Enhanced Cell Migration and Efficient In-vivo Immune-modulation by Combinatorial, Single Formulation Delivery of siRNA, DNA vaccine and Chemokines

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Statement of Purpose: Dendritic cells (DCs) transmit specific signals (through local milieu of secreted cytokines) to T cells, which result in immune responses ranging from cytolytic (T helper type 1 (Th1)) to humoral (Th2). For viral and cancer diseases, a strong cytotoxic response (Th1 mediated) plays an important role in destroying the disease causing cells [1]. Naked DNA vaccines and DNA antigen loaded microparticles, administered intramuscularly, often fail to induce a significant divergence towards Th1 from Th2 response, attributed to high production of interleukin-10 (IL-10) by the DCs and low migration of DCs at the injection site. Hence there is a need to develop strategies that can not only recruit large number of DCs at the injection site but also enhance as well as preferably direct the immune response towards a strong Th1 phenotype, necessary for a robust anti-viral and anti-cancer response. Our recent results show that delivery of IL-10 targeted siRNA along with a DNA-based Hepatitis B surface antigen using a dual-mode siRNA-DNA loaded PLGA microparticles can significantly enhance DC activation and T cell proliferation in-vitro while successfully "switching" the in-vivo immune response towards a strong Th1 type and CTL response [2]. In the present study we present an integrated platform comprising of an in-situ crosslinkable, biodegradable, polymeric hydrogel network as a single delivery system for DC chemo-attractants (MIP3α) and dual-mode siRNA-DNA loaded PLGA microparticles that enhances recruitment of DCs and promotes specific divergence towards Th1 response.

Methods: Cationic PLGA microparticles with or without encapsulated siRNA (IL-10 targeted) were synthesized with PLGA using a w/o/w double emulsion and surface modified with covalently conjugated branched polyethyleneimine (bPEI) as reported by us previously [2, 3]. In vivo immune-modulation to evaluate Th1/Th2 class switching was performed where Balb/c mouse (n=8) were immunized with pDNA-PEI-PLGA microparticles with or without co-encapsulated IL-10 siRNA and CD4+ cells from spleens of these mice were analyzed to determine whether siRNA co-delivery can enhance Th1 cytokine (IFN-γ) production and decrease Th2 cytokine (IL-4) production. To further enhance the recruitment of DCs at injection site, in-situ crosslinkable hydrogels made of fast degrading dextran vinylsulfone (DexVS) and sulfhydryl functionalized four-armed poly(ethylene glycol) (PEG-4SH) molecules were formed using Michael addition reaction at pH 7.4-7.8 and 37ºC. Microparticles and chemokines encapsulated hydrogels were synthesized using Dextran Vinylsulfone (varying degree of vinyl sulfone substitutions (DS)) and various weight percentages of DexVS and PEG4SH. All hydrogels gelled within 2-4 minutes and that degraded ~80 within a week were chosen for further studies. For APC migration chemotaxis assay was performed as detailed earlier [4].

Results: In vivo Th1/Th2 class switching studies in Balb/c mice showed a 64 fold increase in IFN-γ production (Figure 1 (A)) when IL-10 siRNA was co-delivered with the surface-loaded pDNA antigen while IL-4 expression was markedly reduced (not shown). IL-10 siRNA co-delivery further resulted in 90 fold increase in CTL response [2]. Transwell chambers (pore size 5μm) were used to study migration of DCs in response to chemokines released by hydrogels. As shown in Figure 1 (B), DC migration (represented by chemotactic index) towards chemokine source was significantly higher at as low as 10 ng MIP3α dose (2 nM) and increased with increase in MIP3α concentration ($p < 0.05$).

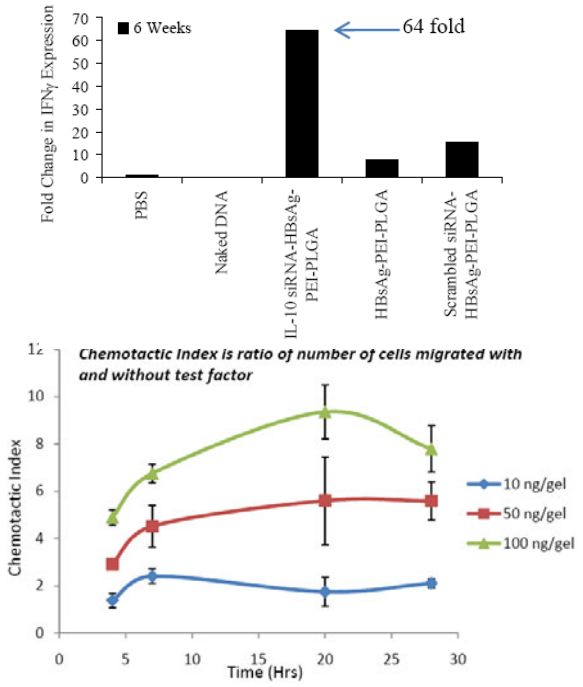

Figure 1: (A) Bar graph represents fold change in expression of IFN-γ by pooled splenocytes from immunized mice. **(B)** Primary DC migration using hydrogels loaded with 10, 50 and 100 ng MIP3a per hydrogel (equivalent to 2, 10 and 20 nM, respectively)

Conclusions: We have successfully demonstrated the capability of preferably diverting the immune response towards Th1 type by combinatorial delivery of IL-10 siRNA and DNA Vaccine using surface functionalized PLGA microparticles. Further, we have demonstrated significant migration of DCs towards the chemokine source (hydrogels with above microparticles). We are now performing studies to determine whether the encapsulated microparticles retain their efficacy and effect on immune response using the in-situ crosslinakble hydrogel based system.

References:

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