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Vaccines for Venezuelan equine encephalitis

Slobodan Paessler*, Scott C. Weaver

Department of Pathology, Center for Biodefense and Emerging Infectious Diseases, and Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555-0609, United States

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ABSTRACT

Arboviruses are capable of causing encephalitis in animals and human population when transmitted by the vector or potentially via infectious aerosol. Recent re-emergence of Venezuelan equine encephalitis virus (VEEV) in South America emphasizes the importance of this pathogen to public health and veterinary medicine. Despite its importance no antivirals or vaccines against VEEV are currently available in the USA. Here we review some of the older and newer approaches aimed at generating a safe and immunogenic vaccine as well as most recent data about the mechanistic of protection in animal models of infection.

1. Venezuelan equine encephalitis virus and its replication

Venezuelan equine encephalitis virus (VEEV, *Alphavirus* in the *Togaviridae* family) is an enveloped virus with a non-segmented, positive-sense RNA genome of approximately 11.4 kb (Fig. 1). The 5′ two-thirds of the genome encodes four nonstructural proteins (nsP1 to nsP4) that form an enzyme complex required for viral replication [1–3]. The full-length RNA then serves as a template for the synthesis of positive-sense genomic RNA and for transcription of a subgenomic 26S RNA [1]. The approximately 4-kb-long, subgenomic RNA corresponds to the 3′ one-third of the viral genome and is translated into a structural polyprotein that is proteolytically cleaved into the capsid and the envelope glycoproteins E2 and E1 [4].

2. Epidemiology of encephalitic alphaviruses

Most of the encephalitic viruses in the Family *Togaviridae*, genus *Alphavirus* are zoonotic pathogens that are transmitted via hematophagous arthropods. These pathogens have a widespread distribution in North, Central and South America (reviewed in [5]). Some of them are highly infectious via the aerosol route, thus have been responsible for numerous laboratory accidents (>150 documented cases without an associated perforating injury) and/or have been developed as a biological weapon in the U.S. and in the former Soviet Union. First virus isolations were reported in the 1930s from diseased horses in California, in Virginia and New Jersey, and from an infected child in Caracas, Venezuela, and were subsequently named based on their region of isolation as Western equine encephalomyelitis virus (WEEV), Eastern equine encephalomyelitis virus (EEEV) and Venezuelan equine encephalomyelitis virus, respectively.

3. Disease in humans

VEEV infection has an incubation period of 2–10 days, which results typically in non-specific flu-like symptoms. Severe encephalitis is a less common outcome of VEEV infection in comparison to EEEV and WEEV infection, although VEEV-associated encephalitis is a more common outcome in children. Neurological disease, including disorientation, ataxia, mental depression, and convulsions can be detected in up to 14% of infected individuals, especially children, although the human case-fatality rate is low (<1%).

4. Mouse model for VEEV infection

The murine model for VEEV-induced disease is established and typically utilizes subcutaneous inoculation [6–9]. Previous studies have demonstrated that the murine model is characterized by biphasic disease, which starts with the productive infection of lymphoid tissue and culminates in the destruction of the CNS by viral replication and a “toxic” neuroinflammatory response that is uniformly lethal [10–16]. By the time the acute encephalitis has developed in an infected mouse, the virus is usually absent from the peripheral organs and blood [10–16]. The mouse model is useful for testing of vaccine and drug efficacy.

* Corresponding author. Tel.: +1 409 7470764; fax: +1 409 7470762.
E-mail address: slpaessl@utmb.edu (S. Paessler).

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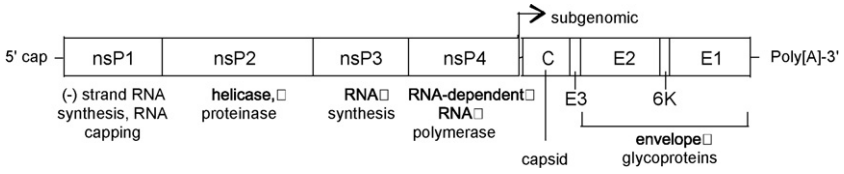

Fig. 1. Genomic organization of Venezuelan equine encephalitis virus.

5. Humoral immunity

Protection from peripheral inoculation or natural alphavirus infection depends mostly on the production of neutralizing antibodies [17,18]. While virus neutralizing antibody is important for the protection against natural (peripheral) challenge mediated by mosquito-borne transmission, more recent studies demonstrate that even relatively high serum titers of polyclonal neutralizing antibody achieved via passive transfer (not achievable with any vaccination known to authors) do not protect mice from intranasal (i.n.) challenge in the mouse model of infection [19,20]. These data supports the conclusion that virus neutralizing antibody plays a significant role in preventing the penetration of the CNS after peripheral challenge with VEEV, while it is relatively ineffective in controlling the rapid onset of CNS disease following i.n. infection [20,21].

