Type 1 Regulatory T-Lymphocytes Modulate Neovascularization in Dermal Wounds

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BACKGROUND

- CD4+ T-lymphocytes are essential in regulating wound healing and dermal fibrosis.
- Interleukin 10 (IL-10) plays a substantial role in the fetal regenerative tissue repair process.
- While many immune cells produce IL-10, little focus has been placed on the role of lymphocytes in wound healing.

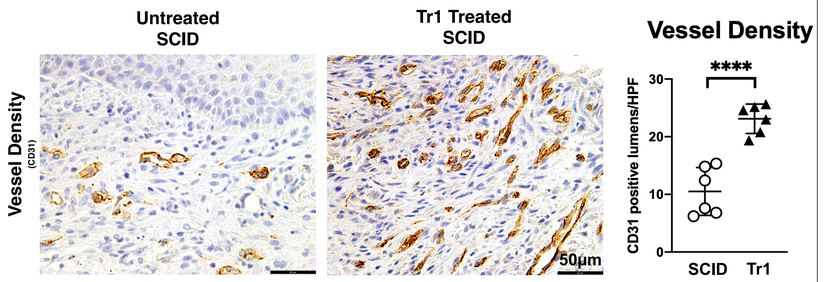


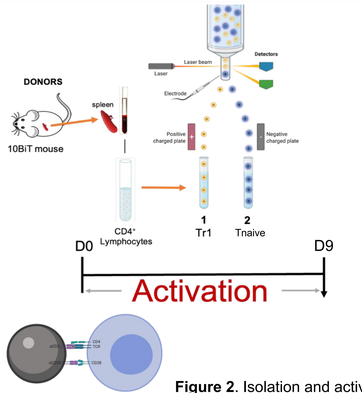
Figure 1. CD31 staining of vessels lumens 7 days post-wounding is improved by adoptive transfer of Tr1 lymphocytes into SCID mice when compared to untreated mice. Scale bar: 50μm. *p<0.05, **p<0.01, ***p<0.001, ****p<0.001.

- SCID mice, which lack lymphocytes, heal with worse angiogenesis and fibrosis compared to WT mice.[1]
- We have demonstrated that IL-10-Producing type 1 regulatory T-lymphocytes (Tr1) improve vascularity in the healing wound (Fig1), accelerate wound closure, and reduce dermal fibrosis in SCID mice.[2]
- WIt is unknown how IL-10oproducing Tr1 regulate angiogenesis in wound healing.

HYPOTHESIS

Tr1 modulate the pro-angiogenic cytokine balance (VEGF; CXCL12; MIF) to improve fibroblast and endothelial cell angiogenic functions, thereby enhancing neovascularization and wound healing outcome.

METHODS



- Splenocytes and dermal fibroblasts were isolated from adult 10BiT and WT mice, respectively.
- Splenocytes were enriched for CD4$^+$ cells via magnetic column separation and sorted into Tnaive and Tr1 precursors using FACS (**Fig 2**).
- Lymphocyte precursors are activated using anti-CD3/28 microbeads in medium containing IL-2
- Functional IL-10 production by lymphocytes was confirmed with flow cytometry and IL-10 ELISA.
- In vitro, dermal endothelial cells and fibroblasts were cultured with conditioned medium from activated lymphocytes and endothelial branching assay and pPCR performed, respectively.
- *in vivo*, bilateral 6mm full thickness wounding was performed on 8wk female SCID mice with simultaneous retro-orbital injection of 10^6 Tr1 cells.
- Wounds were harvested at D3/D7 to evaluate for protein expression and imaging mass cytometry (IMC).

Figure 2. Isolation and activation of Tnaive and IL-10 producing Tr1. Splenocytes are harvested from adult mice and enriched for CD4$^+$ T cells with magnetic column separation. These cells are further sorted into Tnaive and Tr1 precursors using FACS.

RESULTS

in vitro Activation Promote IL-10 Production in Tr1

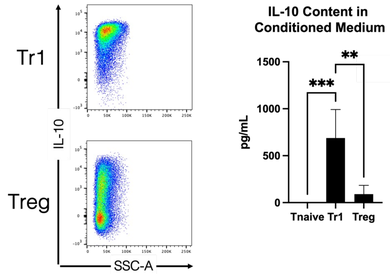


Figure 3. IL-10 producing capacity of 9-day activated Tr1 vs Treg. Gated to living, singlet, CD4$^+$ cells. CD90.1 positivity marks IL-10 production in 10BiT mice. ELISA on conditioned medium following 9 days of activation demonstrates increased IL-10 production by Tr1 compared to naïve T-lymphocytes. *p<0.05, **p<0.01, ***p<0.001.

