Tolerance Induction with Post Transplantation High-Dose Cyclophosphamide relies on Recipient-derived Immune Regulation and Donor Bone Marrow in a Murine Model of Vascularized Composite Allotransplantation

Georg J. Furtmüller, MD, PhD, Byoungchol Oh, DVM, PhD, Madeline L. Fryer, BA, MS, Paul Akre, MS, Sudipto Ganguly, PhD, Jeffrey Dodd-o, MD, PhD, Veronika Malek, BA, Giorgio Raimondi, PhD, W. P. Andrew Lee, MD, Leo Luznik, MD, Gerald Brandacher, MD

Johns Hopkins University, Baltimore, MD

PURPOSE: Developing novel treatment concepts to minimize/avoid immunosuppression by induction of immune tolerance represents the prime task in the field of transplantation. In the clinic to date, this was achieved in highly selected patients in clinical trials of kidney transplantation combined with concomitant stem cell or bone marrow transplantation. Vascularized composite allotransplantation (VCA) is evolving as viable treatment modality for patients suffering from functional and esthetic defects to craniofacial structures, extremities and urogenital tissues. In certain VCAs transplantation of vascularized bone marrow may be inherently part of the allograft designs (i.e. hands and faces) and thereby representing a unique opportunity in combining bone marrow and VCA.

METHODS: Murine skin, heart, and hind limb (VCA) transplants were performed across a full MHC mismatch barrier. Recipients treatment comprised non-myeloablative TBI and T-cell depletion and a single dose of post-transplant cyclophosphamide (PTCy). Donor BM and splenocytes (DBM) were injected at the time of transplantation. Post-transplant multi-lineage and Foxp3 chimerism as well as Vβ-TCR staining was performed. Donor-specific unresponsiveness was tested by MLR and by 2° skin and solid organ transplantation (SOT). Mechanistic studies were undertaken using transgenic mice (DEREG and Foxp3-DTR) to investigate the role of regulatory T cells (Tregs). VCA was performed in animals thymectomized prior to receiving PTCy and the transplant to highlight the role of the thymus in induction and maintenance of tolerance in this murine model.

RESULTS: Untreated animals rejected skin grafts, SOT and VCA acutely within 14±1 days, 9±2 days, and 8±1 days, respectively. The treatment regimen extended skin and SOT graft survival (32±8; 65±4, respectively). Additional DBM augmentation lead to allograft survival of >150 days in skin and SOT. However, indefinite graft survival of >150 days was observed in all animals receiving the induction regimen and a VCA ± DBM. In groups receiving a VCA ± DBM, donor chimerism was detected at 22.51% ± 5.96% and 30.17% ± 8.72%, respectively. Prior or post transplantation depletion of recipient-derived Tregs in VCA recipients did not lead to abrogation of tolerance. In recipients of skin graft ± DBM, chimerism was not achieved after recipient-derived Treg depletion prior to transplantation (POD-2) and consequently the allografts were rejected (MST = 23.5 days, N=4). In-vitro, Vβ-T cell receptor staining indicates clonal deletion as an additional central tolerance mechanism. However, transplantation into a thymectomized host did not abrogate long-term allograft survival (N=6). All long-term survivors showed donor-specific T cell unresponsiveness in-vitro (MLR) while demonstrated proliferation against 3rd party stimulators. In-vivo, tolerant animals accepted donor-matched secondary skin, while 3rd party FVB/N skin was acutely rejected. Donor-matched hearts were accepted long-term.

CONCLUSION: Taken together, robust tolerance and immunosuppression-free long-term allograft survival can be achieved with PTCy in stringent fully MHC mismatched murine models of skin, heart, and vascularized composite allotransplantation. Stable multi lineage chimerism and recipient derived regulatory T cells play a critical role in maintaining tolerance in this model, in particular in the peritransplant time period.

Vascularized Composite Allograft Tolerance Across A Full MHC Mismatch Is Possible With Transient High Dose Tacrolimus

Angelo A. Leto Barone, MD, Howard D. Wang, MD, Edward W. Swanson, MD, Keli Kolegraff, MD, PhD, Joseph Lopez, MD, MBA, Georg Furtmüller, MD, Byoungchol Oh, PhD, Sara AlFadil, MD, Justin M.
Sacks, MD, Steven C. Bonawitz, MD, Giorgio Raimondi, PhD, Jaimie T. Shores, MD, Damon S. Cooney, MD, PhD, W. P. Andrew Lee, MD, Gerald Brandacher, MD

Johns Hopkins, Baltimore, MD

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.

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

Wendy Chen, MD, MS1, Lin He, MD1, Yang Li, MD2, Chiaki Komatsu, MD3, Maxine R. Miller, MD3,4, Yolandi van der Merwe, B. Eng.5,6, Katie Lucy, BS7, Huamin Tang, MD7, Ian Rosner, BS7, Mario G. Solari, MD3, Gadi Wollstein, MD8, Joe S. Schuman, MD8, Kevin C. Chan, PhD8, Kia M. Washington, MD3,9

1University of Pittsburgh Medical Center, Pittsburgh, PA, 2Fourth Military Medical University Department of Plastic and Reconstructive Surgery, Xi’an, China, 3University of Pittsburgh Medical Center Department of Plastic Surgery, Pittsburgh, PA, 4University of Pittsburgh Medical Center Department of Ophthalmology, Pittsburgh, PA, 5University of Pittsburgh Medical Center Department of Ophthalmology, Department of Bioengineering, Pittsburgh, PA, 6University of Pittsburgh Neuroimaging Laboratory, Pittsburgh, PA, 7New York University Langone Eye Center, New York City, NY, 8New York University Langone Eye Center, New York, NY, 9Veterners Administration