Rationale for Modulating Adaptive Immunity: Therapeutic Vaccination, Checkpoint Inhibitor and others

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Rationale for modulating T cell responses in viral hepatitis

Viral elimination

HBV: >95%

Acute hepatitis

HBV: <5%

Virus-specific CD8+ T cell

 Chron. hepatitis

20-30% in 20-30 years

Fibrosis / cirrhosis

1-5% per year

HCC
CD8+ T-cells are the main effector cells against HBV

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

Depletion of CD8+ T-cells prolongs viremia

Role of HBV-specific CD8+ T cells in strategies aiming at virological cure of chronic HBV infection!
Questions

• Are HBV specific CD8+ T cells present during chronic HBV infection?

• What are their phenotypical and functional characteristics?

• Best approach for T cell restoration?
Methods

**Patient cohort 1**
- Chronic HBV infection, genotype D (n=70)
  - mainly low viral loads
  - mainly HBeAg negative
- Resolved HBV infection (n=11)
- Acute HBV infection (n=2)

Rehermann/Thimme, Gastroenterology 2018, in press
Methods

Patient cohort 1
• Chronic HBV infection, genotype D (n=70)
  - mainly low viral loads
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• Resolved HBV infection (n=11)
• Acute HBV infection (n=2)

Experimental approach 1

Stimulation of PBMCs with 223 overlapping peptides (18-mers, genotype D)
• 20% of PBMC loaded with peptide x 1 hour, washed
  • 80% of PBMC added
  • Culture for 10 days

Screen for responses with the overlapping peptides using Elispot

Confirmation of positive responses by intracellular IFN-γ staining using single peptides

HLA-restriction using EBV-transformed B-cells (B-LCL)
Epitope fine-mapping
70 patients with chronic HBV infection (genotype D): 59 CD8+ responses

Functional HBV-specific CD8+ T-cell responses are present in ~50% of chronically infected patients

Patient 7

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Without Peptide</th>
<th>With Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLP175 LIISCSCPTVQASKLCL</td>
<td>3.9%</td>
<td>10%</td>
</tr>
<tr>
<td>OLP51 IPRTPARVTGGVFLVDKN</td>
<td>1.4%</td>
<td>2.1%</td>
</tr>
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</table>
Breadth of CD8+ T-cell responses in chronic infection

Lack of HBsAg-specific responses in chronic infection

► Differential targeting compared to acute-resolving infection?
Breadth of CD8+ T-cell responses in resolved versus chronic infection

- Chronic HBV infection (n=70)
- Resolved HBV infection (n=11)
Breadth of CD8+ T-cell responses in acute / resolved versus chronic infection

Lack of HBsAg-specific responses is unique to persistent infection!
Are HBsAg specific CD8+ T cells really absent or undetectable by functional assays?

<table>
<thead>
<tr>
<th></th>
<th>core&lt;sub&gt;18&lt;/sub&gt;</th>
<th>pol&lt;sub&gt;455&lt;/sub&gt;</th>
<th>env&lt;sub&gt;183&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>0.35%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>enrichment</td>
<td>7.92%</td>
<td>1.22%</td>
<td>0.13%</td>
</tr>
</tbody>
</table>

Core<sub>18</sub>- and pol<sub>455</sub>- but not env<sub>183</sub>-specific CD8+ T cells are detectable in the majority of analyzed patients.
Summary I

- HBV specific CD8+ T cell present at low frequencies in most patients
- Specific lack of HBsAg specific CD8+ T cells in chronic infection
Questions

• Are HBV specific CD8+ T cells present during chronic HBV infection?

• What are their phenotypical and functional characteristics?

