A Biodegradable Antigen-Encapsulating Particle Platform for the Treatment of Immune Dysfunction and the Promotion of Transplant Engraftment

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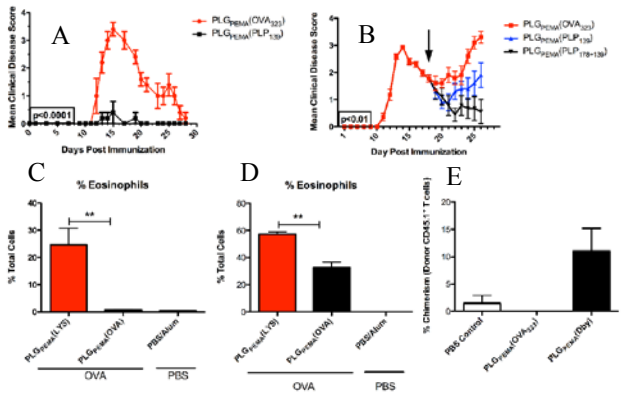
Statement of Purpose: The induction or restoration of antigen (Ag)-specific immune tolerance is crucial for the development of therapies for T-cell mediated autoimmune disorders and allergies and the induction of transplant tolerance. There is currently no universally efficacious method for the safe, facile, and cost-effective induction of Ag-specific immune tolerance. We demonstrate for the first time a clinically-translatable biodegradable microparticle technology platform that can be used to prevent and treat the T_H1/T_H17-driven relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE) murine model of multiple sclerosis (MS), the T_H2-driven allergic airway inflammation (AAI) murine model of allergic asthma, and prevent bone marrow graft rejection caused by minor histocompatibility Ag (miHA)-mismatch.

Methods: Ag-encapsulating microparticles were fabricated using the biodegradable polymer, poly(lactide-co-glycolide) (PLG), by a water-in-oil-in-water (W/O/W) double emulsion-solvent evaporation method. Briefly, Ag was dissolved in water, added dropwise to a solution of PLG in dichloromethane (DCM) and sonicated. The resultant emulsion was then added to an aqueous solution of poly(ethylene-alt-maleic acid) (PEMA) and sonicated. The final W/O/W emulsion was then added to a larger volume of aqueous PEMA and stirred overnight to allow for the evaporation of DCM. The resultant Ag-encapsulating PLG microparticles [PLG(Ag)] were then centrifuged and washed with water thrice. For R-EAE, SJL/J mice were tolerized with PLG($PLP_{139-151}$) or PLG ($OVA_{323-339}$) (irrelevant Ag control) on days -7 and -1 relative to R-EAE disease induction. On day 0 mice were immunized subcutaneously on the flank with $PLP_{139-151}$ peptide emulsified in complete Freund's adjuvant (CFA) to induce R-EAE. For AAI, mice were immunized intraperitoneally with ovalbumin (OVA) in alum adjuvant on days 0 and 14. Mice were tolerized with PLG(OVA) or PLG(LYS) (LYS = lysozyme, control) on either days -7 and 7 (prophylactic) or days 28 and 42 (therapeutic) prior to aerosol challenge. Mice were sacrificed and tissues were harvested. Cells from bronchalveolar lavage fluid (BALF) were cytospun and stained with DiffQuik stain prior to differential cell counts. For the prevention of bone marrow graft rejection, CD45.2 C57BL/6 females were irradiated and transplanted with bone marrow cells from either congenic CD45.1 C57BL/6 females or CD45.1 C57BL/6 males. Transplant recipients of male donor cells were given phosphate-buffered saline (PBS), PLG(Dby) or PLG($OVA_{323-339}$) (control). At weeks 8 post transplant, the graft recipients were bled to examine the level of T cell chimerism.



Results: PLG microparticles containing the encephalitogenic $CD4^+$ epitope, $PLP_{139-151}$, [PLG($PLP_{139-151}$)] completely prevented the development of R-EAE (Fig. A). PLG($PLP_{139-151}$ + $PLP_{178-191}$) and PLG($PLP_{139-151}$) administered to mice during disease remission significantly attenuated the primary R-EAE relapse (Fig. B). PLG microparticles containing ovalbumin [PLG(OVA)], completely prevented the development of T_H2-dependent OVA-specific AAI , as indicated by reduced bronchoalveolar eosinophilia levels in PLG(OVA)-treated mice (Fig. C). Mice with pre-existing AAI, treated with PLG(OVA), exhibited decreased bronchoalveolar eosinophilia (Fig. D) and the T_H2 cytokines, IL-4, IL-5, IL-13, and IL-10 indicating that immune tolerance corrected the T_H2 immune dysregulation. The male-derived Ag, Dby, is a critical determinant in the histocompatibility-Y (H-Y) Ag system of miHA-mismatched transplant rejection. PLG(Dby) induced transplant-specific immune tolerance and enabled male bone marrow cells to engraft in female mice (Fig. E).

Conclusions: Our results provide initial proof-of-concept for the use of PLG(Ag) as a viable platform for the general induction of Ag-specific immune tolerance and the study of the immunological mechanisms underlying tolerance induction.