Dendritic cell subsets interact with CpG-carrying pathogen mimicking particles in a phenotype specific manner

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Statement of Purpose: Biomaterial-based vaccine formulations, often administered through intramuscular, intradermal or subcutaneous routes, are being widely investigated for delivery of antigens and adjuvants. Our current understanding is that these formulations, often in the form of nano or microparticles (NP/MPs) carrying antigens and adjuvants, interact with tissue-resident antigen-presenting cells (most notably dendritic cells (DCs)) at the site of administration. Upon activation and maturation these antigen-adjuvant loaded DCs migrate into the local lymphatic circulation and travel to the draining lymph nodes (dLNs), where DC-T cell interactions result in corresponding adaptive immune responses. Despite numerous reports on many different NP/MP-based vaccine formulations, little is known about the subtypes of tissue-resident DCs important for NP/MP uptake and how specific DC subtypes and their lymphatic transport efficacy affect cellular and humoral immune responses against the antigen.

The design of our pathogen mimicking particle (PMP) vaccines provides a modular, customizable platform that can be modified to mimic specific pathogens. With our control over size and ability to load a wide variety of relevant biological molecules within and onto the surface of our particles, we can tailor our carriers to target specific DC subsets and modulate the immune system in a robust manner. More specifically, we have demonstrated the ability to target tissue-resident conventional and plasmacytoid DCs (cDCs and pDCs, respectively). We hypothesize that this will allow us to direct specific cellular immunological responses downstream as well. By investigating DCs that are known to promote Th1 (pDCs), Th2 (PDL2+ cDCs) or have potential to promote either Th1 or Th2 responses (PDL2- cDCs) using both in vitro and in vivo models, we are able to demonstrate that particle size as a carrier parameter alone can be used to modulate immune responses.

Methods: PMPs were prepared using a double emulsion process then were covanently modified with polyethylenimine to form a cationic shell. Positively charged particles were then incubated overnight at 4°C with negatively charged molecules, including protein and CpG oligos.

In vitro experiments were performed with either primary bone marrow derived dendritic cells (BMDCs) or DC isolated from whole spleens of female C57BL/6 mice. pDCs were isolated from spleens using a magnetic-activated cell sorting system. BMDCs were cultured in GM-CSF supplemented medium with or without IL-4 enrichment. After 6 days in culture each subset was cultured with micro- or nano-PMP formulations for 24 hours. Uptake was determined using a fluorescently-tagged CpG. Activation surface marker expression was analyzed using flow cytometry and cytokine production profiles were determined using intracellular staining procedures. Mixed lymphocyte reactions were conducted using OTI and OTII OVA-specific cells co-cultured with DCs pulsed with OVA and CpG formulations.

In vivo trafficking studies were conducted by injecting various PMP formulations subcutaneously and collecting dLNs at various time points. DC subsets were stained and analyzed using flow cytometry.

Results: Micro- and nano-PMPs were loaded with fluorescent CpG and cultured with three subsets of murine DCs cells for 24 hours to determine uptake and activation. It can be appreciated in Figure 1 that each DC subset has a distinct preference towards either the micro- or nano-formulation. This is also mirrored in the surface activation marker expression of each subset (data not shown).

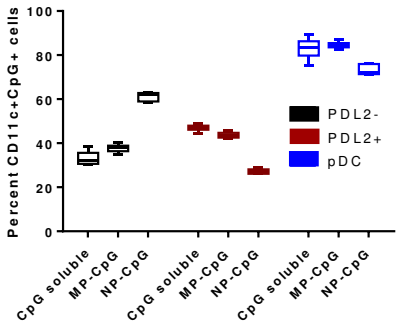

Fig. 1-Uptake: PDL2+ and pDCs preferentially take up MP delivered CpG while PDL2- cDCs take up NP-CpG formulations more efficiently.

Additionally, we find that cultures with majority PDL2- cells stimulate CD8+ T cells faster than cultures with primarily PDL2+ cells and that NP formulations, which target PDL2- cells, promote greater CD8+ T cell activation at later time points (Fig. 2).

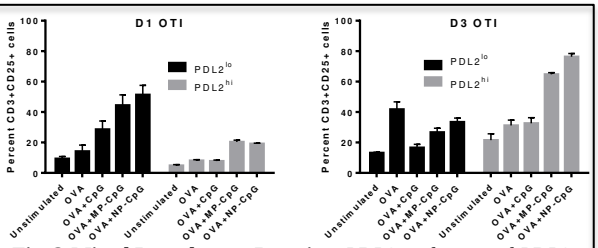

Fig. 2-Mixed Lymphocyte Reaction: PDL2- cultures and PDL2- targeting using nanoparticle formulations promote greater activation of CD8+ T cells.

Conclusions: To ensure efficient vaccine delivery and processing, it is critical to characterize how different DC subsets respond to vaccine carriers of interest. This is especially the case in vivo where many types of DCs will encounter and react to injected vaccine formulations. We have observed that each of three dendritic cell subsets do interact with micro- and nano- sized PMP formulations in a distinct manner. These observations have implications in vaccine targeting for directed cellular immunomodulation.