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Protective efficacy of monovalent and trivalent recombinant MVA-based vaccines against three encephalitic alphaviruses

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ABSTRACT

The three encephalitic alphaviruses, western, eastern, and Venezuelan equine encephalitis viruses (WEEV, EEEV, and VEEV) are potential biothreat agents due to high infectivity through aerosol exposure, ease of production in large amounts, and relative stability in the environment. Currently, there is no licensed vaccine for human use to these three encephalitic alphaviruses, and efforts to move vaccine candidates forward into clinical trials have not been successful. In this study, the modified vaccinia Ankara-Bavarian Nordic (MVA-BN®) vaccine platform was used to construct and produce three monovalent recombinant MVA-BN-based encephalitic alphavirus vaccines, MVA-BN-W, MVA-BN-E, and MVA-BN-V. Additionally, a MVA-BN-based construct was designed to produce antigens against all three alphaviruses, the trivalent vaccine MVA-BN-WEV. The protective efficacy of these vaccines was evaluated *in vivo*. Female BALB/c mice were immunized with two doses of each monovalent MVA-BN-based alphavirus vaccine, a mixture of the three monovalent vaccines, MVA-BN-W + E + V, or the trivalent vaccine MVA-BN-WEV at a four-week interval. Two weeks after the booster immunization, the mice were instilled intranasally with 5×10^3 to 1×10^4 plaque forming units of WEEV, EEEV, or VEEV. All mice immunized with monovalent vaccines survived the respective virus challenge without any signs of illness or weight loss, while all the control mice died. The triple mixture of vaccines or the trivalent vaccine also provided 90 to 100% protection to the mice against WEEV and VEEV challenges, and 60% to 90% protection against EEEV challenge. These data suggest that each monovalent MVA-BN-W, MVA-BN-E, and MVA-BN-V is a potential vaccine candidate against respective encephalitic alphavirus and the three monovalent vaccines can be given in a mixture (MVA-BN-W + E + V) or the trivalent vaccine MVA-BN-WEV can serve as a true multivalent vaccine without significantly reducing efficacy against WEEV and VEEV despite slightly reduced efficacy against EEEV challenge.

1. Introduction

Alphaviruses comprise a group of about 31 enveloped viruses with a positive sense, non-segmented single-stranded RNA genome [1,2]. They share basic structural, sequence, and functional similarities, including a genome with two polyprotein gene clusters [2]. The three encephalitic alphaviruses, western, eastern, and Venezuelan equine encephalitis viruses (WEEV, EEEV, and VEEV) are highly pathogenic for both equines and humans and have caused periodic epizootics throughout North, Central, and South America [1,2].

Although the three viruses are naturally transmitted by mosquitoes, accidental laboratory infections [3] and experimental studies in animals [4] have demonstrated that all three alphaviruses are highly infectious by the aerosol route. They can easily be produced in large quantities and unlike many other pathogenic viruses, they are relatively stable (either liquid or dry) in the environment [5]. These characteristics have made the three viruses suitable for weaponization, and as such, they are potential agents of biological warfare interest [6]. No vaccine for human use is currently available for any of the three encephalitic alphaviruses. Live attenuated or formalin-inactivated vaccines used in horses are not suitable due to their high reactogenicity or low immunogenicity in humans respectively.

Modified vaccinia Ankara (MVA) is an attenuated vaccinia virus [7] that is adapted to chicken embryo fibroblasts. MVA-Bavarian Nordic (MVA-BN®), non-replicating in humans and in other mammals [8], is approved as a smallpox vaccine in Canada and in the EU (under the trade names IMVAMUNE® and IMVANEX®

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respectively). Some of the features that make MVA-BN an excellent vaccine platform include its outstanding safety profile in humans, which was demonstrated in several clinical trials [9–14], and its intrinsic adjuvant capacities to induce both humoral and cellular immune responses [15,16]. Finally, the impact of pre-existing vector immunity to MVA is limited, unlike other viral vectors such as adenovirus-based vaccines [17]. MVA-BN therefore has been used as a vector for many different vaccines ranging from infectious diseases to various cancers [18–21].

In this manuscript, monovalent recombinant MVA-BN vaccines for the three encephalitic alphaviruses, WEEV, EEEV, and VEEV were constructed and produced along with a trivalent construct which expresses the antigens of all three viruses. The protective efficacy of vaccines in monovalent, triple mixture (of the three monovalent vaccines), and trivalent formats was evaluated *in vivo*.

2. Materials and methods

2.1. Reagents

All cell culture reagents were purchased from Gibco (Fisher Sci., Ottawa, ON). Virus DNA for PCR was purified using the NucleoSpin Blood QuickPure Kit (Macherey und Nagel, Düren, Germany) and RNA for reverse transcription (RT)-PCR was purified using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). For PCR and RT-PCR amplification of the inserted transgenes, One Taq Polymerase (NEB, Frankfurt, Germany) and the 5'Polymerase (VWR, Darmstadt, Germany) were used.

