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CHAPTER 25

MOUSE HEPATITIS VIRUS BIOLOGY AND EPIZOOTIOLOGY

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I. INTRODUCTION

Mouse hepatitis virus (MHV) is a highly contagious and ubiquitous coronavirus of laboratory mice. It is present among mice in many commercial breeding colonies and most biomedical research institutions and has been documented in laboratory mice throughout the world. Recognition of the ubiquity of MHV is often not realized because of unfamiliarity with its biology, reliance on insensitive diagnostic methods and the subtle effects of the virus. Its subtle effects should not be underestimated, however, since MHV has been associated with a panoply of effects on mice and research data derived from them. Considering the large financial investment in biomedical research and that mice represent the majority of animals used in such research, the potential scientific and economic impact of this single virus is enormous.

Since the original isolation of MHV in 1949 (1), a body of literature representing nearly 350 citations has been developed on the subject of MHV. The voluminous literature

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on MHV would not have been generated if MHV were simply a pathogen of mice. Interest in MHV has evolved from a number of different perspectives, including emphasis on MHV as a model of viral hepatitis, viral encephalitis and demyelinating disease, genetic mechanisms of host resistance to viral infection, and the molecular biology of coronaviruses, using MHV as a prototype. These have been the subjects of several reviews (2-11).

On the one hand, the literature clearly illustrates the complexity of MHV pathogenesis. Virus strain, passage history, dose, route of inoculation, host genotype, age, immune function and co-infection with other agents all interact to determine the ultimate expression of MHV disease. On the other hand, this complexity interferes with a clear view of the biology of MHV as a natural pathogen of mice. Many of these studies have emphasized different aspects of MHV-host interactions, using artificial routes of inoculation and analysis of only one or a few target organs or effects. As a result, still relatively little is known about the natural pathobiology of MHV in mice. Without such information, the true significance of this agent cannot be assessed and its effect under diverse experimental conditions cannot be predicted. This review will discuss recent updates in MHV pathobiology and incorporate past literature in that discussion wherever possible.

II. PROPERTIES OF THE AGENT

Coronaviruses, including MHV, are enveloped viruses containing single-stranded, non-segmented, infectious RNA with positive (messenger) polarity. The molecular biology of MHV will be discussed in Dr. Holmes' chapter and has been reviewed elsewhere (3,10). Physical resistance properties have also been reviewed (4). Coronaviruses infect many species of birds and mammals, causing respiratory or enteric infections in their hosts (Table 1). The relationship among viruses of this family is complex. They do not share a common group antigen, yet many are antigenically and genetically related (8,10,12). Although "MHV" is a singular term, it refers to many named and unnamed strains of mouse coronavirus (Table 2). Certain prototype strains have served as standards for study of the MHV group, including MHV-1,-2,-3,-A59,-JHM and -S. These strains have been used in most experimental studies and as antigenic or genetic standards for MHV serodiagnosis and comparison to new MHV

Table 1
Coronaviruses and Their Host Species (3)

Human respiratory, enteric coronaviruses	man
Simian enteric coronaviruses	nonhuman primates
Infectious bronchitis virus	chicken
Bluecomb disease virus	turkey
Runde tick coronavirus	tick?
Transmissible gastroenteritis virus	pig
Hemagglutinating encephalomyelitis virus	pig
Neonatal calf diarrhea virus	ox
Ovine enteric coronavirus	sheep
Equine enteric coronavirus	horse
Canine coronavirus	dog
Feline infectious peritonitis virus	cat
Feline enteric coronavirus	cat
Rabbit coronavirus	rabbit
Sialodacryoadenitis virus, rat coronavirus	rat
Mouse hepatitis virus	mouse

Table 2
MHV Strains and Isolates

MHV-1	MHV-RI
MHV-2 [MHV(PRV)]	MHV-(SR3)
MHV-3	MHV-(SR4)
MHV-JHM [MHV-4]	Lethal intestinal virus of infant mice (LIVIM)
MHV-A59	MHV-S/CDC
MHV-S	MHV-D
MHV(C3H)	MHV-Y
EHF 120 [MHV-B]	Diarrhea virus of infant mice (DVIM)
H747	Tettnang virus
ANA	MHV NuA
MHV-C [MHV-BALB/c]	MHV Nu66
MHV (Braunsteiner/Friend)	MHV-Kyoto
MHV (SR1)	
MHV (SR2)	

