Mechanisms of exhaustion of B and T cells and path for functional recovery

Department of Medicine II

Gastroenterology, Hepatology, Endocrinology, and Infectious Diseases

Medical Center – **University of Freiburg, Germany**

Possible disclosures : Roche, Falk Foundation e.V., Dr. Falk Pharma GmbH, med publico GmbH, Gilead, GlaxoSmithKline Research & Development Ldt., GUT, Innovationsausschuss beim G-BA, SCG Cell Therapy Pte Ltd. Singapore, LION TCR Pte., Thieme Verlag, OPASCA, Mannheim, Topas Therapeutics GmbH, F. Hoffmann-La Roche Ltd, Janssen Global Serivces, LLC, Fishawack UK

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RB-RNA

ICE-HBV
International Coalition to Eliminate HBV

IP-cure-B

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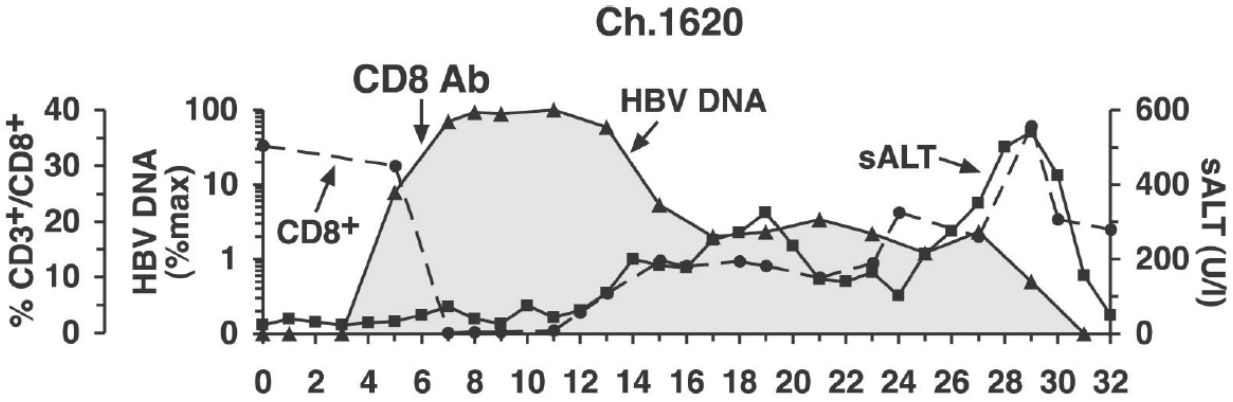
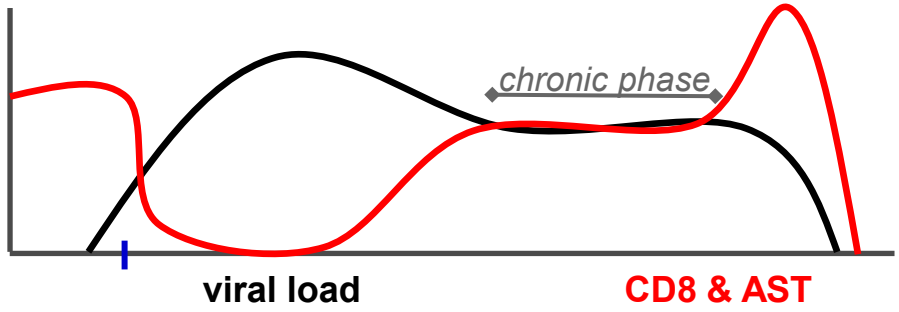
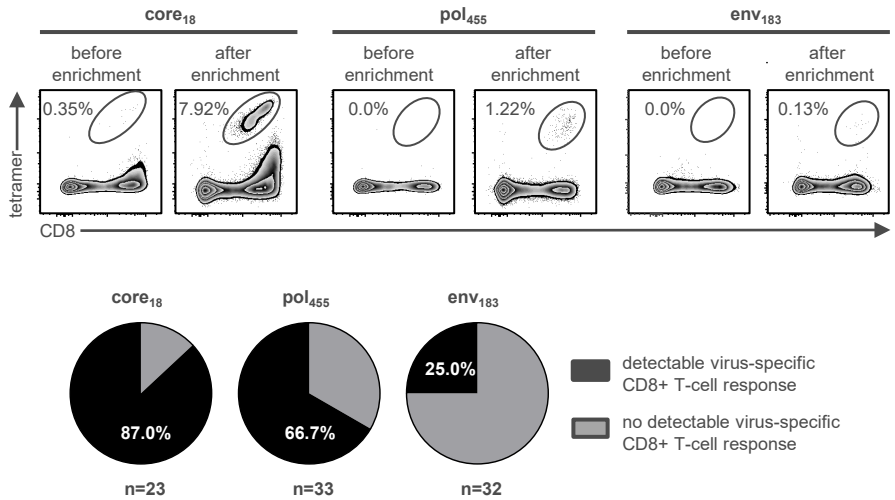
Function of CD8+ T cells



Thimme *et al*. J. Virol. 2003



HBV-specific CD8+ T-cell responses during chronic infection



OPEN ACCESS

ORIGINAL ARTICLE

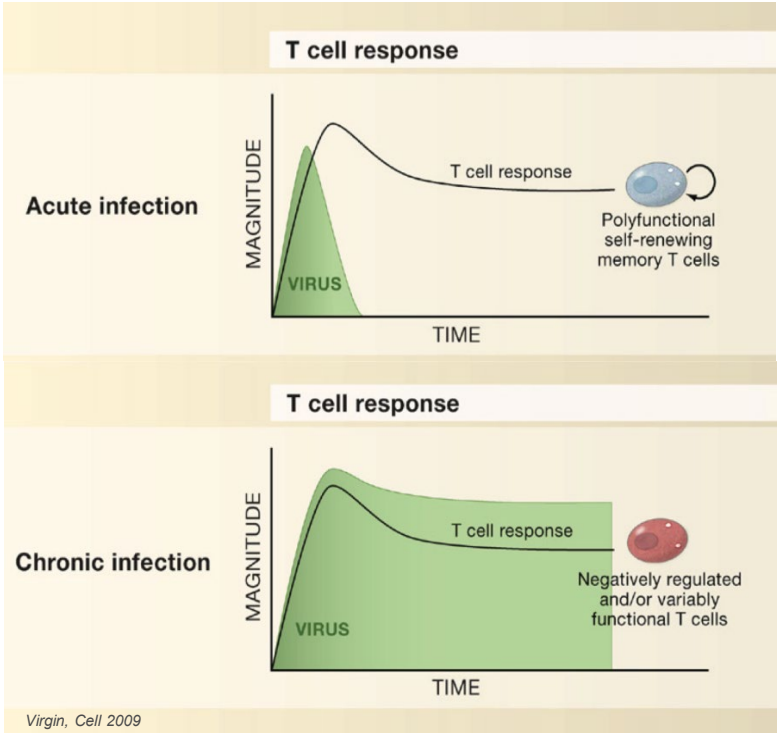
Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load

