REPRODUCING CMV-SPECIFIC CD8+ T CELLS TO TARGET HIV USING TCR GENE TRANSFER

April L Mueller[1], Scott Garforth[1], Steven Almo[1] Harris Goldstein[1]
[1]Albert Einstein College of Medicine, New York, NY, USA

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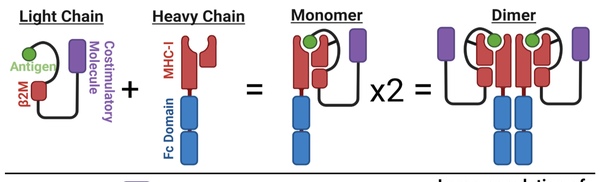
BACKGROUND

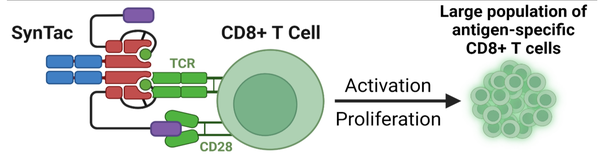
To leverage the **memory inflation** phenotype displayed by some cytomegalovirus (CMV)-specific CTL, we generated **CMV/HIV-bispecific CTL** by selectively transducing CD8 T cells specific for a CMV epitope (NLV) with a **lentivirus** encoding a **T cell receptor (TCR)** against an HIV epitope (SL9). We developed a strategy to efficiently generate bispecific CTL with reactivity towards two epitopes; **NLV(CMV) and SL9(HIV).**

This was accomplished by selectively transducing NLV-specific CTL with lentivirus after specific activation with a novel immunotherapeutic termed **synthetic T cell activation (synTac)**. We hypothesize that the memory inflation phenotype of CMV-specific CTL host cell will enable the generation of CMV/HIV-bispecific CTL displaying prolonged viability *in vivo* with anti-HIV functionality while being resistant to exhaustion.

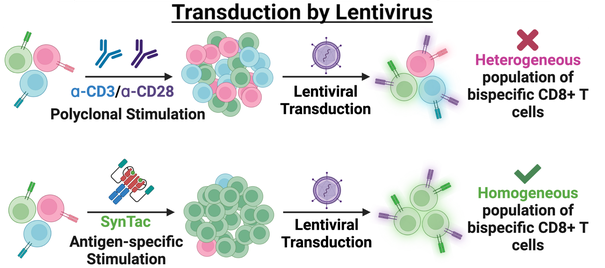
METHODS

SynTac Design Mimics the Immunological Synapse



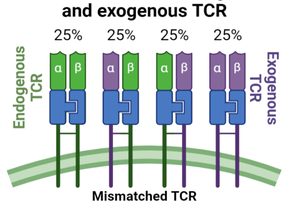


SynTac Stimulation Enables Antigen-Specific CTL Transduction by Lentivirus

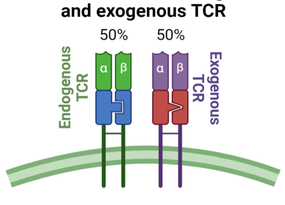


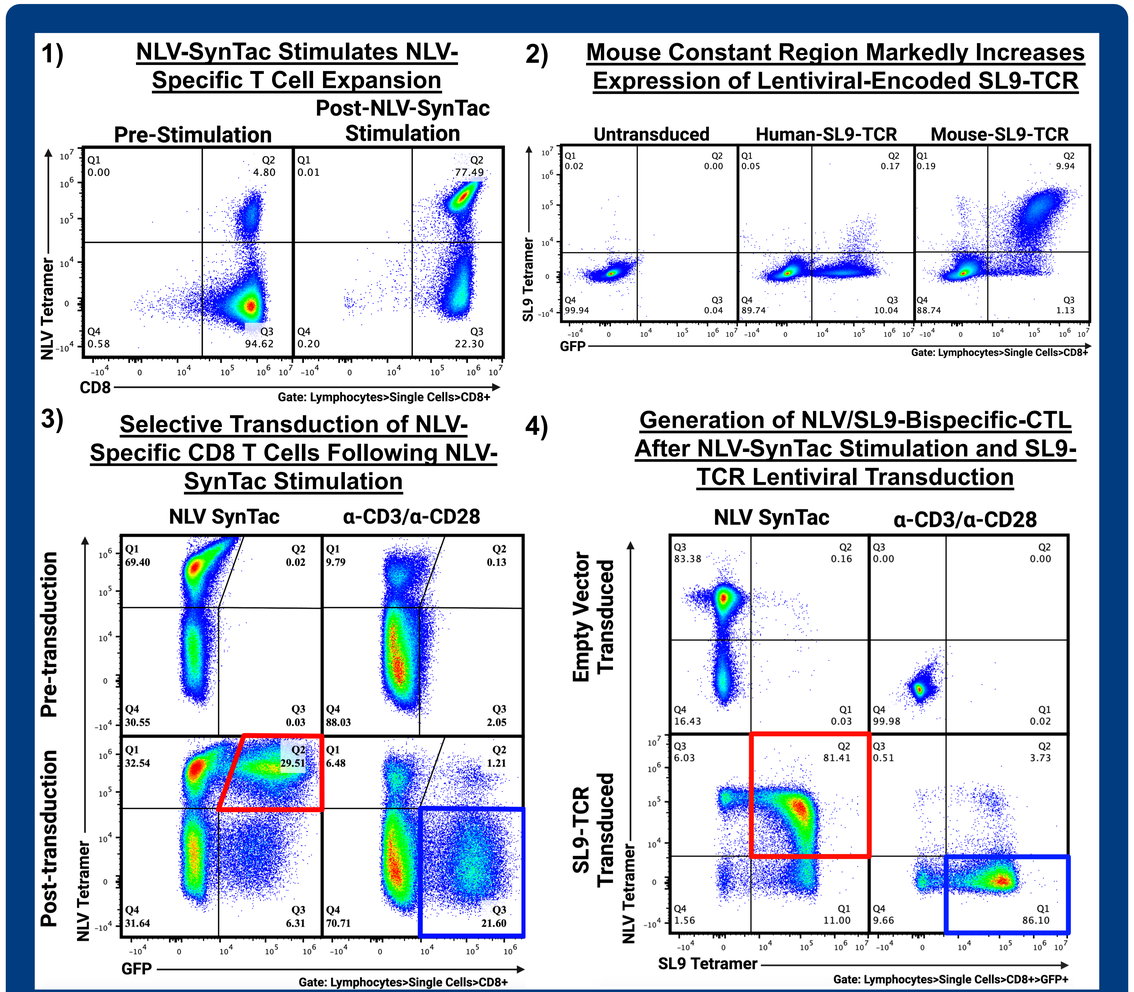
Chimeric TCR with Human Variable and Murine Constant Region Prevents TCR Mismatch

