Immunology of Hepatitis B in Natural History and Therapy

Session on Dec 1, 2015
Scientific and Medical Aspects of Elimination

The National Academies of Sciences, Engineering, Medicine Committee on a National Strategy for the Elimination of Hepatitis B and C
The Keck Center, 500 Fifth Street NW

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I have the following disclosures:

Biogen: Spouse employment
Genentech: Scientific Advisory Board
Alnylam: consulting agreement
Arbutus: Scientific Advisory Board
The situation

• Despite effective vaccine, HBV remains a significant world health issue.

• The available antiviral drugs can efficiently suppress viral replication and liver disease progression in patients with chronic hepatitis B, but without a cure.

  • Since natural HBV clearance is immune-mediated, can we harness host immunity involved in HBV clearance and pathogenesis?

  • Which immune components contribute to HBV clearance and/or disease pathogenesis?
The quick answer:

• Both innate and adaptive immune responses are involved, with **antiviral T cells** providing direct kill/cure of HBV-infected cells.

• HBV persistence is associated with increased immune regulatory pathways—limiting cellular damage **AND** promoting viral survival.

• Potential immune-mediated therapeutic approaches need to balance the curative and damaging aspects—activate but not too much and with good virus control.
Brief overview:

- Innate immune response and HBV
- Adaptive immune response and HBV
- Immune regulatory pathways
- Potential immune modulatory approaches
**HBV: Very brief molecular introduction**

**HBV:** 3.2kb pDS DNA virus
Hepadnaviridae family
Identified in 1965 (HBsAg)
42nm, Geno A-H
Reverse transcription/integration

Newly identified NTCP receptor

**Na taurocholate co-transporting polypeptide**
Course of infection

- Incubation: 6-8 wk
- Viremia
- Clinical Hepatitis
- Innate immune response
- Adaptive immune response

- Resolve
- Chronic
- HBV control (not cure) with Protective immunity
- reactivation
- Disease Progression
- HCC
- Inflammation
- Tolerance
- Control
1. HBV and Innate Immune Responses
HBV is a stealth virus that does not readily induce innate cellular response

Lack of Liver gene expression profile correlating with viremia in chimpanzees inoculated with HBV

Wieland et al, J. Virol 05
Exceptions to this ‘stealthness’

• NK/NKT activation detected very early in HBV infection (Fisicaro Gut 2009).
• IFN-mediated HBV suppression in HepaRG cells in vitro (Lucifora/Zoulim Hepatology 2010).
• HBV induces type III IFN (but not type I IFN) via RIG-I in primary human hepatocytes (Sato Immunity 2015).
HBV can be regulated by innate immune components

- IFN-mediated HBV suppression in HepaRG cells in vitro (Lucifora/Zoulim 2010).
- HBV can be suppressed by IFN lambda in vitro (Robek 2005).
- IFNa and LTβR activation can promote nuclear HBV cccDNA degradation and HBeAg decline via APOBEC3A/B (Lucifora 2014).
HBV regulation via TLR pathways

Detected in HBV-replicating Tg mice:
- TLR3, 4, 5, 7 and 9 (not TLR2) (Isogawa 2005)

Detected in chimps and woodchucks
- TLR7 agonist GS-9620 reduced viral DNA and surface Ag with reversible liver inflammation (Lanford 2013, Menne 2015)

TLR7 agonist GS-9620 in patients:
- Phase 1b: Safe, well-tolerated with the induction of ISG15 without significant IFNα levels or related symptoms (Gane 2015)
- Phase 2 ongoing
Additional roles for innate immune cells

Potential pathogenic roles:

• CD16+ proinflammatory monocytes correlate with liver disease severity and fibrosis in CHB (Zhang JY (PLoS One 2011)


So, despite its apparent ‘stealthness’, innate immune response is involved in HBV pathogenesis.
2. Adaptive Immunity and HBV

Inflammatory recruitment
Induction of regulatory pathways
Virus control vs disease

Cured
Killed

CD4 → CD8

IFNγ
TNFα

HCV

B
B cells

Neutralizing antibody response against HBsAg:
- following natural infection
- following vaccination

Antibody response to HBcAg:
- marker of HBV exposure (not protection)
- may decline over time (with persistent T cell resp)
**B cells: more than just neutralizing Ab?**

- Active virus control: marked HBV reactivation observed with B cell depletion (e.g. Rituxan)
- Pathogenic role—given massive accumulation of plasma cells secreting IgG and IgM (+ complement) in HBV-associated acute liver failure (Fari PNAS 2010)
- Immune regulation via IL10+ regulatory B cells in chronic infection (Das J. Imm 2012).
- More work is needed...
T cell markers appear in the liver of HBV-infected chimpanzees with the onset of hepatitis and viral decline—circumstantial evidence for a causal role.
CD8 T cell depletion delays viral clearance and liver disease in acutely HBV-infected chimpanzees—a causal role in pathogenesis and virus control.

**CD8-depletion**

- Prolonged viremia
- Delayed hepatitis

**Control Ab**

- Prompt virus and hepatitis control animals

*Thimme et al 2003*
CD4 T cell depletion **BEFORE** HBV inoculation promotes HBV persistence in experimentally HBV-infected chimpanzees--CD4 T cells are needed to clear HBV.

