Acta Biomaterialia 8 (2012) 3618–3628

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Chemistry-dependent adsorption of serum proteins onto polyanhydride microparticles differentially influences dendritic cell uptake and activation

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ARTICLE INFO

Article history:
Received 17 February 2012
Received in revised form 29 May 2012
Accepted 1 June 2012
Available online 8 June 2012

Keywords:
Polyanhydride
Microparticles
Serum proteins
Adsorption
Complement receptor 3

ABSTRACT

The delivery of antigen-loaded microparticles to dendritic cells (DCs) may benefit from surface optimization of the microparticles themselves, thereby exploiting the material properties and introducing signals that mimic pathogens. Following in vivo administration microparticle surface characteristics are likely to be significantly modified as proteins are quickly adsorbed onto their surface. In this work we describe the chemistry-dependent serum protein adsorption patterns on polyanhydride particles and the implications for their molecular interactions with DCs. The enhanced expression of MHC II and CD40 on DCs after incubation with amphiphilic polyanhydride particles, and the increased secretion of IL-6, TNF-α, and IL-12p40 by hydrophobic polyanhydride particles exemplified the chemistry-dependent activation of DCs by sham-coated particles. The presence of proteins such as complement component 3 and IgG further enhanced the adjuvant properties of these vaccine carriers by inducing DC maturation (i.e. increased cell surface molecule expression and cytokine secretion) in a chemistry-dependent manner. Utilizing DCs derived from complement receptor 3-deficient mice ($CR3^{-/-}$ mice) identified a requirement for CR3 in the internalization of both sham- and serum-coated particles. These studies provide valuable insights into the rational design of targeted vaccine platforms aimed at inducing robust immune responses and improving vaccine efficacy.

1. Introduction

The design of vaccine adjuvants capable of activating innate immunity is critical for the induction of protective immune responses [1,2]. A key step in the activation of the innate immune system is the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) on the surface of antigen-presenting cells (APCs), including dendritic cells (DCs) [1–3]. DCs can internalize and process soluble antigens, resulting in interactions with other immune cells, such as naïve T cells [2,4,5]. The use of polymer particles to deliver antigen, either encapsulated or bound to the surface, has been shown to enhance antigen presentation compared with the administration of soluble antigen alone [6–8].

The interaction of antigen-loaded microparticles with DCs may benefit from engineering the microparticle surface by exploiting the material properties and introducing motifs that mimic pathogens [9]. For example, it has been demonstrated that cationic surfaces greatly enhance uptake [10]. On the other hand, the presence of certain ligands which bind to specific cellular receptors promotes internalization [3,4,11]. After contact with serum the particles undergo significant changes in their surface properties because of the rapid adsorption of serum proteins [12,13].

Polyanhydride microparticles have been shown to possess immunomodulatory properties [14,15] which, when combined with their ability to stabilize and provide sustained release of protein antigens [16–20], makes them excellent vaccine adjuvants. Our previous work has demonstrated that serum protein adsorption patterns on polyanhydride microparticles are correlated with their surface characteristics (i.e. hydrophobicity), suggesting that the adsorption of serum proteins can be tailored by controlling the particle surface chemistry [13]. Immunoglobulin G (IgG), complement factors, and other proteins (i.e. opsonins) that have been identified on the surface of microparticles likely influence particle uptake by APCs [13,21,22]. Indeed, pathogens such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Mycobacterium leprae* coat themselves with serum proteins [23–26]. Opsonization of the pathogen facilitates host cell phagocytosis by promoting interactions with specific cell surface receptors, including complement, Fcγ, and mannose receptors [23–28]. Therefore, understanding the biological consequences of serum protein adsorption onto particles and its effect on APC activation may provide vital insights for the rational design of improved biomaterial-based adjuvants.

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http://dx.doi.org/10.1016/j.actbio.2012.06.001

This study was designed to investigate the differential adsorption of mouse serum proteins onto the surface of polyanhydride microparticles and to understand the effects of protein adsorption on uptake by and activation of DCs. Polyanhydrides based on sebacic acid (SA), 1,6-bis(p-carboxyphenoxy)hexane (CPH), and 1,8-bis(p-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) (Fig. 1) were evaluated in this work. The profile of serum proteins adsorbed onto the surface of the polyanhydride particles was indeed found to be influenced by the polymer chemistry and subsequently promoted differential effects on DC activation. Moreover, complement receptor 3 (CR3)-mediated pathways were determined to be critical for the internalization of polyanhydride particles by DCs.

