Immune Response to Vascularizing Subcutaneous Engineered Islet Grafts

SEFTON LAB
tissue engineering & regenerative biomaterials

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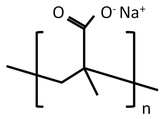
Background

Subcutaneous Space

- Promising alternative islet transplant site to portal vein
- Requires vascularization for utility

Methacrylic acid (MAA) Based Materials

- Semi-intepenetrating polymer network
- 8 arm poly(ethylene glycol) (PEG), 10 kDa and poly(MAA) combined to form a hydrogel with a thiol cross-linker via Michael type addition
- Induce vascularization in the subcutaneous space



Allogeneic Transplantation

- Rodent islets injected in an MAA-poly(ethylene glycol) (MAA-PEG) hydrogel returned diabetic SCID/bg mice to normoglycemia
- A properly tuned peri-operative inflammation suppression protocol is required for immunocompetent mice

Aim

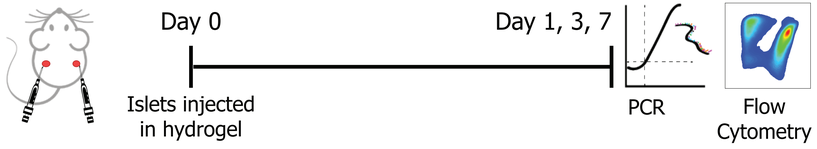
Understand the immune environment of subcutaneously implanted, vascularizing islet allografts and evaluate the efficacy of a short-term immune mitigation strategy by comparison to an immune compromised mouse model.

Methods

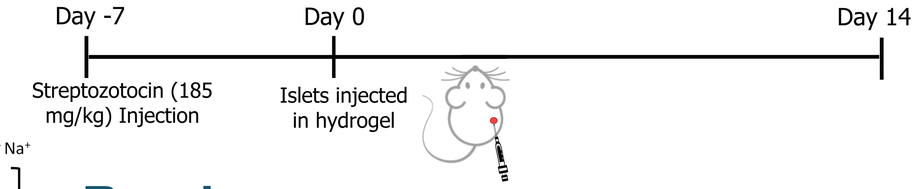
Immune Suppression Regimen

i.p. Rapamycin (0.5 mg/kg/day), i.p CTLA4-Ig (25 mg/kg/day) (Days 0,1,2,4,6)

Non Diabetic Studies: 200 mouse islet equivalents (IEQ) were isolated from C57Bl/6J mice and injected in 100 µl of MAA-PEG hydrogel into Balb/c or SCID/bg mice. Islet grafts were removed on days 1, 3, and 7 for analysis of the immune environment by flow cytometry or qPCR.



Diabetic Studies: 1000 mouse islet equivalents (IEQ) were isolated from C57Bl/6J mice and injected in 200 µl of MAA-PEG hydrogel into streptozotocin induced diabetic Balb/c mice. Non-fasting blood glucose was measured daily.



Results

Immune suppression reduced innate cell recruitment in Balb/C, making it comparable to that of SCID/bg