6. Alpha Beta (αβ) T cell response

The αβ T cells represent the major proportion of T cells that respond to various pathogens and are subdivided into $CD4^+$ helper and $CD8^+$ cytotoxic cells. These "conventional" T cells have been well characterized functionally. Prior studies in mice vaccinated with TC-83 suggest that Th1-type responses predominate [22]. However, in mice vaccinated parenterally with TC-83, cytotoxic T cell activity could not be detected in the spleen or draining lymph node [23]. It was previously demonstrated that host factors contribute to mortality in neurovirulent Sindbis virus (NSV)-induced encephalitis model in mice [24]. Animals deficient in αβ-, but not γδ-T cells had lower mortality rates when infected with neuroadapted Sindbis virus, indicating their different contribution to the outcome of the brain infection [25]. $CD4^+$ T cell effector functions in encephalitis may include a combination of Th1, Th2 and regulatory T cells ($CD25^+$, forkhead family transcription factor, $Foxp3^+$) activities [26–28]. In other models of acute encephalitis, such as experimental autoimmune encephalomyelitis (EAE) as well as in more chronic encephalitis described for toxoplasmic encephalitis (TE), the myelin basic protein specific $CD4^+$ T cells are crucial for the development of the encephalitis. $CD8^+$ T cells contribute to both pathogenesis of and recovery from encephalitic flavivirus infections [29,30] but their role in VEEV-induced encephalitis, immunopathology and protection is less clear. In general, it has been hypothesized that cell-mediated cytotoxicity is less critical for control of cytopathic viruses such as VEEV in comparison to non-cytopathic viruses [31,32]. This can be extended to the CNS where elimination of virus from neurons is thought to be via non-lytic mechanisms due to the poor regenerative capacity of this cell type [24,32–37].

7. Gamma delta (γδ) T cell response

Recent studies suggest an important role for another well studied T cell subpopulation, γδ T cells, in disease development and lethal outcomes of VEEV infection [19]. Specifically, qualitative and quantitative changes in the inflammatory cellular infiltrates in vaccinated and challenged mice suggest a regulatory role in the secondary response to virus. However, direct evaluation of their role in pathology vs. protection is limited by the lack of feasible methods for isolating sufficient quantities for adoptive transfer. This cell population constitutes a relatively minor subset of T cells in lymphoid tissues despite being well represented in other sites including the peripheral blood and in the epithelial and mucosal layers in the respiratory and gastrointestinal (GI) tract [38,39]. The role of γδ T cells in the CNS has not been functionally well-defined, despite studies showing their potential importance in diminishing the neurovirulence of West Nile Virus (WNV) [40] and modulating the progression of neurocysticercosis in TCR-δ chain-deficient mice [41]. In the case of viral infections, γδ T cells can substitute for αβ T cells in a virus model of demyelination [42]. In the neurovirulent Sindbis virus model, animals deficient in αβ, but not γδ T cells have lower mortality rates, indicating the differential contribution of these cell types to the outcome of the brain infection [25].

8. Live-attenuated VEE vaccines

Following upon the success of the 17D yellow fever vaccine by Theiler [43], VEEV was attenuated by 83 serial passages in guinea pig heart cells to produce the TC-83 strain [44]. TC-83 was first tested extensively in equids during the 1971 Texas VEE epizootic/epidemic, where it may have contributed to limiting the spread northward. Although the vaccine produces viremia, fever and leucopenia in horses, robust neutralizing antibodies are generated as well as protection from VEEV challenge [45]. The TC-83 strain continues to be manufactured in Mexico and Colombia for use as a live vaccine in equids, but is currently only marketed in inactivated form in the U.S.

The history of serious laboratory infections by VEEV as well as its development as a biological weapon in the U.S. and former USSR prompted the use of TC-83 in humans as an investigational new drug (IND) product. TC-83 produces seroconversion in about 80% of humans (neutralizing antibody titers $\geq$20) but mild to moderate flu-like symptoms in about 205 of volunteers. Persons who fail to seroconvert in the U.S. Army Special Immunizations program are given boosters of C-84 (inactivated TC-83) [46]. Unlike TC-83, C-84 produces only occasional, mild, reactions. Some rodent studies indicate that TC-83 protects mice better against aerosol challenge than C-84 [47], although neither completely protects nonhuman primates against aerosol exposure [48].

The reactogenicity and limited immunogenicity of TC-83 may be the result of only 2 attenuating mutations among the 12 mutations that accompanied in vitro passage of the Trinidad donkey strain [49]. Presumably, these mutations are subject to reversion in vaccinees, which also presents a risk of epizootic amplification. To produce a more stably attenuated VEEV vaccine candidate, the Trinidad donkey genome was cloned in cDNA form and attenuation was achieved by inserting either point mutations or a PE2 cleavage-signal mutation combined with an E1 gene resuscitating mutation. The latter strain, called V3526, is safe and immunogenic for mice and nonhuman primates, and appears superior to TC-83 in rodents [50–52]. V3526 also appears to have a lower risk for environmental transmission and distribution [53,54]. Although V3526 elicits neutralizing antibodies in nonhuman primates only against homol-