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,[5] Bertram Bengsch,[1,2] Hendrik Luxenburger,[1,2] Christoph Neumann-Haefelin,[1,2] Maike Hofmann,[1,2] Robert Thimme[1,2]

- Lack of HBsAg specific CD8+ T cell responses with increasing duration of infection
- Mechanism unknown

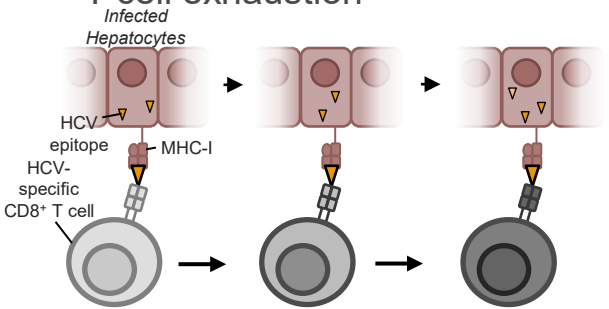
Schuch et al, GUT 2019

What is T cell exhaustion ? – virus-specific T cell responses



T cell effector/memory response

T cell exhaustion



- counteracting immunopathology
- maintaining the T cell response

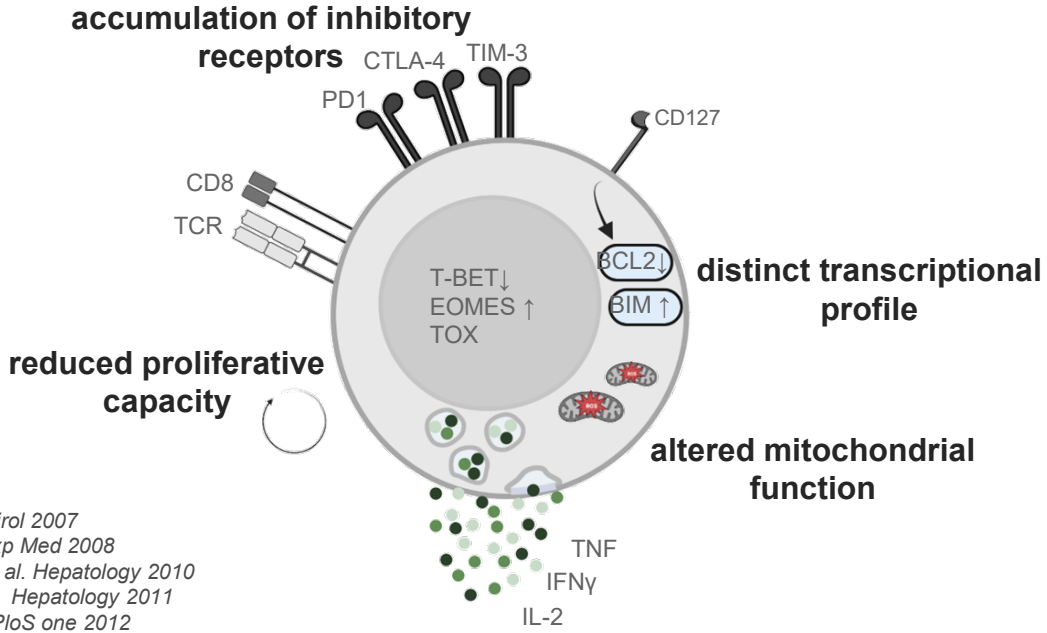
dysfunction

≠ non-functional

→ T cell exhaustion = T cell response in the context of persisting antigen

Is chronic viral infection strictly linked to T cell exhaustion?

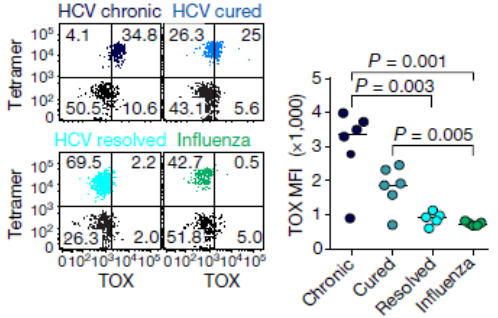
Evidence for T cell exhaustion in chronic HBV



Boni et al. J Virol 2007
Das et al. J Exp Med 2008
Raziorrouh et al. Hepatology 2010
Schurich et al. Hepatology 2011
Nebbia et al. PloS one 2012
Lopes et al. J Clin Invest 2008
Bengsch,et al, J Hepatol 2013
Kurktschiev et al. J Exp Med 2014
Schurich et al. Cell Rep 2016
Fisicaro et al. Nat Med 2017
Heim et al., Gut 2019

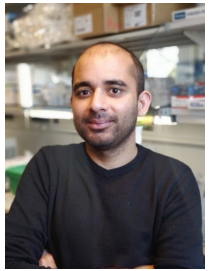
TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection

Francesca Alfei, Kristiyan Kanev, Maike Hofmann, Ming Wu, Hazem E. Ghoneim, Patrick Roelli, Daniel T. Utzschneider, Madlaina von Hoesslin, Jolie G. Cullen, Yiping Fan, Vasyl Eisenberg, Dirk Wohlleber, Katja Steiger, Doron Merkler, Mauro Delorenzi, Percy A. Knolle, Cyrille J. Cohen, Robert Thimme, Benjamin Youngblood & Dietmar Zehn



Nature 2019

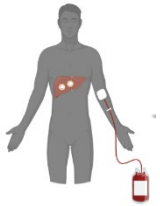
Research question



Are HBV specific CD8+ T cells really exhausted?

Chronic HBV

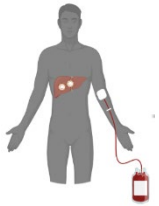
HBeAg- chronic HBV infection



- normal liver enzymes
- low VL

➢ *endogenous viral control*

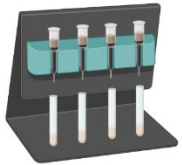
HBeAg- chronic hepatitis



- elevated liver enzymes
- high VL → NUC → low VL

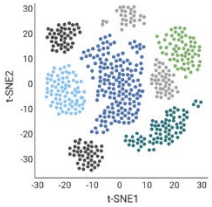
➢ *treatment-mediated viral control*

Enrichment of HBV-specific CD8+ T cells



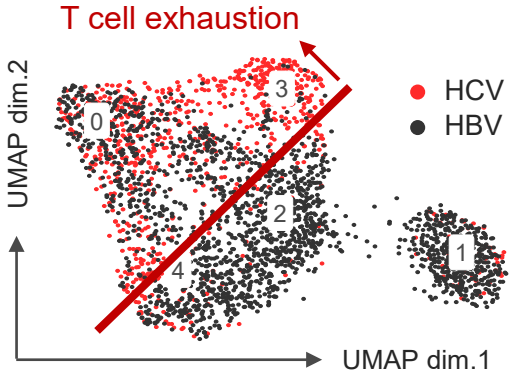
Single-cell sorting in 384 well plates

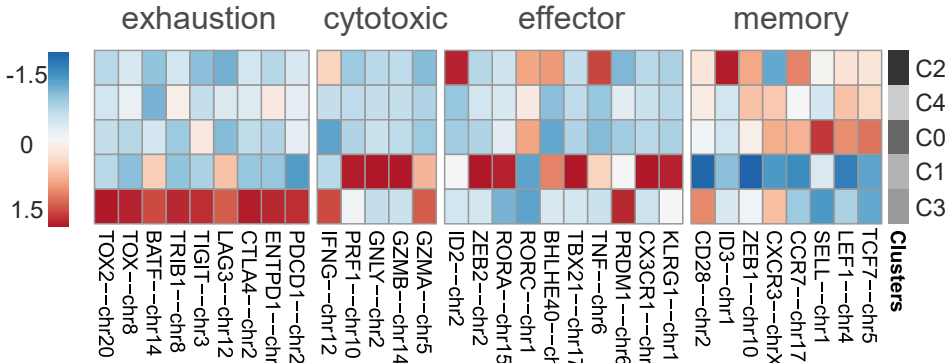
Single-cell RNA sequencing and analysis



- CEL-Seq2 technology
- 10x scRNAseq approach

Comparison of HBV- and HCV-specific CD8+ T cells obtained from chronic infection



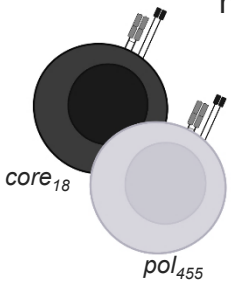


→ HBV-specific CD8+ T cell characteristics distinct from T cell exhaustion in chronic hepatitis B

HBV-specific CD8+ T cell heterogeneity in chronic infection

Targeted viral protein?

HBV-specific CD8+ T cells are not homogenous



Schuch, Salimi Alizei, Heim* et al. Gut 2019*
Hoogeveen et al. Gut 2019
Cheng et al. Sci Immunol 2019
Heim et al. Gut 2020

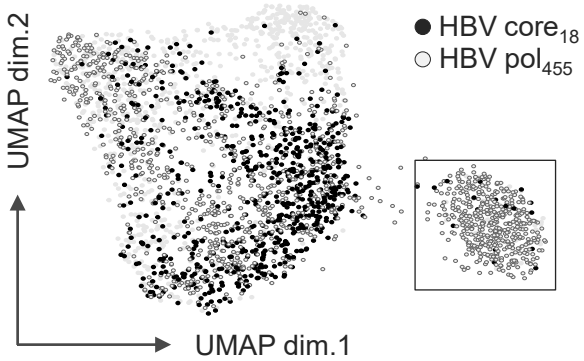
Heterogeneity based on targeted viral protein

	HBV $core_{18}$	HBV pol_{455}
Frequency	↑	↓
Expansion	↑	↓
Dysfunction	↓	↑
Survival	↑	↓

HBV-specific CD8+ T cell heterogeneity in chronic infection

Targeted viral protein?

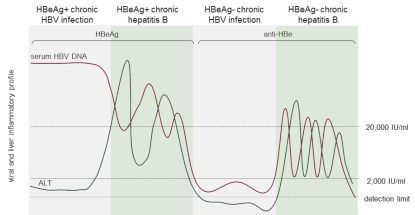
Epitopes



Clinical phases of chronic HBV infection?

Endogenous control phase	Immune-active phase
• normal liver enzymes → no/little liver pathology	• elevated liver enzymes → liver pathology
• low VL	• high VL → NUC → low VL
→ *"endogenous viral control"*	→ *treatment-mediated viral control*

… based on clinical phases



unpublished data, accepted in Nat. Immunol. – PLEASE DO NOT POST

EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017

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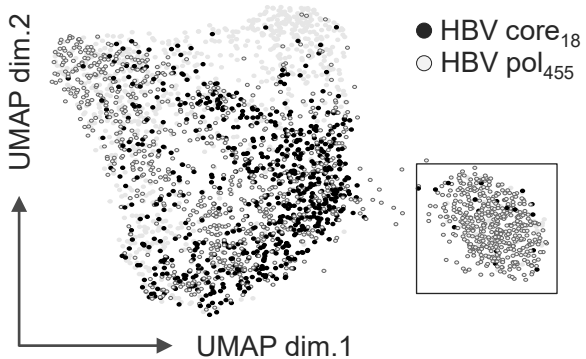
HBV-specific CD8+ T cell heterogeneity in chronic infection

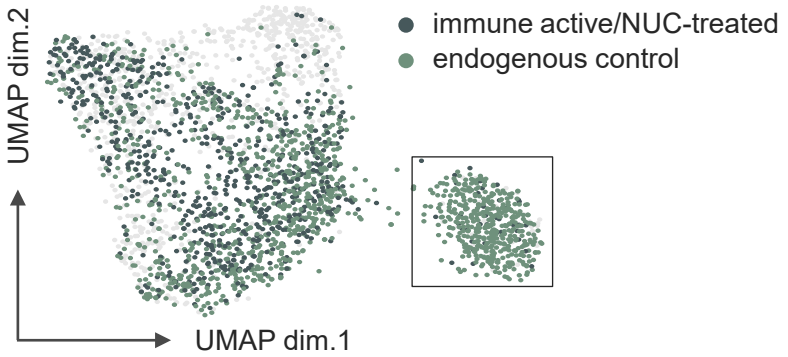
Targeted viral protein?

Epitopes



Clinical phases of chronic HBV infection?

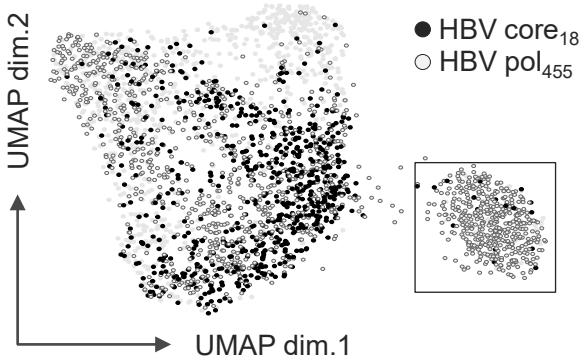
Clinical phases



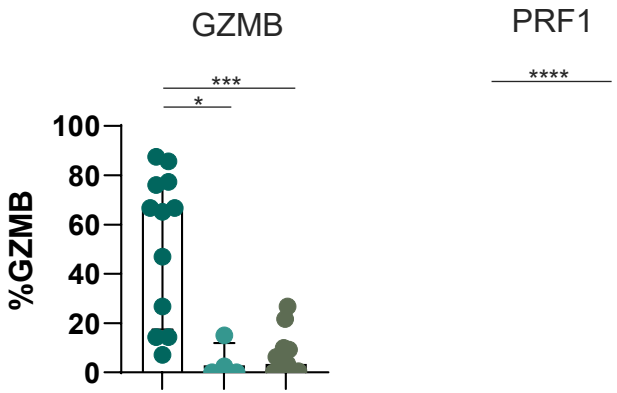
HBV-specific CD8+ T cell heterogeneity in chronic infection

Targeted viral protein?

