Defence Research and Development Canada
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Identification of the 6K-E1 protein of western equine encephalitis virus as a vaccine target

Josh Q.H. Wu; Nicole D. Barabé; Richard D. Swayze
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IMPORTANT INFORMATIVE STATEMENTS

In conducting the research described in this report, the investigators adhered to the 'Guide to the Care and Use of Experimental Animals, Vol. I, 2nd Ed.' published by the Canadian Council on Animal Care.

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Abstract

Vaccination is a cost-effective medical countermeasure against the threat of viral biological agents and endemic viral diseases. Currently, the number of effective vaccines available for the Canadian Foreces (CF) is limited. We have been focusing on the identification of the vaccine targets for western equine encephalitis virus (WEEV), which belongs to the alphaviral agents which also include Venezuelan equine encephalitis virus and eastern equine encephalitis virus. We previously found that the envelope proteins of WEEV elicit rapid immune protection against the lethal challenge of the virus. In this study, we further defined that the 6K-E1 envelope protein of WEEV is a major target for vaccine development. This finding will aid the design of an improved vaccine for WEEV and other alphaviruses.

Résumé

La vaccination est une contre-mesure médicale rentable contre les agents biologiques viraux et les maladies virales endémiques qui constituent une menace. À l'heure actuelle, Les Forces canadiennes (FC) disposent d'un nombre limité de vaccins efficaces. Nos efforts ont principalement porté sur la détermination des cibles vaccinales du virus de l'encéphalite équine de l'Ouest (VEEO) qui appartient à la famille des alphavirus, dont font aussi partie le virus de l'encéphalite équine du Venezuela et le virus de l'encéphalite équine de l'Est. Nous avons déjà observé que les protéines d'enveloppe du VEEO induisaient une protection immunitaire rapide contre une provocation létale par le VEEO. Dans la présente étude, nous avons en outre défini que la protéine d'enveloppe 6K-E1 du VEEO constituait une cible importante pour la mise au point d'un vaccin. Cette constatation aidera à concevoir un vaccin amélioré contre le VEEO et d'autres alphavirus.

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Executive summary

Identification of the 6K-E1 protein of western equine encephalitis virus as a vaccine target

Josh Q.H. Wu; Nicole D. Barabé; Richard D. Swayze; DRDC Suffield TM 2009-201; Defence Research and Development Canada – Suffield; November 2009.

Introduction or background: Western equine encephalitis virus (WEEV) is endemic in western North America where the virus is transmitted via mosquitoes from its reservoir in wild birds to humans and horses. Infected individuals usually present with fever and headache. Severe cases can progress into coma and death, with a case mortality rate of 3–4%. The ease of aerosol transmission of WEEV makes it a potential biowarfare and bioterrorism agent. Currently, no commercial vaccine and no antiviral drugs are available for prevention and treatment of WEEV infection. DRDC Suffield is addressing this deficiency in medical countermeasures against WEEV by developing a vaccine candidate for WEEV. In previous work, it was found that a vaccine consisting of the E3-E2-6K-E1 structural proteins of WEEV elicits fast-acting immune protection against the virus. In this report, a vaccine antigen within E3-E2-6K-E1 was identified.

Results: It was determined that E1 plus the small structural protein 6K are sufficient to confer complete protection against the homologous challenge of WEEV in a mouse infection model of WEEV. For heterologous challenge, the efficacy of 6K-E1 is dependent on the virulence of the WEEV strains. The 6K-E1 protein gives complete protein against the moderately virulent CBA87 strain and partial protection against the highly virulent Fleming strain.

Significance: The major function of the 6K-E1 protein is to help WEEV enter into host cells by promoting membrance fusion between the virus and the host cell. The results from this study demonstrate that the immune response raised against this protein confers rapid and strong protection against WEEV.

Future plans: Future work will be focused on defining the minimal region within 6K-E1 that gives complete protection.

Sommaire

Identification de la protéine 6K-E1 du virus de l'encéphalite équine de l'Ouest comme cible de vaccin

Josh Q.H. Wu, Nicole D. Barabé et Richard D. Swayze; RDDC Suffield TM 2009-201; Recherche et développement pour la défense Canada – Suffield; novembre 2009.

Introduction ou contexte

Le virus de l'encéphalite équine de l'Ouest (VEEO) est endémique dans l'ouest de l'Amérique du Nord où il est transmis par des moustiques, dont les oiseaux sauvages constituent le réservoir, à l'humain et au cheval. Les personnes infectées présentent habituellement de la fièvre et des céphalées. Les cas graves peuvent mener au coma ou à la mort; le taux de mortalité par cas est de 3-4 %. La facilité de transmission du VEEO par aérosols en fait un agent potentiel de guerre biologique et de bioterrorisme. À l'heure actuelle, il n'existe aucun vaccin commercial ni agent antiviral pour prévenir et traiter l'infection à VEEO. RDDC Suffield cherche à combler cette lacune par la mise au point d'un candidat-vaccin contre le VEEO. Dans des travaux précédents, on a observé qu'un vaccin composé des protéines structurales E3-E2-6K-E1 du VEEO induisait une protection immunitaire à action rapide contre le virus. Dans le présent rapport, un antigène vaccinal dans les protéines E3-E2-6K-E1 a été identifié.

Résultats

Il a été établi que la protéine E1 et la petite protéine structurale 6K suffisent à conférer une protection complète contre la provocation avec une souche homologue du VEEO dans un modèle d'infection murin par le VEEO. Pour le test de provocation avec une souche hétérologue, l'efficacité de la protéine 6K-E1 dépend de la virulence des souches du VEEO. La protéine 6K-E1 confère une protection complète contre la souche CBA87, qui est de virulence modérée, et une protection partielle contre la souche Fleming, qui est très virulente.

Importance

La principale fonction de la protéine 6K-E1 est d'aider le VEEO à pénétrer dans les cellules de l'hôte en favorisant la fusion membranaire entre le virus et la cellule hôte. Les résultats de la présente étude démontrent que la réponse immunitaire dirigée contre cette protéine confère une protection rapide et solide contre le VEEO.

