis
TLR2 is not important for hepatic fibrosis after BDL
Gut-sterilization by antibiotics cocktail suppresses plasma endotoxin level after BDL

Antibiotics cocktail (Ampicillin, Neomycin, Metronidazole, Vancomycin) for 4 wks.
Gut microflora contributes to liver fibrosis

**Antibiotics cocktail (Ampicillin, Neomycin, Metronidazole, Vancomycin) for 4 wks**
Kupffer cells and HSCs are the direct targets of TLR4 ligand in the liver.

![Image of microscopic images showing the effects of LPS treatment on liver cells.](image)

- **Non-treated**
  - LPS-: No significant changes.
  - LPS+: Some cells show increased NF-κBp65 and Desmin expression.

- **Clodronate**
  - LPS-: Minimal effects.
  - LPS+: Marked increase in NF-κBp65 and Desmin expression in a subset of cells.

**Legend**
- **Green:** NF-κBp65
- **Red:** Desmin

**Diagram**
- LPS → Kupffer cell → TNF-α → HSC
- LPS directly affects Kupffer cells and HSCs.
Combination of Clodronate, irradiation, and BMT replaces Kupffer cells, but not HSCs

HSCs are not derived from BM 2 weeks after BDL. (Kisseleva et al 2006, J Hepatol)
HSCs, but not Kupffer cells, are the target of TLR4 ligands in hepatic fibrosis

TLR4<sup>WT</sup>BMI→TLR4<sup>WT</sup> 
TLR4<sup>mutant</sup>BMI→TLR4<sup>mut</sup>

Sirius red

<table>
<thead>
<tr>
<th></th>
<th>sham</th>
<th>BDL</th>
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<td>WTBM→WT</td>
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<tr>
<td>TLR4&lt;sup&gt;mutant&lt;/sup&gt;BM→WT</td>
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Hydroxyproline

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TLR4 signaling enhances TGF-β induced HSC activation

Collagen promoter-driven GFP Tg HSCs

Quiescent mouse HSCs

TGF-β reporter
Collagenα1(I) reporter
LPS primes quiescent HSCs for activation by Kupffer cell-derived TGFβ
Kupffer cells are required for hepatic fibrosis after BDL

Green: $\alpha$F4/80/Red: $\alpha$Desmin

TGFβ1

Hydroxyproline

Sirius red
Microarrays identify 121 LPS-regulated genes in quiescent HSCs

Expression levels of other TGFβ signaling regulated molecules (TGFβ receptors, Smad molecules, SnoN, Ski and Sara1) were unchanged.
TLR4 signaling regulates Bambi expression in HSCs

**Quiescent HSCs**

- LPS
  - -
  - +

- BDL
  - -
  - +

**In vivo activated HSCs**

- TLR4<sup>WT</sup>
  - -
  - +

- TLR4<sup>mut</sup>
  - -
  - +

**Type I receptor homodimer**

- Signal transduction

**Type II receptor**

- TGFβ

**Bambi**

- Bambi heterodimer

- No Signal

**TLR4**

- TGFβ

- TGFβR

- Bambi
Bambi regulates LPS-mediated TGF-β induced HSC activation

- TGFβ  + TGFβ
- LPS  - LPS
- LPS  - LPS
- LPS  - LPS

AdLacZ

+ LPS  + LPS
+ LPS  + LPS
+ LPS  + LPS
+ LPS  + LPS

AddnBAMBI

- LPS  - LPS
- LPS  - LPS
- LPS  - LPS
- LPS  - LPS

AdBAMBI

+ LPS  + LPS
+ LPS  + LPS
+ LPS  + LPS
+ LPS  + LPS

Collagen-GFP+ cells (%)
control  dn-BAMBI  BAMBI
- + - +
- + - +
- + - +
- + - +

CAGA-luciferase (fold induction)
control  dn-BAMBI  BAMBI
- + - +
- + - +
- + - +
- + - +

*, p<0.05
n.s.
Bambi expression and HSC activation are regulated in a TLR4-dependent-NF-κB-dependent manner.
Bambi expression and hepatic fibrogenesis are regulated in a TLR4-dependent-MyD88-dependent manner.
Summary

LPS

TLR4

Chemokines

Bambi

TGFb-RI

HSC activation

Collagen deposition

Liver fibrosis

Kupffer cell

quiescent HSC

Adhesion molecules

TGFβ

Collagen deposition

Liver fibrosis
TLR4-mutant mice treated with CDAA diet have a reduction of steatosis and fibrosis.
**TLR2-deficient mice treated with CDAA diet have a reduction of fibrosis, but not of steatosis**

**H-E staining**

WT: [Image of H-E staining for WT mice]

TLR2-/-: [Image of H-E staining for TLR2-/- mice]

**Sirius red staining**

WT: [Image of Sirius red staining for WT mice]

TLR2-/-: [Image of Sirius red staining for TLR2-/- mice]

**Graph**

Sirius red pos-area (%)

- **WT**
  - CSAA: [Value]
  - CDAA: [Value]

- **TLR2-/-**
  - CSAA: [Value]
  - CDAA: [Value]
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