Human constant region permits mismatch between endogenous and exogenous TCR



Mouse constant region restricts mismatch between endogenous and exogenous TCR





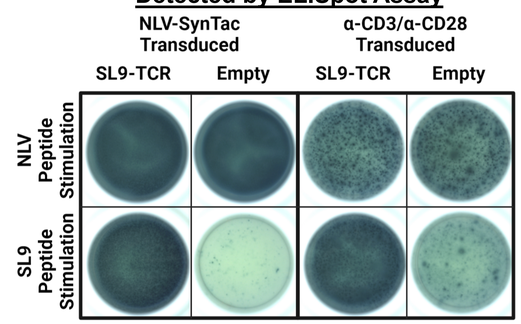
RESULTS

1. **NLV-SynTac enables selective activation and expansion of NLV-specific CTL.** 7-day stimulation of isolated CD8+ cells from donor-derived PBMCs by NLV synTac (0.01nM) results in >10-fold expansion of NLV-specific CTL.
2. **Use of mouse-constant region TCR lentivirus but not human-constant region lentivirus enables highly efficient expression of SL9-TCR in transduced cells.** SL9-TCR is expressed on the surface of CD8+ T cells transduced with a lentivirus encoding SL9-specific with a mouse constant regions which prevents mispairing with endogenous TCR alpha and beta chains.
3. **NLV-SynTac stimulation enables selective transduction by lentivirus.** Stimulation of CD8+ cells by NLV synTac prior to transduction by the empty lentiviral vector results in selective transduction of NLV+ cells (red box), compared to polyclonal stimulation by anti-CD3/anti-CD28 followed by transduction with the same viral vector (blue box).
4. **Generation of bispecific CD8+ T cells.** Donor derived CD8+ cells isolated from PBMC by magnetic bead separation are stimulated by NLV synTac prior to transduction by lentivirus, resulting in generation of T-cells specific for NLV and SL9 as detected by tetramer staining (red box) and flow cytometry. Cells stimulated by anti-CD3/anti-CD28 for 3 days prior to transfection yielded SL9+ but not NLV+ TCR cells (blue box).

RESULTS

5. **Preliminary Data Depicting ELISpot Assay Results.** Donor derived CD8+ cells isolated from PBMCs were transduced with either experimental vector or empty control vector after stimulation with either SynTac or anti-CD3/anti-CD28. 5 days after transduction cells were used for ELISpot assay against NLV or SL9, plated at 250K cells per well and stimulated with 10uM peptide. Images displayed are representative of triplicate wells.

5) NLV/SL9-bispecific T Cells Display IFN-g Response to both NLV and SL9 peptide Detected by ELISpot Assay



CONCLUSIONS

- ✓ **The synTac platform enables efficient antigen-specific activation, expansion, and transduction of CD8+ T cells.**
- ✓ **Functional bispecific-CTL are generated from primary CD8+ T cells using a mouse-constant regions based lentivector.**

Next steps: Test the cytotoxic capabilities of bispecific-CTL cells against peptide-loaded T2 cells, HIV and/or CMV infected cells, assess the memory inflation phenotype of the bispecific-CTL cells using a multiple stimulation assay, and evaluate the ability of these cells to suppress HIV infection in vivo in a humanized mouse model.

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