Targeting IL-15 to CD8 Treg results in their selective expansion and activation and is a potential therapeutic approach to ameliorate autoimmune disease in patients with deficient CD8 Treg populations

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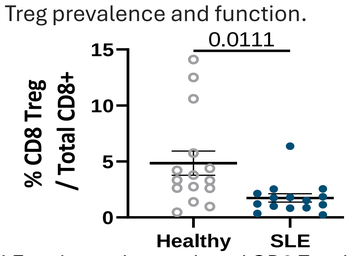
MOZART THERAPEUTICS

Orchestrating The Immune System

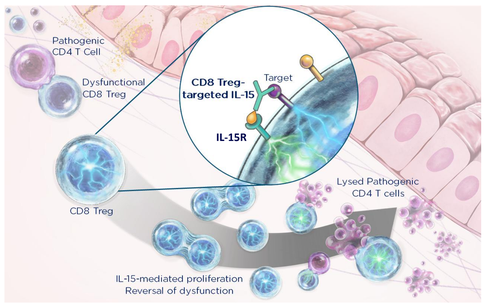
Introduction

CD8 regulatory T cells (CD8 Treg) are characterized by their capacity to kill self-reactive and pathogenic CD4 T cells. Control of pathogenic CD4 T cells appears impaired in autoimmune disease, in part due to reduced CD8 Treg prevalence and function.



SLE patients show reduced CD8 Treg levels

We developed an IL-15 mutein with reduced potency tethered to a targeting antibody to promote selective expansion, activation, and cytolytic capacity of CD8 Treg, with the potential of ameliorating disease. Here we demonstrate that the targeted IL-15 mutein induced selective signaling in CD8 Treg in a pSTAT5 flow cytometry assay using PBMC from healthy donors and SLE patients. Targeted IL-15 mutein also selectively expanded and increased the prevalence of CD8 Treg in healthy donor PBMCs. Using immunocompetent and humanized acute graft versus host disease mouse models, we observed expansion of CD8 Treg as well as increased survival in mice receiving targeted IL-15 mutein.



A targeted IL-15 mutein expands and activates the CD8 Treg population to reduce pathogenic cells

Methods

- Flow cytometry and RNA sequencing were used to characterize CD8 Treg incubated with common gamma chain cytokines and targeted IL-15 muteins
- IL-15 muteins were created by introducing point mutations in wild-type IL-15 (wtIL-15), resulting in mutein variants designated IL-15v0 through IL-15v7 in this poster
- IL-15 muteins were fused with nine different CD8 Treg targeting antibodies, designated T1 through T9, to create CD8 Treg targeted IL-15 muteins
- pSTAT5 signaling titrations and Ki67 proliferation assays were used to assess targeted IL-15 mutein activity across PBMC immune cell subsets
- Human pancreatic organoids were incubated with peptide-stimulated PBMCs from a type-1 diabetes patient to evaluate the effects of targeted IL-15 mutein on the CD8 Treg and Beta cell populations
- The highly inflammatory acute GvHD mouse model was used to assess the effects of targeted IL-15 mutein *in vivo*

