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Vaccine 18 (2000) 2015–2022

Vaccine

www.elsevier.com/locate/vaccine

Boosting with recombinant vaccinia increases HPV-16 E7-specific T cell precursor frequencies of HPV-16 E7-expressing DNA vaccines

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Received 20 July 1999; accepted 25 October 1999

Abstract

We have previously linked the sorting signals of the lysosome-associated membrane protein-1 (LAMP-1) to HPV-16 E7 antigen, creating a chimera, Sig/E7/LAMP-1. We found that both Sig/E7/LAMP-1-containing recombinant vaccinia virus (Vac–Sig/E7/LAMP-1) and Sig/E7/LAMP-1 DNA can generate strong antitumor immunity. To determine whether combination of Sig/E7/LAMP-1 DNA and Vac–Sig/E7/LAMP-1 can further enhance immune responses, sequential vaccination with Sig/E7/LAMP-1 DNA and Vac–Sig/E7/LAMP-1 was given. We found that priming with Sig/E7/LAMP-1 DNA and boosting with Vac–Sig/E7/LAMP-1 generated the strongest E7-specific $CD8^+$ T cell responses. Our results encourage the use of the DNA prime/vaccinia booster regimen in future clinical trials.

Keywords: HPV-16; DNA vaccine; Vaccinia

1. Introduction

Cervical cancer is the second leading cause of cancer mortality in the world [1]. Human papillomavirus (HPV), particularly HPV-16, are associated with most cervical cancers [2]. The HPV oncogenic proteins, E6 and E7, are important in the induction and maintenance of cellular transformation and are co-expressed in most HPV-containing cervical cancers. Several lines of evidence suggest cell-mediated immunity are important in controlling both HPV infection and HPV-associated neoplasms [3]. Therefore, vaccines or immunotherapies targeting E7 and/or E6 proteins may provide an opportunity to prevent and treat HPV-associated cervical malignancies.

We have previously linked the sorting signals of the lysosome associated membrane protein-1 (LAMP-1) to HPV-16 E7 antigen, creating a chimera, Sig/E7/LAMP-1. We found that mice vaccinated with Sig/E7/LAMP-1-containing recombinant vaccinia virus (Vac–Sig/E7/LAMP-1) can generate strong antitumor immunity against the development of HPV-16 E7-expressing tumors (TC-1) subcutaneously [4], and in the lung metastasis [5] and hepatic metastasis models (Chen et al., unpublished data). We also found that gene gun-mediated DNA vaccination with Sig/E7/LAMP-1 DNA can generate potent antitumor immunity against

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0264-410X/00/$ - see front matter
PII: S0264-410X(99)00528-9

subcutaneous growth [6], lung metastasis, or hepatic metastasis of TC-1 tumor cells [7].

Though both Vac–Sig/E7/LAMP-1 and Sig/E7/LAMP-1 DNA can generate potent antitumor immunity, combining routes and vaccine vehicles sequentially is a more effective way of enhancing vaccine-induced immune responses [8]. It has been shown that boosting with recombinant vaccinia can increase the immunogenicity and protective efficacy of vaccines such as the malaria DNA vaccine [8–10] and the human immunodeficiency virus (HIV) DNA vaccine [8,11,12]. Therefore, the aim of our current study is to determine what kind of combinations of Vac–Sig/E7/LAMP-1 and Sig/E7/LAMP-1 DNA can generate the best immune responses.

2. Materials and methods

2.1. Animals

We purchased 6–8-week-old female C57BL/6 mice from the National Cancer Institute (Frederick, MD) and kept them in the oncology animal facility of the Johns Hopkins Hospital (Baltimore, MD). All animal procedures were performed according to approved protocols and in accordance with recommendations for the proper use and care of laboratory animals.

