Linking TCR αβ Chains to Costimulatory Signaling Domains Enhances HIV-Specific CD8 T-Cell Function

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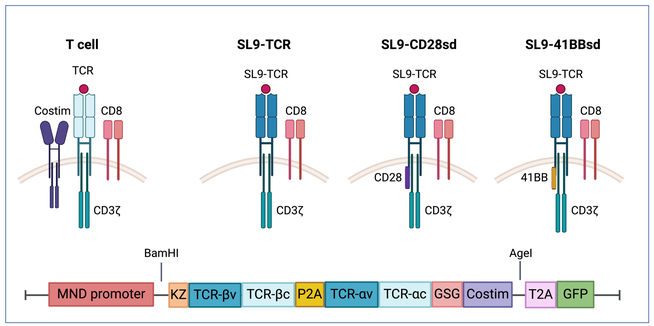
BACKGROUND

The addition of costimulatory signaling domains to CAR constructs has dramatically increased the functional capacity of CAR-T cells. We hypothesized that we could augment the functional anti-HIV activity of cytotoxic CD8 T cells engineered to express HIV-specific TCRs used as an adoptive therapy to provide a functional HIV cure by incorporating the intracellular activation domain of costimulatory signaling domains into the TCR construct, termed TCR-costim.

METHODS

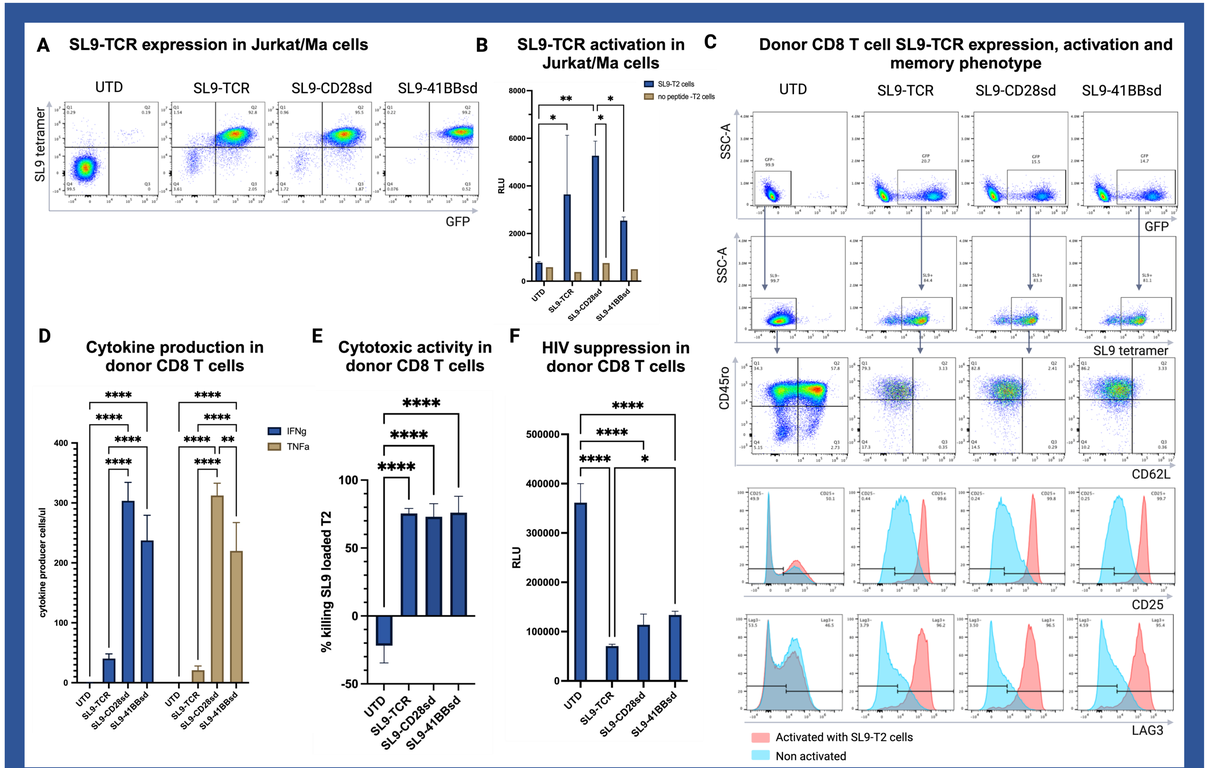
- **Lentiviral vector construction**

We constructed a lentiviral vector (LV) encoding chimeric α and β TCR chains containing the HIV-1 Gag epitope SL9-specific human variable TCR region fused to the murine constant region to prevent TCR mispairing with the endogenous TCR. We added the signaling domain (sd) of either CD28 (SL9-CD28sd) or 41BB (SL9-41BBsd) to the α TCR chains, and Green Fluorescent Protein (GFP). Endogenous CD8 and CD3 signal transducing chains including CD3ζ will be recruited by the TCR.