Wild type IL-15 expands and activates CD8 Tregs *in vitro*

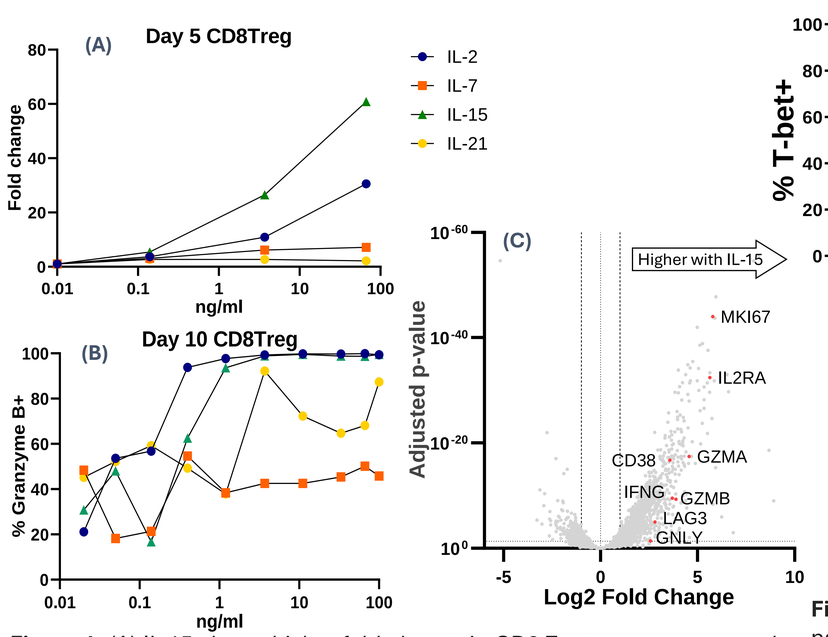


Figure 1. (A) IL-15 shows higher fold change in CD8 Treg counts compared to other γ-chain cytokines after 5 days of culture. (B) IL-2 and IL-15 show highest increase in %Granzyme B+ CD8 Tregs at day 10 of culture. (C) DEG analysis of flow sorted CD8 Treg shows various activation genes upregulated by culture with IL-15 compared to cells in plain culture media.

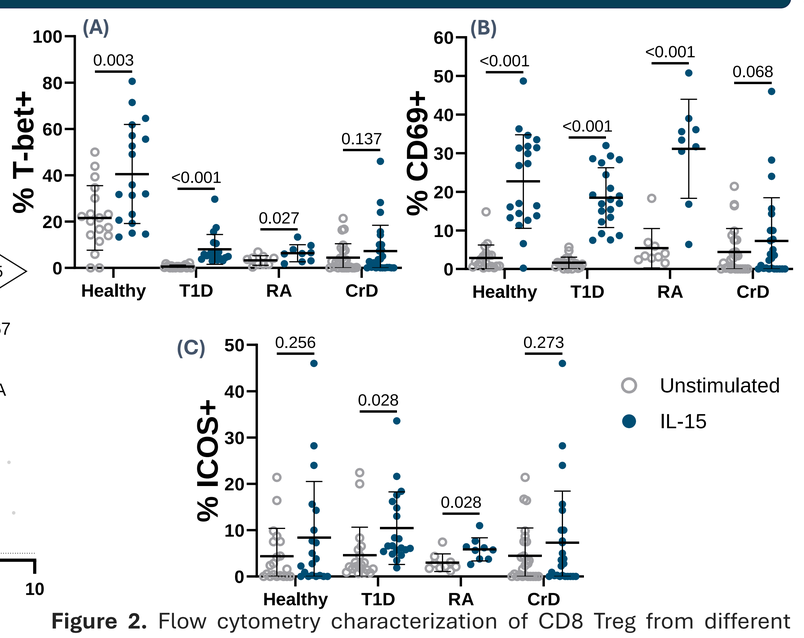


Figure 2. Flow cytometry characterization of CD8 Treg from different patient populations shows increased expression of (A) T-bet, (B) CD69, and (C) ICOS with IL-15 incubation for 24 hours. CD8 Treg from Type 1 Diabetes and Rheumatoid Arthritis patients show statistically significant increases of all three markers. CD8 Treg from Crohn's Disease patients show a trend of increased expression of all three markers

Targeted IL-15 mutein increases survival in a GvHD model

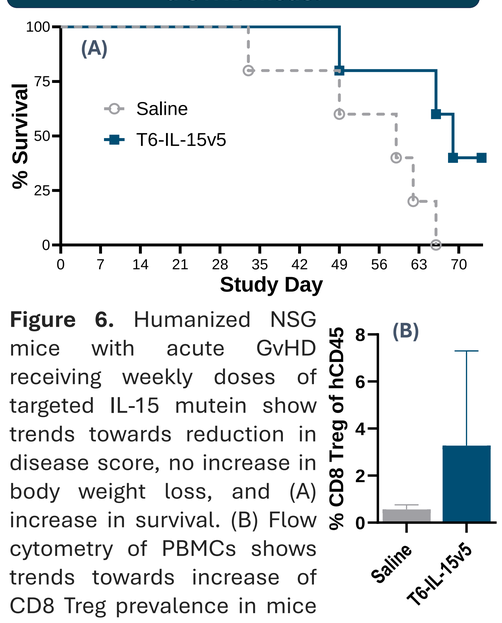


Figure 6. Humanized NSG mice with acute GvHD receiving weekly doses of targeted IL-15 mutein show trends towards reduction in disease score, no increase in body weight loss, and (A) increase in survival. (B) Flow cytometry of PBMCs shows trends towards increase of CD8 Treg prevalence in mice receiving CD8 Treg targeted IL-15 mutein

