Investigation of pathogen-mimicking particles for delivery of vaccine components in murine tumor models: Comparison of micro vs nano

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Statement of Purpose: A promising alternative to current cancer therapies is to "train" the patient's own immune system to recognize and eliminate tumors. This approach requires robust immune response modulation as most tumor antigens are weakly immunogenic. Multi-modal systems that can simultaneously attract sufficient antigen presenting cells (APCs) to the site of immunization, ensure efficient delivery of antigens to the APCs and modulate the resulting immune response could provide significant improvement over current strategies.

Towards this, we have developed pathogen-mimicking particles (PMPs) carrying both antigens and immunomodulating siRNA and pathogen-associated-molecular-patterns (PAMPs) such as toll-like receptors ligands (TLRL). We have also combined these PMPs with an injectable synthetic immune-priming center (sIPC) carrying chemokines to further amplify APC migration, antigen delivery and immune modulation in a single platform. Previously, we have demonstrated the efficacy of this vaccine delivery platform in a murine B cell lymphoma model. However, detailed experiments analyzing how PMP parameters such as size, loading density and biomolecule conjugation techniques, etc. affect immune modulation potential and ultimately protection against tumor challenge have not yet been studied. Here we report detailed investigation of these parameters using primary bone marrow derived dendritic cell (BMDC) cultures in vitro and in vivo murine models of ovalbumin-expressing melanoma or A20 lymphoma.

Methods: PLGA particles were used due to their favorable regulatory history, modifiability and biomolecule loading capacity. PEI-conjugated micro- and nano- sized PLGA particles were prepared using previously published methods.[1] Cationic particles were then incubated overnight at 4°C with negatively charged antigens and immunomodulatory molecules.

In vitro experiments were performed with BMDCs isolated from BALB/c mice as previously reported.[1] Cells were treated with particles for 24-48h before being analyzed via flow cytometry followed by extensive analysis of DC phenotype, DC activation, surface marker expression and cytokine secretion profiles using ELISAs.

In vivo studies (both prophylactic and therapeutic) were conducted by injecting various formulations of the PMPs intramuscularly or subcutaneously in naïve or tumor bearing mice. Evaluation of the resulting immune response and survivability was tracked thereafter.

Results: Micro- and nano- sized PMPs were successfully synthesized with varying amounts of immunostimulatory molecules. Co-delivery of multiple molecules, either on the same or separate PMPs, was also explored.

Figure 1 shows the loading levels of various antigens and immunostimulatory molecules. As expected, nano-sized PMPs of the same total mass have a higher loading potential due to higher surface area to volume ratio.

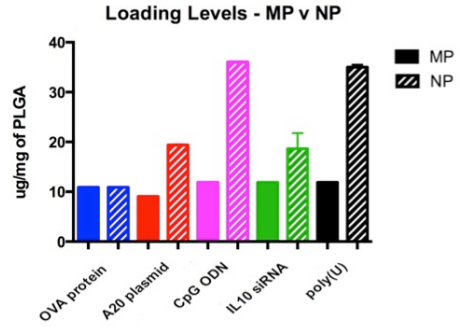

Figure 1: Loading levels on MPs and NPs

In vitro activation studies on BMDCs have produced promising results for micro- and nano- PMPs. Specifically, addition of TLRLs like CpG significantly enhances the expression of surface markers like CD86 and secreted cytokines like IL12p70, indicating efficient DC maturation and activation. These results are mirrored in mixed leukocyte reaction studies, in which PMP vaccines induce greater T cell activation and proliferation than soluble forms of the immunomodulatory molecules.

In vivo prophylactic and therapeutic studies also demonstrate the efficacy and versatility of the PMPs in providing protection against both OVA-melanoma and lymphoma tumors using protein and DNA based antigens, respectively. Figure 2 shows that protein/CpG vaccines delivered using nano-PMPs provided enhanced protection over micro-PMPs in an OVA-melanoma model.

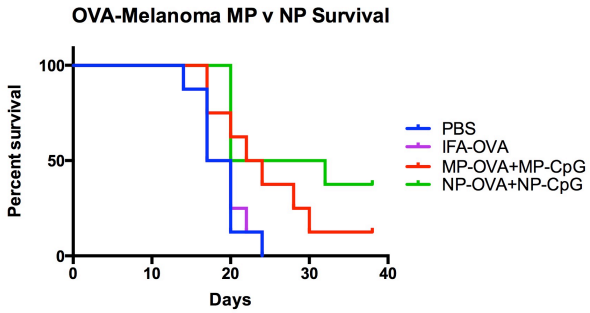

Figure 2. Nano-PMP vaccines provide better survival trends than micro-PMP vaccines.

Conclusions: Our preliminary results suggest that PLGA nano-PMPs enhance immunomodulation potential of tumor-antigen/adjuvant carrying formulations. Nano-PMPs provide a larger surface for loading than micro-PMPs allowing for more flexibility in dosing. Additionally, we shown that nano-PMPs provided enhanced protection of mice challenged with OVA-melanoma tumors. Overall, we have demonstrated the significance of various PMP parameters in potentiating a robust immune response against cancer.

References: [1] Singh A. Mol Ther. 2008;12: 2011-2021