Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vaccine 23 (2005) 2280–2283
Vaccine
www.elsevier.com/locate/vaccine

Efficacy of DNA vaccination against western equine encephalitis virus infection

Les P. Nagata[a,*], Wei-Gang Hu[a], Saad A. Masri[b], George A. Rayner[a], Fay L. Schmaltz[a], Dipankar Das[c], Josh Wu[a], Melissa C. Long[a,1], Christine Chan[a,2], David Proll[d], Scott Jager[a], Lellean JeBailey[a,3], Mavanur R. Suresh[c], Jonathan P. Wong[a]

[a] *Chemical Biological Defence Section, Defence R&D Canada – Suffield, Box 4000, Station Main, Medicine Hat, Alta., Canada T1A 8K6*
[b] *Plant Pathogenesis, Canadian Food Inspection Agency, Sidney, BC, Canada*
[c] *Faculty of Pharmacy, University of Alberta, Edmonton, Alta., Canada*
[d] *Defence Science and Technology Organization, Maribyrnong, Vic., Australia*

Available online 9 February 2005

Abstract

The efficacy of a DNA vaccine against western equine encephalitis (WEE) infection in mice was evaluated. The 26S structural region was expressed, in vitro from an internal T7 promoter using a rabbit reticulysate transcription/translation system; and from a CMV promoter after transfection into Vero cell monolayers. The proteins synthesized were reactive with anti-WEE virus (WEEV) antibodies, both in western blot analysis and histochemical staining, respectively. When the DNA vaccine plasmid, pVHX-6, was administered intraepidermally to mice, followed by challenge in a lethal mouse model, the level of protection obtained ranged from 50 to 100% amongst three strains of WEEV. Preliminary results suggest the protective immunity provided by the DNA vaccine appears to be a cell-mediated immune response, as elevated cytotoxic T lymphocyte activity was detected against the E2 protein in a T-cell proliferation assay. The efficacy results suggest a DNA vaccine may be a promising approach against WEE infection.

Keywords: Western equine encephalitis; DNA vaccine; Alphavirus

1. Introduction

In nature, the alphavirus, western equine encephalitis (WEE), is transmitted from its amplifying hosts or reservoir in wild birds to humans and horses via mosquitoes (*Culex tarsalis*). WEE infections in humans can cause symptoms ranging from fever and headache, to encephalitis with sequelae (3–7% case fatality rate). The young are more susceptible than adults, as is also observed in rodent models of pathogenesis (reviewed in [1]). Since WEE virus (WEEV) is a potential biological warfare agent, we have been interested in the development of subunit vaccines.

DNA immunization is a very promising area of viral vaccine development. Similar to live, attenuated vaccines, DNA vaccines are known to stimulate both humoral and cellular immune responses [2,3], and can be directed to induce mucosal immunity. Although several methods can be used to deliver DNA vaccines, in this study, we used ballistic (intraepidermal) delivery of plasmid DNA coated onto gold particles. High pressure helium gas is used to propel the particles into the epidermis and dermis of animals [4]. In alphaviruses, a subgenomic positive-stranded RNA (the 26S RNA), identical to the 3′ one-third of the genome, serves as the translational template for the structural proteins (capsid, E3, E2, 6K and E1). Previously, we had cloned, sequenced and expressed the 26S region from WEEV strain 71V-1658 [5]. In this report, a construct, pVHX-6, was made which also expressed

* Corresponding author. Tel.: +1 403 544 4628; fax: +1 403 544 3388.
E-mail address: les.nagata@drdc-rddc.gc.ca (L.P. Nagata).
[1] Present address: Invitrogen, San Diego, CA, USA.
[2] Present address: University of British Columbia, Vancouver, BC, Canada.
[3] Present address: The Hospital for Sick Children, Toronto, Ont., Canada.

0264-410X/$ – see front matter.
doi:10.1016/j.vaccine.2005.01.032

the WEEV structural proteins in vitro in mammalian cells. The plasmid was tested for protection in adult mice using intranasal challenge of WEEV in a 100% lethal model.

