Vaccine 27 (2009) 4475–4482

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Harnessing the unique local immunostimulatory properties of modified vaccinia Ankara (MVA) virus to generate superior tumor-specific immune responses and antitumor activity in a diversified prime and boost vaccine regimen

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ARTICLE INFO

Article history:
Received 6 March 2009
Received in revised form 28 April 2009
Accepted 10 May 2009
Available online 29 May 2009

Keywords:
Modified vaccinia Ankara (MVA)
Carcinoembryonic antigen (CEA)
Vaccinia
Costimulation

ABSTRACT

Recombinant poxviruses expressing tumor-associated antigens (TAAs) are currently being evaluated in clinical trials as an approach to treat various cancers. We have previously generated poxviral vectors expressing a TAA and a TRIad of COstimulatory Molecules (B7-1, ICAM-1, and LFA-3; TRICOM) as transgenes, including replication competent recombinant vaccinia (rV) or replication-defective modified vaccinia Ankara (MVA), to prime tumor-specific immune responses, and a replication-defective recombinant fowlpox (rF) to boost these responses. MVA is a potentially safer, replication-defective form of vaccinia virus with unique immunostimulatory properties that could make it a superior priming vaccine. Here, an MVA vector encoding a tumor antigen (CEA) and TRICOM was utilized (rMVA). A single rMVA-CEA/TRICOM vaccination induced greater expression of several serum cytokines associated with enhanced T-cell immunity than that seen with vaccinia. We hypothesized that this effect might "precondition" the vaccination site for a more effective boost. An rMVA-CEA/TRICOM prime followed 7 days later (but not 30 days later) by an rF-CEA/TRICOM boost at the same injection site (but not at a distal site) induced more potent CEA-specific T-cell responses, and superior CEA-specific immunity and antitumor activity, than rV-CEA/TRICOM followed by rF-CEA/TRICOM. This preconditioning effect was also observed using a heterologous antigen model, where priming with rMVA-CEA/TRICOM followed 7 days later by rF-LacZ/TRICOM enhanced β-gal-specific immunity compared to rF-LacZ/TRICOM only. The studies reported here show for the first time that priming with rMVA followed 7 days later by an rF boost at the same injection site, versus a distal site, generates superior tumor-specific immunity and antitumor activity.

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1. Introduction

One current approach to vaccine design includes the insertion of antigens of interest into a wide array of vectors to generate immunity; this is being evaluated in the treatment or prevention of diseases such as malaria, HIV, tuberculosis, influenza, and cancer [1–5]. Vectors used in recombinant cancer vaccines are generally based on DNA, bacteria, viruses, or yeast [6–9]. One approach to cancer vaccine design is to identify potentially immunogenic tumor-associated antigens (TAAs) and optimize epitopes of these antigens to elicit a high-avidity $CD8^+$ T-cell based antitumor response [10]. However, of equal importance is the selection of a vaccine vector that can also induce an innate immune response, and thus potentiate a Th1 immune response. This preclinical study focuses on the use of poxviruses to induce tumor-specific T-cell immunity and antitumor responses, while capitalizing on both the vaccine vectors' innate and adaptive immunostimulatory characteristics.

We and other groups have previously demonstrated (a) that replication-defective attenuated modified vaccinia Ankara (MVA) can be used to deliver various TAAs expressing costimulatory molecules or cytokines to generate tumor-specific immunity [11–15]; (b) generation of host immune responses against MVA or vaccinia virus vectors limits the efficacy of multiple vaccinations [11,16,17]; and (c) the advantages of a diversified strategy employing recombinant MVA (rMVA) or recombinant vaccinia (rV) as a prime, followed by replication-deficient recombinant fowlpox (rF) as a boost [18–21]. Previous studies have also demonstrated that priming with an rMVA vector expressing carcinoembryonic antigen (CEA) and a TRIad of COstimulatory Molecules (B7-1, ICAM-1, and LFA-3, designated TRICOM) as transgenes, then boosting with an rF-CEA/TRICOM vector, more effectively induced CEA-specific

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0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2009.05.017

immunity in human CEA-transgenic (CEA-Tg) mice, compared to a similar rV prime and rF boost [11]. The reasons for this however were unclear.

Previous studies have demonstrated that infecting dendritic cells (DCs) with MVA, but not with vaccinia, induces production of cytokines such as IFN-α by DCs, leading to DC maturation and enhanced antigen presentation, and thereby initiating potent antigen-specific $CD8^+$ T-cell responses [22–25]. We hypothesized that using rMVA to initiate a local innate immune response leading to production of Th1 supportive cytokines and DC maturation at the draining lymph node might "precondition" the vaccination site for a more effective boost. By examining the temporal and spatial relationship of the prime and boost vaccinations, we found that a vaccination strategy of an rMVA prime, followed 7 days later by a fowlpox boost at the same local injection site, harnessed unique immunostimulatory properties of rMVA, prompting the generation of superior tumor-specific immunity and reduced tumor burden.

2. Materials and methods

2.1. Recombinant poxviruses

MVA was generated from a stock (graciously provided by Dr. A. Mayr, Ludwig-Maximilians University, Munich, Germany) through three successive rounds of plaque purification in chicken embryo dermal primary cultures, and amplified further in these cells to produce research stocks. rMVA-CEA/B7-1/ICAM-1/LFA-3 (designated rMVA-CEA/TRICOM) is the recombinant rMVA-TRICOM construct containing the human CEA gene under control of the 40k promoter [11]. The rV and rF viruses containing the human CEA gene and the murine B7-1, ICAM-1, and LFA-3 genes (designated rV-CEA/TRICOM and rF-CEA/TRICOM, respectively) have been previously described [26]. The rF virus containing the gene for murine granulocyte–macrophage colony-stimulating factor (GM-CSF) under control of the 40k promoter has also been described [27]. rF-LacZ-B7-1/ICAM-1/LFA-3 (designated rF-LacZ/TRICOM) has been previously described [28].

