Department of Surgery, Comprehensive Transplant Center

Augmented Delivery of Regulatory T Cells for the Prevention of Transplant Rejection by Using Fucosylation

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Background

- Organ transplantation requires life-long administration of non-specific immunosuppressive agents, all of which have substantial side-effects
- Newer, more targeted therapies are needed that will allow for minimization of such agents
- Regulatory T cells (Tregs) are important mediators of immune homeostasis, promote transplantation tolerance in animals, and are elevated in tolerant human transplant recipients
- Donor-specific Tregs (Ds-Tregs) are more targeted and potent in their effects given they recognize donor-derived MHC:peptide complexes
- Fucosylation of Tregs has been shown to be more effective at graft homing and in preventing graft-versus-host disease in animal models and may improve the efficacy of Ds-Tregs when administered as an adoptive cell therapy

Research Objectives

- To determine the targeting and homing capacity of fucosylated vs non-fucosylated donor-specific Tregs *in vivo*
- To assess whether fucosylated Ds-Tregs are more effective at preventing rejection than non-fucosylated Ds-Tregs

NSG Skin Graft Model Experimental Groups

Table 1. Experimental groups for NSG Skin graft model to test efficacy of fucosylated vs non-fucosylated Ds-Tregs.

Group	Skin Graft (Day 0)	PBMC (Day 42-49)	DsTregs (Day 42-49)	Fcsl-DsTregs (Day 42-49)	# of animals & Repetitions	Expected Graft Result (Group Rationale)
1	Yes	None	None	None	2 animals X 2 times (4)	No Tregs in graft as no Tregs infused; Graft accepted (Acceptance Control)
2	Yes	$10x10^6$	None	None	2 animals X 2 times (4)	No Tregs in graft as no Tregs infused; Graft rejected (Rejection Control)
3	Yes	$10x10^6$	$2x10^6$	None	2 + 2 animals X 2 times (8)	Low Tregs in graft (?); Graft accepted (?) (Testing Efficacy of DsTregs)
4	Yes	$10x10^6$	None	$2x10^6$	2 + 2 animals X 2 times (8)	Highest Tregs in graft; Graft accepted (Testing Superiority of Fcsl-DsTregs)
5	Yes	$10x10^6$	None	$0.5x10^6$	2 + 2 animals X 2 times (8)	Mid-level Tregs in graft; Graft accepted (?) (Testing Efficacy at fewer Fcsl-DsTregs)

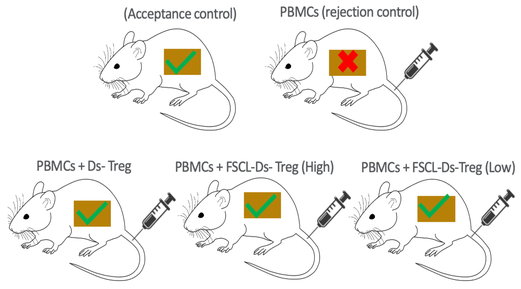

Figure 1. NSG skin graft experimental groups. Spleen and skin are obtained from a deceased donor. Spleen is used to isolate and expand donor B Cells (see Ds-Treg Expansion and Fucosylation). At the same time, deceased donor skin is grafted on to NSG mice at the start of Ds-Treg expansion (Day 0). Groups receive treatment with Ds-Tregs according to **Table 1** ~42-49 days after skin transplantation.

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Ds-Treg Expansion and Fucosylation

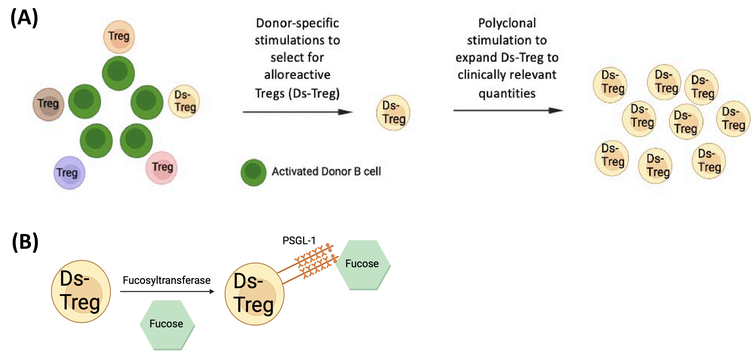


Figure 2. (A) Ds-Tregs are expanded for 21-28 days in culture in the presence of IL-2, TGF-β and everolimus. Activated, expanded B cells isolated from spleen of the deceased donor (i.e., same donor as the skin grafts) are used to stimulate expansion of Ds Tregs. **(B)** Expanded Ds-Tregs are fucosylated utilizing fucosyltransferase in a 1mM solution of GDP-fucose for 30 minutes. Fucosylated Ds-Tregs are assessed for expression of cutaneous lymphocyte associated antigen (CLA) by flow cytometry. Fucosylated and non-fucosylated Ds-Tregs are then administered as an adoptive cell therapy according to **Table 1.**

