**Tunable T cell immunity towards a protein antigen using polymersomes versus solid-core nanoparticles**

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**Statement of Purpose:** Antigen presenting cells (APCs) possess highly efficient mechanisms of endocytosis and unique pathways for processing antigen for MHC presentation [1]. We hypothesized that strategically engineered nanoparticles can take advantage of such mechanisms to achieve productive antigen delivery to APCs for vaccination. Polymersomes (PS), nanoscale vesicles formed from the self-assembly of block copolymers, can encapsulate antigenic molecules, be readily endocytosed by phagocytic cells, and be tailored for degradation under specific biochemical conditions [2]. Here, we use an *in vivo* mouse vaccination model to compare the immunological responses of 120 nm diameter oxidation-sensitive PS encapsulating ovalbumin (OVA) against solid-core 30 nm nanoparticles (NP) conjugated to OVA via a reduction-sensitive disulfide bond. We observed that each particle targeted different proportions of APCs, elicited separate immune responses, and co-injection of both resulted in synergistic responses.

**Methods:** *Nanocarrier preparation:* Dried films containing poly(ethylene glycol)-*bl*-poly(propylene sulfide) (PPS) block copolymers (hydrophilic block weight fraction of 0.28) were rehydrated in buffered solutions containing ovalbumin. Vesicle sizes were specified by subsequent extrusion through nucleopore track-etched membranes (Whatman). Pluronic-stabilized PPS NPs with OVA conjugated to the NP surface via a disulfide bond were synthesized as previously described [3]. *In vivo mouse immunization:* OVA-loaded oxidation-sensitive PS (120 nm) were injected (10 μg of OVA) subcutaneously into footpads of C57BL/6 mice along with free CpG (20 μg) as an adjuvant. Mice were boosted at days 14 and 28 and sacrificed at day 33. $IFNg^+$ $CD4^+$ T cells and $CD8^+$ T cells were extracted from draining lymph nodes, spleen and lung for analysis by flow cytometry. *Biodistribution assay:* Fluorescent OVA (OVA*) was loaded into PSs or conjugated on NP and co-delivered with CpG using the same dose and modality of injection adopted in the immunization protocol. 24 hours after the delivery, the proportions of fluorescent APCs were determined in the draining lymph nodes by flow cytometry.

**Results:** To investigate *in vivo* PS-based antigen delivery, we vaccinated mice with OVA-loaded PS (120 nm) and compared the results to OVA that was conjugated via a disulfide bond to NP. Contrary to reduction-sensitive NP, which have previously demonstrated strong $CD8^+$ antigen-specific responses [4], the oxidation-sensitive PS induced robust $CD4^+$ antigen-specific responses (Fig 1A, B). When antigen-loaded NP and PS were co-injected, both $CD4^+$ and $CD8^+$ activation was enhanced simultaneously (Fig 1A, B). Interestingly, PS were able to enhance elicited Ab titers while NP enhanced $CD8^+$ T cell cytotoxicity, as evidenced by increased CD107a expression. A 24h biodistribution assay that delivered fluorescent OVA via either platform revealed each nanocarrier to target different proportions of APC populations (Fig 1C, D).

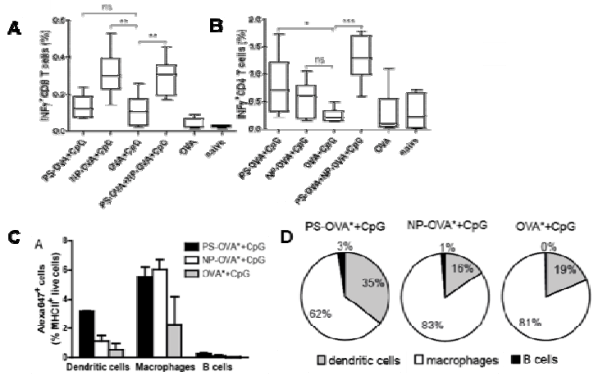


Figure 1. OVA loaded into PS or conjugated on NP is targeted to APCs with different magnitude and quality, generating distinct immune responses. Splenic $CD8^+$ (A) and $CD4^+$ (B) T cell activation after vaccination of mice with either OVA-loaded PS, nanoparticles conjugated to OVA via a disulfide bond, free OVA, or both nanoparticles and PS simultaneously. Free CpG oligodeoxynucleotide was used as an adjuvant. Proportions of (C) dendritic cells, macrophages and B cells that have collected the fluorescent OVA*, and (D) distribution of the total APCs targeted in a pie chart. Data are from two independent experiments, obtained from pooling popliteal lymph nodes of three mice per group.

**Conclusions:** This study highlights the PS platform as an effective antigen delivery system to enhance $CD4^+$ T cell immunity in order to improve subunit vaccines. Additionally, we confirm the enhancement of $CD8^+$ T cell responses elicited by our solid-core NP and demonstrate that the breadth of the T cell response can be improved through the synergistic co-administration of both nanocarrier platforms, obtaining both strong $CD4^+$ and $CD8^+$ T cell responses simultaneously. The ability to elicit different T cell immunity by using two separate yet synergistic nanoscale biomaterial carriers has significant implications for vaccine design and contributes to our understanding of how the physical and chemical properties of nanocarriers can be specified to rationally direct an immune response.

**References:** [1] Savina A et al., Immunol Rev. 2007 Oct;219:143-56. [2] Cerritelli S et al., Biomacromolecules, 2007,8(6):1966–1972. [3] van der Vlies et al., Bioconjug Chem 2010;21:653-62 [4] Hirosue S et al., Vaccine. 2010;28:7897-906.