Vaccine (2008) **26**, 2335–2343

available at www.sciencedirect.com

ScienceDirect

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

Evaluation of a vectored equine herpesvirus type 1 (EHV-1) vaccine expressing H3 haemagglutinin in the protection of dogs against canine influenza

Cristina Rosas[a,1], Gerlinde R. Van de Walle[a,1], Stephan M. Metzger[a], Karin Hoelzer[b], Edward J. Dubovi[c], Sung G. Kim[c], Colin R. Parrish[b], Nikolaus Osterrieder[a,*]

[a] *Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[b] *Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[c] *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

Received 7 December 2007; received in revised form 24 January 2008; accepted 28 February 2008
Available online 31 March 2008

KEYWORDS
EHV-1;
Canine influenza;
H3

Summary In 2004, canine influenza virus (CIV) was identified as a respiratory pathogen of dogs for the first time and found to be closely related to H3N8 equine influenza virus (EIV). We generated a recombinant vectored vaccine that expresses H3 of a recent isolate of EIV using equine herpesvirus type 1 (EHV-1) as the delivery vehicle. This EHV-1 vectored vaccine exhibited robust and stable EIV H3 expression and induced a strong influenza virus-specific response in both mice and dogs upon intranasal or subcutaneous administration. Furthermore, upon challenge with the recent CIV isolate A/canine/PA/10915-07, protection of vaccinated dogs could be demonstrated by a significant reduction in clinical sings, and, more importantly, by a significant reduction in virus shedding. We concluded that the EHV-1/H3 recombinant vector can be a valuable alternative for protection of dogs against clinical disease induced by CIV and can significantly reduce virus spread.
© 2008 Elsevier Ltd. All rights reserved.

Introduction

Interspecies transmission, through genetic reassortment or direct transfer, accounts for most of the evolutionary success of influenza A viruses [1]. Canine influenza virus (CIV) emerged recently as the result of a direct transmission of equine influenza virus (EIV) H3N8 subtype into dogs [2]. EIV infection is the leading cause of respiratory disease in

* Corresponding author. Tel.: +1 607 253 4045; fax: +1 607 253 3384.
E-mail address: no34@cornell.edu (N. Osterrieder).
[1] These authors contributed equally to the work and share first authorship.

0264-410X/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2008.02.064

the horse, and two major virus subtypes, H7N7 and H3N8, have been isolated from affected equids, although the H7N7 has not been isolated for more than two decades [3]. The H3N8 subtype, however, has not been successfully controlled by current vaccination strategies, and continues to pose a serious threat to horse welfare. In addition, the apparent ability of the H3N8 horse virus to jump species results in the threat of clinical disease in other animals such as the dog [3,4].

CIV was first recognized in racing greyhounds in the US in Florida in 2004 and since has rapidly spread among racing and pet dogs throughout several other states in the US [2,5]. The clinical signs associated with CIV infection range from subclinical to acute respiratory disease characterized by pyrexia and cough, which generally last around 10—14 days. Most affected animals will recover from infection, although death has been reported as a result of hemorrhages in the respiratory tract [2]. To date, no effective vaccine against CIV has been licensed, and a specific treatment for the disease is unavailable.

Equine herpesvirus type 1 (EHV-1) is an *Alphaherpesvirus* of the genus *Varicellovirus* and predominates in the horse population as a respiratory pathogen, occasionally causing abortions and neurological disease [6,7]. The EHV-1 modified-live virus vaccine strain RacH is commonly used to vaccinate horses against EHV-1 in Europe and in the US. RacH is innocuous in mice and horses and its attenuation could be attributed to a deletion of both copies of *gene 67* (*IR6*), which arose spontaneously during its 256 passages on primary swine kidney cells. Other genomic alterations such as truncation of the glycoprotein B also contribute to its attenuation in a variety of species [8—10]. RacH-based vaccine vectors stably and efficiently deliver immunogenic proteins, induce both humoral and cellular immune responses, and they may also protect vaccinated animals from heterologous challenge [11—13]. EHV-1 RacH has a broad host range in cultured cells and infects both dividing and non-dividing cells from different hosts including canine cells [14].