Asabe et al 2009
The size of the viral inoculum contributes to the outcome of HBV infection

Asabe et al.
J. Virol. 2009
Patients with chronic hepatitis B display a poor antiviral CD8 CTL response in blood. 

**Chronic**

- cHBV-1
- cHBV-2
- cHBV-3
- cHBV-4
- cHBV-5
- cHBV-6
- cHBV-7
- cHBV-8

**Acute**

- aHBV-1
- aHBV-2

**Convalescent**

- aHBV-1
- aHBV-2

HBV epitopes

Rehermann J Virol 96
HBV-specific T cell response is weak in CHB regardless of clinical phenotype

177 HBsAg+ Chronic Hepatitis B Participants in NIH-sponsored Hep B Research Network

15 HBsAg- vaccinees

Study ongoing in CHB patients on antiviral therapy (NUC +/- PEG IFNα)
T cell response to HBV is weak in CHB patients (compared to S response in vaccinees or to Flu)

Median SI

<table>
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<tr>
<th></th>
<th>RT1</th>
<th>RT2</th>
<th>PreC</th>
<th>C</th>
<th>PreS</th>
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p<0.0001

Median IFNγ

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p<0.0001

Park et al, Gastroenterology (in press)
T cell response to Flu or LPS is also weak in CHB patients (compared to uninfected vaccinees)

![Bar chart showing T cell response to Flu or LPS in CHB patients and uninfected vaccinees.](chart)

- Median SI:
  - RT1: p<0.0001
  - RT2: p<0.00001
  - PreC: p=0.023
  - C: p=0.063
  - PreS: p=0.47
  - S: p=0.0018

- Median IFN\(\gamma\) SFU/M PBMC:
  - RT1: p<0.0001
  - RT2: p<0.0001
  - PreC: p=0.063
  - C: p=0.0018
  - PreS: p=0.0018
  - S: p=0.0018

Park et al, Gastroenterology (in press)
3. Immune regulatory pathways induced in chronic hepatitis B
PD-1 is selectively upregulated in HBV-specific CD8 T cells in chronic hepatitis B.
# Immune regulatory induction in total CD4 T cell compartment in CHB

<table>
<thead>
<tr>
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<th>CHB (n=200)</th>
<th>Normal (n=20)</th>
<th>**p (MWU)</th>
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<tr>
<td><strong>CD4</strong></td>
<td></td>
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<tr>
<td>%FoxP3+</td>
<td>4.9 (3.3, 6.8)</td>
<td>3.6 (3.0, 4.5)</td>
<td>0.027</td>
</tr>
<tr>
<td>(%IQR25,75)</td>
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<tr>
<td>%FoxP3+CD127lo</td>
<td>3.8 (2.7, 5.4)</td>
<td>2.9 (2.5, 3.7)</td>
<td>0.029</td>
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<td>(%IQR25,75)</td>
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<tr>
<td>%PD1+</td>
<td>22.9 (16.8, 27.9)</td>
<td>15.8 (12.5, 23.4)</td>
<td>0.013</td>
</tr>
<tr>
<td>(%IQR25,75)</td>
<td></td>
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<tr>
<td>%CTLA4+</td>
<td>9.2 (7.3, 11.9)</td>
<td>7.2 (3.8, 8.5)</td>
<td>0.003</td>
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<tr>
<td>(%IQR25,75)</td>
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<tr>
<td><strong>CD8</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>%PD1+</td>
<td>18.8 (14, 27)</td>
<td>20 (14, 29)</td>
<td>0.55</td>
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<tr>
<td>(%IQR25,75)</td>
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<td></td>
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<tr>
<td>%CTLA4+</td>
<td>6 (3.9, 9.4)</td>
<td>5.5 (2.3, 7.6)</td>
<td>0.156</td>
</tr>
<tr>
<td>(%IQR25,75)</td>
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</tbody>
</table>

Park et al, Gastroenterology (in press)
HBeAg status impacts HBV-specific T cell response (Core, RT1)

% LPR+

% IFNγ+

% IL10+

Park et al, Gastroenterology (in press)
Mechanisms of HBV persistence in vertical transmission

• High persistence presumed to be due to immature immune system and tolerizing effect of circulating HBeAg that crosses the placenta.

• Emerging studie suggest:
  • a role for age-dependent hepatic lymphoid organization and homing with deficient CXCL13 and IL21 (Publicover, JCI 2011 & 2013)
  • Preserved T cell function in children and young adults with immune tolerant CHB (Kennedy, Gastro 2012)
Additional players in HBV immune pathogenesis

- **IL17+ Th17 cells** increase with severity of liver damage in patients with chronic hepatitis B (Zhang JY Hepatology 2010).

