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The nanomaterial-dependent modulation of dendritic cells and its potential influence on therapeutic immunosuppression in lupus

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ABSTRACT

Targeting dendritic cells with nanoparticles is an attractive modality for instigating immunity or inducing immunosuppression. An important aspect of successful delivery of antigen and immune modulators to these cells is the efficacy of nanoparticle internalization, which can dictate the strength and robustness of immune responses; optimizing particulate uptake is thus key. We compared the internalization of two nanoparticulate platforms: a vesicular "nanogel" platform with a lipid exterior, and the widely-used solid biodegradable poly(lactic-*co*-glycolic acid) (PLGA) system. We found that nanogels were more effectively internalized by dendritic cells *in vitro*, as demonstrated by fluorescent tracer measurements. Additionally, the magnitude of dendritic cell immunosuppression achieved by nanogels loaded with mycophenolic acid, an immunosuppressant, was greater than similarly drug-loaded PLGA. Although both types of particles could mitigate the production of inflammatory cytokines and the up-regulation of stimulatory surface markers, nanogels yielded greater reductions. These *in vitro* measurements correlated with *in vivo* efficacy, where immunosuppressive therapy with nanogels extended the survival of lupus-prone NZB/W F1 mice whereas PLGA particles did not. Our results highlight the importance of material on nanoparticle uptake by dendritic cells, which impacts the quality of therapeutic immunosuppression.

1. Introduction

Dendritic cells are crucial mediators of the immune system, where they instigate or suppress immunity by presenting antigen and providing receptor–ligand interactions and cytokines that lead to T and B cell activation [1]. As such, they have been widely studied for their therapeutic potential in vaccination [2], cancer immunotherapy [3,4], and mediating immunosuppression and tolerance [5–7]. An active area of dendritic-cell based therapies has been to target cells with particles that contain antigen, stimulatory compounds and ligands, or immunosuppressants [8–15]. The observation that antigen presentation by dendritic cells is more effective when antigen is particulate-bound, opposed to soluble [16], demonstrates the potency of particle uptake. The internalization of particles can be augmented with ligands for CD11c [17], DEC-205 [18], other lectins [19–21], or toll-like receptors [22]. This fundamental need to maximize the interaction of particles with dendritic cells is an important design consideration for nanomaterials.

We previously reported the use of a nanogel-based particle for treatment of lupus [23], an autoimmune disease characterized by systemic inflammation and organ damage. Efficacy was accomplished, in part, by attenuating the inflammatory and stimulatory capacity of dendritic cells [23]. Nanogels consisted of an exterior lipid membrane and a gel-like interior primarily of poly(ethylene glycol), and were loaded with the immunosuppressant mycophenolic acid (MPA). While nanogels were efficacious, we questioned if other classes of nanoparticles could be effective, or if there is a property of nanogels that made them unique. Many types of materials could be compared [24], but we focused specifically on biodegradable, solid nanoparticulate matrices made of poly(lactic-*co*-glycolic acid) (PLGA) because of its popularity in a wide-range of drug and antigen delivery applications [25], including the targeting of dendritic cells [26].

This study provides direct comparisons of PLGA nanoparticles with our previously reported data of nanogels mediating immuno

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suppression in dendritic cells [23]. We hypothesized that the therapeutic efficacy of nanoparticles is critically linked to their internalization by dendritic cells. We compared the ability of PLGA and nanogel particles to be internalized, and how well MPA-loaded particles could induce immunosuppression among dendritic cells and *in vivo* in lupus.

2. Materials and methods

2.1. Nanoparticle synthesis and characterization

Nanoparticles were made and characterized according to previously described protocols [23,27]. Briefly, nanogels were made with liposomes extruded from a lipid mixture of 1:2:0.1 M ratio of cholesterol: phosphatidylcholine: 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]. Liposomes were lyophilized, and then rehydrated with a mixture of acrylated lactic acid-poly(ethylene glycol)-lactic acid, MPA complexed in non-methylated β-cyclodextrins, and Irgacure 2959. The particles were cured under UV light, rinsed, and centrifuged. Nanogels were functionalized with avidin using sulfo-*N*-hydroxysuccinimide/1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (sNHS/EDC). For PLGA particles, MPA was dissolved with PLGA in ethyl acetate, and emulsified with poly(vinyl alcohol) and avidin-palmitate using a sonicator probe. PLGA particles were subsequently hardened, washed, and then lyophilized. Biotinylated poly(ethylene glycol) was added to PLGA particles at a ratio of 1.33 μg per mg particle prior to use in experiments.

The MPA amount in particles was measured by fluorescence from the supernatant of dissolved particles. Particle size and molarity (concentration) was measured using Nanosight single particle tracking.

2.2. BMDC culture

BMDCs were cultured from marrow harvested from the femurs and tibias of Balb/c or C57BL/6 mice. BMDCs were cultured with 10 ng/mL GM-CSF and 5 ng/mL IL-4 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. Media was changed every 2–3 days.

2.3. Nanoparticle internalization studies

Nanogels and PLGA particles were titered with biotin-fluorescein so that they had similar amounts of fluorescence, as measured by flow cytometry. Seven-day old BMDCs were incubated with particles (not containing MPA) at 37 °C, washed, and then analyzed for the percentage of fluorescein-positive $CD11c^+$ cells by flow cytometry. For inhibitors, cells were incubated at 4 °C or treated with 10 μg/mL cytochalasin D or 10 mM of randomly methylated β-cyclodextrin. For confocal microscopy, BMDCs treated with particles were washed, incubated onto charged slides, and then stained with phalloidin-Alexa568 and TO-PRO-3.

