3 -Title: Treg-mediated donor-specific hypo responsiveness in a pig-to-nonhuman primate thymokidney xenotransplantation

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Background: The chronic rejection and immunosuppression (IS) associated toxicity remains one of the major obstacles to long-term survival in xenotransplantation. The ultimate solution could be the induction of immune tolerance. In this study, we investigated thymus-dependent tolerance induction across xenotransplantation barriers using a pig-to-nonhuman primate thymokidney (TK) xenotransplantation model.

Methods: A GalT-KO SLA_{hh} miniature swine was used as the TK donor. The recipient baboon underwent total thymectomy and induction therapy with rATG and anti-CD20 mAb. On day 0, the baboon underwent bilateral nephrectomy and splenectomy and received a life-supporting TK xenograft. Posttransplant IS consisted of MMF, rapamycin, anti-CD40 mAb, and CTLA4 Ig. After day 380, IS was gradually weaned off. Kidney function were monitored using biochemistry and biopsies. Donor-specific antibodies (DSA), absolute T-cell, B-cell, and recent thymic emigrant (RTE) counts were monitored. Mixed lymphocyte reaction (MLR) assays were performed to monitor tolerance development.

Results: RTEs were detected within 2 months after the transplant. After day 120, consecutive MLR assays indicated pig-specific hyporesponsiveness. Donor-specific hyporesponsiveness was seen on MLR assay on day 447, mediated by Tregs. Following 380 days of stable graft function, IS was weaned off (MMF in 4 weeks, rapamycin in 2 weeks, and anti-CD40 mAb in 2 weeks). After 60 days of CTLA4 Ig monotherapy, the kidney functions rapidly deteriorated, and the animal was euthanized on day 502. IgM and IgG DSA remained unchanged for the duration of the study, while IgA DSA increased 2-fold after rapamycin was weaned. Before IS weaning, biopsy showed transplant glomerulopathy and fibrosis with no signs of rejection or immunoglobulin and complement deposition, while biopsy at euthanasia showed acute T-cell and chronic antibody-mediated rejection with IgA, IgM, IgG, C4d, and MAC deposition.

Conclusion: Treg-mediated donor-specific hyporesponsiveness in a baboon was achieved by thymokidney xenotransplantation from a single GalT-KO SLA_{hh} swine donor. Even though clinical tolerance was not achieved as the kidney graft was rejected within 2 months of CTLA4 Ig monotheraphy, our study suggests that regulatory tolerance in thymokidney xenotransplantation is achievable.