Perspectives

Des travaux de recherche futurs porteront sur l'identification de la région minimale de la protéine 6K-E1 qui confère une protection complète.

Table of contents

Abstract …….. i
Résumé ….... i
Executive summary iii
Sommaire iv
Table of contents v
List of figures vi
List of tables vi
Acknowledgements vii
1. Introduction 1
2. Materials and Methods 3
   Cells and viruses 3
   Recombinant adenovirus construction 3
   Detection of the 6K-E1 gene in Ad5-WEEV-6K-E1 5
   Detection of the E1 protein expression from Ad5-WEEV-6K-E1 5
   Challenge and protection study in mice 6
   Statistical analysis 6
3. Results 7
   Construction and characterization of Ad5-WEEV-6K-E1 7
   Expression of E1 protein from cells infected with Ad5-WEEV-6K-E1 8
   Protection of mice from lethal challenge of WEEV after a single dose vaccination with Ad5-WEEV-6K-E1 9
4. Discussion 11
5. Conclusion 13
6. References 15
7. List of symbols/abbreviations/acronyms/initialisms 17

List of figures

Figure 1 : Schematic illustration of the synthesis of structural proteins of western equine encephalitis virus. 2

Figure 2 : Construction of recombinant human adenovirus vector expressing the 6K-E1 protein of the 71V-1658 strain of western equine encephalitis virus. 4

Figure 3 : PCR detection of the 6K-E1 gene in Ad5-WEEV-6K-E1. 7

Figure 4 : Expression of the 6K-E1 protein from Ad5-WEEV-6K-E1 8

Figure 5 : Kaplan-Meier survival curves of the mice vaccinated with Ad5-WEEV-6K-E1 and challenged with WEEV. 9

List of tables

Table 1 : Protection of mice against challenge of various strains of WEEV after a single-dose vaccination with Ad5-WEEV-6K-E1 10

Acknowledgements

The authors wish to thank the animal care staff of DRDC Suffield for providing mice for the challenge and protection study.

1. Introduction

Western equine encephalitis virus (WEEV) belongs to the *Alphavirus* genus in the *Togaviridae* family [1]. The alphaviruses are a group of mosquito borne RNA viruses that replicate in invertebrates, birds and mammals, and cause disease in humans and animals. Many Old World alphaviruses, including Sindbis, Chikungunya, Ross River, Semliki Forest viruses, cause an arthralgia syndrome, while the New World alphaviruses, including Venezuelan, eastern, and western equine encephalitis viruses, principally cause infection of the central nervous system [2]. DRDC Suffield conducts research on medical countermeasures against WEEV because the virus is a potential bioterrorism and biowarfare agent, to which no vaccine and no antiviral drug are available for prevention and treatment [3].

WEEV is a single-stranded, positive-sense, enveloped RNA virus that has a protein core containing a genome of approximately 11.5 kb with a 5′ cap and a 3′ poly(A) tail [4] (Figure 1). The 5′ two-thirds of the genome encodes four non-structural proteins, which form the replicase responsible for RNA replication and transcription. The 3′ one-third of the WEEV genome encodes the virus structural proteins, which consist of capsid, E3, E2, 6K, and E1 proteins. The structural proteins are translated as a polyprotein of capsid-PE2-6K-E1 from a subgenomic 26S RNA, which is transcribed by the viral replicase using a promoter in the genome-length minus strand RNA. In the cytosol of the host cell, the capsid protein is autoproteolytically cleaved from the polyprotein by a serine protease present in the N-terminus of the polyprotein. The remaining polyprotein then moves into rough endoplasmic reticulum and is further cleaved by signalases into PE2, 6K, and E1. PE2 and E1 interact to form a heterodimer and are transported to the Golgi complex. Before appearing at the plasma membrane, PE2 is cleaved by a furin-like host protease into E2 and E3. E2-E1 heterodimers further oligomerize into trimers to form the spike proteins in the viral lipid membrane.

The E1 protein of alphaviruses is one of the primary determinants for viral entry into host cells. Extensive studies on Semliki Forest virus, an Old World alphavirus, reveals that the entry of the virus into cells involves the following steps: 1) binding the virus to a cellular receptor(s); 2) taking up the virus into cells through endocytosis in clathrin-coated vesicles; 3) virus trafficking through vesicular compartments; and 4) fusing the viral and cellular membranes within the endosome at low pH, which results in release of the viral nucleocapsid into the cytoplasm where the subsequent steps of the replication cycle take place [5]. The E1 protein of alphaviruses contains a highly conserved hydrophobic domain, which is a putative fusion peptide. The E1 protein is involved in class II membrane fusion [6]. During this type of membrane fusion process, low pH within the endosome triggers conformational changes of E2-E1 heterodimer and exposes the previously masked fusion peptide in E1. The fusion peptide then inserts into membranes, resulting in formation of a highly stable E1 homotrimer that appears to be required for fusion [7, 8].

The function of the 6K protein of alphaviruses is not fully understood. This protein is composed of about 60 amino acid residues, including a long stretch of highly hydrophobic residues that interact with membranes. Immediately after synthesis, the 6K protein forms a complex with E1 and PE2 (precursor of E2 and E3) and the complex is transported to the cell surface. However, 6K is mostly excluded from matured viral particles. It appears that the deletion of 6K does not affect

virus replication, although the yield of virus is reduced [9, 10]. Therefore, 6K could provide functions such as sites for protease cleavage, participation in viral glycoprotein transportation, and enhance membrane permeability [11].

