Aire-Expressing Cells are Novel Mediators of Allograft Tolerance in a Murine Heterotopic Heart Transplant Model

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Introduction: The AutoImmune REgulator (Aire) gene is pivotal in the establishment and maintenance of immune tolerance and homeostasis. Its functional significance has been established in preventing autoimmunity through upregulation of tissue-specific antigen expression in medullary thymic epithelial cells (mTECs). Recently, extrathymic *Aire*-expressing cells (eTACs), found in secondary and tertiary lymphoid organs, were shown to be critical for the maintenance of maternal-fetal immune tolerance. Here, we investigated the role of *Aire*-expressing cells in mediating transplantation tolerance utilizing a MHC class II-mismatched vascularized cardiac transplant model of chronic rejection.

Methods: We previously developed an *Aire*-regulated transgenic mouse line (*Aire*DTR mice) that drives the expression of the diphtheria toxin receptor (DTR) to allow for controlled ablation of *Aire*-expressing cells *in vivo* through diphtheria toxin administration. Bm12 donor hearts (*B6(C)-H2-Ab1^{bm12}/KhEgJ)* were transplanted into recipient *Aire*DTR or wildtype mice, followed by diphtheria toxin treatment. Transplant survival was assessed by transabdominal palpation of the allograft, and cessation of heartbeat was confirmed by echocardiography. At the time of rejection, the allograft and native heart were harvested for histology and the spleen and draining lymph nodes were processed for flow cytometry.

Results: A total of 6 *Aire*DTR and 9 wild-type mice were transplanted and followed for 90 days (**Figure 1A**). Accelerated rejection was observed in *Aire*DTR mice compared to wild-type (Log-rank test $p<0.05$, **Figure 1B**). On the day of harvest, rejected allografts appeared grossly edematous and were friable (**Figure 1C**). On H&E staining, rejected allografts exhibited diffuse cell infiltrate and hemorrhage, indicative of cell-mediated rejection (**Figure 1D**). Flow cytometry showed an increase in activated $CD69^+$ $CD4^+$ T cells in the spleen and draining lymph nodes of rejected grafts.

Conclusion: We demonstrate that ablation of *Aire*-expressing cells leads to accelerated rejection in a mouse heterotopic heart transplant model. These data support a previously undescribed role for *Aire*-expressing cells in maintaining immune tolerance and preventing allograft rejection. Further studies are needed to determine the immune mechanisms, particularly the relative contributions of thymic mTECs and eTACs underlying this rejection phenotype.

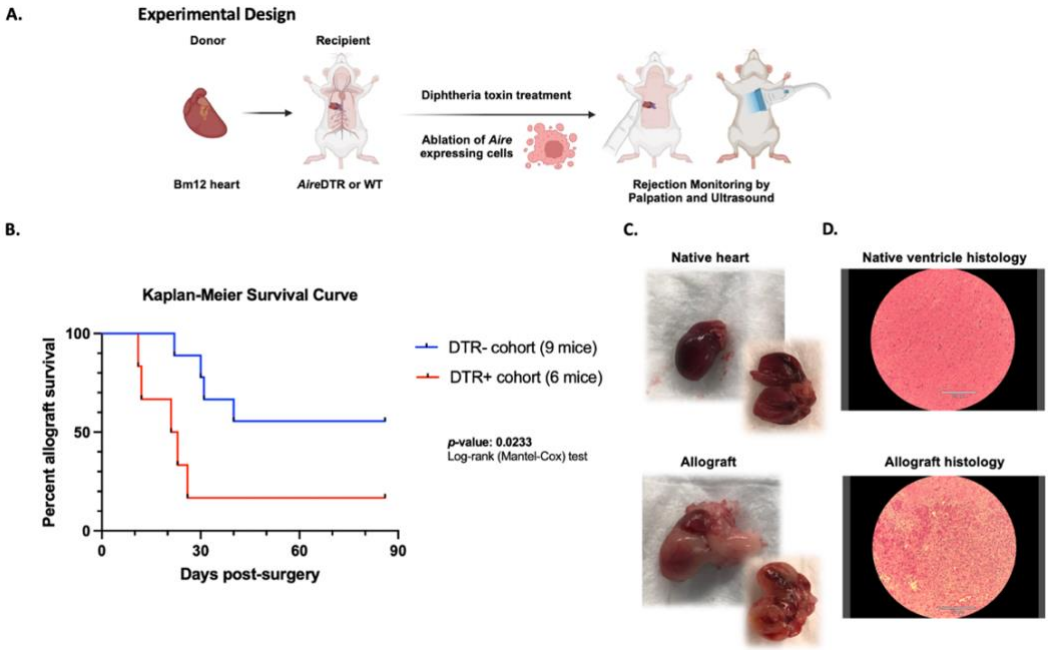

Figure 1. (A) Experimental design (B) Kaplan-Meier graft allograft survival curve. Graft survival differences are significant based on the Log-rank (Mantel-Cox) test, $p<0.05$. (C) Gross anatomy of the native heart and allograft taken on the day of harvest. Sagittal cross-section in outset image. (D) H&E staining of the native ventricle and allograft, displayed at 20X magnification.