Association of the immunodominant HLA-B35:01 restricted CD8+ T cell epitope with clustered viral evolution in HBV polymerase

Elahe Salimi Alizei[1,2], Michael Muthamia Kiraithe[1], Philipp Ehrenmann[1], Maike Hofmann[1], Robert Thimme[1] and Christoph Neumann-Haefelin[1]

[1] Department of Medicine II, University Hospital Freiburg, Germany
[2] Faculty of Chemistry and Pharmacy, University of Freiburg, Germany

1 INTRODUCTION

CD8+ T cell exhaustion and viral escape are considered to be the main mechanisms leading to chronic Hepatitis B (HBV) infection; however, the relative role of viral escape herein is not well defined. Until now, viral escape in HBV infection has been mainly described for the core protein, particularly in the context of HLA-A*02 restriction.

2 AIM

In this study , we aimed to address the role of viral escape from CD8+ T cell responses targeting HBV polymerase. Further, we aimed to analyze the phenotype and function of HBV-specific CD8+ T cells targeting the novel HLA-B*35:01 epitope in polymerase as well as two previously described HLA-A*01:01 and HLA-A*11:01 restricted epitopes located in the core protein.

3 METHOD

- Analysis of 114 patients chronically infected with HBV genotype D by HLA class I typing and HBV full-genome sequencing.
- HLA class I associated HBV polymorphisms were identified.
- Corresponding to a cluster of three HLA-B*35:01 associated HBV polymerase sequence mutations, the optimal epitope peptide was determined.
- Tetramer-based enrichment of CD8+ T cells specific for the novel HLA-B*35:01 polymerase epitope & two previously described HLA-A*01:01 and HLA-A*11:01 restricted core epitopes was performed.
- A cohort of 51 patients with chronic HBV genotype D infection was screened.

4 RESULTS

Three sequence mutations specific for HLA–B*35:01 are present in patients with chronic HBV genotype D infection

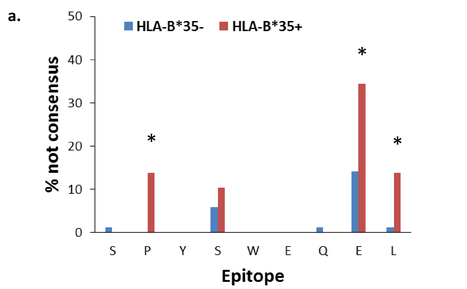
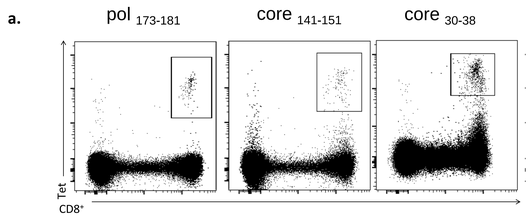


Figure 1. Three HLA-B*35:01-associated HBV polymerase sequence mutations with high significance were identified in HLA-B*35:01 positive patients by sequence analysis of 114 patient chronically infected with HBV **(a)**. The corresponding predicted HLA-B*35:01 restricted CD8+ T cell epitope could be confirmed experimentally **(b).** Statistical significant HLA-associated mutations were determined by *Fisher's exact test*. * = p<0.05

HLA-B*35:01 restricted CD8+ T cells are detectable in chronically HBV-infected patients with low viral load



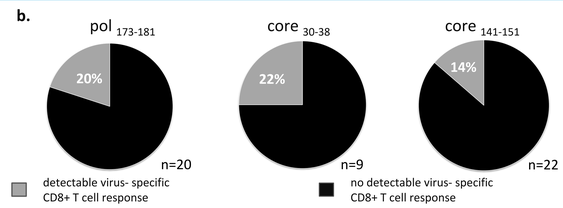


Figure 2. Representative flow cytometry analyses showing pol_{173}-specific CD8+ T cell populations, $core_{30}$-specific CD8+ T cells and $core_{141}$-specific CD8+ T cells of chronically HBV-infected patients after performing peptide/MHC class I tetramer enrichment **(a).** Proportion of analyzed chronically HBV-infected patients with (depicted in grey) and without (depicted in black) detectable epitope-specific CD8+ T cells after performing peptide/MHC class I tetramer enrichment **(b)**.