isolates. All MHV strains or isolates share cross-reacting antigens, but each strain also possesses strain-specific antigenicity, with considerable overlap (6,12-15). Similarly, while there is RNA and polypeptide homology between MHV strains, there is also strain-related heterogeneity (16,17). This suggests ongoing "drift" or mutation as a factor in the biology of MHV. Relatedness of one MHV strain to another is not a useful means of predicting biological behavior. For example, MHV-S and MHV-JHM are relatively unrelated genetically, yet induce similar patterns of disease. Related strains such as MHV-S and MHV-S/CDC produce different patterns of disease (14, 16-20). Furthermore, the behavior of a defined or named MHV strain depends on its passage history and thus varies from laboratory to laboratory. MHV also cross-reacts antigenically with coronaviruses of the rat and some strains of coronavirus of other species, including man (12,21-23). The biological significance of these relationships has not been thoroughly explored.

III. PATHOGENESIS

MHV produces disease in mice by lytic infection of cells, although viral replication and exocytosis can take place in the absence of cytolysis. In addition to cytolysis, cell fusion is a hallmark of MHV, both *in vivo* and *in vitro*, although it is not necessary for viral replication (3,7). MHV pathogenesis is significantly influenced by both virus and host factors. Virus factors include route of inoculation, dose and virus strain (1, 24-27). Virus strain-dependent features include virulence, primary pattern of infection, secondary organotropism and cell tropism. There is a full spectrum of virulence among different MHV strains. Some MHV strains display only weak pathogenic properties, even in athymic nude mice (18,26,28), while other strains such as MHV-2 and MHV-3 are far more pathogenic (24,29). Most naturally encountered MHV isolates are only mildly pathogenic, requiring some other factor for expression of clinical disease (3,6).

Like coronaviruses of other species, strains of MHV cause infections of either the respiratory or enteric systems (3,19,30,31). A respiratory pattern of infection is typical of many MHV strains, including prototype strains (MHV-1,-3,-A59,-JHM,-S) (19). Thus, much of our knowledge about host resistance factors, such as age, genotype and

lymphorecticular function, applies to this group of MHV strains. These strains are most apt to cause illness associated with hepatitis and encephalitis (3,19,30). Other strains of MHV cause an enteric pattern of infection, with lesions and disease referable to the intestine. Identified enterotropic MHV strains include LIVIM, MHV-D, DVIM, MHV-S/CDC and MHV-Y (13,14,32-36). In contrast to respiratory MHV strains, very little is known about host resistance factors to enteric MHV. Most of the MHV strains listed in Table 2 have not been characterized as to their primary pattern of infection, but it is clear that both respiratory and enteric strains exist in contempory mouse populations (19).

Selective organotropism is often stressed as an MHV strain-related phenomenon, such as neurotropism of MHV-JHM (37), hepatotropism of MHV-A59 (38), lymphocytotropism and bone marrow tropism of MHV-3 (39,40). Such differences are only relative, with considerable overlap between MHV strains (19). Many of these differences are simply a reflection of the emphasis an author places on a particular target organ. Nevertheless, recent work is suggesting that selective cell tropism can take place with specific MHV strains and that host cell type can influence the outcome of MHV infection. In primary central nervous system cell cultures, MHV strains exhibit different cell tropism, cytopathic effects and viral assembly, which may account for different patterns of disease (10,41-48).

Understanding the pathogenesis of MHV is best approached by catagorizing infections into the respiratory and enteric patterns, which are determined by virus strain. Relative differences in organo- and cell tropism exist between virus strains in each of these catagories. Furthermore, host factors play a significant role in severity of infection within these basic themes. The following discussion will detail what is known about the pathogenesis of respiratory and enteric patterns of MHV infection with supporting references for each pattern.

IV. RESPIRATORY PATTERN INFECTIONS

These MHV strains rely on upper respiratory mucosa as a site of primary replication and excretion. In weanling or adult outbred mice infected with low-virulence MHV (MHV-S), infection is largely restricted to upper respiratory mucosa (18,20). Secondary involvement of other organs can occur

