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Cationic microparticles consisting of poly(lactide-co-glycolide) and polyethylenimine as carriers systems for parental DNA vaccination

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Abstract

Cationic microparticles for DNA adsorption were formulated by blending poly(lactide-co-glycolide) (PLGA) (50:50), with different cationic agents, either PEI 25 kDa (polyethylenimine) or CTAB (cetyl-trimethyl-ammonium-bromide). The aim was to create adjuvant delivery systems increasing the efficiency of DNA vaccines. Microparticles formulated with 10% PEI exhibited a highly positive ζ-potential, small particle sizes, in contrast to particles prepared with CTAB, which revealed highly aggregated structures in scanning electron micrographs. PEI 10% microparticles efficiently adsorbed DNA and protected DNA from enzymatic degradation.

Microparticles with up to 10% PEI did not affect membrane integrity whereas CTAB particles showed higher LDH release. Transfection efficiencies were assessed using a luciferase reporter gene assay compared to naked DNA and PEI/DNA polyplexes. DNA adsorbed onto microspheres with 10% or 50% PEI generally had higher transfection efficiencies than CTAB but reached lower expression levels than PEI/DNA polyplexes alone. This documented the intact release of DNA. The mechanism of gene delivery to non-phagocytic cells was studied via covalent fluorescence labeling of both the DNA and PEI by confocal microscopy and suggested uptake of DNA.

Immunization of mice was performed using plasmids encoding immunodominant antigens of *Listeria monocytogenes* adsorbed onto RG 502 H+PEI 10% microparticles. The efficiency was tested by intravenous challenge with an otherwise lethal dose of *L. monocytogenes*. PLGA+PEI microspheres can be used as adjuvant delivery systems for DNA but further optimization is necessary to exploit their full potential.

Keywords: Microparticles; Gene delivery; DNA vaccines; Polyethylenimine; Poly(lactide-co-glycolide); Immunization

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1. Introduction

Vaccines are amongst the most effective achievements in medicine [1]. During the past decade, DNA vaccination has been increasingly exploited in an attempt to achieve simpler, safer, and more effective vaccination regimens. DNA vaccination involves inoculation of expression vectors that encode immunogenic proteins. The encoded antigens are then produced in situ and elicit immune responses. Since the introduction of DNA vaccines, studies have shown immunogenicity or protective efficacy of DNA vaccines for a variety of disease targets, including cancer, allergy, autoimmune, bacterial, and viral diseases [2–5]. DNA vaccines effective against intracellular organisms, which are controlled by cell-mediated immunity, such as the agents of tuberculosis, malaria, leishmaniasis, hepatitis, and HIV infection, would be highly desirable. Exogenous protein antigens are taken up by professional antigen presenting cells via endocytosis or phagocytosis and presented by MHC class II molecules to stimulate CD4+T cells, which promote the generation of effective antibody responses [6]. In contrast, MHC class I molecules are associate with antigens synthesized within the cytoplasm of host cells and are generally elicited by live or DNA vaccines [7]. DNA vaccines have the big advantage to be stable at room temperature, easy to handle, economical and relatively safe. However, many DNA vaccines have shown low vaccine efficacy. Therefore, the use of an effective DNA delivery system can enhance cellular uptake of DNA vaccines and/or facilitate intracellular targeting of DNA to the cytoplasm and nucleus, and also reduce the amount of DNA required for injection. Several studies have illustrated that the induction of more efficient immune responses by DNA vaccination could be improved using adjuvant delivery systems [8]. More specifically, the adsorption of DNA onto the surface of preformed cationic microparticles resulted in profound immune responses [9]. The cationic surface charge of these microparticles was obtained by incorporating a cationic detergent, CTAB, into the surface of the microparticles during their preparation. CTAB has been used primarily for DNA isolation from bacteria and plants by precipitation [10].

A microparticulate DNA delivery system based on surface adsorbed DNA is thought to offer the distinct advantage of i) circumventing the degrading effects on DNA during particle preparation; ii) facilitating a rapid delivery of DNA to targeted antigen presenting cells; iii) providing an additional adjuvant effect by the presence of bacterial CpG units of the plasmid on the surface of the delivery system.

In the present study, the potential of cationic microparticles using a blend of PEI or CTAB within a PLGA polymer matrix was investigated. PEI 25 kDa is one of the most powerful non-viral transfection agents used in vitro and in vivo [11]. Thus, it was hypothesized that the adsorption efficiency for DNA, as well as the gene delivery would be increased. The microparticles were characterized with regard to their physicochemical properties, stabilizing effects on DNA integrity, membrane toxicity and gene delivery. Finally, the most promising delivery system was used for immunization against the intracellular bacterial pathogen, *Listeria monocytogenes*, to assess the induction of a protective immunity.

2. Materials and methods

2.1. Materials and DNA

Poly(lactide-co-glycolide) (PLGA) (50:50), Resomer® 502 H, (M_w 15,200, uncapped end-groups) and PLGA (50:50), Resomer® 505 (M_w 80,000) were purchased from Boehringer Ingelheim (Ingelheim, Germany). Partially hydrolyzed poly(vinyl alcohol) (PVA) (Mowiol® 3-83, M_w 14,000) was purchased from Clariant (Frankfurt, Germany). Polyethylenimine (PEI) 25 kDa was purchased from BASF (Ludwigshafen, Germany). Hexadecyltrimethylammonium-bromide (CTAB) was purchased from Fluka (Buchs, Germany). Plasmid DNA, pLuc-CMV, a luciferase encoding plasmid, preceded by a nuclear location signal under the control of a CMV promoter, was kindly provided by Chiron (Emeryville, Ca) and amplified by PlasmidFactory (Bielefeld, Germany). All pLuc-CMV probes used were from one endotoxin free batch in TE-buffer pH 8 (1 mM Na_2EDTA; 10 mM Tris; 143 mM NaCl) and stored at −80 °C until use. All other chemicals were of analytical grade.

Plasmid DNA encoding wild-type antigens of *L. monocytogenes* and mutants thereof were used for in