2.2. Cells and viruses

Vero (CCL-81) and HeLa (CCL-2) cells were obtained from the American Type Culture Collection (ATCC Manassas, VA). Cells were maintained in Dulbecco's modified Eagle media (DMEM) containing 5% (Vero) or 10% (HeLa) heat-inactivated fetal bovine serum (FBS). WEEV Fleming was purchased from ATCC. VEEV Trinidad Donkey (TrD) and EEEV PE6 were kindly provided by Dr. George Ludwig (U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD). Seed stocks of WEEV or EEEV were made by inoculation of Vero cell monolayers with WEEV or EEEV at a multiplicity of infection of 0.1. Stocks of VEEV TrD were made by inoculation of suckling mice and harvesting the brain as a 10% suspension. Supernatants from infected cells were aliquoted and stored at −70 °C. The titers of the alphaviruses were determined by plaque titration on Vero cells. MVA-BN®, deposited at the European Collection of Cell Cultures, Salisbury (UK) under number V00083008 was used for generation of the vaccine candidates. MVA-BN and derived recombinant vaccines were grown in primary chicken embryo fibroblast (CEF) cells (Thermo Fisher/Life Technologies, Darmstadt, Germany) at serum free conditions.

2.3. Construction of MVA-BN-based alphavirus vaccines

The recombinant vaccines encoding the structural proteins E3-E2-6 K-E1 of WEEV (strain 71 V-1658 for vaccine MVA-BN-W), EEEV (strain FL93-939NA for vaccine MVA-BN-E), and VEEV (strain TrD for vaccine MVA-BN-V), respectively, or of all three alphaviruses for the trivalent vaccine MVA-BN-WEV were constructed using the MVA-BN vector.

The cDNAs for the structural protein genes were codon optimized and adapted to avoid large stretches of identity or repeated sequences in or between the genes and synthesized by GeneArt, Regensburg, Germany. For optimal expression, the individual cDNAs were combined with suitable vaccinia early or early/late promoters. For the expression of WEEV and EEEV envelope proteins, the native MVA-BN tandem repeat promoter Pr13.5-long was used [22], whereas for the expression of VEEV envelop protein, the synthetic PrHyb promoter was applied [23]. The trivalent vaccine MVA-BN-WEV was constructed by inserting the envelope genes with their respective promoters of the monovalent constructs into the respective intergenic regions (IGRs) in MVA-BN. This made it necessary to insert both, the WEEV and VEEV envelope cDNAs as a tandem into one IGR, as illustrated in Fig. 1. cDNAs coding for the respective alphavirus envelope proteins E3-E2-6 K-E1 were inserted into the MVA-BN genome following standard methods [24]. The genetically pure stocks were used for produc-

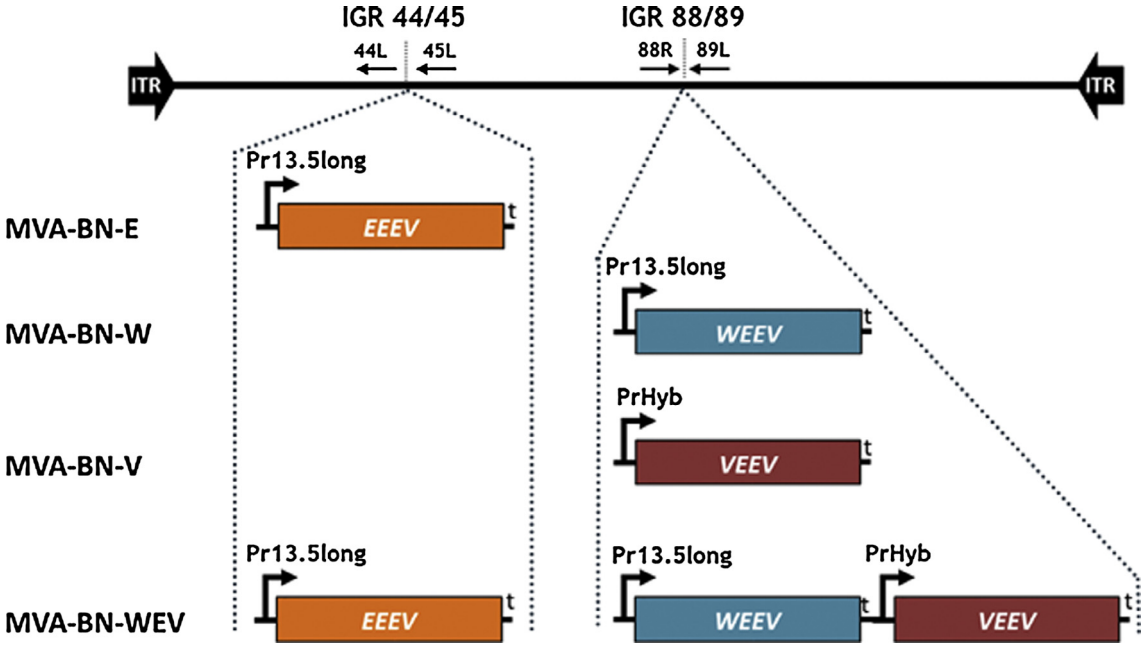

Fig. 1. Overview of vaccines. Four MVA-BN based vaccines were generated using IGR as insertion sites with the flanking MVA genes and directions indicated. The codon optimized sequence of the E3-E2-6 K-E1 envelope proteins of WEEV, EEEV or VEEV was inserted with the pox promoters indicated. t = early transcription termination signal; Pr13.5long = promoter of the MVA 13.5 gene; PrHyb = synthetic hybrid promoter.

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