Comparison of Mouse Hepatitis Virus Strains for Pathogenicity in Weanling Mice Infected by Various Routes

Brief Report

By

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Summary

The pathogenicity for mice of nine strains of mouse hepatitis virus was studied in mice free from the virus by the intracerebral, intraperitoneal, intravenous and intranasal routes of inoculation.

It has been known that there is a wide variation in virulence and organ tropism for mice among many established strains and fresh isolates of mouse hepatitis virus (MHV) (17). However, studies on the pathogenesis of MHV infection have been greatly hampered by difficulty to obtain MHV-free mice. Since we devised a very sensitive checking method for MHV infection (4, 5), MHV-free mice have become available from commercial breeder colonies or laboratory colonies which are routinely checked by this method to ascertain absence of MHV. Using these MHV-free mice we have made some studies on the pathogenicity of MHV strains, i.e., MHV-2 (10, 11, 21), MHV-S (22, 23, 24), JHM (9) and fresh isolates from wasting nude mice (6, 12).

The present study was undertaken to further compare several MHV strains for the pathogenicity in MHV-free mice by different routes of inoculation.

The MHV-strains used were MHV-1 (7), MHV-2 (18), MHV-3 (3), MHV-S (19), MHV-A59 (16), JHM (2) and 3 strains, NuA, NuU and Nu66, which were isolated from nude mice with wasting syndrome and chronic hepatitis in our laboratory (6, 12). These strains were maintained by serial passage in cultures of mouse cell line DBT (10, 13, 14). Virus was purified by plaque cloning (10, 13, 14) and propagated in DBT cells. Culture fluid harvested from infected cell cultures after incubation at 37°C for 24 hours was clarified by centrifugation and stored at...
—70°C until use in the present study. These materials were assayed for infectivity by the plaque count method in DBT cells (10, 13, 14).

ICR mice, 4 weeks of age, were obtained from a commercial breeder colony that was proven by anamnestic serology to have no MHV infection (5). Mice were inoculated by intracerebral (i.c.), intraperitoneal (i.p.), intravenous (i.v.) and intranasal (i.n.) routes with 0.02, 0.2, 0.2 and 0.02 ml amounts of virus, respectively. Serial decimal dilutions of each virus material were inoculated by these routes using 5 mice per virus dilution. Inoculated mice were observed for any clinical manifestations for 2 weeks. The 50 per cent lethal dose (LD₅₀) in plaque-forming units (PFU) was calculated by the method of Kärber. Dead and surviving mice were examined for gross pathological changes. In some experiments 2.5 mg of cortisone acetate was injected subcutaneously shortly after virus inoculation, as enhancement of pathogenicity of MHV by corticosteroids has been reported (17).

The results are summarized in Table 1. Strain MHV-2 was highly virulent for ICR mice, killing 4-week-old mice in 2 to 6 days after i.c., i.p. and i.v. inoculation. Dead mice showed marked swelling and confluent necrosis in the liver and hemorrhage in the duodenum. These results confirm previous findings (10, 11, 21).

| Virus strain | Cortisone treated | LD₅₀ titer obtained by various routes |
|--------------|------------------|-----------------------------------|
|              |                  | i.c. | i.p. | i.v. | i.n. |
| MHV-2        | —                | 0.7  | 0.5  | 0.3  | 4.8  |
|              | +                | NT   | NT   | NT   | 4.0  |
| MHV-3        | —                | 0.6  | <0.9 | >0.9 | 5.4  |
|              | +                | NT   | NT   | NT   | 4.0  |
| JHM          | —                | 0.8  | >6.3 | >6.3 | 5.6  |
|              | +                | NT   | <3.3 | >3.3 | 4.6  |
| NuA          | —                | 0.8  | 5.2  | 3.6  | 5.0  |
|              | +                | NT   | <3.5 | >3.5 | 3.6  |
| MHV-A59      | —                | 3.8  | 4.8  | 4.4  | 5.8  |
|              | +                | <3.3 | <3.3 | <3.3 | 3.0  |
| Nu66         | —                | 3.5  | >6.0 | 5.9  | >5.0 |
|              | +                | <2.0 | 5.1  | 3.9  | 4.7  |
| NuU          | —                | 5.9  | >6.6 | >6.6 | >5.6 |
|              | +                | 4.7  | 4.9  | 5.9  | 4.1  |
| MHV-S        | —                | >6.5 | >6.5 | >6.5 | >6.5 |
|              | +                | 6.6  | >6.5 | >6.5 | >6.5 |
| MHV-1        | —                | >5.5 | >5.5 | 5.4  | >5.5 |
|              | +                | >5.5 | 3.8  | <2.5 | >5.5 |

| Virus strain | Cortisone acetate (2.5 mg/mouse) was injected subcutaneously shortly after virus inoculation | LD₅₀ titer expressed as log₁₀ of PFUs | i.c. — intracerebral; i.p. — intraperitoneal; i.v. — intravenous; i.n. — intranasal | Range of days to death | Not tested |

