Long Term Survival of PEGylated Murine Allogenic Islets using Short Course Immunomodulation

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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] Cell surface PEGylation, the addition of a single coating of poly(ethylene glycol) (PEG), has been shown to reduce inflammation and mitigate immune recognition via generation of a steric barrier.[2-4] Blockade of Lymphocyte Function-associated Antigen 1 (LFA-1), which plays a key role both in lymphocyte trafficking and co-stimulation, has demonstrated partial success in preventing murine allograft rejection.[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 anti-LFA-1 blockade, on the survival of fully-MHC mismatched islet allografts. Alterations of the resulting local microenvironment was 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, 100ug/day, i.p.) on days 0-6 (n=10). Experimental groups received PEGylated islets and either saline (n=10) or anti-LFA-1 antibody (n=9). Graft function was assessed through monitoring of nonfasting 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) on frozen sections by immune-histochemical staining and laser capture microdissection (LCM) for subsequent RNA extraction and quantitative RT-PCR analysis.

Results: In-vitro assessments demonstrated the presence of the PEG coating on the islet surface and no adverse effects of 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% and 60% of cases, respectively, (P=0.022 and 0.0175 vs. controls, 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 under evaluation 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.

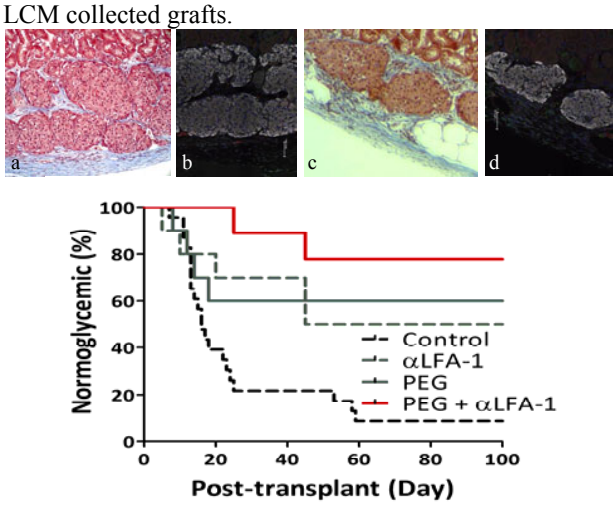

Figure 1. Tri-chrome and immunofluorescent staining of grafts in animals receiving PEGylated islets alone (a,b) or in combination with anti-LFA-1(c,d) therapy. Bottom: Graft survival curves for groups in the study.

Conclusions: Islet PEGylation represents a simple, highly cell compatible procedure to modulate the graft microenvironment and improve allogeneic islet transplant survival. In combination with short-course immunotherapy, murine allograft rejection can be prevented in a large majority of the transplants. This study demonstrates the synergy of the combinatorial use of these strategies. Mechanistic data remains to be evaluated to fully characterize the mechanism of this effect. This work is the first step towards the development of methods for bioactive modification of graft surfaces to attain succesful immunomodulation in the local microenvironment.

Acknowledgements

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