Antigen-specific modulation of immune responses using ovalbumin-poly(lactic-co-glycolic acid) nanoparticles

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Statement of Purpose: Diseases and conditions associated with an underlying immune dysregulation affect millions worldwide. However, there is an unmet demand for the development of innovative immunotherapies for cancer, vaccines, autoimmunity, and allergy. Nanoparticulate-based immunotherapies, specifically polymeric nanoparticles (NP) comprised of poly (D,L-lactic-co-glycolic acid) (PLGA) and poly(D,L-lactic acid) (PLA) have been shown to have inherent immunomodulatory properties and promising results in modulating antigen (Ag)-specific T cell responses. Current methods for Ag association with NPs such as encapsulation and surface conjugation suffer several limitations such as significant burst release, uncontrolled Ag loading, and possible immune recognition. To overcome these limitations and delineate the relationship between NP design parameters on restoring dysregulated immune responses in an Ag-specific manner, we describe the development of ovalbumin (OVA) protein-PLGA bioconjugate NPs (NP-OVA). NP-OVAs offer the ability to induce controllable and Ag-specific differential CD4+ and CD8+ T cell responses.

Methods: PLGA-OVA conjugates were synthesized by coupling the carboxylic acid end group of PLGA to the 20 available lysine groups on OVA using EDC/NHS chemistry. Spectral and thermal analysis was employed to confirm successful conjugate synthesis and Ag concentration was quantified by BCA Protein Assay. Precise Ag loadings of 2, 8 and 25 µg OVA/mg NP-OVA was achieved by combining PLGA-OVA conjugates with unmodified PLGA and prepared using a single-emulsion method. NP-OVA formulations were incubated with bone marrow-derived dendritic cells (BMDCs) and stained with MHC-I bound to SIINFEKL, MHC-II, and CD80 antibodies and measured using flow cytometry to evaluate Ag presentation and cell phenotype. Soluble OVA at high (100 µg/mL) and low (10 µg/mL) concentrations was used as a control. To further evaluate the immunological response of NP-OVA, Ag-specific T cell proliferation studies were conducted using carboxyfluorescein succinimidyl ester (CFSE)-labeled OT-I ($CD8^+$) and OT-II ($CD4^+$) T cells co-cultured with NP-OVA-treated BMDCs for 72 hours. CFSE dilution and T cell activation ($CD25^+$) were analyzed using flow cytometry. IFN-γ secretion was evaluated via ELISA.

Results: PLGA-OVA conjugates were synthesized at various stoichiometry ratios. The 30:1 PLGA-OVA conjugate demonstrated full solubility in DMSO, and a coupling efficiency (µg of OVA per mg of PLGA) of approximately 70 µg/mg and therefore chosen to formulate the NP-OVAs (Figure 1).

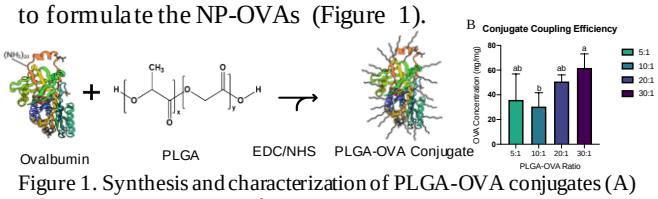

Figure 1. Synthesis and characterization of PLGA-OVA conjugates (A) Schematic representation of OVA-PLGA conjugate (B) OVA quantification of PLGA-OVA conjugates synthesized at 4 various ratios.

NP-OVA formulations were 500-600 nm in size, and zeta potentials ranged from -40 to -50 mV. Each formulation displayed precise Ag loading, reduced burst release, and negligible immune recognition compared to OVA-encapsulated NPs. NP-OVAs at high Ag loading (8 and 25 µg OVA/mg) upregulated MHC-I: SIINFEKL and MHC-II presentation while reducing CD80 expression. Flow cytometry analysis also demonstrated NP-OVA ability to induce OVA-specific $CD8^+$ and $CD4^+$ T cell proliferation in an Ag loading-dependent manner. A similar trend was observed in IFN-γ secretion (Figure 2).

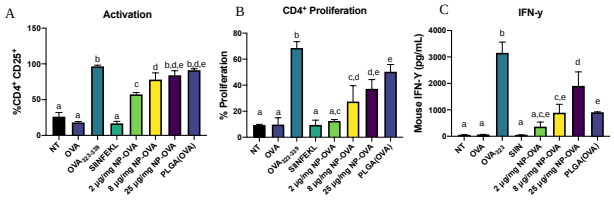

Figure 2. Evaluation of NP-OVA-treated BMDCs co-cultured with CFSE labeled OT-II ($CD4^+$) T cells. After 72 hours, T cells were measured for A) activation ($CD4^+$ $CD25^+$) B) proliferation (CFSE) measured via flow cytometry and C) IFN-γ cytokine secretion.

Conclusion: The ability to precisely control Ag delivery to Ag presenting cells to induce Ag-specific $CD4^+$ and $CD8^+$ responses offers the ability to investigate the fundamental relationships between NP designs and innate and adaptive immune cell responses. Precise delivery of protein Ag using bioconjugate NPs has the potential to be used to tailor specific immune responses for a multitude of therapeutic applications, ranging from vaccines, autoimmune diseases, and allergy.

Reference: Pearson, Ryan M., Casey, L.M., Hughes, K.R., Wang, L.Z., North, M.G., Getts, D.R., Miller, S.D., and Shea, L.D. Molecular Therapy 2017. 25(7), pp 1655-1664.