Mouse Hepatitis Virus Strain — Related Patterns of Tissue Tropism in Suckling Mice

By

S. W. Barthold[1] and Abigail L. Smith[2]

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

With 3 Figures

Summary

The pattern of tissue tropism for several prototype and uncharacterized strains of mouse hepatitis virus (MHV) was studied by intranasal inoculation of each virus strain into groups of neonatal Swiss mice under otherwise identical conditions. Mice were killed at intervals up to 18 days after inoculation, and their tissues were examined for the presence of MHV antigen by indirect immunofluorescence. Two patterns of infection were apparent. Prototype MHV strains 1, 3, A 59, JHM, S and uncharacterized MHV strains Tettnang and wt-1 produced a respiratory pattern, in which nose and lung were consistently involved with dissemination to other organs in a vascular distribution. Pulmonary vascular endothelium and alveolar septal cells, but not airway epithelium, were infected. An enteric pattern was observed with MHV-Y and wt-2 in which MHV antigen was largely restricted to the nose and bowel, with limited dissemination to other abdominal organs but not lung. Intestinal lesions in these mice were severe compared to those manifesting the respiratory pattern of infection. These results indicate that, like coronaviruses of other species, different strains of MHV possess different primary and secondary organotropisms following a natural route of inoculation in a susceptible host.

Introduction

Coronaviruses are an important group of human and animal pathogens which cause primarily respiratory and enteric disease, depending on virus

strain and host species. Different strains of coronavirus from a single host species, such as man, can have either respiratory or enteric tropism (25). Laboratory mice are commonly infected with mouse hepatitis virus (MHV), a singular name which actually represents many strains that can be partially differentiated biologically or antigenically. MHV has served as a model for the molecular biology of coronaviruses, viral hepatitis, encephalitis and demyelination, as well as a model for genetic and age-related resistance to viral disease (2, 21, 23, 25, 31). MHV can cause significant clinical disease in mice (4) or a number of more subtle but equally significant effects, including modified immune responsiveness (27, 36), an effect upon tumor kinetics (1, 7, 12, 20, 22) and effects upon macrophage function (6, 34) which can negate the research usefulness of the infected animal.

Despite extensive studies on MHV, little is known about its pathogenesis in mice following natural routes of exposure. Selective organotropism of certain MHV strains, such as neurotropism and hepatotropism, has been demonstrated (23, 25, 31). However, true differences between MHV strains are difficult to assess, since in most studies, virus has been inoculated by artifical routes, only a few target organs have been examined and mice of different ages, genotypes and microbial status have been used. In the present report, we compare patterns of tissue tropism following intranasal inoculation of pathogen-free mice with several prototype and uncharacterized MHV strains under otherwise identical conditions. Since resistance to MHV is age-related, neonatal mice were utilized to allow maximal expression of infection.

Materials and Methods

Mice

Late pregnant outbred CD-1 Swiss mice were purchased (Charles River Breeding Laboratories, Portage, MI) and shipped in filtered boxes, placed in sterile flexible film isolators and allowed to whelp. Mice from this source were seronegative to all detectable murine viruses, including MHV and rotavirus, by serological methods previously described (3, 28, 30). Each experimental group was established by placing two dams and their combined litters into a Micro-Isolator cage (Lab Products, Maywood, NJ) with sterile food, water and corncob bedding. Cages were kept in separate rooms and handled with disposable gloves to prevent cross-contamination among treatment groups.

Virus

Prototype MHV strains 1, 3, A 59, JHM and S were obtained from the American Type Culture Collection, Bethesda, MD. Four wild-type MHV isolates were also utilized. Tettnang virus is a recently characterized MHV strain which is antigenically related to JHM and MHV-1 (29). MHV-Y was isolated from infant mice with typhlocolitis, and is antigenically related to MHV-S and MHV-1 (4). The other two unnamed wild type (wt) isolates herein referred to as wt-1 and wt-2, were isolated from natural cases of MHV infection in mice. Antigenic relationships for the MHV strains were determined by cross neutralization with prototype and test strain

reagents as previously described (4, 29). MHV 1, 3, A 59, JHM, S, Y, and wt-1 virus stocks consisted of tissue culture fluid from infected, mycoplasma-free NCTC 1469 cell cultures. Tettnang and wt-2 virus stocks were in the form of 10 percent infected mouse liver suspensions. Ten µl of inoculum, containing approximately 10^4 median tissue culture infectious doses of MHV, was placed on the external nares of each mouse pup at 2—4 days of age. Virus stocks were not disclosed until completion of the study.

