Immune Response to Allogeneic Islets in a Methacrylic Acid (MAA)-induced, Vascularized Subcutaneous Space

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Introduction

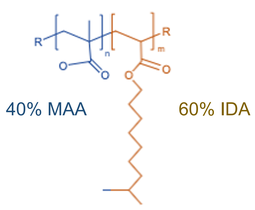
- Type I diabetes mellitus (T1DM) is an autoimmune disease that results in the destruction of pancreatic beta-cells[1]
- Cell transplantation can reverse incurable diseases like T1DM, but current transplant methods use portal vein infusion where few islets will engraft– multiple donors are therefore required further limiting the availability of this treatment option[2, 3]
- The subcutaneous space is a promising alternative transplant site
  - less hostile, accessible, & large enough to support large transplant volumes[4]
- Minimal vascularization of the site has prevented its use, but our lab has sufficiently vascularized this tissue using a methacrylic acid (MAA)-coated silicone tube[5]
- By injecting islets in collagen into this prevascularized site our lab has successfully returned diabetic immune-compromised mice to normoglycemia[5]

This has not yet been replicated in immunocompetent mice, and it is our goal to characterize the subcutaneous immune response to the MAA-coated tube, and to the allogeneic cells in this prevascularized subcutaneous space with and without systemic immune suppression to fully harness the potential of this transplantation site.

Methods

- Silicone tubes (3cm long) were dip-coated in a 40% MAA-co-isodecyl acrylate (IDA) (Diagram1) solution in tetrahydrofuran (50mg/mL), gas-sterilized, then inserted into the upper dorsum of BALB/c mice
- After 14 days, 250 islet equivalents (IEQ) isolated from C57Bl/6J mice were suspended in 20 µl of neutralized type 1 collagen
- Mixture was drawn into PE90 tubing and gelled at 37°C for 1 hour
- Incision made in upper dorsum of the prevascularized BALB/c mice to access transplant site
- The tube was flushed with PBS, and used as a guide to inject islets in tubing into the prevascularized site
- Both tubes were removed upon injection; the incision was sutured
- Fingolimod (1mg/kg) was administered intraperitoneally, daily for 7 days following transplantation to suppress the immune response
- Grafts were removed and digested at timepoints between days 0 (tube-only response) and 7 for analysis of the immune response by flow cytometry

Diagram 1: 40% MAA-co-IDA polymer coating on silicone tubes



Methods (cont.) & Results

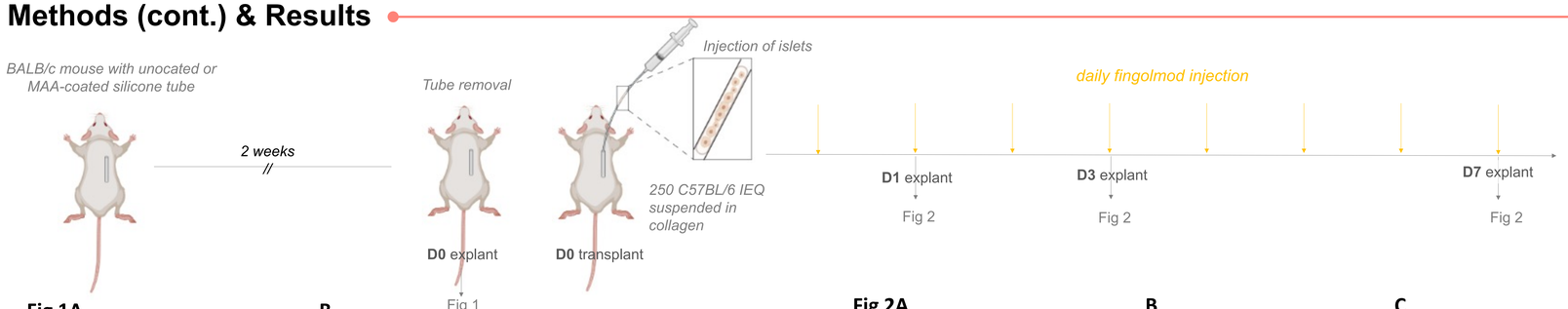
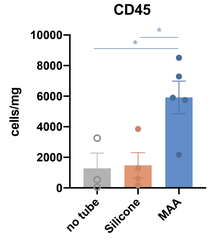