2.2. Vaccines

2.2.1. Preparation and immunization of Vac–Sig/E7/LAMP-1

The generation of Vac–Sig/E7/LAMP-1 was described previously [13]. The viral stock was preserved at −70°C prior to vaccination. Before use, the virus was thawed, sonicated in liquid phase for 30 s, trypsinized with trypsin/EDTA in 37°C water bath for 30 min, and diluted with minimal essential medium containing 2.5% fetal bovine serum to the final concentration of 1×10^8 plague forming units (PFU)/ml. Each mouse was vaccinated with 10^7 PFU of vaccinia (0.1 ml of the diluted vaccine) intraperitoneally.

2.2.2. Preparation of Sig/E7/LAMP-1 DNA

The Sig/E7/LAMP-1 chimeric gene was generated as described earlier [13]. The chimeric gene and HPV-16 E7 gene were then cloned sequentially into the unique BamH1 and EcoR1 cloning sites of the pCMVneoBam expression vector downstream of the cytomegalovirus promoter [13]. The recombinant plasmid DNA was transfected into subcloning efficient DH5α^{TM} cells (Life Technologies, USA). The DNA was then amplified and purified by using double CsCl purification (BioServe Biotechnologies, Laurel, MD). The integrity of plasmid DNA and the absence of *Escherichia coli* DNA or RNA was checked for each preparation using 1% agarose gel electrophoresis. DNA concentration was determined by optical density at 260 nm. The presence of an inserted Sig/E7/LAMP-1 fragment was confirmed by restriction enzyme digestion and gel electrophoresis.

2.2.3. Gene gun-mediated Sig/E7/LAMP-1 DNA immunization

Gene gun-mediated DNA vaccination was performed using a helium-driven gene gun (Bio-rad, Hercules, CA) according to the protocol provided by the manufacturer. In brief, DNA-coated gold particles were prepared by combining 25 mg of 1.6 μm gold microcarriers (Bio-rad, Hercules, CA) and 100 μl of 0.05 M spermidine (Sigma, St. Louis, MO). Plasmid DNA (50 μg) and 1.0 M $CaCl_2$ (100 μl) were added sequentially to the microcarriers, while mixing by vortex. This mixture was allowed to precipitate at room temperature for 10 min. The microcarrier/DNA suspension was then centrifuged (10,000 r.p.m. for 5 s) and washed three times in fresh absolute ethanol before resuspending in 3 ml of polyvinylpyrrolidone (0.1 mg/ml) (Bio-rad, Hercules, CA) in absolute ethanol. The solution was then loaded into tubing and allowed to settle for 4 min. The ethanol was gently removed and the microcarrier/DNA suspension was evenly attached to the inside surface of the tubing by rotating the tube. The tube was then dried by 0.4 l/min flowing nitrogen gas. The dried tubing coated with microcarrier/DNA was then cut to 0.5-in. cartridges and stored in a capped dry bottle at 4°C. As a result, each microcarrier/DNA cartridge (bullet) contains 1 μg of plasmid DNA and 0.5 mg of gold. The DNA coated gold particles (1 μg DNA/bullet) were delivered to the shaved abdominal region of the mice using a helium-driven gene gun (Bio-rad, Hercules, CA) with a discharge pressure of 400 p.s.i.

Table 1
Vaccination schedule and immunization regimen[a]

Vaccination group	Immunization (day 0)	Immunization (day 7)
C	none	none
D	none	Sig/E7/LAMP-1 DNA
D + D	Sig/E7/LAMP-1 DNA	Sig/E7/LAMP-1 DNA
D + V	Sig/E7/LAMP-1 DNA	Vac–Sig/E7/LAMP-1
V	none	Vac–Sig/E7/LAMP-1
V + D	Vac–Sig/E7/LAMP-1	Sig/E7/LAMP-1 DNA
V + V	Vac–Sig/E7/LAMP-1	Vac–Sig/E7/LAMP-1

[a] Seven groups (five mice/group) of C57BL/6 mice received various combinations of Sig/E7/LAMP-1 DNA or Vac–Sig/E7/LAMP-1 recombinant vaccinia. For DNA vaccination, 2 μg Sig/E7/LAMP-1 DNA was administered intradermally via gene gun. For vaccinia vaccination, 1×10^7 PFU Vac–Sig/E7/LAMP-1 were given intraperitoneally. The mice were bled and sacrificed at day 19.