with more virulent MHV strains or in susceptible hosts, such as neonates, adult susceptible genotypes and immunocompromised hosts, including nude mice. Under these circumstances, there is dissemination to multiple organs in a vascular distribution (19). Lungs are frequently infected early in the course of disease, with antigen present in vascular endothelium and alveolar septal cells (18,19,49). This explains the frequency of pulmonary vascular syncytia in infected athymic nude mice (50) and perivascular and interstitial inflammatory changes in recovered mice (3,18,49). Vascular endothelium in other organs is also infected. In susceptible neonates, virtually any other tissue can become focally infected with MHV, including brain, spinal cord, ganglia, spleen, thymus, lymph nodes, intestine, brown fat, bone marrow and mesothelium (3,19,30,31). Respiratory-type MHV strains are often isolated from feces of susceptible hosts but not resistant hosts in which infection is restricted to the upper respiratory tract (3,6,18-20,51). Multinucleate syncytia of enterocytes are also seen in susceptible hosts, including nude mice (19,20,37,52) but lesions are inconsistently present and minor compared to those caused by enterotropic MHV strains (3,19,30,31). With respiratory strains of MHV, transmission takes place among adult mouse populations in the absence of intestinal excretion (3). Thus, although intestinal infection may be present, it does not play a major role in the replication strategy of respiratory MHV strains.

Since brain is often infected with several strains of MHV in susceptible hosts, neurotropism is not a particularly unique feature of any single MHV strain. However, some MHV strains (S and JHM) seem to possess the relatively unique ability to extend directly from the nasal lining into the olfactory tracts of the brain, even in older, resistant mice (18-20,37,53). Within 48 hours after intranasal inoculation, MHV-JHM causes meningitis and necrosis of mitral cells in the olfactory bulbs, with extension into the olfactory tracts, meninges, piriform cortex, septum pelucidum, anterior commissure, and hippocampus within 120 hours. During this phase, both neurons and glia are infected. At 144 hours, virus is present in the pons, medulla and spinal cord and by 168 hours, severe demyelination is present. At this time, titers are diminishing (53). A similar course of events probably occurs with MHV-S (18,20). The demyelinating component has been shown to be due to selective destruction of oligodendroglia (54-57) and reportedly does not seem to occur with other encephalitogenic strains of MHV, such as MHV-3 (41,47,58). Virus

strain tropism for different nervous system cell types *in vitro* correlates with disease phenotype (41-48). The pattern of nervous system disease that occurs is also dependent on host age, genotype as well as dose and route of virus inoculation (45,57-59).

Duration of MHV infection is a subject of considerable contention. The behavior of the virus under natural conditions has created the impression that infections are chronic and latent, since many experimental manipulations such as immunosuppression, tumor transplantation or co-infection with other agents will precipitate acute disease (3). It appears, however, that this impression is created by the generally subclinical (but not latent) nature of MHV infection and that experimental manipulations that exacerbate disease are coincidentally applied during active infection. An overwhelming body of literature with a number of MHV strains clearly supports the concept of short-term infection, from which the host fully recovers within 2-3 weeks of infection (18,19,40,48,57,60-66). Aggressive immunosuppression after the acute phase of MHV infection will not reactivate the virus, even when residual MHV antigen and lesions remain in the brain (66,67). A smaller number of reports exist, however, that uphold the hypothesis of persistence. Mice of the semisusceptible C3H genotype that survive intraperitoneal inoculation of virulent MHV-3 can have persistent infections, in which virus can be recovered in low titer from liver, brain and lymphoid organs for up to 42 days (68). Chronic brain infections have also been established in mice following intracerebral inoculation of MHV-3, intracerebral or intranasal inoculation of brain-passaged MHV-JHM and selected temperature sensitive JHM mutants (58, 69-71). However, others have shown neurotropic MHV is eventually cleared from the brain (18,19,48,57,67). These studies suggest that MHV can potentially cause chronic persistent infections, particularly under artificial experimental conditions, but limited infections, without a chronic carrier state, are the norm. An obvious exception is the athymic nude mouse, which suffers from a wasting syndrome due to chronic, persistent MHV infections. In contrast to their heterozygous euthymic counterparts, which recover within 2-3 weeks, homozygous athymic mice develop increasingly high titers of virus in multiple organs, ultimately dying from hepatitis or encephalitis. During the early acute phase of infection, euthymic and athymic mice have parallel titers and distribution of virus (28,60, 72,73).

Persistent MHV infections incontestably take place *in vitro* in cultured cells in the absence of immune surveillance in the live mouse. This has been demonstrated repeatedly with a variety of mouse and rat cell types (47,74-79). The addition of a low concentration of antiviral antibody to the culture medium was found to modulate infection, producing a carrier state with intracytoplasmic antigen, but no virus production or expression of viral antigens on the cell surface. The carrier state continued in the absence of antibody, and virus could not be rescued by co-cultivation with susceptible cells. Virus could only be recovered following fusion of persistently infected cells to permissive cells with polyethylene glycol (78,79). The significance of these observations for the mouse remain to be determined.

