Veterinary Microbiology 195 (2016) 70–77

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Mouse model for the Rift Valley fever virus MP12 strain infection

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ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form 25 August 2016
Accepted 20 September 2016

Keywords:
Rift Valley fever virus
Mouse model
MP12 vaccine strain

ABSTRACT

Rift Valley fever virus (RVFV), a Category A pathogen and select agent, is the causative agent of Rift Valley fever. To date, no fully licensed vaccine is available in the U.S. for human or animal use and effective antiviral drugs have not been identified. The RVFV MP12 strain is conditionally licensed for use for veterinary purposes in the U.S. which was excluded from the select agent rule of Health and Human Services and the U.S. Department of Agriculture. The MP12 vaccine strain is commonly used in BSL-2 laboratories that is generally not virulent in mice. To establish a small animal model that can be used in a BSL-2 facility for antiviral drug development, we investigated susceptibility of six mouse strains (129S6/SvEv, STAT-1 KO, 129S1/SvlmJ, C57BL/6J, NZW/LacJ, BALB/c) to the MP12 virus infection via an intranasal inoculation route. Severe weight loss, obvious clinical and neurologic signs, and 50% mortality was observed in the STAT-1 KO mice, whereas the other 5 mouse strains did not display obvious and/or severe disease. Virus replication and histopathological lesions were detected in brain and liver of MP12-infected STAT-1 KO mice that developed the acute-onset hepatitis and delayed-onset encephalitis. In conclusion, the STAT-1 KO mouse strain is susceptible to MP12 virus infection, indicating that it can be used to investigate RVFV antivirals in a BSL-2 environment.

1. Introduction

Rift Valley fever virus (RVFV), the causative agent of Rift Valley fever (RVF), belongs to the genus *Phlebovirus*, in the *Bunyaviridae* family and is classified as a Category A pathogen designed by the NIH/NIAID (Bishop and Shope, 1979; Georgiev, 2009). RVF is an acute, fever-causing zoonotic disease that affects humans and animals such as cattle, buffalo, sheep, goats, and camels. RVFV was first reported in 1931 from a farm of Rift Valley in Kenya (Daubney et al., 1931). The virus has been circulating in the countries of the African continent and Madagascar, causing great economic loss and human deaths. The first RVF outbreak outside Africa was confirmed in 2000 in the Arabian Peninsula, resulting in loss of livestock and more than 200 human deaths out of approximately 2000 hospitalized patients (Balkhy and Memish, 2003; Shoemaker et al., 2002). This fact raised concern that the disease could spread worldwide (Ahmed et al., 2009).

The RVFV MP12 attenuated strain provides a BSL-2 model virus for preliminary investigations of RVFV prior to using virulent RVFV strains that are required to be handled in an expensive high biosecurity BSL-3 facility (Gaudreault et al., 2015). This attenuated strain was obtained through 12 serial passages of a virulent isolate ZH548 in the presence of 5-fluorouracil and has been shown to be safe and efficacious against virulent RVFV challenge in sheep (Morrill et al., 1991, 1987), cattle (Morrill et al., 1997a,b) and macaques (Morrill and Peters, 2003). However, a previous study showed that this vaccine still can cause abortion in pregnant ewes (Hunter et al., 2002). MP12 is classified as Risk group 2 pathogen and commonly used in BSL-2 laboratories (Ikegami et al., 2015). MP12 is highly attenuated in mice at immunogenic doses (Caplen et al., 1985). However, it may cause tissue pathology in some strains of mice such as BALB/c and C57BL/6 mice when administered at high doses (Indran et al., 2013; Lihoradova et al., 2013). When RVFV natural hosts, such as cattle and lambs,

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http://dx.doi.org/10.1016/j.vetmic.2016.09.009
0378-1135/

were infected with a higher dose (10^6 PFU) of MP12 virus, clinical disease and viremia were not observed in inoculated animals (Wilson et al., 2014), indicating that they are not good models for evaluating antivirals using RVFV MP12 virus in a BSL-2 environment. It has been reported that all strains of RVFV remain closely related at the nucleotide and amino acid level (Ikegami, 2012), suggesting that antiviral drugs or vaccines effective against one strain may also be effective against all strains. To date, there is no fully licensed vaccine for human or animal use in the U.S. and effective antiviral drugs have not been identified (Eddy et al., 1981; Hunter et al., 2002; Ikegami, 2012; Liu et al., 2008). It is urgent to develop efficacious antivirals and vaccines to protect public and animal health. However, limited laboratories can perform efficacy testing of RVF antivirals and vaccines and have access to the virulent RVFV strains, since high biocontainment facilities are required and the cost for performing antiviral test is also very expensive. Based on above knowledge, our objective was to establish a mouse model susceptible to infection with the MP12 vaccine strain in order to identify and evaluate antiviral compounds against RVFV in a BSL-2 level facility. BALB/c mice are highly susceptible to the virulent RVFVs such as the ZH501 strain, displaying acute-onset liver disease and delayed-onset encephalitis (Smith et al., 2010). Mouse strains including NZW/LacJ, 129S1/SvlmJ, and C57BL/6J have been reported to be susceptible to infection with Punta Toro virus, another phlebovirus in the *Bunyaviridae* family (Ashley et al., 2011; Mendenhall et al., 2009). Furthermore, MP12 is able to infect and cause mortality in Sv129 IFN α/β receptor −/− (interferon α/β receptor knockout) mice that have been used to study vaccine efficacy (Boshra et al., 2011; Bouloy et al., 2001; Lorenzo et al., 2010). Therefore, we selected and screened six strains of mice [129S6/SvEv, STAT-1 KO (129S6/SvEv-Stat1^{tm1Rds}), 129S1/SvlmJ, C57BL/6J, NZW/LacJ, and BALB/c] to test their susceptibility to the MP12 strain in this study. The mouse strain most susceptible to the MP12 vaccine strain infection will likely be selected for further testing the efficacy of antiviral compounds in future studies.

