Development of a Humanized Mouse Model for Evaluation of Autologous HIV-Specific T-cell Therapies

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Background

- Immunotherapy with *ex vivo* expanded HIV-Specific CD8⁺ T-cells (HSTs) may contribute to functional or sterilizing cures.
- A small animal model would enable the rapid and cost-effective pre-clinical evaluation of multiple T-cell therapies.
- Currently, humanized mouse models of HIV infection rely on fetal human stem cell and organ tissue transplants.
  - Key limitations:
    - Inability to test T-cell therapy products from HIV-infected individuals against **autologous graft**
    - Experimental timelines limited by graft versus host disease (GvHD)
    - Expensive and labor/resource intensive
- **Our approach:** Engraft NSG mice with only the **memory** subset of CD4⁺ T-cells from ARV-treated HIV-infected donors
  - Allows for testing T-cell therapy products from HIV-infected donors in autologous setting
  - Use of **memory** vs **total** CD4⁺ T-cells greatly mitigates GvHD allowing for prolonged experimental timelines
  - Model shows sustained viral loads along with CD4⁺ T-cell depletion, and responsiveness to ARV therapy

Methods

- NSG mice were engrafted with 5-10x10^6 memory CD4⁺ T-cells isolated from HIV- (model characterization) or HIV+ (HST evaluation) donor leukapheresis samples.
- Autologous HIV-Specific T-cells were generated by stimulating T-cells with pools of overlapping Clade B consensus peptides spanning Gag, Pol, and Nef (**HST**) or peptides representing only conserved epitopes (**HSTconsv**; Ondondo et al, *Mol Ther*, 2016).
- Four to six weeks post humanization, mice were simultaneously infected with HIV-JRCSF and treated with autologous HSTs.
- Weekly blood samples were analyzed by flow cytometry to measure changes in the human CD4⁺/CD8⁺ levels as well as qRT-PCR to measure plasma viral load. Plasma RNA was subsequently sequenced for the presence of viral escape mutations.

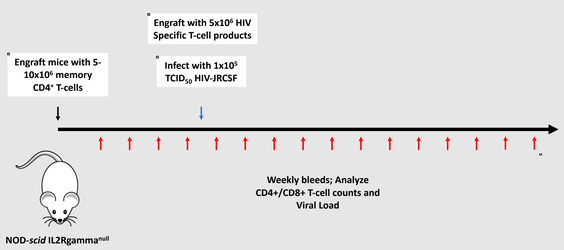


Figure 1. Schematic of *in vivo* analysis of HSTs in memory CD4⁺ T-cell humanized mouse model.

Results

Characterization of Memory CD4⁺ T cell Humanized Mouse Model of HIV Infection

- Mice engrafted with only the memory CD4⁺ T-cell subset survived significantly longer than mice engrafted with Total CD4⁺ T-cells. (**Figure 2**).

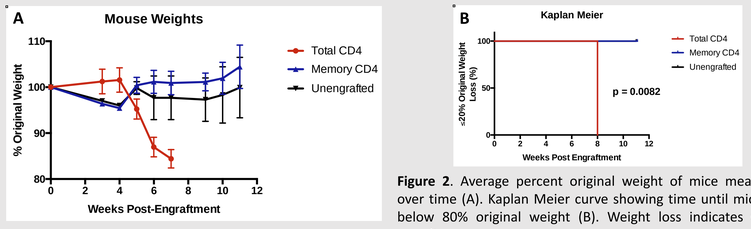


Figure 2. Average percent original weight of mice measured over time (A). Kaplan Meier curve showing time until mice fell below 80% original weight (B). Weight loss indicates GvHD severity.

- Mice engrafted with memory CD4⁺ T-cells were able to support robust HIV infection with rapid viral expansion reaching a set point of ~1x10^6 HIV RNA copies/ml blood sustained out to 20 weeks post engraftment (**Figure 3**).

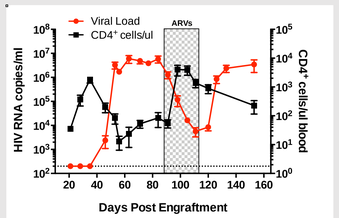


Figure 3. Plasma viral load and CD4⁺ T-cell counts of mice engrafted with memory CD4+ T-cells and infected with 1x10^5 $TCID_{50}$ HIV-JRCSF (n=5). Checkered box represents time in which mice were administered daily injections of ARV cocktail.

- Daily ARV injections resulted in viral suppression and CD4⁺ T-cell reconstitution, followed by viral rebound and subsequent CD4⁺ T-cell loss upon ARV cessation (**Figure 3**).