2.2. Animals and cells

Female C57BL/6 mice were obtained from the National Cancer Institute, Frederick Cancer Research Facility (Frederick, MD). Mice were housed and maintained in pathogen-free conditions in microisolator cages until used for experiments at 6–8 weeks of age. CEA-Tg C57BL/6 mice were obtained from a breeding pair provided by Dr. John Thompson (Institute of Immunobiology, University of Freiburg, Freiburg, Germany). The generation and characterization of CEA-Tg mice have been previously described [29]. These studies used murine colon adenocarcinoma cells expressing human CEA (MC-38-CEA) [30]. Before transplantation to mice, the cells were trypsinized, dispersed through a 70-μm cell strainer (Falcon; Becton Dickinson, Franklin Lakes, NJ), and washed twice in HBSS before final suspension in HBSS.

2.3. Efficacy of MVA or vaccinia to prime CEA-specific immune responses

CEA-Tg mice (three per group) were primed s.c. with 1×10^8 pfu rMVA-CEA/TRICOM or rV-CEA/TRICOM, then boosted s.c. 1, 2, or 3 times at 7- or 30-day intervals with rF-CEA/TRICOM. Control mice received HBSS buffer. Three to four weeks after the last vaccination, spleens were removed, dispersed into single-cell suspensions, pooled, and immune responses were analyzed. Purified splenic $CD4^+$ T cells were isolated and tested for reactivity to CEA or β-galactosidase (β-gal) protein in an in vitro lymphoproliferation assay, as previously described [9]. Briefly, purified $CD4^+$ T cells were cultured with irradiated antigen-presenting cells (APCs) and CEA or β-gal protein for 5 days. [^{3}H] thymidine was added to the wells (1 μCi/well) for the last 24 h; proliferation was assayed by measuring incorporated radioactivity. Results from triplicate wells were averaged and reported as mean CPM ± one standard error (SE). To evaluate $CD8^+$ T-cell responses, pooled splenocytes were coincubated with 10 μg/ml of the H-$2D^b$-restricted 8-mer peptide $CEA_{526–533}$ (EAQNTTYL) [31] for 6 days. Bulk lymphocytes were recovered by centrifugation through a Ficoll-Hypaque gradient. T cells were restimulated with fresh, irradiated, naive splenocytes and 10 μg/ml of either CEA peptide or VSV-$N_{52–59}$ peptide (vesicular stomatitis virus, RGYVYQGL) [32] for 24 h. Supernatant was collected and analyzed for murine IFN-γ by cytometric bead array (BD PharMingen, San Jose, CA) according to the manufacturer's instructions.

2.4. Serum cytokine studies

C57BL/6 mice were vaccinated s.c. once with 1×10^8 pfu of either rMVA-CEA/TRICOM or rV-CEA/TRICOM; serum was collected at 0, 2, 3, 4, or 7 days following vaccination. Murine IFN-γ was measured using ELISA (Biosource; Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. Serum CXCL-1, G-CSF, GM-CSF, IL-6, IL-12p70, and MCP-1 levels were determined by LICNOplex analysis (Millipore, Billerica, MA).

2.5. Ability of MVA priming to enhance LacZ-specific immune responses from a rF-LacZ boost

CEA-Tg mice (three per group) were vaccinated s.c. with 1×10^8 pfu of either rMVA-CEA/TRICOM or HBSS, and vaccinated s.c. 7 days later with 1×10^8 pfu rF-LacZ/TRICOM. Four weeks after the last vaccination, immune responses were analyzed. Purified splenic T cells were tested for reactivity to β-gal protein in an in vitro lymphoproliferation assay, as described above. For tetramer analysis, 1×10^6 cells were incubated on ice with FITC-labeled anti-CD8 (BD Biosciences, San Jose, CA) and PE-labeled H-$2K^b$ β-gal tetramer (Tetramer Core Facility, National Institutes of Health, Bethesda, MD) for 30–45 min, washed twice, then analyzed on a FACSCalibur (BD Biosciences). Background staining was assessed by isotype control antibodies (BD Biosciences).

2.6. Tumor therapy studies

Before transplantation to CEA-Tg mice, MC38-CEA^+ cells were trypsinized, dispersed through a 70-μm pore size cell strainer (Falcon; Becton Dickinson), and washed twice in HBSS before final suspension in HBSS. Mice were injected s.c. in the right flank with 3×10^5 MC38-CEA^+ tumor cells. Four days following tumor transplant, mice were primed s.c. with 1×10^8 pfu of either rMVA-CEA/TRICOM or rV-CEA/TRICOM admixed with 1×10^7 pfu rF-GM-CSF, then boosted 7 and 14 days later with 1×10^8 pfu rF-CEA/TRICOM admixed with 1×10^7 pfu rF-GM-CSF. Additional groups of mice were injected with 1×10^8 pfu of either control vector rMVA-TRICOM or rV-TRICOM admixed with 1×10^7 pfu rF-GM-CSF, then boosted 7 and 14 days later with 1×10^8 pfu rF-TRICOM admixed with 1×10^7 pfu rF-GM-CSF.

2.7. Statistical analysis

Results of tests of significance are reported as *P*-values, derived from Student's *t*-test using a 2-tailed distribution. *P*-values were calculated at 95%. For graphical representation of data, *Y*-axis error bars representing ±1SE are shown. In some cases, the variation is such that error bars are obscured by the plot symbol.