CD4 Targeted Nanoparticle Delivery of Eggmanone for T Cell Modulation in Autoimmunity.

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Statement of Purpose: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by dysregulated T and B cell responses and production of anti-nuclear autoantibodies. Treatment options are limited due to a lack of clear understanding of disease mechanisms. One example is the formation of spontaneous germinal centers (SGCs). A potential mechanism for SGC formation in SLE is phosphodiesterase-4 (PDE4) activation. PDE4 activity is essential for the activation of T cells and PDE4 inhibitors are used to treat chronic inflammatory diseases such as psoriasis and psoriatic arthritis. We propose the use of a novel PDE4 inhibitor, Eggmanone (Egm), delivered by targeted nanoparticles prepared from FDA-approved polymers. We hypothesize that overactivation of PDE4 facilitates the production of disease potentiating autoantibodies in SLE and that targeted delivery of Egm can decrease SLE disease severity.

Methods: Whole splenocytes were isolated from OT-II mice and incubated with OVA or $OVA_{323-339}$ peptide in the presence of Egm dissolved in DMSO (vehicle). T cell activation and cytokine production were evaluated via flow cytometry and ELISA, respectively. Egm and a fluorescent surrogate compound, DiD, loaded PEGylated PLGA nanoparticles (Egm-NPs and DiD-NPs) were synthesized using oil in water emulsion. Particle size was characterized via nanoparticle tracking analysis. Egm localization within nanoparticles was investigated using elemental analysis of scanning electron transmission energy dispersive X-ray spectroscopy (STEM-EDS). Celltiter glo viability assays and IFN-γ ELISAs were performed to determine the biocompatibility and therapeutic potential of Egm-NPs, respectively. CD4 targeting antibody fragments were conjugated to PEGylated PLGA nanoparticles using maleimide-thiol conjugation chemistry and targeting efficacy was quantified via flow cytometry. [1]

Results: At concentrations of $OVA_{323-339}$ above 25 µg/mL, Egm significantly reduced the percent of helper T cell populations expressing the activation/memory phenotype $CD44^{hi}CD62L^{lo}$ (**Figure 1A**). Significant reduction of IFN-γ was observed for Egm concentrations as low as 0.63 µM, and IFN-γ was undetectable at concentrations above 2.5 µM (**Figure 1B**). For all concentrations of Egm evaluated, Egm-NPs significantly inhibited the production of IFN-γ from OVA stimulated OT-II splenocytes (**Figure 1B**) with no significant decreases in cell viability (**Figure 1C**). STEM-EDS analysis of Egm-NPs revealed a concentration of sulfur, a unique element found only in the chemical structure of Egm in our formulations, in the core of Egm-NPs that was not observed for empty-NPs (**Figure 1D**). Anti-CD4 decorated DiD-NPs achieved ~83% $CD4^+$ T cell staining, and significantly increased targeting specificity for $CD4^+$ T cells compared to isotype and undecorated controls. Anti-CD4 decorated particles targeted $CD4^+$ T cells significantly more than $CD8^+$ T cells and non-T cells, and targeting specificity was dependent on particle concentration. In all cases, undecorated and isotype-decorated control particles exhibited low levels of nonspecific binding. [1]

Conclusions: In this work, we synthesized and characterized anti-CD4 targeting PEGylated PLGA nanoparticles for specific delivery of Egm to $CD4^+$ T cells. Nanoscale elemental analysis supported the notion that emulsion mediated fabrication localized Egm in nanoparticle cores. Egm-NPs were biocompatible and capable of inhibiting antigen-specific $CD4^+$ T cell cytokine responses. Using maleimide-thiol conjugation chemistry, we were able to achieve high levels of $CD4^+$ T cell targeting specificity *ex vivo* with minimal non-specific binding. Collectively, this work represents the first characterization of Egm's immunomodulatory potential and the development of a rationally designed nanoparticle delivery vehicle intended for systemic Egm administration for the treatment of SLE. [1]

References: 1. (Haycook CP et al. Int J Nanomedicine. 2020;15:1215-1228).

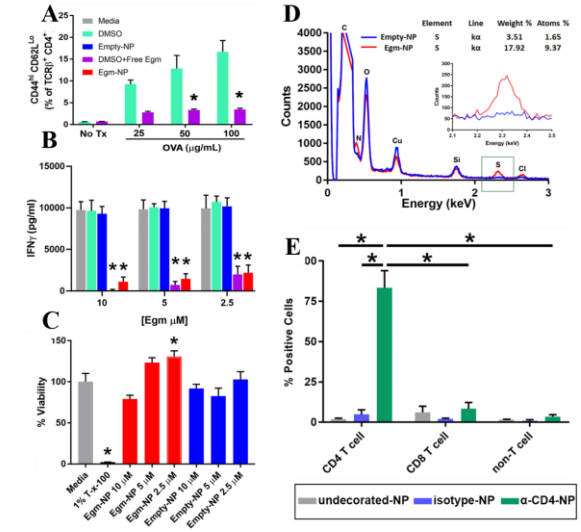

Figure 1. A) Flow cytometry analysis of inhibited OT-II $CD4^+$ T cell activation *ex vivo*. **B**) IFN-γ production measured by ELISA 72 hrs after nanoparticle delivery of Egm to OVA stimulated OT-II whole splenocytes. **C**) Cell viability following Egm delivery to FVB derived whole splenocytes. **D**) STEM-EDS elemental analysis of Egm- and Empty-NPs. **E**) Flow cytometry analysis of undecorated, isotype control-decorated (isotype-NP), and anti-CD4-decorated (α-CD4-NP) DiD-NPs. (*P<0.05).