Tissue Collection and Processing

Two pups from each group were killed by decapitation on each of days 3, 6, 11, 14 and 18 after inoculation. In some groups, pups were also examined on days 1 or 2 after inoculation and moribund pups were also examined. Depending on virulence of the inoculated virus, some samples were not available at later intervals (days 11, 14 or 18). The calvarium and body cavities were opened and tissues were fixed in 10 percent neutral buffered formalin, pH 7.2. Tissues were processed for light microscopy and immunohistochemistry as described earlier (8). All immunofluorescence tests were performed with MHV-positive and negative antiserum and tissue controls. A control group was given sterile inoculum and processed similarly. The distribution of MHV antigen in all major organs of each mouse at each interval was recorded.

Results

Cross neutralization tests (Table 1) indicated that both wt-1 and wt-2 were reciprocally related to JHM and unilaterally related to A 59 (wt-1) or MHV-S (wt-2).

Table 1. *Antigenic comparisons of field (wild type) MHV isolates with prototype MHV strains by cross neutralization*

Virus	Sera						
	MHV 1	MHV 3	MHV S	A 59	JHM	wt-1	wt-2
MHV-1	*16*[a]					—[b]	—
MHV-3		*8*				—	—
MHV-S			*16*			—	—
A 59				*8*		—	—
JHM					*16*	8	8
wt-1	—	—	—	8	16	*8*	—
wt-2	—	—	8	—	16	—	*8*

[a] Homologous neutralization reactions are in italics
[b] — Indicates neutralization did not occur with sera diluted 1:2

All MHV isolates infected multiple tissues of suckling mice. However, two basic patterns of organ involvement were apparent (Table 2). The prototype MHV strains, Tettnang and wt-1 all infected upper respiratory mucosa and lung, with generalization to other organs in a vascular distribution (respiratory pattern). MHV strains Y and wt-2 also infected upper respiratory mucosa, but not lung or vascular endothelium. These strains

were largely restricted to the intestine, where they induced significant lesions (enteric pattern). In all experimental groups, organ distribution and staining intensity of MHV antigen was greatest on days 3 and 6 after inoculation, corresponding to necrosis detected histologically. In mice beyond day 6, MHV antigen was reduced or absent, suggesting recovery. Neither MHV antigen nor lesions were found in control mice.

Table 2. *Patterns of MHV antigen distribution in neonatal mice following intranasal inoculation of different MHV isolates*

| | MHV isolate and pattern | | | | | | | | | |
| | | Respiratory | | | | | | | Enteric | |
Organ	Control	1	3	A 59	JHM	S	Tett	wt-1	Y	wt-2
Upper respiratory mucosa	−a	+	+	+	+	+	+	+	+	+
Lung	−	+	++	++	+	+	+	+	−	−
Nonpulmonary vessels	−	+	++	++	−	−	+	+	−	−
Mesothelium	−	−	+	+	+	−	+	+	−	−
Bone marrow	−	++	++	+	+	+	+	+	−	−
Thymus	−	+	+	−	+/−	−	+/−	−	−	−
Spleen	−	+	++	+	+	0	+	+	−	+/−
Peripheral lymph node	−	++	++	+	+	−	−	+	0	0
Abdominal lymph node	−	++	++	+	+	+	++	+	+	+/−
Peyer's patch	−	+/−	+	+/−	+/−	−	+	+	0	+/−
Olfactory bulb	−	+/−	−	−	+	+	−	−	−	−
Brain, other	−	+/−	+/−	−	++	++	++	++	−	+
Spinal cord	−	−	−	−	−	+	−	−	−	−
Dorsal root nerve, ganglia	−	−	−	+/−	−	+/−	+/−	−	−	−
Kidney	−	−	−	−	+/−	−	−	−	−	−
Liver	−	++	+	++	+	+	+	++	−	+
Pancreas	−	−	−	−	−	−	−	−	−	−
Stomach	−	−	−	−	−	−	−	−	−	−
Small intestine	−	−	+	+/−	+/−	+	+	+	++	++
Large intestine	−	−	−	+/−	+/−	−	+	+	++	++

a All mice negative
+/− Single mouse positive, mild involvement
+ Most mice positive, mild-moderate involvement
++ Most mice positive, marked involvement
0 Tissue not available

