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Uptake characteristics of NGR-coupled stealth PEI/pDNA nanoparticles loaded with PLGA-PEG-PLGA tri-block copolymer for targeted delivery to human monocyte-derived dendritic cells

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Abstract

We have investigated the *in vitro* uptake, toxicity, phenotypic consequences and transfection efficiency of a stealth NGR/PEG/PDBA-coupled-SHA-PEI/pDNA targeting polyplex loaded with PLGA-PEG-PLGA tri-block copolymer in human monocyte-derived dendritic cells (DCs). Modification with PEG effectively shielded and reduced non-specific phagocytosis by immature DCs to approximately 20%. Coupling the NGR cell-specific peptide to the PEGylated polyplex (NGR/PEG/PDBA-SHA-PEI/pDNA) however resulted in specific and enhanced phagocytosis in DCs without any observable toxicity at the optimum concentration of 0.25% of the copolymer. DNase treatment had no effect on DNA integrity in the encapsulated polyplex. Confocal microscopy confirmed intracellular localization of the targeting NGR/PEG/PDBA-SHA-PEI/pDNA microparticles, resulting in more enhanced uptake of the radiolabeled plasmid DNA and approximately 5- and 10-fold increase over the control tri-block Pluronic F68 copolymer and the non-targeting polyplex, respectively. More importantly, phagocytosis of the targeting microparticles neither altered the functionality of immature DCs nor the phenotypic expression of DC-specific cell surface molecules, CD80, CD86, CD40 and CD54 (ICAM-1), suggesting that uptake of the targeting microparticles by themselves did not induce DC maturation. Taken together, these results suggest that PLGA-PEG-PLGA encapsulation of this stealth targeting polyplex has no negative effects on key properties of immature DCs and should pave the way for targeting DCs for vaccination purposes.
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1. Introduction

Dendritic cells (DCs) are potent antigen presenting cells with a unique capacity to induce an immune response and have thus been identified to play a crucial role in DNA vaccination (Coombes and Mahony, 2001). Antigen uptake and processing by DCs triggers a cascade of signaling network resulting in activation and maturation of DCs, phenotypically characterized by increased expression of CD40, ICAM-1, CD80, CD86 costimulatory molecules, MHC molecules, secretion of cytokines (e.g. IL-12) and chemokines (e.g. CCL19 and CCL22). DCs display 10–100 times more MHC molecules than other antigen presenting cells (APCs) and hence offer a powerful tool to manipulate the immune system (Banchereau and Steinman, 1998) for DNA vaccination. However, inherent difficulty in transfecting DCs, in combination with low numbers of DCs found in the epidermis (Bergstresser et al., 1980) is considered to be one of the major bottlenecks of this vaccination approach.

Gene delivery systems using non-viral polymeric carriers have received much attention because they have conceivably far less safety problems. Emphasis has been placed on microparticulate DNA vaccine delivery systems formed between DNA and polycations due to the inherent capability of DCs to efficiently phagocytose particles in the micrometer range (Thiele et al., 2001; Foged et al., 2002). Polycations including synthetic polymers (Mumper et al., 1996), lipids (Felgner et al., 1987) and polymer–lipid hybrids (Han et al., 2001) have been utilized in these formulations to improve non-viral gene delivery. Previous

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studies have shown that PLGA microparticles are readily phagocytosed by monocyte-derived DCs *in vitro* (Jilek et al., 2004). In other common approaches, non-ionic polymers consisting of tri-block copolymers of polyethyleneoxide and propyleneoxide (PEO-PPO-PEO) pluronics have been used to enhance gene delivery (Lemieux et al., 2000). While encapsulation of DNA in biodegradable PLGA microparticles leading to protection and controlled release of DNA seems to be an attractive strategy for gene delivery, there is a paucity of information on extensive characterization and formulation of PLGA-encapsulated nanoparticles for gene delivery to DCs.

Previous studies have shown that drug release from PLGA microspheres is very low due to the hydrophobicity of both drug and PLGA (Mu and Feng, 2001; Feng et al., 2004). It is therefore conceivable that incorporation of a hydrophilic segment such as poly(ethylene glycol) (PEG) into the hydrophobic PLGA chain forming the tri-block copolymer PLGA-PEG-PLGA would greatly facilitate the drug release. Indeed, earlier studies (Deng et al., 1990; Li et al., 2000) have demonstrated that this tri-block copolymer increases the release rate of hydrophilic proteins. Another potential advantage provided by the hydrophilic PEG would be improvement of the biocompatibility of the delivery vehicle. This is because most of the biological environment is hydrophilic in nature and biocompatibility appears to be correlated directly with the degree of hydrophilicity that a surface exhibits (LaPorte, 1997). Consequently, we utilized PLGA-PEG-PLGA in the present study to encapsulate the PEGylated polyplex to yield a stealth gene delivery vehicle for evaluation of transfection efficiency in DCs.

