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Nanoparticles built by self-assembly of amphiphilic γ-PGA can deliver antigens to antigen-presenting cells with high efficiency: A new tumor-vaccine carrier for eliciting effector T cells

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Summary Nanotechnology is a fundamental technology for designing and generating innovative carriers for biomacromolecular drugs. Biodegradable poly(γ-glutamic acid)-based nanoparticles (γ-PGA NPs) are excellent vaccine carriers capable of delivering antigenic proteins to antigen-presenting cells (APCs) and eliciting potent immune responses based on antigen-specific cytotoxic T lymphocytes. In mice, subcutaneous immunization with γ-PGA NPs entrapping ovalbumin (OVA) more effectively inhibited the growth of OVA-transfected tumors than immunization with OVA emulsified using Freund's complete adjuvant. In addition, γ-PGA NPs did not induce histopathologic changes after subcutaneous injection or acute toxicity through intravenous injection. Importantly, γ-PGA NPs efficiently delivered entrapped antigenic

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proteins into APCs, and these antigen-capturing APCs migrated to regional lymph nodes. Our results demonstrate that a γ-PGA NP system for antigen delivery will advance the clinical utility of vaccines against cancer.

Introduction

Cancer vaccines are a promising approach for anticancer therapy as fewer side effects are induced than with other therapies and, more importantly, there is an opportunity for developing long-term immunity [1,2]. In contrast to general medicines, which are directly targeted to specific molecules, cancer vaccines initiate a cascade of antigen-specific immune responses against tumor-associated antigen (TAA)-expressing tumor cells. Activating the immune system to trigger a specific response sufficient for the eradication of tumor cells, however, is a major challenge in the development of cancer immunotherapy.

Nanotechnology is a new multidisciplinary field that is expected to lead to breakthroughs in bio-engineering, molecular biology, diagnostics, and therapeutics. The recent developments in nanotechnology have considerably increased the interest in particulates as a platform for the delivery of antigens [3]. An attractive motif for this approach is the postulate that particulate delivery systems mimic pathogens that are commonly recognized, phagocytosed, and processed by professional antigen-presenting cells (APCs). APCs, such as dendritic cells and macrophages, represent the foremost sentinels of the immune system and orchestrate antigen-specific T cell-mediated immune responses [4,5]. When APCs encounter particulates, they are activated to migrate to regional lymph nodes (rLN) where they present the antigen to T cells, thereby triggering cellular and humoral immunity. The development of particulate vaccines is also motivated by safety concerns, e.g., to avoid the risk of infection induced by live attenuated vaccines and to suppress the excessive inflammation that is frequently caused by the use of Freund's adjuvant or aluminum salts adjuvant [3]. Thus, there is an urgent need for the development of potent and safe antigen-delivery systems.

Our studies are aimed at promoting the clinical application of nanoparticles (NP) as antigen delivery carriers for cancer immunotherapy through the development and study of self-assembled NPs using a biodegradable polymer derived from a natto mucilage, poly(γ-glutamic acid) (γ-PGA), as ''vaccine carriers'' [6,7]. We recently developed a technique to prepare uniform nanoparticles (γ-PGA NPs) using amphiphilic γ-PGA (γ-PGA-L-PAE), in which L-phenylalanine ethyl ester (L-PAE) is introduced as a hydrophobic residue into the α-position group carboxyl of γ-PGA [8—10]. γ-PGA NPs entrapping antigenic proteins enhance antigen-specific cellular immunity. Basic information about the effectiveness and safety of this nanovaccine, however, is still insufficient for its clinical application.

In the present study, we assessed the usefulness and versatility of γ-PGA NPs as antigen delivery carriers for cancer immunotherapy by verifying the validity, safety, and mechanism of action of this approach. The effectiveness of γ-PGA NPs entrapping antigens as vaccine and the scientific basis of γ-PGA NPs as antigen delivery carriers were analyzed to assess the feasibility of using this approach for cancer immunotherapy.

Materials and methods

Cell lines and mice

EL4 (murine thymoma cells; H-2^b), E.G7-OVA cells (ovalbumin [OVA] cDNA-transfectant of EL4 cells), and YAC-1 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA). EL4 cells and YAC-1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 50 μM 2-mercaptoethanol (2-ME), and antibiotics. E.G7-OVA cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 50 μM 2-ME, and 400 μg/ml G418. Female C57BL/6 mice and BALB/c-nude mice were all purchased from Japan SLC Inc. (Hamamatsu, Japan), and used at 6—8 weeks of age. Animal experimental procedures were performed in accordance with the Osaka University guidelines for the welfare of animals in experimental neoplasia.

Synthesis of γ-PGA-L-PAE

γ-PGA (MW 300 000) was kindly provided by Meiji Seika, Co. Ltd. (Tokyo, Japan). L-Phenylalanine ethyl ester, OVA, and fluorescein-isothiocyanate-conjugated OVA (FITC-OVA) were purchased from Sigma Chemical Co. (St. Louis, MO). The γ-PGA-L-PAE co-polymers were synthesized as described previously [8—10]. Briefly, 4.7 mmol of γ-PGA was incorporated with 4.7 mmol of L-PAE in 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

Preparation of protein-entrapping γ-PGA NPs

The protein-entrapping γ-PGA NPs were prepared as described previously [8—10]. Briefly, the γ-PGA-L-PAE (10 mg/ml in dimethyl sulfoxide) was added to an equal volume of protein solution (2 mg/ml in saline), and then mixed gently. The mixture was centrifuged at 18 000 × *g* for 20 min, resuspended in distilled water, followed by centrifugation at 18 000 × *g* for 20 min. This washing step was repeated twice. The resulting pellet was resuspended in phosphate-buffered saline (PBS). The amount of protein entrapped in the γ-PGA NPs was estimated using a Bio-Rad DC Protein Assay (Bio-Rad Laboratories Inc., Hercules, CA) after dissociation of the γ-PGA NPs using 2% sodium dodecyl sulfate solution. The size and ζ-potential of the γ-PGA NPs were determined using photon correlation spectroscopy (Zetasizer 3000; Malvern Instruments Ltd., Malvern, UK).