Video Article

A Murine Model of Dengue Virus-induced Acute Viral Encephalitis-like Disease

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Abstract

Dengue virus (DENV), an arthropod-borne virus transmitted by mosquitoes, may cause the severe disease known as dengue hemorrhagic fever, which is characterized by lethal complications due to plasma leakage, ascites, pleural effusion, respiratory distress, severe bleeding, and organ impairment. A few cases of DENV infection present neurological manifestations; however, studies have not explored DENV-induced neuropathogenesis further. In this study, we present a protocol to use an immunocompetent outbred ICR (Institute of Cancer Research) mouse for investigating the induction of central nervous system (CNS) infection with DENV, followed by the progression of acute viral encephalitis-like disease.

Introduction

DENV, an arthropod-borne virus of the *Flaviviridae* family, contains a positive-sense RNA genome that encodes three viral structural proteins (capsid, premembrane, and envelope) and seven viral nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The four serotypes of DENV (DENV1-4), which infect approximately 390 million people annually, cause a global burden even though governments have directed substantial efforts toward mosquito vector and disease control[1]. Currently, protective vaccines and therapeutic antiviral drugs are under development and require further long-term validation[2]. In clinical practice, although a dengue patient with CNS infection is rare, it needs to be further explored to understand the diversity of dengue disease development[3]. Further investigation and validation are needed; notably, the World Health Organization (WHO) has included the involvement of CNS impairment, such as cognitive impairment, convulsions, encephalopathy, and encephalitis, in the classification of severe dengue[3,4]. Constructing animal models of DENV infection is indispensable for exploring the neuropathogenesis of DENV infection.

For generating CNS infection by DENV, several studies have executed different routes of DENV infection, including (1) intracerebral inoculation of C57BL/6 mice who received 4 x 10^3 plaque-forming units (PFU) of nonadapted DENV3[5,6], (2) intraperitoneal inoculation of BALB/c mice who received 7 x 10^4 PFU of in vitro neuroadapted DENV4[7], (3) intracerebral inoculation of Swiss mice who received 1 x 10^5 PFU of in vivo neuroadapted DENV1[8], and (4) intracerebral and intraperitoneal co-inoculation of ICR suckling mice who received 1 x 10^6 PFU of nonadapted DENV2[9]. According to the findings of these studies[5,6,7,8,9], DENV infection in mice result in viral replication in the brain, leading to acute viral encephalitis-like syndromes, behavioral changes accompanied by limb paralysis and postural instability, CNS neurotoxicity and inflammation, general and localized plasma leakage through the blood-brain barrier (BBB), and lethality. All the results from these studies[5,6,7,8,9] have shown the ability of DENV to infect the CNS and the induction of acute viral encephalitis-like disease following infection.

Based on our current findings[9,10,11,12,13,14,15], we have created a murine model of DENV infection as an in vivo platform to examine the therapeutic efficacy of targeted agents/factors against viral replication, as well as neurotoxicity. Here, we report the protocol utilized to create an immunocompetent outbred ICR mouse to study CNS infection and to monitor the development of neuropathies with different severities caused by DENV. The results show the significant progression of encephalitis-like disease in DENV-infected mice in a time-dependent manner.

Protocol

Experimental protocols of animal study were approved by the Institutional Animal Care and User Committee of the National Defense Medical Center (IACUC number: 16-261), according to guidelines established by the Ministry of Science and Technology, Taiwan.

1. Infection procedure

1. Prepare nonadapted DENV2 (strain PL046) stocks[9] (originally obtained from the Centers for Disease Control in Taiwan, ranging from 2.5 x 10^7 to 1 x 10^9 PFU/mL).
2. Dilute the virus stock to 1 x 10^6 PFU with Roswell Park Memorial Institute (RPMI) 1640 medium to a total volume of 40 µL.
3. Fill one 0.3 mL syringe fitted with a 30 G needle with 10 µL (2.5 x 10^5 PFU) of diluted virus, and fill another 0.3 mL syringe fitted with a 30 G needle with 30 µL (7.5 x 10^5 PFU) of diluted virus.

4. Hold the 7-day-old ICR suckling mouse and conduct the following steps.
    1. For the intracerebral injection, hold the mouse in a prone position by pressing the auricle between the index finger and thumb, and intracerebrally inject 10 µL of diluted virus into the lambda area, the point at the intersection of the sagittal and lambdoid suture[16].
    2. After the intracerebral injection, hold the mouse in a supine position by using the index finger and thumb and intraperitoneally inject 30 µL of diluted virus gently into the murine abdomenl.
    NOTE: To avoid cannibalism, 75% alcohol is used to create a temporary loss of the mother's olfactory sensation. Additionally, covering the litters with their mother's stool and urine is suggested.

