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Mouse Hepatitis Virus Nasoencephalopathy is Dependent upon Virus Strain and Host Genotype

By

S. W. Barthold, Deborah S. Beck[1], and A. L. Smith[2]

[1,2] Section of Comparative Medicine, and
[2] Department of Epidemiology and Public Health,
Yale University School of Medicine, New Haven, Connecticut, U.S.A.

With 2 Figures

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Summary

Mouse hepatitis virus (MHV) S induced typical MHV spongiform lesions in brainstem 28 days following intranasal inoculation of adult A/J, BALB/cByJ, CBA/J, C 3 H/HeJ and C 3 H/RV, but not SJL mice. In all but SJL mice, brain lesions occurred at or near the infectious dose level, based on seroconversion by the indirect immunofluorescence assay. During the acute phase of infection (day 5), lesions were limited to the nose and brain in most genotypes. Exceptions were BALB mice, which had mild hepatitis and SJL mice, which had lesions restricted to the nose. No mortality occurred in any genotype. Following intranasal inoculation of adult mice, MHV-1, -3, -A 59, -JHM and -S all caused brain lesions at 28 days after inoculation. MHV-1 and -3 caused lesions that were usually restricted to the anterior olfactory tracts, while MHV-A 59, -S and -JHM also caused more generalized and pronounced lesions involving the midbrain and pons. These studies suggest that avirulent MHV-S given intranasally to most mouse genotypes is a good model for induction of brain infection in the absence of mortality. They also confirm observations made by others in which MHV-JHM, -S and -A 59 are relatively more neurotropic than other MHV strains, such as MHV-1 and -3.

Introduction

Mouse hepatitis virus (MHV) is a coronavirus with several antigenically and biologically distinguishable strains (3). MHV-JHM has been studied extensively as a model for virus-induced encephalitis and demyelination in mice and rats (1, 9, 10, 13, 14, 16, 17, 21, 22, 27, 31, 37). The relevance of this

model has recently been augmented by the association of coronaviruses with some cases of human multiple sclerosis (6, 8). MHV-JHM is emphasized as a relatively unique neurotropic strain of MHV, but neurotropism may be a common biological feature of several other MHV strains. It has been shown that three antigenically distinguishable MHV strains (MHV-JHM, -S and -A 59) can induce demyelination (1, 4, 12, 19, 39). At least 2 of these strains (MHV-JHM and -S) can infect the brain by direct extension from the nasal mucosa to the olfactory tracts, apparently in the absence of other organ involvement (4, 9, 32, 33). It remains unclear if other MHV strains can cause nasoencephalopathy in adult mice, but most MHV strains are at least encephalitogenic following oronasal inoculation of suckling mice (5). Confusion over the relative neurotropic properties of this group of viruses may emanate from differences in disease caused by factors such as virus dose, route of inoculation, passage history, host age and genotype (14).

The purpose of this study was to determine if low-virulence, neurotropic MHV-S can induce brain lesions in adult mice of different genotypes following intranasal inoculation and if brain infection is dose-dependent by this route of exposure. We also investigated the ability of several different prototype MHV strains to infect brain following intranasal inoculation of adult mice.

Materials and Methods

Mice

Inbred A/J, BALB/cByJ, BALB/cJ, CBA/J, C 3 H/HeJ and SJL/J mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Outbred Cr 1 : CD-1 (ICR) BR Swiss mice were purchased from Charles River Breeding Laboratories, Portage, MI. Inbred C 3 H/RV mice were obtained from a pathogen-free breeding colony at Yale. Mice from all sources were free of serum antibody to MHV as determined by indirect immunofluorescence (26). Mice were transferred from their source in filtered boxes at 6 weeks of age and placed immediately on arrival into autoclaved, filter-top Micro-Isolator cages (Lab Products, Maywood, NJ) with sterile food, water and bedding in a room separate from other laboratory rodents. Cages were handled aseptically as previously described (4) to preclude contamination by experimental or adventitious MHV. Uninoculated, sentinel mice in open cages were maintained in the experimental room and periodically tested for seroconversion to MHV. Mice were killed with carbon dioxide gas and exsanguinated by cardiac puncture.

Virus

MHV-S, -JHM, -1, -3 and -A 59 were obtained from the American Type Culture Collection, Rockville, MD and passaged in mycoplasma-free NCTC 1469 cell cultures. Inocula consisted of cell culture fluid containing approximately 10^4 median tissue culture infectious doses ($TCID_{50}$)/100 µl of each virus strain or dilutions thereof. Unanesthetized mice were inoculated intranasally with 10 µl of virus stock in a Class II B biological safety cabinet.

