Neuropathogenicity of Mutant Strains of Mouse Hepatitis Virus, JHM

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ABSTRACT. Two plaque purified mutants, JHM-1a and JHM-2c of mouse hepatitis virus were examined on neuropathogenicity for mice. After intracerebral inoculation with 10⁴ PFU of JHM-1a virus 30% of weanling ICR mice died within 7 days showing viral growth in the brain equivalent to that of the original JHM, while all JHM-2c infected mice survived with very low-titered virus. At early stage of JHM-1a infection mice showed much less extensive encephalomyelitis than in case of the original JHM although almost the same amount of viral antigen was detectable in neurons and glial cells of animals infected with both viruses. Infected mice showed fusion of oligodendrocytes and neurons without significant inflammatory response. With JHM-2c of lower-neurovirulence viral antigen was seen in glial cells rather than neurons. At 4 weeks postinfection viral antigen was detected within oligodendrocytes and demyelination was produced in mice infected with both mutant viruses.—KEY WORDS: inclusion body, MHV, mutant, oligodendrocyte.

Demyelinating encephalomyelitis was produced in mice and rats by inoculating a neurotropic JHM strain of mouse hepatitis virus (MHV) [1, 7, 19, 28], and several mutant strains of JHM virus were used for production of persistent infection in the central nervous system (CNS) [22, 23]. The mutant viruses were temperature sensitive variants [15, 23, 27], isolates from latently infected cell lines [12, 24], plaque purified variants on cell cultures [22] or antigenic variants identified by monoclonal antibodies [5]. The mutants were shown to have lower pathogenicity than the original virus while they were capable of producing persistent infection with demyelination. The pathogenicity of mutant viruses was suggested to be related to the viral peplomeric E2 protein [2, 22].

Recently, Makino et al. [17] isolated two strains of small plaque mutants, JHM-1a and JHM-2c, from DBT cell culture persistently infected with JHM virus [12]. JHM-1a strain had a specific polypeptide being uncommon to other JHM virus strains, and JHM-2c had a specific polypeptide in the peplomer. These mutants showed slower growth in DBT cells than did the original JHM virus without causing apparent fusion of host cells and were found to be less encephalitogenic for mice [9].

This paper describes pathogenicity of the two plaque mutant strains, JHM-1a and JHM-2c, for the CNS of weanling mice as compared with the original JHM virus.

MATERIALS AND METHODS

Mice: ICR mice were purchased from a commercial breeder (Shizuoka Laboratory Animals, Shizuoka) and used at 4 weeks of age. The breeder colony was checked for being MHV-free by seromonitoring [6].

Virus: Two mutant strains, JHM-1a and JHM-2c, of JHM virus from persistently infected DBT cells [17] were propagated on DBT cells and stored at -70°C before use, and 10⁴ PFU/0.04 ml were inoculated intracerebrally. For virus titration the brain and spinal cord of inoculated mice were
asectically sampled and titration was carried out on DBT cells as described earlier [13].

Immunofluorescence: According to Sainte-Marie [21] samples were fixed in 95% ethanol and dehydrated at 4°C and embedded in paraffin at 58°C. Sections were treated first with anti-MHV-2 rabbit serum and then with fluorescein-isothiocyanate (FITC) conjugated anti-rabbit goat IgG (Cappel, PA, USA).

Histopathology: The brain and spinal cord of infected mice were fixed in 10% neutral buffered formalin, and 4 to 5 μm paraffin sections were made and subjected to hematoxylin and eosin stain, luxol-fast blue stain for myelin, Holms stain for nerve fibers and periodic acid Schiff reaction.

Electron microscopy: Small blocks of the cerebral cortex and medulla, peduncle of cerebrum, lower part of midencephalon, medulla of cerebellum and medulla oblongata around the nucleus trigemini were sampled and fixed with 5% glutaraldehyde and postfixed with osmium tetroxide, dehydrated by ascending ethanol series and embedded in epoxy resin (Epok 812, Oken, Tokyo). After monitoring with semi-thin sections stained with toluidine blue, ultrathin sections were cut and stained with uranyl acetate and lead nitrate, and observation was made using an electron microscope, JEM 100CXII.

