OCTOBER 11-12, 2024 MILAN, ITALY
DeltaCure 3rd International Meeting
P54
Poster for you

Development of a rapid test for HDV-specific T cell characterization in whole-blood

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Introduction

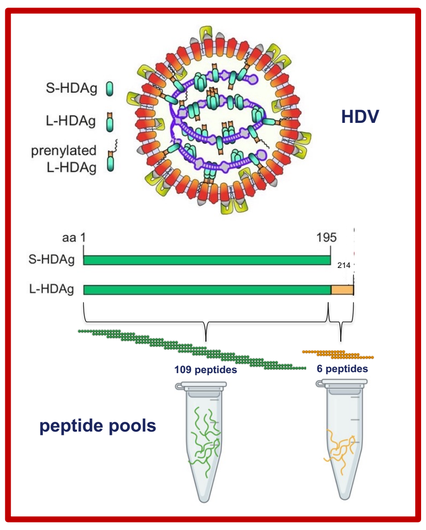
- The ability to cure HBV and HBV-HDV co-infection is linked with the presence of functional virus-specific T cells that directly recognize and lyse infected cells (CD8 T cells) and help the production of high-affinity antibodies (CD4 T cells).
- Yet, clinical management of patients with chronic HBV-HDV co-infection (CHB/HDV) relies exclusively on the assessment of virological and biochemical markers.

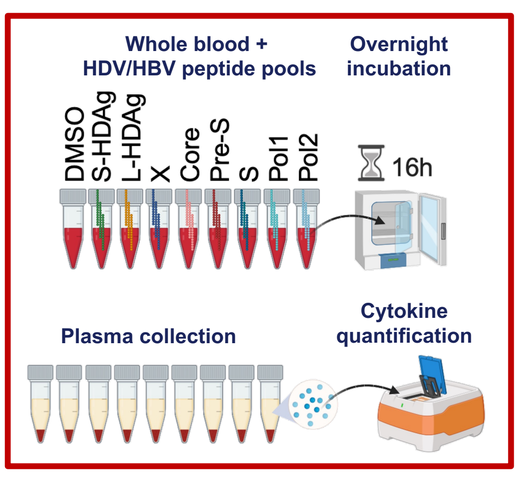
Aim

- We aim to develop a robust and simple cytokine release assay (CRA) to measure in parallel HBV and HDV-specific T cell frequency and function with efficient throughput and minimal invasiveness to allow integration of these immunological biomarkers in CHB/HDV clinical management.

Methods

- We designed 15-mer peptides covering the sequence of Small HDAg (S-HDAg) of genotypes 1, 2, and 4 (109 peptides) and of Large HDAg (L-HDAg) covering the 19 AA C-terminal extension (6 peptides).
- The 109 S-HDAg and the 6 L-HDAg peptides were pooled and used to stimulate whole blood (400 μl for each peptide pool).





- In parallel, peptide pools covering the envelope, core, polymerase, and X proteins of different HBV genotypes were also used to stimulate whole blood and measure HBV-specific T cells.
- After overnight incubation, supernatants were collected and analyzed for the secretion of different cytokines (IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-17A, Granzyme B and/or TNF-α).
- We first evaluated the correlation between HBV-CRA and ex-vivo ELISpot to assess its sensitivity.
- Next, we examined whether HBV-CRA could provide reproducible results in longitudinal samples from NUC-treated CHB patients, while capturing dynamic changes in T cell function during acute HBV infection.
- Finally, we assessed if HDV/HBV-specific responses in CHB/HDV patients (n=17).

Results

HBV-CRA Correlates with Ex-Vivo ELISpot Assay but Demonstrates Higher Sensitivity for Detecting HBV-Specific T Cells in CHB Patients

