J. Comp. Path. 2017, Vol. 157, 67–74

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EXPERIMENTALLY INDUCED DISEASE

Effect of Mouse Strain on Equine Herpesvirus 9 Infection

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Summary

The infectivity of equine herpesvirus (EHV)-9 has been studied in different animal models including immunocompromised animals. The current study focused on the infectivity of EHV-9 in different mouse strains (C3H, C57BL, DBA, BALB/c-nu/nu, BALB/c and ICR) by intranasal inoculation of 2×10^6 plaque forming units (PFU). Various organs, including head and lungs, were collected 7 days post infection (dpi) to investigate microscopical lesions and the distribution of EHV-9 antigen. Immunopositivity of tissue sections was scored using ImageJ software. Open reading frame (ORF) 30 expression in lung tissues was quantified using quantitative reverse transcriptase polymerase chain reaction. Pathological examination revealed different degrees of rhinitis in the different mouse strains. Severe rhinitis was detected in C3H and BALB/c-nu/nu strains, moderate rhinitis was observed in C57BL and DBA strains and no lesions were detected in BALB/c mice. Immunopositivity for EHV-9 antigens was detected in the olfactory epithelium of C3H and BALB/c-nu/nu strains. Compared with C57BL, DBA, BALB/c-nu/nu, ICR and BALB/c strains, the C3H strain showed greater expression of EHV-9 antigens in the brain. The proportion of areas with high positive to positive immunoreactivity for EHV-9 were 7.57, 3.42, 3.12, 2.51, 1.79 and 0.03% for C3H, C57BL, DBA, BALB/c-nu/nu, ICR and BALB/c strains, respectively. The proportions of areas with low positive to negative immunoreactivity were 92.42, 96.70, 96.87, 97.48, 98.16 and 99.96%, respectively. The highest relative expression levels for EHV-9 ORF30 in the lungs were in C3H mice. No significant differences in the expression of ORF30 were observed in other strains. In conclusion, of the strains examined, C3H, C57BL, DBA, BALB/c-nu/nu and ICR were the most susceptible to EHV-9 infection, and the BALB/c strain was less susceptible.

Keywords: equine herpesvirus-9; immunohistochemistry; infectivity; mouse strain

Introduction

Equine herpesvirus (EHV)-9 is considered to be the newest member of the family Herpesviridae. It was isolated from an outbreak in Thomson's gazelles (*Gazella thomsoni*). Initially, the infectivity of EHV-9 was evaluated by intranasal inoculation of different experimental animals (Yanai *et al.*, 1998, 2003; Fukushi *et al.*, 2000; Narita *et al.*, 2000; Taniguchi *et al.*, 2000a,b; Kodama *et al.*, 2007; El-Habashi *et al.*, 2011a).

The characteristic lesion associated with EHV-9 infection is fatal encephalitis. Recently, the virus has been detected in polar bears and giraffes in the USA (Schrenzel *et al.*, 2008; Donovan *et al.*, 2009). The wide host range of EHV-9 facilitates transmission by the natural route (El-Habashi *et al.*, 2011a), thereby raising the risk of the virus spread.

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2017.06.002

The aim of this study was to compare and evaluate the susceptibility of six different mouse strains (C3H, C57BL, DBA, BALB/c-nu/nu, ICR and BALB/c) to EHV-9 infection. The virus susceptibility was assessed by quantifying the immunoexpression of EHV-9 antigens and molecular expression of EHV-9 open reading frame (ORF) 30 in lung tissues using ImageJ analysis and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), respectively.

Materials and Methods

Virus Culture

EHV-9 was propagated in Madin–Darby bovine kidney (MDBK) cells. EHV-9 stocks used for the inoculation were obtained from cultures of MDBK cells infected with the virus seed stock P19 (5^{th} passage). The virus titre was determined by plaque-forming assays on MDBK cells.

Mice and Virus Inoculation

A total of 48 male mice (C3H, C57BL, DBA, BALB/c-nu/nu, BALB/c and ICR strains), all aged 4 weeks, were divided into six equally-sized groups; one strain for each group. Six mice from each group were inoculated with a single dose of 2×10^6 plaque-forming units (PFU) of EHV-9 and the remaining two were designated as controls. The mice were killed at 7 days post infection (dpi). The experimental protocol was approved by the Animal Experiment Committee, Faculty of Applied Biological Science, Gifu University, Japan.

Pathology and Immunohistochemistry

Sagittal sections from the head and lungs were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE). Immunohistochemistry (IHC) was performed using the avidin–biotin complex (ABC) method (Yanai *et al.*, 1998).

Image Analysis

Images of sections of the head subjected to IHC were captured using a digital camera (Leica, DM2500M, Leica, Wetzlar, Germany). Examination and analysis of labelling was performed using a freeware version of ImageJ (1.51d) (Varghese *et al.*, 2014). The ImageJ software was used to measure the integrated intensities (in pixels) of EHV-9 labelling in the olfactory areas.

Polymerase Chain Reaction

RNA was extracted from formalin-fixed and paraffin wax-embedded (FFPE) sections of lung using the RNeasy FFPE Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions. Total RNA (1.5 μg) was added to a reverse transcriptase master mix consisting of 2 μl of 10× RT buffer (TOYOBO, Osaka, Japan), 5 mM dNTP (TAKARA Bio Inc., Otsu, Japan), 25 pmol of random primers (TOYOBO), 40 U of RNase inhibitor (TOYOBO) and 50 U of reverse transcriptase (TOYOBO). The reaction mixture was incubated at 30°C for 10 min and subsequently at 42°C for 40 min. The reaction was stopped by incubation at 99°C for 5 min. qRT-PCR assays were performed using 12.5 μl of SYBR Premix EX Taq™ (TAKARA Bio), 10 μl of specific primers, and 10 ng of cDNA in a thermal cycler Dice™ Real Time System (TAKARA). The primers used were ORF30A (5′-GTC AGG CCC ACA AAC TTG AT-3′) and ORF30B (5′-ACT CGG TTT ACG GAT TCA CG-3′), which were specific for EHV-9 ORF30. Conditions for the qRT-PCR were 37°C for 15 min and 98°C for 1 min. The ΔΔCt method (cross-point method) was used to determine the relative quantities. The PCR products were assessed by 3% agarose gel electrophoresis and ethidium bromide staining. The expression levels were normalized to that of the internal control gene β-actin (El-Nahass *et al.*, 2014).

Statistical Analysis

Expression of data was as mean ± standard deviation (SD). The difference in intensity of immunohistochemical labelling and expression levels of ORF30 were determined by ANOVA followed by post-hoc testing. $P < 0.05$ was considered significant.

Results

Clinical Features

At 4 dpi, none of the inoculated mice exhibited obvious clinical signs. The initial appearance of clinical signs was at 5 dpi in C3H, C57BL and DBA mice, and at 6 dpi in BALB/c-nu/nu and ICR strains. No obvious clinical signs were detected in BALB/c mice. Mortality rates were 83.33, 50.0, 33.33 and 16.66% for C3H, C57BL, DBA and BALB/c-nu/nu mice, respectively. The ICR and BALB/c mice exhibited no mortality. All deaths occurred at 6–7 dpi. Percentages of body weight loss at 7 dpi (compared with the initial weight at 0 dpi) were 26.66, 20.17, 22.72, 16.03, 23.88 and 1.49% in C3H, C57BL, DBA, BALB/c-nu/nu, ICR and BALB/c mice, respectively.