Biomaterials-based Immunomodulation and Co-Stimulatory Blockade for Allograft Survival Enhancement in Murine Vascularized Composite Allotransplantation

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Statement of Purpose: Vascularized composite allotransplantation (VCA) is a promising reconstructive strategy for patients suffering from devastating tissue loss requiring major reconstruction. VCA is unique in that combinations of muscle, bone, nerve, and skin developed from different germ layers are transplanted as a unit allowing for "like-with-like" replacement and functional restoration. One of the most important elements for the clinical advancement of VCAs is to develop regimens that prevent rejection by targeting immune mechanisms that influence the allotransplant tolerance. Major limitations include the tissue damage and inflammation caused by ischemia inherent to the surgery, downstream toxicity and adverse effects caused by the administration of long-term, high dose immunosuppressants to mitigate alloreactivity.

Biologic scaffolds derived from tissue extracellular matrix (ECM) are used clinically for tissue repair in a variety of scenarios. Recent studies demonstrated that ECM materials modulate macrophage expression of CD86 and CD206 towards an M2-biased macrophage phenotype[1,2]. CTLA4-Ig was developed as a costimulatory blockade to specifically block the T cell CD28:B7 costimulation instrumental in the immune response leading to graft rejection, however, it has minimal impact on long-term graft failure when used as a sole therapy[3]. Here, we investigate the potential of systemic CTLA4-Ig combined with a biological porcine-derived extracellular matrix (ECM) scaffold that elicits a type 2 response to promote allograft survival and regulate the inflammatory microenvironment in a mouse orthotopic hind limb VCA model.

Methods: To test the effects of systemic CTLA4-Ig and ECM (ACell) implantation on VCA survival, we used a murine orthotopic hind limb allogeneic transplant model (Balb/C [H-2d] to C57BL6 [H-2b])[4] with ECM delivered locally to the transplantation site during surgery. Survival curves were generated analyzed with Mantel Cox tests for significance ($p < 0.05$). For flow cytometry donor and recipient quadriceps femoris and biceps femoris interface were processed with a mechanical and enzymatic dissociation into a single cell suspension. Cells were stained and data collected on an Attune NxT Flow Cytometer (ThermoFisher) or LSR II (BD Bioscience) and analyzed with FlowJo (BD Bioscience). Significance was determined using two-way ANOVA with Sidak's multiple comparison test ($p < 0.05$).

Results: Locally delivered ECM with systemic CTLA4-Ig prolonged VCA graft survival, representative VCA image Figure 1A. While ECM treatment alone did not increase median survival time (MST) of the VCA compared to untreated control, the combination therapy of ECM with CTLA4-Ig significantly increased VCA survival to 24.5 days relative to surgery alone, Figure 1B. Analysis of the myeloid infiltration on postoperative day (POD) 14 shows increased macrophage infiltrate into the ECM ($2.3 \pm 0.2 \times 10^6$ [Mean ± SEM] to $4.2 \pm 0.5 \times 10^6$ cells/g), Figure 1C. To determine macrophage polarization, CD206 (pro-regenerative), CD86 (pro-fibrotic), and MHC-II (antigen presentation) mean fluorescence intensity (MFI) was normalized to expression in CTLA4-Ig controls. While the CD86 and MHC-II were not significantly different between treatments, CD206 was 1.7-fold higher with ECM treatment, Figure 1D. These results show the addition of ECM not only increases the myeloid population of immune cell infiltration but promotes a more pro-regenerative associated phenotype.

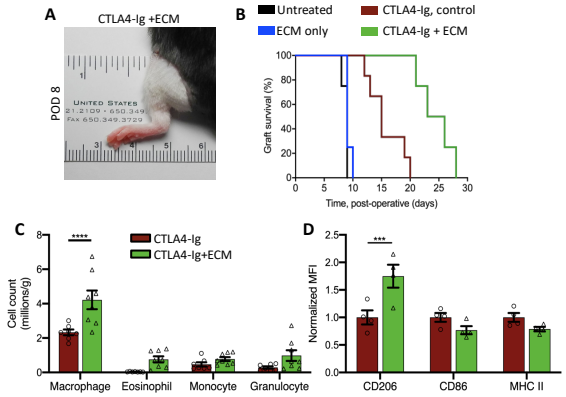

Figure 1: (A) Representative VCA (B) VCA survival curve (C) Myeloid cell recruitment on POD 14 (D) relative expression of macrophage polarization markers

Conclusion: There is a substantial, unmet need to develop novel strategies to increase the efficacy and reduce the toxicity of current treatment regimens for VCA. We have shown that although implantation of ECM alone in murine VCA does not delay graft rejection, when treated in combination with CTLA4-Ig, graft median survival time can be significantly increased. Data on myeloid recruitment suggests a shift in Type 2 response and with further investigation into the T cell phenotype we aim to show the effects of ECM on prolonging VCA graft survival. This study demonstrates ECM with co-stimulatory blockade prolongs allograft survival.

References:[1]K. Sadtler, *Science* (2016) [2] J. Dziki *Tissue Eng. Part A* (2016) [3]C.P. Larsen, *Am. J. Transplant.* (2006) [4] G.J. Furtmüller, *J. Vis. Exp.* (2016)

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