Antibody Functionalized Peptidic Membranes Modulate Rejection of Skin Allografts

Y. Wen 1, E. S. Gawalt 1, N. Giannokakis 2, W. S. Meng 1
1 Duquesne University, 2 University of Pittsburgh

Purpose
The purpose of this study is to characterize the performance of an in situ forming membrane (or gel) to attenuate rejection of allogeneic transplants. Acute rejection is a function of donor antigen presenting cells (dAPC) accumulating in recipient lymph nodes. Previously we have reported an injectable platform (Figure 1) by which retention of IgG molecules in vivo is enhanced with EAK16-II and its analogue EAKIIH6 (Biomaterials, 2011, 32:249-257; Molecular Pharmaceutics, 2013, 10:1035-1044). Herein we report new data supporting the use of the system to display anti-MHC-II antibody (anti I-A\(^d\)) specific to dAPCs.

Methods
The dorsal ear skins of BALB/c (MHC: I-A\(^d\)) mice were grafted onto C57BL/6 (MHC: I-A\(^b\)) mice as the rejection model. Solution of amphiphilic peptides, intermediate proteins, and anti-MHC-II antibodies (anti I-A\(^d\) membrane) were administered prior to skin grafting. Retention of antibodies was monitored in live mice using a fluorescence conjugated IgG. APCs were enriched from recipient draining lymph nodes, and flow cytometry was used to quantify the number of I-A\(^d\)+ dAPCs. Splenic T cells from recipient mice were analyzed for interferon-gamma (IFN-\(\gamma\)) production using ELISA.

Results
The self-assembling system was able to retain superior fluorescence conjugated IgG. On day 3 and day 6, the system rendered 2-fold and 4-fold higher fluorescent intensity compared to the antibody alone (n\(\geq\)3, p<0.01). Flow cytometric analyses indicate that the anti I-A\(^d\) membrane treated C57BL/6 mice had only 2.0\%\pm0.18 of dAPCs in the draining lymph nodes, significantly lower than that (3.5\%\pm0.52) of C57BL/6 mice received only the anti I-A\(^d\) antibody delivered in saline (n\(\geq\)3, p<0.05). Splenic T cells recovered from mice received the anti I-A\(^d\) membrane produced 4.4 ng/ml\pm1.7 IFN-\(\gamma\) in contrast to 15.1 ng/ml\pm3.4 and 22.1 ng/ml\pm5.1 from mice received only anti I-A\(^d\) antibodies in saline or the amphiphilic peptides (EAK16-II/EAKIIH6) respectively (n\(\geq\)3, p<0.001).

Conclusion
Anti I-A\(^d\) membrane can attenuate anti-transplant T cells responses by impeding the trafficking of dAPCs in situ.