HBV and the immune response

C. Ferrari

Unit of Infectious Diseases and Hepatology
Laboratory of Viral Immunopathology
Azienda Ospedaliero-Universitaria di Parma
Italy
List of topics

- Kinetics of immune responses: from the early stages of infection to HBV control or persistence
- Features of T cell and NK responses in chronic infection
- Mechanisms of T cell dysfunction in chronic HBV infection
- Effect of virus control on T and NK cell responses in chronic patients
- Potential strategies to reconstitute the anti-viral T cell function
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HBV is a ‘stealth virus’ poorly sensed by the innate immune system

Wieland S et al. PNAS 2004
HBV is a poor inducer of innate responses

- Cytokine and chemokine production in acute HBV infection is significantly more modest and delayed compared with acute HIV infection *(Stacey AR J. Virol. 2009)*

- Low production of type I IFN, IL-15 and IFN-λ1, associated with high serum IL-10 levels, at the early stages of HBV infection *(Dunn C. et al Gastroenterology 2009)*
Is HBV able to inhibit innate responses?

Extracellular sensing (TLR)
- TLR2
- TLR4
- TLR3

Intracellular sensing (RIG-1)
- RIG-1
- HBx
- HBVpol
- IFN-β

- NF-kB target genes
- PRD
Is HBV able to inhibit innate responses?
Summary of the early events in HBV infection

- Poor induction of early intracellular innate responses
- Efficient and timely induction of adaptive responses
- Early non cytolytic clearance of HBV
- Delayed NK cells activation
- T cell inhibition to avoid excessive damage

**Clinically overt infections**
Dunn C et al Gastroenterology 2009

Weeks from infection

- HBV-DNA
- NK cells
- %IFNγ+ NK cells

Weeks

- Dunn C et al Gastroenterology 2009

HBV-DNA

0 5x10⁷ 1x10⁸

%IFNγ+ NK cells

0 5 10 15 20 25

ALT IU/L

2,000

1,000

0
Maturation of long-lasting memory T cell responses in self-limited HBV infections

HBV INFECTION

Strong, multi-specific, T1 oriented T cell responses

Self-limited

Long-lasting protective responses

Virus control / occult infection

ACUTE PHASE

RECOVERY PHASE
(20 years from recovery)

% CD8-mediated cytotoxicity

HLA-A2 restricted HBV peptides
Progressive T cell functional impairment in chronically evolving acute HBV infections

HBV INFECTION

Weak and narrowly focused T cell responses

Chronic evolution

Persistent and progressive impairment of protective responses

CD4 RESPONSES (to core peptides)

CD8 RESPONSES (to HBV peptides)

HBV peptides

Virus persistence
HBV-specific T cells in chronic infection
HBV-SPECIFIC CD8 CELLS ARE PREFERENTIALLY CONCENTRATED WITHIN THE LIVER IN PATIENTS WITH CHRONIC HBV INFECTION
(Fisicaro P. et al. Gastroenterology 2010)
INTRAHEPATIC HBV-SPECIFIC T CELLS ARE MORE DEEPLY EXHAUSTED THAN THEIR PERIPHERAL BLOOD COUNTERPARTS IN CHRONIC HBV INFECTION

(Fisicaro P. et al. Gastroenterology 2012 and personal communication)

![Graph showing CD4 and CD8 T cells in blood and liver with IFN-γ, TNF-α, and IFN-γ/IL2](image)

![Graph showing correlation between HBV liver and HBV-DNA IU/ml](image)
NK cells in chronic infection
NK cell functional dichotomy in chronic HBV infection

**Impaired IFN-\(\gamma\) production with normal cytotoxicity (I)**

Peppa D. et al Plos Pathogens 2010

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**IFN-\(\gamma\)**

![Graph showing % IFN-\(\gamma\) production in Healthy vs. CHB]

- Healthy: N=29
- CHB: N=46

P<0.0001

**CD107a**

![Graph showing % CD107a production in Healthy vs. CHB]

