

English abstract

Based on previous experimental studies in various animals inoculated via the nasal route, it was confirmed that the olfactory pathway (i.e. through the olfactory nerves), as well the trigeminal pathway (through the trigeminal nerve), were the major route of transmission of EHV-9 into the CNS. However, our recent study, in which different routes of inoculation were compared, clearly indicated that the virus can enter the CNS after administration of EHV-9 via the oral, peritoneal, and ocular routes, and that there are differences in the distribution of antigen-positive cells and in the location and severity of the cerebral lesions. Thus, EHV-9 may gain access to the CNS through a non-olfactory route, as animals inoculated via these non-nasal routes did not exhibit EHV-9 induced rhinitis, and the olfactory bulbs showed milder lesions and fewer viral antigen-positive cells than were observed in the animals infected via the nasal route . These findings spurred the author to perform the investigation on intraperitoneal inoculation of EHV-9 described in Chapter 1. In this part, I first used the adult Syrian hamster as the animal model for evaluating the kinetics of EHV-9 induced encephalitis . The results of this study showed the essential role of the spinal cord in the propagation and transmission of EHV-9. However, the study failed to determine the following :

- ١ The primary sites for virus attachment and propagation
- ٢ Time scheduled pathogenesis
- ٣ Whether or not the hematogenous routes play a role in virus transmission

To elucidate these points, suckling Syrian hamsters were used in Chapter 1. In this part, using this animal model, it was possible to definitely determine the actual

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pathogenesis of EHV-9 following intraperitoneal inoculation of EHV-9. This study showed that the virus gained access to the brain through the neuronal pathway rather than the haematogenous pathway, with this finding being confirmed by performing PCR on blood, brain and spinal cord samples. Fingerprints of EHV-9 DNA were found

in the spinal cord samples at 36 h PI, in the brain samples at 96 h, and in the PI blood samples at 48 h PI. The results clearly showed that EHV-9 DNA was detected earlier in the spinal cord than in the blood. EHV-9 induced encephalitis following intraperitoneal inoculation of EHV-9 may occur initially through primary attachment and propagation of EHV-9 virus in peritoneal cells, mainly macrophages (which was confirmed by applying immunocytochemistry in an abdominal wash), following which two possible pathways might be proposed (Plate I):

-1) Infection of the peripheral nerve axons and coeliac plexus within the abdominal cavity, followed by propagation of the virus within the dorsal root (spinal ganglia). That would be followed by transmission and propagation of EHV-9 in the spinal cord. The latter plays an essential role in ascending transmission of the virus to the brain.

-2) Infection of the myenteric plexus with EHV-9, leading to spreading of the virus to the brainstem via the vagus nerve.

Also, as described in Chapter 1, it was possible for the first time to identify the tendency of EHV-9 to infect the livers of suckling animals, and consequently to identify the role of the liver in virus replication, especially during the initial stages of infection.

In Chapter 2, the detailed pathogenesis of EHV-9 following oral inoculation is illustrated, first in adult ICR and then in suckling Syrian hamsters. In the former, it

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seemed that the primary sites for virus attachment and propagation were lingual macrophages, as the virus was detected immunohistochemically starting from 12 h PI.

These cells help in the propagation, transmission and spreading of the EHV-9 virus to target cells, or may serve as reservoirs for long-term infection that is followed by the development of encephalitis, as well as detection of the virus immunohistochemically at 72 h PI, mainly in the pons, in the hippocampus, midbrain and cerebellum at 96 h PI.

and finally in the olfactory bulb (mainly the granular layer) at 120 h PI .

The distribution of EHV-9 in the granular layer and mitral layer of the olfactory bulb seems to indicate that the virus travels through non-olfactory pathways. One of the predominant features that is found in adult ICR is the effect of EHV-9 virus infection on the gastrointestinal tract in the form of hyperkeratosis, moderate to severe gastritis and multifocal ulceration in the forestomach. This is considered very important in terms of future studies on the effect of EHV-9 on other systems of animal bodies, particularly the gastrointestinal system .

In Chapter 2, using a suckling animal model, I tried to cover points relating to the pathogenesis of EHV-9 infection that could not be achieved using adult ICR mice . In this section, the role of oral and lingual submucosa was confirmed, as well as the role of macrophages in the propagation and transmission of EHV-9 to the mandibular and maxillary branches of the trigeminal nerve at 36 h PI and at 48h PI in the nuclei and cytoplasm of pseudounipolar neurons of the trigeminal ganglia, the meninges and the brainstem (the root of the trigeminal nerve entrance). That was followed by the occurrence of encephalitis in the midbrain and pons from 48 h PI until the end of the experiment (plates II and III). At the same time, EHV-9 DNA was detected in the brains of EHV-9 inoculated hamsters at 36 h PI, in the spinal cord at 96 h PI and finally

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in the blood samples at 48 h PI, thus confirming that the EHV-9 virus is transmitted through neuronal pathways following oral inoculation.

In Chapter 3, I discussed the manner of EHV-9 infectivity in two mouse strains :

the congenitally athymic strain (BALB/c-nu/nu) and phenotypically normal mice (BALB/c). The infectivity of these two mouse strains was found to be quite different . BALB/c-nu-nu mice are more susceptible to EHV-9 infection than BALB/c mice , which were found to be relatively resistant. That was confirmed through weak EHV-9 propagation in the olfactory epithelia, followed by complete virus clearance within the

olfactory epithelia at 96 h PI in BALB/c mice. Furthermore, the application of RT-PCR of EHV-9 in formalin fixed tissues on the olfactory epithelia of BALB/c mice produced increases in the relative transcription activity of ORF30 in the olfactory epithelia until 48 h PI, followed by a sharp decrease in transcription activity in this gene at 96 h PI (plate IV). In contrast, in immunohistochemical testing, BALB/c-nu-nu mice demonstrated high levels of EHV-9 antigen within the olfactory epithelia from 24 h PI until the end of the experiment. In addition, the virus was detected immunohistochemically not only in the olfactory nerves of all inoculated animals but also within the olfactory bulb in one animal. A proportional increase in mRNA expression levels was seen until 48 h PI, followed by a gradual slowing until the expression level reached 20-fold at 96 h PI. Comparison of the relative quantity of ORF30 gene expression using the cross point method (CP) each hour post inoculation between BALB/c and BALB/c-nu-nu mice strains showed no statistical differences in relative gene expression values of ORF30 in the brain tissues. In addition, significant gene expression was observed in olfactory epithelia in BALB/c-nu-nu mice compared to BALB/c mice at 24, 36, 48, 72 and 96 h PI .