Surface-functionalized Polymer Microparticles for Combinatorial Delivery of Nucleic Acid Vaccines against Cancer

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Statement of Purpose: DNA vaccines have emerged as a safer and potent alternative to traditional vaccines involving killed and live attenuated pathogens[1,2]. Synthetic polymeric delivery systems have shown considerable promise in terms of their safety, biodegradability and enhanced adjuvant activity to improve the potency of DNA vaccines[1,2]. We believe, based on our previous work as well as recent publications, significant opportunities exist in improving polymer-based delivery systems for genetic vaccines[2,3]. We have developed a novel cationic microparticle formulation by covalent conjugation of branched and linear PEI on the surface of biodegradable PLGA microparticles. Here we present in-vitro particle characterization demonstrating efficient activation of antigen presenting cells and *in-vivo* tumor challenge data showing the efficacy of these microparticles in pre-clinical prophylactic model of B cell lymphoma. Immunization with the PEI-PLGA formulations significantly enhanced the survival rates of mice following lethal challenge with the A20 B cell tumor. In vitro results in antigen-presenting cells indicate efficient maturation upon particle phagocytosis suggesting enhanced adjuvant activity which could lead to efficient protective immune response. We believe that further optimization of the microparticles and combination of these formulations with additional immunostimulatory molecules, would significantly enhance the anti tumor effects.

. **Methods:** Branched PEI of molecular weights 70kd and 25kd, Linear PEI, (25kd, Polysciences Inc, PA) and were conjugated to the surface of PLGA microparticles using a carbodiimide chemistry. These microparticles were characterized for size and zeta potential using Dynamic Light Scattering and electrophoretic mobility (ZetaPlus Brookhaven NY). Increase in buffering capacity was evaluated using acid titration experiments. Adjuvant property of formulations was studied using flow cytometric evaluation of maturation-specific cell surface markers in RAW 264.7, murine macrophage cells. *In vivo* animal studies were conducted with an A20 B cell lymphoma tumor challenge model in Balb/c mice. Formulations were injected either intradermally or intramuscularly 3 times at two week intervals before challenge with 2.5X the minimal lethal dose. Survival was followed upon tumor challenge and significant differences in survival were evaluated using a log-rank test.

Results/Discussion: We have previously published particle characterization and efficient transfection of antigen-presenting cells (APC) using PEI-PLGA microparticles[3]. Figure A shows surface charge (zeta potential) comparison of PEI-conjugated and PEI-adsorbed microparticle batches indicating efficient conjugation of PEI to the particle surface. We tested our hypothesis that surface adsorption of pDNA would significantly enhance APC maturation and activation as demonstrated by the up regulation of surface maturation markers I-A^b, CD80 and F4/80 (Figure B)

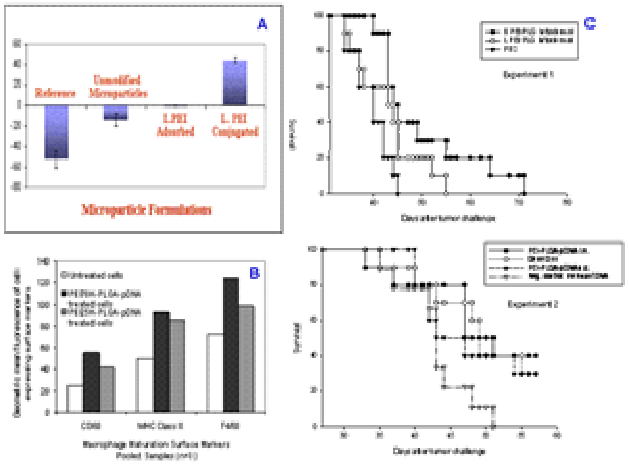

Figure C shows survival curves from two independent experiments of particle-mediated immunotherapy against B cell lymphoma using a chemokine fused plasmid DNA antigen (B cell idiotype). Gene gun mediated administration of plasmid coated gold pellets has been shown previously to be the most optimal method to induce anti tumor effects this model[4]. As shown, PEI conjugated PLGA microparticles administered either by the intramuscular or the intradermal route can generate significant protective immunity (comparable to gene gun mediated delivery) against lethal tumor challenge. *Our current work focuses on co-delivery of immunostimulatory nucleic acids (siRNA and CpG ODN) and chemokines (e.g. MIP3 alpha) for further optimization of anti-tumor effects.*

Conclusions: In conclusion we have developed a cationic microparticle formulation that can (a) deliver multiple therapeutic molecules (nucleic acids or proteins and peptides) in a single platform (b) efficiently transfect and activate (i.e. induces maturation) antigen presenting cells and (c) significantly enhance tumor survival in a murine model of B cell lymphoma. Such a formulation could offer a more relevant parenteral delivery option for a wide range of immunotherapy against cancer and infectious diseases.

References:

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Acknowledgements: National Institute of Health and the Charles and Judith Tate foundation