- Healthy: N=21
- CHB: N=33

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<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
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<tbody>
<tr>
<td>Healthy</td>
<td>29</td>
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<tr>
<td>CHB</td>
<td>46</td>
</tr>
</tbody>
</table>

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Peppa D. et al Plos Pathogens 2010
NK cell functional dichotomy in chronic HBV infection

*Impaired IFN-γ production with normal cytotoxicity (II)*

**IFN-γ**

- IL2+IL12 18h

- p=0.0045

**CD107a**

**anti-NKp30**

**anti-NKp46**

**anti-NKG2D**

Oliviero B et al *Gastroenterology* 2009
NK cell functional dichotomy in chronic HBV infection

Impaired IFN-γ production with normal cytotoxicity (III)

Tjwa E. et al. Journal of Hepatology 2011
NK cell functional dichotomy in chronic HBV infection

*Impaired IFN-γ production with normal cytotoxicity (IV)*

NK cells seem to be more pathogenic than protective in chronic HBV infection
List of topics

- Kinetics of immune responses: from the early stages of infection to HBV control or persistence
- Feature of T cell and NK responses in chronic infection

**Mechanisms of T cell dysfunction in chronic HBV infection**

- Effect of virus control on T and NK cell responses in chronic patients
- Potential strategies to reconstitute the anti-viral T cell function
Different levels of T cell functional efficiency in different conditions of HBV control


![Graph showing different levels of T cell functional efficiency in different conditions of HBV control. The graph compares CD4+ and CD8+ T cell responses across different stages of HBV infection: Naïve HBeAg negative CHB, Inactive carriers, Occult infections, and Acute hepatitis B (resolution phase). The graph illustrates the mean % IFN-γ, IL-2, and TNF-α of T cells.](image-url)
PUTATIVE MECHANISMS OF T CELL EXHAUSTION IN HBV INFECTION

MODEL FOR HIERARCHICAL LOSS OF CD8 FUNCTIONS DURING CHRONIC VIRAL INFECTIONS

<table>
<thead>
<tr>
<th>Antigen Persistence</th>
<th>Antigen clearance</th>
<th>Proliferation</th>
<th>IFN-γ</th>
<th>TNF-α</th>
<th>IL-2</th>
<th>Cytotoxicity</th>
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<tbody>
<tr>
<td>Naïve CD8 cell</td>
<td>Effector CD8 cell</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Acute infection</td>
<td></td>
<td></td>
<td></td>
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</table>

Are the virus-specific T cell defects of chronic HBV infection reversible?

Effect of antigen decline

- Acute Self-limited Infection
  - Efficient T cell function/differentiation
  - Effector memory
  - Central memory

- Chronic infection
  - Inefficient T cell function/differentiation
  - Antigen persistence

- Antigen decline

Rapid Proliferation / Differentiation

Naïve CD8 cell

+Ag

Effector CD8 cell

Restored T cell function/differentiation
Effect of long-term NUC therapy on T cell responses
T cell restoration following long-term NUC treatment is efficient in vitro

![Graph showing percentage of CD8+ T cells producing IFN-γ, IL-2, and CD107a in different groups: Naive CHB, NUC treated HBsAg neg, NUC treated HBsAg pos, Acute hepatitis B (follow-up).]

Boni C. et al. Gastroenterology 2012
T cell restoration following long-term NUC treatment is partial ex vivo

Restoration of the T cell function is efficient in vitro but only partial ex vivo even following complete control of virus replication and decline of antigen.
List of topics

- Kinetics of T cell responses: from the early stages of infection to HBV control or persistence

- Additional mechanisms of T cell dysfunction in chronic HBV infection

- Effect of virus control on T cell responses in chronic patients

- Potential strategies to reconstitute the anti-viral T cell function

- Implications for future therapies
INTRAHEPATIC INHIBITORY MECHANISMS

Modified from U. Protzer et al. Nature Reviews in Immunology 2012
THE INTRAHEPATIC MILIEU IMPAIRS IL-2 PRODUCTION BY T CELLS