Epitopes

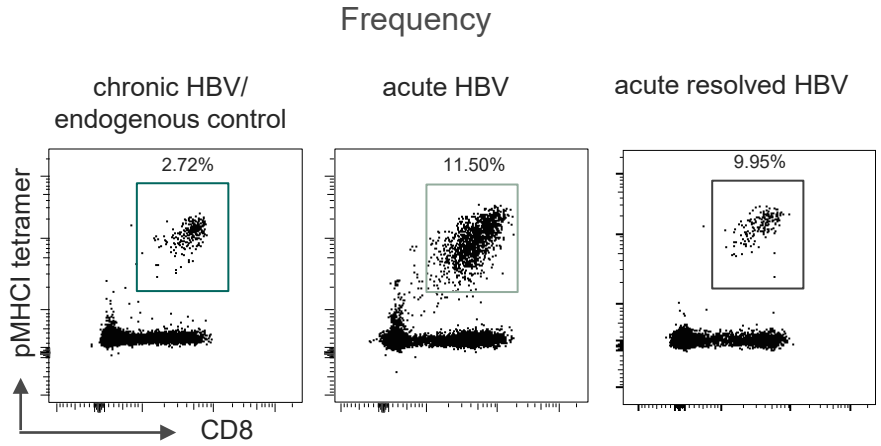


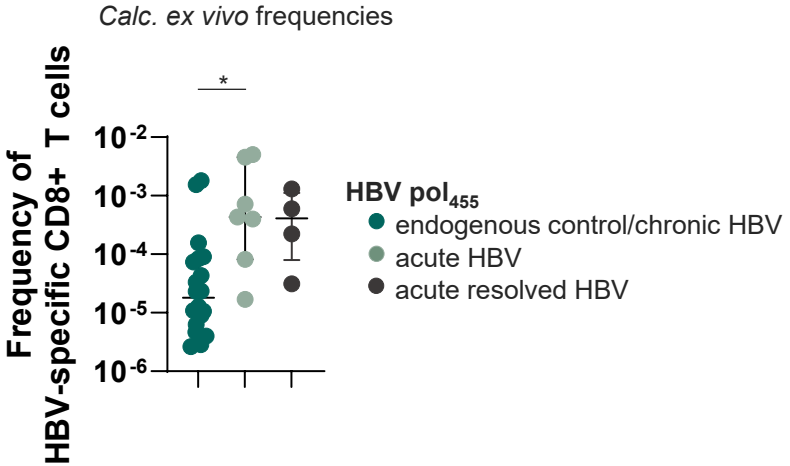
Clinical phases of chronic HBV infection?



→ Cytotoxic effector-like subset targets HBV polymerase and is associated with the endogenous control phase of chronic HBV infection

Do we find a bona fide effector T cell response in chronic HBV infection?

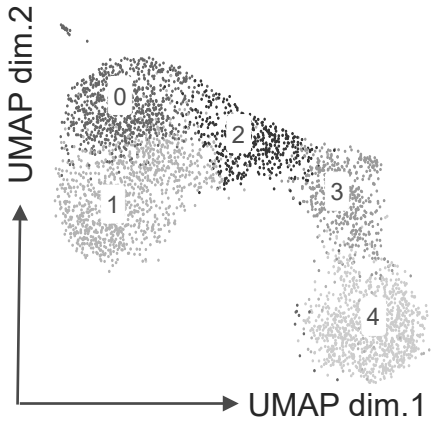




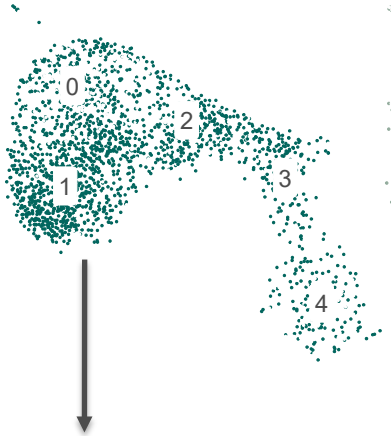
→ The strength of the HBVpol-specific CD8+ T cell response appears to be attenuated in patients with endogeneous control

Do we find a bona fide effector T cell response in chronic HBV infection?

HBVpol-specific CD8+ T cells



chronic HBV/ endogenous control



C1: cytotoxic, effector-associated genes and TRM genes

acute HBV

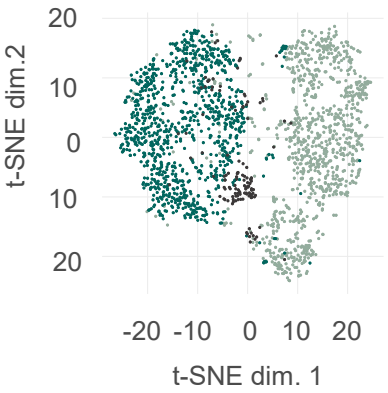
C0: genes associated with terminal differentiated effector cells

acute resolved HBV

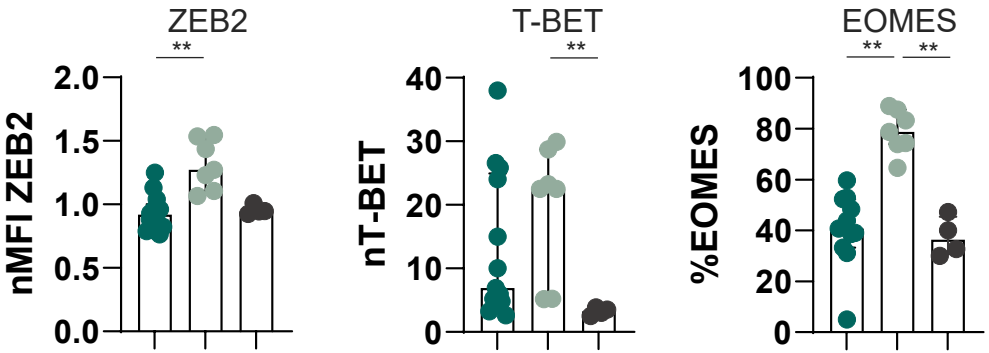
C3/4: stem-like associated genes

Do we find a bona fide effector T cell response in chronic HBV infection?

HBV-specific CD8+ GZMB+ T cells



Effector differentiation program



HBV pol$_{455}$
- endogenous control/ chronic HBV
- acute HBV
- acute resolved HBV

→ Attenuated T cell differentiation program in chronic HBV infection

Mechanisms of attenuated effector T cell response? TGFβ?

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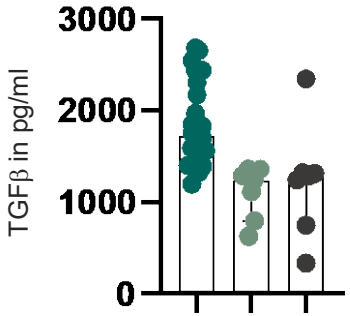
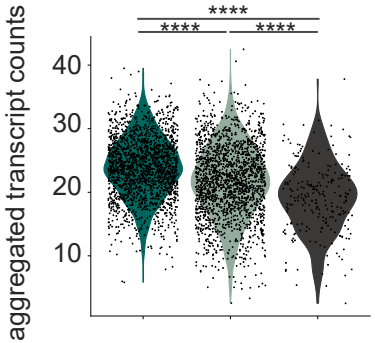
unpublished data, accepted in Nat. Immunol. – PLEASE DO NOT POST

Is TGFß linked to T cell attenuation?

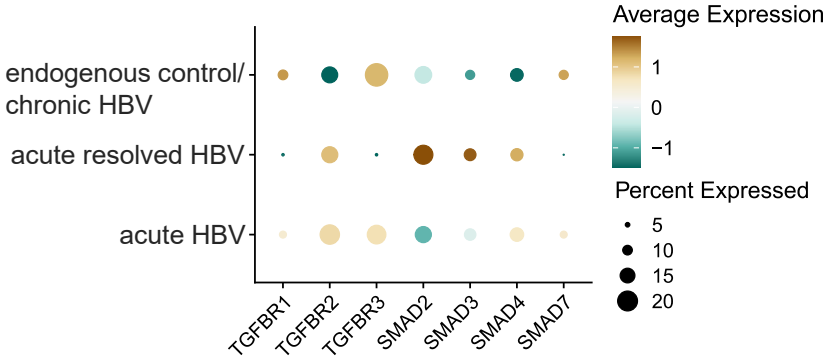
TGFβ plasma levels



TGFβ signaling pathway (KEGG)



TGFβ pathway gene expression



HBV pol$_{455}$
- endogenous control/chronic HBV
- acute HBV
- acute resolved HBV

→ TGFβ signaling pathway is augmented in patients with endogenously controlled chronic HBV infection

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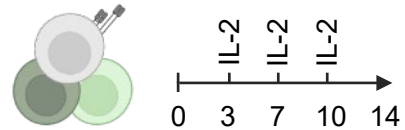
Is TGFß linked to T cell attenuation?

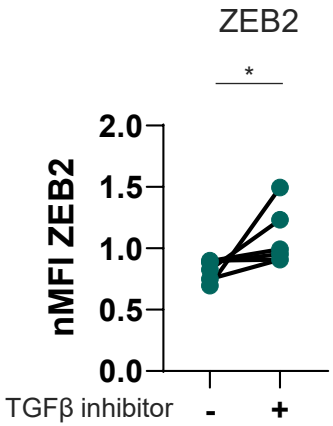
Blocking TGFβ signaling

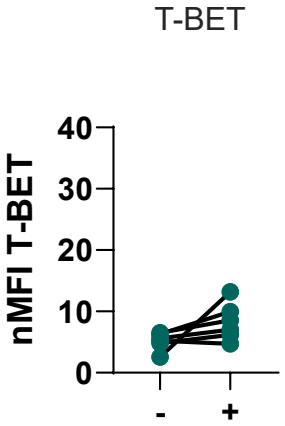
Peptide-specific in vitro expansion

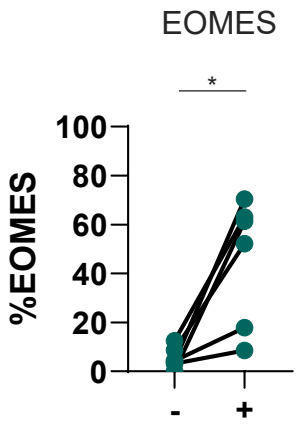


+ peptide
+ anti-CD28
+TGFβ receptor kinase II inhibitor

Transcription factor analysis







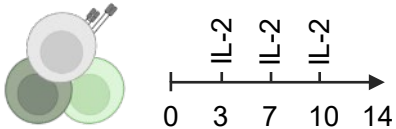
→ TGFβ signaling is linked to attenuated HBVpol-specific effector CD8+ T cell characteristics

Is chronic antigen stimulation strictly linked to T cell exhaustion?

Is TGFß linked to T cell attenuation?

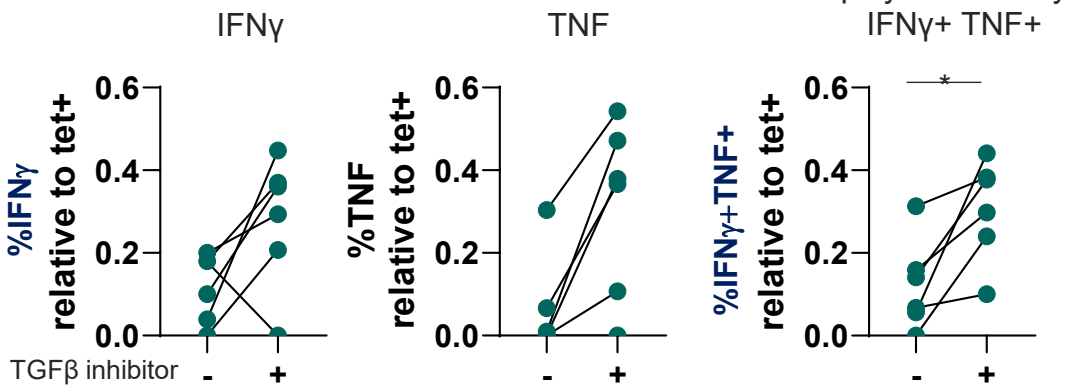
Blocking TGFβ signalling

Peptide-specific in vitro expansion



Cytokine Assay after restimulation

+ peptide
+ anti-CD28
+TGFβ receptor kinase II inhibitor

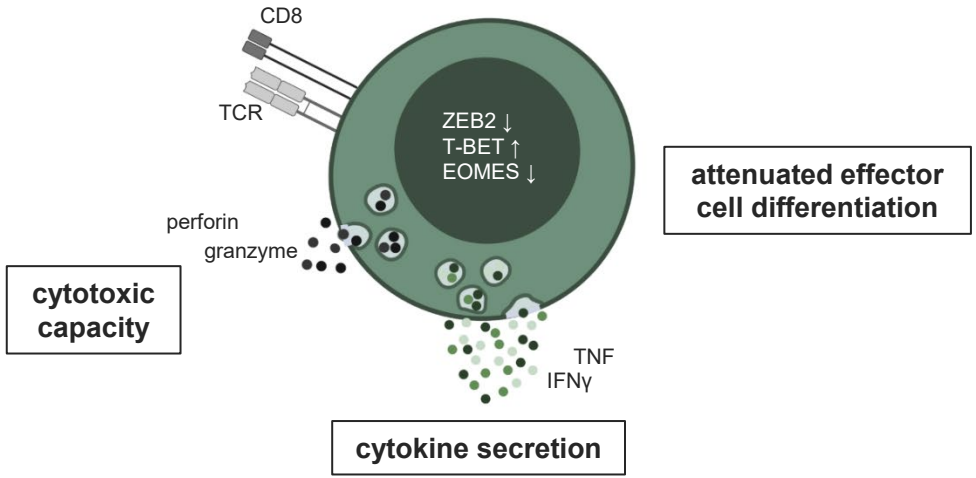


→ TGFβ signalling inhibition leads to an increased cytokine response of HBVpol-specific CD8+ T cells

Summary: Beyond T cell exhaustion – HBV-specific CD8+ T cell attenuation

Functional adaptation of HBV-specific CD8+ T cells is not purely restricted to "classical" T cell exhaustion.