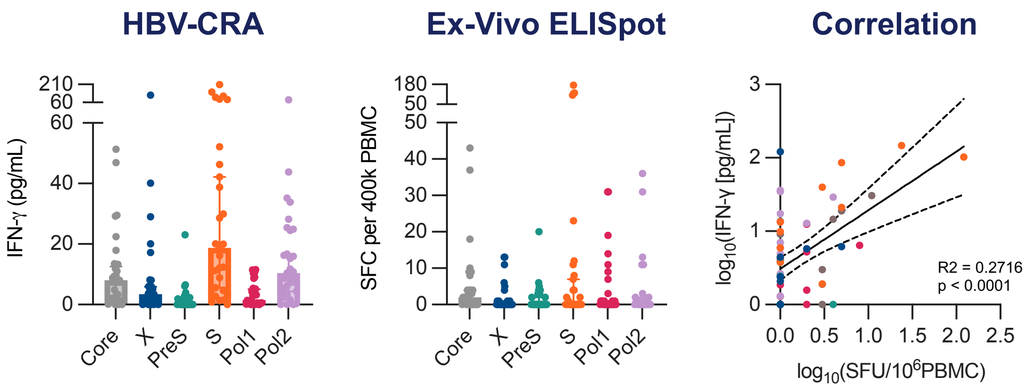


Figure 1: *Fresh whole blood from CHB patients (n=10) was either stimulated directly with HBV peptide pools for the HBV-CRA (left) or processed for PBMC isolation and ex-vivo ELISpot assay (middle). Secreted IFN-γ levels from HBV-CRA were correlated with the number of IFN-γ–SFU from ELISpot assays (right).*

HBV-CRA Provides Reproducible Measurements of HBV-Specific T Cell Function in Longitudinal Samples from NUC-Treated CHB Patients

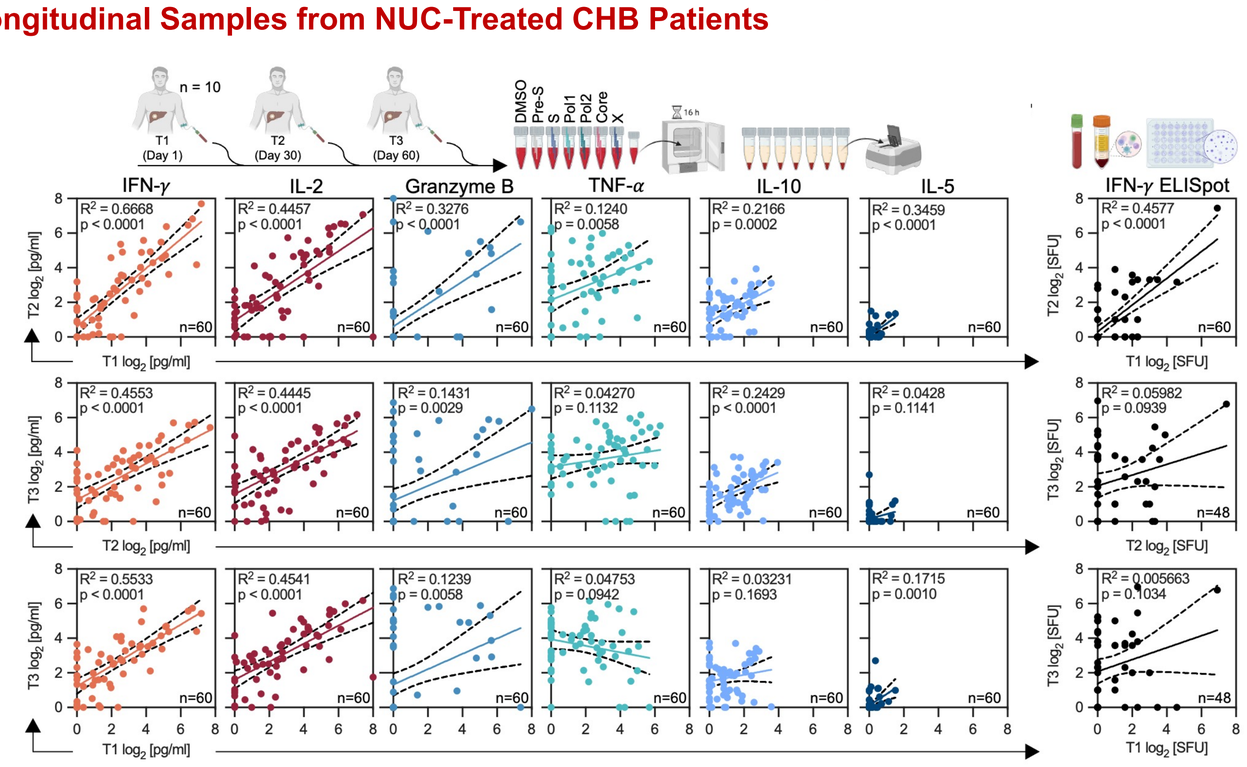


Figure 2: *CHB patients treated with NUC therapy (n=10) were followed longitudinally, and their HBV-specific T-cell profile was analyzed monthly with the HBV-CRA. Secreted cytokine levels measured at different time points were better correlated than those of ex-vivo ELISpot assays.*

HBV-CRA Measures Dynamic Kinetics of HBV-Specific T Cells in Patients with Acute HBV Infection