2. Materials and methods

WEEV strains used in this study were kindly provided by Dr. Nick Karabatsos, Centers for Disease Control, Fort Collins, CO (71V-1658); by Dr. George Ludwig, United States Army Medical Research Institute of Infectious Disease, Frederick, MD (B11 and CBA87); or purchased from American Type Culture Collection (ATCC), Manassas, VA (Fleming). Virus strains were propagated, and plaque assays performed roughly as described [6] using Vero cells, ATCC CCL 81. The 26S insert of pcDWXH-7 [5] was subsequently engineered into the pVAX mammalian expression vector (Invitrogen, La Jolla, CA) and the resulting isolate, pVHX-6, was verified by DNA sequence analysis as showing no changes in primary amino acid sequence. Individual gene segments (C, E2, E1) were also cloned into pRSET (Invitrogen) for the expression of individual proteins in *E. coli*.

In vitro transcription and translation of pCXH-3 using TNT™ T7 rabbit reticulysate system (Promega, Madison, WI) with or without added canine microsomes, demonstrated synthesis of ^{35}S-methionine-labelled proteins of the correct size [7]. Expression of pVHX-6 in vitro transcription/translation using the internal T7 promoter (Fig. 1, lane 2) produced identical protein profiles when compared to pCXH-3 (Fig. 1, lane 3). The pVAX negative control is shown in Fig. 1, lane 1. Relative amounts of protein expressed were higher from pVHX-6, as compared to pCXH-3 when identical amounts of DNA were translated. The pVHX-6 lysates also reacted with monoclonal antibodies to WEE in western blot and immunoprecipitation analysis (data not shown). Testing of the fidelity of expression of the insert from the CMV promoter utilized liposomal transfection of pVHX-6 plasmid into Vero cell monolayers. Cells expressing the E1 or E2 proteins were detected through the use of specific anti-E1 or anti-E2 monoclonal antibodies [7], followed by histochemical staining with the HRP substrate Tru-Blue™ [5]. Fig. 1B-1 demonstrates reactivity of pVHX-6 transfected Vero cells with the WEE specific E1 monoclonal antibody 11D2, while the pVAX control cells (Fig. 1B-2) showed no reactivity.

Large-scale plasmid preparations for mouse immunization/challenge studies were made using the Endo-free Giga kit (Qiagen). DNA solutions or an inactivated WEEV vaccine in Hank's Balanced Salts Solution (HBSS), were administered to the mice by ballistic or intramuscular (IM) routes, respectively. Female BALB/c mice, 17–25 g, were obtained from the pathogen-free mouse breeding colony at DRDC Suffield, with the original breeding pairs purchased from Charles River Canada (St. Constant, Que., Canada). The use of these animals was reviewed and approved by Animal Care Committee at DRDC Suffield. Care and handling of the mice followed guidelines set out by the Canadian Council on Animal Care. For the intranasal (IN) route of infection, mice were anaesthetized with sodium pentobarbital (50 mg/kg body weight) given intraperitoneal (IP), and the virus suspension applied to the nostrils of the unconscious mouse [8].