Ds-Treg Expansion and Phenotypic Analysis

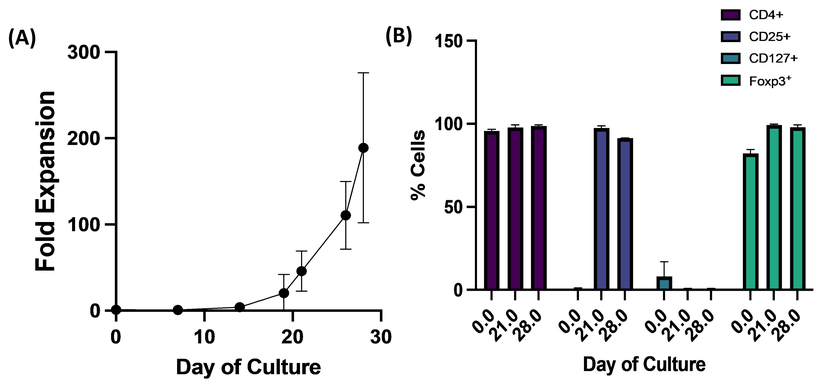


Figure 3. Ds-Treg Expansion. **(A)** Ds-Tregs expanded ~200 fold after 28 days in the presence of IL-2, TGF-β, and everolimus. **(B)** Ds-Tregs were phenotypically characterized by flow cytometry at varying time points. After expansion, Ds-Tregs maintained their phenotype with >90% CD4+CD25+CD127-Foxp3+.

Ds-Treg Functional Testing

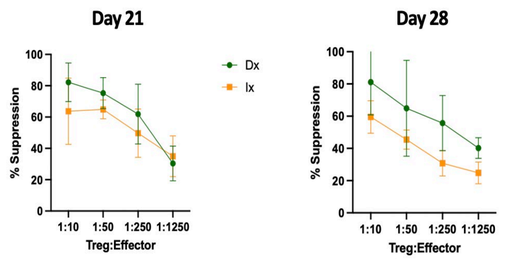
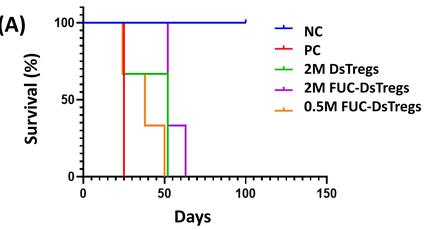


Figure 4. MLR assay. Ds-Tregs demonstrated donor-specific suppression of alloimmune responses with >50% suppression at a Treg:effector cell ratio of 1:250. Ds-Tregs were more potent in their response to stimulators from the B cell donor as compared to a third-party not related to the "recipient."

Skin Allograft Survival



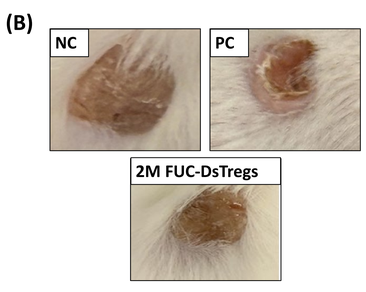


Figure 5. Fucosylated Ds-Tregs Prolong Skin Allograft Survival. (A) Kaplan-Meier survival curve showing the effect of different treatments on skin allograft survival. High-dose fucosylated Ds-Tregs (2M FUC-DsTregs) prolonged graft survival compared to all other groups. The positive control (PC) group, which received no treatment, showed rapid rejection. Non-fucosylated (2M Ds-Tregs) and low-dose fucosylated (0.5M FUC-DsTregs) groups provided an intermediate, but less robust, protective effect. This data suggests that fucosylation enhances the ability of Ds-Tregs to prolong allograft survival by improving "homing" to the targeted area. (B) Representative images of grafts at the time of sacrifice. Images from the negative control (untreated), positive control, and the 2M FUC-DsTregs treated groups are shown, illustrating the visual difference in graft viability between the groups.

Conclusions

- Ds-Tregs can be expanded and fucosylated with upregulation of cutaneous lymphocyte antigen (CLA) while maintaining adequate viability (>90%) for adoptive cell transfer experiments.
- Fucosylation enhances the ability of Ds-Tregs to prolong allograft survival by improving "homing" to the targeted area.