Nanoparticle vaccines: antigen and adjuvant delivery to lymph node dendritic cells

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Statement of Purpose: Conventional vaccines are limited in that they cannot effectively treat many diseases that evade the immune system. A key to developing the next generation of vaccine technology is the ability to deliver antigen to dendritic cells while also maturing them with an adjuvant, which then leads to subsequent activation of T cell immunity. We have previously shown that 20 nm poly(ethylene glycol) (PEG) -stabilized poly(propylene sulfide) nanoparticles (NPs) perfuse efficiently and quickly into the lymphatic capillaries and into the draining lymph nodes following intradermal injection. Moreover these NPs remain within the lymph node for prolonged durations (up to 120 h post-injection), and are specifically internalized by DCs and other antigen-presenting cells (APCs) there.

Methods: A model antigen, ovalbumin (OVA) is conjugated to Pluronic vinylsulfone (PL-VS) in order to synthesize OVA functionalized NPs (OVA-NPs). Lipopolysaccharide (LPS) is used as a molecular adjuvant or positive control for DC maturation. Mice are injected intradermally in the tail and/or front footpads with the following: fluorescent OVA-NPs (300µg), NPs (300µg), PBS (25µl), and OVA+LPS (25µl+10ug) at 24 h post-injection, the draining lymph nodes are harvested. Lymph node cells are then isolated. With the lymph node cell suspension, DCs are stained for with with anti-CD11c-allophycocyanin (Pharmingen) and the maturation markers are stained with R-PE labeled CD86, CD80, and CD40 (Pharmingen). Flow cytometry is performed on cells to determine DC maturation. CD4 T cell proliferation is measured by using an adoptive transfer model with OT-II mice. Humoral immunity is detected by performing an anti-OVA IgG ELISA on mouse serum at 7, 14, and 21 d post-injection.

Results/Discussion: We have conjugated ovalbumin (OVA) protein as a model antigen to the surface of the NPs. OVA delivered by NPs is retained in lymph nodes for much longer time points compared to free OVA.

We show that lymph node DCs become mature by upregulation of CD40, CD80, CD86 following NP uptake. NPs induce in-vivo DC maturation levels similar to that of LPS injections – demonstrating the ability for NPs to serve a dual role as both a DC-specific antigen delivery vehicle and adjuvant. Delivery of OVA-NPs leads to proliferation of CD4 T cells in an OT-II adoptive transfer model at levels equal to OVA+LPS, with nearly 7 cycles of proliferation measured at 3 d post-injection. Humoral immunity is also verified following injections of OVA-NPs through detection of positive titers in serum of anti-OVA IgG. OVA-NPs induced similar titers as that of the positive control OVA+LPS.

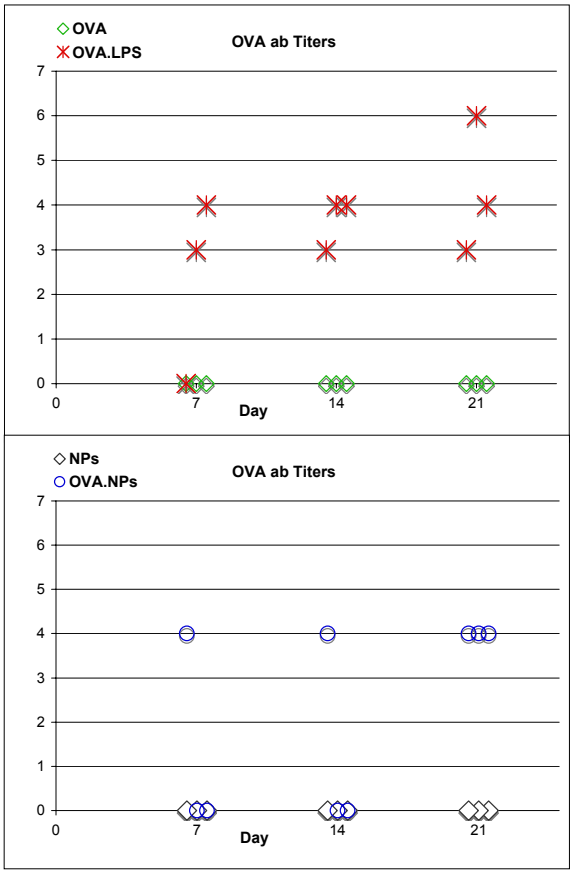

Figure1: OVA ab Titers.

Finally, we investigate the *in vivo* mechanism of NP induced maturation of lymph node dendritic cells. Specifically, the role of Toll-like receptor (TLR) 4 and complement are studied with TL4 -/- and C3 -/- mouse models, respectively.

Conclusions This work integrates biomaterials with immunology and lymphatic physiology and is very unique in the field. Specifically, the development of a polymer nanoparticle system that efficiently gets taken up into lymphatics from peripheral injection sites and travel to the lymph nodes where they target immature dendritic cells there. The polymer NPs act as a potent adjuvant as well as being able to deliver a specific antigen; this system is able to sufficiently deliver antigen to dendritic cells, mature them, and induce T cell immunity. Moreover, the molecular mechanisms responsible for activation of dendritic cells by NPs are being studied. Such a multi-discplinary approach shows exciting potential and is a great example of combining bioengineering and immunology to develop new therapies and also elucidate complex mechanisms of DC activation.