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Towards programming immune tolerance through geometric manipulation of phosphatidylserine

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ABSTRACT

The possibility of engineering the immune system in a targeted fashion using biomaterials such as nanoparticles has made considerable headway in recent years. However, little is known as to how modulating the spatial presentation of a ligand augments downstream immune responses. In this report we show that geometric manipulation of phosphatidylserine (PS) through fabrication on rod-shaped PLGA nanoparticles robustly dampens inflammatory responses from innate immune cells while promoting T regulatory cell abundance by impeding effector T cell expansion. This response depends on the geometry of PS presentation as both PS liposomes and 1 micron cylindrical PS-PLGA particles are less potent signal inducers than 80 × 320 nm rod-shaped PS-PLGA particles for an equivalent dose of PS. We show that this immune tolerizing effect can be co-opted for therapeutic benefit in a mouse model of multiple sclerosis and an assay of organ rejection using a mixed lymphocyte reaction with primary human immune cells. These data provide evidence that geometric manipulation of a ligand via biomaterials may enable more efficient and tunable programming of cellular signaling networks for therapeutic benefit in a variety of disease states, including autoimmunity and organ rejection, and thus should be an active area of further research.

1. Introduction

Recent advances in multiple scientific fields, including materials science and immunology, are now converging to enable targeted, pathology-specific biological programming to promote health in living animals [1–4]. This is in part due to our increased understanding of the signaling pathways involved in a variety of disease states as well as our fundamental appreciation of the molecular interactions which underlie initiation and maintenance of immune responses. This information has been used to generate a new wave of therapeutic tools that program biological responses using rationally-designed biomaterials, including nanoparticles and hydrogels. Some examples of the programming potential of this new class of tools include more potent and sustained vaccine responses to pathogens [5–8], enhanced drug delivery and immunomodulation in the setting of cancer [9–18], amelioration of both acute and chronic inflammatory responses [19–22], and

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dampening autoimmunity and allergies through promotion of antigen-specific tolerance [23–25].

While the potential for nanotechnology to transform therapeutic approaches to the diseases that burden 21st century societies is readily apparent at this stage, there remain many unanswered mechanistic and design questions as relates to nanoparticle-mediated programming of biologic responses. In this report, we aimed to address one such fundamental question regarding the particulate presentation of a ligand that binds a cell surface receptor. This is a mechanistically critical question, as programming of biological responses will often require interface with cell surface receptors yet little is known as to how the geometric manipulation of ligands via biomaterials augments downstream biological signaling from the cell surface. Here, we use the term geometry to broadly define the three dimensional presentation of a ligand that can be manipulated through varying the size, shape, composition and biophysics of a particulate delivery vehicle. Given the recent finding that geometric manipulation of the erythropoietin cell surface receptor using diabodies can fundamentally alter downstream signaling effects, there is precedent for this concept [26].

To address this issue, we chose to use the cell membrane phospholipid phosphatidylserine (PS) as our model ligand for a cell surface receptor. PS is primarily localized on the inner membrane of cells until apoptosis triggers it to re-localize to the outer membrane where it can serve as an "eat-me" signal to phagocytes [27–29]. This is a critical means by which the body avoids aberrant immune response to self-antigens because the PS signal induces an anti-inflammatory program in the engulfing phagocyte which promotes tolerance to components of the dying cell. This is thought to be in part due to TGF-β1 release as well as triggering of cell surface receptors, including the PS receptor (PSR) and MERTK, a member of the TAM family of receptor tyrosine kinases [27,28], [30–32]. We hypothesized that particulate presentation of PS on a nanoparticle would mimic apoptotic cells and thus drive an anti-inflammatory program in innate immune cells. There is evidence for this using liposomal preparations of PS however we broadened our approach to specifically address how the geometry of PS presentation augmented its signaling effects and subsequent therapeutic potential [33–35]. In particular, we addressed how PS liposomes versus delivery of PS on 80 × 320 nm PLGA nanorods or 1 micron cylindrical PLGA particles affected downstream immune responses.

This latter feature of our paper – design control of particulate PS presentation – was made possible through using the Particle Replication in Non-Wetting Template (PRINT) process, which enables fabrication of particles of a homogenous size, shape, and charge composed of both lipids and polymers through a molding process [36–39]. We have previously shown that the PRINT process enables fabrication of nano- and microparticles made of poly lactic-co-glycolic acid (PLGA) or PEG (poly-ethylene glycol) that do not induce immune activation at baseline in mouse and human cells. We have also shown these particles can be designed to target mammalian innate immune cells *in vitro* and *in vivo* or remain extracellular, thus making them an attractive tool for programming immune responses for therapeutic benefit [40–43].