Targeting IL-15 mutein to a CD8 Treg surface antigen and using precise point mutations provides selective signaling in CD8 Treg

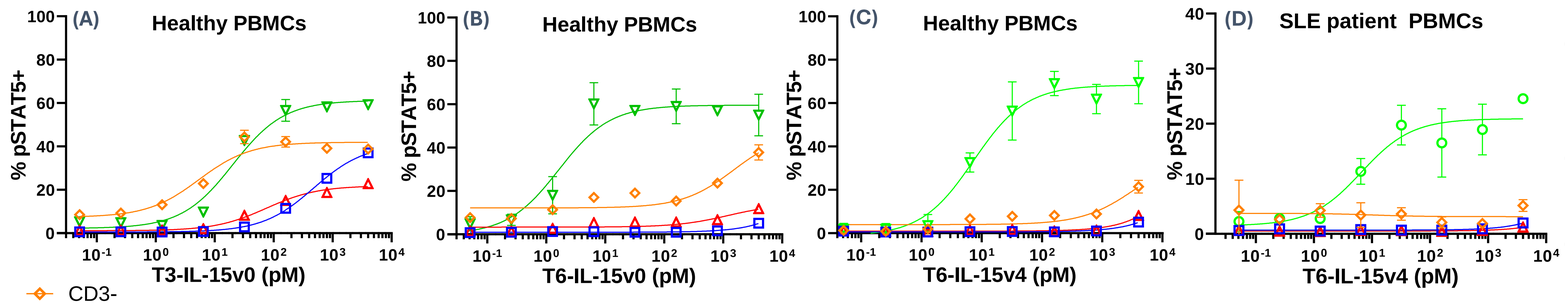


Figure 3. pSTAT5 titrations of mAb-fused IL-15 muteins in human PBMCs from healthy donor or SLE patient. Combinations of Targeting mAb (T1-T9) fused to IL-15 mutein variants (v0-v7) were tested. In healthy PBMCs, (A) IL-15v0 fused to anti-Target3 mAb (T3) shows high signaling in CD8 Treg and CD3- cells; (B) IL-15v0 fused to anti-Target6 mAb (T6) shows high signaling CD8 Treg and moderate signaling in CD3- cells. (C) T6-IL-15v4 shows selective signaling in CD8 Treg in healthy and (D) SLE patient PBMCs.

Targeted IL-15 mutein expands CD8 Treg *in vitro* with greater selectivity than wtIL-15

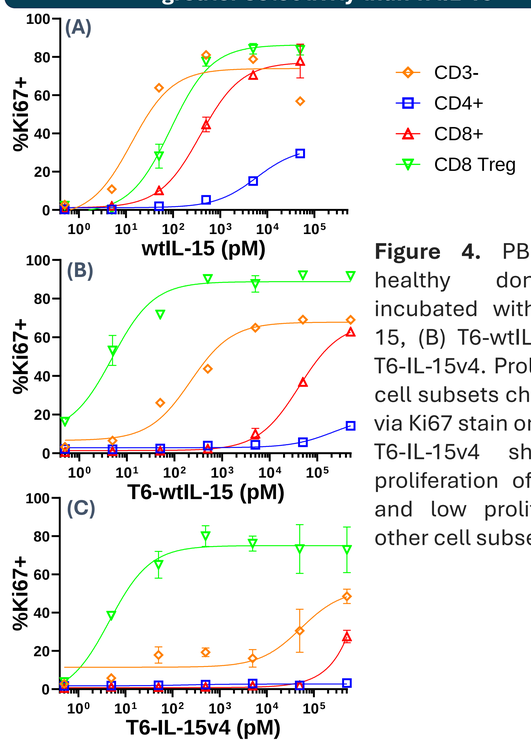


Figure 4. PBMCs from healthy donors are incubated with (A) wtIL-15, (B) T6-wtIL-15, or (C) T6-IL-15v4. Proliferation of cell subsets characterized via Ki67 stain on Day 7. T6-IL-15v4 shows high proliferation of CD8 Treg and low proliferation of other cell subsets.

