Combined Co-Stimulatory Blockade and Donor Bone Marrow Cells Induce Robust Immune Tolerance in a Fully MHC-Mismatched Swine Hind Limb Transplant Model

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Abstract:

Background: Vascularized Composite Allografts (VCA), such as hand and upper extremity transplants, contain vascularized bone marrow (BM) and a BM niche representing a constant source of donor-derived stem cells and hence can favor chimerism and tolerance induction. This study investigates the immunological effects of vascularized BM within VCA under co-stimulation blockade-based regimen and its impact on allograft survival and tolerance induction.

Methods: Fully MHC- and gender mismatched MGH miniature swine (n=3-5 per group) underwent heterotopic hind-limb transplantation containing intact vascularized BM component (Figure 1).

Recipient animals received a short course (30 days) of tacrolimus monotherapy with or without donor BM infusion (60x10⁶ cells/kg) and CTLA4Ig. Short course tacrolimus only and untreated animals served as controls. Chimerism was assessed by SRY PCR analysis. Sequential skin and muscle biopsies were performed for histology. Alloreactivity against donor antigens was assessed in vitro using mixed lymphocyte reaction (MLR) assays. Challenge with secondary skin grafts was utilized to demonstrate robust immune tolerance in vivo.

Results: The co-stimulation blockade based immunomodulatory protocol resulted in indefinite graft survival (>150 days) in 3 out of 5 animals whereas control and tacrolimus only groups rejected allografts at days 7+/-1 and 29+/-2 respectively (Figure 2).
Figure 2: Kaplan-Meier survival curve demonstrating rejection free survival of skin component of hind limb allograft

Combined costimulation blockade with augmented donor BM infusion resulted in indefinite graft survival in 2 out of 3 animals (>150 days). Long-term survivors demonstrated stable micro-chimerism in various graft and recipient tissues including skin, lymph node, bone marrow, and spleen. MLR data showed unresponsiveness to donor but not to third party allogeneic controls. Secondary skin grafting demonstrated advanced rejection of third party grafts on day 7 while donor-matched grafts were accepted indicating donor-specific immune tolerance. There was no evidence of donor specific antibody formation in long-term survivors. Donor unresponsiveness in MLR was lost five weeks post-graftectomy, which demonstrated that persistent antigenic stimulation was required for operational tolerance.

Conclusion: Combined costimulation blockade and donor BM cell infusion can induce robust immune tolerance in a fully MHC mismatched hind limb transplant model. Such targeted immunomodulatory protocols might eliminate the need for long-term multi-drug immunosuppression after reconstructive transplantation.

References:
