FRI354

Characterization of the HBV-Specific T-Cell Pool Reveals Shared Epitope Usage, But Different Phenotypes of HBV-Reactive T Cells Across Patients

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Introduction

- Development of effective therapeutic strategies for hepatitis B virus (HBV) infection requires a better understanding of the nature and biology of cluster of differentiation (CD)–8+ T-cell responses, including epitopes driving protective responses and associated T-cell phenotypes
- HBV-specific T-cell characteristics could be used as a measure of clinical and biological activity of the candidate immunotherapy[1]
- Identifying and characterizing HBV-specific CD8+ T cells in patients with chronic HBV has, however, been historically challenging due to the rare frequencies and functional impairment of such cells, and multiple potential T-cell epitope targets in the context of diverse HBV genotypes

Objectives

- To define and validate a list of relevant human leukocyte antigen (HLA)–A*11:01-restricted HBV epitopes to be widely applied for longitudinal monitoring of CD8+ T-cell responses in HBV immunotherapy clinical trials
- To assess the relation between clinical readouts and HBV-specific T-cell epitope usage and profile in a pilot cohort of patients with chronic HBV treated with the T-cell vaccine GS-4774 in combination with tenofovir disoproxil fumarate (TDF)

Methods

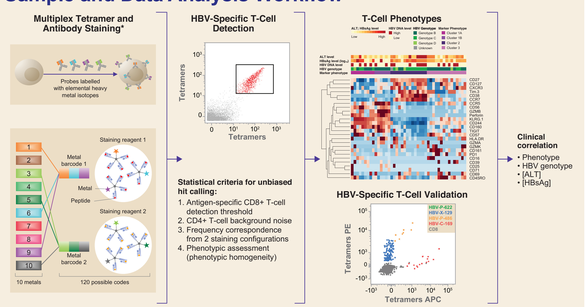
Study Population

- Baseline and on-treatment peripheral blood mononuclear cell (PBMC) samples from 22 patients recruited in GS-US-330-1401 (ClinicalTrials.gov NCT02174276), a study designed to evaluate the safety and efficacy of GS-4774 in combination with TDF in patients with chronic HBV, were investigated by high-dimensional immune profiling
  - Median age 40 y, 16/22 men, and 21/22 Asian race
  - HLA type: A*11:01
  - Median baseline hepatitis B surface antigen (HBsAg): 1693 IU/mL
  - Median baseline HBV DNA: 3830 IU/mL
  - Median baseline alanine aminotransferase (ALT): 34 U/L
  - HBV genotype distribution (n): A (1), B (10), C (8), D (1), and unknown (2)

Workflow

- A mass cytometry-based, highly multiplexed peptide-major histocompatibility complex tetramer-staining strategy[2-5] was applied to simultaneously screen >100 HLA-A*11:01- and HLA-A*02:01-restricted HBV CD8+ T-cell candidate epitopes, and to further phenotypically profile HBV-specific T cells in PBMCs from patients with HBV
  - Influenza-, cytomegalovirus-, and Epstein-Barr virus (EBV)–specific CD8+ T cells were tracked as controls, and used as benchmarks
- Peptide clusters were automatically generated, grouping HBV peptide variants and virus-related epitopes
- 2 technical replicates of triple metal-coded tetramers were used for the same epitopes and for each donor to validate the detected specificities
- Samples were barcoded and acquired on a mass cytometer

Sample and Data Analysis Workflow



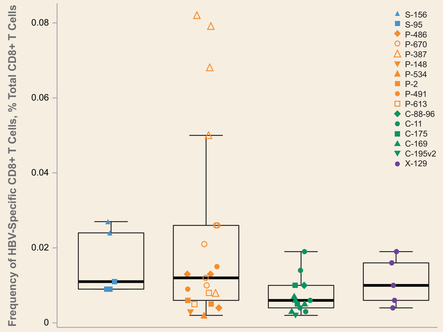
*Tested 49 peptide clusters for HLA-A*11:01 (total of 110 peptides); tested 30 peptide clusters for HLA-A*02:01 (total of 33 peptides); peptide clusters have same metal code. APC, allophycocyanin; PE, phycoerythrin.

