Differential T cell signaling in memory-like and severely exhausted HCV-specific CD8⁺ T cells in chronic HCV infection revealed by highly multiplexed mass-based phosphoflow analysis

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Background

T cell exhaustion is a major contributor to CD8⁺ T cell dysfunction in chronic viral hepatitis and linked to persistent antigen stimulation and altered cellular metabolism. TEX integrate signals from the T cell receptor and multiple inhibitory receptors (e.g., PD-1). The consequences of PD-1 signaling on the metabolism of exhausted T cells are profound, and include rapid regulation of glycolysis and attenuation of TCR-driven mTOR and Akt signaling. Thus, TCR signaling and coregulatory signaling are involved in the regulation of metabolic pathways at various levels. However, the signaling determinants of distinct exhausted CD8⁺ T cell subsets in chronic HCV infection remain unclear.

Methods

To investigate the signaling dynamics and heterogeneity of CD8⁺ T cell subsets in chronic HCV, we established a highly multiplexed phosphoflow mass cytometry panel comprising 21 signaling proteins. To assess the time-dependent signaling response, PBMCs from chronic HCV patients were stimulated with CD3/CD28 for 0-60 minutes.

Results

We used time-lapse phosphoflow mass cytometry to examine signaling interactions at the subset level. In cHCV patients, PD1⁺CD127⁺ TPEX and PD1⁺CD127⁻ TEX cells showed distinct proximal TCR signaling. Baseline conditions already showed reduced pPLCγ activity, calcium signaling (pNFATc1) in TEX cells. Furthermore, TCR stimulation with CD3/28 elicited a diminished signaling response in TEX cells relative to TPEX cells.

Conclusion

By establishing a multiplexed, time-resolved phosphoflow mass cytometry platform we were able to perform a detailed dissection of dynamic signaling network in CD8+ T cells subsets of chronic HCV samples. This approach revealed a different signaling architectures in HCV-specific TPEX and TEX. Our findings demonstrate that exhausted CD8+ T cells display altered signaling, which likely contributes to their metabolic dysregulation and functional impairment.