Immuno-suppressive Microparticle Vaccines for Type 1 Diabetes

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Introduction: The debate on the cause of autoimmune diseases, particularly diabetes, has shifted recently from auto-reactive cell escape of clonal deletion to reduction in number and function of regulatory T cells (T regs) which suppress low levels of physiologic auto-reactive cells.[1] This new opinion has inspired the development of moderately successful T1D therapies that seek to generate greater numbers of functional regulatory T cells in patients afflicted with insulin-dependent diabetes.[2] Of particular interest to our group is dendritic cell-based approaches. Dendritic cells (DCs) are often called 'natural adjuvants' due to their ability to recruit the adaptive arm of the immune system in response to 'foreign' material, but it has been recognized that they also play a critical role in the maintenance of peripheral tolerance.[3] To this extent, scientists have developed vaccines in which DCs prompt Treg induction and ultimately curtail excessive immune responses. However, problems such as the plasticity and complexity of DC maturation, donor specificity, ex-vivo stability, shelf-life, and cost abrogate the benefits of this therapeutic approach.[4]

A rational design strategy might focus on the development of a synthetic particle-encapsulated vaccine, or *vaccine particle* that can be easily administered with simultaneous delivery of both prime & boost doses using time-release materials (e.g., poly lactide-co-glycolide).[5] This flexible approach greatly simplifies issues related to manufacturing, storage, and shipping as biomaterial encapsulation provides vaccine stability and improved shelf-life. Furthermore, they can be engineered to target specific antigen presenting cells, and provide both intracellular and extracellular delivery of immunomodulatory agents. Our *long-term goal* is to develop a novel synthetic microparticle vaccine capable of prevention and reversal of the onset of type 1 diabetes. In these preliminary studies, we researched (i) the ability of surface-modified PLGA microparticles (MPs) to target to DCs for phagocytosis, (ii) induction of tolerogenic DCs (tDCs) and Tregs using drug-loaded MPs and (iii) antigen presentation of diabetogenic peptide by DCs fed GAD1040-55 peptide-loaded MPs.

Materials and Method: Immature DCs (iDCs) were obtained by culturing precursors isolated from bone marrow of C57BL/6j mouse in the presence of 20 ng/ml of growth factor GM-CSF for 10 days with half-media change every alternate day.

A 50:50 polymer composition of poly(d,l lactide-co-glycolide) (PLGA) (Lactel, AL, USA) was used to generate microparticles. Microparticles were formed using a standard oil-water solvent evaporation technique.

Microparticle uptake was determined by measuring total fluorescence of rhodamine-loaded microparticles prior to and after exposure to DCs for 1 hr using a plate reader (excitation: 532 nm; emission: 585 nm). This assay is repeated for MΦs derived from bone marrow of BL/6 mice to compare how specific our particles are for DCs compared to MΦs.Cell culture supernatants were collected after 24 hours of cell culture with various surface-modified MPs. The IL-12 cytokine subunit, IL-12p40, and IL-10 cytokine production was analyzed using sandwich enzyme-linked immunosorbant assay (ELISA) kits (Becton Dickinson) according to manufacturer's directions.

To determine the level of expression of Treg-inducing indoleamine deoxygenase (IDO), DCS were collected and stained with fluorescently-tagged anti-IDO antibodies following manufacturer's instructions and analyzed as using flow cytometry. Additionally, T cell suppression was analyzed using standard allogenic MLR procedures flowed by immuno-staining and flow cytometry.

Results: Microspheres decorated with various ligands have differential levels of DC and Mφ uptake (**Fig. 1**). Further we demonstrated using cytokine profiles that our microsphere formulations do not induce DC maturation (**Fig. 2**). Finally, drug-loaded microparticles demonstrate the ability to induce expression of IDO in DCs (**Fig. 3**) and further suppress proliferation of T cell in a MLR (**Fig. 4**).

