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PURPOSE: The achievement of tolerance induction in Vascularized composite allografts (VCA) could potentially enhance the quality of life for patients with severe facial or extremity injuries while avoiding the risk of immunosuppression. The achievement of tolerance would help transitioning VCA from an experimental to a routine practice worldwide. The purpose of this study was to investigate strategies for tolerance induction in a large animal model and to assess whether current VCA recipients could potentially be weaned off of immunosuppression while maintaining their intact graft using only a co-stimulation blockade agent.

METHODS: A total of 19 MGH miniature swine underwent heterotopic osteomyocutaneous hind limb transplantation across full swine leukocyte antigen mismatch. All animals received non-myeloablative conditioning with 50cGy total body and 350cGy thymic irradiation for induction. Group I was treated with high-dose tacrolimus (15-20ng/ml) maintenance therapy. Group II was treated with low-dose tacrolimus (4-6ng/ml). Group III received low-dose tacrolimus and 20 mg/kg of CTLA4-Ig administered on POD2, 7, 14, 30, 60, 90, and 120. Group IV received transient high-dose tacrolimus until POD60. Group V received transient high-dose tacrolimus until POD60 and was switched to CTLA4-Ig administered on POD60, 85, 100, 120 and 150. Graft rejection was monitored by clinical assessment and protocol skin biopsies. Alloreactivity against donor antigens was assessed using a lymphocyte reaction (MLR).

RESULTS: Prolonged high-dose tacrolimus led to maintenance of VCA in 3/3 animals but was associated with major infectious complications and death of animals with intact grafts. 2/3 animals in group II rejected their grafts by POD46 and 217. In group III, 2/5 animals demonstrated rejection prior to POD150, while 3/5 animals achieved long-term survival of their VCA beyond POD300. 3/3 animals in group IV and 4/5 animals in group V achieved indefinite graft survival (beyond POD300) despite weaning of all immunosuppression. The one animal in group V that rejected its graft began to show evidence of rejection on POD277. One animal in group V rejected its graft on POD277. Donor specific unresponsiveness was confirmed in all long-term survivors in vitro by CFSE-MLR. The addition of CTLA4-Ig to subtherapeutic (low-dose) CNI does not prevent graft rejection. The use of transient high-dose tacrolimus +/- CTLA4-Ig allows long-term graft survival.

CONCLUSIONS: A conditioning regimen consisting of peritransplant high-dose tacrolimus without myeloablative conditioning leads to tolerance of VCA containing vascularized bone marrow. These encouraging findings hint to the potential use of an induction regimen to eliminate the need for long-term immunosuppression and its complications in reconstructive transplantation.

Whole Eye Transplantation in the Rodent: Long-Term Survival and Effects on the Unoperated Partner Eye

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**PURPOSE:** Globally, 39 million people suffer from blindness. Whole eye transplantation (WET) offers the opportunity to provide a viable optical system to recipients with irreversible vision loss. We have previously established a viable orthotropic model for vascularized whole eye transplant in the rat. The purpose of our study is to evaluate gross morphology, structural integrity and aqueous humor dynamics in a long-term WET survivor, as well to evaluate for any adverse effects of WET in the unoperated, contralateral eye.

**METHODS:** Syngeneic whole eye transplants were performed with Lewis rats. The donor flaps included ocular tissues anterior to the optic chiasm, eyelid and periorbital tissue, and external ear. The recipient site was prepared by removing a similar region of skin and ocular tissue, with optic nerve division at its exit from the globe, vascular anastomoses, and optic nerve coaptation. Optical coherence tomography (OCT), gadolinium-enhanced magnetic resonance imaging (Gd-enhanced MRI) and electroretinography (ERG) were performed to evaluate the viability and structural integrity of the eyes of the long-term WET survivor and compared with a naive, age-matched control. In a subsequent series of syngeneic transplants, we evaluated the unoperated, contralateral eye with OCT, slit lamp exam, fundoscopy, and histology.

**RESULTS:** The long-term WET survivor and corresponding control animal were >400 days old at time of evaluation. Corneal opacification prohibited OCT imaging of the retina of the transplanted eye. OCT of the cornea and retina of the contralateral eye corresponded with the naive eyes of the control rat. Gd-enhanced MRI imaging revealed existing aqueous humor dynamics in the contralateral unoperated eye in the transplanted eye had a normal electrical response with ERG, which was similar to the control. In the transplanted eye, aqueous humor dynamics was compromised, and there was no evidence of electrical response with ERG analysis. As for our follow-up series of syngeneic transplants (n=6, sacrifice at 3mos), we continued to find no abnormalities in the contralateral eye with respect to OCT, slit lamp exam, fundoscopy, and histology.

**CONCLUSION:** In this study, we demonstrate findings in a long-term survivor. We did not identify any adverse changes in structural integrity and function in the contralateral eye >400 days after WET in comparison to the age-matched control eyes. This preservation of the structure and function of the contralateral eye was recapitulated in a subsequent series of animals.

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**Delayed Tolerance Induction Protocol for Vascularized Composite Allografts in Non-Human Primates: The Immunomodulatory Effect of Donor Bone Marrow Transplantation Does Not Prevent the Development of Chronic Rejection in the Absence of Durable Mixed Chimerism**

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**PURPOSE:** Our laboratory is currently developing a delayed tolerance induction protocol for vascularized composite allografts (VCAs) in a clinically relevant non-human primate (NHP) model using donor bone marrow transplantation (DBMT) after VCA to induce durable mixed chimerism and tolerance. DBMT has a demonstrated immunomodulatory effect in our previous experimental work and in clinical VCA, enabling some patients to be maintained on low-dose tacrolimus monotherapy. With longer-term follow-up of clinical VCA, recent reports of chronic rejection have emerged. We sought to investigate in further detail the immunomodulatory effect of DBMT on acute and chronic rejection of VCAs in NHPs.

**METHODS:** Following VCA transplantation and maintenance on standard triple immunosuppression (IS) for 2 months, donor bone marrow cells that were previously harvested from the vertebrae, minimally processed and cryopreserved were thawed and infused into MHC-mismatched recipient NHPs (n=6) conditioned with irradiation (total body and thymic), T cell depletion, co-stimulatory blockade and anti-inflammation (with anti-IL-6 receptor monoclonal antibody). A bridging course of calcineurin inhibition (CNI) was given for 4 weeks before IS withdrawal and assessment of the VCA for tolerance. Observed rejection episodes of the VCA were biopsied and treated with CNI and a tapering