Data Analysis

- Virus-specific CD8+ T cells were identified by triple metal-coded tetramers after gating on singlets+DNA+cisplatin–CD45+Lin–CD3+CD8+ T cells
- Bonafide antigen-specific T cells were identified by different objective statistical criteria and phenotypes were assessed using high-dimensional analytical tools
- Longitudinal changes in HBV-specific CD8+ T-cell response and associations with clinical parameters were assessed

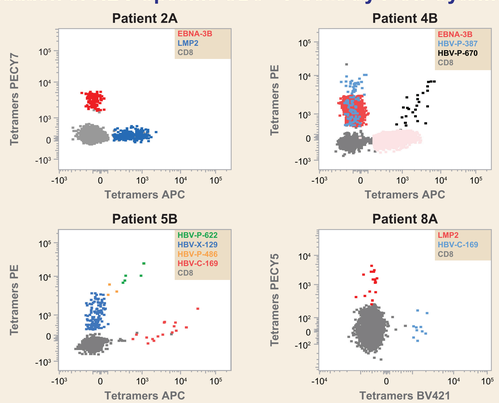
Results

Identification and Quantification of HBV-Specific CD8+ T Cells by Mass Cytometry



- Most HBV-specific CD8+ T cells detected in this cohort were specific for HBV polymerase, with reactivities detected against core, X, and surface proteins, as well
- Frequencies of HBV-specific T cells varied from 0.002 to 0.08% of total CD8+ T cells
- 4/7 hits recognizing the polymerase HBV-P-387 epitope presented higher frequencies compared with T cells recognizing other HBV epitopes (>0.05% of CD8+ T cells)

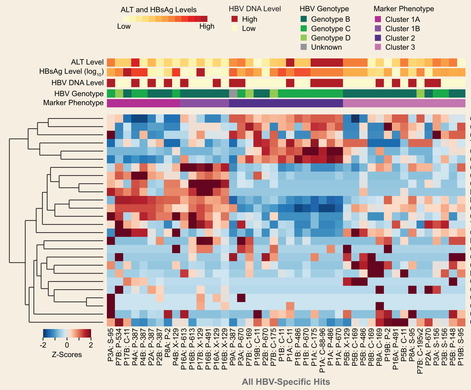
Validation of HBV-Specific CD8+ T Cells by Flow Cytometry



BV421, Brilliant™ Violet 421 (BD Biosciences, San Jose, California, USA); EBNA-3B, Epstein Barr nuclear antigen 3B; LMP2, latent membrane protein 2.

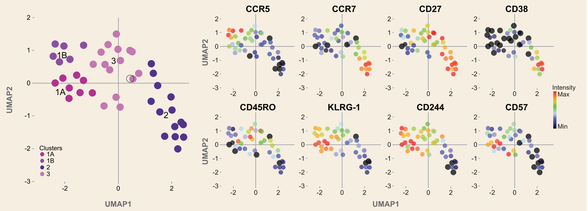
- Patients with chronic HBV infection were selected according to HBV-specific hits detected by CyTOF® staining (Fluidigm, San Francisco, California) and availability of duplicate samples
- Flow cytometry data corroborated CyTOF data for overall detection of viral-specific T cells in the subset of patients tested

Phenotypes of HBV-Specific CD8+ T Cells and Associations With Clinical Parameters



- Frequencies of each phenotypic marker for all HBV-specific CD8+ T cells (hits) were normalized and are represented as Z-scores in a cold-to-hot heatmap
  - Markers and hits were clustered in an unsupervised fashion
- The heatmap visualizes co-expression of markers by HBV-specific CD8+ T cells across patients and identifies/groups 4 main phenotypic profile clusters:
  - Cluster 1A: late differentiated “senescent” T cells (KLRG1+CD244+CD160+ CD57+)
  - Cluster 1B: late differentiated “functional” T cells (KLRG1+CD244+CCR5+ GZM−B+)
  - Cluster 2: naïve-like T cells (CD45RO–CD27+CD127+CCR7+CD38+)
  - Cluster 3: transitional memory T cells (CD45RO+CD27+CD244+CD69+)