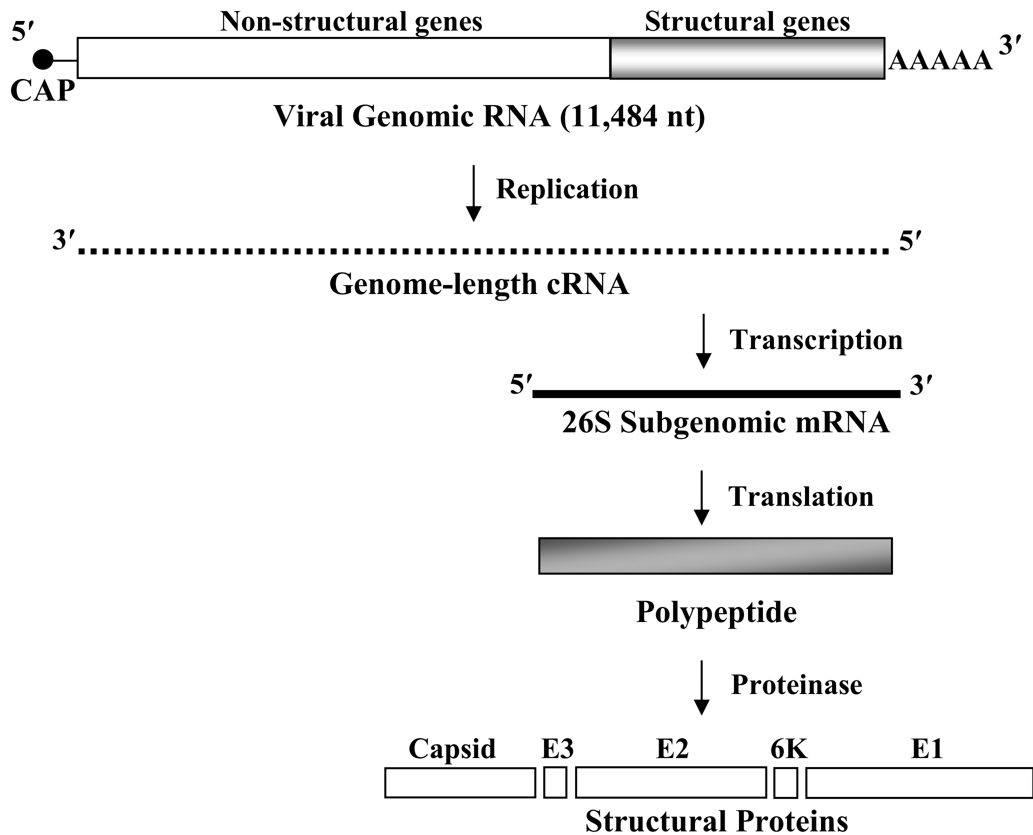

Figure 1 : Schematic illustration of the synthesis of structural proteins of western equine encephalitis virus.

It has been demonstrated previously that a vector based on human adenovirus type 5 (HAd5) expressing the E3-E2-6K-E1 structural proteins of WEEV provides rapid cross-protection against a lethal dose challenge of WEEV after a single dose vaccination in a mouse model [12]. In this study, the effectiveness of an HAd5 vector expressing 6K-E1 alone was assessed to determine whether it would give the same protection that was shown by the HAd5 vector expression E3-E2-6K-E1.

2. Materials and Methods

Cells and viruses

Human embryonic kidney 293A (HEK 293A) cells were purchased from Invitrogen (Burlington, ON; Catalog number R705-07). Cells were grown in Dulbecco's modified Eagle media (DMEM) containing 10% fetal bovine serum and antibiotics. Recombinant adenovirus Ad5-WEEV was made as previously described [13]. Three WEEV strains were used throughout the study. The 71V-1658 strain originally in a 10% suckling mouse brain (SMB) suspension was provided by Nick Karabatsos (U.S. Centers for Disease Control and Prevention, Fort Collins, CO). The Fleming strain was purchased from American Type Culture Collection (ATCC; Manassas, VA). The CBA87 strain was provided by George Ludwig (United States Army Medical Research Institute of Infectious Disease, Frederick, MD). Seed stocks of WEEV were made from the original vials by the inoculation of Vero cells with the viruses at a multiplicity of infection (MOI) of less than 0.1. The supernatants of the infected cells were collected, aliquoted, and stored at –70 °C for further use in animal challenge studies. The titers of the WEEV stocks were determined by plaque assay in Vero cells. All of the experiments with WEEV were carried out in the Biosafety Level 3 laboratory at Defence Research and Development Canada – Suffield (DRDC Suffield) in compliance with guidelines set by Health Canada and the Canadian Food Inspection Agency.

Recombinant adenovirus construction

Two recombinant adenoviruses (Ad5-WEEV-6K-E1 and Ad5-Empty) were constructed in this study. To construct Ad5-WEEV-6K-E1 (Figure 2), the 6K-E1 gene of the 71V-1658 strain of WEEV was PCR isolated from plasmid pVHX-6. Primers for PCR amplification of the 6K-E1 gene were forward primer JQW6 (5′-CAC CAT GGA AAC ATT TGG AGA AAC TTT GAA CC- 3′) and reverse primer JQW4 (5′-CCG CGC TCA GTC ATC TAC GTG TG- 3′). These primers were designed based on the GenBank sequence (NC_003908) of the 71V-1658 strain [4]. The PCR was carried out using *Pfu*Turbo DNA polymerase (Stratagene, La Jolla, CA) with 2 min of initial denaturation at 95°C, 25 cycles of 0.5 min of denaturation at 94 °C, 0.5 min of annealing at 55 °C, and 1 min of extension at 70 °C. A step of 7 min of extension at 72 °C was added immediately after the final cycle. The PCR fragment was separated by 0.8% agarose gel and purified by QIAquick Gel Extraction kit (QIAGEN, Mississauga, ON). The purified PCR fragment was cloned into linearized plasmid vector pcDNA3.1D/V5-His-TOPO (Invitrogen) to produce plasmid pcD3-WEEV-6K-E1. The nucleotide sequence of the 6K-E1gene cloned in the plasmid was verified by automated sequencing. To insert the 6K-E1 gene into the full-length HAd5 genome, a transfer plasmid (pSCMV-WEEV-6K-E1) was made by the ligation of a 1.6-kb *Kpn*I-*Xba*I fragment from pcD3-WEEV-6K-E1 into the compatible sites of pShuttle-CMV (Qbiogene, Carlsbad, CA). The resultant plasmid pSCMV-WEEV-6K-E1 was linearized with *Pme*I and co-transformed with pAdEasy-1 plasmid (Qbiogene) into *E. coli* strain BJ5183 (Qbiogene). The pAdEasy-1 plasmid contains a full-length of HAd5 DNA genome with deletions in E1 and E3 coding regions [14]. Through homologous recombination in BJ5183 [15], the gene encoding WEEV 6K-E1 was inserted into the deleted E1 region of HAd5 genome to yield infectious plasmid pAd5-WEEV-6K-E1.

