Carbohydrate Modification of Polyanhydride Nanoparticles and Polymer Chemistry Influence Interactions with Dendritic Cells

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Statement of Purpose: Vaccines that actively target the pattern recognition receptors (PRRs) on antigen presenting cells (APCs) stimulate the innate immune system by activating the APCs.[1] This can ultimately enhance the immunogenicity of antigens.[2,3] Herein, polyanhydride nanoparticles were functionalized with di-mannose in order to mimic carbohydrate moieties found on the surface of pathogens and target C-type lectin receptors. C-type lectin receptors are a class of endocytic PRRs found on the surface of APCs that can modulate the immune response.[4] Our studies showed that carbohydrate-functionalized nanoparticles influenced the interactions with dendritic cells (DCs) *in vitro*.

Methods: Anhydride copolymers based on a 20:80 molar ratio of 1,8-bis-(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis-(*p*-carboxyphenoxy)hexane (CPH) or a 20:80 molar ratio of CPH and sebacic acid (SA) were copolymerized using melt polycondensation. Blank nanoparticles or 1% quantum dot loaded nanoparticles were synthesized using anti-solvent nanoprecipitation. Nanoparticles were surface-functionalized with di-mannose or glycolic acid using an amine-carboxylic acid coupling reaction. Particles were incubated in C57BL/6 mouse serum to mimic parenteral injection or incubated in phosphate buffered saline (PBS) (i.e., non-serum coated particles) as a control.

DCs were differentiated from the bone marrow of C57BL/6 mice. DCs were stimulated with 125 μg/mL of nanoparticles on the 11th day of culture. After 48 hours of incubation, the cells were harvested and their surface marker expression was analyzed using flow cytometry. Quantum dot loaded nanoparticles were used to measure cellular internalization and pro-inflammatory cytokine secretion was analyzed in the cellular supernatant.

The identity of specific proteins from mouse serum as well as the quantity of protein adsorbed was also analyzed. Adsorbed proteins were eluted from the nanoparticles. They were identified using 2-D gel elctrophoresis and quantified by the bicinchoninic acid (BCA) assay.

Results: The studies showed that coating polyanhydride nanoparticles with mouse serum proteins enhanced DC internalization. Polymer chemistry also greatly affected nanoparticle uptake by DCs as 20:80 CPH:SA nanoparticles were internalized by a greater percentage of cells than 20:80 CPTEG:CPH nanoparticles. Surface functionalization did not significantly influence DC internalization. The surface expression of MHC II, CD86, and CD206 by DCs stimulated with nanoparticles was mediated by both polymer chemistry and surface functionalization. Specifically, the more hydrophobic 20:80 CPTEG:CPH nanoparticles enhanced the surface expression of MHC II and CD86 on DCs compared to DCs stimulated with the 20:80 CPH:SA nanoparticles (Figure 1). Cytokine secretion was also influenced by polymer chemistry and surface functionalization. Serum-coated 20:80 CPTEG:CPH nanoparticles that were functionalized with di-mannose induced higher amounts of pro-inflammatory cytokine secretion than their non-functionalized counterparts. On the other hand, DCs stimulated by serum-coated non-functionalized 20:80 CPH:SA nanoparticles had higher amounts of cytokine secretion than DCs stimulated by di-mannose-functionalized 20:80 CPH:SA nanoparticles.

2-D gel electrophoresis of the eluted mouse serum proteins revealed that albumin was the most abundant protein to be adsorbed onto the nanoparticles. Each nanoparticle formulation also showed significant amounts of IgG and complement adsorbed to their surface. Finally, the more hydrophobic 20:80 CPTEG:CPH nanoparticles adsorbed more proteins than the 20:80 CPH:SA nanoparticles.

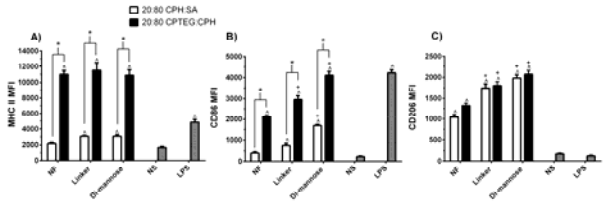

Fig. 1. Nanoparticle chemistry and surface functionalization influenced the surface expression of A) MHC II, B) CD86, and C) CD206 on DCs.

Conclusions: These results indicate that nanoparticle chemistry, carbohydrate functionalization, and serum protein adsorption onto polyanhydride nanoparticles influenced DC stimulation and nanoparticle uptake. Even though the more hydrophobic 20:80 CPTEG:CPH nanoparticles were internalized less efficiently by DCs than the 20:80 CPH:SA particles, these particles stimulated DC activation more effectively. These data support the concept that hydrophobicity is a "danger signal" that can trigger effective immune responses.[5]

References:

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