2.4. Functional assays for BMDCs

BMDCs from Balb/c mice were treated with MPA-loaded particles beginning on day 1 of culture, with the media changed every 2 days with fresh immunosuppressant. On day 6 of the culture, 50 ng/mL LPS was added for 18 h. The supernatant was subsequently assayed for cytokines by ELISA, and cell surface markers were measured by flow cytometry. For mixed lymphocyte reactions, purified CD4 T cells from C57BL/6 mice were co-cultured with irradiated BMDCs. T cell proliferation was measured by CFSE dilution.

2.5. Lupus animal studies

NZB/W F1 female mice (The Jackson Laboratory, stock #100008) were treated with weekly intraperitoneal injections of 0.625 mg MPA/kg weight animal. For prophylactic studies, treatment was initiated at 18–20 weeks of age. Proteinuria (≥300 mg/dL) was assayed with Uristix, using urine acquired by bladder compression. For studies beginning at later-stage disease, mice were placed on therapy once they had two consecutive days of proteinuria.

2.6. Statistical analysis

All statistical comparisons were performed on GraphPad Prism (version 5.03). Survival was analyzed with log-rank (Mantel–Cox) tests. Two-tailed *t*-tests and ANOVA with Bonferonni's post test were performed as indicated. A *p*-value of 0.05 or less was considered statistically significant.

2.7. Study approval

All animal studies were performed with the approval of the Yale University Institutional Animal Care and Use Committee (New Haven, CT).

3. Results

3.1. Fabrication and characterization of nanoparticles

Nanogels and PLGA nanoparticles encapsulating MPA were prepared using previously reported methods [23,27]. Nanogels contain an aqueous poly(ethylene glycol)-*co*-lactic acid (PEG-PLA) core where MPA, which is hydrophobic, is solubilized by complexation to non-methylated β-cyclodextrins (Fig. 1a). These particles have a greater drug loading than conventional liposomes (Table 1). The nanogels possess a lipid membrane fabricated from a mixture of 2:1 M ratio phosphatidylcholine and cholesterol, and approximately 3.2% (mol/mol) amount of PEGylated DSPE lipid. In

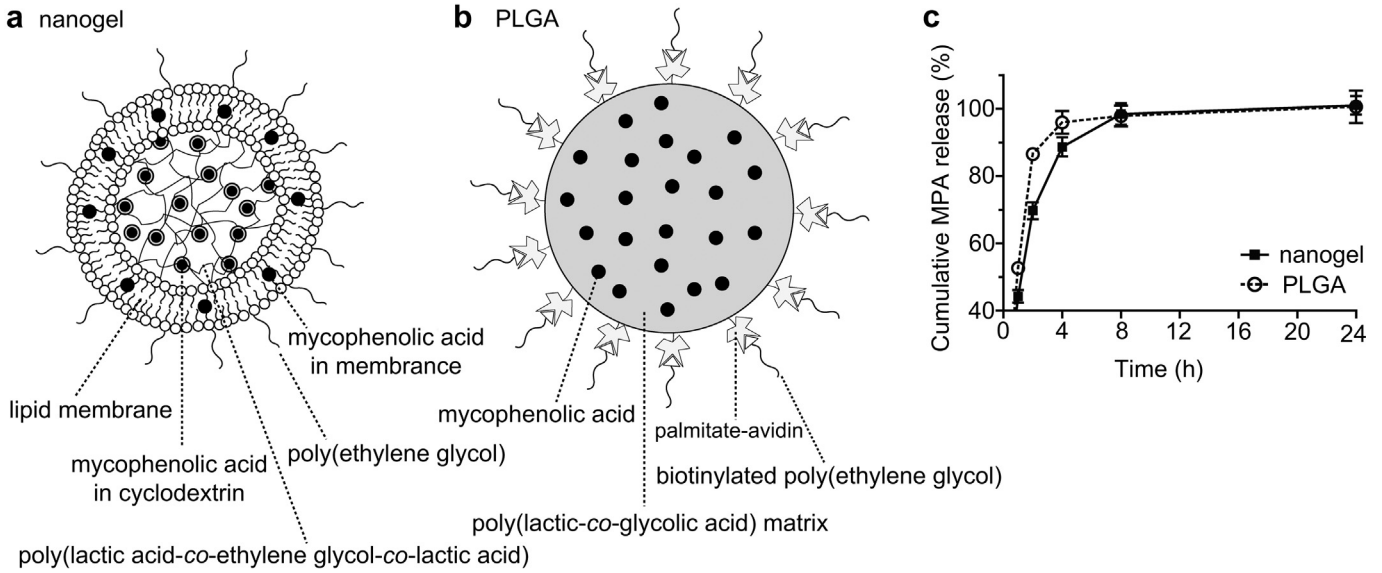

Fig. 1. Description of nanoparticles. (A) Nanogels contain a hydrophilic interior core composed of photo-linked poly(lactic acid-*co*-ethylene glycol-*co*-lactic acid). A lipid membrane surrounds the nanogel. MPA was primarily complexed within β-cyclodextrins located in the core, but may also partition within the lipid membrane. (B) PLGA particles are a solid matrix, with MPA embedded throughout the matrix. Avidin-palmitate was incorporated onto the surface of PLGA particles to permit the attachment of biotinylated poly(ethylene glycol). (C) The release of MPA from particles in 10% FBS at 37 °C. Results are representative of three experiments, where data showing MPA release from nanogels previously reported in Ref. [23]. Error bars represent the standard deviation. Drug release profiles were not significantly different by two-tailed *t*-test.