(PS2308) CO-RECEPTOR ATTENUATION DEFINES A CONVERGENT TRANSCRIPTIONAL STATE OF LONG-TERM PERSISTING CAR-T CELLS DRIVEN BY INTERMITTENT ANTIGEN STIMULATION

Topic: 25. Gene therapy, cellular immunotherapy and vaccination - Biology & translational research

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Background

Long-term CAR-T persistence after CD19-directed therapy is associated with durable clinical responses, however the cellular states that sustain persistence and the stimuli that drive them remain undefined. Post-infusion CAR19 dynamics have been characterized within single diseases or constructs, but whether long-term persisting CAR-T cells converge on shared adaptive programs across clinical contexts is unknown. While chronic antigen stimulation induces exhaustion, CAR19 cells likely encounter intermittent antigen exposure following disease eradication as evidenced by sustained deep B-cell aplasia reflecting ongoing deletion of regenerating B-cell precursors. The adaptive states induced by such stimulation are unknown.

Aims

To define the characteristics of long-term persisting CAR-T cells and determine the mechanisms driving their adaptive states.

Methods

We analyzed 10 patients from two cohorts (7 LBCL, 3 CLL) treated with distinct CD19 CAR-T products (CD28 vs 4-1BB costimulatory domain), who remained in complete remission with persistent CAR-T cells (19–55 months, ongoing B-cell aplasia). Rare (<1%) long-lived CAR-T cells were sorted 10–40 months post-infusion and profiled using multimodal single-cell sequencing (RNA, surface proteins, TCR). Transcriptional programs were compared across diseases and constructs, and lineage relationships inferred by TCR tracking. Repetitive stimulation assays modeled intermittent antigen exposure in vitro, followed by high-dimensional flow cytometry and functional assays.

Results

Long-lived CAR-T cells from patients with LBCL and CLL clustered distinctly from endogenous immune cells and converged on a shared transcriptional program despite differing constructs and disease contexts. This state was characterized by a T-effector memory (TEM) phenotype, differential expression of GZMK, FXYD2, CD95, HMOX1, and GYPC, among others, and enrichment of interferon-γ response, oxidative phosphorylation, and cell cycle pathways. Comparison with independent single-cell datasets from children with ALL treated with obe-cel confirmed reproducibility of this signature across clinical settings.
Notably, a hallmark of this convergent program was co-receptor attenuation. Long-lived CAR-T cells in CLL were predominantly $CD4^-CD8^-$ (double negative, DN), whereas LBCL samples showed heterogeneity ranging from DN to CD4-low states. The shared transcriptional profile resembled naturally occurring $TCR\alpha\beta^+$ DN T cells in healthy blood. TCR lineage tracing demonstrated minimal overlap between peak expansion and long-term persisting clones. However, DN CAR-T cells shared TCR sequences with endogenous $CD4^+$ or $CD8^+$ populations, consistent with co-receptor downregulation rather than a distinct developmental lineage. Gene regulatory network analysis identified strong MYB regulon activity across cohorts.
In vitro modeling of intermittent antigen exposure using huCAR19 led to progressive enrichment of DN CAR-T cells after day 30. These cells maintained a TEM phenotype, upregulated MYB and TCF7, displayed reduced exhaustion features compared with $CD8^+$ counterparts, and retained cytotoxicity with enhanced cytokine production at late timepoints.

Summary/Conclusion

Long-term persisting CAR-T cells across diseases and constructs converge on a shared co-receptor attenuated state arising from $CD4^+$ and $CD8^+$ T cells through adaptive downregulation. Intermittent antigen stimulation drives this adaptive program, generating less terminally differentiated, functionally preserved DN CAR-T cells.