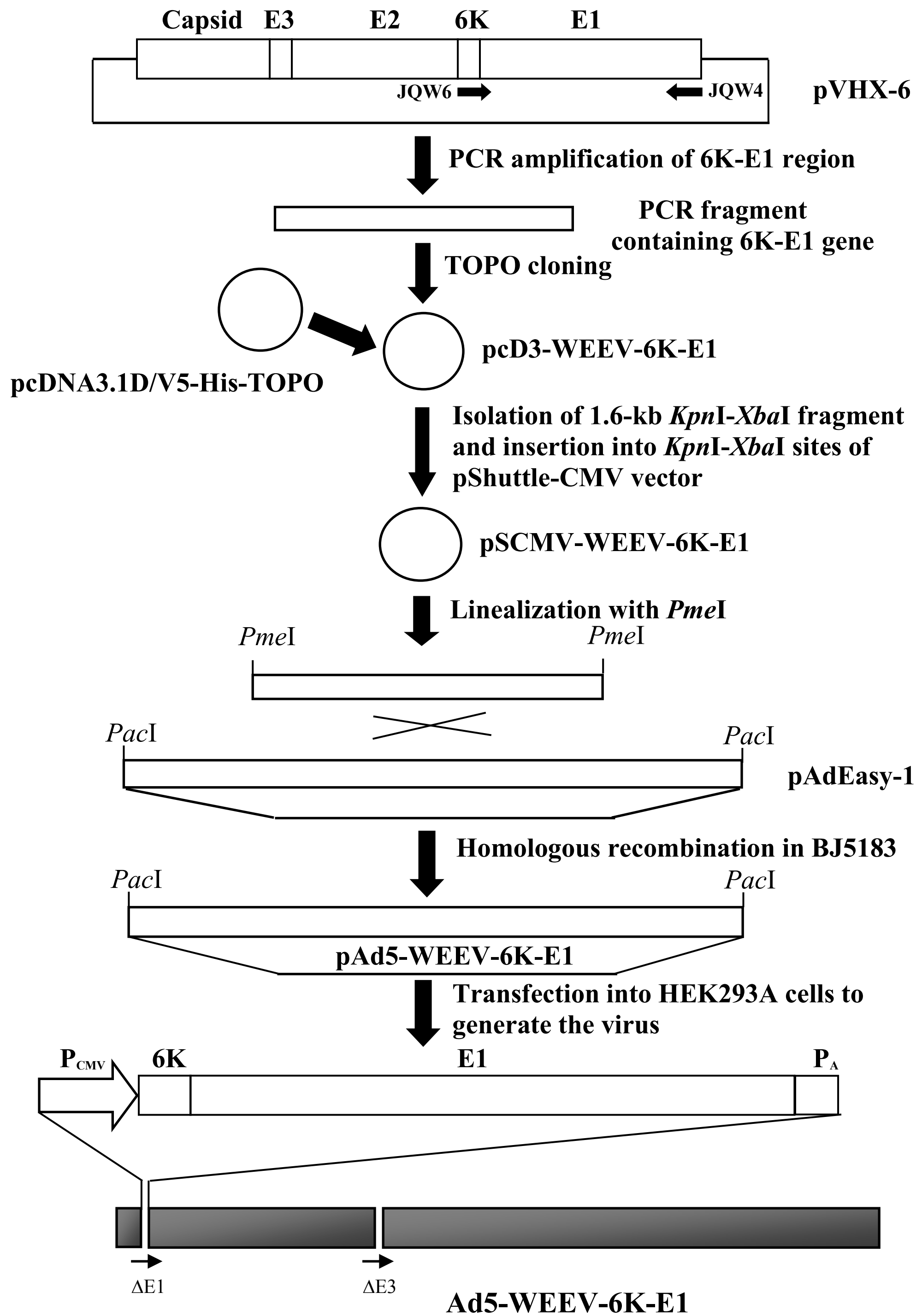

Figure 2 : Construction of recombinant human adenovirus vector expressing the 6K-E1 protein of the 71V-1658 strain of western equine encephalitis virus.

To generate a recombinant adenovirus, the infectious plasmid pAd5-WEEV-6K-E1 was transfected into HEK 293A cells using Lipofectamine 2000 (Invitrogen). To do this, the plasmid was first purified by QIAGEN Plasmid Maxi kit (QIAGEN). The purified plasmid DNA (40 µg total) was digested with *Pac*I and purified by ethanol precipitation. Then, 8 µg of the purified DNA was incubated with 60 µL of Lipofectamine 2000 (Invitrogen) at room temperature for 20 min. The mixture of the DNA-Lipofectamine 2000 was added dropwise onto HEK 293A cells seeded in a T25 flask. The flask was incubated at 37°C in a CO_2 incubator for 1 to 2 weeks.

Recombinant adenovirus Ad5-Empty (a HAd5 vector with deletions in the adenovirus E1 and E3 regions) was used as a control virus and constructed as follows. An infectious plasmid, pAd5-Empty, was made by co-transformation of *Pme*I-linearized pShuttle-CMV and pAdEasy-1 into *E. coli* BJ5183 strain. Ad5-Empty was generated by transfection of *Pac*I-digested pAd5-Empty into HEK 293A cells using Lipofectamine 2000. All of the recombinant adenoviruses were amplified in HEK 293A cells and purified by Adeno-X Mega Purification Kit (Clontech, Mountain View, CA). The purified viruses were resuspended in 1X Formulation Buffer (2.5% glycerol (w/v), 25 mM NaCl, and 20 mM Tris-HCl, pH 8.0) provided by the kit. The titers of the purified recombinant adenoviruses were determined by a 50% tissue culture infectious dose assay [16].