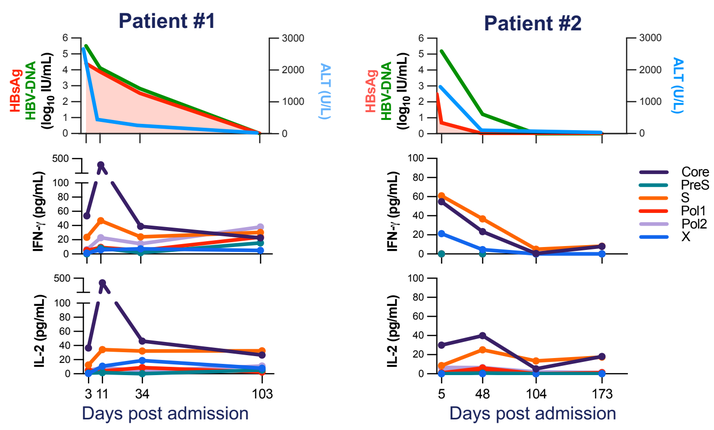


Figure 3: *Two patients with an acute HBV infection were followed longitudinally until the resolution of the infection. Their HBV-specific T-cell profile was analyzed with the HBV-CRA at 4 time points. Both patients cleared HBsAg levels within two months of symptom onset and the assay depicted the dynamic changes in the HBV-specific T cell response over time.*

HDV-CRA Detects HDV-Specific T Cell Responses Targeting the Short C-Terminal Extension of L-HDAg, Characterized by Strong Granzyme B Secretion

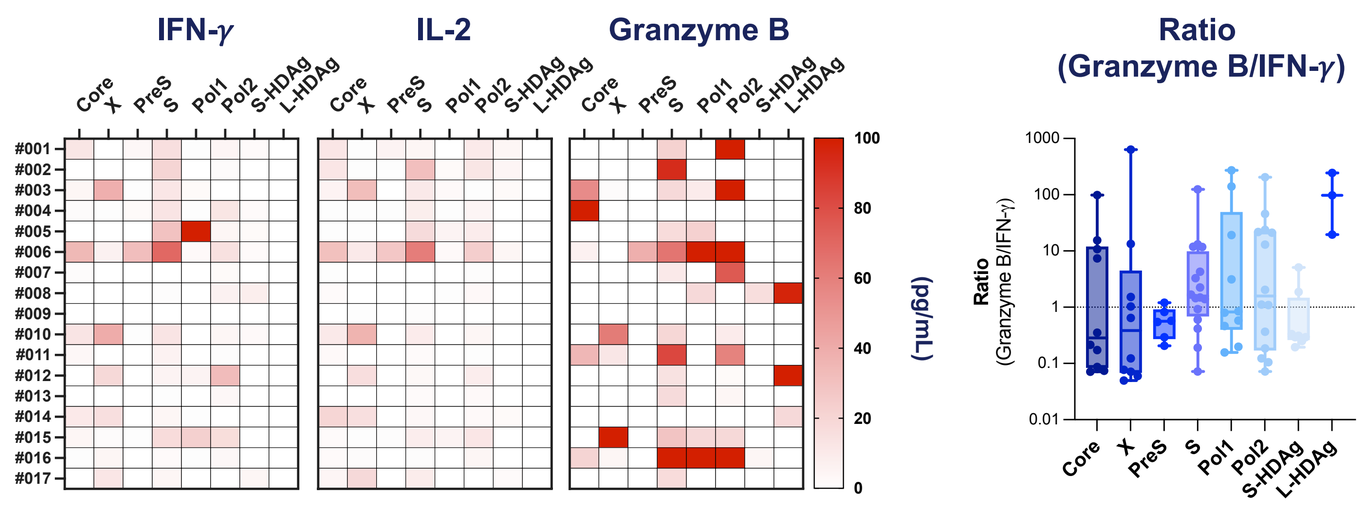


Figure 4: *Blood samples from CHB/HDV patients (n=17) were stimulated with peptide pools covering HBV (Core, X, Pre-S, S, Pol1, Pol2) and HDV antigens (S-HDAg, L-HDAg). Secreted IFN-γ, IL-2 and Granzyme B levels are shown in the heatmap for each patient (left). The ratio of Granzyme B vs IFN-γ, in samples with detectable responses, is shown in the box plot (right).*

Conclusions

- We demonstrated that we can accurately assess the quantity and multi-functionality of HBV/HDV-specific T cells in patients without complex in-vitro manipulation in a small volume of whole blood.
- Global measurement of cytokines secreted or induced by HBV/HDV-specific T cells shows high heterogeneity among patients. Assessment of virological and biochemical biomarkers cannot define the immune heterogeneity in chronically infected patients.
- Deciphering the functional secretome of HBV/HDV-specific T cells might provide a novel biomarker for the interpretation of host-viral interactions and can signpost the selection of novel immunotherapies.

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