- **In vitro functional assays:**

Jurkat/MA-NFAT-luciferase reporter (Jurkat/Ma) cells were transduced with the LV to evaluate SL9-TCR activation by quantification of the luciferase reporter. Primary CD8 T cells were activated with αCD3/CD28 for transduction. SL9-TCR expression in CD8 T cells from seronegative donors was determined by tetramer staining; and their cytotoxic activity, activation, and IFNγ and TNFα production by flow cytometry. Their HIV suppression capacity was determined by co-culture with HIV-luciferase infected CD4 T cells.



RESULTS

A. TCR-costim LV constructs were successfully expressed in Jurkat/Ma cells. TCR-costim LV transduction efficiency reached over 95%, of which 95-99% expressed SL9-TCR.

B. SL9-CD28sd displays the highest TCR activation. Jurkat/Ma cells expressing NFAT-regulated luciferase reporter gene transduced with SL9-CD28sd LV demonstrated 1.47 or 2.2-fold greater TCR activation than SL9-TCR or SL9-41BBsd, respectively, after coculturing with SL9-peptide loaded T2 cells.

C. Donor CD8 T cells expressing TCR-costim are specifically activated and differentiated into effector memory T cells. Donor CD8 T cells transduced with the indicated LV (GFP+, 15-30% transduction efficiency) expressed SL9-TCR in ~80% of the transduced cells and had an effector memory (EM) (CD62L- CD45Ro+) phenotype. Activation was demonstrated by increased LAG3 and CD25 expression of TCR-costim transduced CD8 T cells incubated with SL9-loaded T2 cells and not T2 cells alone.

D. SL9-CD28sd and SL9-41BBsd T cells produce more IFNγ or TNFα than SL9-TCR CD8 T cells. Donor CD8 T cells expressing SL9-TCR followed by CD28sd or 41BBsd produce 10 or 8.3-fold increased IFNγ and TNFα, respectively, compared as SL9-TCR alone after SL9-peptide presentation by T2 cells.

E. SL9-TCR and TCR-costim CD8 T cells display similar cytotoxicity. The SL9-T-TCR +/- CD28sd or 41BBsd CD8 T cells killed >75% of the SL9-loaded T2 cells as compared to T2 cells at a 1:10 E:T ratio.

F. SL9-TCR and TCR-costim CD8 T cells suppress HIV suppression equivalently. SL9-TCR and TCR costim CD8 T cells recognized HIV infected CD4 T cells and markedly suppressed HIV infection at similar levels, as compared to untransduced CD8 T cells (UTD) that do not express SL9-TCR.

CONCLUSIONS

- Incorporating **CD28sd** with the SL9-TCR construct provides **increased T cell activation through the TCR**.
- TCR-costim CD8 T cells present an **EM phenotype** which plays a crucial role in providing an immediate response to limit the spread of infection.
- Activation of the engaged TCR and second costimulatory signaling solely by engagement of the TCR-peptide-MHC complex **increased the cytokine production of SL9-CD28sd and SL9-41BBsd** HIV-specific CD8 T cells.
- Adding CD28sd and 41BBsd to TCR does not change cytotoxicity and HIV suppression capability, suggesting the pre-activation of CD8 T cells with αCD3/CD28 for transduction may reduce the additive effect of adding the costim-sd to the TCR due to their EM phenotype.
- TCR activation requires >100-fold fewer antigen engagements than CAR-T cells, indicating **TCR adoptive transfer therapy could be effective** for recognizing and **killing latent HIV** infected cells which may express lower levels of HIV proteins than detectible by CAR-T cells.

FUTURE EXPERIMENTS

- Measuring cytotoxicity and HIV suppression later after attenuation of αCD3/CD28 activation might identify differences between adding costim-sd to the SL9-TCR.
- Expressing CD28sd and 41BBsd together, as multiple copies or with sd from other costimulatory molecules, such as OX40, GITRL, ICOS, CD27, may potentiate anti-HIV CD8 T cells functionality as compared to TCR alone.

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