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 roleof the spinal cord in the propagation

and transmission of EHV-9. However, the study failed to determine the following:

1- The primary sites for virus attachment and propagation

2- Time scheduled pathogenesis

3- Whether or not the hematogenous routes play a role in virus transmission

To elucidate these points, suckling Syrian hamsterswere used in Chapter 1. In

this part, using this animal model, it was possible to definitely determine the actual

123

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 brainsamples at 96 h, and in the PI blood

samples at 48 h PI. The results clearly showed thatEHV-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

124

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 andmitral 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 formof 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 ponsfrom 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

125

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 bycomplete 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 olfactoryepithelia of BALB/c mice produced

increases in the relative transcription activity ofORF30 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 showedno statistical differences in

relative gene expression values of ORF30 in the brain tissues. In addition, significant

gene expression was observed in olfactory epitheliain BALB/c-nu-nu mice compared

to BALB/c mice at 24, 36, 48, 72 and 96 h PI.