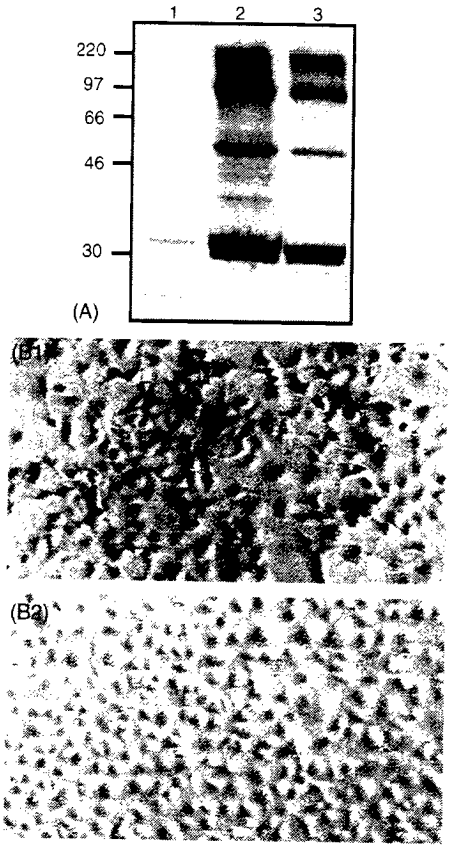

Fig. 1. Expression from WEEV DNA vaccine vector VHX-6. The WEE 26S gene segment in pCXH-3 was excised cloned into the *Hind*III and *Xba*I sites of the mammalian expression vector, pVAX (Invitrogen, La Jolla, CA). The resulting plasmid, pVHX-6 was analyzed for expression: (A) In vitro using the TNT system and [^{35}S]-methionine labeling, followed by SDS-PAGE. Lane: (1) pVAX; (2) pVHX-6; (3) pCXH-3. (B) After cationic liposome, Effectene™-mediated (Qiagen, Chatsworth, CA) transfection into Vero cell monolayers. Expression of WEE antigen was detected by immunohistochemistry with a monoclonal antibody to the WEE E1 protein (11D2). (1) pVHX-6; (2) pVAX (control plasmid).

3. Results and discussion

When adult BALB/c mice were infected IP with WEE strains Fleming, CBA87 and 71V-1658 diluted in HBSS to 10^4 PFUs in 100 μL, the virus did not induce encephalitis or show overt symptoms of disease. However, if the adult BALB/c mice were inoculated with the same strains using an IN route, 100% of adult mice succumbed to a lethal infection using 1.5×10^3 PFU (date not shown). The pVAX control group survival curves (Fig. 2) mirror those of the untreated, infected control groups. The different strains of WEE

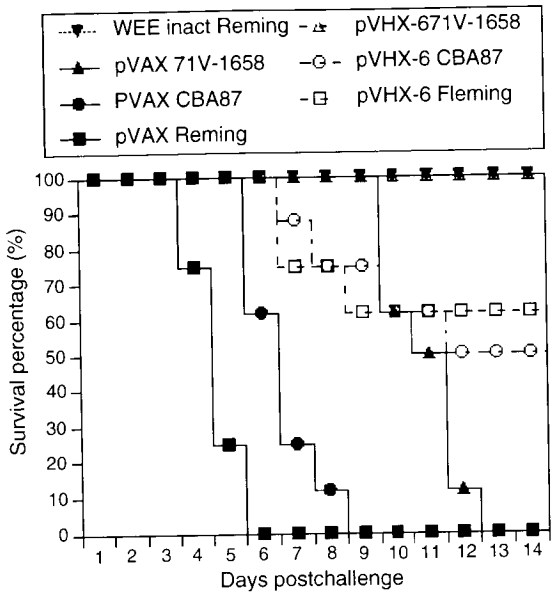

Fig. 2. Protection using ballistic delivery of pVHX-6. Groups of eight mice were immunized with four doses (2 shots × 2.5 μg precipitated on 0.5 mg gold, 1 μm diameter) of pVAX control plasmid or pVXH-6 using the Helios Gene Gun (Biorad, Mississauga, ON) at a pressure setting of 400 psi. A group of eight mice were immunized with 50 μL of inactivated WEE vaccine (SALK WEE inactivated vaccine), IM, 3 doses. The interval between boosters or challenge was 2 weeks. After the mice were rendered unconscious using sodium pentobarbital (50 mg/kg) administered IP, mice were challenged IN with 50 μL of WEE Fleming, CBA 87 or 71V-1658 (1.5×10^3 PFU). The mice were monitored for 14 days and the survival rates of the treatment and non-treatment control groups were compared using the two-tailed *t*-test and one-way analysis of variance. Graph-Pad Prism ver. 2.0 (GraphPad Software, San Diego, CA). Differences were considered statistically significant at P values <0.05.

