Veterinary Microbiology 203 (2017) 117–124

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Equine herpesvirus type 1 induces both neurological and respiratory disease in Syrian hamsters

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ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 2 March 2017
Accepted 3 March 2017

Keywords:
EHV-1
Brain
Encephalitis
Rhinopneumonitis
Horses
Neurovirulence

ABSTRACT

The equine herpesvirus type 1 (EHV-1) is an important cause of myeloencephalopathy and respiratory disease in horses. Animal models for EHV-1 infection have been specially developed using mice and Syrian hamsters (*Mesocricetus auratus*). However, few studies have attempted to evaluate the pathogenesis of EHV-1 infection in the central nervous system (CNS) and respiratory system of hamsters. Therefore, the aim of this study was to evaluate the pathogenesis of four Brazilian EHV-1 strains within the CNS and lungs of Syrian hamsters. Hamsters intranasally infected with A4/72, A9/92, A3/97, and Iso/72 EHV-1 strains developed severe neurological and respiratory signs and died during acute EHV-1 infection within 3 to 5 days post-inoculation. However, neurological signs were more severe in A4/72 and A9/92-infected hamsters, whereas respiratory signs were more prominent in A3/97 and Iso/72-infected hamsters. In the latter, lesions in the CNS were predominantly inflammatory, whereas in A4/72 and A9/92-infected hamsters, neuronal and liquefactive necrosis were the predominant lesions. EHV-1 infected hamsters also developed an interstitial pneumonia with infiltration of alveolar septa by macrophages, neutrophils, and lymphocytes, with the exception of A9/92-infected hamsters, which developed severe hemorrhages within the airways. EHV-1 antigens were detected along with CNS and pulmonary lesions. EHV-1 was also recovered from CNS of all infected hamsters, whereas the virus was recovered from the lungs of A4/72, A9/92, and Iso/72-infected hamsters. Brazilian EHV-1 strains caused both severe CNS and respiratory disease in hamsters, thus making this species an interesting model for EHV-1 infection in the CNS and respiratory system.

1. Introduction

Equine herpesvirus type 1 (EHV-1) is an alphaherpesvirus that causes a broad range of manifestations in horses, including a central nervous system (CNS) disease involving the spinal cord and brain (myeloencephalopathy), respiratory disease, abortions, and perinatal death (Ostlund, 1993). EHV-1 was classified by the US Department of Agriculture (USDA) as a potentially emerging pathogen, since there have been an increase in the frequency of myeloencephalopathy outbreaks in North America and Europe (USDA APHIS, 2007).

In horses, EHV-1 initially infects nasal epithelial cells, which could result in respiratory disease (Pusterla and Hussey, 2014). After replication in the nasal epithelium, EHV-1 infects leukocytes in the local lymph nodes and establishes a cell-associated viremia, which contributes to infection of endothelial cells within the CNS, resulting in thrombosis, inflammation, and necrosis (Pusterla and Hussey, 2014).

Several animal models for EHV-1 infection have been developed, with many of them using mice and Syrian hamsters (Stokes et al., 1989; Awan et al., 1990). Suckling hamsters have primarily been used for virus isolation purposes and to study the possible effects of the equine abortion virus, later known as EHV-1

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http://dx.doi.org/10.1016/j.vetmic.2017.03.007
0378-1135/

(Anderson and Goodpasture, 1942; Doll et al., 1956). Since then, few studies have evaluated EHV-1 pathogenesis in hamsters. EHV-1 can replicate within the liver and lungs, reaching a peak at 4 days post-inoculation (dpi) in hamsters inoculated intranasally or intraperitoneally (Stokes et al., 1989). Hamsters infected with EHV-1 developed neurological signs, whereas mice exhibited only weight loss (Tsujimura et al., 2006), which demonstrates that hamsters might be more susceptible to some EHV-1 strains in comparison to mice. In contrast to EHV-1 studies, Syrian hamsters have been widely used to study the pathogenesis of equine herpesvirus type 9 (EHV-9), which is closely related to EHV-1 (Fukushi et al., 2000; El-Habashi et al., 2011a, 2011b, 2013; El-Nahass et al., 2011, 2012). Hamsters inoculated intranasally with EHV-9 developed a lymphocytic meningoencephalitis with neuronal degeneration, gliosis, and perivascular aggregates of lymphocytes, plasma cells, and neutrophils (El-Habashi et al., 2011b). These hamsters also developed a moderate interstitial pneumonia with bronchitis and bronchiolitis (El-Habashi et al., 2011b). The lesions described in EHV-9-infected hamsters (El-Habashi et al., 2011b) were very similar to those observed in mice infected with different Brazilian EHV-1 strains (Mori et al., 2012). In addition to showing a high neurotropism and neurovirulence in a mouse model, Brazilian EHV-1 strains also caused lesions in the lungs of mice intranasally infected with EHV-1 (Mori et al., 2012).