- **Pathological functions of IL22** in chronic liver inflammation and fibrosis (Zhang Y Gastro 2011, Zhao Hepatology 2014)

- **γδT cells** with variable frequencies and relevance reported
Antiviral T cells are tolerized by multiple regulatory mechanisms in chronic hepatitis B

- DC
- Tregs
- Regulatory cytokines
- NK
- HBsAg, HBeAg
- HBV DNA

Poor signaling
Cell intrinsic Regulation
Apoptosis

Arg
CTLA-4
PD-1
CD3ξ
Arginase
Tim3
Bim
T-bet↓

Adapted from Dr. FS Wang
4. Can we reverse T cell dysfunction in chronic hepatitis B?

- with therapy
- with therapeutic vaccination
- with immune inhibitory blockade
  in vitro
  in vivo
Therapeutic virus suppression can lead to improved HBV-specific T cell function

**Initial Enhancement**

- **HBcAg**
- **HBeAg**

Weeks: 0 4 12 20 24 25 26 28 32 36 40

S.I.: 0 1 10 100

**But not sustained!**

- %Patients with HBV-pol-specific IFNγ+ T cell responses

**Responses to HBV polymerase**

- Grp 2
- Grp 3
- Grp 4, treated for 1-3 yrs
- Mean HBV DNA [GE/ml]
  - 0 2x10⁵
  - 3x10⁵ 4x10⁷ 1x10⁸

- Patients with Lamivudine-resistant HBV Mutants [%]
  - 30 92
  - 0 71 68

- Mean ALT [U/l]
  - 56 62 70

*Bonj JCI 1998*

*Mizukoshi J Immunol 2004*
HBV-specific T cell response can emerge in patients with HBsAg loss on NUC following in-vitro expansion

HBeAg-neg CHB (naïve)

Spontaneously resolved CHB

Nuc-Rx HBsAg-neg

Boni Gastro 2012
Therapeutic Vaccine Approaches (since 80’s)

- HBsAg +/- IFNα, NUC and/or rIL2
- Yeast-derived rHBsAg/HBIG immune complex
- CTL epitope vaccine (HBc epitope)
- Plasmid DNA vaccine:
  - preS2/S + NUC
  - PreS/S/C/P +/- X, with NUC and IL12
- Generally well-tolerated and immunogenic but inconsistent therapeutic efficacy.
Plasmid DNA encoding HBV S/S1/S2/X/C/P + IL12, and 3TC

- Plasmid DNA HB-100 (adr) encoding S/S1/S2/X/C/Pol/hIL-12
  - Phase I study in 12 Caucasian patients
  - Monthly HB-100 IM injection + daily 3TC x 52 wk
  - Safe, well-tolerated, immunogenic with 50% showing HBV suppression at 1y post treatment cessation (Yang, Gene Therapy 2006)

**Responder**

**NonResponder**

Yang, Gene Therapy 2006
DNA vaccine encoding more HBV regions + IL12, and NUC
Blockade of PD1 or CTLA4 can enhance T cell responses to both HBV AND Flu (and in both CHB and uninfected controls)

Park et al, Gastroenterology (in press)
HBeAg status impacts responsiveness to PD1/CTLA4 blockade

<table>
<thead>
<tr>
<th>medium</th>
<th>αPDL1</th>
<th>αCTLA4</th>
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<tbody>
<tr>
<td>S</td>
<td>0.80</td>
<td>0.32</td>
</tr>
<tr>
<td>Core</td>
<td>0.93</td>
<td>0.03</td>
</tr>
<tr>
<td>RT2</td>
<td>0.90</td>
<td>0.74</td>
</tr>
<tr>
<td>Flu</td>
<td>0.34</td>
<td>0.56</td>
</tr>
</tbody>
</table>

HBeAg status
(HBeAg+: n=11, HBeAg-: n=8)

Park et al, Gastroenterology (in press)
PD-1/PD-L1 blockade promotes functional restoration for HBV-specific T cells from both liver and blood.

Further impact also seen by stimulating CD137 (41BB) via its ligand.

Fisicaro Gastro 2010
Effect of PD-1 blockade + DNA immunization + ETV in WHV

Degranulation

C: control
E: ETV alone
ED: ETV + DNA imm
EDA: ETV + DNA Imm + aPDL1

Summary

- Both innate and adaptive immune responses are involved, with **antiviral T cells** providing direct kill/cure of HBV-infected cells.
- HBV persists with increased immune regulatory pathways (e.g. PD-1, CTLA-4, Tregs, Bim)—limiting cellular damage **AND** promoting viral survival.
- While antiviral therapy may reverses some immune inhibition, sustained virus control is not common with oral antivirals.
- Immune-mediated therapeutic approaches may provide enhanced virus control, provided we can activate the immune system but not too much and in the setting of good virus control.
Potential therapeutic options

• Treat indefinitely ($$$)
• Treat for a defined duration hoping that some will achieve HBV control on their own
  – Risk of ALT flare/decompensation
• Consider immune modulation to harness the underlying immunity with careful consideration of:
  – Potential risk of worsened liver inflammation
• Research needed to better define safe and effective therapeutic approaches:
  • DNA, HBeAg, HBsAg titer, % HBV+ hepatocytes
  • ALT activity, cirrhosis
  • viral coinfections, comorbidities
Thank you for the invitation and for your attention!