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Antigen-coated poly α-hydroxy acid based microparticles for heterologous prime-boost adenovirus based vaccinations

Caitlin D. Lemke, Sean M. Geary, Vijaya B. Joshi, Aliasger K. Salem*

College of Pharmacy, University of Iowa S228 PHAR, 115 S. Grand Avenue, Iowa City, IA 52242, USA

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ABSTRACT

Adenoviruses show promising potential as vectors for cancer vaccines, however, their high immunogenicity can be problematic when it comes to homologous prime-boost strategies. In the studies presented here we show that heterologous prime-boost vaccinations involving ovalbumin (OVA)-antigen-coated microparticles as a prime, and adenovirus encoding OVA (AdOVA) as a boost, were equally as effective as homologous AdOVA prime-boosts at generating OVA-specific $CD8^+$ T-cell responses, which translated into effective tumor protection. OVA-coated biodegradable poly α-hydroxy acid-based microparticles of varying chemistries, when used as primes in heterologous prime-boost vaccinations, were comparable in terms of promoting OVA-specific $CD8^+$ T cells as well as providing protection against subsequent tumor challenge. These findings auger well for using poly α-hydroxy acid-based microparticles in prime-boost viral vaccination strategies geared toward the safer, and potentially more efficient, generation of anti-tumor immunity.

1. Introduction

Activation of tumor-targeting cytotoxic $CD8^+$ T cells is a primary goal of cancer vaccines given the long-recognized key role these cells play in protective anti-tumor responses [1–3]. Both timely initial expansion and generation of memory populations are important outcomes of cancer vaccines since primary malignancy as well as subsequent recurrences would need to be targeted for sustained protection. Many vaccination strategies have been tested for their ability to give rise to T cell activation and memory, but the prime-boost approach is most consistently effective [4–7]. A novel short-interval prime-boost can generate significant $CD8^+$ T-cell expansion and rapid memory, demonstrating that the dictates of conventional prime-boost vaccination timing can be manipulated [8]. We have previously focused our attention on tumor antigen (Ag)-encoding recombinant, replication-deficient adenoviruses because they have been successfully used in our mouse tumor models [9–15] and in clinical trials for prostate cancer therapy [16]. However, using viral vectors as cancer vaccines is limited by the pre-existence/generation of anti-vector immunity in patients which reduces their effectiveness when applying them in homologous prime-boost vaccination regimens [10]. Adapting our adenovirus cancer vaccine into a heterologous prime-boost system could be advantageous for both the magnitude and kinetics of generating anti-tumor responses.

Heterologous (or diversified) prime-boost vaccinations, wherein the prime consists of a different agent/formulation from the boost, have shown promise in various pre-clinical cancer models [17–23]. While the majority of pre-clinical heterologous prime-boost cancer vaccines rely on antigen (Ag) delivery directly via plasmid DNA or viral vectors, a strong case is being made for employing biodegradable microparticles as vehicles for protein or peptide Ag-based vaccinations [24–28].

2. Materials and methods

2.1. Mice and tumor cell lines

Studies involving mice were approved by and performed according to guidelines established by the University of Iowa Institutional Animal Care and Use Committee. Inbred 6- to 8-week-old C57BL/6 and male Balb/c were obtained from Jackson Laboratories and maintained in filtered cages. E.G7-OVA tumor cell lines were obtained from American Type Culture Collection (Manassas, VA) and grown in RPMI-1640 (GIBCO, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 1 mM sodium pyruvate (GIBCO), 10 mM HEPES (GIBCO), 0.05 mM 2-mercaptoethanol, and 50 μg/mL gentamicin sulfate (Mediatech, Inc., Manassas, VA), with 0.4 mg/mL G418 (GIBCO) for selection maintenance.

2.2. Biodegradable particle fabrication

Three polymers were used to prepare particles: polylactic acid (PLA), 50:50 end-capped poly(lactide-co-glycolide) (PLGA) (Birmingham Polymers, Birmingham, AL) and 65:35 PLGA with a free carboxylic acid terminus (PLGA-COOH) (Sigma–

* Corresponding author. Tel.: +1 319 335 8810; fax: +1 319 335 9349.
E-mail address: aliasger-salem@uiowa.edu (A.K. Salem).