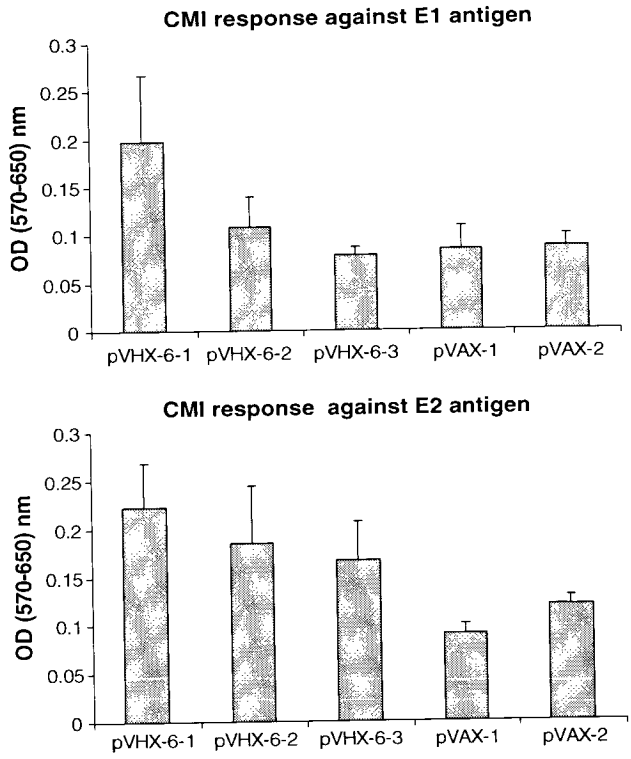

Fig. 3. Cytotoxic T lymphocyte proliferation assay. Spleens were removed three weeks after the final immunization booster. Three groups of mice were used. The first group (Naïve mice – no immunization) was used as stimulator cells. The second and third groups were used as responder cells. Group 2 was immunized with pVAX vector only and Group 3 was immunized with pVHX-6. The stimulator cells (spleen cells from the first group of mice) were incubated for 1 h after E1 or E2 antigen addition, followed by treated with Mitomycin C. The two groups of cells, responder and stimulator, were mixed at suitable ratios and incubated for 2 days. Substrate (MTT) was then added and the cells incubated a further 2 h

were shown to vary in their range of time to death of the infected mice. The Fleming strain generally killed the mice in 4–6 days, CBA87 in 6–9 days and 71V-1658 in 10–12 days (Fig. 2). All the mice immunized with inactivated virus vaccine survived, in addition to 100% survival of the pVHX-6 DNA immunized mice infected with WEEV 71V-1658 ($P < 0.01$) (Fig. 2). Although significant, the pVHX-6 immunized mice did not protect completely against other WEEV strains, Fleming and CBA87, with 62% ($P < 0.01$) and 50% ($P < 0.05$) survival, respectively (Fig. 2).

The lack of complete protection against WEEV Fleming and CBA87 other than 71V-1658 could be due to limited strain cross-reactivity between the Fleming or CBA87, in comparison to 71V-1658. The cloning and addition of a second strain of WEEV (Fleming) and repeating cross-protection studies, may allow better coverage of a DNA vaccine to WEEV isolates. Of interest, polyclonal mouse ascites to Fleming did not cross neutralize against the 71V-1658 strain (data not shown), adding support to this notion. The more rapid rate of killing of the mice with WEEV Fleming may also play a role in the difficulty of providing protection against this virus. All mice inoculated with the pVAX control did not survive infection with the three strains of WEEV, indicating the protection obtained was specific for WEE, and not due to the protective effect of nonspecific adjuvant-like action of CpG motifs from the vector [10].

Both humoral and T-cell mediated immune responses potentially induced by the WEE DNA vaccine were examined. An antibody-capture ELISA for quantitation of mouse antibody titres had been previously described [7]. Mice vaccinated with pVHX-6 did not show significant levels of anti-E1, anti-E2 (<1/20) or anti-WEE inactivated whole virus (<1/10) in serum by ELISA (data not shown). A cytotoxic T lymphocyte proliferation assay was used as described [9], using *E. coli* expressed E1 or E2 protein as the stimulating antigen. In this study, spleens from mice were removed, macerated and the cells were used in a tetrazolium-based colorimetric assay. These mice showed significantly strong T-cell proliferation response to purified E2 protein and a partial response to E1 protein as compared with pVAX controls (Fig. 3).

