<Comprehensive Transplant Center, Northwestern University Transplant Surgery >

Myd88-Areg axis in Regulating Host Responses to Vascularized Composite Allotransplantation

YN Guo, JY Cai, W. Yuan, S. HAN, J. Wang, L. VanOsdol, Z. Zhang
Comprehensive Transplant Center, Northwestern University, Chicago, Northwestern University Transplant Surgery, Chicago, IL

Background

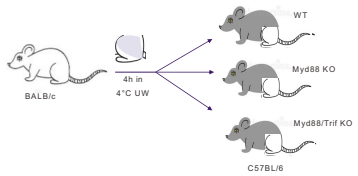
The rejection of Vascularized Composite Allotransplantation (VCAs) is known for being a more complicated process compared to solid organ transplant. The inherent discrepancy of immunogenicity among tissues has led to a phenomenon named “split tolerance”. The skin component, where antigen presenting cells and high levels of glycoproteins, has been viewed as highly immunogenic. When subjected to ischemic injury, the inflammation of skin grafts would fuel the immune rejection. Myd88 signaling have been documented in ischemia reperfusion injury, chronic graft dysfunction and rejection of solid organ (kidney, heart and lung) and skin transplantation. It has also been found to dampen inflammation through Amphiregulin (Areg). Areg, an EGF receptor ligands could also be induced in recipient regulatory T cells (Tregs), which would help reduce graft rejection and fibrosis. We sought to examine the role of Myd88-AREG axis in VCA.

Research Objectives

Unveiling the mechanisms and exploring the potential strategy that targets Myd88-Areg axis to improve the graft survival of VCA

Methods

Full MHC-mismatched hindlimb allografts procured from BALB/c mice were subjected prolonged cold ischemic storage (4 hours at 4°C UW) and then orthotopically transplanted onto wt C57BL6, Myd88-/- C57BL6 and Trif/Myd88-/- C57BL6 was performed. Graft survival was monitored and documented. Graft, spleen and lymph node were harvested at POD1 and POD7 in designated group. H&E staining, mRNA expression and flowcytometry was performed. Cellular infiltration and proinflammatory cytokines were evaluated.



Results

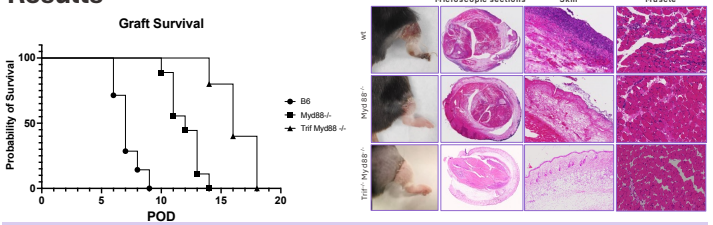


Figure 1. Graft survival. The median graft survival of wt C57BL6, Myd88-/- C57BL6 and Trif Myd88-/- C57BL6 were respectively 7d, 12d and 16d (Log-rank test p<0.0001). Representative photos from each group on POD7 are demonstrated above.

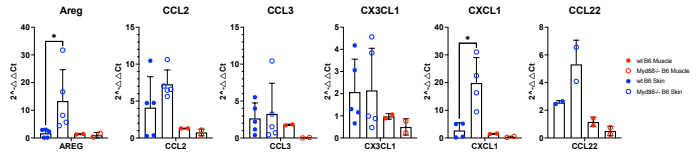


Figure 2. PCR. the Areg and CXCL1 are found significantly upregulated in Skin sample harvested on POD1. CCL22 were found to have the trend to elevate in graft skin but not muscles of MyD88-/- Recipients.

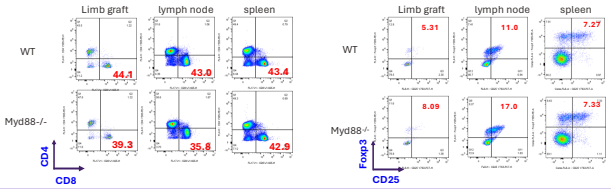
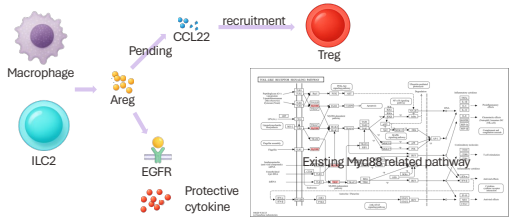


Figure 3. MyD88 deficiency Recipients show less CD8 T cells but more Regulatory T cells ($CD4^+CD25^+Foxp3^+$) On Day 7 after VCA

Working Hypothesis

We hypothesize that the deficient of Myd88 has led to a modulation of either Type 2 Innate Lymphoid Cells (ILC2) or Macrophage within skin component, which has been reported to be sources of Areg. Amphiregulin may function through EGFR or CCL22, the former has protective role in skin, the later help recruit regulatory T cells.

Our on-going experiments are aimed to test this hypothesis. We have performed additional limb transplants in mice and the VCA samples are collected at POD 1,3, and 7 for cell phenotypical and molecular analyses. In addition. We will explore treatments targeting Myd88, using a novel Lipid nanoparticle encapsuled the Myd88 inhibitor to examine its therapeutic potential.



Conclusion

Recipient deficiency of Myd88 has significantly reduced inflammation and extended full MHC-mismatched hindlimb graft survival. The early graft protection is associated with increased influx of Tregs that corresponds with increased CCL22 and Areg in the skin of in Myd88-/- recipient, suggesting a role of Myd88 in regulating mediated differentiation and recruitment of Treg through CCL22 and Areg. Further studies are warranted to determine precise mechanisms and potential strategy that targets Myd88-Areg axis for the improvement of VCA outcome.