Host-related factors in MHV pathogenesis have been elucidated using several respiratory prototype MHV strains. Mice less than 2-3 weeks of age are susceptible, regardless of genotype (2,26,80-82). Other workers have suggested that resistance to intracerebral JHM infection further evolves at 6-12 weeks of age (83,84). Genotype plays an important role in expression of susceptibility and resistance to MHV in adult mice (2,45,46,64,80,81,84-89). A simple list of genetically susceptible and resistant mouse strains cannot be generalized, since disease expression seems to be dependent on interaction of host genotype with virus strain. For example, adult C3H mice have been reported to be resistant to MHV-2 (2,64,80,81,85,86,90,91), but susceptible to MHV-JHM and MHV-3. Similarly, A/J mice have been reported to be resistant to MHV-3, but susceptible to MHV-JHM (46,89).

Genetic expression of susceptibility *in vivo* often, but not always, parallels intrinsic susceptibility of cultured cells *in vitro*. This has been demonstrated with cultured macrophages (2,45,46,64,80,81,84-89) hepatocytes (92) and neurons (46). Others report that spleen cells, but not macrophages, reflect genetic susceptibility (93). Resistance at the cellular level *in vitro* is also age dependent, evolving at the same interval as *in vivo* (2,80-82,94). Resistant cells adsorb virus, but may or may not express limited antigen and tend not to release virus, while susceptible cells express antigen and release replicating virus (2,45,64,82,84,86,87,89,92). Again, virus strain is important in determining outcome of cell infection. MHV-JHM productively infects both neuronal and non-neuronal cells *in vitro*, while laboratory-created temperature-sensitive mutants of JHM cause non-productive infection of non-neuronal cells only, with expression of

antigen without virus replication. On the other hand, MHV-A59 selectively infects non-neuronal cells in a productive fashion (41). Differences among various MHV strains have been observed in their ability to productively infect adherent cells (macrophages) from liver explant cultures (88). Intrinsic susceptibility of cells in vitro can be modified with lymphokines (90,91) and interferon (82,96,97).

Lymphoreticular function in the whole animal also influences the course of MHV infection. Agents that interfere with or stimulate macrophage activity alter the course of MHV infections (65,90,95,98-100). Interferon (IF) plays a disputed role in MHV pathogenesis. Serum IF levels are low in MHV-infected immature mice and elevated in adults (82), but others have found that resistant mouse genotypes may produce lower levels of IF than susceptible genotypes in response to MHV infection (88,101). Others have found the same degree of IF levels (93). Tissue IF levels have also been variably correlated with resistance (88,102). It is apparent that IF plays a role in the initial response of mice to MHV (97), and becomes elevated in adult, susceptible genotypes because of permissive infections, also resulting in increased natural killer (NK) cell activity in some mice. In resistant adults, virus titers, IF and NK cells may all remain low (101). Immunosuppression by a variety of means, including x-irradiation, neonatal thymectomy, anti-lymphocyte serum, graft vs host reaction, cyclophosphamide and corticosteroid treatments, renders resistant mice susceptible to MHV. Immunosuppression, however, does not modify intrinsic genetically determined resistance of cells cultured in vitro or abrogate a secondary immune response to virus challenge (62,64,94,103,104).

Cell-mediated immunity is crucial in recovery from MHV infections. This fact is most evident in T cell deficient, athymic nude mice, in which MHV titers initially parallel their heterozygous euthymic counterparts, but continue to rise as heterozygotes recover (73). Resistance can be transferred to nude mice with unprimed spleen cells from euthymic mice and abrogated by treatment of spleen cells with anti-theta but not anti-IgG serum (105,106). Similarly, resistance in neonatal mice can be transferred with macrophages, T lymphocytes and macrophages, whole spleen cells and macrophages, T lymphocytes and bone marrow-derived cells with NK cell features (82,83,94,107). T lymphocytes alone do not seem to be effective (62,83,94).

Antibody does not confer intraperitoneal MHV challenge resistance (62, 83,106), although vaccination does (60,108). Only a single study has demonstrated delayed-type hypersensitivity in MHV immunized mice (62).