Severely exhausted subsets of HLA–B*35:01 restricted CD8+ T cells are present in chronic HBV-infected patients with low viral load

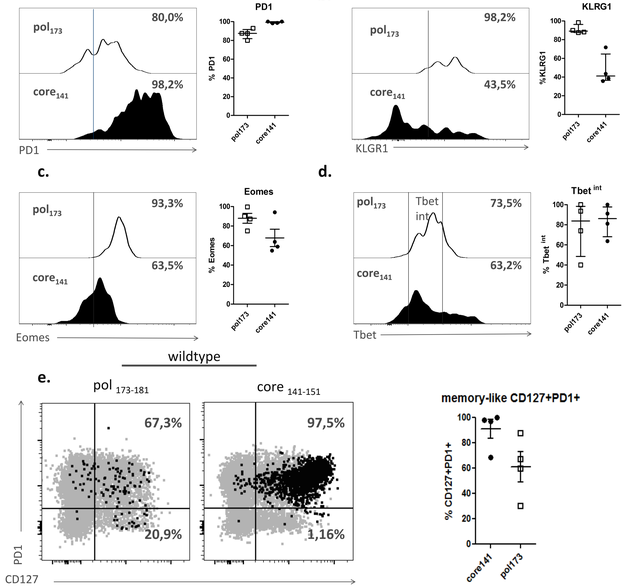


Figure 3. *Ex vivo* expression analyses of inhibitory receptor PD1 did not show different expression in pol_{173}- specific CD8+ T cells which targeted wildtype epitopes in cHBV- infected patients compared to $core_{141}$- specific CD8+ T cells **(a)** while pol_{173}-specific CD8+ T cells highly express the inhibitory receptor KLRG1 **(b).** *Ex vivo* expression analyses of the transcription factors Eomes and Tbet revealed higher Eomes expression in pol_{173}- versus $core_{141}$-specific CD8+ T cells **(c)** while the intermediate expression of the transcription factor Tbet was similar for pol_{173}- versus $core_{141}$- specific CD8+ T cells **(d)**. CD127/PD1 co-expression of pol_{173}-specific CD8+ T cells **(e)** revealed less memory-like phenotype $core_{141}$- specific CD8+ T cells **(f)**.

5 CONCLUSIONS

- A novel HLA-B*35:01 restricted polymerase epitope could be identified.
- Viral escape mutations are evident in the novel HLA-B*35:01 restricted polymerase epitope.
- pol_{173}-specific CD8+ T cells of chronically HBV-infected patients detected *ex vivo* also revealed phenotypic features of exhaustion.

→ **Failure of polymerase-specific CD8+ T cells is due to viral escape *and* exhaustion**

6 REFERENCES

1. A. Schuch, E. Salimi Alizei*, K. Heim*, D. Wieland M.M. Kiraithe, J. Kemming, S. Llewellyn-Lacey, O. Sogukpinar, Yi Ni, S. Urban, P. Zimmermann, M. Nassal, F. Emmerich, D.A. Price, B. Bengsch, H. Luxenburger, C. Neumann-Haefelin, M. Hofmann* and R. Thimme*. Phenotypic and functional differences of HBV core-versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load. *GUT 2019*

2. M. Kiraithe#, E. Salimi Alizei#, E. Gostick, J. Sidney, M. Marget, B. Raziorrouh, M. Cornberg, J. Lang, P. Ehrenmann, A. Sette, D. Price, T. Böttler, R.Thimme, C. Neumann-Thimme: Differential role of HLA-B*27 restricted CD8+ T cell responses and viral escape in acute and chronic HBV infection. " *In Preparation*

7 CONTACT

Elahe.salimi.alizei@uniklink-freiburg.de

8 AFFILIATIONS

[1] Department of Medicine II, University Hospital Freiburg, Germany
[2] Faculty of Chemistry and Pharmacy, University of Freiburg, Germany

Poster presented at: EASL The International Liver Congress 2019
DOI: 10.3252/pso.eu.ILC2019.2019
FRI-150
ILC2019