Effect of plasma protein adsorption on *in vitro* activation of dendritic cells by polyanhydride microparticles

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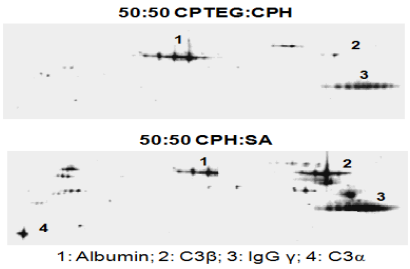
Statement of Purpose: In order to overcome the poor immunogenicity of recombinant proteins used for vaccination, researchers have focused on the development of new adjuvants. Polyanhydride microparticles have shown immunomodulatory properties that, when combined with their ability to stabilize and provide sustained and slow release of antigens, make them excellent candidates as adjuvants for the design of single dose vaccines[1]. A characteristic of this class of polymer carriers is that their adjuvant effect can be tailored by polyanhydride chemistry. It has been suggested that the biodistribution and biocompatibility of antigen carriers, and therefore their effectiveness as vaccine adjuvants, can be influenced by the adsorption of serum proteins *in vivo*[2]. Our previous work has shown that plasma protein adsorption patterns on polyanhydrides microparticles are correlated to their surface properties (i.e., hydrophobicity) suggesting that the plasma adsorption can be tailored by controlling the particle surface chemistry[3] Activation of dendritic cells (DCs) plays a major role in inducing protective immunity and polyanhydrides particles have been shown to activate DCs[4]. The main goal of this work is to understand the effect of plasma protein adsorption on the adjuvant properties of polyanhydride carriers by evaluating the in vitro activation of bone marrow-derived DCs.

Methods: *Microparticles.* Polymers based on sebacic acid (SA), 1,6-bis(*p*-carboxyphenoxy)hexane (CPH), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were synthesized. Cryogenic atomization was used to fabricate 50:50 CPH:SA and 50:50 CPTEG:CPH microparticles. *In vitro protein adsorption*. Whole mouse serum as well as specific plasma proteins (albumin, immunoglobulin G, and fibrinogen) were absorbed on the surface of polymer microparticles. 2-D gel electrophoresis and western blot were used to characterize protein adsorption. *DCs.* Bone marrow derived DCs from C57BL/6 mice were cultured for 48 h in the presence of protein-adsorbed microparticles. *Cell response evaluation.* Flow cytometry was used to assess for the surface expression of MHC I, MHC II, CD86, CD40 on DCs co-incubated with the microparticles. Culture supernatants were analyzed for the presence of cytokines (IL-6, IL-10, IL-12p40, and TNFα) by Luminex®.



1: Albumin; 2: C3β; 3: IgG γ; 4: C3α

Figure 1. (a) 2-D electrophoresis pattern of protein adsorbed on polyanhydride microparticles after incubation with mouse serum.

Results: C3 (β and α chains) complement components were identified on the surface of 50:50 CPH:SA microparticles suggesting activation of the complement system by an alternative pathway (**Figure 1**). Opsonization of 50:50 CPH:SA microparticles increased the expression of cell surface markers (i.e., MHC II, CD40, and CD86), while opsonization of 50:50 CPTEG:CPH microparticles decreased the expression of cell surface markers (**Figure 2**). The presence of serum proteins on both 50:50 CPH:SA and 50:50 CPTEG:CPH led to an increase in the secretion of TNF-α.

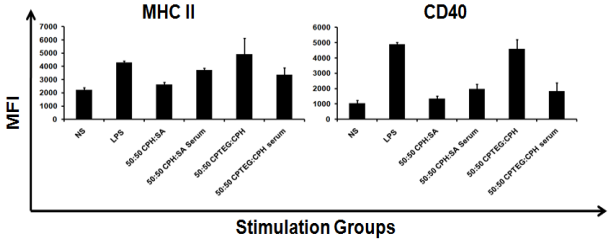


Figure 2. Cell surface expression of MHC II, and CD40 on dendritic cells after incubation with microparticles or LPS.

Conclusions: Absorption of complement components (i.e., C3) on 50:50 CPH:SA microparticles may mediate particle uptake by receptor-mediated endocytosis and direct DC activation. However, the proteins adsorbed on 50:50 CPTEG:CPH microparticles inhibited cell surface marker expression on DCs. These results indicate that protein adsorption on microparticle surfaces can dictate their adjuvant properties.

References: [1]Kipper MJ, et al. J Biomed Mater Res A. 2006;76:798-810; [2]Soppimath K, et al. J Control Release. 2000;70:1-20; [3]Carrillo-Conde B, et al. J Biomed Mater Res A. 2010;95A(1):40-48; [4]Petersen LK, et al. Biomaterials. 2009;30:5131-5142