Serology

Sera were diluted 1 : 10 and tested for MHV antibody by an indirect immunofluorescence assay, using a bivalent antigen consisting of cultured L cells infected with MHV-S

mixed with L cells infected with MHV-JHM (26). Median infectious dose based on seroconversion was calculated by the method of REED and MUENCH (23).

Pathology

Tissues were fixed in neutral buffered formalin, pH 7.2 and processed by routine histological technique. Nose, olfactory bulb, brain, liver and intestine were examined on day 5 after inoculation. This interval has been determined to be best suited for examination of the peak acute phase of MHV infection (4, 5). Sections of brain consisted of a coronal hemisection through the olfactory bulbs; anterior cerebrum; mid-cerebrum and brainstem; and cerebellum and pons. Spongiosis with demyelination is most apt to be detected in the mesencephalon and pons following intranasal inoculation of MHV (1, 4, 9, 33). Median infectious dose based on brain stem lesions was calculated by the method of REED and MUENCH (23). Statistical evaluations were performed using the Chi-square method (28).

Results

Influence of Host Genotype on Intranasal Infection with MHV-S

The susceptibility of several selected genotypes of mice to the effects of different doses of neurotropic MHV was tested using MHV-S. This virus strain was chosen because of its low virulence and proven ability to induce nasoencephalopathy in the absence of other organ involvement, thus minimizing mortality (4, 9, 32, 33). Mouse genotypes were selected to represent a spectrum of strains with susceptibility and resistance to various strains of MHV described by others (2, 14, 15, 17, 18, 20, 25, 29, 30, 34–36, 38). Groups of 5 mice of each genotype were inoculated with serial 100-fold dilutions of virus stock and the median infectious dose was established based on seroconversion and presence of brain stem spongiform lesions at 28 days. In addition, an extra 5 mice of each genotype were inoculated with the highest dose level (1×10^3 $TCID_{50}$) of MHV-S and killed at 5 days for comparison of acute disease patterns among genotypes.

As expected, MHV-S induced only mild acute disease in the inoculated adult mice (Table 1). No mortality was observed. Mice of all genotypes developed mild necrotizing rhinitis on day 5. Only BALB/cByJ mice had

Table 1. *Distribution of acute lesions in key target organs of different adult mouse genotypes, 5 days after intranasal inoculation of* 1×10^3 $TCID_{50}$ *MHV-S*

Genotype	Prevalence of MHV lesions			
	Nose	Brain	Liver	Intestine
A/J	5/5[a]	5/5	0/5	0/5
BALB/cByJ	6/6	5/6	4/6	0/6
CBA/J	5/5	5/5	0/5	0/5
C3H/HeJ	5/5	5/5	0/5	0/5
C3H/RV	5/5	5/5	0/5	0/5
SJL	4/4	0/4	0/4	0/4

[a] 5 with MHV-related lesions/5 examined

evidence of generalized infection, with a few foci of mild necrotizing hepatitis. With the notable exception of SJL mice, all other genotypes developed a high prevalence of mild acute encephalitis of the olfactory bulb, anterior olfactory tracts and piriform cortex.

At 28 days after inoculation, all genotypes except SJL developed brain stem spongiform lesions. When compared to the ID_{50} established by seroconversion, spongiosis occurred at or near the infectious dose level (Table 2). Chi square analysis rejected independence of the 2 variables of seroconversion and spongiosis ($P \leq 0.001$). Furthermore, no differences in severity of spongiosis were noted between dose levels or genotypes. SJL mice were an exception, since they required a remarkably higher dose of MHV-S for seroconversion, and none developed brain lesions at any dose level.

Table 2. *Median infectious MHV-S dose for different adult mouse genotypes, based on seroconversion and brain stem spongiosis*

Genotype	Median infectious dose[a]	
	Seroconversion	Spongiosis
A/J	0.1	0.1
BALB/cByJ	0.3	10.0
BALB/cJ	0.5	1.0
CBA/J	0.5	1.5
C 3 H/HeJ	0.1	0.4
C 3 H/RV	1.0	2.0
SJL	562.0	>1000[b]

[a] Expressed in $TCID_{50}$

[b] No demyelination at any dose

Induction of Nasoencephalopathy by Different Prototype MHV Strains

Five prototype MHV strains were evaluated for their ability to induce brain lesions in adult susceptible (BALB/cByJ) mice by the intranasal route. Because of the high virulence of MHV-3 and the high susceptibility of BALB mice, outbred CD-1 were inoculated with MHV-3. Strains JHM and S were used as positive controls, since both cause nasoencephalopathy with demyelination (4, 9, 21, 32, 33). Strain A 59 has been reported to cause demyelination by intracerebral inoculation (12, 19, 39), but its pathogenicity by the intranasal route has not been established. Strains 1 and 3 can infect brain following intracerebral or intraperitoneal inoculation (7, 36) but spongiosis or demyelination have not been observed with these strains.