Attenuated HBV-specific CD8+ T cells…

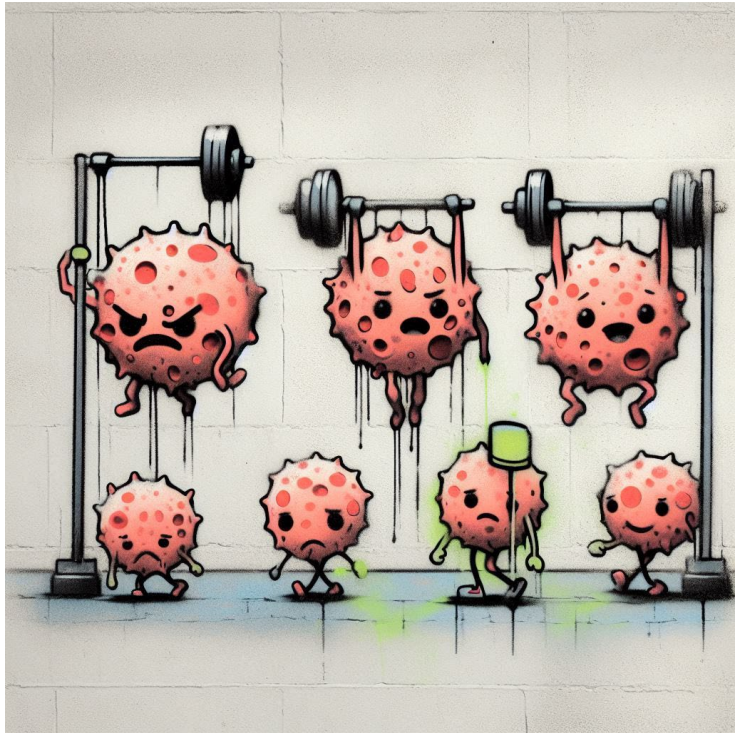


…enriched in HBV pol-specific CD8+ T cells.

… associated with endogenous control (HBeAg- chronic HBV infection) phase

… "ready-to-go", but blocked by TGFβ signaling

New concept: not all HBV-specific CD8+ T cells are exhausted!



Not all of us are exhausted.

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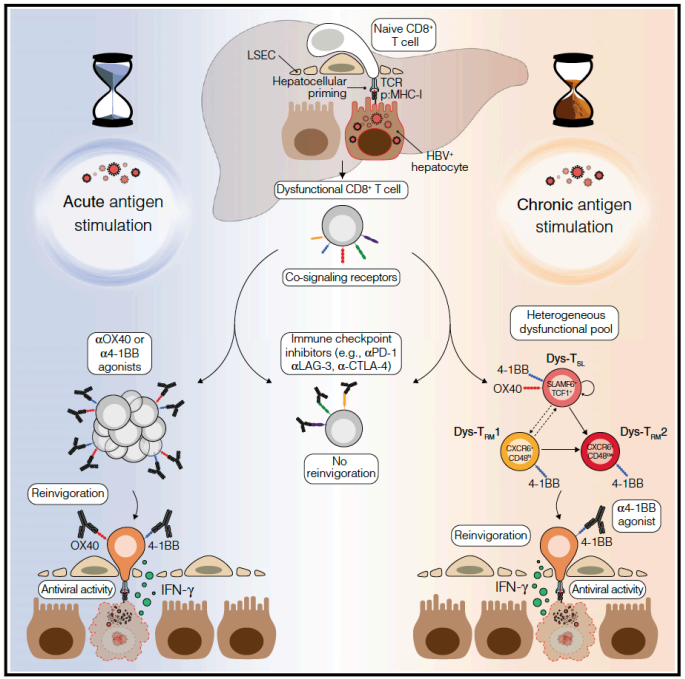
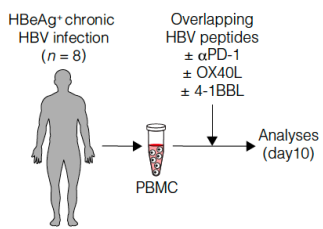
Cell

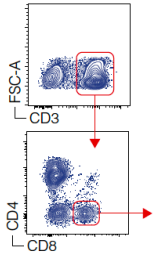
Therapeutic potential of co-signaling receptor modulation in hepatitis B

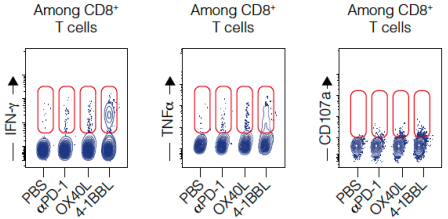
Francesco Andreata,[1,2,8] Chiara Laura,[1,2,3,8] Micol Ravà,[1,2,8] Caroline C. Krueger,[1,2,8] Xenia Ficht,[1,2,8] Keigo Kawashima,[1,8] Cristian G. Beccaria,[1,2,8] Federica Moalli,[1] Bianca Partini,[1,2] Valeria Fumagalli,[1,2] Giulia Nosetto,[1,2] Pietro Di Lucia,[1,2] Ilaria Montali,[4] José M. Garcia-Manteiga,[1,3] Elisa B. Bono,[1] Leonardo Giustini,[1] Chiara Perucchini,[1] Valentina Venzin,[1] Serena Ranucci,[1] Donato Inverso,[1,2] Marco De Giovanni,[1] Marco Genua,[5] Renato Ostuni,[2,5] Enrico Lugli,[6] Masanori Isogawa,[7] Carlo Ferrari,[6] Carolina Boni,[6] Paola Fisicaro,[6] Luca G. Guidotti,[1,2] and Matteo Iannacone[1,2,9,*]

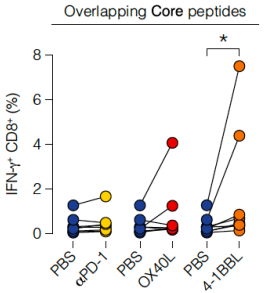
Graphical abstract

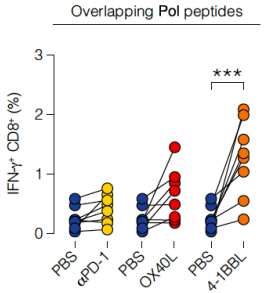


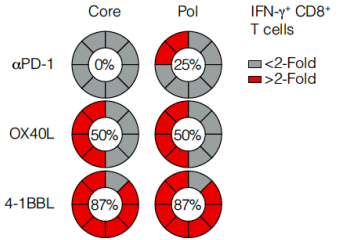












Immune restoration is possible

- Restoration of dysfunctional immunity (checkpoint inhibitor)
- Induction of new immunotherapeutic approaches (T cell vaccine)