Tr1 promotes VEGF/CXCL12 Expression in Fibroblasts and Endothelial Sprouting

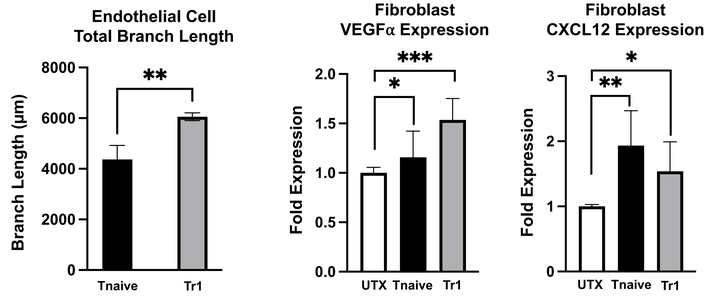


Figure 4. *in vitro* culture of dermal endothelial cells or dermal fibroblasts with lymphocyte CM. Endothelial cells cultured with CM from Tr1 demonstrated significantly greater total branch length on tube formation assay compared to endothelial cells treated with Tnaive CM. Fibroblasts treated with Tr1 CM for 24hrs demonstrated increased gene expression of angiogenic mediators VEGF and CXCL12. *p<0.05, **p<0.01, ***p<0.001.

Tr1 Improve the Balance of Angiogenic Growth Factors in Day 3 Wounds

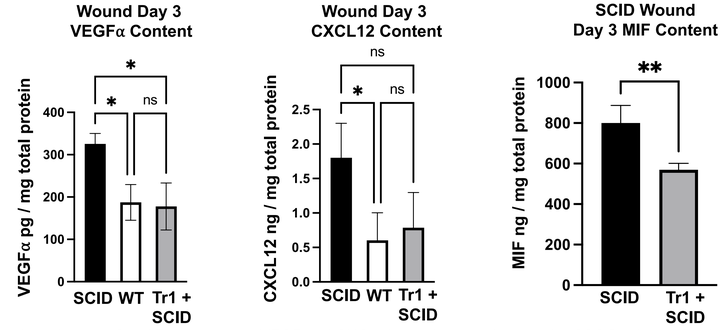


Figure 5. Adoptive transfer of Tr1 normalizes VEGF and CXCL12 protein levels in wounds of SCID mice at day 3 post-wounding. Wounds of SCID mice treated with Tr1 also demonstrated significantly decreased levels of MIF compared to untreated SCID. *p<0.05, **p<0.01

RESULTS

Tr1 Spatially Associated with αSMA+ Vessels at Day 7 Post-Wounding

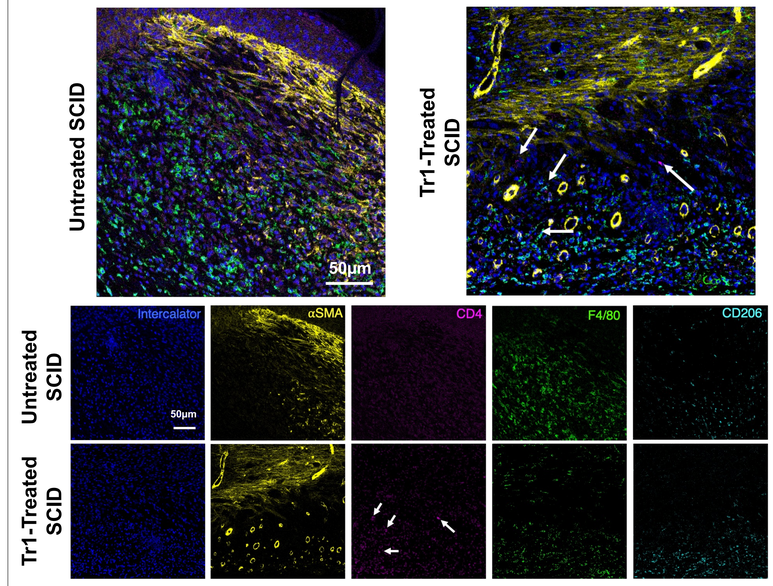


Figure 6. Imaging mass cytometry (IMC) of day 7 wounds of untreated SCID mice compared to those of SCID mice treated with Tr1. Tr1 demonstrate more mature vasculature, indicated by lumens positive for αSMA (yellow) These vessels are spatially associated with cells positive for CD4 (Purple) which represent adoptively transferred Tr1.

CONCLUSION

- We have demonstrated that Tr1 improve the balance of angiogenic modulators VEGFα, CXCL12, and MIF which promotes improved vascularization of wounds in SCID mice.
- Culture of WT endothelial cells in Tr1 conditioned medium increases total branch length of endothelial networks
- Culture of WT dermal fibroblasts in Tr1 conditioned medium increases VEGFα and CXCL12 expression.
- Treatment of SCID mice with Tr1 increases density of mature blood vessels.

REFERENCES

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2. Short WD, et al. Interleukin-10 Producing T Lymphocytes Attenuate Dermal Scarring. Ann Surg. 2021 Oct 1;274(4):627-636.

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QUESTIONS AND SUGGESTIONS

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