Assessment of CL075-loaded polymersomes for neonatal vaccination: biodistribution and benchmarking against conventional vaccine formulations

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Statement of Purpose: Due to deficiencies in the neonatal immune system, a significant window of vulnerability exists that spans from several months to over a year during which infants are highly susceptible to viral infection. In the developing world, this window contributes to the death of over two million infants annually (1). One reason for the ineffectiveness of neonatal vaccination is that the activation of neonatal dendritic cells (DCs) by currently approved adjuvants is impaired relative to adult DCs (2). DCs are the most potent antigen presenting cell and are essential to the initiation of immune responses. Several new adjuvants have been found to effectively activate both neonatal and adult DCs, including the toll-like receptor 8 (TLR8) agonists, CL075 (3). CL075 is an imidazoquinoline derivative that has limited water solubility and a high potential to induce autoimmunity and anaphylaxis when not delivered in a controlled fashion *in vivo*. We have previously developed a nanoscale polymersome (PS) platform for the stable encapsulation and delivery of imidazoquinoline-based adjuvants to DCs (4). These polymeric vesicles were self-assembled from poly(ethylene glycol)-*bl*-poly(propylene sulfide) (PEG-PPS) copolymers possessing oxidation-sensitivity for controlled payload release within DC endosomes. Here, we demonstrate that subcutaneous injection of PEG-PPS PSs allows targeting of DC subpopulations linked to effective vaccination against viruses, and that CL075-loaded PSs achieve superior DC activation when benchmarked against conventional vaccine formulations.

Methods: Fluorescently tagged copolymers were synthesized from amine functionalized PEG-PPS-NH2 that was reacted with Dy647-N-hydroxysuccinimide. Dried films containing both CL075 and PEG-PPS block copolymers (hydrophilic block weight fraction of 0.28) were rehydrated in buffered solutions to permit self-assembly of vesicles. Vesicle diameters were specified to 120 nm by subsequent extrusion through nucleopore track-etched membranes, and CL075 loading was quantified with UV/Vis/Fluorescence-HPLC. For biodistribution assays, Dy647-PSs were injected subcutaneously into the footpads of C57BL/6 mice, after which lymphocytes were extracted from spleens and lymph nodes at time points for analysis by flow cytometry. Activation of human monocyte-derived DCs was assessed *in vitro* by cytokine production and surface marker upregulation that were respectively quantified using multiplex cytokine assays and flow cytometry.

Results: Polymersome formulations with concentrations up to 1.4 mM of CL075 were achieved with loading efficiencies of 5 µg CL075/1mg PEG-PPS. Loading was found to be highly stable with no leakage of CL075 detectable (detection limit of ≈1 ng/mL) for over 1 year at 4°C. Biodistribution assays revealed Dy647-PSs to highly target plasmacytoid DCs (up to 80% of the total population) while minimizing uptake by eosinophils and neutrophils (Fig. 1a). CL075-PSs increased the expression of key cytokines by DCs when benchmarked against conventional vaccine formulations supplemented with aluminum salt adjuvants, with notable increases in the release of T_H1 cytokines IL-12p70 and TNF by over 100 fold (Fig. 1b). A pronounced increase in IL-1β by neonatal DCs without exogenous ATP suggested activation of the typically suppressed inflammasome.

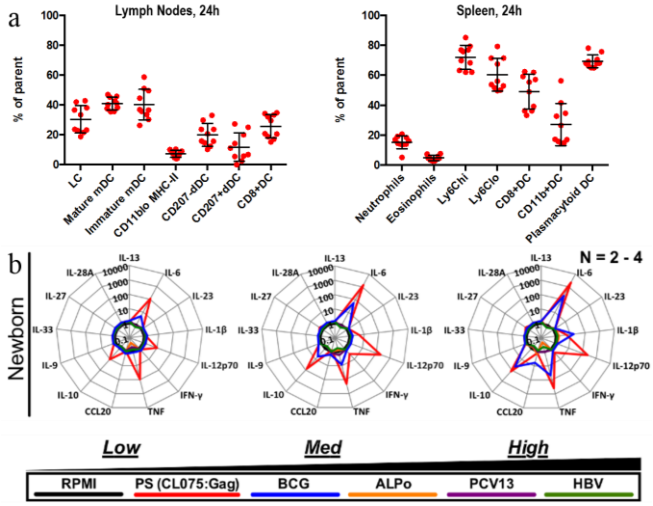

Figure 1. Assessment of a PS-based neonatal vaccine formulation. (a) *In vivo* targeting of monocyte and DC subpopulations by subcutaneously injected Dy647-PSs and (b) cytokine release by neonatal DCs following 24h stimulation with CL075-PSs (1, 5, 10 µM CL075) benchmarked against alum-adjuvanted vaccines.

Conclusions: The majority of conventional vaccine formulations were designed and validated for the adult immune system and are not effective for infants immediately following birth. To address this issue, we have developed a PSs delivery system that targets the potent adjuvant CL075 to vital immune cell populations and elicits robust responses from both neonatal and adult DCs. Targeting was a result of the physical and chemical properties of the nanomaterials, and, when benchmarked *in vitro* against conventional vaccine formulations, CL075-PSs were superior for neonatal DC activation. Our work highlights the potential for improved immunotherapy and vaccination via the rational design and engineering of nanobiomaterials.

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