In the present study, we developed a RacH-based vaccine that expresses H3 derived from the EIV subtype H3N8 and that was named rH_EIV. We showed that rH_EIV retains expression of H3 over several passages without impairing its ability to replicate in cultured cells. Furthermore, rH_EIV induces robust immune responses in mice and dogs, and partially protects dogs against challenge with virulent CIV by significantly reducing clinical signs and virus shedding.

Materials and methods

Cells and viruses

Rabbit kidney (RK13) and Madin—Darby bovine kidney (MDBK) cells were maintained in Earle's minimum essential medium (EMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 0.1 mg/ml streptomycin (1% Pen/Strep; Mediatech, Inc.). Parental virus HΔgp2 (EHV-1 strain RacH in which gene 71 was replaced with mini-F sequences) [15] and rH_EIV (recombinant EHV-1 expressing H3) were propagated in RK13 cells. The influenza virus used for challenge, A/canine/PA/10915-07 (H3N8) (Dubovi, unpublished observation), was isolated from an outbreak of canine influenza in Pittsburgh, PA, in 2007 and was propagated in chicken eggs.

Generation of vaccine virus

The H3 gene of equine influenza (EIV) strain A/equine/OH/03 (H3N8), was commercially synthesized after codon-optimization (sequence available upon request, Geneart, Regensburg, Germany) and cloned into shuttle plasmid pEP_goi-in [13,16]. Two-step Red recombination [16] was used to insert EIV H3 into the infectious bacterial artificial chromosome (BAC) clone of the EHV-1 vaccine strain RacH (pRacH1). This recombinant pH_EIV and parental pRacH1 DNA was isolated, digested, and analyzed by 0.8% agarose gel electrophoresis after visualization of DNA fragments with ethidium bromide (EtBr) as described previously [13]. Recombinant and parental viruses were reconstituted after transfection of 2 μg of pH_EIV or pRacH1 DNA into RK13 cells by calcium phosphate precipitation. Two days post-transfection, virus-containing supernatants were clarified from cellular debris, viruses were harvested, titered by plaque assay as described below, and stored frozen at −80 °C [13,15].

Indirect immunofluorescence (IF) analysis

For IF analyses, RK13 cells were infected at a multiplicity of infection (MOI) of 0.0001 with rH_EIV. One hour post-infection (hpi), medium was removed and infected cells were overlaid with 0.25% methylcellulose in EMEM-10% FBS. Two days post-infection (dpi), cells were fixed with 90% ice-cold acetone for 10 min at −20 °C. After re-hydration with PBS, cells were blocked using phosphate-buffered saline (2.5 mmol NaH_2PO_4, 7.5 mmol Na_2HPO_4, 145 mmol NaCl, pH 7.2) (PBS)-0.5% bovine serum albumin (BSA) for 30 min at room temperature (RT). Cells were incubated for 30 min at RT with monoclonal antibody (mAb) 3E5.2 specific for EIV H3 at a 1:5 dilution (kindly provided by Dr. Judy Appleton, Cornell University) or mAb F7 directed against EHV-1 gM (1:50 dilution) [15] for 30 min at RT. After extensive washing with PBS, a 1:500 dilution in PBS of Alexa Fluor488-conjugated goat anti-mouse immunoglobulin (Ig) G (Molecular Probes) was added for 30 min at RT. Plaques were inspected and photographed after thorough washing using an inverted fluorescent microscope (Zeiss Axiovert 25 and Axiocam). Viruses were continuously passaged using an MOI of 0.01 until all cells exhibited cytopathic effect (CPE), and expression of H3 was verified by IF every three passages until passage level 10.

In vitro replication assays

To determine replication of the recombinant virus, single-step replication kinetics and plaque areas were determined. Plaque areas on RK13 cells were measured after infection of cells seeded in a 6-well plate at an MOI of 0.0001 and overlay with EMEM-10% FBS containing 0.25% methylcellulose at 1 hpi. At 3 dpi, plaques were analyzed by IF using mAb F7; 50 plaques were photographed, and average plaque areas were determined using the *ImageJ* soft-

Download English Version:

https://daneshyari.com/en/article/2408250

Download Persian Version:

https://daneshyari.com/article/2408250

Daneshyari.com