• Best approach for T cell restoration?
Exhausted CD8+ T cells

Expression of exhaustion markers (e.g. PD1$^{\text{high}}$, CD39$^{+}$)

Functionally impaired (e.g. cytokines, proliferation)

Impaired T-cell homeostasis (e.g. CD127$^{\text{low}}$)

Eventually depleted by apoptosis

Heterogeneous populations

References:
Moskophidis, Zinkenagel Nature 1993
Gallimore, Zinkernagel, JEM 1998
Zajac, Ahmed JEM 1998
Paley, Wherry Science 2012
Doering, Wherry, Immunity 2013
Gupta, Haining PloS Path 2015
Utzschneider, Zehn Immunity 2016
Im, Ahmed Nature 2016
Bengsch, Wherry, Immunity 2018
Are exhausted HBV-specific CD8+ T cells comprised of different subsets?

What is meant by different subsets?

HCV-specific CD8+ T cells

Phenotypic/functional analyses

Memory-like phenotype

- TCF1+
- CD127+
- BCL2\text{high}
- PD1+
- Eomes\text{lo} / T-bet\text{lo}

Associated with expansion capacity in vitro

Severely exhausted

- TCF1-
- CD127-
- BCL2\text{+}
- PD1\text{high}
- Eomes\text{high} / T-bet\text{lo}
- CD39+
Loss of terminally exhausted HCV-specific CD8+ T cells after antigen elimination

Wieland et al., Nat Commun 2017

Maintenance of a memory-like T-cell population
• Antigen-independent survival
• Increased functionality
Loss of terminally exhausted HCV-specific CD8+ T cells after antigen elimination

Rehermann/ Thimme, Gastroenterology 2018, in press
Are HBV-specific CD8+ T cells comprised of different subsets?

CD127+ PD1+ TCF1++
CD127- PD1+ Eomes\textsuperscript{hi} / T-bet\textsuperscript{lo}

HCV specific CD8+ T cells are more exhausted compared to HBV
Cor and polymerase show phenotypical differences
Central role for Tox in T cell exhaustion

Central role for Tox in virus-specific T cells in chronic vs acute infection

High Tox expression in exhausted T cell subsets

LCMV

Doering/Wherry Immunity 2013

Bengsch/Wherry Immunity 2018
Different Tox expression in HBV versus HCV

TOX expression also reveals less severe exhaustion of HBV-specific CD8+ T cells
Differences between cor and pol-specific CD8+ T cell responses

Expansion of core\textsubscript{18/141} but not of pol\textsubscript{455/173}-specific CD8+ T-cell populations is linked to the memory-like subset
What are the differences of memory-like core18/141- vs. pol455/173-specific CD8+ T cells?

Similar TCF1 expression in memory-like core18/141- and pol455/173-specific CD8+ T cells
What are the differences of memory-like core18/141- vs. pol455/173-specific CD8+ T cells?

Decreased BCL2 expression of memory-like pol\textsubscript{455/173}-specific CD8+ T cells...

...correlates with the decreased expansion capacity.
HBV-specific CD8+ T cells are heterogeneous

- consisting of distinct subsets:

  - Memory-like phenotype

  - Severely exhausted

- differing with respect to their targeted epitopes:

<table>
<thead>
<tr>
<th></th>
<th>Core$_{18/141}$</th>
<th>Pol$_{355/173}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>expansion</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>severely exhausted</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>memory-like</td>
<td>TCF1↑BCL2↑</td>
<td>TCF1↑BCL2↓</td>
</tr>
</tbody>
</table>

Schuch et al., GUT 2018, in press

Relevance? – immunotherapies targeting T cells
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Strategies to boost HBV-specific T cells

**T cell boosting**
- Vaccine therapy
- Inhibition inhibitory signals (e.g., anti-PD-1)

**T cell engineering**
- Engineering HBV-T cells

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*BERTOLETTI/FERRARI, GUT 2012*

*BERTOLETTI, GUT LIVER 2017*
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Epitope specific CD8+ T cell failure may lead to specific therapeutic strategy
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Hepato-Regio-Net
Thank you for your attention!

Impressions from Freiburg in the black forest!