Supplementing cell membrane-coated PLGA nanoparticles with exogenous phosphatidylserine reduces inflammatory cytokine production in macrophages

Chelsea Kraynak[1], Laura Suggs[1]
[1]Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX

Statement of Purpose: Macrophages are a ubiquitous and critical cell type in maintaining tissue homeostasis by playing a variety of important roles in injury and repair depending on their phenotype. Extracellular cues drive macrophage phenotype toward inflammation (M1) or regeneration and repair (M2), though cells uniquely retain phenotype plasticity after stimulation[1]. Unchecked macrophage inflammatory response can be highly detrimental to tissue, resulting in cytotoxicity and tissue injury[2]. Dysregulated macrophage phenotype has been implicated in arthritis, wound healing, and skeletal muscle regeneration, among others. Here, we aim to harness the anti-inflammatory effect of one important extracellular cue, apoptotic cell efferocytosis. Apoptotic cell engulfment reduces inflammatory response, which has been shown to be mediated in part by phosphatidylserine (PS)[3]. PS is a phospholipid found on the inner leaflet of the plasma membrane, only exposed to the exterior during apoptosis. We aim to mimic the macrophage response to apoptotic cell binding and engulfment through the development of a nanoparticle coated in phosphatidylserine-supplemented cell membrane. These membrane-coated nanoparticles (MNPs) act to reduce inflammatory macrophage signaling solely through this apoptotic-mimicry mechanism, without the use of small molecule inhibitors or other drugs.

Methods: Primary bone marrow-derived macrophages from FVB/n mice were used in all experiments. Cell membranes were isolated from 3T3 fibroblasts using differential centrifugation[4]. 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine liposomes were synthesized, added to isolated cell membrane, and extruded through a polycarbonate membrane to incorporate exogenous PS. Bare PLGA nanoparticles of approximately 100nm diameter were synthesized through an emulsion-evaporation method. PLGA particles were then co-extruded with cell membrane or PS-supplemented membrane to generate MNPs. Macrophages were treated with 750µg/ml MNPs for 4 hours, then stimulated to an inflammatory phenotype with 500pg/ml LPS. MNP treatment was assessed with qPCR, ELISA, imaging, and flow cytometry.

Results: Membrane coating of PLGA nanoparticles was confirmed with DLS size measurements and TEM imaging (Fig. 1A). PS incorporation to the membrane was further confirmed with zeta potential measurements. Particles coated with PS-supplemented cell membrane reduce production of key inflammatory cytokine TNFα in LPS-stimulated macrophages. The PS-supplemented MNPs reduce secreted TNFα to a greater extent than membrane coating alone. mRNA levels of TNFα, as well as other inflammatory cytokines IL-1β, IL-6, and iNOS, were also significantly reduced; again, PS-supplemented MNPs had a greater effect than membrane coating alone (Fig. 1B). Further, secreted TNFα levels upon LPS stimulation decreased with increasing proportions of PS in the cell membrane coating, with no further changes past 10% PS (Fig. 1C). Similarly, secreted TNFα was reduced in a dose-dependent manner to total MNP concentration (data not shown).

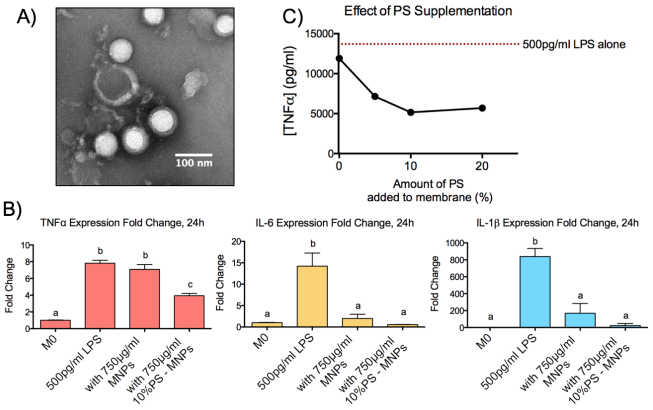

Figure 1. A) TEM image of MNPs showing membrane coating around PLGA interior. B) Gene expression of inflammatory cytokines TNFα, IL-6, and IL-1β is reduced from MNP treatment before LPS stimulation. 10% PS-supplemented MNPs result in a more dramatic reduction in expression levels than membrane alone. Letters indicate significantly different groups, $p<0.05$. C) TNFα in culture media is reduced 24 hours after LPS stimulation in macrophages treated with PS-supplemented MNPs. As proportion of PS increases, secreted TNFα is reduced, plateauing at approximately 10% PS.

Conclusions: Here, we have shown that PS-supplemented, membrane-coated nanoparticles reduce an inflammatory phenotype in macrophages. By exploiting the macrophage response to apoptotic body engulfment, we are able to modulate inflammatory response to an LPS stimulus and reduce the expression of key inflammatory cytokines TNFα, IL-1β, and IL-6, among others. Moving forward, we aim to better understand contributions of different parts of the membrane coating through the design of other fully or partially synthetic membrane-coated particles. We are also evaluating the mechanism of action of this particle through analysis of transcription factors and suppressive cytokine production. This unique nanoparticle design can be used to investigate and better understand the relationship between macrophages and apoptotic cells. Further, we aim to use PS-supplemented MNPs to modulate inflammation *in situ* in an arthritis model, but believe this platform can be adapted to therapeutically address a wide variety of tissue injuries where inflammatory macrophage infiltration is present.

References: [1] Mosser DM & Edwards JP. *Nat Rev Immunol*. 2008;8(12):958-69. [2] Tidball J. *Nat Rev Immunol*. 2017;17(3):165-178. [3] Hunyh M et al. *J Clin Invest*. 2002;109(1):41-50. [4] Thamphiwatana S et al. *PNAS*. 2017;114(43):11488-93.