In Vivo Evaluation of HIV-Specific T cell Therapies

- Injection of autologous HSTs resulted in significant, sustained decreases in plasma viral load compared to No Treatment (p<0.0001; **Figure 4**).

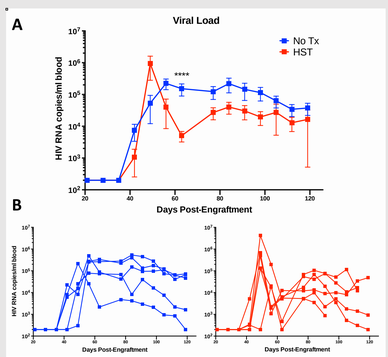


Figure 4. Plasma viral loads of memory CD4⁺ T-cell engrafted mice infected with HIV-JRCSF and treated with HIV-Specific T-cells; plotted as group averages (A) and individual mice (B).

In Vitro Evaluation of HSTs vs. HSTconsv

- HSTs and HSTconsv demonstrate similar degrees of recognition and killing of HIV-infected cells *in vitro* (**Figure 5**).

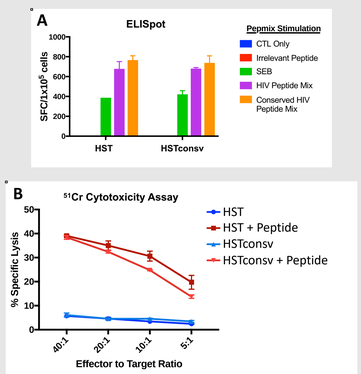


Figure 5. ELISpot data measuring INF-γ release from HST or HSTconsv stimulated with various antigens (A). Results from ^{51}Chromium release assay demonstrating similarly efficient killing of peptide-pulsed cells by HSTs and HSTconsv (B).

Results

HSTconsv Therapy Demonstrates Increased Viral Suppression *in vivo* Compared to HSTs due to Reduced Viral Escape

- 33% (1/3) of mice receiving HSTs and 66% (2/3) of mice receiving HSTconsv had undetectable viral loads 4 weeks post therapy initiation.
- Both mice in the HST group with detectable plasma viremia had viral escape mutations in the TW10 epitope of Gag, which were not present in mice receiving HSTconsv or controls.

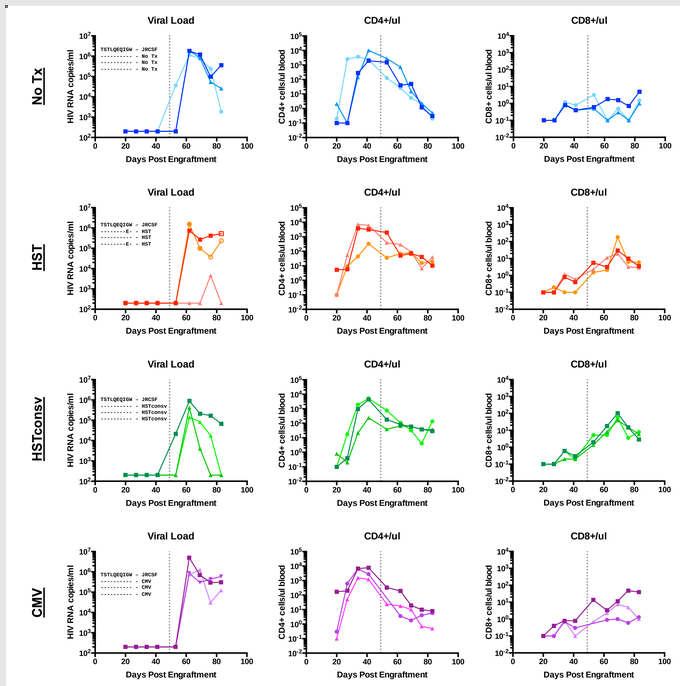


Figure 6. Viral Load (qRT-PCR) and CD4⁺/CD8⁺ T-cell counts (flow cytometry) of memory CD4⁺ engrafted mice infected with HIV-JRCSF and treated with HIV-specific T-cell products or controls (grey dotted line). Insets show sequencing results of escape mutation variants found only in the HST group (open symbols).

Conclusions

1) We have demonstrated that our novel memory CD4⁺ T-cell humanized mouse model accurately recapitulates many aspects of natural HIV infection while significantly reducing the effects of GvHD.
2) Using this model, we have observed significant decreases in viral load in mice receiving clinically relevant HIV-specific T-cell products.
3) This platform provides opportunities to assess a variety of immunotherapeutic strategies as well as immunomodulatory approaches in an *in vivo*, autologous target/effector setting.
4) Results from these studies could better inform future human clinical trials using patient-derived T-cell therapies.