Olfactory neural pathway in mouse hepatitis virus nasoencephalitis*

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Summary. The mechanism of brain infection with mouse hepatitis virus-JHM was studied in BALB/cByJ mice following intranasal inoculation, and found to be a consequence of direct viral spread along olfactory nerves into olfactory bulbs of the brain. Infection was followed sequentially from nose to brain, using microscopy, immunohistochemistry and virus quantification. Lesions, antigen and virus were observed in the olfactory bulb and anterior brain as early as 2 days and posterior brain by 4 days after inoculation. Viral antigen extended through nasal mucosa into submucosa, then coursed along the olfactory nerve perineurium and fibers, through the cribiform plate into the olfactory bulbs. On days 4 and 7, viral antigen was found in the antero-ventral brain, along ventral meninges, olfactory tracts and anterior ramifications of the lateral ventricles. Virus was cleared from nose by 10 days and anterior brain by 20 days, but persisted in posterior brain for 20 days after inoculation. Mice also developed disseminated infection, with viremia and hepatitis. Infection of brain did not correlate with presence of viremia. In contrast to intranasally inoculated mice, orally-inoculated mice did not develop encephalitis, despite evidence of disseminated infection.

Material and methods

Mice

Three-week-old BALB/cByJ mice were purchased from the Jackson Laboratory (Bar Harbor, Me) and Crl:CD1BR mice were purchased from Charles River Laboratories (Portage, Mich). Both sources were MHV free. Mice were shipped in filtered boxes and transferred on arrival into sterile Microisolator (Lab Products) cages containing pine shavings, food (Purina Laboratory Chow) and water. Mice were inoculated i.n. or p.o. with 10^3 median tissue culture infectious doses of MHV-JHM.

Virus

MHV-JHM was obtained from the American Type Culture Collection (Bethesda, Md), passaged twice in NCTC-1469 cells, once in BALB/cByJ brain and once in 17 CI 1 cells [16]. Infectious virus was quantified in tissues by infant mouse infectivity assay. Frozen tissues were thawed, weighed and diluted 10% (w/v) in Dulbecco’s minimal essential medium containing 5% fetal bovine serum. They were homogenized, then clarified in a refrigerated centrifuge at 2,000 rpm for 20 min. Serial tenfold dilutions of supernates in 0.025 ml were inoculated intracerebrally into 2-day-old Crl:CD1BR mice in groups of four dilution. Mortality was established at 72 h and the log10LD50/gram of tissue was determined [15].

Histology/immunohistochemistry

Tissues were immersion fixed in neutral buffered formalin, pH 7.2, and paraffin embedded. Skulls were demineralized in a solution containing 1.8 g tetrasodium ethylenediaminetetra-acetic acid, 0.7 g sodium tartrate, 125 ml hydrochloric acid and 875 ml distilled water. Tissues were sectioned at 5 µm and stained with hematoxylin and eosin. Selected tissues were stained for MHV antigen using a streptavidin-biotin-horseradish peroxidase (Bethesda Research Laboratories) method and tissue treatment as previously described [5]. Primary antibody was hyperimmune mouse ascitic fluid prepared in multiparous Crl:CD1BR mice by 3-weekly intraperitoneal injections of

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MHV-JHM-infected infant mouse brain emulsified in Freund's complete adjuvant.

**Tissue samples**

Groups of five BALB mice were killed at intervals (3 and 12 h; 1, 2, 3, 4, 5, 7, 10 and 20 days) after i.n. MHV-JHM inoculation. Mice were randomly selected for necropsy and killed with carbon dioxide gas followed by cardiac exsanguination. Following removal of the lower jaw and skin, the head was sagitally hemisectioned. One hemisection was placed in formalin. The nasal turbinates and brain were removed for virus recovery from the other hemisection. The brain was then cut coronally into anterior and posterior segments. Nose, olfactory bulb, brain, and liver were collected for histology and immunohistochemistry. Nasal turinate, anterior brain, posterior brain and whole blood were collected and frozen at −70°C for virus determination. In addition, groups of five BALB mice were inoculated i.n. or p.o. and the subsequent patterns of infection in nose, brain and liver were compared microscopically on day 5 after inoculation.

**Results**

**Histology/immunohistochemistry**

The distribution of lesions suggested a sequential progression of infection from nose to posterior brain (Fig. 1). Necrosis of nasal respiratory and olfactory epithelium was observed as early as 12 h after MHV inoculation, with extension into submucosa by 24 h. By day 2, necrotizing lesions were visible in the anterior olfactory bulb. At subsequent intervals, necrotizing inflammation extended posteriorly along the meninges and olfactory tracts of the anteroventral brain, as well as into the rostral ramifications of the lateral ventricles and hippocampus. Brain appeared to be infected by spread from meninges and ventricles, as well as by extension along olfactory tracts. Antigen was seen nonselectively in meningeal, neuronal, glial and ependymal cells.

**Virus**

Virus detection confirmed morphological impressions of an anterior to posterior progression of MHV from nose to posterior brain, as well as anterior to posterior regression of infection (Fig. 4). At 3 h after i.n. inoculation, only one of five mice had detectable virus in nasal tissue, which probably represented residual inoculum. By 12 h, all mice had demonstrable virus in nasal tissue, which rose in titer through day 2, then declined. Virus was first detected in anterior brain on day 4 after inoculation and cleared by day 20. In contrast, virus was not detected in posterior brain until day 4 and was still present in brains of all infected mice at day 20. Viremia was confirmed in four of five infected mice at 24 h and all mice at 2, 3, 4 and 5 days after i.n. inoculation.

**Intranasal versus oral inoculation**

To confirm that brain infection resulted from direct extension of virus along olfactory nerves and not due to viremia, groups of five mice were inoculated either i.n. or p.o. with MHV-JHM. At 5 days after inoculation, the distribution of MHV lesions was compared between the two groups (Table 1). Both groups developed the same prevalence and severity of hepatitis,