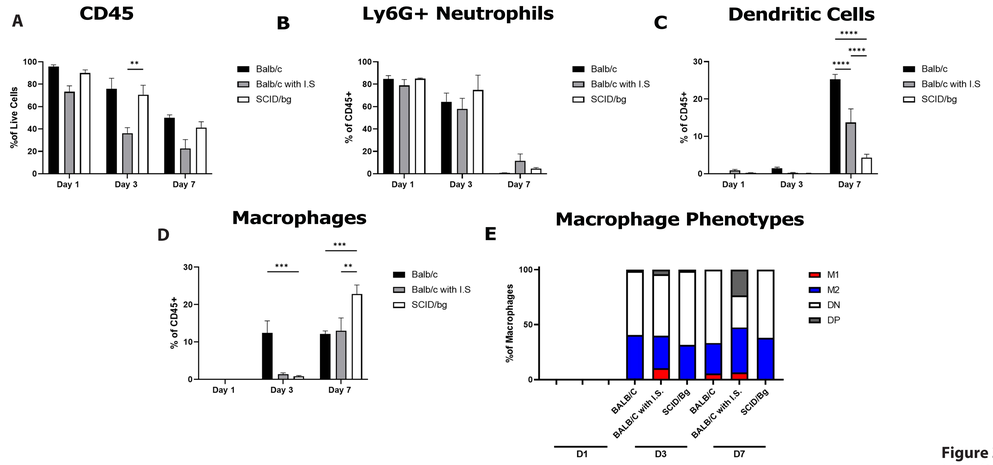


Figure 1 - Differences in innate cell recruitment between Balb/c and SCID/bg mice as well as Balb/c with immune suppressants (I.S) CTLA4-Ig and rapamycin. Subcutaneous allograft sites were digested and analyzed by flow cytometry. A) CD45+ immune cells (n=5-6) are further gated into B) Ly6G+ neutrophils C) CD11b+CD11c+Ly6G-Ly6C- dendritic cells D) Ly6G- F480+ CD11b+ macrophages and E) their phenotypes (DP: CD206+MHCII+, DN: CD206-MHCII-, M2: CD206+MHCII-, M1: CD206-MHCII+), Data shown as mean ± SEM. SCID/bg Day 1 (n=4), SCID/bg Day 3 and 7 (n=5), Balb/c (n=3), Balb/c with I.S. (n=3)

Results Cont'd

Immune suppression decreased expression of common inflammatory markers at early times

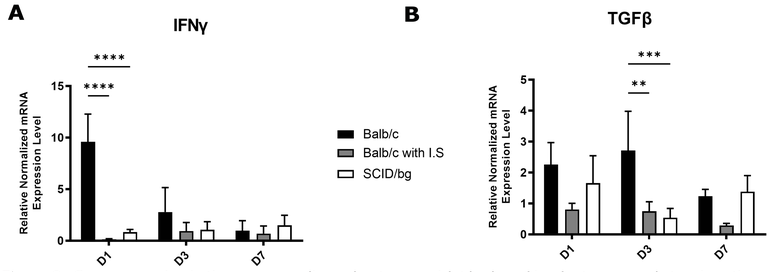


Figure 2 - Gene expression in tissue surrounding subcutaneous islet hydrogel implants removed at various time points after transplantation, analyzed via qPCR (n=2-3).* p<0.05, ***p<0.001

Initial drop in blood glucose levels of diabetic Balb/c mice treated with immune suppression similar to response observed in SCID/bg

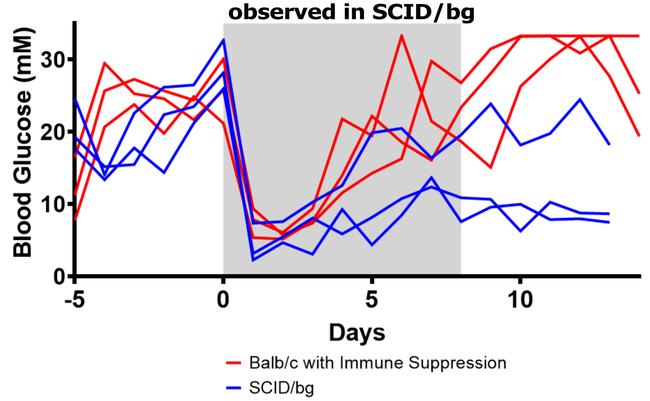


Figure 3 - Non-fasting blood glucose levels for STZ-induced diabetic Balb/c mice or SCID/bg mice with rodent islets (1000 IEQ) delivered in an MAA-based hydrogel. Immune suppressed animals treated with rapamycin (0.5 mg/kg/day) and CTLA4-Ig (25 mg/kg/day) delivered from Day 0 to Day 8 (shown on graph as shaded region). n=3

Conclusion

Immune suppression reduced innate immune cell recruitment in Balb/c mice but was insufficient to induce sustained reversal of hyperglycemia in diabetic animals.

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