It becomes apparent from this overview that MHV strains interact at different levels with mice of different ages and genotypes. Expression of susceptibility or resistance is mediated first through the intrinsic ability of the target cell to support or restrict MHV replication. This can be further modified by IF and NK cells, among other factors. Secondly, infection is restricted by a specific immune response to the virus, resulting in susceptibility, attenuated disease or recovery. This is influenced by immunosuppressive regimens or impaired immune responsiveness. Both intrinsic resistance and development of an effective immune response are age and genotype dependent (29,62,64,84, 97,101,103,109). This is why susceptibility to MHV can be predicted in genotypes that allow high-titer virus replication early in infection, prior to mounting an immune response (62,64,97,109). It also explains the confusion over whether genetic susceptibility or resistance are inherited as one or two genes, recessive or dominant, H-2 haplotype linked or unlinked with different MHV strains, different routes of inoculation and different mouse genotypes (2,45, 46,59,80,109).

The course of MHV infection is notoriously sensitive to modification of lymphoreticular function. Normally resistant mice are rendered susceptible by x-irradiation, cyclophosphamide, cortisone, neonatal thymectomy, antilymphocyte serum and graft vs host reaction, among other manipulations (3). Co-infection with other infectious agents also potentiates MHV disease. Eperythrozoon coccoides and K virus are normally minimally pathogenic, but they exacerbate MHV in resistant hosts (104,110). Leukoviruses and even Schistosoma mansoni also enhance MHV disease. These diversely related infectious agents have been suggested to have a common mechanism by blocking IF responsiveness of the host to MHV (111-113).

V. ENTERIC PATTERN INFECTIONS

Several enterotropic strains of MHV have been identified. Like other MHV strains, they can be differentiated antigenically, but also share antigenic and genetic homology with other members of the MHV group (13,14,17,34-36). The

upper respiratory mucosa is often a target of enterotropic MHV (19), but intestinal mucosa is the major target for virus replication and excretion. Unlike respiratory MHV strains that infect intestine only in highly susceptible hosts, enterotropic MHV universally infects intestine, regardless of host age. In neonatal mice, epithelial degeneration, necrosis and syncytia predominate, resulting in erosions, ulceration and villus attenuation. Virus-induced syncytia, or "balloon cells," are diagnostic. Intracytoplasmic inclusions have also been described. Lesions can be fully developed within 24-48 hours (32), but are usually most severe at three to five days (14,19,33). In surviving mice or mice exposed at older ages, there tends to be less necrosis and more compensatory mucosal proliferation, or hyperplasia. Adult mice are also susceptible, but usually have mild infections with only minor lesions (3,13, 14,31,32,34). Lesions can be found anywhere from pylorus to anus, but stomach is not involved. Distal small intestine, cecum and ascending colon are preferential sites (3,13,14, 19,31-33). Dissemination beyond the intestine can also occur. Some MHV strains are highly restricted to intestinal mucosa and mesenteric lymph nodes, while others spread to liver and other organs (3,13,14,19,31,32). MHV-D, like MHV-JHM and -S, appears to share the ability of infecting the olfactory bulbs of the brain (33). These differences in behavior have not been carefully examined and may be either virus strain or host related. The influence of host genotype and immune response have not been well studied with enterotropic MHV. However, clinical symptoms and lesions associated with intestinal protozoal infestations are exacerbated by enterotropic MHV (3,31) and x-irradiation exacerbates enterotropic MHV infection.

Duration of intestinal MHV infections is limited, with no known carrier state beyond two weeks after exposure (3,13,19,31,32). An exception is the athymic nude mouse, which develops persistent infections of the intestinal and nasal mucosa. Enteric mucosa tends to be segmentally hyperplastic with syncytia. Involvement of other organs is minimal or absent. These mice suffer from progressive emaciation, but lesions and distribution of lesions differ markedly from the typical wasting syndrome caused by respiratory MHV strains (3,31,114).