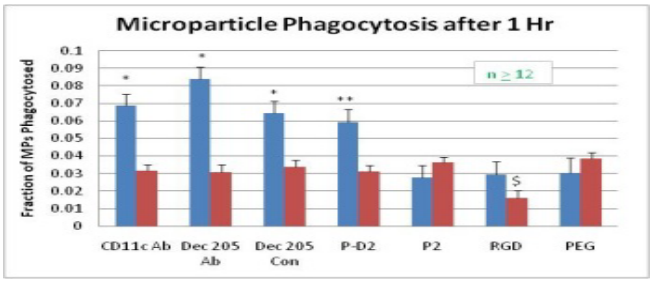

Fig. 1: Comparison of surface-modified microparticle phagocytosis between DCs (blue) and Mφ (red)

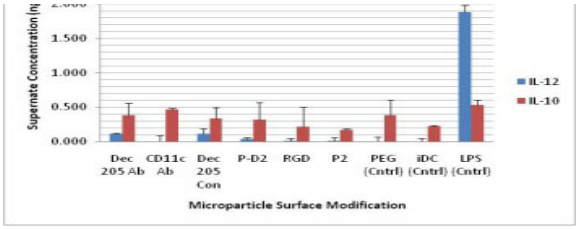

Fig. 2: IL-10 and IL-12 Cytokine Analysis of DCs treated with surface-modified microparticles

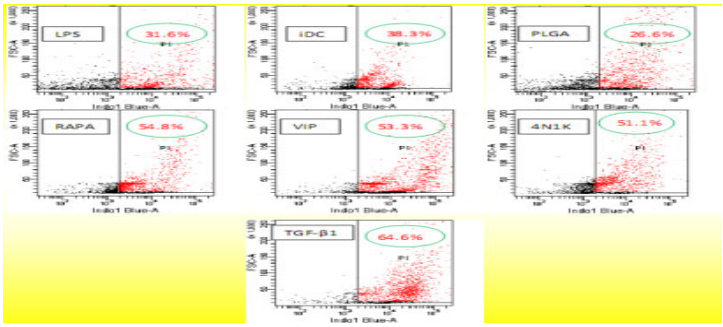

Fig. 3: Showing percentage of positively-stained DCs (after treatment with drug-loaded microspheres) for IDO as determined by immunofluorescent quantification

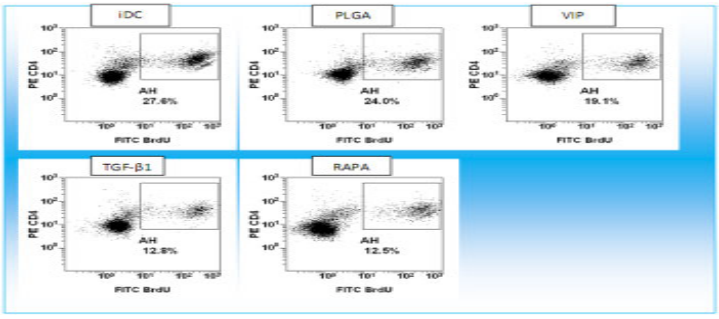

Fig. 4: Showing Percentage of T cells positive for BrdU incorporation as determined by flow cytometry. In contrast to controls (iDC, PLGA), DCs treated with immunosuppressive particles failed to support T cell proliferation in response to allogenic stimuli.

Conclusions:
These preliminary studies demonstrate engineered microparticle vaccine formulations that: (a) target DCs in vitro for phagocytosis (b) induce DCs with 'tolerogenic' phenotype (i.e. low MHC and co-stimulatory molecules, and production of suppressive agents (indoleamine deoxygenase [IDO]) and (c) reduce T-cell proliferation. The potential impact of optimized vaccines for the prevention and reversal of auto-immune diseases worldwide is quite significant.

References: (1) Kabelitz et al, Trends Immunol. (2008); (2) Lo et al, N. Y. Acad. Sci. (2006); (3) Steinman et al, Annl. Rev. Immunology (2003); (4) Morelli et al, Seminars in Immunology (2001); (5) Tamber et al, Advanced Drug Delivery Reviews (2005).