Detection of the 6K-E1 gene in Ad5-WEEV-6K-E1

The presence of the 6K-E1 gene in the viral genome of Ad5-WEEV-6K-E1 was confirmed by PCR. The viral DNA of Ad5-WEEV-6K-E1 was isolated using the QIAamp DNA Blood Mini Kit (QIAGEN; Cat# 51104) as per manufacturer's instructions. Briefly, a total of 200 µL of purified Ad5-WEEV-6K-E1 containing 10^7 plaque forming units (PFU) of the virus was mixed with 20 µL of QIAGEN protease. Then 200 µL of Buffer AL was added to the mixture, followed by incubation at 56°C for 10 min. The sample was then mixed with 200 µL of pure ethanol, and loaded onto a spin column. The column was sequentially washed with 500 µl of Buffer AW1 and AW2, and viral DNA was eluted with 100 µL of DNA grade water. PCR was performed using 200 ng of purified Ad5-6K-E1 DNA. The purified DNA from Ad5-Epmpty virus was included in PCR as a negative control or the control Ad5 empty vector as DNA templates. The primers used for PCR were the forward primer F-6K-E1 (5′-GTA CCC TTC ACC ATG GAA AC-3′) and the reverse primer R-6K-E1 (5′CGC GCT CAG TCA TCT ACG TG-3′), synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). HotStar*Taq* polymerase (QIAGEN) was used to amplify the 6K-E1 gene with an initial polymerase activation step of 15 min at 95 °C, followed by 35 cycles of 45 sec denaturation at 94 °C, 45 sec annealing at 50 °C, and 1 min extension at 72 °C. After 35 cycles, a final extension at 72 °C was done for 10 min. The PCR products were resolved on a 0.8% agarose gel and visualized by ethidium bromide staining.

Detection of the E1 protein expression from Ad5-WEEV-6K-E1

The expression of the E1 protein from Ad5-WEEV-6K-E1 was detected by western blot. HEK 293A cells grown to 100% confluency in T25 flasks were infected with purified Ad5-WEEV-6K-E1 or Ad5-Empty control at an MOI of 2. The infected cells were harvested at 24 h after infection and pelleted by centrifugation at 1500 rpm for 5 min at room temperature. The pellet was washed once with 1 mL of phosphate buffered saline (PBS) and resuspended in 300 µL of 1X NuPAGE sample buffer. The samples were extensively vortexed and passed through a 28 gauge syringe 3 times to shear the DNA. Samples were then boiled at 100 °C for 5 min and further diluted 40X in

1X sample buffer. A total of 20 µL of each sample was loaded onto a 4 to 12% NuPAGE Bis Tris gel. In addition, 10 µL of Invitrogen's MultiMark standard was used as the protein marker for assessing the molecular mass. Proteins were separated in a MOPS buffering system at 180 volts for 50 min. Resolved proteins were transferred to a nitrocellulose membrane using the iBlot apparatus from Invitrogen. The E1 protein was probed by 11D2 monoclonal antibody (MAb) specific for E1 [17] and visualized using Invitrogen's WesternBreeze Chromogenic Immunodetection Kit. As per manufacturer's instructions, the membrane was incubated with 11D2 for 1 hr, followed by incubation with alkaline phosphatase-conjugated anti-mouse antibody for 30 min at room temperature. The membrane was developed by brief incubation with the chromogenic substrate.

Challenge and protection study in mice

Female BALB/c mice (17 to 20 *g*) were used throughout the study. The mice were obtained from pathogen-free breeding colonies at the animal care facility of DRDC Suffield. The original breeding pairs were purchased from Charles River Canada (St. Constant, QC). Animal experiment protocols were approved by the DRDC Animal Care Committee. The guidelines of the Canadian Council on Animal Care were followed for animal care and experimentation.

To determine the efficacy of Ad5-WEEV-6K-E1 against the challenge of different WEEV strains, mice were divided into 9 groups of 5 mice each. Each mouse in Groups I, IV, and VII was given intramuscular (IM) injection of 10^7 PFU of Ad5-Empty for each mouse. Each mouse in Groups II, V, and VIII was given IM injection of 10^7 PFU of Ad5-WEEV for each mouse. Each mouse in Groups III, VI, and IX was given IM injection of 10^7 PFU of Ad5-WEEV-6K-E1. At day 7 after injection, mice in Groups I, II and III were challenged intranasally (IN) with a 25 lethal dose 50 (LD_{50}) of the 71V-1658 strain of WEEV. Mice in Groups IV, V, and VI were challenged IN with a 25 LD_{50} dose of the CBA87 strain. Mice in Groups VII, VIII, and IX were challenged IN with a 25 LD_{50} dose of the Fleming strain.

To do the challenge, mice were anaesthetized intraperitoneally with sodium pentobarbital (50 mg/kg body weight). When the animals were unconscious, they were supported by hand with their nose up. Then a total of 25 LD_{50} doses of the virus diluted in 50 µL of 1X Hank's balanced salt solution (HBSS, Invitrogen) was distilled with a micropipette into the nostrils. The challenged mice were examined daily for 14 days for survival and the severity of infection using the following scoring system: 0, normal; 1, slightly ruffled hair, very active, no visible signs of infection; 2, very ruffled hair, definite signs of infection, not as active, but still fairly mobile; 3, very ruffled hair, hunched posture, reduced mobility; and 4, very ruffled hair, hunched posture, little or no mobility, rapid breathing. Mice scored at the scale of 4 were considered terminally ill and were euthanized.