2. Materials and methods

2.1. Materials

The chemicals needed for monomer synthesis and polymerization, sebacic acid (99%), p-carboxy benzoic acid (99%), and 1-methyl-2-pyrrolidinone anhydrous (99%), were purchased from Aldrich (Milwaukee, WI); 4-p-hydroxybenzoic acid, 1,6-dibromohexane, 1-methyl-2 pyrrolidinone, and triethylene glycol were purchased from Sigma Aldrich (St Louis, MO); 4-p-fluorobenzonitrile was obtained from Apollo Scientific (Stockport, UK); potassium carbonate, dimethyl formamide, toluene, sulfuric acid, acetic acid, acetonitrile, acetic anhydride, methylene chloride, and petroleum ether were purchased from Fisher Scientific (Fairlawn, NJ). Materials for SDS-PAGE and two-dimensional (2-D) electrophoresis, which included 12% Tris–glycine precast gels, unstained protein standards, Flamingo gel stain, 11 cm immobilized pH gradient (IPG) strips (pH 3–10, nonlinear), and 4–15% polyacrylamide gels were purchased from BioRad Laboratories (Richmond, CA). Phosphatase substrate was purchased from Aldrich (St Louis, MO). β-Mercaptoethanol, *Escherichia coli* lipopolysaccharide (LPS) O111:B4, and rat immunoglobulin (rat IgG) were purchased from Sigma Aldrich. Materials required for the DC culture medium included: granulocyte–macrophage colony-stimulating factor (GM-CSF), purchased from PeproTech (Rocky Hill, NJ); HEPES buffer, RPMI 1640, penicillin/streptomycin, and L-glutamine, purchased from Mediatech (Herndon, VA); heat inactivated fetal bovine serum, purchased from Atlanta Biologicals (Atlanta, GA). Materials used for flow cytometry included: BD stabilizing fixative solution purchased from BD Bioscience (San Jose, CA); unlabeled anti-CD16/32 FcγR, purchased from Southern Biotech (Birmingham, AL); unlabeled hamster IgG, fluorescein isothiocyanate (FITC)-conjugated anti-mouse MHC II (I-A/I-E) (clone M5/114.15.2), PE-conjugated anti-mouse MHC Class I (H-2Kd/H-2Dd) (clone 34-1-2S), allophycocyanin (APC) anti-mouse CD40 (clone 1C10), phycoerythrin-Cy7 (PE/Cy7) anti-mouse CD86 (clone GL-1), Alexa Fluor® 700 anti-mouse CD11c (clone N418) and the corresponding isotypes, FITC-conjugated rat IgG2bκ, PE-conjugated rat IgG2a (clone eBR2a), APC rat IgG2aκ (clone eBR2a), PE/Cy7-conjugated rat IgG2b (clone KLH/G2b-1-2), Alexa Fluor® 700-conjugated Armenian hamster IgG (clone eBio299Arm), all purchased from eBioscience. Cadmium selenide quantum dots (QDs) (emission at 630 nm) were a kind gift from Dr. Aaron Clapp of Iowa State University.

2.2. Monomer and polymer synthesis

Diacids of CPH and CPTEG were synthesized as described previously [29,30]. SA and CPH prepolymers were synthesized by the methods described by Shen et al. [31] and Conix [29], respectively. Subsequently 50:50 CPH:SA and 50:50 CPTEG:CPH co-polymers were synthesized by melt polycondensation as described by Kipper et al. [32] and Torres et al. [30], respectively. ^{1}H NMR spectroscopy was used to characterize the polymer structure, and the resultant spectra were consistent with previous work [30,32]. The synthesized 50:50 CPH:SA co-polymer had a M_w of 12 kDa with a polydispersity index (PDI) of 2.0, while the 50:50 CPTEG:CPH co-polymer had a M_w of 8 kDa with a PDI of 1.8. These values were obtained from ^{1}H NMR and corroborated with GPC and are consistent with previous works [8,30,32].

2.3. Microparticle fabrication and characterization

Cryogenic atomization was used to fabricate 50:50 CPH:SA and 50:50 CPTEG:CPH microparticles, as described elsewhere [13,16,19,20]. Briefly, 100 mg of the polymer was weighed and dissolved in methylene chloride. For QD-loaded microparticles, QDs were added to the dissolved polymer and dispersed by sonication at 40 Hz for 30 s. The polymer solution was then pumped through an 8700–1200 MS ultrasonic atomizing nozzle (SonoTek Corp., Milton, NY) into 200 ml of frozen ethanol (with an excess of liquid nitrogen). Microparticles were fabricated at 4 °C. Compositions were stored at −80 °C for 3 days. For 50:50 CPTEG:CPH, after the first 24 h 200 ml of cold ethanol was added to reduce aggregation, the solutions were stirred at 300 r.p.m. for 15 min and placed back in the freezer at −80 °C. After 3 days vacuum filtration was used to collect the microparticles and they were dried overnight under vacuum. Scanning electron microscopy (SEM) (JEOL 840 A, JEOL Peabody, MA) was used to observe the morphology of the micro-

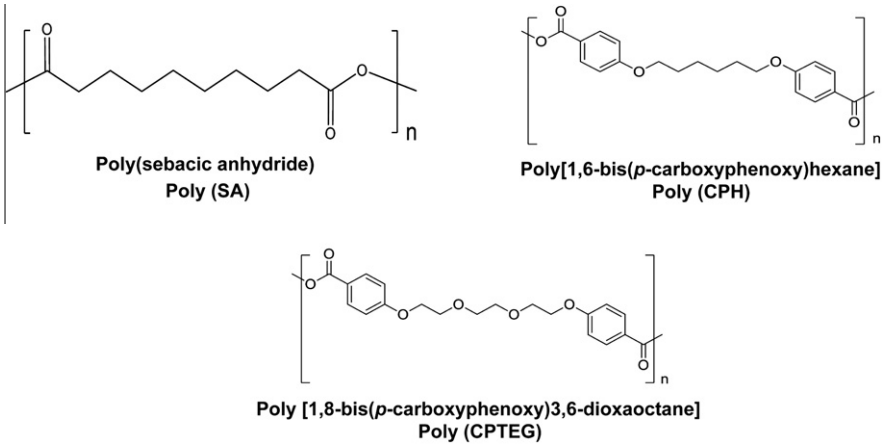

Fig. 1. Chemical structures of the SA, CPH, and CPTEG repeat units. Upon degradation these polyanhydrides produce dicarboxylic acids in which the anhydride bonds are replaced by –COOH groups on both ends.