Targeting Dendritic Cells with “Pathogen-Like” Polyanhydride Nanoparticles

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Statement of Purpose: The design of single dose vaccines is a key strategy in providing immunological defense against infectious agents. Polyanhydride nanoparticles are promising platforms for use as single dose vaccine delivery systems. These vehicles have been shown to: a) stabilize protein antigens; b) provide sustained release of antigens; and c) enhance the adjuvant effect by modulating the immune response[1]. Dendritic cells (DCs) are antigen presenting cells that play a major role in connecting the innate and adaptive immune systems, which is a necessary step to induce protective immunity against infectious agents. Receptors involved in the recognition and uptake of pathogens into DCs are crucial for establishing a robust immune response. C-type lectin receptors (CLRs) (e.g., mannose receptor and DC-SIGN) recognize carbohydrate structures on pathogens and are important for recognition and internalization of antigens leading to processing and presentation of antigens on MHC I and II molecules[2]. In this work, a novel approach to target CLRs on DCs was designed by decorating the surface of polyanhydride nanoparticles with specific carbohydrates (i.e. lactose and di-mannose) to mimic pathogen surfaces. Characterization and evaluation of the targeting capabilities of these nanomaterials by *in vitro* activation and uptake of DCs were performed.

Methods: *Nanoparticle fabrication and modification.* An anti-solvent nanoencapsulation method was used to fabricate nanoparticles based on a 50:50 ratio of 1,8-bis(*p*-carboxyphenoxy)-3,6-dioaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH). The surface of polyanhydride nanoparticles was modified by attaching either lactose or di-mannose residues by an amine-carboxylic acid coupling reaction[3]. Particles with attached glycolic acid groups were employed as a control. *Particle characterization.* Particle morphology was imaged by scanning electron microscopy. The presence of carbohydrates on the surface was quantified by X-ray photoelectron spectroscopy (XPS) and a phenol-sulfuric acid assay. *Dendritic cells.* Bone marrow derived DCs from C57BL/6 mice were cultured for 48 h in the presence of sugar-modified and unmodified nanoparticles. *Cell response evaluation.* Flow cytometry was used to assess for the expression of MHC I, MHC II, CD86, CD40, mannose receptor (CD206), and DC-SIGN (CD209) on DCs co-incubated with the various nanoparticles. Particle uptake was studied by epifluorescence and laser scanning confocal microscopy.

Table 1. XPS analysis of atomic concentrations and ratios of elements present on 50:50 CPTEG:CPH nanoparticles

Samples	C%	N%	O%	O/C%	N/C%
Unmodified	76.3	0.3	23.5	0.31	0.0034
Lactose	73.8	5.1	21.1	0.28	0.0691
Di-mannose	72.8	5.3	21.9	0.30	0.0728

Results: From **Table 1**, XPS shows an increase in the nitrogen concentration of the modified particles and is related to the presence of the amine linker on the particle surface. This experiment was corroborated by a phenol-sulfuric acid assay that showed ca. 2% (w/w) of sugar. An increase in the surface expression of MHC II, CD86, CD206, and CD209 was observed for di-mannose modified nanoparticles when compared with unmodified 50:50 CPTEG:CPH nanoparticles. The greatest increase in expression was seen for the mannose receptor CD206 and DC-SIGN (**Figure 1**). Results from epifluorescence microscopy showed that particle uptake was enhanced by carbohydrate modification of the nanoparticles.

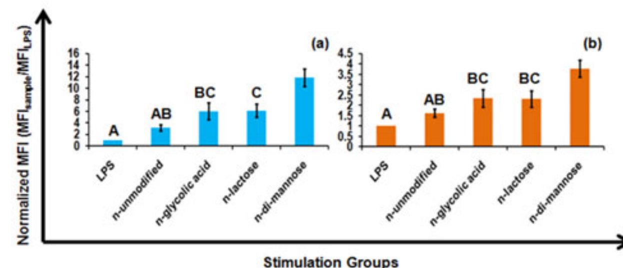

Figure 1. Relative cell surface expression of (a) CD206, and (b) CD209 on dendritic cells after incubation with nanoparticles or LPS. Treatments marked with the same letter are not significantly different ($p < 0.05$). Values were normalized to the response induced by LPS.

Conclusions: The targeted activation of murine DCs by novel “pathogen like” polyanhydride nanoparticles was demonstrated by the increased expression of CD206 and CD209 as well as enhanced particle uptake. Such a targeted and enhanced uptake of antigen-loaded nanoparticles is an important determinant of antigen processing and presentation by DCs, which ultimately affects the nature of the immune response. This is also highlighted by the accompanying increased expression of MHC II and CD86 on the DCs. These studies provide key insights into the rational design of targeted nanovaccine platforms that will enhance the induction of antigen-specific immune response

References: [1]Kipper MJ, et al. J Biomed Mater Res A. 2006;76:798-810;[2]van Kooyk Y, et al. Biochem Soc Trans. 2008;36;1478-81; [3]Sheehan J, et al. J Org Chem. 1961;26;2525-2528.