Therapeutic Immune-modulation using siRNA: combinatorial, single carrier delivery of IL-10 silencing siRNA and DNA vaccine

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Statement of Purpose: Immune modulation is a fundamental aspect for effective immunotherapy. For viral infections and cancer, a strong cytotoxic response (Th1 mediated, Class I restricted) plays an important role in destroying the disease causing cells. A vigorous cytotoxic (CTL) and helper T (Th) cell response to Hepatitis B virus (HBV) is weak or undetectable in patients with chronic infection [1]. Similarly for lymphoma, Th1 type response, specifically IFN- γ mediated events, is necessary for adequate protection or reversal of tumors [2]. Thus it is critical for an immunotherapeutic strategy to "drive" the antigen specific immune response towards a Th1 phenotype. Recently, short-interfering RNAs (siRNAs) have emerged as potential therapeutic tools [3] subsequently conferring knockdown of gene expression in a sequence specific manner. RNA interference mediated gene knockdown of specific Th1 suppressor genes (e.g Interleukin 10 (IL-10)) in antigen presenting cells (APCs) is a potential immuno-modulatory tool for tuning dendritic cells (DC) activation and function in-vivo, thereby driving the immune response towards the Th1 pathway and enhance cellular immunity. We have developed a novel, surface functionalized poly(lactide-co-glycolide) (PLGA) based, multi-tiered delivery system for combinatorial administration of IL-10 cytokine targeted siRNA and plasmid DNA antigens in a single gene carrier system.

Methods: Cationic PLGA microparticles with or without encapsulated siRNA (IL-10 targeted) were synthesized with an acid end-capped PLGA using a w/o/w double emulsion, solvent evaporation technique and surface modified with covalently conjugated branched polyethylimine (bPEI) as reported by us previously [4,5], The particles were characterized for encapsulation and biostability of siRNA and pDNA loading on the surface In vitro siRNA release studies as well as cellular studies in primary bone marrow derived dendritic cells were performed to evaluate gene silencing efficacy, pDNA transfection efficacy, DC activation/maturation and effect on T cell responses using mixed lymphocyte reaction. In vivo immune-modulation to evaluate Th1/Th2 class switching was performed where Balb/c mouse (n=8) immunized with pDNA-PEI-PLGA microparticles with or without co-encapsulated siRNA. Specifically CD4+ cells from spleens of immunized or control mice were analyzed to determine whether siRNA delivery can enhance Th1 cytokine (IFN-γ) production and decrease Th2 cytokine (IL-4) production and enhance CTL response.

Results: siRNA release from PLGA microparticles indicated a biphasic release over a period of 35 days. Transfection with IL-10 siRNA-PEI-PLGA microparticles resulted in significant gene silencing (Figure 1 (A) up to 80% compared to untreated cells). The silencing effect was sequence specific and for microparticle-treated cells, gene silencing efficacy remained unchanged even after 15 days. Flow cytometry studies confirmed significantly enhanced activation of DCs by the combinatorial delivery system with upregulation of phenotypic surface markers. In vivo Th1/Th2 class switching studies in Balb/c mice indicated a 10 fold increase in IFN-γ production (Figure 1 (B)) when IL-10 siRNA was co-delivered with the surface-loaded pDNA antigen while IL-4 expression was markedly reduced (latter not shown).

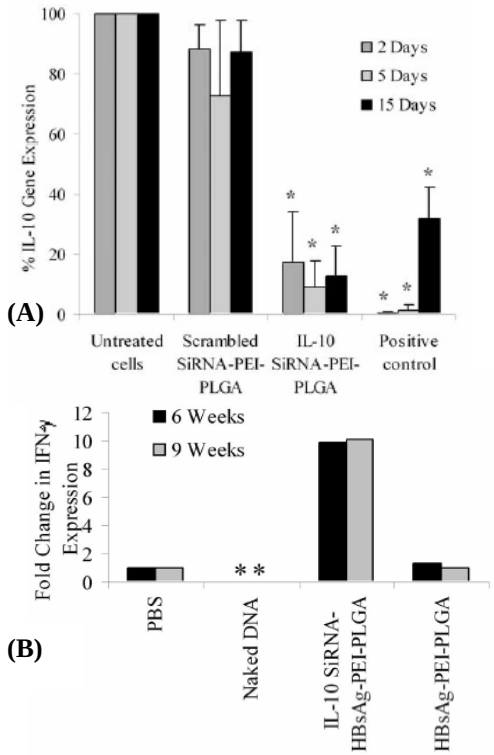

Figure 1: (A) Microparticles mediated Interleukin10 (IL-10) gene knockdown in Antigen presenting cells.* represents $p<0.05$.**(B)** Bar graph represents fold change in expression of IFN-γ by pooled splenocytes from immunized mice. * *Represents failure to detect any T cell activity by a group.*

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. Surface functionalized PLGA microparticles co-delivering IL-10 siRNA and DNA Vaccine demonstrated efficient, prolonged gene silencing, DNA transfection and a "switch" in the CD4+ T helper cell response from Th2 to a Th1 phenotype and was further supported by a vigorous improvement in CTL response with the with co-delivery system.

References:

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