Statistical analysis

PRISM® 4 program (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. A P value of less than 0.05 is considered to be significant. Differences in mean survival time between the mice given Ad5-WEEV-6K-E1 and the mice given Ad5-Empty control were assessed by a two-tailed paired t test. Differences in percentage of survival among the different injection groups after the challenge were assessed by comparing Kaplan-Meier survival curves with use of the log rank test.

3. Results

Construction and characterization of Ad5-WEEV-6K-E1

As shown in Figure 2, the gene encoding the 6K-E1 protein of the 71V-1658 strain of WEEV was PCR isolated from pVHX-6 plasmid which contains the gene encoding the entire structural proteins of 71V-1658 [18]. The 6K-E1 gene was then inserted into the deleted E1 region of the HAd5 genome through homologous recombination in *E. coli* [14, 15]. The gene was placed between the immediate-early promoter of cytomegalovirus (CMV) and the polyadenylation signal of Simian Virus 40 (SV40). The recombinant adenovirus vector Ad5-WEEV-6K-E1 was generated by transfection of the infectious plasmid pAd5-WEEV-6K-E1 into HEK 293A cells. The presence of the 6K-E1 gene in Ad5-WEEV-6K-E1 was detected by PCR using primers specific for the 6K-E1 gene. As shown in Figure 3, a 1.5 kb DNA fragment was amplified from the viral DNA of Ad5-WEEV-6K-E1 (lane 3), which is consistent with the anticipated size of the 6K-E1 gene of 71V-1658 [4].

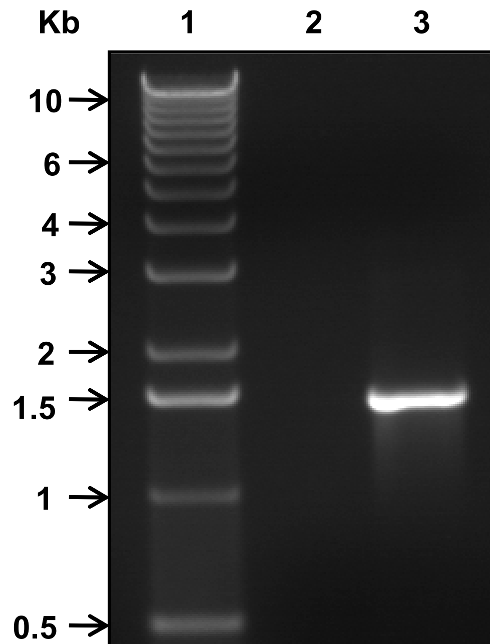

Figure 3 : PCR detection of the 6K-E1 gene in Ad5-WEEV-6K-E1.

Lane 1: Invitrogen TrackIt DNA Ladder; Lane 2: viral DNA from Ad5-empty; Lane 3: viral DNA from Ad5-WEEV-6K-E1.

Expression of E1 protein from cells infected with Ad5-WEEV-6K-E1

Having demonstrated the presence of the 6K-E1 gene in the viral genome of Ad5-WEEV-6K-E1, the expression of the E1 protein by Ad5-WEEV-6K-E1. HEK 293A cells were infected with Ad5-WEEV-6K-E1 or Ad5-Empty control vector was assessed next. Expression of E1 from cells infected with Ad5-WEEV-6K-E1 was detected by western blot using 11D2 MAb specific for the E1 protein of WEEV [17]. As shown in Figure 4, a 52 kDa protein band, which is in good agreement with the calculated molecular mass of 53 kDa of the E1 protein, was detected in the cell lysates collected from HEK 293A cells infected with Ad5-WEEV-6K-E1 (lane 4). The E1 protein was not detected in mock-infected 293A cells (lane 2) or 293A cells infected with Ad5-Empty control (lane 3), confirming that the E1 protein was specifically produced in cells infected with Ad5-WEEV-6K-E1.

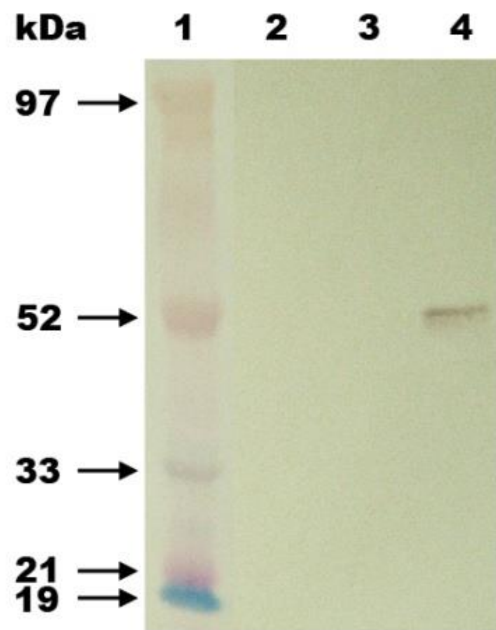

Figure 4 : Expression of the 6K-E1 protein from Ad5-WEEV-6K-E1

Total protein was extracted from mock-infected HEK 293A cells (lane 2), HEK 293A cells infected with Ad5-Empty control vector (lane 3), or HEK 293A cells infected with Ad5WEEV-6K-E1 (lane 4). Protein extracts were resolved in a 4–12% NuPAGE Bis-Tris gel, transferred to nitrocellulose, and probed with the MAb 11D2 specific for the E1 protein. Lane 1: the MultiMark molecular weight standard from Invitrogen.

Protection of mice from lethal challenge of WEEV after a single dose vaccination with Ad5-WEEV-6K-E1

DRDC Suffield found previously that mice were completely protected from a lethal dose challenge of different strains of WEEV at 1 week after vaccination with a single dose of Ad5-WEEV, a HAd5-vectored vaccine expressing the E3-E2-6K-E1 protein of the 71V-1658 strains of WEEV [12]. In this study, it was determined whether Ad5-WEEV-6K-E1 expressing the 6K-E1 protein would provide the similar protection as Ad5-WEEV. Mice were IM vaccinated with a single dose injection of 10^7 pfu of Ad5-WEEV-6K-E1. One week after the vaccination, mice were challenged IN with 25 LD_{50} doses of WEEV strain 71V-1658, CBA87, or Fleming and monitored 14 days for the severity of the infection.