5. Lay the suckling mice back in their cages and wait for 5 min to check their safety poststimulation with the avidity of mice, including walking and mother milk sucking.
    NOTE: Most often, mice demonstrate normal activity poststimulation and there does not seem to be any harmful effects following the technical challenge. During this experiment, the animals were maintained by the Institutional Animal Care and User Committee of the National Defense Medical Center, Taiwan.
6. Evaluate the daily progress of the mice in terms of body weight (by using a microgram balance), acute viral encephalitis-like disease (by disease scoring as described in section 2), and survival rate[9,10,11,12,13,14,15].

2. Disease scoring

1. Monitor the grade of acute viral encephalitis-like illnesses. Assign a score of 0 to healthy mice; 1 to mice with minor illness symptoms, including weight loss, reduced mobility, and a hunchback body orientation; 2 to mice that exhibit limbic seizure; 3 to mice the exhibit limbic weakness, including moving with difficulty and anterior limb or posterior limb weakness; 4 for paralysis; 5 for death.
    NOTE: Once the score reaches 3, the disease symptoms are considered to be evolving rapidly.
2. Plot the scoring of the disease, according to step 2.1, for each day as a curve-based figure by using the mean ± SD of the daily test scores in each group.

Representative Results

Severe dengue-associated neurological complications have been reported in patients for dengue pathogenesis[4]. Although these cases are rare in the clinic, creating an immunocompetent murine model of DENV infection can be used not only for studying immunopathogenesis but also for exploring CNS infection, neuroinflammation, neurotoxicity, and acute viral encephalitis-like disease. In this study, according to our current model[9,10,11,12,13,14,15], 7-day-old ICR suckling mice were inoculated concurrently with DENV2 through an intracerebral microinjection (2.5 x 10^5 PFU) and an intraperitoneal injection (7.5 x 10^5 PFU) (**Figure 1**). Following infection, we performed numerous analyses to show the presence of dsRNA replication, viral protein expression, and virus release in the DENV-infected brain[9,10,11,12,13,14,15]. These experiments demonstrated that a successful in vivo model of DENV infection, replication, and release has been established.

To evaluate the induction of acute viral encephalitis-like illnesses[9,10,11,12,13,14,15], we monitored the changes in body weight in DENV-infected mice. Compared with the body weight changes in the mock group (where the mice were inoculated with RPMI medium only), **Figure 2** shows an increase in the mock group but a significant ($P < 0.01$) decrease in body weight in the infected mice. According to the symptoms of encephalitis-like illnesses, including hunchback posture, limbic seizure, limbic weakness, paralysis, and death, as manifested in DENV-infected mice, **Figure 3** shows a significant ($P < 0.05$) increase in clinical score in the DENV-infected mice. The survival assay in **Figure 4** shows a time-dependent reduction in the survival rate ($P < 0.001$) of the DENV-infected mice. The data indicate the establishment of an infectious model of DENV in the CNS, which shows the progression of acute viral encephalitis-like illnesses.

