How does HBV interact with the immune system?

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Course of HBV

Acute HBV

95%
Elimination

5%
Persistence

Chronic hepatitis B
Components of immune responses against HBV

- Dendritic cells
- NK cells
- CD4+ T cells
- CD8+ T cells
- Hepatocytes
- Immunoregulatory cytokines (IL10, TGFβ)
- Innate immunity (IFNα)
- NK cells
- Treg
CD8+ T-cell responses in HBV infection

Temporal association of CD8+ T-cell response and viral load

Intrahepatic accumulation of CD8+ T-cell responses during viral clearance

Depletion of CD8+ T cells prolongs viremia

Maini, Bertoletti Gastro 2001
Boettler, Thimme, J Virol 2005

Thimme, Chisari, J Virol 2003
Function of CD8+ T cells in HBV

cytolytic mechanisms

Ch.1620

Thimme et al. J. Virol. 2003
Identification of a novel immunological cell culture model

Untreated control  Direct co-culture  Transwell cultures

HBV infected hepatoma cell

CD8+ T cell  core18-27 peptide

stimulation
Reduction of viral loads requires cell killing

\[ \Delta t = 96 \text{ h} \]
\[ E: T = 1:1 \]

- cytoplasmic HBV DNA
- AST

untreated vs. direct
Reduction of viral loads requires cell killing

$\Delta t = 96\ h$
$E:T = 1:1$

Strong reduction (95%) of viral loads requires:

- Direct cell-cell contact
- Cytotoxic effector mechanisms

Function of CD8+ T cells in HBV

- Granzyme
- Perforin

CD8+ H

IFN-γ
TNF-α

cytolytic
non cytolytic
Immune responses in chronic HBV infection

CD8+ T-cell failure

- Viral escape
- Immunoregulatory cytokines (IL10, TGFβ)
- Exhaustion

- Priming
- Antigen
- TCR

- CD8+ T cells
- CD4+ T cells
- NK cells
- Treg cells
Immune responses in chronic HBV infection

**CD8+ T-cell failure**

- Priming
- Viral escape
- Antigen
- TCR
- CD8+ T cells
- Exhaustion
- CD4+ T cells
- NK cells
- Treg cells
- Immunoregulatory cytokines
  - IL10
  - TGFβ
Fate of virus-specific CD8+ T cells in acute and chronic infections

**Acute:**
- **Effector:** short-lived, antigen-dependent, polyfunctional

**Chronic:**
- **Memory:** long-lived, antigen-independent, polyfunctional
- **Exhausted:** short-lived, antigen-dependent, functionally exhausted

**Heterogeneous populations - lessons from mouse model:**
- **Tbet^{dim}Eomes^{hi} / Tbet^{hi}Eomes^{dim}**
  (Paley, Science 2012)
- **TCF1+** memory-like
  (Utzschneider, Nature Immunology 2013)
- **CXCR5+** progenitor
  (Im & He, Nature 2016)
Heterogeneity of virus-specific CD8+ T-cell populations in humans: chronic HBV and HCV infection.
Patient cohorts

**cHBV (n=12):**
- 7 inactive carriers
  - treatment naive
  - low viral load: 779 IU/ml
    (136 – 2149)
  - no cirrhosis
- 5 patients with NUC therapy
  - low viral load: 19 IU/ml
    (<10 – 33)
  - no cirrhosis

**cHCV (n=8):**
- HCV genotype 1
- Viral load: 3213771 IU/ml
  (42241 – 15x10^6)
- no cirrhosis
Magnetic bead enrichment of virus-specific CD8+ T cells

Enhanced resolution and potential for in-depth analysis of rare T-cell populations

Alanio et al., Blood 2010
Schmidt et al., J Virol 2011
Nitschke et al., J Virol 2015
Magnetic bead enrichment of virus-specific CD8+ T cells

Enhanced resolution and potential for in-depth analysis of rare T-cell populations

Alanio et al., *Blood* 2010
Schmidt et al., *J Virol* 2011
Nitschke et al., *J Virol* 2015
HBV-specific CD8+ T cells lack terminal exhaustion markers

