Innate Immune Responses to Hepatitis C Virus Infection

3rd ACHA
10 May, 2014

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HCV History

Recognized as PT-NANBH in 1970s

Cloned and sequenced: 1989

Antibody screening tests available: 1990

Infectious clone: 1997

HCV RNA replicon: 1999

Pseudovirus particles: 2003

Culture HCV in hepatoma cells: 2005
Therapy for Hepatitis C

Pegylated Interferon + Ribavirin

+ Direct-acting Antivirals (since 2011)

  NS3/4A Protease inhibitors (2011)

  NS5B polymerase inhibitor (2013)

Ultimate control of HCV will require a vaccine!
Hepatitis C Virus

~9.6 kb Single-stranded, positive-sense RNA genome

7 major genotypes, many subtypes

3000-aa polyprotein

5' cis replication signal

IRES

3' cis replication signal
The Hepatitis C Virus Life Cycle

Challenges in Working with HCV

- Lack of convenient small animal models
  chimpanzee: availability; $$$$$$;
  a higher rate of spontaneous resolution
  SCID mice engrafted with human hepatocytes (2001)
  Transgenic mice expressing human CD81 and occludin (2013)

- Study closely related viruses: GB virus B

- Can not grow HCV in cell culture until 2005
  HCV RNA replicons (since 1999)
  only JFH-1 strain (genotype 2a) efficiently produces infectious HCV
  in cell culture until recently: J6 (2a); J8 (2b); H77S & TN (1a)
  Efficient propagation of HCV is confined to the Huh7 cell line

Sorry, I am about to retire!
Factors Associated with HCV Clearance

- Humoral immune responses are not able to clear HCV infection.

- Vigorous, broadly-directed, and sustained CD8$^+$ and CD4$^+$ T cell responses are critical in HCV clearance.

- Innate immune responses offer early viral control and help orchestrate the development of adaptive immunity.

  Host genetic variation in the IL28B (IFN-λ3) gene is critical for predicting response to IFN therapy as well as spontaneous clearance in patients.

  Multiple HCV proteins disrupt innate immunity through distinct mechanisms.
Cellular Control of Early Antiviral Responses
- Induction of IFNs & inflammatory cytokines

**PAMP**
- **PRR**
- Adaptor
- Kinases
- Transcription Factors
- IFNs, cytokines, chemokines

Li & Lemon, Semin Immunopathol 2013
How do Liver cells Detect HCV Infection and Initiate Innate Immune Responses?

- Microarray analysis has demonstrated that HCV infection is generally associated with induction of a strong interferon-stimulated gene (ISG) response in the liver in vivo.

- Intrahepatic chemokines such as the CCR5 ligands, RANTES, MIP-1β and MIP-1α, and the CXCR3 ligand, IP-10, are elevated in hepatitis C patients.

- Yet, the cellular sources of IFN(s) and cytokines/chemokines in HCV-infected liver are not known, and the signaling pathways responsible for their induction are just being uncovered.
Liver Microanatomy

Productively infected

Bystanders responding to virus exposure

Modified from Jenne & Kubes, Nat Immunol 2013
TLR3 and RIG-I Constitute Two Distinct Viral RNA Sensing Pathways in Non-neoplastic Hepatocytes

**Figure:** Activity of IFN-β and Luciferase in PH5CH8 cells following treatment with Mock, M-PLC, and SeV. The graphs show the relative luciferase activity with Ctrl and TLR3 siRNA for both IFN-β and Luciferase assays.

Li et al., JBC 2005
Huh7.5 Cells Highly Permissive for HCV Replication Are Defective for RIG-I Signaling

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<tr>
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<th>SG-Neo</th>
<th>Huh-7</th>
<th>Huh-7.5</th>
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<td>S2204I</td>
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<td>1 x 10^4 cells</td>
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<td>5AΔ47</td>
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<td>2.5 x 10^4 cells</td>
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Sumpter et al, *J. Virol.* 2005
Reconstitution of RIG-I Signaling Restricts HCV RNA Replication in Huh7.5 cells

Sumpter et al., *J. Virol.*, 2005
Saito et al., *Nature*, 2008
Although the RIG-I defect likely contributes to the highly permissiveness phenotype of Huh7.5 cells, it does not explain why parental Huh7 cells, which contain an active RIG-I pathway, are already unique among the hepatoma cell lines for the ability to efficiently support HCV replication.

