Acta Biomaterialia 57 (2017) 136–145

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Precise manipulation of biophysical particle parameters enables control of proinflammatory cytokine production in presence of TLR 3 and 4 ligands

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ARTICLE INFO

Article history:
Received 9 September 2016
Received in revised form 3 January 2017
Accepted 5 January 2017
Available online 6 January 2017

Keywords:
Silica particle
Poly(amino acid)
Dendritic cell
T cell
Vaccine
Immunotherapy

ABSTRACT

The biophysical parameters governing nanoparticle (NP)-cell interactions significantly affect biological responses, particularly in the application of NP-based immunotherapeutics. Modulation of the surface biophysical character of NPs can be achieved via introduction of amino acids, which offer the ability to fine tune a range of biophysical parameters of interest. We employed this approach using monodisperse silica NPs coated with numerous poly(amino acid)s (PAAs). The NPs were incubated with dendritic cells (DCs) in conjunction with TLR ligands and production of IL-1β from DCs and IFNγ from T cells primed by these DCs were measured. These key cytokines can prognosticate the efficacy of the NP platform as a potential vaccine or active cellular immunotherapy carrier. IL-1β production showed a correlation with both NP size and degree of hydrophobicity. High IFNγ secretion from T cells was shown to be correlated with both the hydrophobicity and charge of the NPs used to activate the DCs. Other cytokines were also screened in order to compare the immune responses. The results of this study highlight the importance of nanoparticle biophysical parameters and the selection of TLR ligands to the rational design of nanoparticle-based vaccines and immunotherapies.

Statement of Significance

The manuscript describes a systematic investigation into the effects of biophysical parameters of nanoparticles (NPs) on immune cells. Modulation of the biophysical character of the NP surface can be achieved by introduction of amino acids on monodisperse silica NPs, introducing a range of tunable biophysical parameters of interest, i.e. distinct sizes, different surface charges and varying degrees of surface hydrophobicity. We examine internalization of the NP in dendritic cells (DCs) and measure a myriad of cytokines, including IL-1β and IFNγ, which prognosticate the efficacy of the NPs as a potential vaccine (IL-1β metric) or active cellular immunotherapy carrier (IFNγ metric). Two different TLR ligands (a viral TLR3 ligand and a bacterial TLR4 ligand) were used along with the PAA NPs to compare their costimulatory immunogenicity. We strongly believe that this study will provide crucial information to many readers of ***Acta Biomaterialia*** and further drive the use of nanoparticle platforms in modulating immune responses.

1. Introduction

Although nanoparticle-based vaccines and active immunotherapy have gained significant momentum in academic, clinical and industrial settings, very little is known about the ways in which nanoparticle biophysical parameters affect the immune response [1–4]. Modulating the immune system through the use of NPs

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http://dx.doi.org/10.1016/j.actbio.2017.01.025
1742-7061/

opens the door to new treatment modalities with tremendous potential in ameliorating a wide range of infectious diseases and cancers. Delivery of antigens and immune-stimulatory molecules is one of the major objectives of nanomaterial-based immunoengineering platforms because local presentation of these immunogenic agents can greatly affect the body's immune response [5,6]. Additionally, NPs can be used to stimulate proinflammatory cytokines to regulate immunity, due to their similarity to pathogens in both size and shape [7,8]. With their ability to target their immunogenic payload to specific antigen-presenting cells (APCs), NPs could revolutionize traditional immunization methods, reducing the number of necessary doses [9].

Despite many studies existent in the literature that investigate the application of NPs to immunotherapy, there is still a gap in understanding the effect that particles have on the complex machinery of the immune system. For example, there are some articles in which the surface charge and size of particles were shown to have a significant effect on immunological activity; however, the results are oftentimes inconsistent, possibly due to variations in other biophysical parameters and differences in the assay methods [10]. While investigating the effect of decorating the surface of NPs with various molecules, one thing often overlooked is the change in size of the NPs that occurs, which can be a 2 or even 4-fold increase, leading to an incomplete interpretation of the results [11]. There are examples in the literature in which the surface modification of polystyrene particles (~1 μm) with cationic groups significantly enhanced particle uptake by macrophages and DCs, but a different trend was evident when surface modifying nanoparticles (100 nm) [12]. This difference highlights the importance of controlling for the size of the particles when examining the effects of surface modification.

In this study, we evaluated the immunological activity of a library of particles with well-defined surface characteristics. Silica NPs coated with PAAs were used to study the influence of surface character on cytokine production in immune cells. Silica particles were employed as a core substrate for PAA coatings because they are available in a wide range of sizes, each having a very narrow size distribution [13,14]. They were also chosen for their stability in most organic solvents that are used in surface modification reactions. The particles were coated with 12 different PAAs to generate a surface hydrophobicity gradient and different surface charges, while keeping diameter change to a minimum (the majority changing less than 10%). A peptide bond that is known to be highly stable in aqueous solutions was used to immobilize PAAs on the silica particles [15]. PAAs have been widely used as biomaterials and drug delivery systems because of their biological origin, negligible toxicity of degradation products and facility of chemical modification [16–18].