RESULTS

After intracerebral (i. c.) inoculation with 10^4 PFU of the original JHM virus all mice died within 7 days postinoculation (p. i.),

Table 1. Response of ICR mice (4-week-old) to i.c. inoculation with MHV-JHM and its mutant JHM-1a and JHM-2c

<table>
<thead>
<tr>
<th>Virus (10^4PFU)</th>
<th>Survivals/tested on day 7</th>
<th>Lesions on day 7</th>
<th>Encephalitis</th>
<th>Demyelination</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHV-JHM</td>
<td>0/20</td>
<td>—</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>JHM-1a</td>
<td>18/25</td>
<td>17/25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JHM-2c</td>
<td>25/25</td>
<td>25/25</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

Fig. 1. Growth of MHV-JHM (●), JHM-1a (○) and JHM-2c (■) in the brain of 4-week-old ICR mice after i. c. inoculation (1×10^4 PFU). Each point represents the titer of individuals.

Table 1. Distribution of viral antigen, virions and inclusions in the brain of ICR mice after i.c. inoculation with MHV-JHM, JHM-1a and JHM-2c

<table>
<thead>
<tr>
<th>Virus (10^4PFU)</th>
<th>Immunofluorescence</th>
<th>Electron microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neuron</td>
<td>Oligodendrocyte</td>
</tr>
<tr>
<td>MHV-JHM</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>JHM-1a</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>JHM-2c</td>
<td>—</td>
<td>++</td>
</tr>
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</table>

a) Viroplasmic.
b) Granular.
while only 30% of mice infected with JHM-1a virus died in the same period (Table 1). With JHM-2c virus all mice survived for 4 weeks p. i. Most of mice surviving the inoculation with JHM-1a and JHM-2c showed no clinical signs.

The infective titers in the brain of mice inoculated with JHM-1a or JHM virus reached a peak at 72 to 96 hr p. i., while the peak titer of JHM-2c was at 120 hr p. i. being much lower (Fig. 1).
After infection with JHM-1a, virus antigen was shown to be distributed in neurons, glial cells, meningocytes, and ependymal cells (Table 2) though much less in amount as compared with JHM infection. In the spinal cord it was detected mostly in oligodendrocytes. In mice infected with JHM-2c viral antigen was present mainly in microglial cells and oligodendrocytes (Fig. 3).

During the early stage of JHM-1a infection degeneration and disappearance of neurons were prominent in the cerebral cortex. Marked degeneration with inflammatory response was produced in the peduncles of cerebrum, lower parts of midencephalon and peduncles of cerebellum. Small accumulations of microglial cells and polymorphonuclear cells were scattered (Figs. 4a and 4b). In a more advanced stage spongy lesions appeared and glial cells were
accumulated around degenerated neurons with small perivascular cuffs and demyelination. There were degeneration of meningoocytes and ependymal cells, collapse of perivascular matrix and mild meningitis.

Some oligodendrocytes had a small number of virions located closely to electron-dense granular cytoplasmic inclusions (Figs. 5 and 6). A few virions were seen in extracellular spaces. Occasionally large perinuclear inclusions were produced, and many inclusion-bearing oligodendrocytes were poor in cytoplasmic matrices and nuclear chromatin. Neurons being in contact with inclusion-bearing oligodendrocytes were moderately degenerated, and fusion occurred between the two cells, producing a viroplasmic structure within the neuron (Fig. 7). Dissociation of myelin sheath in many axons was often seen (Fig. 8). There were degenerated axons with slightly or moderately degenerated myelin sheath. At 4 week p. i. atrophy and disappearance of neurons and oligodendrocytes with marked demyelination were seen.

In mice infected with JHM-2c neurons and glial cells were degenerated and neuropil around the inoculated site was destroyed. Later the changes subsided leaving perivascular cuffing and degenerated glial cells in the cerebral white matter, cerebral peduncles, midencephalon and medulla oblongata. Demyelination was limited to some areas in the cerebral peduncles, midencephalon and around the nucleus trigemini of the medulla oblongata (Figs. 9a and 9b). At this stage demyelination was seen without significant damage of neurons (Fig. 10).

In mice infected with JHM-2c electron microscopy revealed cytoplasmic inclusions in oligodendrocytes similar to those seen in JHM-1a infected mice (Fig. 11), while only a small number of virions were seen within cells in collapse. Some of neighbouring axons were demyelinated (Fig. 12), but most neurons being in contact with degenerated oligodendrocytes remained intact. These changes were less extensive than in case of JHM-1a infection.

