A microparticle-based vaccine for the amelioration 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. Recent research has implicated the cause of auto-immune diseases, particularly type 1 diabetes (T1D), to be a reduction in number and function of regulatory T cells (Tregs) which suppress low levels of physiologic auto-reactive cells. Dendritic cells (DCs) play a critical role in the maintenance of peripheral tolerance including induction of Tregs. We are developing a multifunctional, synthetic microparticle-encapsulating vaccine that can be easily administered with simultaneous and continuous delivery using controlled-release materials (poly lactide-co-glycolide) for the amelioration of T1D. Moreover, these microparticle-based vaccines are engineered to target DCs, and provide both intracellular and extracellular delivery of immunomodulatory agents (Vitamin D3 [VitD3], Transforming growth factor- beta 1[TGF-β1], and Granulocyte macrophage colony stimulating factor [GM-CSF]) as well as antigen. Our ultimate goal is to develop a novel synthetic microparticle (MP) vaccine capable of reversal of T1D. To date, we have demonstrated (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. Current investigative work is focused on espousing the cellular mechanisms behind the observed prevention in NOD mice and enhancing the prevention efficacy of this formulation for potential translation into humans.

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 48 h with relevant tolerogenic markers (e.g. 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. Finally, purified splenocyte populations from NOD mice treated with the particle vaccine were adoptively co-transferred with diabetic splenocytes in NOD.SCID mice to determine the cellular elements involved in the observed protection.

Results: We fabricated two classes of MPs sized ~ 1μm (phagocytosable) and 30 μm (un-phagocytosable). The phagocytosable MPs were loaded with 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. Diabetes was prevented in 60% mice injected with GM-CSF+D3+TGF-B1+insulin MP formulation, compared to the blank MPs at 10% (**Figure 1**).

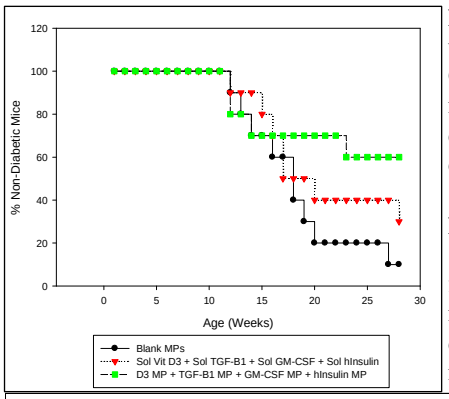
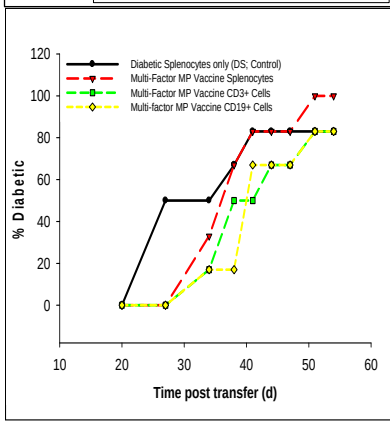


Figure 1 (Top left). MP vaccine with loaded D3, GM-CSF and TGF-B1 may be capable of delaying the onset of diabetes in NOD mice.

Figure 2 (Below). Splenocytes populations from MP vaccine-treated mice delayed the onset of diabetes in NOD.SCID mice.



Cells Transferred	Mean Survival Time (d)
Diabetic Splenocytes (Control)	35.7
Multi-factor MP Vaccine Splenocytes	41.5
Multi-factor MP Vaccine CD3+ Cells	43.2
Multi-factor MP Vaccine CD19+ Cells	43.7

We have also begun to investigate the immune cell populations responsible for the protection diabetes in particle vaccine-treated NOD mice using an adoptive transfer model into NOD.SCID mice. In this adoptive transfer model that is yet to be optimized, all splenocytes populations from MP vaccine-treated mice delayed the onset of diabetes in NOD.SCID mice, particularly purified CD3+ cells and purified CD19+ cells (**Figure 2**).

Conclusions: These preliminary studies demonstrate that our engineered microparticle vaccine formulation can: (a) modulate DC phenotype and further promote allogenic T cell hyporesponsiveness to exposed DCs in vitro; (b) prevent the onset of diabetes in NOD mice if treated at an age that is therapeutically relevant; (c) modulate immune cells in vivo and potentially induce protective subpopulations of T and B cells. In the future, we intend to improve vaccine formulation efficacy at T1D prevention in mice and further, identify the subsets of immune cells responsible for protection.