Biomaterial-based Microparticle Vaccine Modulates Cellular Tolerance for Type 1 Diabetes

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Statement of Purpose: Type 1 Diabetes (T1D), characterized by T-cell mediated destruction of insulin producing β-cells, is linked to the dysfunction and number of dendritic cells and antigen-specific regulatory T-cells (Tregs) [1]. Dendritic cells (DCs) are professional antigen-presenting cells that play an intimate role in regulating the adaptive immune system, capable of initiating inflammatory or anti-inflammatory responses. Dendritic cells can modulate immune tolerance through the induction of Tregs, T-cell anergy, and production of anti-inflammatory cytokines [2]. Dendritic cells exogenously treated with tolerogenic factors have shown promise as an autoimmune therapy, but present numerous challenges for implementation [3]. It is possible to circumvent these challenges by fabricating a tunable, biodegradable biomaterial delivered in vivo. We are developing a synthetic particle-based vaccine using poly(lactide-co-glycolide) microparticles (MPs) encapsulating tolerogenic factors (Vitamin D3 [VitD3], Transforming Growth Factor- beta 1[TGF-β1], and Granulocyte Macrophage Colony Stimulating Factor [GM-CSF]) along with antigen specific for T1D (denatured insulin) for delivery to DCs via subcutaneous injection. While previous prevention studies in our lab using the multi-factor MP vaccine showed efficacy in preventing diabetes in 60% of NOD mice the underlying mechanisms of action are still under investigation.

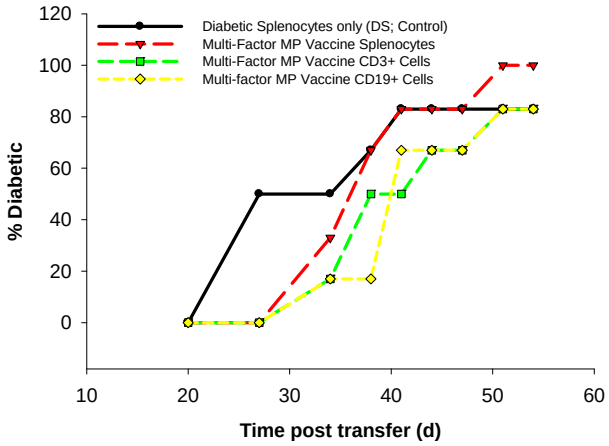
Methods: A vaccine was developed by fabricating two classes of MPs. Phagocytosable MPs were loaded with VitD3 and insulin and un-phagocytosable MPs were loaded with TGF-β1 and GM-CSF. Female, 8 week old NOD mice were given three subcutaneous injections total of the multi-factor MP vaccine every other day. Spleens were harvested 14 days post-injection from MP-treated mice and cellular subpopulations isolated for 3 experimental groups: all splenocytes, CD3+ splenocytes only, and CD19+ splenocytes only. Diabetic splenocytes from hyperglycemic NOD mice along with purified, MP-treated splenocytes were adoptively transferred intra-peritoneally into immunodeficient NOD.SCID mice. The blood glucose levels were then monitored once weekly for the next 32 weeks. Mice were considered diabetic when blood glucose levels were consecutively above 240 mg/dL and sacrificed from the study. Spleen and blood were also harvested from these mice to identify cellular components of tolerance.

Results: Large non-phagocytosable and small phagocytosable MPs were sized to ~30 μm and ~ 1 μm, respectively. Loading efficiency and release kinetics showed encapsulation efficiency to be ~ 60% for loaded MPs (data not shown). Splenocytes harvested from MP-treated mice delayed the onset of T1D and increased mean survival time by over 16% compared to the diabetic splenocyte control group. Survival was further improved to over 22% and 21% by transferring CD19+ B-cells and CD3+ T-cells, respectively.

Conclusions: Preliminary data indicates that our MP vaccine (a) modulates immune cells towards tolerogenic phenotypes (b) allows transferrable tolerance (c) affects cell populations (CD3+ and CD19+) more selectively. Future studies will first optimize the adoptive transfer study and then pinpoint specific cellular subpopulations within CD3+ and CD19+ phenotypes

References:

[1] J.H. Buckner, *Nat. Rev. Immunol.* **10**, 849 (2010)
[2] S. Rutella, et al. *Blood*. **108**, 1435 (2006)
[3] S.K. Mallapragada, B. Narasimhan, *Int. J. Pharm.* **364**, 265 (2008)



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

Figure 1. Survival rates of NOD.SCID mice adoptively transferred with MP-treated splenocyte subpopulations and diabetic splenocytes.