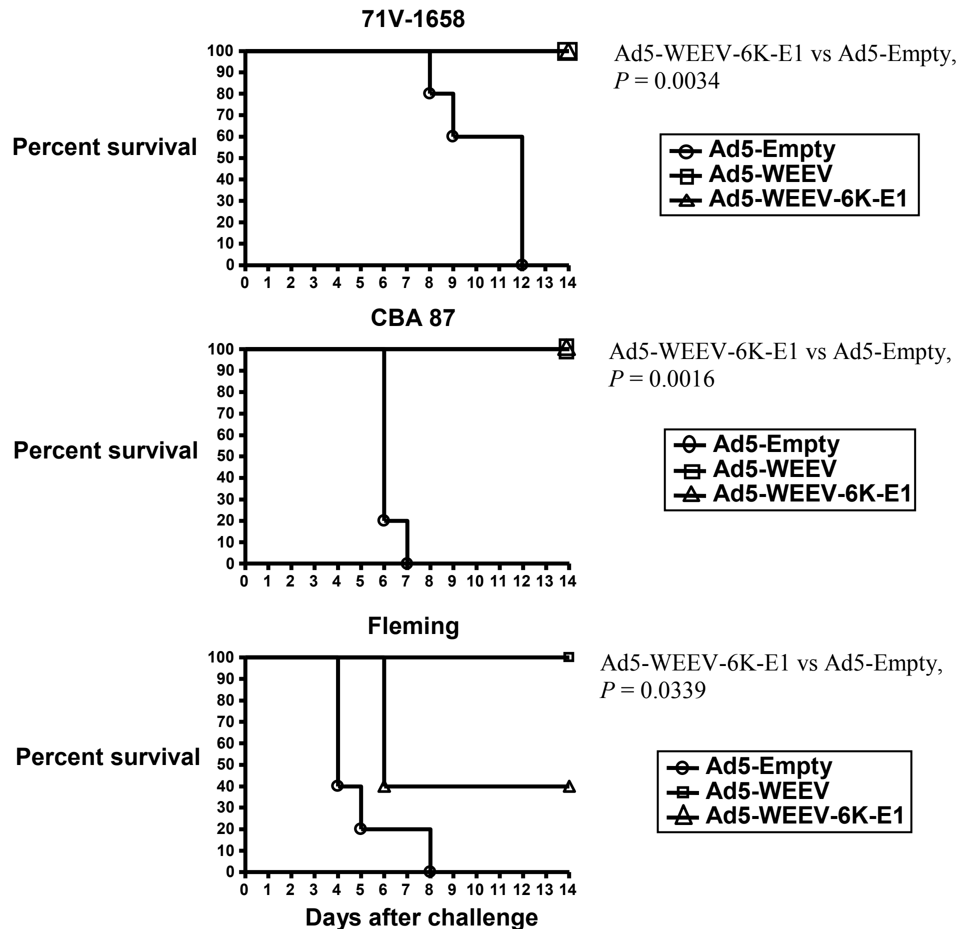

Figure 5 : Kaplan-Meier survival curves of the mice vaccinated with Ad5-WEEV-6K-E1 and challenged with WEEV. The vaccinated mice (5 per group) were challenged intranasally with WEEV strain of 71V-1658, CBA87 or Fleming. The log rank test was used to assess differences in survival rates of the challenged mice.

Figure 5 shows that Ad5-WEEV-6K-E1 provided the same level of protection against the challenge of 71V-1658 or CBA87 as Ad5-WEEV. All the mice vaccinated with Ad5-WEEV-6K-E1 survived at day 14 after the challenge. These mice were completely protected with no signs of infection throughout the 14-day observation period (Table 1). In contrast, all the mice given Ad5-Empty control showed signs of infection as early as day 8 after the challenge (Table 1) and died by day 12. Although Ad5-WEEV-6K-E1 extended the survival time of the Fleming-challenged mice (Table 1), it failed to protect the mice from the death. About 50% of the mice died at day 14 after the challenge (Figure 5) and 80% of the mice had signs of infection by day 8 (Table 1). The result demonstrated that Ad5-WEEV-6K-E1 provided partial protection against the highly virulent Fleming strain of WEEV.

Table 1 : Protection of mice against challenge of various strains of WEEV after a single-dose vaccination with Ad5-WEEV-6K-E1

Challenge virus SD)	Survival time (Mean day ± SD)			Clinical score on day 8 after (Mean ±		
	Ad5-WEEV-6K-E1	Ad5-WEEV	Ad5-Empty	Ad5-WEEV-6K-E1	Ad5-WEEV	Ad5-
71V-1658	14.0 ± 0.0 [a]	14.0 ± 0.0	10.6 ± 1.9	0.0 ± 0.0	0.0 ± 0.0	1.6 ±
CBA-87	14.0 ± 0.0 [b]	14.0 ± 0.0	6.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	4.0 ±
Fleming	9.2 ± 4.4 [c]	14.0 ± 0.0	5.0 ± 1.7	2.8 ± 1.8	0.0 ± 0.0	4.0 ±

Each data point represents the arithmetic mean ± standard deviation for 5 mice.

[a] Ad5-WEEV-6K-E1 vs Ad5-Empty control, $P = 0.0175$.
[b] Ad5-WEEV-6K-E1 vs Ad5-Empty control, $P < 0.0001$.
[c] Ad5-WEEV-6K-E1 vs Ad5-Empty control, $P = 0.0424$.