Is there a role for TLR3 in hepatocellular innate responses to HCV?
Hepatoma Huh7 Cells are Defective in TLR3 Signaling due to Lack of TLR3

Li et al, JBC 2005
Ectopic Expression of TLR3 Reconstitutes the Signaling Defect to Extracellular dsRNA in Huh7 cells

Wang et al, J Virol 2009
Primary Human Hepatocytes Express TLR3 *in situ*

Primary Human Hepatocytes Contain a Robust TLR3 Signaling Pathway
(Induction of ISGs)

Wang et al, J Virol 2009
Primary Human Hepatocytes Contain a Robust TLR3 Signaling Pathway (Induction of cytokines)

Li et al, Hepatology 2012
Reconstitution of TLR3 Signaling in Huh7.5 Cells Inhibits HCV Infection

H539E & N541A:
TLR3 mutants defective for dsRNA binding
TLR3 mediates Production of Chemokines and Proinflammatory Cytokines in HCV-infected Hepatoma cells

H539E & N541A:
TLR3 mutants defective for dsRNA binding

Li et al, Hepatology 2012
TLR3-mediated Chemokine Induction by HCV infection in Hepatocytes

- depends on viral replication and first occurs at 36-48 h post-infection
- depends on activation of NF-κB
- HCV dsRNA intermediates generated during viral replication is the ligand for TLR3
- HCV dsRNAs need to be ≥ ~80-100 bp for TLR3 activation, while are independent of the genome position or nucleotide composition (as opposed to the RIG-I ligand)

Li et al, Hepatology 2012
Short-range Exosomal Transfer of HCV RNA from Infected Cells to pDCs Triggers TLR7-dependent Type I IFN Production

Huh7.5

HCV RNA+

Co-culture

pDC

TLR7

↑ IFN-α

HCV RNA Exosome

Exosome release inhibitor

Takahashi et al, PNAS 2010
Dreux et al, Cell Host Microbe 2012
HCV dsRNAs Released from Infected Cells Triggers TLR3-dependent Type III IFN Production in co-cultured BDCA3+ mDC2 Cells

Co-culture

Huh7.5

HCV dsRNA+

mDC2

TLR3

IFN-λs

Inhibitors of endocytosis (cytochalasin D or chlorpromazine)

Inhibitors of endosome acidification (chloroquine or bafilomycin)

Zhang et al, Gastroenterology 2013
Phagocytosis of HCV virions containing viral RNAs Activates the NLRP3 Inflammasome in Kupffer cells Leading to IL-1β Secretion
Summary

1. RIG-I and TLR3 constitute two parallel innate antiviral pathways in human hepatocytes that act to fend off HCV infection.

2. In addition to its antiviral role in sensing and restricting HCV replication, TLR3 also mediates inflammatory cytokine induction in HCV-infected hepatocytes, which may contribute to immune responses to the virus.

3. HCV ssRNAs and dsRNAs released from infected hepatocytes can be sensed by infiltrating pDCs and mDC2, respectively, leading to production of differing types of IFNs via distinct TLR pathways.

4. Intrahepatic IL-1β production by liver resident macrophages (Kupffer cells) after phagocytosis of HCV virions may contribute to liver inflammation.
Questions Remain Open

1. How do multiple arms (pathways) of the innate immune system interact to coordinately respond to HCV infection? (depending on novel culture systems)

2. How do various liver cell types act in concert to drive immunity against HCV? (depending on immuno-competent, permissive mouse models)

3. How do the innate immune responses to clinical isolates (especially the ones difficult to treat) differ from those to JFH-1 virus?

4. How do genetic variations (e.g., IL28B genotype) alter spontaneous viral clearance and treatment responses?
## Acknowledgement

**Li Lab @ Univ of TN HSC**

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NIH: $$$
Hepatitis C is a Global Health Problem

170-200 million infected worldwide

~70-85% people infected progress to chronic liver diseases
Dependence on miR-122 for HCV Replication

Protect uncapped HCV RNA from exonuclease attack
Sheild 5’-end triphosphate from innate immune recognition by RIG-I
Stimulate translation of HCV RNA

Scheel & Rice, Nature Med 2013