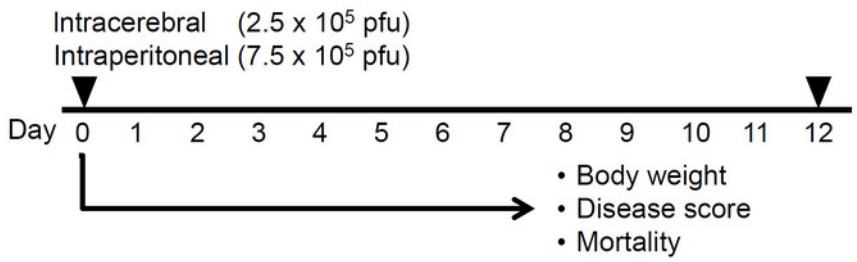

Figure 1: Experimental design for dengue virus (DENV) infection in suckling mice. Seven-day-old ICR suckling mice were inoculated with DENV2 PL046 by concurrent intracranial and intraperitoneal injections. We measured changes in body weight, disease score, and mortality.
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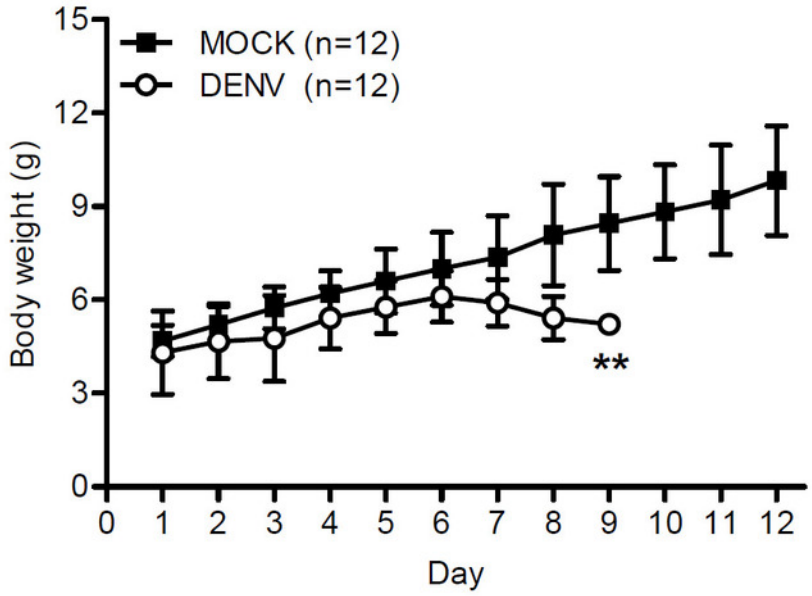

Figure 2: The body weight in suckling mice during dengue virus (DENV) infection. In DENV2-infected ICR suckling mice (*n* = 12), changes in body weight were measured per day. Following a Wilcoxon signed-rank test, the values are presented as the mean ± SD. ***p* < 0.01. Please click here to view a larger version of this figure.

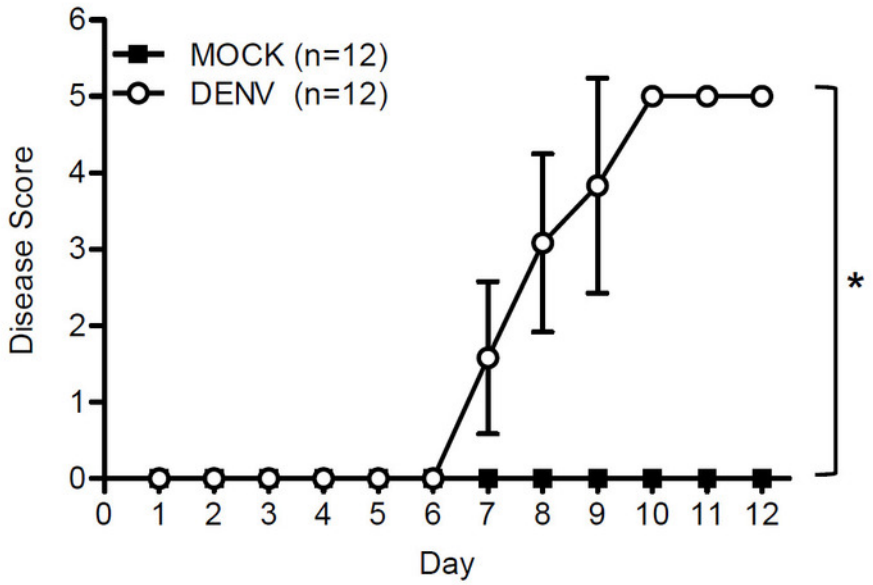

Figure 3: The encephalitic disease score in suckling mice during dengue virus (DENV) infection. Following DENV2 infection in ICR suckling mice (*n* = 12), time-kinetic changes in clinical scores for hunchback posture (score 1), limbic seizure (score 2), limbic weakness (score 3), paralysis (score 4), and death (score 5) were measured. Following a Wilcoxon signed-rank test, the values are presented as the mean ± SD. **p* < 0.05. Please click here to view a larger version of this figure.

