CD8⁺ T CELL REPROGRAMMING TO BOOST ANTI-HIV POTENTIAL

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NIAID/NHLBI/NIDDK/NINDS/NIDA **1UM1AI164562-01**

Our results demonstrate that targeting the signaling pathways Wnt/TCF-1 and mTORC to reprogram HIV-specific CD8⁺ T cells from non-controllers promotes superior response to antigen, enhanced survival, polyfunctionality, metabolic plasticity, and better response to γ-chain cytokines.

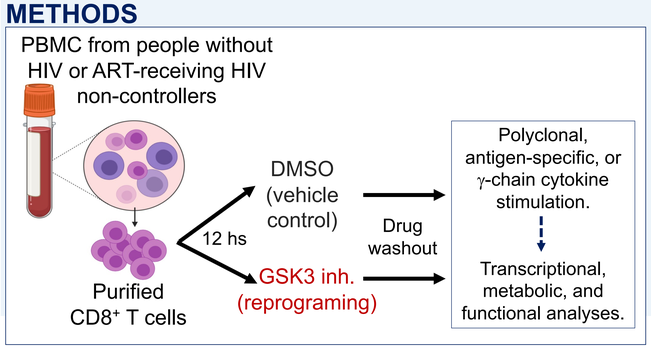
BACKGROUND

- HIV-specific CD8⁺ T cells from HIV controllers have an imprinted stem-like profile, characterized by enhanced polyfunctionality, survival, and metabolic plasticity.
- The development and maintenance of such qualities thus appear crucial to achieving natural HIV-1 control.
- The signaling pathways Wnt/TCF-1 and mTORC regulate stemness properties of CD8⁺ T cells, including longevity, self-renewal, and the potential to differentiate into multiple subsets
- We hypothesized that reprogramming HIV-specific CD8⁺ T cells from non-controllers by targeting these signaling pathways could boost their antiviral potential.

AIM

To evaluate the potential of CD8⁺ T cell reprogramming for improving the functional properties in cells derived from non-controller individuals, via the manipulation of the TCF-1 and mTORC pathways.