Phagocytic cells such as DCs recognize injected polymeric particles as foreign materials and remove them quickly and efficiently from the blood stream. The non-specific nature of the phagocytosis by DCs represents one of the main hurdles that need to be overcome in order to achieve selective targeting of particulates to selected cells. PEG surface coatings have been investigated for a wide variety of biomedical applications where their immobilization on surfaces have been shown to decrease protein adsorption (Norman et al., 1993; Kenausis et al., 2000) and the non-specific uptake by phagocytes (Norman et al., 1993; Luck et al., 1998).

We recently reported a novel formulation of a salicyl hydroxamic acid (SHA)-derivatized PEI/pDNA and a PEGylated PDBA linkage for enhanced *in vivo* transfection (Moffatt et al., 2005; Moffatt et al., 2006a,b). In an attempt to use this coupling strategy for DNA vaccination, the first aim of the present study was to investigate if the disulphide-bridged NGR (Asparagine-Glycine-Arginine) domain-containing CNGRC cell-specific peptide coupled to the PEGylated polyplex (NGR/PEG/PDBA-SHA-PEI/pDNA) would enhance receptor-mediated phagocytosis, since NGR receptors are over-expressed on the surface of immature phagocytic DCs (Arap et al., 1998; Dong et al., 2000). Secondly, because the availability of functionalized PEG-PLGA permits the preparation of target-specific nanoparticles by conjugation of cell surface ligands, this study was undertaken to investigate the effect of PEGylating the surface of the encapsulated microparticles containing the derivatized SHA-PEI/pDNA on alleviating non-specific phagocytosis by DCs. We also performed a comparative evaluation of the *in vitro* uptake, toxicity profile and functional characteristics of immature DCs transfected with a control Pluronic F68-encapsulated and PLGA-PEG-PLGA-encapsulated NGR/PEG/PDBA-SHA-PEI/pDNA targeting polyplex. Even more importantly, we examined the influence of the encapsulated targeting polyplex on the phenotypes of immature DCs and compared it to the matured stage upon LPS challenge. Our results reveal this coupling strategy and delivery vehicle as a potent and efficient platform for targeting particulate vaccine delivery systems to professional antigen presenting cells like immature DCs, without any significant adverse effects on the phenotypes and functionality of the cells.

2. Materials and methods

2.1. Materials

PLGA-PEG-PLGA tri-block copolymer (MW = 4200; PEG MW 1450) was obtained from MacroMed Inc. (Sandy, UT, USA); PEG (MW 3000) was from Sigma and PDBA-x-NHS was obtained from Prolinx Inc. (Seattle, WA, USA). The cell-specific CNGRC and the control CARAC peptides were gifts from Drs. Arap and Pasqualini (MD Anderson Cancer Center, Department of Genitourinary Medical Oncology). Branched polyethylenimine (MW 25000) was purchased from Aldrich (Milwaukee, WI, USA). Pluronic F68 (MW 8400) was obtained from BASF (Parsippany, NJ, USA). RPMI medium and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Petri dishes (Costar, Cambridge, MA); Glutamine, 1000 U/ml penicillin–streptomycin (Life Technologies, Gaithersburg, MD); GM-CSF (1000 U/ml) and IL-4 (200 U/ml) were obtained from Schering Plough Research Institute (Kenilworth, NJ, USA); 2 units/μl TURBO DNase^{TM} (Ambion, Inc., Austin TX); Alexa Fluor 488, 4′-6-diamidino-2-phenylindole (DAPI) and PicoGreen quantification assay kit (Molecular Probes, Eugene, OR). Propidium iodide (PI) and FITC-annexin V were from Becton Dickinson (Mountain View, CA); BCA protein assay kit (Pierce, Rockford, IL, USA) and the [α-^{32}P]dATP radioactive label kit were from Amersham (Piscataway, NJ, USA). Lipopolysacharide (LPS) (1 μg/ml, *Escherichia coli* 0.55:B5) was purchased from Sigma.

2.2. Methods

2.2.1. Establishment of DCs from human peripheral blood mononuclear cells (PBMCs)

After mixing whole blood from healthy donors (>10 participants) with Ficoll-Hypaque, the mononuclear cells was collected after centrifugation and plated in petri dishes for 1 h at 37 °C to remove non-adherent cells. After washing with PBS, adherent cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM glutamine, 1000 U/ml penicillin–streptomycin, 800 U/ml GM-CSF and 500 U/ml IL-4. The culture medium was changed every other day with 300 μl of fresh medium containing 2400 U of GM-CSF and 1500 U of IL-4. The detached cells, the main population of CD1a^{+} cells, were used for experiments after culture for 7 days. During this period of time, the exam-

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