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Review

Novel vaccination approaches against equine alphavirus encephalitides

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ABSTRACT

The current production of inactivated vaccines for the prevention of equine alphavirus encephalitides caused by Eastern, Western and Venezuelan Equine Encephalitis viruses (EEEV, WEEV, VEEV) involves the manipulation of large quantities of infectious viral particles under biosafety level 3 containment laboratories with the potential risk of transmission to the operators. Moreover, these vaccines are not capable of inducing a long-lasting immunity. Modified live vaccines, which were also attempted, maintain residual virulence and neurotropism, causing disease in both horses and humans. Therefore, the production of an efficacious second generation vaccine which could be used in the prevention of alphavirus infection without the need to manipulate infectious viral particles under high biocontainment conditions could be of great benefit for the worldwide horse industry. Furthermore, equine alphaviruses are considered as biological threat agents. Subunit, chimeric, gene-deleted live mutants, DNA and adenovirus-vectored alphavirus vaccines have been evaluated; such approaches are reviewed in this work.

Climate changes, together with modifications in bird and vector ecology, are leading to the arise of emerging pathogens in new geographical locations, and these zoonotic New World arboviruses are gaining concern. Novel vaccine development does show a promising future for prevention of these infections in both horses and humans.

Contents

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1. Introduction

The alphavirus equine encephalitides are mosquito-borne infections that cause severe neurologic disease and fatality in horses and humans in the Americas [1]. Due to their negative impact on public and equine health, and also because of the potential of Venezuelan Equine Encephalitis virus to spread through movement of infected horses (and people) across borders [2], these diseases are of considerable worldwide concern and are notifiable to the World Organisation for Animal Health (OIE) [3–5]. In addition, equine alphaviruses are considered a potent biological weapon, adding further impetuosity to develop an effective vaccine [6–12].

The disease is caused by Eastern Equine Encephalitis (EEEV), Western Equine Encephalitis (WEEV) or Venezuelan Equine Encephalitis viruses (VEEV), which are related members of the Alphavirus genus in the *Togaviridae* family, but genetically and antigenically distinct [13]. The disease is characterized by fever, anorexia, depression and clinical signs of encephalomyelitis. Case fatality may be up to 90% for both humans and horses, particularly in the case of EEE and VEE [3,14–16]. Surviving horses develop lifelong immunity, but may have permanent neuropathology.

The aim of the present work is to analyze the available scientific information on the development of EEE, WEE and VEE second generation vaccines. Publications regarding experimental approaches on subunit, live chimeric recombinants, gene deleted live mutants, recombinant vectored, DNA and IRES-based vaccines are included.

1.1. Alphavirus epidemiological cycles

EEEV and WEEV are maintained in nature within a sylvatic or enzootic transmission cycle between ornithophilic mosquitoes and birds (passerine and others), where birds participate as amplifying viral hosts (natural reservoir). More than 20 mosquito species are implicated in viral transmission, being *Culiseta melanura* and *Culex tarsalis* the typical EEEV and WEEV vectors, respectively. When a disruption of the endemic cycle occurs, EEEV and WEEV are transmitted to equines and humans *via* mosquitoes, causing an epidemic outbreak. Both horses and humans act as dead-end hosts, and do not develop a high titer viremia [1,3,13,17–20].

In contrast, the enzootic cycle of VEEV takes place between mosquitoes (*Aedes* spp. and *Culex* spp.) and small mammals (principally rodents) [3,16]. It is also important to consider that for VEEV, horses and humans act as amplifying hosts, and develop a high titer viremia capable of transmitting the disease *via* mosquitoes to other horses or humans [1,3,5,16,18–22].

1.2. Diversity of alphaviruses

Due to their antigenic characteristics, different variants and subtypes of EEEV, WEEV and VEEV can be recognized. EEEV is classified into two variants: North (NA) and South (SA) American variants, NA EEEV being more virulent for horses and humans than SA EEEV [3,13,15,20,23,24].

Virulence and genotypic differences have been identified among the WEEV complex [15,25].

The VEEV complex is subdivided into 6 subtypes (I–VI). Subtype I has different variants (IAB, IC, ID, IE and IF). Subtypes IAB and IC are considered epizootic, causing disease in humans and horses. Subtypes ID, IE, IF, II, III, IV, V and VI are enzootic and are not virulent to horses, but may cause disease in humans [16,22,26]. However, VEEV subtype IE was isolated from encephalitic horses in Mexico, and may eventually be considered as a new epizootic variant [2,27]. Cross-immunity between subtypes has been demonstrated [10,13,20,28,29].

1.3. Alphaviruses and their replication cycle

Alphavirus virions have a lipid envelope, which contains glycoprotein spikes constituted by E1 and E2 viral proteins in a heterodimeric conformation and with two important glycosylation sites, surrounding an icosahedral nucleocapsid, formed by repeated subunits of the C protein, which encapsidates the viral genome, without any matrix intermediate [3,18,19,30,31].

The viral genome is a single-stranded, positive-sense RNA which includes two polyprotein gene clusters. The 5′ and 3′ ends encode the four nonstructural (NSP1-4) and viral structural proteins, respectively. The heterodimer formed by glycoproteins E1 and E2 is strongly immunogenic and induces the production of neutralizing antibodies associated with protection [3,18–21,32–36]. A schematic view of the viral structure, genomic arrangement and E1 and E2 heterodimer is shown in Fig. 1.

After viral attachment, mediated by E2, endocytosis and viral envelope-endosome membrane fusion mediated by E1, the nucleocapsid is released into the cytoplasm. Genomic RNA is released, and RNA replication and protein synthesis take place. Structural proteins are synthesized from a subgenomic 26S positive RNA strand, and are translated as a single polyprotein (Capsid-E3-E2-6K-E1), which is post-translationally cleaved into capsid protein (C) and PE2-6K-E1, which suffers posterior cleavage into E1 and E2 glycoproteins. Nucleocapsids are assembled and moved to areas of plasma membrane containing E1 and E2 viral glycoproteins, from where they emerge, obtaining the lipid envelope [18].

1.4. Current marketed vaccines

Control and prevention of equine alphavirus encephalitides is mainly based on vector control, reduction of vector exposure, and vaccination. Current available vaccines for horses are inactivated, and there are no licensed vaccines for general human use [6,16,20,24,37]. Vaccines for human use are limited to US military forces or laboratory workers [6,38,39]. Even though these vaccines generate an acceptable antibody response which ensures protection [34,35], the manufacturing process requires the growth and manipulation of high titer ($>10^6$ $TCID_{50}$) viral preparations, that must be performed in a biosafety level 3 containment laboratory (BSL-3) [4,36], with the consequent high production costs and potential risk of infection by the operators. Therefore, the production of an efficacious second generation vaccine without the need to manipulate infectious viral particles could be of great benefit for both the horse industry and human health (Table 1).

Regarding modified live vaccines, there was a marketed vaccine against VEEV developed for horse and human use, the VEE TC-83, derived from the Trinidad donkey strain (subtype IAB TrD), with 2 effective attenuating mutations [5,13,19,40]. TC-83 vaccine induces protective and durable immunity in horses, but it may cause adverse effects, and nearly 40% of the vaccinated people