In mice from all groups, the upper respiratory mucosa usually had foci of MHV antigen-positive bipolar olfactory epithelial cells or columnar nasal epithelial cells. Apparent extension of MHV antigen into neurons of the adjacent olfactory bulb was seen with MHV-S and JHM and a single MHV-1 pup. Mice inoculated with most of the strains contained MHV antigen in neurons (Fig. 1) but not glia with scattered foci in the cerebral

cortex, midbrain and brainstem. The cerebellar cortex was usually spared. The pattern suggested a vascular distribution, although MHV antigen was not found in brain vessels. Random involvement of cranial and spinal nerves or ganglia was observed in a few mice.

With the exception of groups inoculated with MHV-Y and wt-2, lung was a frequent site of MHV antigen localization. A few pyknotic cells in alveolar walls and hemorrhage into alveoli were evident in these mice. Fluorescence of vascular endothelial cells and unidentified alveolar septal cells (Fig. 2) was usually noted in a patchy distribution. Antigen in vascular endothelium was often observed in several other organs, especially the great vessels at the base of the heart.

Fig. 1   Fig. 2

Fig. 1. Indirect immunofluorescence micrograph, depicting MHV antigen in the cytoplasm of cerebral cortical neurons of a neonatal mouse inoculated intranasally with MHV ($\times 630$)

Fig. 2. Indirect immunofluorescence micrograph, depicting MHV antigen in endothelium (vessel in upper right) and alveolar septal cells in the lung of a neonatal mouse inoculated intranasally with MHV. Grey background material represents erythrocytes in alveolar spaces ($\times 630$)

Foci of hepatocellular necrosis and MHV antigen were visible in all but MHV-Y-infected mice. Necrosis was remarkably severe in the MHV-3-inoculated mice but with relatively weak fluorescence of MHV antigen in involved hepatocytes. Intensity of MHV antigen staining in the liver was usually mild compared to other organs and antigen-positive hepatocytes were often found without necrosis. Kupffer cells only rarely contained antigen. A single JHM-infected mouse had one focus of interstitial MHV antigen in the renal medulla. In all other mice, kidney, pancreas and salivary gland (when represented) did not contain detectable MHV antigen.

MHV antigen was found in intestine (but not stomach) of all groups except MHV-1-infected mice. Antigen was usually confined to a few randomly distributed single and small multinucleate enterocytes in the small or large intestine. Histopathological changes were minimal. Exceptions to this pattern were apparent in mice infected with MHV-Y and wt-2. These mice had extensive MHV antigen in the intestinal mucosa, especially in the colon and cecum (Fig. 3). This form of infection was associated with mucosal necrosis, hyperplasia and inflammation.
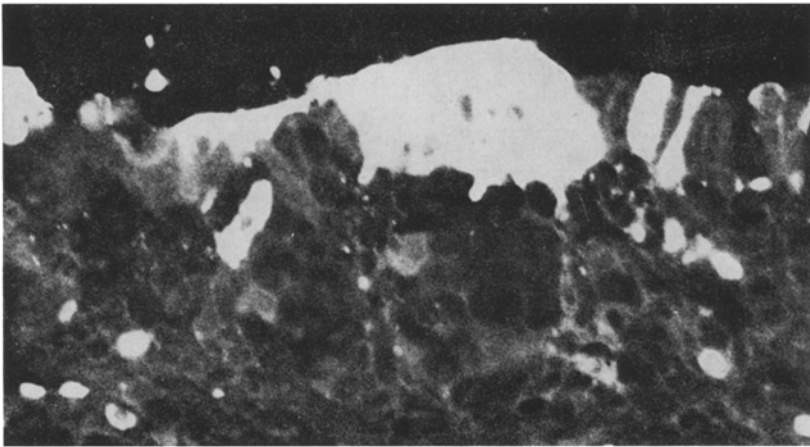

Fig. 3. Indirect immunofluorescence micrograph, depicting MHV antigen in single and syncytial colonic enterocytes of a neonatal mouse inoculated intranasally with enterotropic MHV. Most of the antigen is present in surface epithelium. Fluorescent structures in the underlying mucosa represent autofluorescing erythrocytes (× 630)