VI. DIAGNOSIS

Clinical disease is usually absent or mild and lesions are often restricted to the primary target organ (nose or intestine), except in highly susceptible hosts. Disease and lesions are most apt to be seen in naive mouse populations experiencing an initial epizootic. Suckling, genetically susceptible or immunologically compromised mice are the best candidates for diagnostic evaluation. Suckling mice naturally infected with respiratory MHV suffer moderate to high mortality between 4-10 days of age. They may have signs of encephalitis, but more often signs are non-specific. Gross necropsy findings include multiple white spots on the liver (30,51). Flaccid paralysis was described in adult Swiss mice naturally infected with MHV-JHM (1), but this is uncommon. A variety of experimental manipulations will exacerbate otherwise mild infections in resistant mice (3). Enterotropic MHV strains tend to produce explosive epizootics of diarrhea and high mortality among infants but these signs may be absent in enzootically-infected colonies. Necropsy findings include enteritis, usually in the absence of liver lesions. Surviviors are often runted and pot-bellied (3,14,19,31-34).

Histopathology is a useful means of confirming a diagnosis of MHV, but only in susceptible mice during the active phase of infection. In susceptible hosts, particularly neonates and athymic nude mice, MHV induces multinucleate syncytia in multiple tissues, which are pathognomonic for MHV. Syncytia are especially obvious in enteric mucosa of mice infected with enterotropic MHV. Histopathology details are given elsewhere (3,30,31). A more definitive diagnosis can be made with the use of immunohistochemical techniques such as immunofluorescence and immunoperoxidase. Formalin-fixed, paraffin-embedded tissues can be ultilized for this purpose if subjected to prior trypsinization. Antigen is very stable in formalin-fixed tissue, allowing retrospective analysis of archival cases (13,18,19,115). Electron microscopy can also be utilized. MHV has characteristic coronavirus morphology and buds into cisternae of endoplasmic reticulum. It can also produce intracytoplasmic aggregates of viral products (3,13,31).

Virus recovery from actively infected tissues is difficult but can be accomplished using a variety of effective cell lines, such as NCTC 1469, DBT, 17Cl-1 or L929 cells. These cell lines, however, may not be successful substrates for some enterotropic MHV strains. Syncytium formation is the hallmark MHV cytopathic effect. Identification of a

suspect agent as an MHV strain can be accomplished by immunocytochemistry. Reciprocal serum neutralization is used as a means of characterizing the antigenic relatedness of a new isolate to prototype MHV strains. However, because of extensive and complex antigenic inter-relationships, this method is not definitive for strain classification (13-15, 19,116).

Several serological assays can be used for detection of antibody in MHV-recovered mice. The complement fixation test was used extensively at one time, but is usually too insensitive to detect seroconversion following natural exposure (13,117-120). As mentioned above, serum neutralization against prototype MHV strains grown in vitro can be used, but it tends to be highly strain-specific. The neutralizing profiles of sera from different mouse populations or from different MHV outbreaks within a population can be compared to determine if the same or unrelated MHV strains are involved (13-15). Hemagglutination inhibition is used for detecting antibody to coronaviruses of other species, but only one MHV isolate (DVIM) has been found to possess a hemagglutinin (35,36). Two very sensitive and specific assays for MHV antibody are an indirect immunofluorescence assay (IFA), using MHV-infected cells and an enzyme-linked immunosorbent assay (ELISA) (118-120). Both offer the advantage of using multiple MHV strains simultaneously as antigens in order to cover a broad range of reactivity to MHV serotypes. Under experimental conditions, these two tests are nearly equally sensitive and both are more sensitive than serum neutralization (120). Nude mice are not good candidates for serology. Although they can produce neutralizing antibody to MHV, titers are widely variable and sometimes absent (60,141).

Morphological, immunohistochemical, virus recovery and serological methods can be embellished with the use of susceptible MHV hosts. For example, if MHV is suspected but cannot be confirmed in a mouse population, athymic nude mice can be placed in the animal room as sentinels. When these mice become infected, lesions and antigen are easily confirmed and virus is easier to isolate. Infant mouse brain inoculation will amplify virus titers from suspect tissues, allowing easier antigen detection or virus recovery. Similarly, inoculation of cortisonized mice will serve the same purpose. The mouse antibody production test can also be effectively utilized. Inoculation of adult, virus antibody free mice with suspect material will result in seroconversion if MHV is present.

VII. EPIZOOTIOLOGY

Under natural conditions, MHV is highly contagious and is transmitted by the respiratory or oro-fecal routes. Expression of overt disease requires immature, immunocompromised or genetically susceptible mice. Under experimental conditions, mice seroconvert by IFA or ELISA between 7 to 14 days after inoculation (120). Under natural conditions, seroconversion occurs in 100% of mice within two weeks after introduction to an MHV-infected population, underscoring the rapidity of exposure that takes place (unpublished observations). Outbreaks of clinical disease among susceptible neonatal mice are also very rapid with high morbidity (13,14). As already discussed, infections last less than two weeks with seroconversion at the time of recovery (18,120). Maintenance of infection in a mouse population (enzootic infection) requires continual exposure of new, susceptible mice, either through newly introduced mice or breeding populations. In the absence of susceptible mice, MHV will die out of a population.

