Will Antigen Depletion Restore HBV-specific Immunity?

Adam J. Gehring, Ph.D.
Biology Lead
Toronto Centre for Liver Disease
University Health Network (UHN)

Assistant Professor
Department of Immunology
University of Toronto
**HBsAg Burden in Chronic HBV Patients**

**HBsAg load in peripheral blood**
- 1,000 IU/ml HBsAg = 5 μg/ml
  - Serum = 55% of whole blood (5,000 ml)
  - 5 μg/ml x 2,750 ml of serum

1,000 IU/ml = 13,750 μg of HBsAg in circulation
10,000 IU/ml = 137,500 μg = 137 mg

+ HBsAg in liver = Lots of HBsAg

**Prophylactic vaccine dose = 10 μg HBsAg**

---

Persistent Antigen Exposure Exhausts Virus-specific T Cells
Dysfunction of the HBV-specific T cell Response

Antigen specific dysregulation of T and B cells

HBV Specific T cells
1. HBV-specific T cells are prone to apoptosis

2. Co-express inhibitory receptors – PD-1, CTLA-4, Tim-3

3. Metabolic dysfunction

HBV-specific B cells
1. Up-regulation of inhibitory receptors = impaired antibody production

2. Atypical B cell phenotype

3. Produce IL-10
**Key questions**

1. Is reversal of immune exhaustion possible?

2. Will just reducing antigen exposure reverse exhaustion?

3. How long will it take?

4. What immune function will recover with antigen reduction?

5. If immunity recovers, will it be effective?
   1. Is recovery fragile? Transient?
   2. Does recovery need a boost?
      - Vaccine or immunomodulator
Imprinting Antigen-specific T cell Exhaustion?

chronic LCMV

1st c13
4 or 8 weeks

2nd c13
Day 8

Naive recipients

CD8+ T cells

CD45.1+CD45.2+

Acute

chronic

2nd Arm
Day 8

PD-1

Total CD8+ T cells (%)

1st: c13 c13 c13 c13
2nd: Arm Arm Arm Arm

chronic = PD-1

chronic = impaired function

Liver tolerance = no proliferation

Donor: Naive Memory Tolerant

Host: B6 B6 GAG B6

Cell number

Listeria strain

0 10 10

Imprinting Antigen-specific T cell Exhaustion?

**chronic LCMV**

1\(^{st}\) c13 4 or 8 weeks

Acute

CD8\(^{+}\) T cells

CD45.1\(^{+}\)CD45.2\(^{+}\)

2\(^{nd}\) c13

Day 8

Naive recipients

2\(^{nd}\) Arm

Day 8

chronic = PD-1

Total CD8\(^{+}\) T cells (%)

Day 0

Day 8

Arm c13 Arm c13 c13 c13 c13

Arm Arm Arm c13 c13

chronic = impaired function

Liver tolerance = no proliferation

Tolerant/Alb-Gag

Gag

B6

Liver tolerance = no proliferation

Lymphodeplete

Forced proliferation = function

Transient

Donor: Host: Naive Memory Tolerant

HP = homeostatic proliferation

Gag B6 GAG B6

HP = homeostatic proliferation

IFN-γ


Strategies for HBV Antigen Reduction
Strategies for HBV Antigen Reduction
- Antisense Oligos -

**Single-stranded, DNA Oligo**
- Single strand DNA
- Complementarity to 3’ end of HBV transcripts
- GalNac targeting to hepatocytes
- Forms RNA-DNA heteroduplex
- RNA degradation by RNaseH activity

**Results**
- Good antigen reduction
- No immunology presented
Strategies for HBV Antigen Reduction - RNAi -

**Results**

- HBsAg, RNA, HBeAg, HBcrAg all respond similarly to a single dose of ARC-520
- HBV DNA shows synergistic reduction in response to ARC-520 plus entecavir

**Single patient**
- Antigen decline coincides with ALT elevation
- No immunology presented
Strategies for HBV Antigen Reduction
- Nucleic Acid Polymer (NAP)-

REp 401 study - HBeAg negative chronic HBV mono-infection
40 patients received 48 weeks of REP 2139-Mg or REP 2165-Mg, TDF and pegIFN
Interim analysis from July 7, 2018

Results
• NAPs reduce HBsAg in most patients
• Appearance of anti-HBs
• Unmasking or T/B cell stimulation?

REP 2139-Mg = REP 2165-Mg
4/40 non-responders
8/40 HBsAg > 1 log reduction but > 1 IU/mL
28/40 HBsAg < 1 IU/mL
24/40 HBsAg loss (≤ 0.05 IU/mL)

Anti-HBs dramatically increased with the introduction of pegIFN
(but only in patients with HBsAg declines to < 1 IU/mL)
HBV-specific T Cell Responses After HBsAg Loss

**In vitro expanded T cell response**
- Increased T cell immunity with HBsAg loss
- Recovery highest in Nuc treated patients

**Cross-sectional**
- Did T cell response cause Ag loss?
- HBsAg loss allow for T cell recovery?

HBV Mouse Model Data on Immune Recovery After Antigen Reduction

Antibody-mediated HBsAg depletion

AAV-HBV Model

Days 0 40 54 138
NAb ↓ NAb+EnxB

HDS-HBV T cell transfer Model

Acute vs. Chronic Infection

Acute vs. Chronic Infection

Only eliminates HBsAg

HBsAg (ng/ml)

Days

mIU/ml

wks

NAb EnxB NAb+EnxB

HBeAg

NTC NAb EnxB/CpG NAb/EnxB/CpG

# of ENV159-specific spots in 10^6 lymphocytes

Spots

AAV/HBV NAb EnxB CpG

Acute Challenge

Impaired HBV control
New HBV therapies can effectively reduce HBV antigen concentration in the blood
  • different approaches clear HBV antigen through different mechanisms
    • prevent production = RNAi and antisense DNA oligos
    • prevent secretion = NAPs
  • May alter the immunological response to infected hepatocytes

No immune recovery data in chronic hepatitis B patients receiving RNAi or NAPs
  • ALT elevation ≠ antiviral immunity
  • anti-HBs become detectable with NAPs = unmasking or B cell expansion?
  • Partial T cell recovery in chronic HCV patients after SVR

Animal data indicates that functional exhaustion is a stable phenotype
  • Antigen reduction alone will likely not be sufficient for immune recovery
    • Combination with immunotherapy may improve responses

  • Real caveats with HBV mouse models = AAV & HDI
    • Duration of “infection”, amount of antigen produced all impact immunotherapy

The experiment can, and needs, to be done in chronic patients treated with drugs that reduce HBV antigen