METHODS



RESULTS

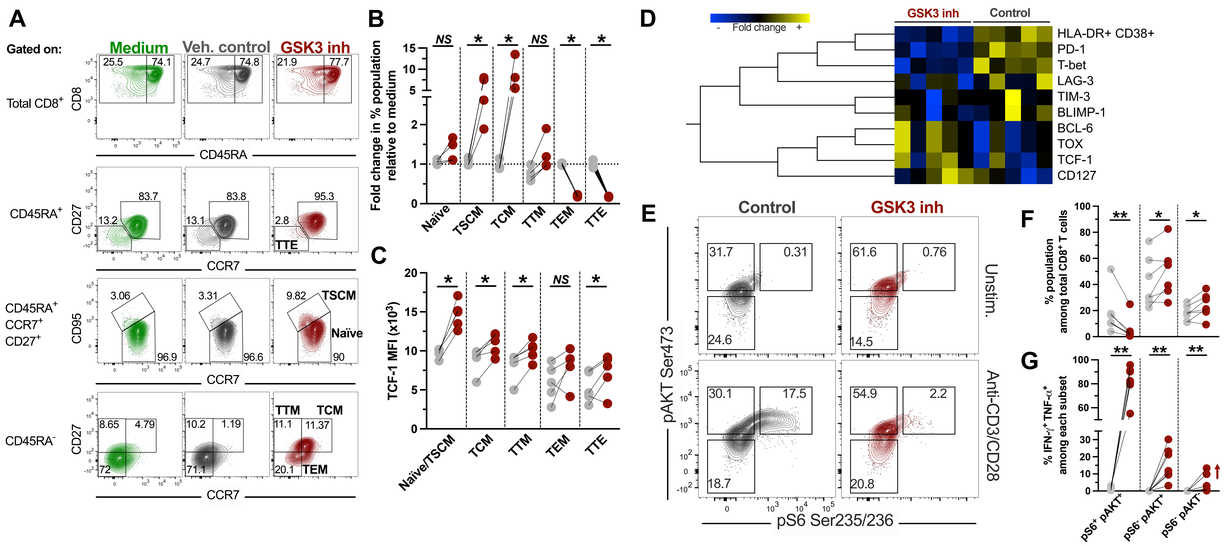


Figure 1. Induction of polyfunctional stem-like CD8⁺ T cells by reprogramming. A. CD8⁺ T cells from people without HIV were incubated with medium, vehicle control, or a GSK3 inhibitor, for 12 hs and the phenotype was evaluated by flow cytometry. **B.** Fold change of CD8⁺ T cell subpopulations after treatment with vehicle control or a GSK3 inhibitor, relative to medium alone condition (n=4). **C.** Expression of TCF-1 in CD8⁺ T cell subpopulations (n=4). **D.** The expression of the indicated markers in cells pre-treated with vehicle control or a GSK3 inhibitor was evaluated by flow cytometry. The fold change in the expression induced by 48 hs anti-CD3/CD28 stimulation, relative to unstimulated cells, is shown (n=5). **E.** Flow cytometry analysis of the expression of pS6 (mTORC1 marker) and pAKT (mTORC2 marker) in total CD8⁺ T cells after vehicle control or GSK3 inhibitor treatment, followed by resting or anti-CD3/CD28 stimulation. **F.** Frequencies of pS6⁺ pAKT⁺, pS6⁻ pAKT⁺ and pS6⁻ pAKT⁻ subsets among total CD8⁺ T cells (n=7). **G.** Frequency of IFN-γ⁺ TNF-α⁺ cells among the indicated subsets (n=7). *P<0.05; **P≤0.01. NS: Not statistically significant; Wilcoxon test. TSCM: Stem cell memory; TCM: Central memory; TTM: Transitional memory; TEM: Effector memory; TTE: Terminal effector.