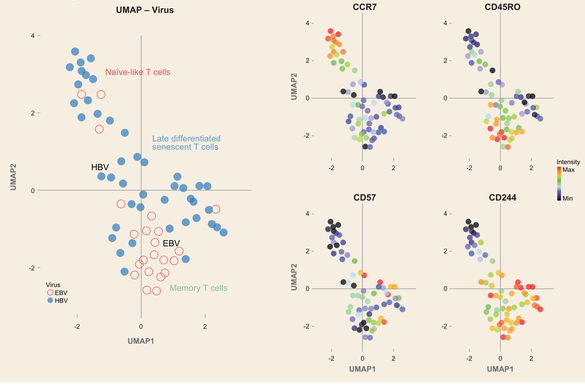
High-Dimensional Phenotypes of HBV-Specific CD8+ T Cells



CCR, C-C chemokine receptor; KLRG, killer cell lectin-like receptor subfamily G; Max, maximum; Min, minimum; UMAP, uniform manifold approximation and projection.

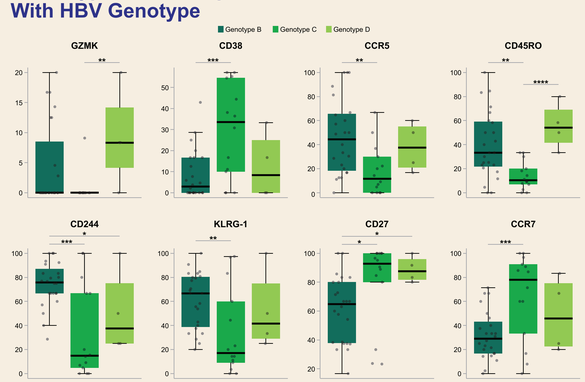
- Phenotypes of all HBV-specific CD8+ T cells (hits) were visualized in a UMAP representation and clustered into 4 phenotypic profiles
- The position of each hit in the UMAP projection is determined by phenotypic marker expression

Phenotypes of HBV-Specific CD8+ T Cells Differ From EBV-Specific CD8+ T Cells



- EBV-specific T cells clustered together and were characterized by a more homogeneous memory T-cell phenotype (CD45ROhigh, CCR7low) compared with HBV-specific CD8+ T cells

Phenotypes of HBV-Specific CD8+ T Cells and Association With HBV Genotype



*Mann-Whitney U test: *p <0.05, **p <0.01, ***p <0.001, and ****p <0.0001. GZMK, granzyme K.

- Frequencies of each marker assessed on HBV-specific T cells were compared across patients infected by HBV genotypes B, C, and D
- HBV-specific T cells in patients infected with HBV genotype C had significantly higher expression of CD38, CD27, and CCR7, and lower expression of CD45RO, CCR5, and KLRG-1 compared with hits found in patients with genotype B

Conclusions

- The feasibility of simultaneously identifying and deeply characterizing rare HBV-specific T cells was demonstrated in heterogeneous HBV patient cohorts and a list of relevant HLA-A*11:01-restricted HBV epitopes was derived that could be widely applied for longitudinal monitoring of CD8+ T-cell responses in HBV immunotherapy clinical trials
- Most HBV-specific T cells detected recognized epitopes derived from the polymerase protein, as described in previous reports[2]
- HBV-specific T cells grouped into 4 main phenotypic clusters, corresponding to late differentiated—either senescent or functional—T cells, naïve-like T cells, and memory T cells
  - However, antigen-specific T cells were phenotypically heterogeneous across patients and epitopes
  - No effect of treatment on T-cell phenotypes could be measured in this cohort
- Phenotypes of HBV-specific T cells differed from those of EBV-specific T cells identified in the same cohort
- HBV-specific T-cell phenotypes showed a potential association with the HBV genotype, with naïve-like hits mostly found in patients with HBV genotype C

References: 1. Bertoletti A, et al. J Hepatol 2016;64:S71-83; 2. Becht E, et al. Nat Biotechnol 2019;3738-44; 3. Cheng Y, et al. Sci Immunol 2019;4:eaau6905; 4. Levine JH, et al. Cell 2015;62:184-97; 5. Newell EW, et al. Nat Biotechnol 2013;31:623-9. Acknowledgments: We extend our thanks to the patients, their families, and all participating investigators. This study was funded by Gilead Sciences, Inc.