T-Cell mediated delay of B cell Lymphoma tumor onset after immunization with *in situ* crosslinkable hydrogel vaccine

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Statement of Purpose: Tumor development occurs over a long period of time and undergoes limited immune-editing [1]. Once removed from the patient's body through surgical excision, the recurrence of tumor at metastatic sites also takes significant time. It is at this intermediary stage when a therapeutic vaccination against cancer can be used to either delay the onset of recurring tumor or completely prevent tumor development. Non Hodgkin's B cell malignancies result from a clonal expansion of B cells synthesizing an immunoglobulin (Ig) with unique variable regions in the heavy and light chains, called idiotype (Id) [2]. These Id molecules of a B-cell lymphoma can be recognized as a potential tumor marker for immunotherapeutic vaccine development against B cell malignancies. However, since the Id antigen is a patient-specific self-antigen, using recombinant protein based immunization approaches are time consuming, expensive and would be difficult to translate into a viable clinical approach [2]. On the other hand, use of plasmid DNA (pDNA) encoding for the idiotype antigen is significantly more feasible. These "self-antigens" remain poorly immunogenic and require use of manipulative additions of cytokines, chemokines to recruit more number of immune cells and adjuvants, to boost up the tumor specific immunity up to desirable levels and drive the immune response more towards T helper type 1 (Th1) [3]. We have developed an injectable, *in situ* forming, synthetic immune center that (a) attracts large number of dendritic cells (DCs) to the site of injection, (b) efficiently delivers DNA antigens to those DCs, (c) uses RNAi mediated cytokine silencing approach to "force" the immune response to a specific direction and (d) delay the onset of tumor in mouse model of A20 B cell Lymphoma.

Methods: Cationic PLGA microparticles with encapsulated siRNA (interleukin-10 (IL-10) targeted) were synthesized with PLGA and surface modified with covalently conjugated polyethyleneimine as reported by us [4, 5]. By using siRNA against IL-10 we drive the response towards Th1 by suppressing production of IL-10 by the DCs. To further enhance the recruitment of DCs at injection site, in-situ crosslinkable hydrogels made of dextran vinylsulfone (DexVS) and poly(ethylene glycol) tetrathiol (PEG-4SH) molecules were formed using Michael addition reaction at pH 7.8 and 37ºC and co-delivered microparticles and chemokines [6].

Ten Balb/C mice per group were immunized thrice in 2 week intervals as described earlier [5]. Hydrogel solutions co-encapsulating DC chemokine MIP3a and MCP3-sFv20 pDNA-IL10 siRNA loaded microparticles were injected intramuscularly at dose equivalent of 50 µg pDNA. After 9 weeks, spleens were isolated to perform T cell assays. In a separate experiment, mice were challenged i.p. 2 weeks post last immunization with lethal dose of 2×10^5 A20 tumor cells. Tumor onset was followed for 35 days.

Results: In vivo Th1/Th2 class switching studies showed a significant 17 fold increase in production of Th1 cytokine IFN-γ by CD4+ T cells from mice immunized with fast degrading hydrogels delivering MIP3a, DNA and IL10-siRNA. The formulation further resulted in 53% cytotoxic T cell (CTL) response as compared to 0.09 % with PBS control (Figure 1A). Tumor onset data revealed

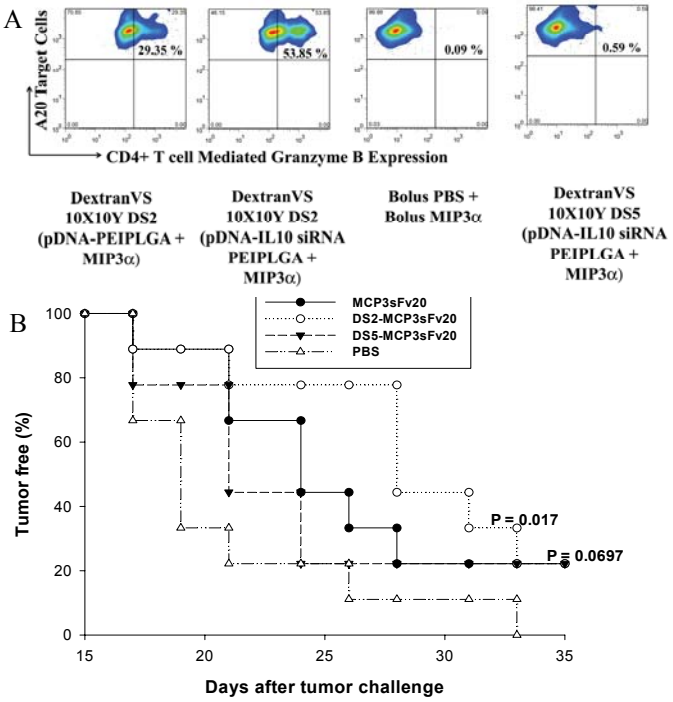

Figure 1. A) Expression of Granzyme B injected into target tumor cells by T cells from immunized mice, indicative of CTL activity. Data acquired using Flow cytometry. B) Tumor free mice upto 35 days from tumor challenge indicating tumor onset.

a significant delay in onset of tumor in mice immunized with hydrogels delivering MIP3a, DNA and IL10-siRNA over PBS (p = 0.017) as compared to using a slow degrading hydrogel system or naked DNA (Figure 1B).

Conclusions: We have successfully demonstrated the capability of generating and diverting nti-tumor immune response towards Th1 type by combinatorial delivery of chemokines, IL-10 siRNA and DNA Vaccine using a hydrogel-microparticle based hybrid biomaterial system. The strategy, which comprises of a single injectable formulation, also resulted in significant delay in tumor onset.

References:

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