Case Study: Clinical observations

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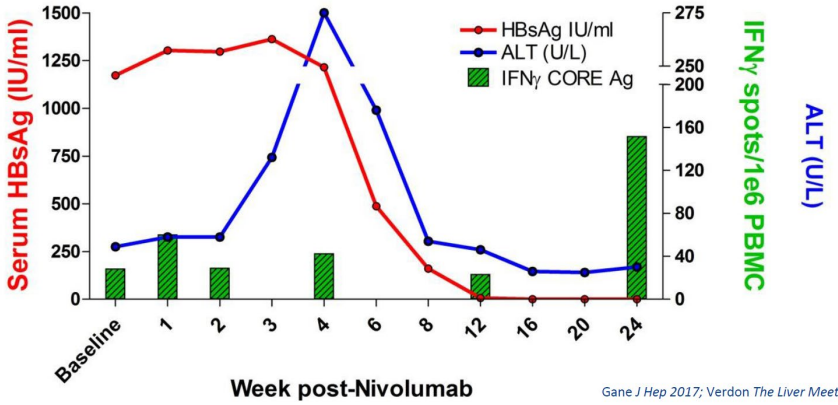


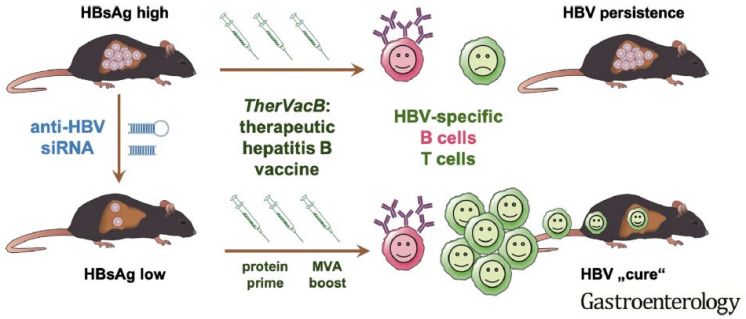
Gane *J Hep 2017;* Verdon *The Liver Meeting* 2017.

Combination of antiviral strategy with vaccination

- Restoration of dysfunctional immunity (checkpoint inhibitor)
- Induction of new immunotherapeutic approaches (T cell vaccine)
- Combine these methods with novel antiviral strategies

Knockdown of Virus Antigen Expression Increases Therapeutic Vaccine Efficacy in High-Titer Hepatitis B Virus Carrier Mice

Thomas Michler,[1,2,*] **Anna D. Kosinska**,[1,2,*] Julia Festag,[1] Till Bunse,[1,2] Jinpeng Su,[1] Marc Ringelhan,[1,3] Hortenzia Imhof,[1] Dirk Grimm,[2,4] Katja Steiger,[5] Carolin Mogler,[6] Mathias Heikenwalder,[6] Marie-Louise Michel,[7] Carlos A. Guzman,[2,8] Stuart Milstein,[9] Laura Sepp-Lorenzino,[9] Percy Knolle,[2,10] and Ulrike Protzer[1,2]



Maintenance of a chronic molecular scar

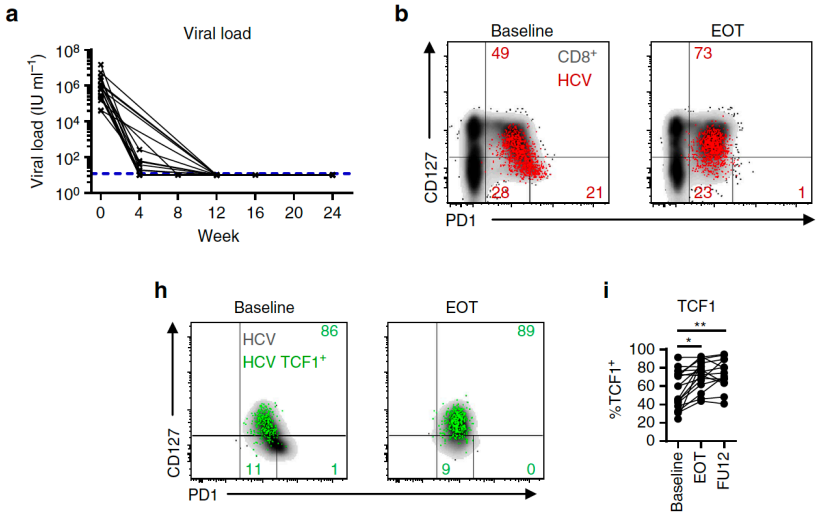
ARTICLE

Received 3 Sep 2016 | Accepted 23 Feb 2017 | Published 3 May 2017

DOI: 10.1038/ncomms15050 OPEN

TCF1$^+$ hepatitis C virus-specific CD8$^+$ T cells are maintained after cessation of chronic antigen stimulation

Dominik Wieland[1,2,3], Janine Kemming[1,3], Anita Schuch[1,3], Florian Emmerich[4], Percy Knolle[5], Christoph Neumann-Haefelin[1], Werner Held[6], Dietmar Zehn[7], Maike Hofmann[1,*] & Robert Thimme[1,*]