Myeloid suppressor cells + ARGINASE + L-arginine depletion

Hepatocytes

CD3ζ down-regulation

CD3ζ dependent impairment of IL2 production by intra-hepatic T cells

Das et al J.Exp.Med. 2008
INTRAHEPATIC INHIBITORY MECHANISMS

Modified from U. Protzer et al. Nature Reviews in Immunology 2012
MECHANISMS OF HEPATIC TOLERANCE: IMMUNOSUPPRESSIVE CYTOKINE MILIEU

Hepatocytes

SPECE OF DISSE

Sinusoidal endothelial cells

Dendritic cell

Kupffer Cell

Stellate Cell

Dendritic cell

TGF-β

IL-10

HEPATIC SINUSOID
THE IMMUNOSUPPRESSIVE CYTOKINE MILIEU CAN IMPAIR IFN-γ PRODUCTION BY NK CELLS LIMITING THEIR ANTI-VIRAL ACTIVITY

Preserved cytolytic activity

Dunn et al J.Exp.Med 2007
Peppa et al PloS Pathogens 2010
NK CELL MEDIATED DELETION OF HBV-SPECIFIC T CELLS

TRAIL-mediated T cell deletion

**INTRAHEPATIC INHIBITORY MECHANISMS**

- Bim mediates premature death of CD8 T cells following intrahepatic antigen presentation (Holtz et al Gastroenterology 2008)

*Modified from U. Protzer et al. Nature Reviews in Immunology 2012*
INTRAHEPATIC INHIBITORY MECHANISMS

Modified from U. Protzer et al. Nature Reviews in Immunology 2012
Expression of various inhibitory receptors on circulating and intrahepatic virus-specific CD8 cells of patients with chronic HBV infection
T CELL CO-INHIBITORY MOLECULES IN THE LIVER

PD-1 is up-regulated on HBV-specific T cells

Kupffer, LSEC and stellate cells express PD-L1
TIM-3 is up-regulated on HBV-specific T cells

Kupffer cells express galactine-9

T CELL CO-INHIBITORY MOLECULES IN THE LIVER
Expression of various inhibitory receptors on circulating and intrahepatic virus-specific CD8 cells of patients with chronic HBV infection

Fisicaro P. et al. Gastroenterology 2012
Bengsch et al. J. Hepatol. 2014

T cell restoration by Tim-3 blockade

Nebbia G. et al. Plos One 2012

T cell restoration by CTLA-4 blockade

Control
Anti-TIM-3
Anti-PD-L1

Raziorrouh B et al, Hepatology 2010
Schurich A et al. Hepatology 2011

T cell restoration by 2B4 blockade

T cell restoration by CTLA-4 blockade

p = 0.01

HBV-specific T cells

Genome-wide expression profiling

Mysregulated genes and pathways associated with T cell exhaustion

Correction strategies to restore anti-viral T cell functions
## TRANSCRIPTOME STUDY IN ACUTE AND CHRONIC HBV INFECTION

### Isolation of HBV/FLU-specific CD8+ T cells by cell sorting

### RNA extraction and amplification

### Gene expression by microarray analysis (4x44K Agilent)

### VALIDATION AND DISCOVERY OF NEW TARGETS

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<thead>
<tr>
<th>Patient</th>
<th>Infection</th>
<th>LT</th>
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<tbody>
<tr>
<td>1</td>
<td>ACUT E</td>
<td>785</td>
</tr>
<tr>
<td>2</td>
<td>ACUT E</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
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<td>118</td>
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<tr>
<td>5</td>
<td>ACUT E</td>
<td>11</td>
</tr>
<tr>
<td>1</td>
<td>RESO LVED HEP B</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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<tr>
<td>CONTROLS</td>
<td>CELL SPECIFICITY</td>
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</tr>
<tr>
<td>1</td>
<td>THY HEAL LU</td>
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</tr>
<tr>
<td>2</td>
<td>THY HEAL LU</td>
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</tr>
<tr>
<td>3</td>
<td>THY HEAL LU</td>
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</tr>
</tbody>
</table>
A deep metabolic and energetic impairment is typical of exhausted T cells. Multiple levels of correction will certainly be needed to restore an efficient anti-viral T cell function. Is restoration of an efficient anti-viral T cell function an achievable objective?
Potential strategies to reconstitute the anti-viral T cell function and implications for future therapies
Clinical needs in HBV therapy for CH-B:

to shorten NUC therapy by accelerating HBsAg clearance
SEQUENCIAl NUC/IFN-α THERAPY
Potential strategy to to shorten NUC therapies

Modified from: Ferrari C. Gastroenterology 2008
EFFECT OF ANTI-PD-1 THERAPY ON HCV INFECTED CHIMPANZEEES

Effect on viral load

Effect on magnitude of T cell responses

Fuller MJ et al. PNAS 2013
PD-1 PATHWAY BLOCKADE

*Proof of concept of α-PD-1 in Chronic HCV*

- Blinded, PBO controlled, SAD study
- α-PD-1 in 54 HCV infected patients, IFN failures and treatment naive
- 0.03mg/kg -10mg/kg
- 3 subjects w/ > 4 log HCV RNA decline: All 3 received 10mg/kg dose
  - 1 subject (A) had isolated, transient Grade 4 ALT increase to ~17x ULN
- 1 subject (B) undetectable > 1 year post treatment

SEQUENTIAL NUC/IFN-α THERAPY
Potential strategy to optimize IFN-α efficacy and to shorten NUC therapies

Modified from: Ferrari C. Gastroenterology 2008
SEQUENCIAL NUC/IFN-α THERAPY
Potential strategy to optimize IFN-α efficacy and to shorten NUC therapies

- NUC treatment
-Decline of antigen load

Antigen load

T CELL DYSFUNCTION

RECOVERY OF T CELL RESPONSIVENESS

HBsAg CLEARANCE

ANTI-HBs SEROCONVERSION

SPECIFIC VACCINES
- Recombinant (Gilead)
- DNA (Transgene)
- Peptides (Immune Targeting System)

CD4

CD4

CD8

CTL

IFN-γ

IL-2

Proliferation

-/+     ++     ++     ++

Modified from: Ferrari C. Gastroenterology 2008
Synergistic effect of PD-L1 blockade and therapeutic vaccination on T cell responses and viral control

*(Liu J. et al. PLOS Pathogens 2014)*

T cell immunity

WHA replication

![Graphs showing T cell immunity and WHA replication](Image)
Synergistic effect of PD-L1 blockade and therapeutic vaccination on T cell responses and viral control

(Ha S-J. et al. J. Exp. Med. 2008)
SEQUENCIAL NUC/IFN-α THERAPY
Potential strategy to optimize IFN-α efficacy and to shorten NUC therapies

Modified from: Ferrari C. Gastroenterology 2008
TLR8 agonists can trigger potent activation of innate immune cells in human liver

J. Jo et al. PLOS Pathogens 2014
SEQUENTIAL NUC/IFN-α THERAPY
Potential strategy to optimize IFN-α efficacy and to shorten NUC therapies

Modified from: Ferrari C. Gastroenterology 2008
FUTURE POTENTIAL IMMUNE MODOLATORY STRATEGIES TO TREAT HBV INFECTION

NUC therapy
Decline of antigen load
Blockade of inhibitory pathways
T cell stimulation

Virus/antigen load

T cell functional efficiency

Full T cell exhaustion → Partial T cell exhaustion → Partial T cell restoration
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Laboratory Viral Immunopathology
Unit Infectious Diseases and Hepatology
Azienda Ospedaliero-Universitaria di Parma, Italy

A. Bertoletti
Singapore Institute for Clinical Sciences, A*STAR, Singapore

P. Lampertico
M. Colombo
University of Milano, Italy

M. Levrero
La Sapienza University
Rome, Italy