Fig 1A



B

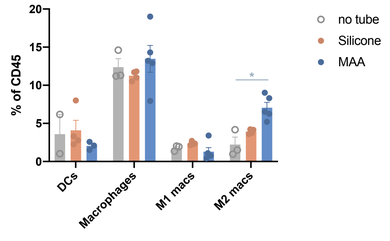


Figure 1: Greater immune cell recruitment to the subcutaneous space around the MAA-coated tube than the silicone tube after 2 weeks of prevascularization (before transplant). B. Dendritic cells (DCs) were gated as F480-CD11c+; macrophages as F480+CD11b+; M1 macrophages as (CD206-MHCII+); M2 macrophages as (CD206+MHCII-). Data shown as mean ± SEM; n=2-6; analyzed using two-way ANOVA.

Discussion & Conclusion

- MAA-coated tube induced a greater immune response in the subcutaneous space of immunocompetent mice (Fig1)
- Significant increase of M2 macrophages with the MAA-coated tube reveals the alternatively-activated macrophage phenotype that has been previously observed[6]
- Fingolimod is expected to dampen the immune response by preventing lymphocyte egress from lymph nodes as well as skew T cell responses towards a regulatory phenotype [7-9], and in a functional study returned one streptozotocin-induced diabetic mouse to normoglycemia (n=1 of 3; data not shown)
- Upon transplantation of allogeneic islets, fingolimod did not significantly decrease the number of recruited immune cells, but altered the distribution of immune cell population after 1 dose:
  - neutrophil response at D1 was decreased at the transplant site as compared to a no drug control (Fig2B)
  - dominant macrophage and the NK cell response at D3 dampened by daily fingolimod (Fig2C,D)
- Recruited DCs at D7 are present at the transplant site, but fingolimod does not significantly alter this response (Fig2E)

This characterization reveals a dampened innate response following daily fingolimod injection at early timepoints post-transplantation. It provides greater understanding into the allogeneic, subcutaneous, immune response and will help drive decisions about the immunosuppressive drugs that will allow for greater cell acceptance.

Fig 2A

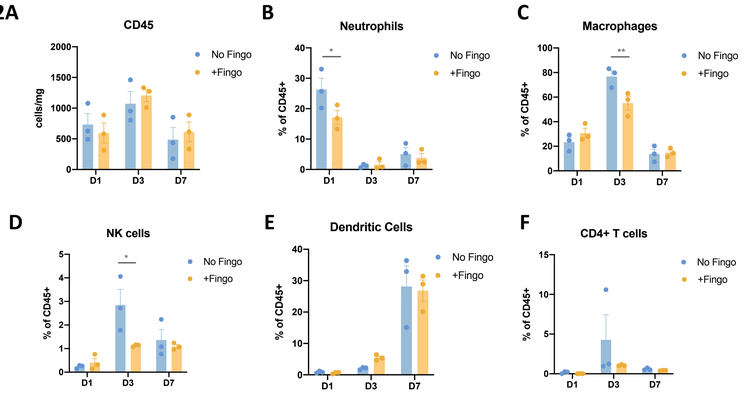


Figure 2: Fingolimod alters the distribution of immune cell populations at the transplant site at D1, D3, and D7 after transplantation of 250 allogeneic IEQ. A. CD45+ immune cells were gated from live, single cells of digested, subcutaneous tissue. B. Neutrophils (further gated as Ly6G+), C. macrophages (F480+CD11b+), D. natural killer (NK) cells (CD49b+), E. dendritic cells (DCs) (F480-CD11c+), and F. CD4+ T cells (CD3+ CD4+) are all shown as a percentage of the total CD45+ immune cells. Data shown as mean ± SEM; n=3; analyzed using two-way ANOVA.

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