Histological evidence of brain infection was present at 28 days after intranasal inoculation of mice with all MHV strains tested (Table 3). In some of the mice (5/14) inoculated with MHV-1 and most of the mice (8/9) inoculated with MHV-3, very mild nonsuppurative meningitis, gliosis and spongiosis selectively involved the tractus olfactorius of the olfactory bulb and of the anteroventral cerebral cortex (Fig. 1), but not other areas of brain.

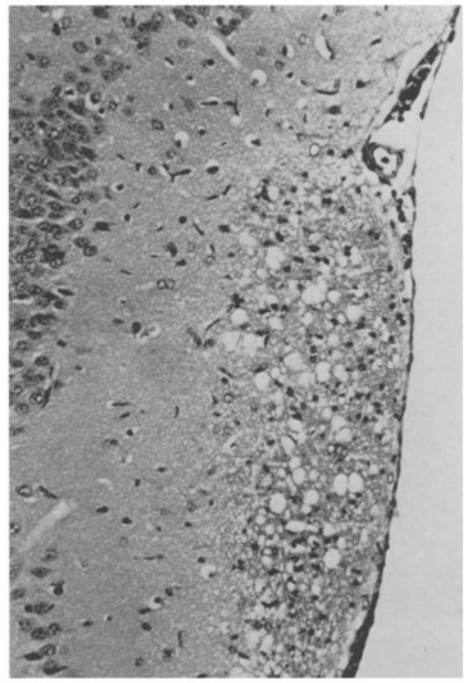

Fig. 1. Inflammation and spongiosis selectively involving the olfactory tract of the anteroventral cerebral cortex in a mouse infected intranasally with MHV-3 (H and E × 135)

Most of the mice inoculated with MHV-A 59, -JHM and -S and a single mouse inoculated with MHV-3 also developed prominent patches of spongiosis in the midbrain and pons (Fig. 2). Chi square analysis of the prevalence of brainstem spongiosis among treatment groups revealed significant differences between virus strains ($P \leq 0.001$). Mortality varied markedly among treatment groups (Table 3). Neither MHV-S nor MHV-1 caused mortality; MHV-A 59 caused a low prevalence of deaths; MHV-JHM caused death in half of the BALB mice inoculated between days 5–11. MHV-3 caused moderate mortality among the outbred Swiss mice.

Table 3. *Prevalence of brain lesions in adult BALB/cByJ mice 28 days after intranasal inoculation of different MHV strains*

MHV strain	Mortality	Prevalence of brain lesions	
		Olfactory tracts	Brain stem
1	0/14[a]	5/14	0/14
3	4/13[b]	8/9	1/9
A 59	2/11	8/9	8/9
JHM	7/14	6/7	6/7
S	0/13	12/13	12/13

[a] 0/14 = zero mortality among 14 mice infected

[b] Swiss mice

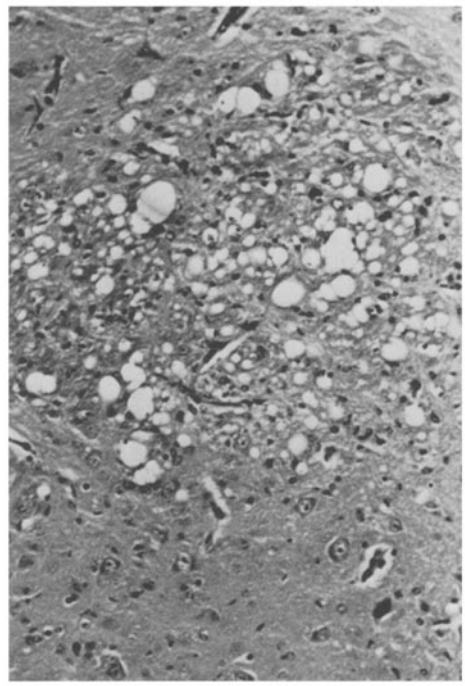

Fig. 2. Spongiosis of the brain stem in a mouse infected intranasally with MHV-S (H and E × 135)

