Encapsulation within Reductive Polymersomes Enhances the Adjuvant Effect of Gardiquimod on Murine Splenocytes

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Statement of Purpose: Polymersomes have not been previously utilized in vaccine development and may present several advantages over current adjuvant and antigen delivery strategies. Polymersomes are stable vesicles composed of block copolymers that allow delivery of either hydrophilic or hydrophobic molecules respectively within an aqueous interior or amphiphilic outer shell [1]. The aqueous interior may permit the secure transport of molecules without the need for chemical modification. We have developed a polymersome system using poly(ethylene glycol) (PEG) and poly(propylene sulfide) (PPS) blocks linked via a disulfide bond (PEG-SS-PPS) to allow disassembly under reducing conditions, and we have previously demonstrated that this synthetic approach permits the controlled release of molecules within the early endosome of phagocytic cells [2]. We hypothesized that the delivery of unmodified adjuvant or antigen to the cytoplasm of antigen presenting cells prior to the exposure of the molecules to the harsh conditions of the lysosome may enhance the activation of antigen presenting cells. Here, we demonstrate the activation of murine splenocytes using reductive polymersomes loaded with gardiquimod, a water soluble TLR7 agonist.

Methods: *Polymer synthesis*: PEG-SS-PPS was synthesized from the reaction of thioacetate functionalized PEG-monomethyl ether (MW 750, Fluka) with pyridyl disulfide terminated PPS (27 units). Synthesis of the PEG thioacetate was performed as described previously [2]. The living ring-opening polymerization of propylene sulfide (Fluka) was initiated with s-phenyl thioacetate (Sigma) and capped with aldrithiol (Sigma). *Polymersome formation*: Calcein (Sigma) or Gardiquimod (InvivoGen) was loaded into vesicles using thin film rehydration of dessicated PEG-ss-PPS films. Polymersomes were purified with Sepharose 6B and Gardiquimod loading was quantified using fluorescence measurements (Ex/Em = 270/356 nm). *Cell uptake and release*: DY-649 labeled polymersomes (white) loaded with calcein (green) at a self-quenching concentration (150 mM) were incubated with RAW 264.7 macrophages for 30 min, 2h, 12h, or 24h. The cell membrane and endosomes were stained with Vybrant DiD (red) and nuclei with DAPI (blue). Cells were fixed prior to analysis by confocal microscopy (LSM 710, Zeiss). *Splenocyte activation assay*: Splenocytes were harvested from C57BL/6 mice and incubated for 24 h with RPMI medium or medium containing CpG, gardiquimod, empty polymersomes, or polymersomes encapsulating gardiquimod. Cells were stained with fluorescent antibodies specific for CD80, CD86 or CD40 prior to analysis by flow cytometry.

Results: *Polymersomes enter macrophage endosomes and release payloads within 12 h.* Confocal scans of cells at 12h and 24 h demonstrated colocalization of fluorescently tagged PEG-SS-PPS and calcein within the majority of DiD labeled endosomes (Figure 1A). Calcein was additionally found to be present throughout the cytoplasm, indicative of endosomal release. MTS assays of macrophages incubated with polymersomes for 72 h demonstrated no decrease in cell viability. *Encapsulation within polymersomes enhances the potency of gardiquimod*: Murine splenocytes incubated with gardiquimod-loaded polymersomes showed increased expression of dendritic cell activation and maturation surface markers relative to control cells exposed solely to medium or medium containing empty polymersomes (Figure 1B). Only 40 ng/mL of encapsulated gardiquimod was required to achieve maturation levels comparable to 2 µg/mL and 0.2 µg/mL of free CpG and gardiquimod respectively.

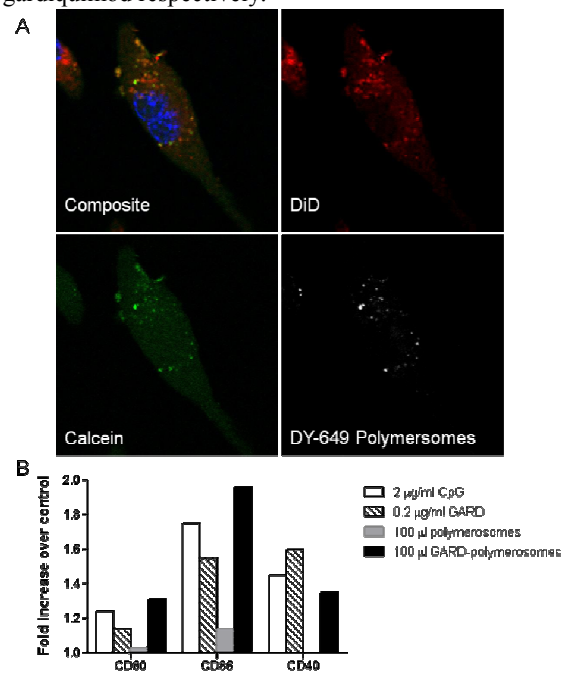

Figure 1.

Conclusions: The strong endosomal and cytoplasmic fluorescence of macrophages after incubation with calcein-loaded polymersomes at a self-quenching concentration suggests that PEG-SS-PPS polymersomes released their payloads after exposure to the reductive endosomal environment. Utilization of the polymersome vehicle significantly decreased the concentration of gardiquimod required to elicit splenocyte activation relative to free CpG and gardiquimod. Future experiments will assess the ability of peptide-loaded reductive polymersomes to enhance expression of MHC-I/peptide complexes on the surfaces of dendritic cells for the elicitation of cytotoxic T-cell responses.

References:

[1] Ahmed, F., J Control Release, 2006.116(2): p.150-58
[2] Cerritelli, S., Biomacromolecules, 2007. 8(6): p. 1966-72