Although some studies have evaluated some aspects of EHV-1 infection in hamsters (Anderson and Goodpasture, 1942; Doll et al., 1956; Stokes et al., 1989; Tsujimura et al., 2006), few studies have attempted to evaluate the pathogenesis of EHV-1 that might affect both the CNS and respiratory system of hamsters. Therefore, the aim of the present study was to evaluate the pathogenesis of four EHV-1 strains within the CNS and lungs of Syrian hamsters.

2. Material and methods

2.1. Equine herpesvirus type 1 (EHV-1)

The EHV-1 strains A4/72, A9/92, A3/97, and Iso/72 used in this study were isolated from aborted equine fetuses in Brazil. All isolates were confirmed to be EHV-1 by PCR amplification and DNA sequencing of the unique transcriptional regulator and gH genes as previously reported (Varrasso et al., 2001; Mori et al., 2012).

Stock viruses were propagated in E. Derm cells (CCL-57, ATCC) provided by the Biological Institute, Sao Paulo, Brazil, and then maintained in Eagle's Minimal Essential Medium (EMEM) supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2.

EHV-1 strains Iso/72, A9/92, and A3/97 were passaged in vitro a maximum of 3 times and were consequently described as low-passage strains. EHV-1 strain A4/72 was propagated in vitro at passage number 14.

2.2. Animals and experimental design

Sixty-five, 3-week-old, male Syrian hamsters (*Mesocricetus auratus*) were obtained from the animal facility of the Department of Pathology, School of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, Brazil. All animals were housed at the animal facility at temperatures of 22 to 24 °C, humidity of 40–60% and a 12:12 h light:dark cycle. Control hamsters were housed separately from experimentally infected animals in polycarbonate cages with filtered tops. Commercial pellets and autoclaved water were given *ad libitum*. All procedures were approved by the Ethics Committee for the Use of Animals of FMVZ-USP (protocol number 3101/2013).

Animals were separated into four different experimental groups according to the viral strain used. Thirteen hamsters from each group were intranasally inoculated with four different EHV-1 strains (A4/72, A9/92, A3/97, Iso/72). Animals were anesthetized with sevoflurane and inoculated with 50 μl of EMEM containing 10^4 $TCID_{50}$ of each virus. Control animals were inoculated with 50 μl of EMEM identically as described for the experimental group. Hamsters were monitored twice daily until 5 dpi, and the body weight of each hamster was recorded daily. Hamsters showing severe neurological or respiratory signs were euthanized by xylazine and ketamine overdose.

2.3. Necropsy and sampling

Five hamsters from each group were euthanized as described previously, and samples of CNS, lungs, liver, spleen, and thymus were submitted to virus isolation. Samples of CNS and lungs from these animals were also submitted to histology. In addition, eight euthanized hamsters were used to perform a bronchoalveolar lavage (BAL). The CNSs from these animals were submitted to histology. A complete necropsy was performed in all hamsters and gross changes were recorded.

2.4. Virus isolation and identification

Virus isolation was attempted with samples from lungs and brains collected at the time of necropsy examination. Homogenates of tissue were prepared by maceration and resuspended in EMEM. Isolation was performed by inoculation of E. Derm cell monolayers. Inoculated cells were incubated at 37 °C, and the monolayers were then observed daily for 7 days for the cytopathic effect (CPE). When these cells exhibited CPE, the identification of isolates was performed by PCR as previously reported (Varrasso et al., 2001; Mori et al., 2012).

A single nucleotide polymorphism in ORF30 (G/A_{2254}) showed a strong association with neuropathogenic and non-neuropathogenic EHV-1 isolates (Nugent et al., 2006). Therefore, to verify if these viruses belong to the neuropathogenic or non-neuropathogenic subtypes, a genetic sequencing of ORF30 (encoding the catalytic subunit of DNA polymerase) from the different EHV-1 isolates was performed as previously described (Mori et al., 2015)

2.5. Histology and immunohistochemistry

The CNS, lungs, liver, and spleen from control hamsters and hamsters inoculated with different EHV-1 strains were fixed in 10% neutral buffered formalin for 48 h and were processed by the routine methods for histology. Sections of 5 μm were stained with hematoxylin and eosin. CNS sections were taken from the olfactory bulb (OB), pyriform cortex, septo-striatal, rostral and caudal diencephalon, and rostral mesencephalon and cerebellum with cerebellar peduncles. Histological lesions were classified as mild, moderate, or severe.

Immunohistochemistry for EHV-1 antigens were performed on CNS and lungs of control hamsters and hamsters inoculated with EHV-1 strains similar as previously described (Mori et al., 2012).

2.6. Bronchoalveolar lavage (BAL)

After euthanasia, the lungs of control animals and hamsters inoculated with different EHV-1 strains were washed twice. For this purpose, a needle was inserted and fixed within the trachea. On the first lavage, 2 ml of phosphate-buffered saline (PBS) pH 7.4 was introduced within the lungs, and the BAL fluid was recovered. On the second wash, 1 ml of PBS was used. The total amount of recovered BAL fluid was homogenized into one sample, and the