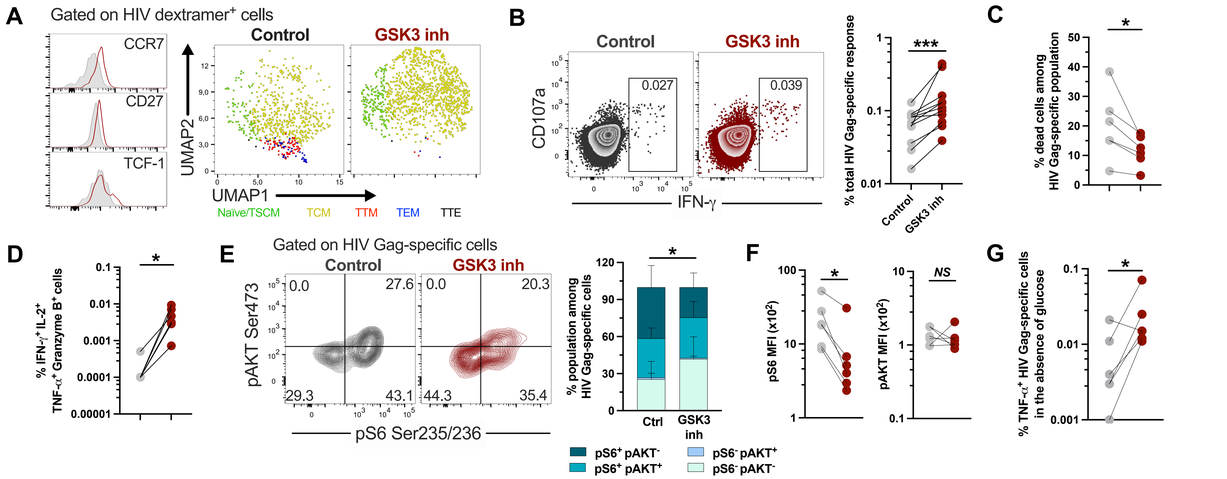


Figure 2. Enhanced functionality, survival, and metabolic plasticity of reprogrammed HIV-specific CD8⁺ T cells. A. Left: Expression of CCR7, CD27 and TCF-1 in non-reprogrammed (gray histograms) and reprogrammed (red histograms) HIV dextramer⁺ CD8⁺ T cells. Right: UMAP plots generated from HIV dextramer⁺ CD8⁺ T cells after data concatenation (n=4) showing CD8⁺ T cell subpopulations in the respective conditions. **B.** Frequency of HIV Gag-specific CD8⁺ T cells after vehicle control or GSK3 inhibitor treatment (upon Gag peptides stimulation for 6 hs; n=11). **C.** Frequency of dead HIV Gag-specific CD8⁺ T cells after Gag peptides stimulation for 6 days (n=6). **D.** Frequency of polyfunctional CD8⁺ T cells after Gag peptides stimulation (n=6). **E-F.** Expression of pS6 and pAKT in HIV Gag-specific CD8⁺ T cells. **G.** Frequency of TNF-α⁺ HIV Gag-specific CD8⁺ T cells after Gag peptides stimulation for 6 hs in the absence of glucose (n=6). *P<0.05; ***P≤0.001. NS: Not statistically significant. Wilcoxon test.

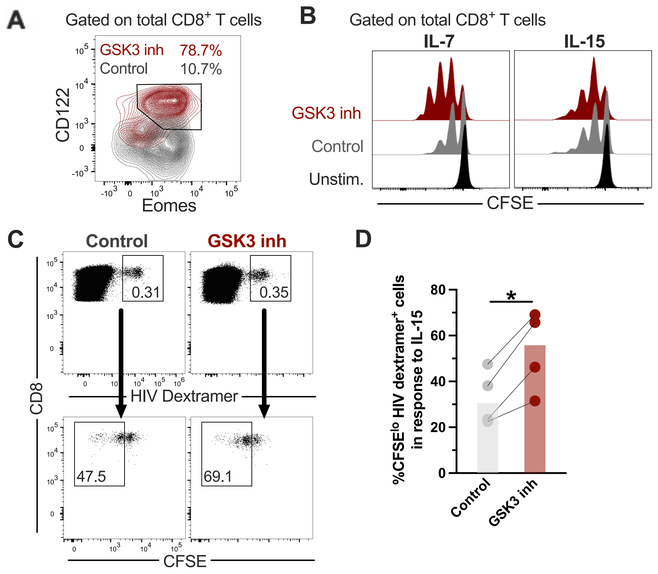


Figure 3. Superior response to γ-chain cytokines by reprogrammed HIV-specific CD8⁺ T cells. A. Expression of Eomes and CD122 in total CD8 T cells after vehicle control or GSK3 inhibitor treatment. **B.** Proliferation of total CD8⁺ T cells from a person without HIV after vehicle control or GSK3 inhibitor treatment, followed by stimulation with IL-7 or IL-15 for 6 days. **C-D.** Proliferation of HIV dextramer⁺ CD8⁺ T cells from a HIV-1 non-controller individual, after vehicle control or GSK3 inhibitor treatment, followed by stimulation with IL-15 for 6 days. *P<0.05. Wilcoxon test.

CONCLUSIONS

- *Ex vivo* treatment of virus-specific CD8⁺ T cells from non-controllers with a GSK3 inhibitor resulted in reprogramming and acquisition of phenotypic, transcriptional, metabolic, and functional attributes associated with natural control of HIV-1.
- Such CD8⁺ T cell reprogramming, combined with other available immunomodulators, might represent a promising strategy for adoptive cell therapy in the search for an HIV-1 cure.

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Acknowledgements:
This work was funded by ANRS, and NIH UM1AI164562.