4. Discussion

In a previous DRDC Suffield study, it was demonstrated that a single dose vaccination with an HAd5 vector expressing the E3-E2-6K-E1 proteins of WEEV offers rapid, complete protection of mice against both homologous and heterologous challenge of WEEV [12]. The current study addressed the question of whether HAd5 vector expressing 6K-E1 would give the same protection that shown by the HAd5 vector expressing E3-E2-6K-E1. It was found that the HAd5 vector expressing the 6K-E1 protein offered the same rapid, complete protection as the HAd5 vector expressing the E3-E2-6K-E1 proteins against the less virulent WEEV strains such as 71V-1658 and CBA87 and partial protection against high virulent WEEV strain such as Fleming.

Das et al. evaluated the potential use of recombinant E1 protein as a vaccine target for WEEV [19]. They found that although the recombinant E1 protein induced both humoral and cellular immune responses after vaccination, it did not provide protection against a lethal dose challenge of WEEV. Recently, DRDC Suffield constructed a DNA vaccine encoding the 6K-E1 protein from the 71V-1658 strain of WEEV. The vaccine provided complete protection of mice against homologous challenge of WEEV after two-dose vaccination (unpublished data). The result from the current study demonstrated that the HAd5-vectored 6K-E1 vaccine rapidly protected mice against both homologous and heterologous challenges of WEEV after given a single dose vaccination, suggesting that the efficacy of WEEV vaccine can be significantly improved when HAd5 vector is used for delivery. In addition, it is unknown whether the inclusion of the 6K coding region in both DNA and HAd5 vectored vaccines enhances efficacy.

WEEV can be grouped into high- and low- virulence pathotypes based on the percentage mortality and mean time to death [20, 21]. This study shows that the HAd5 vector expressing 6K-E1 completely protected mice against low-virulence strains of 71V-1658 and CBA87 but only partially protected against the high-virulence strain of Fleming (Table 1; Figure 5). Compared to the previous DRDC Suffield HAd5 vector expressing the E3-E2-6K-E1 proteins of WEEV [12], Ad5-WEEV-6K-E1 appears to be less effective for cross-strain protection against Fleming. These results indicate that expression of the entire E3-E2-6K-E1 from the HAd5-vectored vaccine to form the heterodimers of E1/E2 proteins may be required to elicit broad immune response against highly virulent WEEV strains such as Fleming.

Currently, an investigational new drug vaccine based on formalin-killed WEEV has been developed and used for laboratory workers at the risk of exposure to WEEV [22]. However, the vaccine only had 50% responder rate in humans after three-dose vaccination [23]. In addition, the immune response induced by the vaccine was maintained in only 20% of recipients by 1 year after the vaccination [23]. Live attenuated vaccine candidates for WEEV have shown to protect animals from WEEV challenge after a single-dose immunization [24–26], but there are concerns of the potential side effects and the risk of reversion to virulent wild-type WEEV. Results from this and previous studies at DRDC Suffield demonstrate that adenovirus-vectored WEEV vaccine is a better alternative for WEEV vaccine development.

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5. Conclusion

The availability of advanced technology to isolate and produce a wide variety of viral agents has increased the possibility of using these agents as biological weapons. In addition, viral endemic diseases compose a significant percentage of non-battle field injuries during operations. For example, dengue fever had a significant impact on the CF in previous operations in East Timor and Haiti. Vaccination is a cost-effective approach to mitigate the threat of viral biotheat agents. However, there are limited number of effective vaccines available for the CF against viral biothreats. DRDC Suffield has been focusing on the development of an improved vaccine for WEEV, a potential biowarfare agent. DRDC Suffield previously developed a single-dose, fast-action vaccine candidate effective against WEEV. The target in the vaccine candidate is the entire envelope proteins of WEEV. This study is designed to define the region of the envelope proteins that confer the protection against WEEV. The results from this study demonstrated that the 6K-E1 envelope protein is sufficient to provide protection against WEEV. DRDC Suffield is in the process of dissecting the minimal portion of 6K-E1 that plays a role in the immune protection against WEEV.

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7. List of symbols/abbreviations/acronyms/initialisms

CAF	Canadian Forces
CMV	cytomegalovirus
DMEM	Dulbecco's modified Eagle media
HAd5	human adenovirus type 5
HBSS	Hank's balanced salt solution
HEK 293A	human embryonic kidney 293A cells
IN	intranasally
LD_{50}	lethal dose in 50% of a population
MAb	monoclonal antibody
MOI	multiplicity of infection
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFU	plaque forming unit
SMB	suckling mouse brain
SV40	simian virus 40
WEEV	western equine encephalitis virus

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Vaccination is a cost-effective medical countermeasure against the threat of viral biological agents and endemic viral diseases. Currently, the number of effective vaccines available for the Canadian Foreces (CF) is limited. We have been focusing on the identification of the vaccine targets for western equine encephalitis virus (WEEV), which belongs to the alphaviral agents which also include Venezuelan equine encephalitis virus and eastern equine encephalitis virus. We previously found that the envelope proteins of WEEV elicit rapid immune protection against the lethal challenge of the virus. In this study, we further defined that the 6K-E1 envelope protein of WEEV is a major target for vaccine development. This finding will aid the design of an improved vaccine for WEEV and other alphaviruses.

La vaccination est une contre-mesure médicale rentable contre les agents biologiques viraux et les maladies virales endémiques qui constituent une menace. À l’heure actuelle, Les Forces canadiennes (FC) disposent d’un nombre limité de vaccins efficaces. Nos efforts ont principalement porté sur la détermination des cibles vaccinales du virus de l’encéphalite équine de l’Ouest (VEEO) qui appartient à la famille des alphavirus, dont font aussi partie le virus de l’encéphalite équine du Venezuela et le virus de l’encéphalite équine de l’Est. Nous avons déjà observé que les protéines d’enveloppe du VEEO induisaient une protection immunitaire rapide contre une provocation létale par le VEEO. Dans la présente étude, nous avons en outre défini que la protéine d’enveloppe 6K-E1 du VEEO constituait une cible importante pour la mise au point d’un vaccin. Cette constatation aidera à concevoir un vaccin amélioré contre le VEEO et d’autres alphavirus.

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vaccine, western equine encephalitis virus, envelope protein