Functionalized polyanhydride nanoparticles preserve protein stability and activate antigen presenting cells

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Statement of Purpose: One of the focus areas in current vaccine development is the design of novel adjuvant formulations that can elicit strong and balanced immune responses towards a variety of pathogens. Biodegradable polymers have shown promising characteristics as adjuvants and/or delivery vehicles by enhancing antigen presentation compared with soluble protein.[1,2] The chemical and physical properties of these materials can be tailored to enhance their interactions with antigen presenting cells (APCs). In particular, polyanhydride nanoparticles exhibit desirable characteristics as adjuvants such as immunomodulation, sustained antigen release, and stabilization of protein antigens.[2-4] Pattern recognition receptors like C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) are key components of innate immunity, and also fundamental in the modulation of adaptive immune responses. In these studies, the effect of carbohydrate functionalization of antigen-loaded polyanhydride nanoparticles on their ability to activate APCs was investigated.

Methods: The antigen of interest in the current studies was gp41-GHC, an HIV polypeptide, which was encapsulated into polyanhydride nanoparticles based on sebacic acid (SA), 1,6-bis(*p*-carboxyphenoxy)hexane (CPH), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG). The nanoparticles were functionalized with carbohydrates (i.e., di-mannose, galactose). The structural stability of the released antigen was studied using SDS-PAGE and circular dichroism and the antigenicity of the released protein was evaluated using an ELISA. Following stimulation of murine dendritic cells, activation of these cells was investigated using cell surface marker expression (by FACS) and cytokine secretion (by a Luminex-based bead assay).

Results: Sustained antigen release kinetics was observed using different polyanhydride chemistries, as a function of polymer hydrophobicity (data not shown). Polyanhydride nanoparticles based on CPH:SA and CPTEG:CPH preserved the antigenicity of the released protein (Fig. 1), compared to non-encapsulated gp41-GHC, using the monoclonal HIV antibodies Z13e1, 2F5 and 4E10. These nanoparticles also preserved the primary and secondary structure of the antigen upon release (data not shown). Bone marrow derived dendritic cells were harvested and cultured for 14 days, and stimulated with CPTEG:CPH nanoparticle formulations (w/ and w/o di-mannose functionalization) targeting CLRs on the APCs. After stimulation with nanoparticles, the cell surface markers MHC I, MHC II, CD40 and CD86 were up-regulated (Fig. 2). The expression of the CD206 marker by the di-mannose-functionalized nanoparticles suggested the ability to activate APCs using CLR pathways. These data were consistent with the enhanced internalization of the functionalized nanoparticles by receptor-mediated mechanisms (data not shown). In addition, polymer chemistry and functionalization were observed to differentially enhance the secretion of the cytokines IL-6, IL-12p40, IL-10, TNF-α, and IL-1β.

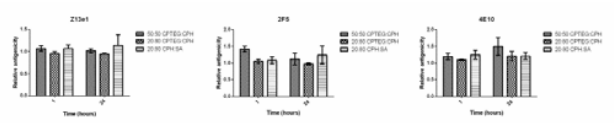

Figure 1. Relative antigenicity of released gp41-GHC from polyanhydride nanoparticles using different polymer chemistries, compared to non-encapsulated antigen. The monoclonal HIV antibodies Z13e1, 2F5 and 4E10 were used in these experiments.

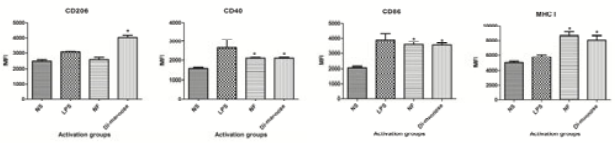

Figure 2. Dendritic cell activation by carbohydrate functionalized 1% antigen loaded (gp41-GHC) polyanhydride nanoparticles. Analysis of cell surface marker expression is presented as mean fluorescence intensity (MFI). Data are expressed as the mean ± the SEM of two independent experiments performed in triplicate. * represents groups that are statistically significant ($p \leq 0.05$) compared to the no stimulation (NS) group.

Conclusions: These studies have shown that carbohydrate-functionalized nanoparticles provide sustained release of antigenically active protein. The functionalized nanoparticles were internalized by CLR-mediated pathways. Cell surface marker expression and cytokine secretion were differentially enhanced, with the CLR-functionalized particles suggesting a more balanced immune response. Analysis of the combined effects on antigen stabilization and APC activation of polyanhydride nanoparticles functionalized by CLR ligands will enable rational design of efficacious vaccine formulations.

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