MHV antigen was found in one or more lymphoreticular organs in mice of all groups and bone marrow of many mice. Foci of MHV antigen were evident in the thymic medulla and only rarely cortex, peripheral lymph nodes or lymph nodes draining abdominal organs (hepatic and/or mesenteric). The splenic white pulp and foci of red pulp generally contained few MHV antigen-positive cells. Lymphocytic necrosis and occasional syncytia were seen in these organs. Bone marrow involvement was focal but present

in many bones. MHV antigen was seen in hematopoeitic cells including megakaryocytes. MHV antigen was not observed in bone marrow of MHV-Y or wt-2-infected mice.

Discussion

These results help to clarify the interaction of MHV strains with their host species, the mouse. Hepato- and neurotropism following intraperitoneal and intracranial inoculation of MHV have been emphasized in the literature (21, 23, 25, 31), providing little insight into pathogenesis following natural routes of infection. This study suggests that strains of MHV produce two basic patterns of infection, respiratory and enteric, following intranasal inoculation. Secondary involvement of other organs, such as liver, brain, bone marrow and lymphoid organs, is common in a susceptible host such as the neonate. Coronaviruses of other species also manifest these two primary patterns, often with secondary involvement of other organs (25).

The most common pattern of infection, as seen with the prototype MHV strains and with two other strains, Tettnang and wt-1, is the respiratory pattern. Others have shown preferential infection by the intranasal over oral route with MHV-2 (17), MHV-S (32), and JHM (10). Lesions or antigen have also been shown in the nasal mucosa of mice infected with MHV-S and JHM (3, 14, 33) and lungs of mice inoculated with MHV-1, A 59, MHV-S and JHM (3, 9). Endothelial syncytia have been described in the pulmonary vasculature of athymic nude (nu/nu) mice (13). In lungs, MHV antigen is restricted to vascular endothelium and unidentified alveolar septal cells. The lung is therefore probably important in clearance or dissemination of virus within the host, rather than as a route of virus excretion. Weanling Swiss mice inoculated intranasally with MHV-S have lesions, viral antigen and infectious virus in the nose and lung early in the course of infection, with occasional dissemination to other organs at later intervals (3). The findings reported here and previously (3) suggest that the bowel plays a minimal role in virus replication and excretion with MHV strains which cause the respiratory syndrome.

Enteric MHV syndromes have been recognized for some time (5), but the relationship of the causative viruses to the MHV group has been confusing. Like coronaviruses of other species, enteric MHV strains produce significant lesions and resultant clinical signs referable to the bowel, particularly in neonates. Some of these strains appear to be highly enterotropic, with little dissemination to other organs, as seen with MHV-Y (4), while others (L.I.V.I.M., MHV-S/CDC, MHV-D) spread to liver and brain depending on virus strain and host factors (5, 15, 18).

The data generated in this study are intended for relative comparison between virus strains under parallel conditions, but should be interpreted

with caution. They do not necessarily mean that a given MHV strain possesses or lacks tropism for a particular tissue. Tropism for any organ, tissue or cell type depends not only on virus strain and passage history, but on route of inoculation, host age and genotype as well. For example, MHV A59 did not infect nervous tissue in this study or as an original isolate (22), but others have shown A59 neurotropism (19). Temperature-sensitive mutants of neurotropic JHM may have selective tropisms for glia, while parental (wild type) JHM replicates in both neurons and glia (11). Different patterns of brain lesions occur with MHV-3 in different mouse genotypes (35). Both MHV-S and JHM were markedly neurotropic in the present study, a feature which was obvious when both of these strains were first isolated (10, 26). Furthermore, direct extension of MHV from the nose to the olfactory bulbs has been described only with these two strains (3, 14, 33), an observation which we have reproduced here.

There are clearly MHV strain-related patterns of primary target tissue and secondary organotropism, but these patterns overlap considerably. Based on the nine strains compared here, tropism for bone marrow and lymphoreticular organs, as described for MHV-3 (16, 24), may be common for most MHV strains. Even basic patterns can overlap, since an entero-tropic MHV strain, MHV-D, has been shown to infect nose and lung as well as liver and brain (18). However, the current study provides useful generalities for understanding the pathogenesis of infection with this very common virus of laboratory mice.

Acknowledgments

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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.