Transplant tolerance to minor antigen mismatched bone marrow grafts using PLG particles

Kelan A. Hlavaty, Derrick P. McCarthy, Woon Teck Yap, W. Kelsey Song, Stephen D. Miller, Lonnie D. Shea.
Northwestern University, Chicago, IL.

Statement of Purpose: Non-autologous cell and organ transplantation has become standard procedure for the treatment of numerous conditions, yet life-long immunosuppressive therapies are required to delay graft rejection. An antigen-specific therapy is a highly desirable solution to resultant toxicities from immunosuppressive drugs, as this would selectively allow tolerance to donor antigens without compromising the immune system as a whole. Using antigen-loaded (surface coupled and encapsulated) biodegradable particles, we have investigated transplant tolerance in a histocompatibility Y chromosome antigen (Hya) model of bone marrow transplantation, where Dby and Uty are the respective CD4 and CD8 male-specific minor Hy antigens that are responsible for rejection in major MHC matched donor recipient/pairs.

Methods: Poly(lactide-co-glycolide) (PLG) particles were fabricated using a single emulsion technique. Size was determined by dynamic light scattering (DLS). Particles were coupled to either Dby, Uty, or a sham $OVA_{323-339}$ peptide using the chemical crosslinker 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDC). PLG particles encapsulating Dby, Uty, or a sham $OVA_{323-339}$ peptide were fabricated using a double emulsion technique. Coupling and encapsulation efficiencies of Dby and Uty peptides were determined by BCA protein assay.

C57BL/6 CD45.2 female recipient mice were irradiated (non-lethal dose) one day before transplant of C57BL/6 CD45.1 male bone marrow cells (Day 0). Recipients receiving CD45.1 female bone marrow served as a positive control. Surface-coupled or encapsulated particles (Dby, Uty, or Dby+Uty) were injected i.v. into recipients on Day -7 and/or Day +1, where the timing of doses and dose amount was investigated. Transplant recipients receiving PBS injections served as a negative control. Recipients were evaluated up to 20 weeks post-transplant for the presence of engrafting donor CD45.1 donor bone marrow by tail blood samples and FACS. Additionally, tolerance was examined through proliferation of recipient splenocytes and production of IFN-γ in response to Dby, Uty, and $OVA_{323-339}$ peptides.

Results: Both single and double emulsion PLG particles produced were 400-500 nm with a zeta potential ~ -45 mV. The amount of peptide encapsulated ranged from 0.5 - 2.5 μg/mg and encapsulation efficiencies were ~8%.

Mice receiving Dby coupled particles (Dby-PLG) demonstrated tolerance to minor antigen mismatched bone marrow grafts through 20 weeks post-transplant **(Fig 1)**. Dby-PLG mice showed ~ 40% engraftment, compared to 25% engraftment from the positive control group and zero engraftment from the negative and sham controls. Furthermore, tolerizing on Day +1 yielded similar engraftment to tolerizing on both Days -7 and +1, where Day -7 alone was less efficacious.

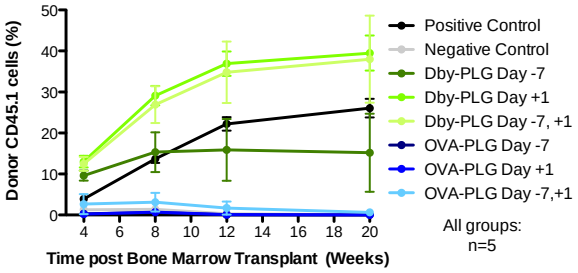

Figure 1.

Mice tolerized with particles encapsulating Dby peptide, PLG(Dby), also demonstrated engraftment 8 weeks post-transplant **(Fig 2D)** compared to the mice receiving sham PLG(OVA) or PBS (**Fig 2A-B**). Encapsulated Uty peptide alone was not sufficient to induce tolerance to bone marrow grafts. Additionally, isolated splenocytes from tolerized recipients showed reduced proliferation and IFN-γ production in response to the Dby peptide compared to sham OVA-PLG tolerized mice.

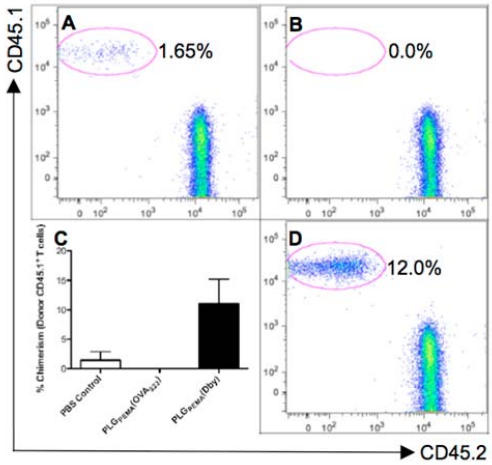

Figure 2.

Conclusions: The CD4 male-specific Dby epitope induces tolerance to minor HY antigen mismatched bone marrow grafts, both when coupled to the surface of PLG particles and encapsulated within PLG particles. The percentage of engrafting donor bone marrow cells in tolerized recipients exceeded 50% in several animals, and a single particle dose on Day +1 was sufficient for tolerance. Conversely, the CD8 Uty epitope did not induce tolerance, indicating that CD4 T cells are the primary mediator of graft rejection in this model. Future studies will address transplant tolerance in a fully MHC mismatched allogeneic model, where the donor antigens are numerous and unidentified. Using PLG particles as a platform for inducing tolerance in minor HY antigen mismatched bone marrow transplantation is a key step in developing a translatable, donor-specific tolerance strategy for modern transplantation procedures.