Experimental intranasal infection of equine herpesvirus 9 (EHV-9) in suckling hamsters: Kinetics of viral transmission and inflammation in the nasal cavity and brain

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Equine herpesvirus 9 (EHV-9), the newest member of the equine herpesvirus family, is a highly neurotropic herpesvirus that induces encephalitis in a variety of animals. To access transmission of EHV-9 in the nasal cavity and brain, a suckling hamster model was developed so that precise sagittal sections of nasal and cranial cavities including the brain could be processed, which proved useful in detecting viral transmission as well as extension of pathological lesions. Suckling hamsters were inoculated intranasally with EHV-9, and were sacrificed at 6, 12, 18, 24, 36, 48, and 60 h post inoculation (PI). Sagittal sections of the entire head, including nasal and cranial cavities including the brain, were made to assess viral kinetics and identify the progress of the neuropathological lesions. At 12 to 24 h PI the virus attached to and propagated in the olfactory epithelium, and infected adjacent epithelial cells. At 48 h PI, immunohistochemistry for EHV-9 viral antigen showed that virus had extended from the site of infection into the olfactory bulb and olfactory nerve. These results indicate that EHV-9 rapidly invades the brain via the olfactory route after experimental intranasal infection. Journal of NeuroVirology (2010) 16, 242–248.

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Introduction

Equine herpesvirus 9 (EHV-9), the newest member of the equine herpesvirus family, is a highly neurotropic herpesvirus firstly described in an outbreak of disease in Thomson's gazelles (Gazella thomsoni) that died of fulminant encephalitis (Fukushi et al., 1997). Serologically, EHV-9 is most closely related to the recently emergent neurotropic pathogen, EHV-1, but its DNA fingerprint is different from that of EHV-1 and other equine herpesviruses.

Recently, the EHV-9 virus and a virus serologically similar to EHV-9 were proven to have a high seroprevalence of 60% in free-living zebras in Serengeti National Park, which suggests that zebras might be a natural host of EHV-9, and that there might be a possibility of widespread exposure to the EHV-9 virus within zebra populations in the wild (Borchers et al., 2005).

Many experimental studies on the infectivity of EHV-9 using rodents, domestic animals, and a new-world monkey have been conducted. Emerging EHV-9 infection was considered a concern, because of the wide range of susceptible hosts and the ease of transmission by the nasal route, which is thought to be the most probable route of transmission in the field. Recently, EHV-9 was detected in a polar bear with progressive encephalitis as well as in a giraffe.
(Schrenzel et al., 2008; Donovan et al., 2009; Kasem et al., 2008), raising fears of emerging infections in various wild and domestic animal species. Although EHV-9 was shown to infect a broad range of animals, including mice, rats (Fukushi et al., 1997), hamsters (Fukushi et al., 2000), goats (Taniguchi et al., 2000b), pigs (Narita et al., 2000), dogs and cats (Yanai et al., 2003a, 2003b), and common marmosets (Kodama et al., 2007), it remains unknown how the virus travels from the nasal cavity to the brain, or how long this progression takes. Because the Syrian hamster has been reported to be a useful model for studying the pathogenesis of EHV-9 infection by the nasal route (Fukushi et al., 2000), we recently conducted a study in this model to clarify acute infection of EHV-9. The results showed that it took 48 h for the virus to travel from the nasal mucosa to the olfactory bulb (data not yet published), but it was still not clear how EHV-9 moved from the olfactory epithelium, through the ethmoid turbinate and septa, to the olfactory bulb. Precise sagittal sections of the nasal cavity, the ethmoid turbinate and septa, and the olfactory bulb were needed to accurately detect and stage the kinetics of viral infection from the nasal cavity to the brain, but the calcified cranial bone in adult hamsters created significant technical obstacles. Thus, to resolve this technical difficulty, we used suckling hamsters, since they have a very thin and soft skull and bones making precise histologic sagittal sections of the nasal and cranial cavity feasible. This model provided a panoramic view of the entire head, including the nasal and cranial cavities and the brain in the same section. Suckling hamsters have been used in some studies in the past to investigate pathogenesis in various viruses. The suckling hamster was used for studying mumps virus pathogenicity and the development of hydrocephalus through intracerebral inoculation (Kilham and Overman, 1952; Takano et al., 1991), and Doll et al. (1953) adapted EHV-1 to suckling hamsters.

In the present study, we used the suckling hamster to enable sagittal sectioning of the head, including the nasal cavity, the ethmoid turbinate and septa, and the brain. The panoramic view obtained from sagittal sections enabled a focused study on the dynamic kinetics of EHV-9 from nasal infection to induction of acute encephalitis.

Results

The majority of inoculated hamsters showed various degrees of clinical signs that included depression and uncoordinated movement starting at 48 h post inoculation (PI). By 60 h PI, the end of the in-life portion of the study, all of the animals had severe incoordination of the movement and depression.

Histopathology

Sagittal sections of suckling hamster made it easy to view the nasal cavity and brain in a single histologic section (Figure 1). Histopathological changes in the olfactory epithelium and the brain in EHV-9 inoculated animals are summarized in Table 1 and as follows:

- At 6 h PI: There were no significant findings except for detachment of the superficial microvillus in some areas, along with a few infiltrations of inflammatory cells, including neutrophils and lymphocytes, between olfactory epithelial cells and in the lumen of the nasal cavity.
- At 12 h PI: There were varying degrees of necrosis observed in the columnar olfactory epithelial cells. In addition, there was a slight degree of irregularity on the surface of the olfactory epithelium, as well as varying degrees of ablation of the superficial microvillus.
- At 18 h PI: Prominent necrosis of the olfactory epithelial cells was observed, as well as vacuolization of the olfactory receptor neurons and infiltration of the lamina propria by inflammatory cells, including neutrophils and lymphocytes.
- At 24 h PI: The surface of the olfactory epithelium had become more irregular, with frequent necrosis of the olfactory epithelial cells, which became desquamated into the lumen in some areas. A complete ablation of the microvillus was often observed. In and around the olfactory mucosa of the nasal cavity, there were frequent clusters of neutrophils, some of which were desquamated into the lumen.

Figure 1 Sagittal section showed the head from the nose to the whole brain, it is easy to check the connection between olfactory bulb (OB), brain (Br), and olfactory epithelium (OE) and to examine the cranial nerves and ganglion. Inset: Higher magnification showing normal intact olfactory epithelium (OE) of control uninfected animals.