Frequently, MHV-free mice are received into enzootically infected mouse rooms. If they are neonatal mice, they suffer high mortality (51). However, extramural mice are usually weanlings or older, developing only subclinical infections upon exposure, but perpetuating the infectious cycle. Use of these newly acquired exposed mice within the first one to two weeks after arrival creates a high potential for exacerbation of overt disease from subclinical infection when experimentally immunosuppressed or otherwise stressed. In enzootically infected populations, disease or lesions are most apt to be encountered in weanlings, rather than neonates, because neonates are protected by passive immunity. Colostral IgG from immune dams is partially protective under experimental conditions and seems to occur naturally (14,122). Therefore, when MHV initially infects a naive mouse population, neonatal mice suffer high mortality, particularly with enterotropic MHV strains (13,14). Once infection is enzootic, adult mice confer protection to neonates, so the infection cycle is perpetuated among older mice, when their passive immunity wanes.

Vertical transmission of MHV is possible, but unlikely in the short time span of an acute infection relative to the breeding life of a female mouse. Transplacental transmission to fetuses has been shown in mice inoculated with MHV-JHM intravenously, but depended upon stage of gestation at the time of inoculation (123). Neither transplacental transmission to fetuses nor vaginal transmission to newborns

could be demonstrated in dams inoculated intraperitoneally with MHV-3 (124). Thus, the potential is there, but it is not a likely consideration.

One aspect of MHV epizootiology that is not well understood is whether repeated infections can occur with the same MHV strain or different MHV strains. Challenge resistence to MHV has been accomplished by vaccination of mice with denatured virus or surface peplomers, but not with virus membrane or ribonucleoprotein subcomponents, suggesting that the most immunogenic components of the virion are virus-strain specific (60,108).

The host range of MHV has not been defined. Under natural conditions, rats and mice develop serum antibody to coronaviruses of the heterologous species but this is not surprising because of the antigenic cross-activity of rat and mouse coronaviruses (21,23). Mice are experimentally susceptible to rat sialodacryoadenitits virus (SDAV) (21). Suckling mice infected with SDAV develop encephalitis, but not hepatitis or enteritis. Weanling mice develop interstitial pneumonia, with antigen in alveolar lining cells (type 1 pneumocytes). In contrast, MHV infects alveolar septal cells, but not lining cells in the mouse (19). Suckling rats are susceptible to MHV by intranasal inoculation. Lesions are restricted to the nasal mucosa and antigen is present for only 2-3 days (125). Intracereberal inoculation of rats with MHV-JHM causes encephalitis and demyelination (9). *In vitro* growth characteristics of viruses from these two species also differ. SDAV will grow in primary rat kidney but not mouse cells and MHV will grow in mouse but not rat cells (23,126). SDAV passaged through infant mouse brain remains unable to grow in mouse cells *in vitro* (126). It therefore appears that cross-species infections are laboratory, rather than natural, phenomena. The antigenic relatedness of MHV to coronaviruses of other species, including man, has unexplored biological significance.

VIII. IMPLICATIONS FOR RESEARCH

MHV has a long history of interference with research. Most MHV strains were isolated and identified as inadvertant contaminants that were exacerbated by experimental protocol. Subclinical MHV infections are potentiated by a variety of experimental manipulations that modify immune or

macrophage function (3). Co-infections with other infectious agents have been mentioned as potentiators of MHV disease. Treatment of mice with urethane, methylformamide (27), halothane (128), ferric salts (99) and necrogenic yeast diets (130) also precipitate more severe MHV disease. Transplantable tumors, especially ascites and lymphoreticular tumors, can be contaminated or become contaminated with MHV. Tumor transplantation can exacerbate MHV disease, but MHV can also influence tumor kinetics. Abnormal tumor invasion patterns, abnormal tumor passage intervals and spontaneous regression of normally stable transplantable tumor lines have all been observed (38,127,131-133). MHV-infected, athymic nude mice will reject human tumor xenografts (131-134). Growth of MHV in vitro is enhanced by cell transformation, which may explain its intimate association with tumors (135). MHV can form pseudotypes with murine leukemia virus in vitro, but whether this occurs in vivo has not been determined (136). In addition to contaminating tumors, MHV can contaminate established cell lines. Latent infections, without cytolysis, occur under these circumstances and can interfere with growth of other viruses, including MHV itself (47,74-79).