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Aldrich®, St. Louis, MO). Particles were fabricated using a standard oil-in-water (o/w) single emulsion technique. Briefly, polymer was dissolved in dichloromethane (DCM), followed by sonication into 1% polyvinyl alcohol. This solution was then stirred for 2 h to allow for evaporation of DCM; then, particles with an average size of 1–3 μm were collected by differential centrifugation and lyophilized.

2.3. Particle size and zeta potential analysis

Comparative particle size and surface charge (zeta potential) measurements were conducted using the Zetasizer Nano ZS (Malvern, Southborough, MA), as previously described [29]. Briefly, lyophilized particles were suspended in deionized water at a concentration of 1 mg/mL. The size measurements were performed at 25 °C with a 173° scattering angle. The mean hydrodynamic diameter was determined by cumulative analysis. Average diameter measurements were also confirmed by scanning electron microscopic (SEM) examination of particle preparations. Zeta potential determinations were based on electrophoretic mobility of the particles in the aqueous medium, which were performed using folded capillary cells in automatic mode.

2.4. Ag coating of particles

PLA, PLGA or PLGA-COOH particles were coated with full-length ovalbumin (OVA) or MHC Class I PSA peptide ($HPQKVTKFML_{188-197}$) by passive adsorption. Briefly, lyophilized particles were resuspended in a 1 mg/mL solution of either OVA or PSA peptide dissolved in sterile 1× PBS. This mixture was then rotated continuously overnight at room temperature. After allowing for adsorption, the particles were centrifuged and resuspended in 1× PBS immediately prior to use. The extent of OVA protein and PSA peptide coating was analyzed by comparative zeta potential measurement (see above), and furthermore was confirmed by detecting fluorescence after absorption of OVA-FITC onto particles by flow cytometry.

2.5. In vitro stimulation of BMDCs with biodegradable microparticles

Primary murine bone marrow-derived dendritic cells (BMDC) were generated as previously described [30]. Briefly, bone marrow cells were harvested from C57BL/6 or Balb/c mouse femurs by flushing with complete culture medium. To enrich for BMDCs, isolated cells were grown in complete DMEM culture medium supplemented with 20 ng/mL of recombinant murine GM-CSF (PeproTech, Rocky Hill, NJ) for 9–13 days. Resulting cells (~90% DCs as determined by CD11c staining) were then seeded at 10^6/well in complete DMEM culture medium and particle preparations added in varying concentrations for 24 h. BMDC activation after culturing with microparticles was measured by mAb staining for surface CD86 (eBioscience).

2.6. Short-interval prime-boost vaccination

Mice received subcutaneous prime or boost immunizations spaced 7 days apart. Prime immunizations consisted of either PBS (2.5 mg of biodegradable particles with surface adsorbed OVA) or 10^8 pfu of adenovirus encoding OVA. Boost immunizations consisted of either PBS or 10^8 pfu of adenovirus encoding OVA. OVA-encoding, replication-deficient adenovirus serotype 5 (AdOVA) was obtained from the University of Iowa Gene Transfer Vector Core, as previously described [14,15].

2.7. Analysis of Ag-specific $CD8^+$ T-cell frequency

For analysis of Ag-specific circulating T cells, peripheral blood leukocytes (PBL) were isolated from a small volume of blood obtained by submandibular bleeding at the indicated time points. The H-$2K^b$ SIINFEKL Class I iTAg™ MHC Tetramer (K^b-OVA_{257}) (Beckman Coulter, Fullerton, CA) was used as previously described [14]. For general staining, PBLs were incubated with Fc block (2.4G2; BD Biosciences, San Diego, CA), followed by the appropriate peptide:MHC class I tetramer, anti-CD8a (53-6.7; eBioscience, San Diego, CA) and anti-CD3 (145-2C11; eBioscience). Samples were acquired on a FACScan™ flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and data analyzed with FlowJo software (TreeStar, Ashland, OR).