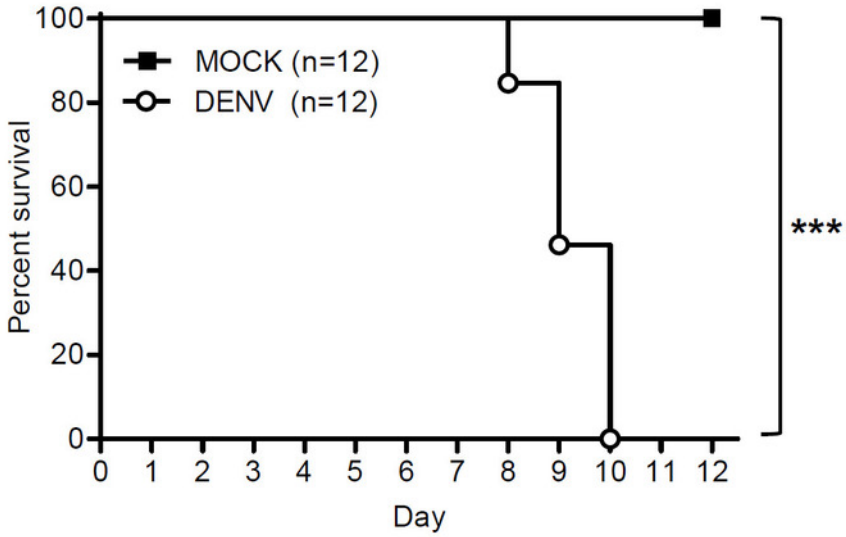

Figure 4: The survival rate in suckling mice during dengue virus (DENV) infection. Time-kinetic changes in survival rates were measured in DENV2-infected ICR suckling mice (*n* = 12). Following a log-rank test, the values are presented as the mean ± SD. ***$p < 0.001$. Please click here to view a larger version of this figure.

Discussion

DENV infection has been detected in the CNS of severe dengue patients[3,17], suggesting the possibility of the manifestation of acute viral encephalitis occurred during dengue pathogenesis. Here, we report an in vivo murine model of DENV infection for studying the involvement of CNS dysfunction in severe dengue, particularly with a focus on DENV-induced acute viral encephalitis-like illnesses. As compared with the previous models, particularly for one-route infection (intravenous only, intraperitoneal only, or intracerebral only), an extremely high titer (1×10^8 PFU) of DENV is utilized in immunocompetent mice. However, in immunocompromised mice, a relatively low but variant titer (1×10^5 to 1×10^8 PFU) of DENV can be performed[18,19,20]. In this study, we used two-route infection to induce concurrent CNS and systemic infection with a low titer (intracranial: 2.5×10^5 PFU; intraperitoneal: 7.5×10^5 PFU) of DENV in immunocompetent ICR suckling mice. The progression of encephalitis-like disease is completely reproducible[9,10,11,12,13,14,15]. The possible limitation of this study is, therefore, mitigated by using a concurrent intracranial and intraperitoneal injection. Although it is artificial-however, consistent with previous works by others[5,6,7,8] and us[9,10,11,12,13,14,15]-DENV is actively replicated in the experimental murine brain[9,10,11,12,13,14,15], and the possible effects of viral factors and host responses on neuroinflammation, as well as neurotoxicity, are, therefore, of interest.

For neuropathogenesis, the stimulation of neuronal cell apoptosis is triggered by DENV infection as well as CNS inflammation, such as microglial cell activation followed by proinflammatory cytokine tumor necrosis factor-α (TNF-α) production[8,12,15]. Direct neurotoxicity caused by DENV infection and indirect neuroinflammation initiated by host immune responses are speculated to be involved in dengue encephalitis. Acute viral encephalitis is caused by virus-induced CNS inflammation of the brain[3,21]. Common clinical symptoms are a high fever, headache, stiff neck and back, vomiting, and confusion, while severe cases also develop seizures, paralysis, and coma[21]. By monitoring the changes in scores with hunchback posture, limbic seizure, limbic weakness, paralysis, and death, the development of these illnesses shows the progression of acute viral encephalitis-like disease in DENV-infected mice[9,10,11,12,13,14,15]. Generally, those DENV-infected mice with a score of 3 (according to the disease scoring method presented in the protocol) rapidly progressed to paralysis and death. The pathogenic mechanisms causing these illnesses require further investigation; however, it is speculated that neuronal dysfunction followed by neuropathy induction is involved[21]. Targeting viral replication, as well as TNF-α-mediated CNS inflammation[10,11,12,13,14], at least in part, confers therapeutic effects against the progression of dengue-associated acute viral encephalitis-like disease. The protocol of this study could be used for creating an encephalitis-like pathological model of DENV infection.

Some studies have described the induction of intracerebral hemorrhage induced by DENV infection in the brain[22,23]. We previously showed the induction of BBB disruption in DENV-infected mice[9]. Although DENV is not recognized as a neurotropic virus, DENV can produce an effective infection in neuronal cells in vivo and in vitro[8,11]. Dopamine D2 receptor (D2R) is speculated to be a neuronal receptor for DENV infection, and pharmacologically targeting D2R effectively reduces DENV infection in neuronal cells in vivo and in vitro, as well as DENV-induced encephalitic illnesses[11,24]. Possible therapeutic strategies against acute viral encephalitis are important to develop so that severe dengue cases with neurological complications can be treated. For its future application, this protocol of a disease model using DENV infection in the brain may become an in vivo platform to screen not only the viral and host factors associated with neuropathogenesis but also possible antiviral and anti-encephalitic drugs. Regarding the incidence of variant neurological disorders caused by DENV infection[25] by this established protocol, the difference between the variable types and/or strains of DENV for causing neurological dysfunction is needed for validation.

Disclosures

The authors have nothing to disclose.

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