Table 1. Pathogenicity of various strains of mouse hepatitis virus for weanling ICR mice

a Cortisone acetate (2.5 mg/mouse) was injected subcutaneously shortly after virus inoculation
b LD₅₀ titer expressed as log₁₀ of PFUs
c i.c.—intracerebral; i.p.—intraperitoneal; i.v.—intravenous; i.n.—intranasal
d Range of days to death
e Not tested
However, large amounts of virus were required for i.n. inoculation to kill mice, in agreement with our previous results (11). Cortisone treatment somewhat enhanced virulence by this route.

Strain MHV-3 demonstrated essentially the same pathogenicity for weanling mice as did strain MHV-2. Dick et al. (3) reported that strain MHV-3 was highly virulent, causing acute hepatitis in weanling mice.

Strain JHM was highly neurotropic, killing weanling ICR mice in 5 to 9 days after i.c. inoculation even with very small amounts of virus. Infected mice produced nervous disorders resulting in limb paralysis. On the other hand, the strain caused no death by i.p. or i.v. inoculation even with an inoculum of $10^6$ PFU. However, when treated with cortisone, mice infected by these routes developed acute hepatitis even with $10^3$ PFU and died in 3 to 5 days. When inoculated i.n. with $10^5$ or $10^6$ PFU, some mice died of cerebral disorders. The cortisone treatment somewhat enhanced virulence by i.n. route. Neurotropism of this strain has been studied by previous workers (1, 2, 9, 26).

Strain NuA, like strain JHM, was shown to be highly neurotropic. The strain killed weanling ICR mice in 2 to 6 days even with an inoculum as small as 10 PFU by i.c. inoculation. No hepatitis was observed in dead mice. When inoculated i.v. or i.p., strain NuA caused fatal hepatitis, but death occurred erratically in relation to virus dose. These findings are somewhat different from our previous reports (6, 12) that without cortisone the strain could not produce acute fatal hepatitis in weanling ICR mice even with $10^6$ PFU by i.p. route. The reason for this discrepancy is obscure, but seems to be due to advanced serial passage in DBT cells. The cortisone treatment enhanced virulence by these routes of inoculation. The strain caused acute fatal hepatitis after i.n. inoculation, although relatively large amounts of virus were required. The cortisone treatment increased virulence by this route. Marked enhancement of pathogenicity by cortisone has been reported for this strain (6, 12).

Strain MHV-A59 caused fatal hepatitis by all routes of inoculation tested, although relatively large amounts of virus were required. Enhanced virulence was shown by the cortisone treatment. Similar results were obtained with strain Nu66. Previously we reported that, without cortisone, strain Nu66 could not produce acute fatal hepatitis in weanling ICR mice even with $10^6$ PFU by i.p. inoculation (6, 12). The reason for this discrepancy is obscure, but seems to be due to passages in DBT cells.

Strain NuU did not cause acute fatal hepatitis by all the routes tested even with $10^5$ or $10^6$ PFU. The cortisone treatment enhanced virulence, causing fatal hepatitis. Previously we reported similar results after i.p. inoculation with this strain (6, 12).

Strain MHV-S did not cause fatal hepatitis by these routes of inoculation even with $10^6$ PFU, confirming our previous results (22). However, fatal hepatitis could be produced by cortisone treatment as previously reported by us (22).

Strain MHV-1 showed very low pathogenicity by these routes as did strain MHV-S, in agreement with previous observations (8). The cortisone treatment enhanced virulence of MHV-1, confirming previous results (15, 20, 25).

The data presented herein indicate that these MHV strains are widely varied in virulence and organ tropism. In the present study we used ICR mice, 4 weeks...
of age. Previous workers reported differences in the pathogenicity of MHV according to the strain and the age of mice (11, 21, 26). Another factor involved in the pathogenicity of MHV is the adaptation through passage in vivo or in vitro as shown for NuA and Nu66 strains in the present study. Further studies are needed to solve these problems and to elucidate the pathogenesis of MHV infections.

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