In this report we show that geometric manipulation of PS programs the immune response towards an anti-inflammatory state that dampens effector T cell expansion while promoting T regulatory cell abundance in a tunable fashion. We show that this anti-inflammatory response may possibly be co-opted for therapeutic benefit in a variety of disease states, including autoimmunity using a mouse model of multiple sclerosis and transplantation medicine approaches using a model of allogeneic human T cell activation. These results strongly suggest that particulate delivery of a cell surface receptor ligand can trigger unique signaling effects through geometric manipulation of the surface membrane. Therefore, geometric and biophysical properties of particles should be included as part of baseline experiments that seek to further the field of programming biological responses as it is highly likely each ligand of therapeutic interest will have its own unique 'best-in-class' particle design parameters.

2. Materials and methods

2.1. PRINT particle fabrication and characterization

For the incorporation of PS (Avanti Polar Lipids, 840032) in the PRINT platform, PS and poly(D,L-lactide-co-glycolide) (PLGA) of lactide:glycolide 85:15 (Sigma Aldrich) were dissolved separately in chloroform. The solutions of PS and PLGA were mixed at ratios of 10:90 (PS:PLGA), and the sample was diluted to 2 wt% (mass/mass) solution with chloroform. A thin film of PS and PLGA was deposited on a 6″ × 12″ sheet of poly(ethylene terephthalate) (PET) by spreading 200 μL of solution using a #5 Mayer Rod (R.D. Specialties). The solvent was evaporated with heat. Fluorocur®, d = 80 nm × h = 320 nm, d = 1000 nm × h = 1000 nm prefabricated molds and 2000 g/mol polyvinyl alcohol (PVOH) coated PET sheets were provided by Liquidia Technologies. The PET sheet with the film was then placed in contact with the patterned side of a mold and passed through heated nips (Chem Instruments Hot Roll Laminator) at 130 °C and 80 psi. The mold was split from the PET sheet as they both passed through the hot laminator. The patterned side of the mold was then placed in contact with a sheet of PET sheet coated with 2000 g/mol PVOH. This was then passed through the hot laminator to transfer the particles from the mold to the PET sheet. The mold was then peeled from the PET sheet. The particles were removed by passing the PVOH coated PET sheet through motorized rollers and applying water to dissolve the PVOH to release the particles. To remove excess PVOH and unbound PS, the particles were purified and then concentrated by tangential flow filtration (Spectrum Labs). PS was resuspended in deionized, distilled water and did not undergo more than 1 freeze–thaw cycle during experimentation. The PLGA particles were imaged by scanning electron microscopy (SEM) by pipetting a 3 μL sample of particle on a glass slide. The sample was then dried and coated with 3 nm gold palladium alloy using a Cressington 108 auto sputter coater. Images were taken at an accelerating voltage of 2 kV using a Hitachi model S-4700 SEM. For size and zeta potential measurement, dynamic light scattering (DLS) (Malvern Instruments Nano-ZS) was used. The particles were suspended in 10 mM KCl solution for zeta potential measurements. PS was measured using an Agilent Technologies Series 1200 HPLC with a C18 reverse phase column (Zorbax Eclipse XDB-C18, 4.6 × 100 mm, 3.5 micron). A linear gradient from 85:15 of methanol with 0.1% trifluoroacetic acid (TFA): water with 0.1% TFA to 100% methanol with 0.1% TFA was run over 25 min at a flow rate of 1 ml/min. Particle samples were dissolved in an acetonitrile:water solution, and the PS was quantified using an ELSD detector. Liposomal PS formulations were generated through resuspension of unadulterated PS in sterile water.

2.2. Murine bone marrow-derived dendritic cell activation studies

Red blood cell-depleted bone marrow cells were harvested from the femurs and tibias of C57BL/6 mice as previously described [44]. These cells were cultured with 5 ng/ml GM-CSF in complete media (RPMI 1640 media containing 10 mM HEPES, 1 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM β-mercaptoethanol and 10% heat inactivated FBS) at 37 °C for 6 days and purified using anti-CD11c$^+$ beads (MACS). The six-day old bone marrow-