Discussion

Although MHV-S has not been studied as extensively as MHV-JHM as a neurotropic MHV strain, MHV-S offers the distinct advantage of possessing equal neurotropism but low virulence. Both MHV-JHM and MHV-S appear to follow similar olfactory pathways into the brain and induce a similar distribution of spongiform brain lesions in intranasally-inoculated mice (4, 9, 32, 33). Results of this investigation indicate that following intranasal inoculation, MHV-S induces a reproducibly high prevalence of brainstem lesions with minimal involvement of other organs and no mortality in most adult mouse genotypes. Intranasal inoculation of MHV-S has been shown to be equally effective as intracerebral inoculation for inducing brain infection (32), but this strain is remarkably avirulent, regardless of the route of inoculation (11). The spongiosis with minimal encephalitis and mortality seen in mice of the several genotypes tested parallel the effects of non-encephalitogenic, temperature-sensitive mutants of MHV-JHM following either intracerebral or intranasal inoculation. In contrast, as we noted in this study, wild-type MHV-JHM produces a high mortality due to encephalitis (10, 11, 16, 17, 24). Following intranasal inoculation of MHV-S, brain stem spongiosis occurred in most genotypes at or near the infectious dose level,

based on comparison of brain lesions with seroconversion. The indirect immunofluorescence assay utilized in this study is very sensitive for detecting seroconversion to MHV following natural or experimental exposure of adult mice to MHV (26). Our studies suggested that once an infectious intranasal dose of MHV-S is achieved, higher doses do not influence the subsequent severity of infection. This suggests that brain infection occurs after initial infection and replication in the nasal mucosa, which has been shown for both MHV-S and MHV-JHM (4, 9, 32, 33). Thus, MHV-S given intranasally appears to be a good model for induction of nasoencephalopathy that does not require laboratory attenuation of virulence, has a wide effective dose range and can be used in many genotypes without excessive mortality.

Genotypic resistance to infection has been demonstrated with several MHV strains, usually using intraperitoneal or intracerebral inoculation. The genotypes examined for susceptibility to intranasal MHV-S were selected on this basis. BANG *et al.* noted resistance of C 3 H mice to MHV-2 (2) and later developed congenic C 3 H mice which were susceptible to MHV-2 (38)). The C 3 H/HeJ mice and C 3 H/RV mice utilized in this study are congenic for this resistance locus. Although C 3 H/HeJ mice are resistant to MHV-2, they have been found to be semi-susceptible to MHV-3 (36). Resistance of A/J mice has been demonstrated to MHV-3 (20, 25), but this genotype seems to be susceptible to MHV-JHM (15, 29). A marked dichotomy of resistance and susceptibility to neurotropic JHM has been noted between BALB and SJL mice (14, 15, 17, 29, 30). Mechanisms of resistance are multifactorial and vary with host age and genotype, virus strain, dose and route of inoculation (14). In our studies with low-virulence MHV-S given intranasally to adult mice a spectrum of resistance and susceptibility among genotypes was not particularly apparent. Strains A, BALB, CBA and C 3 H were all infected at a similar virus dose level and all developed a similar degree of nasoencephalopathy. BALB/cByJ mice developed mild hepatitis, while other strains did not, suggesting slightly higher susceptibility. SJL mice were remarkably resistant to MHV-S and did not develop brain lesions, as noted by others with more virulent MHV-JHM (14, 15, 17, 29, 30).

Most strains of MHV display neurotropism following oronasal inoculation of susceptible suckling mice (5). On the other hand, brain stem spongiosis with demyelination has been observed with only a relatively few strains of MHV, including MHV-JHM (1), MHV-S (33) and MHV-A 59 (19, 39). Reports of histological studies in mice intracerebrally inoculated with MHV-1 and MHV-3 mention inflammatory changes but not spongiosis or demyelination (7, 36). Results of our studies confirm the ability of MHV-JHM, -S and -A 59 to cause widespread brain lesions following intranasal inoculation. Our data also indicate that MHV-1 and -3 are also capable of infecting brain following intranasal inoculation, but lesions are generally

restricted to the anterior olfactory tracts. The mechanism of this virus strain-related restriction requires further investigation. It does not appear to be related to virulence, since virulent MHV strains can cause both restricted (MHV-3) and generalized (MHV-JHM) patterns and avirulent MHV strains likewise cause both restricted (MHV-1) and generalized (MHV-S) patterns.

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Authors' address: Dr. S. W. Barthold, Section of Comparative Medicine, Yale University School of Medicine, 375 Congress Avenue, New Haven, CT 06510, U.S.A.

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