The two cytokines highlighted most extensively are IL-1β and IFNγ, chosen for their pertinence to vaccine development and tumor immunotherapy, respectively. While the study has a deliberate focus on these two cytokines, a myriad of others were also investigated to demonstrate the broad scope of effects that modulating the biophysical characteristics of particles has on immune cells. Two costimulatory molecules (polyinosine-polycytidylic acid (polyI:C, TLR 3 ligand) and lipopolysaccharide (LPS), TLR 4 ligand) were used to study their synergistic immunostimulatory effect with PAA NPs. NPs are known to be DC stimulatory through NALP3 inflammasome activation; however, in order to activate DCs, the NPs must be used in conjunction with a costimulatory molecule, because DC activation occurs through the simultaneous or sequential stimulation of multiple pathways [19]. The immunostimulatory effect on DCs was investigated by monitoring the production of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17, MCP-1, IFNγ, TNFα, MIP-1α and RANTES. T cells were activated by NP-treated DCs and their IFNγ production was measured.

2. Materials and methods

2.1. Materials

Silica particle *N*-hydroxysuccinimide (NHS) (Sicastar, 300 nm, 1 μm and 10 μm) was purchased from Micromod (Rostock, Germany). Amino acids, poly-L-histidine (molecular weight (MW) 5000 – 25,000 g/mol), poly-L-arginine (MW 5000 – 15,000 g/mol), poly-L-lysine (MW 1000 – 5000 g/mol), Rose Bengal and dimethylformamide (DMF) were supplied by Sigma-Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO, anhydrous) was purchased from Alfa Aesar (Lancashire, UK). PyBOP and *N*,*N*-diisopropylethylamine (DIPEA) were obtained from Acros Organics (Geel, Belgium). Lipopolysaccharide (LPS, *Escherichia coli* strain 0111:B4), fluorescein isothiocyanate conjugated LPS (FITC-LPS), polyinosine-polycytidylic acid (polyI:C), and cytochalasin D were purchased from Sigma-Aldrich. Chicken egg ovalbumin (EndoFit) was received from InvivoGen (San Diego, CA). Cell Titer Blue was purchased from Promega (Madison, WI). CA-074Me was supplied by Enzo Life Sciences (Farmingdale, NY). EasySep mouse CD8+ T cell isolation kit was purchased from Stemcell technologies (Vancouver, Canada). Antibodies used in ELISA for IL-1β and IFNγ were received from R&D systems (Minneapolis, MN). Multiplex ELISA, Mouse cytokine 16-Plex, was purchased from Quansys Biosciences (Logan, UT). Granulocyte-macrophage colony-stimulating factor (GM-CSF) was obtained from eBioscience (San Diego, CA).

2.2. Animals

Female C57BL/6 mice (8–12 week old) were obtained from Charles River (Kingston, NY). Female OT-I mice were supplied by Taconic (Hudson, NY) and used at the age of 6–8 weeks. Animals were maintained and used in accordance with protocols that were approved by the Institutional Animal Care and Use Committee at Yale University.

2.3. Surface modification of silica particles with PAAs

Twelve amino acids were selected based on the simplicity of synthesis while keeping diversity of side chains and hydrophobicity. For example, we chose Leu as a representative of alkyl chain side groups such as Leu, Ile and Val. Five mg of amino acid monomer (Ala, Phe, Leu, Gly, Asp, Asn, Tyr, Trp, or Ser) was polymerized by dispersing in 500 μL of DMF in a 1.5 mL tube [20]. To the monomer solution, 5 mg of silica particles dispersed in 200 μL of DMF was added. PyBOP (15 mg) was dissolved in 300 μL of DMF and added to the mixture. After the addition of DIPEA (25 μL), the mixture was incubated at 37 °C for 18 h. The particle dispersion was centrifuged at 16,000 rcf for 3 min (Eppendorf Centrifuge 5415D) and the supernatant was discarded. The particles were washed with 1 mL of PBS once and 1 mL of DMSO twice. The particles were dispersed in 1 mL of DMSO and stored at 4 °C until use.

For the amino acids with positively charged side groups (e.g. His, Arg, and Lys), their polymers (poly-L-histidine, poly-L-arginine, and poly-L-lysine, respectively) were directly conjugated to the silica particles because their side groups may have interrupted the polymerization reaction. Conjugation of poly-L-histidine is described here as a typical example [21]. Five mg of silica particles (Sicastar from Micromod) was dispersed in 200 μL of DMSO. Poly-L-histidine (5 mg) was dissolved in 500 μL of DMSO and added to the particle suspension. After the addition of DIPEA (25 μL), the mixture was incubated at 37 °C for 18 h. The particles were washed with 1 mL of 0.01 M HCl once and 1 mL of DMSO twice. The particles were dispersed in 1 mL of DMSO and stored at 4 °C until use.