**PD1**

- **Eomes**
- **CD39**

**PD1**

- **Eomes**
- **CD39**

**HBV**

- inactive carriers
- patients with NUC therapy

**HCV**

- NUC therapy
- HCV

**HBV**

- MFI PD1
- % Eomes high
- % CD39+

**HCV**

- MFI PD1
- % Eomes high
- % CD39+
HBV-specific CD8+ T cells are predominantly CD127+PD1+

- CD127+PD1+ subset dominates in HBV-specific CD8+ T-cell populations

HBV: inactive carriers
HBV: patients with NUC therapy
HCV
Two subsets of exhausted virus-specific CD8+ T cells in HCV but not HBV

Fate of terminally exhausted CD8+ T cells after antigen elimination?

T Cell Factor 1-Expressing Memory-like CD8+ T Cells Sustain the Immune Response to Chronic Viral Infections

Immunity 2016
DAA mediated antigen removal leads to disappearance of terminally exhausted HCV-specific CD8+ T cells

Dominik Wieland, in revision
The memory marker TCF1 determines proliferative capacity of virus-specific CD8+ T cells

High proliferative capacity of HBV-specific CD8+ T cells correlates with increased TCF1 expression
Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy

Se Jin Im1, Masao Hashimoto1, Michael Y. Gerner2,3, Junghwa Lee1, Haydn T. Kissick1,4, Matheus C. Burger5, Qiang Shan6, J. Scott Hale1, Judong Lee1, Tahseen H. Nasti1, Arlene H. Sharpe7,8, Gordon J. Freeman9, Ronald N. Germain1, Heider I. Nakaya5, Hai-Hui Xue6,10 & Rafi Ahmed1

Nature 2016
Research Article

Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation

Bertram Bengsch1,2,3, Bianca Martin1,2,3, Robert Thimme1,4

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→ PD-L1 blockade has the strongest effect
T-cell subpopulations in chronic HBV and HCV infection

**chronic HCV**
- Less differentiated/memory-like
- Terminal differentiated/exhausted

**Antigen recognition**

**chronic HBV**
- Less differentiated/memory-like
- Terminal differentiated/exhausted

**Determinant?**
Characteristics of T-cell subpopulations in chronically HBV-infected patients with high VL

- Lack of terminally exhausted HBV-specific CD8+ T cells is not due to absent antigen.

**VL: 30 405 IU/ml**
Immune responses to HBV

CD8+ T-cell failure

priming

viral escape

antigen

TCR

CD8+ T cells

CD4+ T cells

NK cells

Treg cells

immunoregulatory cytokines

IL10

TGFβ

exhaustion
NK cells in chronic viral hepatitis

**NK cell functional dichotomy in cHBV**
- response to cytokine stimulation \(\downarrow\downarrow\)
- response to antibody-coated target cells \(\uparrow\)

**Oliviero et al., Gastroenterology 2009**
NK cells in chronic viral hepatitis

**NK cell functional dichotomy in cHBV**

- response to cytokine stimulation ↓↓
- response to antibody-coated target cells ↑

**Increased frequencies of NKG2C+ NK cells in chronic viral hepatitis**

→ linked to CMV seropositivity

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Oliviero et al., Gastroenterology 2009
Béziat et al., EJI 2012
Conventional vs. adaptive NK cells

**conventional NK cell**
- ADCC $\uparrow$
- IFN$\gamma$ $\uparrow$
- CD16
- Helios
- PLZF
- cytokine responsiveness $\uparrow\uparrow$

**adaptive NK cell**
- ADCC $\uparrow\uparrow$
- IFN$\gamma$ $\downarrow$
- CD16
- Helios $\downarrow$
- PLZF $\downarrow$
- NKG2C $\uparrow$
- cytokine responsiveness $\downarrow$

$\rightarrow$ **epigenetic regulation**

Lee J et al., Immunity 2015
Schlums H et al., Immunity 2015
Aim of Study

Is altered NK cell function in cHBV patients linked to an expansion of adaptive NK cell subsets?
Study approach