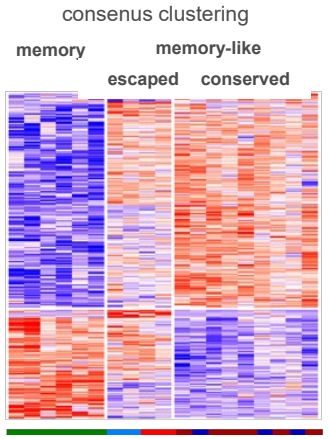


nature immunology ARTICLES

https://doi.org/10.1038/s41590-020-00817-w

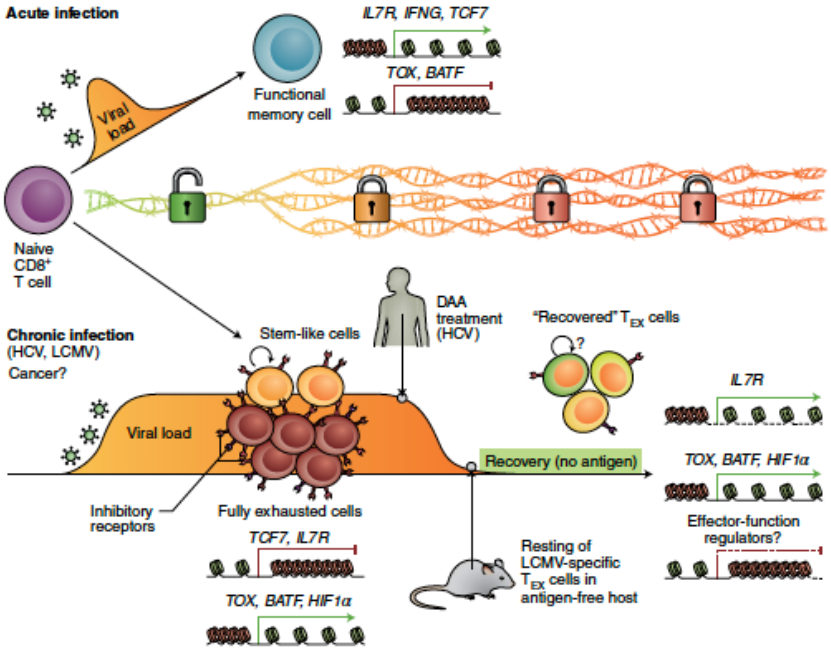
Memory-like HCV-specific CD8$^+$ T cells retain a molecular scar after cure of chronic HCV infection

Nina Hensel[1,2,3,15], Zuguang Gu[4,15], Sagar[1,5,15], Dominik Wieland[1,2], Katharina Jechow[6], Janine Kemming[1,2,3], Sian Llewellyn-Lacey[7], Emma Gostick[7], Oezlem Sogukpinar[1,2], Florian Emmerich[2,8], David A. Price[7,9], Bertram Bengsch[1,2,10], Tobias Boettler[1,2], Christoph Neumann-Haefelin[1,2], Roland Eils[4,11], Christian Conrad[4,6], Ralf Bartenschlager[12,13,14], Dominic Grün[3,10], Naveed Ishaque[4,16], Robert Thimme[1,2,16] ✉ and Maike Hofmann[1,2,16] ✉



Before and after DAA therapy

Summary molecular scar



IMMUNE EXHAUSTION

T cell exhaustion—a memory locked behind scars

Following clearance of chronic infections, virus-specific CD8+ T cells recover a subset of memory-related transcriptome features. Yet their unique open chromatin landscape largely reflects an exhausted or dysfunctional state, limiting their protective memory potential.

Amir Yousif and Hazem E. Ghoneim

Henselt et al
Yates et al
Tonnerre et al
Abdel-Hakem et al

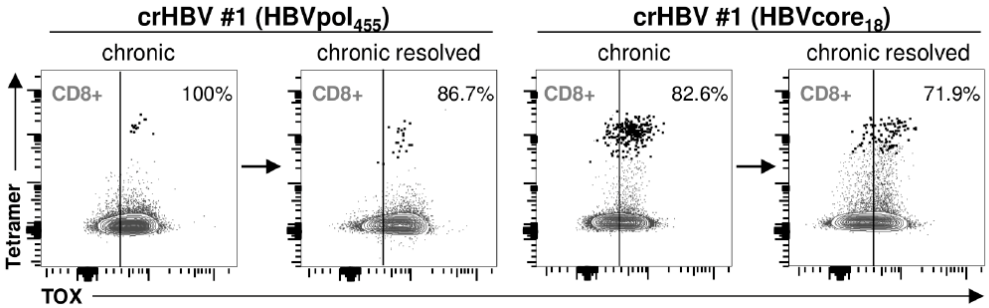
Nature Immunology 2021

Maintenance of a chronic molecular scar

Original research

TOX defines the degree of CD8+ T cell dysfunction in distinct phases of chronic HBV infection

Kathrin Heim,[1,2] Benedikt Binder,[1] Sagar ,[1] Dominik Wieland,[1] Nina Hensel,[1,2] Sian Llewellyn-Lacey,[3] Emma Gostick,[3] David A. Price ,[3,4] Florian Emmerich,[5] Hildegard Vingerhoet,[6] Anke R M Kraft,[7,8] Markus Cornberg ,[8,9] Tobias Boettler,[1] Christoph Neumann-Haefelin,[1] Dietmar Zehn,[10] Bertram Bengsch ,[1,11] Maike Hofmann ,[1] Robert Thimme [1]



Transcriptional scar of HBV specific CD8+ T cell exhaustion after antigen elimination

Heim et al, GUT 2021

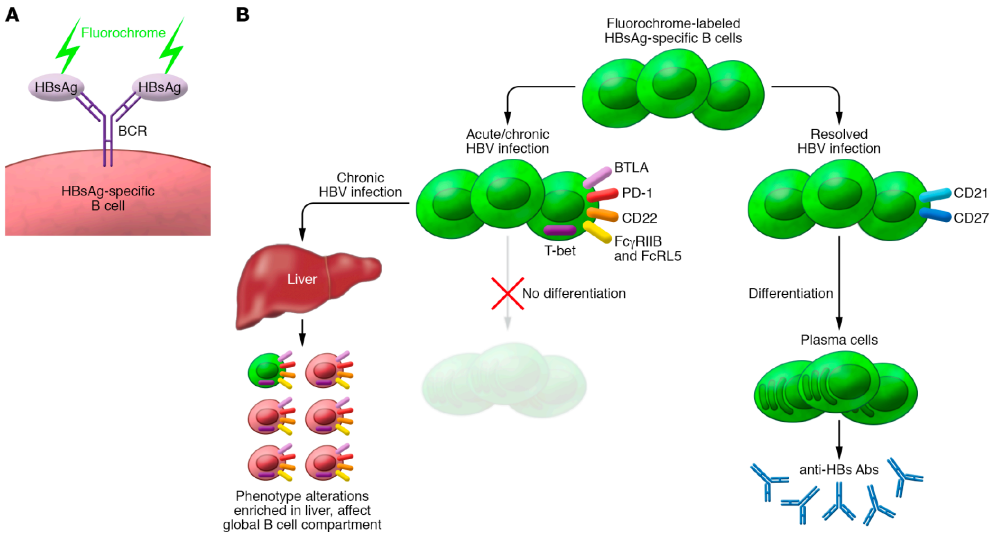
UNIVERSITÄTS KLINIKUM FREIBURG

The Journal of Clinical Investigation

Entering the spotlight: hepatitis B surface antigen–specific B cells

Christoph Neumann-Haefelin and Robert Thimme

Department of Medicine II, University Medical Center Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany.



Salimzadeh et al, JCI 2018
Burton et al, JCI 2018

Conclusion: mechanisms of exhaustion and path for functional recovery

- T cell heterogeneity driven by virus (HBV / HCV) and targeted antigen (cor / pol)
- Absence of classical T cell exhaustion in chronic HBV (attenuated effector function)
- Attenuated effector function linked to endogeneous control
- Relevance for functional recovery?
- Molecular scar in HBV?

Thank you!

TRANSLATIONAL EXPERIMENTAL IMMUNOLOGY LAB, Department of Medicine II, Medical Center – University of Freiburg

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All physicians, patients and volunteers!