In conclusion, the plasmid, pVHX-6 shows promise as a vaccine candidate for WEE. This is especially important for protection against an aerosol challenge of WEEV, an event that would be envisioned in a potential biological warfare attack using WEEV as a BW agent. Information available on

WEE laboratory-associated/acquired infections: "7 reported cases with 2 deaths (associated with egg cultures, suckling mice and aerosols – broken lyophilized material)" from the Office of Biosafety, Health Canada (1989), indicates the aerosol hazard to humans could be serious. New cross-neutralization and cross-protection studies will be carried out to investigate these possibilities. A significant cytotoxic T-cell response against the E2 protein was detected in the third week, following the final booster. In summary, the ballistic method of delivery is quick (3 doses over 28 days), and is able to provide complete protection against the identical 71V-1658 strain of WEEV.

Acknowledgements

M. Long was supported with Natural Science and Engineering Research Council visiting fellowship to a government laboratory, and W.-G. Hu was supported with a DRDC – Canadian Institutes for Health Research postdoctoral fellowship.

References

[1] Griffin DE. Alphavirus pathogenesis and immunity. In: Schlesinger S, Schlesinger MJ, editors. The Togaviridae and Flaviviridae. New York: Pleum Press; 1986. p. 209–49.

[2] Deck RR, DeWitt CM, Donnelly JJ, Lui MA, Ulmer JB. Humoral immune responses to influenza hemagglutinin by DNA vaccination. In: Brown F, Chanock RM, Ginsberg HS, Norby E, editors. Vaccines 95. NY: Cold Spring Harbour Laboratory Press; 1995. p. 91–4.

[3] Whalen RG, Schirmbeck R, Reimann J, Davis HL. Induction of a strong CTL response after DNA-mediated immunization to the envelope protein of HBV. In: Brown F, Chanock RM, Ginsberg HS, Norby E, editors. Vaccines 95. NY: Cold Spring Harbour Laboratory Press; 1995. p. 117–21.

[4] Prayaga SK, Fuller DH, Haynes JR, Murphey-Corb M. Particle-mediated nucleic acid immunization. In: Brown F, Chanock RM, Ginsberg HS, Norby E, editors. Vaccines 95. NY: Cold Spring Harbour Laboratory Press; 1995. p. 105–9.

[5] Netolitzky DJ, Schmaltz FL, Parker MD, Rayner GA, Fisher GR, Trent DW, et al. Complete genomic RNA sequence of western equine encephalitis virus and expression of the structural genes. J Gen Virol 2000;81:151–9.

[6] Greenway TE, Elridge JE, Ludwig G, Staas JK, Smith JF, Giley RM, et al. Enhancement of protective immune responses to Venezuelan equine encephalitis (VEE) virus with microencapsulated vaccine. Vaccine 1995;13:1411–20.

[7] Long MC, Nagata LP, Ludwig GV, Alvi AZ, Conley JD, Bhatti AR, et al. Construction and characterization of monoclonal antibodies against western equine encephalitis virus. Hybridoma 2000;19:121–7.

[8] Wong JP, Zabielski M, Schmaltz FL, Brownlee GG, Bussey LA, Marshall K, et al. DNA vaccination against respiratory influenza virus infection. Vaccine 2001;19:2461–7.

[9] Das D, Gares SL, Nagata LP, Suresh MR. Evaluation of a western equine encephalitis recombinant E1 protein for protective immunity and diagnostics. Antiviral Res 2004;64:85–92.

[10] Krieg AM, Yi A-K, Schorr J, Davis HL. The role of CpG dinucleotides in DNA vaccines. Trends Microbiol 1998;6:23–7.