Combination of Cell Surface PEGylation and Short-Course Immunotherapy for Prolonged Survival of Pancreatic Islets in an Allogeneic Murine Model

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Statement of Purpose: Clinical islet transplantation has shown promise for Type 1 diabetes treatment. Nonetheless, inflammatory and immunological host responses to the implant lead to islet dysfunction and destruction, in spite of systemic immunosuppression.1 Cellular PEGylation, the addition of a single coating of poly(ethylene glycol) (PEG) to the cell/tissue surface, has been shown to reduce inflammation and mitigate immune recognition via generation of a steric barrier.2,4 Furthermore, blockade of Lymphocyte Function-associated Antigen 1 (LFA-1), which plays a key role both in lymphocyte trafficking and co-stimulation, has demonstrated success in partially preventing murine allograft rejection.3,5 In this study, we sought to evaluate the effect of surface modification of the islet transplant through PEGylation, alone and in combination with a short-course immunotherapy, on the survival of fully-MHC-mismatched islet allografts. Furthermore, alterations of the resulting local microenvironments as a result of such treatment(s) were characterized.

Methods: Islets were coated with a single layer of PEG (SVA-mPEG, MW 5000 Da, Laysan Bio). Surface modification and its effect on islet viability and function was evaluated in-vitro by confocal microscopy and glucose-stimulated insulin release. PEGylated or unmanipulated DBA/2 islets (H2d) were then transplanted into the renal subcapsular space of chemically-induced diabetic C57BL/6J mice (H2b). Control animals received untreated islets and either saline (n=20) or anti-LFA-1 antibody (KBA, 100μg/day, i.p.) on days 0-6 (n=10). Experimental groups received PEGylated islets and either saline (n=10) or anti-LFA-1 antibody (KBA, 100μg/day, i.p.) on days 0-6 (n=9). The effect of the various treatments was evaluated by graft survival rates through monitoring of blood glucose levels. Graft bearing kidneys were collected following euthanasia for histological evaluation. Mechanistic studies to characterize the effects of different treatments on mitigating inflammatory and immunological attack were also performed. Cellular infiltrates, as well as gene expression within the graft, was assessed at early time points (< 15 days post-transplant) by flash-freezing graft bearing kidneys in OCT medium and processesing for immunohistochemical staining and laser capture microdissection (LCM) for subsequent RNA extraction and quantitative RT-PCR analysis.

Results: In-vitro assessments demonstrate the presence of the PEG coating on the islet surface and no adverse effects of said coating on cell viability or function when compared to untreated control islets. Ninety percent of the control islet transplants rejected within 60 days. Both the short course of LFA-1 blockade or PEGylation of islets alone resulted in long term (>100 days) function of the allograft in 50% or 60% of cases, respectively, statistically different from control animals (P=0.022 and 0.0175, respectively). Combination of islet PEGylation with LFA-1 blockade resulted in 78% of the transplants functioning long-term (Fig 1). Nephrectomy of the graft bearing kidney resulted in prompt return to hyperglycemia for all transplants. Histological evaluation of grafts functioning long-term exhibited healthy, robust islets with minimal cellular infiltrate (Fig 1). The mechanism behind these protective effects is currently being studied through characterization of the local graft site microenvironment early post-implantation (< 15 days) through assessment of leukocyte populations via immunohistochemistry and RT-PCR analysis (e.g. IFN-γ, TNF-α, TGF-β, and IL-10) of LCM collected grafts.

Figure 1. Tri-chrome and immunofluorescent staining of grafts in animals receiving PEGylated islets alone (a,b) or in combination with anti-LFA-1(c,d) therapy. Bottom: Graft survival curves for groups in the study.

Conclusions: Islet PEGylation represents a simple, highly cell compatible procedure to prevent allograft rejection. In combination with short-course immunotherapy, murine allograft rejection is prevented in a large majority of the transplants. This study demonstrates the potency found with the combination of these two mild strategies, indicating a synergistic effect. Mechanistic data remains to be evaluated to fully characterize the mechanism of this effect. This work is the first step in developing methods for bioactive modification of graft surfaces for immunomodulation of the local microenvironment.

References: