Poly(lactic acid) Nanoparticle Modulation of Macrophage Inflammatory Responses

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Statement of Purpose: Inflammation plays a key role in protecting the body against foreign invaders. When the triggering event is poorly controlled, severe inflammation and global dysregulation of immune responses can occur. These overreactive responses are often tied to infection, autoimmune disorders, cancer, and other chronic conditions. Our group [2,3] and others [4] have developed cargo-less immunomodulatory nanoparticles (iNPs), lacking peptides, proteins or small molecules, and successfully demonstrated that the physicochemical properties of the nanoparticles were the driving factors to the observed therapeutic effects. However, the physical and biological mechanisms that affect iNP-mediated modulation of macrophage activation by TLR agonists have not been clarified. We hypothesize that the anti-inflammatory effects of iNPs are multimodal, such that their design parameters can be manipulated to elicit differential innate immune responses. Herein, we assessed iNPs composed of poly(lactic acid) (PLA) with either poly(vinyl alcohol) (PVA) or poly(ethylene-alt-maleic acid) (PEMA) as stabilizing surfactants and investigated the mechanisms by which they exert their inherent anti-inflammatory effects.

Methods: Nanoparticles were prepared using the oil-in-water single emulsion-solvent evaporation technique and characterized via dynamic light scattering (DLS). Fluorescence microscopy and flow cytometry were used to determine the levels of iNP uptake by cells and the subsequent interaction of cells with FITC- tagged pathogen associated molecular patterns (PAMPS), specifically FITC-tagged lipopolysaccharide (LPS) and FITC-tagged CpG ODN (CpG DNA). Modulation of macrophage proinflammatory responses were assessed using iNP-treated bone marrow-derived macrophages (BMMΦs) exposed to LPS. Cytokine secretions from BMMΦs were measured using ELISA and cytotoxicity was evaluated using flow cytometry using DAPI as an exclusion dye. Immunoblotting was used to evaluate iNP-mediated effects on BMMΦ transcriptional activity. Both the NF-κB p65 and various MAPK pathways were probed and evaluated. A novel inhibitor of GPR68, Ogremorphin (OGM), that blocks the GPR68-mediated inhibition of inflammation, was also used to test the mechanism by which PLA iNPs inhibit LPS-induced inflammation.

Results: iNPs were generated with diameters in the range of 400-600 nm and zeta potentials that were highly negative to neutral for the PEMA and PVA surfactants, respectively. We found that iNPs interfered with the interactions between PAMPs and BMMΦs, without directly interacting with the PAMPs themselves (**Fig 1A**). Further, iNPs attenuated proinflammatory cytokine secretions induced by LPS via a composition- and time-dependent reduction of NF-κB p65 and p38 MAPK activation; both particle formulations caused a decrease expression of activated NF-κB p65, but PLA-PEMA did so at a faster rate (**Fig 1B**). The PLA composition of iNPs was found to be a driving force for reducing NF-κB p65 activation, as both polystyrene-COOH (PS) and poly(methyl methacrylate) (PMMA) particles did not produce a similar effect. Lastly, it was determined that the protective function of the PLA-based iNPs was dependent on GPR68, a pH-sensing G-protein-coupled receptor, as demonstrated through specific inhibition studies.

Conclusion: These results provide support for the multimodal mechanism of action of iNPs and establish their potential use as a novel therapeutic for the treatment of severe inflammation, including sepsis.

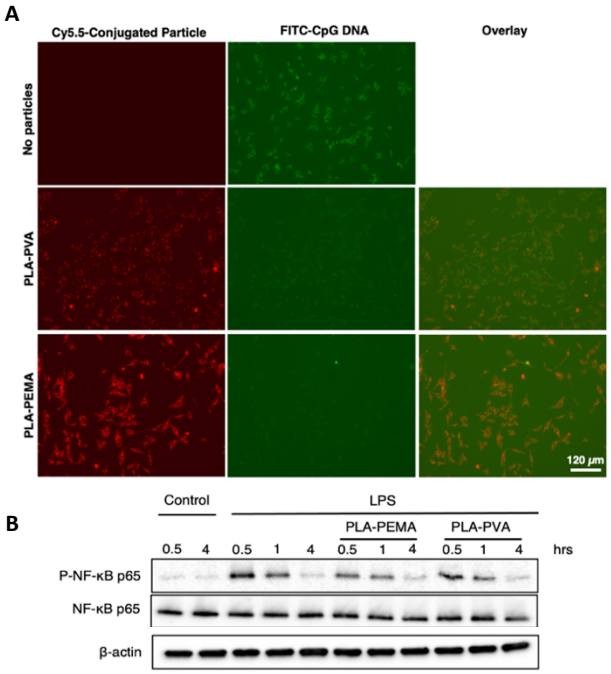

Figure 1: iNPs invoke multiple physical and biological mechanisms to elicit a protective effect in BMMΦs. (A) Fluorescence microscopy images of BMMΦ pretreated with iNPs and stimulated with FITC-CpG DNA. (B) Western blots of BMMΦ pretreated with iNPs and stimulated with LPS. Probed for activated and total NF-κB p65.

References: [1] Furman, D, *Nat Med* **2019**, 25, 1822-1832. [2] Casey LM. *Biomaterials*. **2019**;218. [3] Getts, D.R. *Sci Transl Med* **2014**, 6, 219ra7. [4] Allen, R.P *ACS Biomater Sci Eng* **2018**, 4, 900-918.