Targeted IL-15 mutein expands CD8 Treg in a T1D organoid model and results in protection of Beta cells

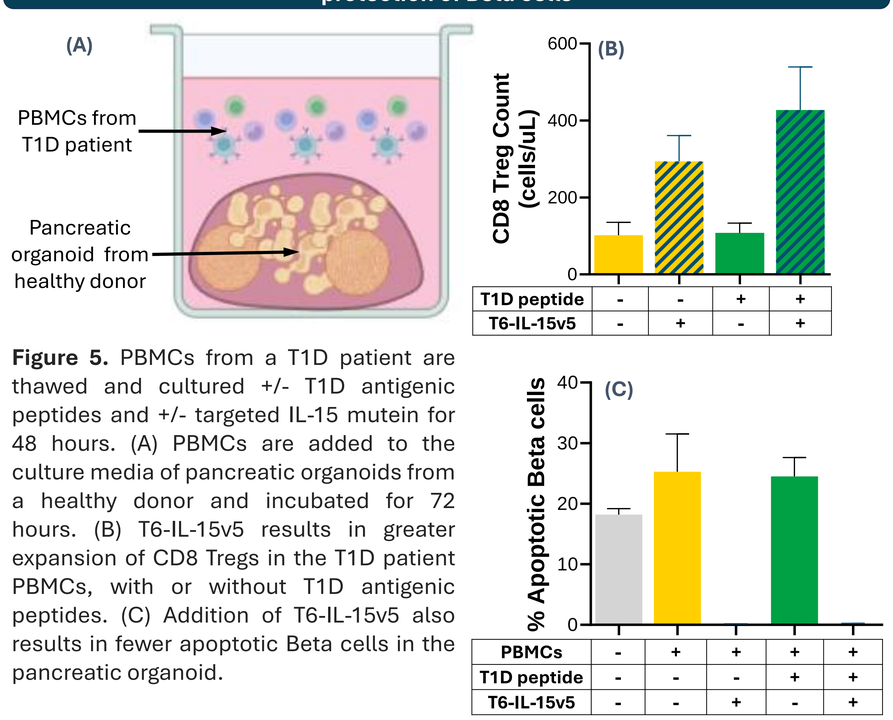


Figure 5. PBMCs from a T1D patient are thawed and cultured +/- T1D antigenic peptides and +/- targeted IL-15 mutein for 48 hours. (A) PBMCs are added to the culture media of pancreatic organoids from a healthy donor and incubated for 72 hours. (B) T6-IL-15v5 results in greater expansion of CD8 Tregs in the T1D patient PBMCs, with or without T1D antigenic peptides. (C) Addition of T6-IL-15v5 also results in fewer apoptotic Beta cells in the pancreatic organoid.

Results

- Wild-type IL-15 demonstrates higher potential for expansion and activation of the CD8 Treg population compared to other γ-chain cytokines
- Selective signaling of IL-15 mutein in the CD8 Treg population can be achieved by fusion to an antibody for an appropriate CD8 Treg surface marker
- Selective signaling of a CD8 Treg targeted IL-15 mutein can be enhanced with select combinations of point mutations
- CD8 Treg targeted IL-15 mutein demonstrates selective expansion of the CD8 Treg population over other PBMC subsets *in vitro*
- PBMCs incubated with CD8 Treg targeted IL-15 mutein show an increased CD8 Treg population and prevent Beta cell death in a human T1D pancreatic organoid model
- Humanized NSG mice with acute GvHD receiving CD8 Treg targeted Il-15 mutein show trends towards increased survival and CD8 Treg prevalence in peripheral blood

Conclusions

1. Targeted IL-15 mutein demonstrates selective signaling in the CD8 Treg population, resulting in their proliferation and expansion *in vitro*, *in vivo* and *ex vivo*.
2. In the presence of targeted IL-15 mutein, amelioration of disease symptoms and improved survival is observed *ex vivo* and *in vivo*.
3. Targeted IL-15 mutein may have therapeutic potential in autoimmune diseases characterized by a deficiency of the CD8 Treg population.

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