DISCUSSION

The wild type JHM virus is known to cause acute fatal encephalomyelitis in susceptible mice affecting neurons, glial cells and mesenchymal elements with characteristic demyelination [1]. Viral antigen can be detected in the whole CNS.

For producing subacute or chronic encephalitis with demyelination a mutant strain MHV-JHM-CC was isolated from DBT cells persistently infected with JHM virus [11, 12]. In the present study another
mutant JHM-1a from persistently infected DBT cells with JHM virus [17], showed infective titers in the mouse brain nearly equivalent to those of JHM virus infected mice. The in vitro growth of both wild and mutant viruses was shown to be comparable [17], but encephalitogenicity of the mutant viruses in mice was much lower, particularly JHM-2c showing low titers in the brain of infected mice (Fig. 1).

The both low-virulence mutant viruses seemed to have much less neurotropism than the original JHM. The cell tropism and maturation of JHM and other MHV strains in the CNS have been investigated in vitro [18] and in vivo [14, 19]. Dubois-Dalcq et al. [4], using in vitro system, reported that the wild type JHM virus produced smaller number of virions in neurons than in non-neuronal cells, despite both equally showing acute cytopathic changes. In mice infected with the original JHM virus antigen was shown to be abundant in neurons of the cerebral cortex, pyramidal cells of the hippocampus and Purkinje cells of the cerebellum. After infection with JHM-1a viral antigen was detectable but much less abundant while atrophied and degenerated neurons were seen at later stage of infection. In case of JHM-2c viral antigen was present only in oligodendrocytes without showing histological evidence of the neurotropism.

The MHV-A59, Ts8-JHM and the original JHM are known to produce cytoplasmic inclusions in vitro [3, 4] or in vivo [8]. MHV-A59 forms reticular and tubular inclusions releasing virions, whereas Ts8-JHM produces granular inclusions in infected cell culture. In oligodendrocytes of the spinal cord infected with the original JHM virus, dense granular viroplasmic inclusions appeared to be producing free virions at the periphery [25]. The mutant viruses of lower virulence also produced detectable inclusions with mild cytopathogenicity (Table 2). The fusion activity was common in the CNS of mice infected with JHM virus [1, 10], while it was not ascertained with the mutant JHM-1a and JHM-2c on DBT cells. The fusion of neurons and oligodendrocytes observed in the present study may represent a means of spreading of low virulent viruses in the CNS.

Demyelination, which is caused by degeneration of glial cells or immunologic processes, is a characteristic feature of the JHM virus infection [16, 20, 26, 28]. In the present study myelinated axons near to inclusion-bearing oligodendrocytes frequently became naked with dissociated myelin lamellae. At least in early demyelination there might be no participation of immunologic processes. In addition to the demyelination, in JHM-1a infection axonal degeneration was followed by the destruction of myelin, indicating that the secondary collapse of myelin also occurred.

The present study showed that the low virulent mutants JHM-1a and JHM-2c had tropism to oligodendrocytes rather than neurons, producing useful animal models for subacute to chronic viral demyelinating encephalomyelitis.

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REFERENCES

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要約

神経親和性マウス肝炎ウイルス JHM 株ブラック変異株の中枢神経系に対する病原性について: 後藤正彰・堤 嘉隆・佐藤昭夫・藤原公策 (山口大学農学部家畜病理学教室, 東京大学農学部家畜病理学教室) —— マウス肝炎ウイルス JHM 株のブラック変異株 JHM −1a および JHM −2c のマウスの中枢神経系に対する病原性について検討した。両変異株 10^4 PFU を 4 週齢 ICR マウスに接種すると JHM −1a 接種マウスは接種後 7 日以内に 30% が観察死し、脳のウイルス価は親ウイルス JHM 株のそれに近い高い価を示した。JHM −2c 接種マウスは全例生存し、ウイルス価も低かった。JHM −1a 感染初期には、マウスは軽度の脳脊髄炎を示し、ウイルス抗原は神経細胞および希突起細胞に分布していた。神経細胞と希突起細胞の融合も認められた。JHM −2c 接種マウスでは病変は著しく軽度で、ウイルス抗原は主として希突起細胞にみられた。両変異株感染希突起細胞の細胞質に顆粒集合物状の封入体形成を認めめた。接種 4 週後では、両変異株接種マウスで希突起細胞にウイルス抗原が検出され、脱髄を観察された。