Targeted Activation of Antigen Presenting Cells with Mannose-Modified Polyanhydride Nanoparticles

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Statement of Purpose: Acute respiratory infections, including bacterial pneumonia and influenza, are responsible for 4.5 million deaths annually[1]. There is a critical need for the development of robust, immunologically protective, single dose vaccines against respiratory pathogens and polyanhydride nanoparticles represent a dynamic platform upon which to develop a novel class of efficacious vaccine delivery vehicles.[2,3] For a mucosal vaccine to be efficacious, it must activate dendritic cells (DCs) and alveolar macrophages (AMs). DCs are considered professional antigen presenting cells (APC) that are primarily responsible for initiating immune responses while AMs are the first phagocytic cells to encounter respiratory pathogens and transport them to the lymph nodes draining the lung[4]. Ligation of a specific family of cell surface receptors known as C-type lectin receptors (CLRs), including dectin-1, mannose receptor and DC-SIGN, is known to enhance antigen presentation and modulate cytokine production of APCs[5]. These CLRs specifically recognize carbohydrates present on the surfaces of many pathogens. The goal of this work was to target the ligation of CLRs on APCs using "pathogen-mimicking" mannose-modified polyanhydride nanoparticles.

Methods: Polyanhydride nanoparticles were fabricated using a 50:50 ratio of 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy) hexane (CPH) by an anti-solvent nano-precipitation method.[6] The nanoparticle surface was modified by attaching di-mannose residues by an amine-carboxylic acid coupling reaction. Non-modified, glycolic acid-, and ethylene diamine-modified particles were used as controls. The surface chemistry of the nanoparticles was quantified by X-ray photoelectron spectroscopy (XPS) and a phenol-sulfuric acid assay. Bone marrow derived DCs from C57BL/6 mice and the MH-S murine alveolar macrophage cell line were cultured in the presence nanoparticles for 48 h. In separate experiments, monoclonal antibodies against CD206 and/or CD209 were added to cultures prior to stimulation with the nanoparticles. Expression of the cell surface markers MHC I, MHC II, CD86, CD40, CD206 (mannose receptor), CD209 (DC-SIGN), and Dectin-1 was assessed via flow cytometry. Cytokine secretion was measured via a multiplexed bead assay. Nanoparticle uptake was assessed by laser scanning confocal microscopy.

Results: XPS analysis showed that modified particles had an increased nitrogen concentration, presumably due to the presence of the amine linker on the particle surface. The addition of di-mannose-modified nanoparticles to cell cultures increased the surface expression of CD206, CD209, and Dectin-1 on AMs (**Figure 1**) and also enhanced CD206 and CD209 expression on DCs. Confocal microscopic analysis revealed that nanoparticle uptake by DCs was enhanced by glycolic acid-modified nanoparticles but not by di-mannose-modified nanoparticles (**Figure 2**), indicating that di-mannose functionalization did not improve particle uptake despite enhancing APC activation.

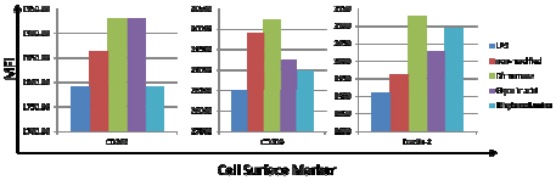

Figure 1. Surface expression of CD209, CD206, and Dectin-1 on AMs after stimulation with nanoparticles.

Additional studies demonstrated that blocking the CD206 receptor before addition of the di-mannose -modified nanoparticles inhibited the increased expression of MHC II, CD40 and CD86, indicating that engagement of the mannose receptor is a key mechanism by which di-mannose-modified nanoparticles activate DCs (**Figure 3**).

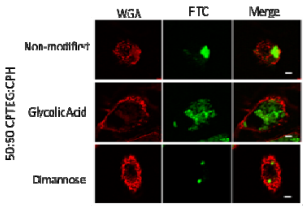

Figure 2. Photomicrographs of internalized nanoparticles. DC cultures were fixed 2 hrs after internalizing FITC-labeled non-modified, glycolic acid- or di-mannose-modified nanoparticles. Plasma membrane was visualized by staining with wheat germ agglutinin (red). Scale bar = 5 μm.

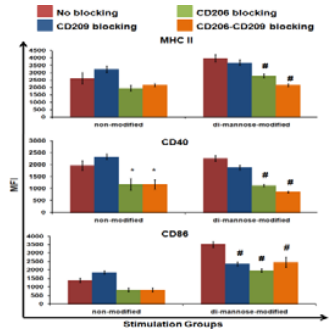

Figure 3. Surface expression of MHC II, CD40, and CD86 after blocking with CD206 and/or CD209 receptors before stimulation. *, # represent statistical significance ($p \leq 0.05$) compared to the no blocking group.

Conclusions: This work demonstrates that targeting CLRs on murine DCs and AMs using mannose-modified polyanhydride nanoparticles enhances APC activation by engaging the mannose receptor. These studies provide key insights into the rational design of targeted nanovaccines against respiratory pathogens.

References:

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