High resolution of exhausted HBV specific CD8+ T cells in chronic HBV infection

Robert Thimme

Clinic for Gastroenterology, Hepatology, Infectious Diseases and Endocrinology
University of Freiburg
Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B

Fisicaro et al, Nature Med 2017
Characteristics of exhausted CD8+ T cells

- High expression of inhibitory receptors (e.g. PD1\textsuperscript{high})
- Impaired T-cell homeostasis (e.g. CD127\textsuperscript{low})
- Distinct transcriptional signature
- Distinct epigenetic profile
- Functionally impaired (e.g. cytokines, proliferation)
- Altered metabolism
- Eventually depleted by apoptosis

Relevant in defining therapeutic targets

Moskophidis, Nature 1993
Galimore, JEM 1998
Zajac, JEM 1998
Wherry, J Virol 2003
Wherry, Immunity 2007
Paley, Science 2012
Doering, Immunity 2012
Buggert, PloS Path 2014
Utzschneider, Immunity 2016
Im, Nature 2016
Fisicaro, Nat. Med. 2017
Bengsch, Immunity 2017/2018
Wieland, Nat. Communication 2016
Subsets of exhausted virus-specific CD8+ T cells

What is meant by different subsets? - Lessons from HCV

HCV-specific CD8+ T cells

Phenotypic/functional analyses

- Memory-like phenotype
  - TCF1+
  - CD127+
  - BCL2\text{high}
  - KLRG1+
  - PD1+
  - Eomes\text{lo} / T-bet\text{lo}
  - associated with expansion capacity in vitro

- Severely exhausted
  - TCF1-
  - CD127-
  - BCL2+
  - KLRG1+
  - PD1\text{high}
  - Eomes\text{high} / T-bet\text{lo}
  - CD39+
  - TOX

Wieland et al., Nat Commun 2017
Alfei et al, Nature 2019
Heterogeneity of HCV specific CD8+ T cells by single cell RNA sequencing

Differentiation pathway:
memory-like → intermediate → terminally exhausted
Fate of HCV specific CD8+ T cells after DAA elimination

**Chronic HCV infection**
- CD127+ PD1+
- HCV

**DAA-mediated HCV clearance**
- CD127− PD1−
- HCV

Antigen removal

**Maintenance of a memory-like T-cell population**
- With remaining chronic signature
- With specific gene regulation

Wieland et al., Nat Commun 2017
Fate of HCV specific CD8+ T cells after DAA elimination

Quadratic programming
HCV-specific CD8+ T cells after versus before DAA therapy (measure of similarity)

Memory-like cells before and after DAA therapy are quite similar → maintenance?!
Fate of HCV specific CD8+ T cells after DAA elimination

Maintenance of a memory-like T-cell population
- Antigen-independent survival
- Increased functionality
- Recall expansion

Rehermann/Thimme, Gastroenterology 2019
HCV specific CD8+ T cells display chronic scar after DAA elimination

Low input RNAseq - unsupervised clustering of CD127/PD1 subsets

Cluster 1

Cluster 2

conventional memory

severely exhausted memory-like

During and after chronic HCV infection

conventional memory

Also different subsets in HBV infection?
Are HBV-specific CD8+ T cells comprised of different subsets?

Memory-like subsets dominate HBV-specific CD8+ T-cell populations with different frequencies depending on the targeted epitope.
Epitope specific differences in HBV

HBV-specific CD8+ T cells are heterogeneous

- consisting of distinct subsets:

- 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 2019
Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load

Anita Schuch,1,2,3 Elahe Salimi Alizei,1,2,4 Kathrin Heim,1,2,3 Dominik Wieland,1,2 Michael Muthamia Kiraithe,1,2 Janine Kemming,1,2,3 Sian Llewellyn-Lacey,5 Özlem Sogukpinar,1,2 Yi Ni,6 Stephan Urban,6,7 Peter Zimmermann,1,2,3 Michael Nassal,1,2 Florian Emmerich,8 David A Price,3 Bertram Bengsch,1,2 Hendrik Luxenburger,1,2 Christoph Neumann-Haefelin,1,2 Maike Hofmann,1,2 Robert Thimme1,2

Phenotype and function of HBV-specific T cells is determined by the targeted epitope in addition to the stage of infection

Ruben C Hoogeveen,1,2 Maxwell P Robidoux,1 Tatjana Schwarz,3 Laura Heydmann,4 James A Cheney,1 Daniel Kvistad,1 Jasneet Aneja,1 Juliana G Melgaço,5 Carlos A Fernandes,6 Raymond T Chung,1 Andre Boonstra,2 Arthur Y Kim,7 Thomas F Baumert,4 Jörg Timm,3 Lia L Lewis-Ximenez,5 Pierre Tonnerre,1 Georg M Lauer1

HBV antiviral immunity: not all CD8 T cells are born equal

Antonio Bertoletti,1,2 Patrick T F Kennedy3
How different are core- and pol-specific CD8+ T cells?