Patient cohorts

cHBV (n=21):
• no cirrhosis
• age: 41 (18 – 68)
• viral load: 8657512 IU/ml (0 – 1.64x10^8)
• ALT: 43 IU/ml (11 – 304)
• treatment: 16 naïve, 5 NUC

HD (n=30):
• age: 42 (24 – 63)

Methods

Multiparametric flow cytometry
• Phenotypic analyses
• Functional analyses of cytokine production and degranulation after
  – cytokine stimulation with IL-12/IL-15 and IL-12/IL-18
  – CD16 crosslinking (plate-bound)

Epigenetic analyses (EpigenDx)
• FCER1G promoter methylation
• IFNG promoter methylation
Downregulation of FcεRιγ in CD56dim NK cells of cHBV patients

Adaptive NK cells lack FcεRιγ expression

Lee et al., Immunity 2015
Schlums et al., Immunity 2015

%FcεRιγ+

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HD cHBV

Adaptive NK cell

NKG2C↑
Helios↓
PLZF↓

ADCC↑↑

IFNγ↓
Downregulation of FcεRIγ in CD56dim NK cells of cHBV patients

Adaptive NK cells lack FcεRIγ expression

Lee et al., Immunity 2015
Schlums et al., Immunity 2015

%FcεRIγ+

HCMV: pos neg pos neg

HD  cHBV
Downregulation of FcεRIγ in CD56dim NK cells of cHBV patients

Adaptive NK cells lack FcεRIγ expression

Lee et al., Immunity 2015
Schlums et al., Immunity 2015

%FcεRIγ+
FcεRIγ deficiency in CD56dim NK cells of CMV+ cHBV patients correlates with other protein downregulations

Transcriptional regulation of adaptive FcεRIγ- NK cells

Lee et al., Immunity 2015
Schlums et al., Immunity 2015

Helios is down-regulated in FcεRIγ- NK cells in chronic HBV
FcεRιγ deficiency in CD56dim NK cells of CMV+ cHBV patients correlates with other protein downregulations

Transcriptional regulation of adaptive FcεRιγ- NK cells

Lee et al., Immunity 2015
Schlums et al., Immunity 2015

Helios is down-regulated in FcεRιγ- NK cells in chronic HBV

% FcεRιγ/Helios subsets

CD56dim NK cells of HCMV+ cHBV
Are these FcεR1γ-Helios- CD56dim NK cells in CMV+ cHBV patients adaptive NK cells?

Analysis of the master regulator PLZF

Transcriptional regulation of adaptive FcεR1γ- NK cells

Lee et al., Immunity 2015
Schlums et al., Immunity 2015

%PLZF+

conventional NK cells

adaptive NK cells

PLZF
Are these FcεR1γ-Helios- CD56dim NK cells in CMV+ cHBV patients adaptive NK cells?

Functional analysis (cytokine responsiveness)

cytokine stimulation

IL-12/IL-15

IL-12/IL-18

CD16 crosslink
CMV-induced adaptive NK cells are present in the majority of cHBV patients

**CMV-seropositive donors**

<table>
<thead>
<tr>
<th></th>
<th>HD:</th>
<th>80%</th>
<th>20%</th>
<th>cHBV:</th>
<th>30%</th>
<th>70%</th>
</tr>
</thead>
</table>

- HD: CMV-seropositive donors
- cHBV: CMV-seropositive donors
CMV-induced adaptive NK cells are present in the majority of cHBV patients.
CMV-induced adaptive NK cells are present in the majority of cHBV patients.

CMV-seropositive donors

HD: 80% 20%

C HBV: 30% 70%

Functional dichotomy:

- Increased/conserved cytotoxicity
- Decreased IFNγ production

Cytokine responsiveness:

- IL-12/IL-15/IL-18

IFNγ ↑↑
Novel insights into immune responses against HBV

CD8+ T cells

- memory-like
- TCF1\textsuperscript{high}
- promising target for PD1 blockade
- determinant?

NK cells

- expansion of epigenetically regulated adaptive NK cells
- driving force?
- cross-talk with CD8+ T cells?
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