Encapsulation of antigen in chitosan particles enhances activation and antigen specific response by antigen presenting cells

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Statement of Purpose: Subunit antigens are much safer, but also much less immunogenic than whole pathogens. However, subunit antigens are rapidly degraded by proteases and lack the immune stimulus, i.e. co-stimulation and/or danger signals, required for the generation of antigen-specific immunity. The encapsulation of subunit and polypeptide antigens in nano- and/or microparticles has been explored extensively as a strategy to enhance their immunogenicity. Encapsulation of proteins offers protection from degradation as well as an opportunity to deliver an immune-potentiating signal. The objective of current project is to understand how antigen encapsulation into chitosan particles (AgCPs) affects antigen uptake and presentation by antigen presenting cells (APC). Specifically, we explore the effect of particle size on APC activation/ maturation and antigen presentation.

Methods: Chitosan particles, 300nm, 1µm, and 3µm in size, with encapsulated model antigen Fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) or ovalbumin were prepared via precipitation coacervation (Berthold et al 2001). Uptake of AgCPs by RAW 264.7 macrophages and bone marrow dendritic cells (BMDC's) was determined by spectrophotometry (Synergy2, Biotek, Winooski, VT) and flow cytometry (FACSCantoII, BD biosciences, San Jose, CA). Cell activation/maturation in response to particulate antigens was determined by flowcytometry evaluating the expression of surface activation markers including MHC I, MHC II, CD11b, CD11c, CD80, CD86, CD40, and CD54. Cytokine release by macrophages and BMDC's in response to particulate antigens including IL-1β, IL-6, TFN-α, MCP-1, and MIP-1 was determined through cytometric bead array analysis (CBA) (BD Biosciences, San Jose, CA). The antigen presenting ability of BMDCs co-incubated with AgCPs was evaluated by quantifying proliferation of antigen specific CD4+ and CD8+T-cells. Proliferation of T-cells in response to BMDC antigen presentation was assessed by CellTiterGlo (Promega, Madison, WI) cell proliferation assay.

Results: Particle uptake studies showed that the antigen uptake was dependent on particle size with 1µm size particles showing significantly higher uptake compared to 300nm and 3µm particles. Incubation of APCs with soluble and particulate antigens resulted in activation of both macrophages and BMDC's as evident by the upregulation of antigen presenting markers MHC I, MHC II, and co-stimulatory markers including CD40, CD80, and CD86. While activation was observed with soluble FITC-BSA and chitosan particles without encapsulated antigen, strongest activation was observed with AgCPs. Also, a higher up regulation of surface

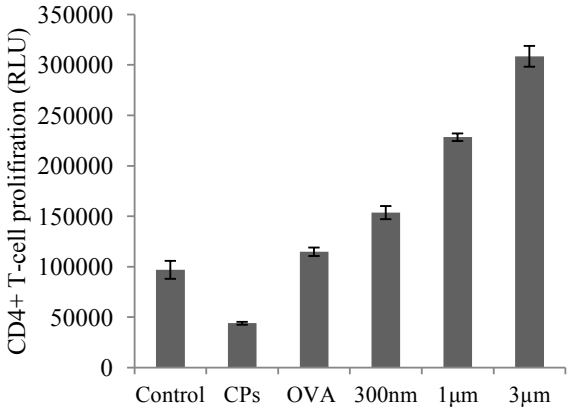

Figure 1. Proliferation of CD4+ T-cells in response to the cross presentation of antigens by BMDC's

activation markers was observed with 1 µm samples compared to 300 nm and 3 µm AgCPs. CBA analysis revealed that APCs co-incubated with AgCPS release greater levels of pro-inflammatory cytokines including IL-1β, IL-6, MCP-1, MIP-1, and TNF. Although APC activation was observed with soluble antigen, no resultant cytokine secretion was observed. Antigen presentation studies showed that BMDCs pulsed with AgCPs induced significantly higher level of proliferation in antigen specific CD4+ and CD8+ T-cells compared to BMDCs pulsed with soluble antigen.

Conclusion: In this study, we explored the effects of antigen-encapsulated chitosan particles on APC uptake and function. Our results indicate that encapsulation of subunit antigen in chitosan particles enhances antigen delivery and subsequent activation of APCs. In all measures of APC activation and presentation, AgCPs outperformed soluble antigen. The ability of BMDC's pulsed with AgCPs to present both MHC I and MHC II epitopes is particularly encouraging. Modification of chitosan or incorporation of additional immune response modifiers may permit the direction of a vaccine response toward either humoral or cell-mediated immunity. Taken together, our results indicate that AgCPs are a promising vaccine delivery platform deserving of continued exploration. Future studies will evaluate the in vivo immune response to chitosan particle-based antigen delivery systems.

References:

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