Single cell RNAseq

core\textsubscript{18}-specific CD8+ T cells
pol\textsubscript{455}-specific CD8+ T cells

core- and pol-specific CD8+ T cells show different transcriptomes
Transcriptomic profiles of core- versus pol-specific CD8+ T cells

Single cell RNAseq – compiled data of 4 patients

Transcriptomes of core- and pol-specific CD8+ T cells are clearly distinct
Differentiation status of core- and pol-specific CD8+ T cells

core-specific CD8+ T cells

pol-specific CD8+ T cells

→ precursor CTL differentiation

→ terminal CTL differentiation
TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection

Francesca Allei1, Kristiyan Kanev1, Maike Hofmann1, Ming Wu1, Hazem E. Ghoneim1,2, Patrick Roelli1,4,5, Daniel T. Utschneider6, Madlaine von Hoesslin7, Jolie G. Cullen1, Yiping Fan1, Vasily Eisenberg1, Dirk Wohlleber4, Katja Steiger10, Doron Merkler11, Mauro Delorenzi4,5, Percy A. Knolle7, Cyrille J. Cohen8, Robert Thimme2,8, Benjamin Youngblood1,8 & Dietmar Zehn1

TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion

Omar Khan1,2,3,4, Josephine R. Giles1,2,3, Sierra McDonald1,2,3, Sasikanth Mann1,2, Shin Foong Ngio1,2,3, Kunal P. Patel1,2,7, Michael T. Werner4,6, Alexander C. Huang2,3,2, Katherine A. Alexander2,3,6, Jennifer E. Wu1,2,3,1, John Attanasio2,1, Patrick Yan1,3, Sangeeth M. George1,2, Bertram Bengsch2,3,4, Ryan P. Staupe2,3, Greg Donahue1,2,3, Wei Xu4,5, Ravi K. Amaravadi1,3, Xiaowei Xu1,6, Giorgos C. Karakousis1,2,1, Tara C. Mitchell2,1, Lynn M. Schuchter3,2, Jonathan Kaye3,2, Shelley L. Berger4,5,6 & E. John Wherry1,2,3

Nature 2019
Is there an exhaustive signature in core- and pol-specific CD8+ T cells?

→ low TOX expression...

...is associated with epitope

...is associated with clinical state
Summary

High resolution of exhausted virus-specific CD8+ T cells in chronic HBV and HCV infection reveals:

• Heterogeneity of HCV specific CD8+ T cells linked to antigen recognition

• Differential subset distribution in HBV versus HCV with lack of terminally exhausted CD8+ T cells in HBV

• HBV specific CD8+ T cells differ in transcriptional, phenotypical and functional profile on an antigen-specific level (core versus pol)

• Impact of clinical state (HbeAg, viral load) on TOX expression of HBV specific CD8+ T cells

Complex immunological and virological determinants of virus-specific CD8+ T cell exhaustion/ differentiation in chronic HBV
Acknowledgements

Department of Medicine II - University of Freiburg

Maike Hofmann
Franziska Daul
Katharina Dörnbrack
Katharina Grützmann
Katharina Dörnbrack
Katharina Grützmann
Kathrin Heim
Nina Hensel
Sebastian Merker
Laura Polcik
Anita Schuch
Özlem Sogukpınar
Catrin Tauber
Dominik Wieland
Elisa Wüstrich
Sebastian Zehe

Christoph Neumann-Haefelin
Janine Kemming
Valerie Oberhardt
Elahe Salimi Alizei
Isabel Schulien

Tobias Böttler
Benedikt Csérmalabics
Sabine Ehrlich
Rafael Käser
Saskia Killmer
Maike Smits
Katharina Wild
Katharina Zoldan

Nico Büttner
Peter Hasselblatt
Anna Globig

Bertram Bengsch
Patricia Otto-Mora
Frances Winkler
Zheng Zhang

Ralf Bartenschlager
Naveed Ishaque / Zuguang Gu
Dominic Grün / Sagar
Christian Konrad / Katharina Jechow

All patients and volunteers!
Thank you for your attention!

Impressions from Freiburg in the black forest!