2.8. In vivo tumor challenge

For tumor challenge, C57BL/6 mice were anesthetized by intraperitoneal injection of a ketamine/xylazine mix, at a final concentration of 87.5 mg/kg ketamine and 2.5 mg/kg xylazine (provided by the Office of Animal Resources, University of Iowa). Each mouse was challenged subcutaneously in the right flank with 10^6 (E.G7-OVA) tumor cells. Tumor outgrowth, determined by size as a function of time, was measured three times a week. Tumor volume was calculated as previously described [12] and mice were sacrificed when tumor diameter became greater than 20 mm.

2.9. Statistical analysis

Statistical analysis of CD86 expression levels was performed using two-way ANOVA with a Bonferroni post-test. Comparative analysis of Ag-specific $CD8^+$ T-cell levels was performed using one-way ANOVA with a Tukey post-test. Comparison of survival curves was performed using the Log-rank (Mantel–Cox) test. The confidence interval for all tests was set at 95% and a P value of <0.05 was accepted as significant (*); $P < 0.01$ (**); $P < 0.001$ (***). All analyses were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA (www.graphpad.com).

3. Results

3.1. Effect of microparticle chemistry on DC activation

Preparations of particles from either PLA, PLGA 50:50 or PLGA-COOH 65:35 yielded microparticles that were an average of 2–3 μm in diameter and spherical in nature (Fig. 1A, B). All three types of particles were highly negatively charged prior to full-length OVA adsorption (−34, −34.5, −34.3 mV, respectively) and became almost neutral after adsorption (−2.9, −3.5, −3.4 mV, respectively) (Fig. 1C). To confirm that the loss of surface negativity was directly related to protein coating we adsorbed PLA, PLGA and PLGA-COOH microparticles with OVA conjugated to FITC (OVA-FITC) and detected fluorescent particles by flow cytometry (Fig. 1D). We consistently observed that PLA particles had the highest proportion that was fluorescent and the greatest reduction in surface negativity after adsorption. Furthermore, net loss of surface negativity was seen after adsorption of PSA peptide onto all three particle preparations (data not shown), which demonstrated that surface coating of microparticles could be achieved regardless of protein length or source.

Biodegradable microparticles are readily phagocytosed by APCs, particularly DCs, which have been shown to induce their activation [31–35]. Because the hydrophobic nature of microparticles can influence their immunostimulatory capacity and uptake by phagocytic cells [36,37], we wanted to comparatively examine the impact of PLA, PLGA and PLGA-COOH microparticles on murine BMDCs (mBMDC). Incubation of C57BL/6 mBMDCs with PLGA-COOH microparticles resulted in a slightly higher, but not significant, upregulation of CD86 as compared to PLA or PLGA microparticles (Fig. 2A). The effect on Balb/c mBMDCs, however, was not altered by polymer chemistry (Fig. 2B). Overall, we observed a similarly dose-dependent immunostimulatory effect on BMDCs, regardless of polymer.

3.2. $CD8^+$ T-cell responses to microparticle prime-adenovirus boosts

To determine if particle polymer chemistry impacts $CD8^+$ T-cell priming in a heterologous prime-boost system, mice were primed with either OVA adsorbed onto PLA, PLGA or PLGA-COOH microparticles followed by boosting with AdOVA. OVA-specific $CD8^+$ T-cell responses detected in PBL were not significantly different from naïve mice in any of the groups 7 days after priming. One week after the AdOVA boost (d14) PLA-OVA (Fig. 3A) and PLGA-OVA (Fig. 3B) primed mice had significantly higher responses than naïve mice, whereas AdOVA-primed mice did not develop a significantly higher response as compared to naïve until two weeks after the boost (d21) (Fig. 3D). Interestingly, PLGA-COOH-OVA-primed mice also developed higher responses compared to naïve mice, but the difference was not significant at any of the time points measured (Fig. 3B). The peak response for all of the heterologous particle-primed groups was on day 14 (7 days after boost), which was followed by a gradual contraction phase over the next 21 days. In contrast, the homologous AdOVA-primed group's response did not peak until day 28 (21 days after boost) and was then followed by a rapid contraction over the next 7 days. Despite the slightly varied kinetics and magnitudes of the early effector phase responses, all of the prime-boost groups had comparable levels of OVA-specific $CD8^+$ T cells during the late memory phase on day 100 (93 days after boost).

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