2. Materials and methods

2.1. Virus and cells

African green monkey kidney epithelial (Vero) E6 cells (ATCC-CRL 1586) were grown in 1 x DMEM medium containing 10% fetal bovine serum (FBS, Atlanta Biologicals). The RVFV MP-12 strain was kindly provided by the US Army Medical Research Institute for Infectious Diseases. The virus was propagated and passaged 3 times in Vero E6 cells. Cells were infected with 0.01 multiplicity of infection (MOI) of the virus in the medium of DMEM with 10% FBS. Cell cultures were collected when 80–95% of the infected cells showed cytopathogenic effect (CPE). Titer of viral stock was determined and calculated as tissue culture infective dose 50 ($TCID_{50}$) based on the presence of cytopathic effect.

2.2. Animals

Six groups (14 mice/group) of seven-week-old female mice were obtained from different companies. 129S6/SvEv and STAT-1 KO mice were purchased from Taconic, Hudson, NY; 129S1/SvlmJ, C57BL/6J and NZW/LacJ mice were purchased from Jackson Lab, Harbor, ME; BALB/c mice were purchased from Charles River Laboratories, Wilmington, MA. All animal procedures used in this study complied with guidelines set by the USDA and were approved by Kansas State University Animal Care and Use Committee.

2.3. Mouse infection experiment

Mice were maintained in a mouse vivarium at the USDA, ARS, ABADRU, Manhattan, KS. Twelve mice of each group were intranasally inoculated with 1.58×10^6 $TCID_{50}$ of the MP12 virus in a volume of 70 μl (35 μl per nostril) under anesthesia using isoflurane. The remaining two mice of each group served as negative controls. Mice were weighed every 2 days and observed daily for clinical symptoms. On 3 and 6 days post inoculation (dpi), three inoculated mice and one negative control from each group were euthanized and necropsied. The remaining six mice of each group were kept for 14 days. If a mouse has more than 25% weight loss or showed signs of neurologic disease, it was humanely euthanized and necropsied. During necropsy, livers, brains, spleens, and blood from each mouse were collected under sterile conditions. The half of liver or brain from each mouse collected and stored at −80 °C for later virus detection, and the part of these tissues and spleen was fixed in 10% formalin for histopathologic analysis (Liu et al., 2011). Tissues were homogenized twice in PBS for 1 min in a Mini BeadBeater-8 (Biospec Products) to make 10% tissue homogenates. The homogenate was centrifuged at 640*g* for 5 min, and the supernatant was transferred to 1.5 ml reaction tubes for virus isolation and titration in Vero E6 cells as described previously (Richt et al., 2003). The serum was isolated from collected blood samples for further viremia analysis using the real-time RT-PCR assay.

2.4. RNA extraction and real-time RT-PCR

Total RNA was extracted from serum samples collected from each mouse using the Applied Biosystems MagMAX-96 total RNA Isolation Magnetic-bead capture kit according to the manufacturer's protocols. Briefly, 130 μl of lysis/binding buffer was mixed with 50 μl of sera followed by shaking in a 96-well plate. Then 20 μl bead mix was added and the mixture was shaken for 5 min. After washing (150 μl each) for four times, the RNA was eluted in 50 μl of elution buffer at 65 °C. RNA was quantitated using UV spectroscopy.

Real-time RT-PCR was performed to detect viral RNA of serum samples using the AgPath ID rRT-PCR Kit (Life Technologies, Inc., Grand Island, NY) as described previously (Wilson et al., 2013). Briefly, the RVFV triplex rRT-PCR procedure was designed to contain L, M and S primers and probes and one of the external RNA control combinations using the following cycling conditions: 45 °C for 10 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 sec and 60 °C for 1 min. The Ct value of at least two of the three RVFV segments from one sample below 35 cycles was considered positive.

2.5. Histopathological and immunohistochemical analysis

Tissues including liver, brain and spleen in 10% formalin buffer were routinely processed, sections were cut at 4 μm thick on a microtome and stained with hematoxylin and eosin. The amount of necrosis and inflammation were graded microscopically. In the spleen and liver samples, a 5 point scale was used: 0 = No necrosis, 1 = Occasional scattered single cell necrosis, 2 = Scattered 0.5–2 mm foci of random necrosis, 3 = Scattered larger random area of necrosis, often coalescing with each other, 4 = Diffuse lobular necrosis. In the spleen, necrosis was seen as lymphocytolysis in periarteriolar sheaths. In the brain samples, a 4-point scale was used: 0 = No necrosis, 1 = one focal area of necrosis and inflammation, 2 = 2-5 areas of necrosis and inflammation, 3 ≥ 6 areas of necrosis and inflammation.

For immunohistochemical analysis, tissues were deparaffinized and rehydrated, antigen retrieval was performed in pH 9.0 EDTA