A dendritic cell-targeting microparticle vaccine for treatment of Type 1 Diabetes

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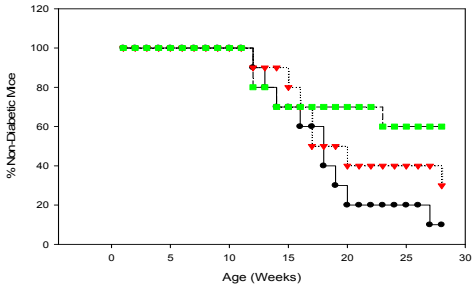
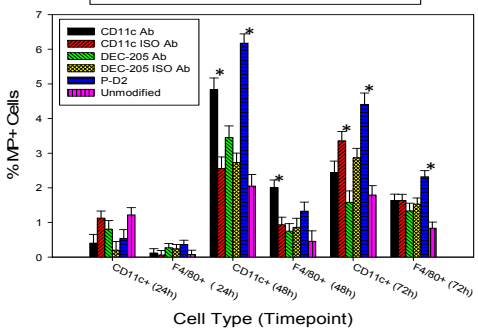
Introduction: Current paradigms for diabetes treatment are inadequate at responding accurately to short term homeostatic imbalances and cannot prevent chronic diabetes-related complications. Predictably, novel approaches to re-establish homeostatic conditions in patients afflicted by T1D, including generation of immunological tolerance to auto-reactive diabetogenic epitopes, are being investigated. Notably, the exogenous generation and injection of tolerance-promoting dendritic cells (DCs) is being pursued in clinical trials for applications in diabetes. While instructive, exogenously-conditioned DC-based vaccines for T1D treatment have numerous limitations including isolation and storage of DCs, and high manufacturing costs that prohibit widespread application. Therefore, alternatives are being pursued for the targeted delivery of conditioning factors to DCs *in vivo*. One design strategy focuses on *in vivo* targeting of DCs with injectable, synthetic particulate systems that can deliver vaccine components including immunomodulatory agents. We are developing a multifunctional, synthetic microparticle-encapsulated vaccine that can be easily administered with simultaneous and continuous delivery using controlled-release materials (poly lactide-co-glycolide). Further, these microparticle-based vaccines are engineered to target DCs, and provide both intracellular and extracellular delivery of Vitamin D3 [Vit D3], Transforming growth factor- beta 1[TGF-β1], and granulocyte macrophage colony stimulating factor [GM-CSF]). Our long-term goal is to develop a novel synthetic microparticle (MP) vaccine capable of reversal of T1D. To date, we have investigated (i) the ability of targeted MPs to improve *in vivo* DC uptake and translocation, (ii) the effect of our non-targeted MP vaccine on bone marrow-derived DC phenotype and downstream effects on allogenic T cells, and (iii) the efficacy of the non-targeted MP vaccine to prevent diabetes onset in NOD mice.

Methods: A 50:50 polymer composition of poly (d lactide-co-glycolide) (PLGA) was used to generate microparticles via a standard oil-water solvent evaporation technique and sized using standard DLS equipment. To determine the tolerogenic nature, MP-fed DCs were immuno-fluorescently stained after 72 h with antibodies for maturation (MHCII, CD80, CD86) and tolerance-inducing markers (IDO) followed by flow cytometric analysis. Additionally, T cell suppression and Treg induction was analyzed using standard allogenic MLC procedures followed by immuno-staining and flow cytometry. We studied the ability of our particle vaccine approach to prevent diabetes in a cohort of NOD mice given injections of our formulation at 8 weeks of age. The blood glucose levels of mice were then monitored once weekly for the next 28 weeks. Fluorescent dye-loaded MPs were injected into the footpad of mice to determine the DC targeting efficacy of our ligand-modified MPs in an in vivo environment following excision of draining lymph nodes.

Results: We fabricated two classes of MPs sized ~ 1μm (phagocytosable) and 30 μm (un-phagocytosable). The phagocytosable MPs were loaded with Vit D3 and insulin. The un-phagocytosable MPs encapsulated TGF-B1 and GM-CSF. We confirmed loading and release kinetics of these drug-loaded using conventional particle degradation and drug detection methods. The effects of the Vit D3/ TGF-β1 dual MP system on the expression of stimulatory molecules on DCs were studied. In comparison to iDCs, all of these activating markers are down-regulated significantly by incubation with the combination of VitD3/ TGF-β1 MPs (GM-CSF in media). One of the downstream effects of the DCs exposed to Vit D3/ TGF-β1 MPs is that these DCs considerably inhibit proliferation of allogenic T cells (not shown). These MPs were injected into a cohort of 8 wk old, female NOD mice to investigate their efficacy at diabetes prevention. Kaplan-Meier analysis at this point reveals an overall p-value of 0.153 for survival proportions, with the GM-CSF+Vit D3+TGF-B1+insulin MP formulation having 60% non-diabetic mice, compared to the blank MPs at 10% non-diabetic after 28 wks (**Figure 1**).



← **Figure 1**. MP vaccine with loaded D3, GM-CSF and TGF-B1 may be capable of delaying the onset of diabetes in NOD mice.



← **Figure 2**. Uptake and trafficking of MPs by APCs is enhanced through MP surface modification.

Finally, we investigated how MP functionalization influenced the extent of MP translocation from injection site to proximal lymph node. After 48 h, CD11c Ab and P-D2 peptide surface-modified MPs increased the rate and extent of MP uptake and trafficking of DCs and MΦs to the draining LN compared to unmodified MPs (**Figure 2**).

Conclusions: These preliminary studies demonstrate that our engineered microparticle vaccine formulations can: (a) modulate DC phenotype and further promote allogenic T cell hyporesponsiveness to exposed DCs; (b) prevent the onset of diabetes in NOD mice if treated at an age that is therapeutically relevant; (c) target DCs in vivo for uptake and trafficking. In the future, we will investigate diabetes prevention with the targeted MPs, as we expect improved uptake to further promote diabetes prevention.