A number of direct effects of MHV on certain target tissues have been described. Effects on the liver include alteration of hepatic enzyme and other biochemical markers (3,6), induction of alpha-fetoprotein (137) and increased iron uptake (138). The mitotic response of liver to injury is altered by MHV. Regeneration in response to partial hepatectomy is delayed during acute MHV infection and the mitotic index is markedly elevated during recovery (139), which is also seen in natural infections (3,30). The increased proliferative activity of liver and intestine during or after MHV infection could potentially alter response to chemical carcinogenesis (3). Bone marrow infection is common with many MHV strains (19). Mice develop anemia, thrombocytopenia and leukopenia (140) and peripheral blood monocyte procoagulant activity can be induced in MHV infections (141).

The ubiquity of MHV among laboratory mice creates a high potential for contamination of other agents by MHV or confusion with MHV when passed in mice. Examples are not hard to find. Tettnang virus was an unclassified virus isolated from ticks and man in Germany, Egypt and Czechoslovakia. The agent was unrelated to a variety of other tick-born arboviruses, and was eventually found to be MHV. Retrospective study revealed that there was a relationship among

European and North African mouse colonies in which Tettnang virus isolations were made. Passage of diagnostic material through infant mice in MHV-enzootically infected colonies was responsible for the problem (15). Other examples include isolation of MHV when human encephalitis brain biopsy material was passed in infant mice (142), confusion over the human or mouse origin of coronaviruses isolated from multiple sclerosis material passed in mice or mouse tissue (143) and confusion over avian or mouse origin of coronavirus isolated from Manx Shearwaters suffering from puffinosis (144).

MHV has a number of effects on immunologic and non-specific host responses to antigens. Following intraperitoneal injection of MHV-3, mice had a modified immune response to sheep red blood cells. During acute infections, if mice were infected prior to antigen exposure, immunodepression occurred, while simultaneous injection with MHV and antigen resulted in immunostimulation. Chronic immunodepression occurred in semisusceptible (C3H and A2G) mice with chronic MHV infections (145). Although conditions of this study were somewhat artificial, including route of MHV inoculation, they are being reinforced by ongoing studies. Mice inoculated oronasally with MHV-JHM and an enterotropic MHV isolate display transient but significant functional disturbances in T and B lymphocyte populations, in mitogenesis assays with splenocytes from susceptible (BALB/cByJ) but not resistant (SJL) genotypes (146). It remains to be determined if these effects are due to direct or indirect action of the virus on lymphoid tissue. MHV certainly has the potential of direct action on lymphoid organs (19,39). Pre-infection of mice with MHV, either naturally or experimentally, alters the ability of normally permissive respiratory tract tissues to support replication of pneumonia virus of mice and also reduces susceptibility of DBA/2J mice to Sendai virus (147). Regardless of the mechanisms involved, these observations have broad implications for experimental virology and laboratory animal science, since MHV-infected mice respond abnormally to respiratory and possibly other viruses. MHV has been shown to activate NK cells and alter the IF responsiveness of infected mice (101,145). Macrophage function can be modified in a number of ways by MHV infection and effects have been shown to continue in MHV-recovered mice (100,117,148, 149). Athymic nude mice infected with MHV develop a number of immunological peculiarities. They develop cells with T-cell markers (150,151) and have altered IgM and IgG

responses to sheep red blood cells, including a secondary immune response (121,151). In addition, they are able to reject tumor xenografts (131,134).

IX. PREVENTION AND CONTROL

Since MHV causes acute infections and because its contagiousness results in almost simultaneous infection of an entire population, it can be controlled like coronaviruses of the rat by simply breaking the infectious cycle (3). Cessation of breeding or quarantine without introduction of new mice will achieve this objective. Selection of seropositive breeding stock for re-establishment of a breeding colony will ensure that mice are recovered from infection. Judgement on the success of this approach must be made only under conditions that prevent re-infection of the population.

Prevention of infection is extremely difficult in facilities receiving mice from outside sources at various intervals. Such conditions can allow introduction of subclinically infected animals that can infect resident stock or, conversely, perpetuation of the infectious cycle by introduction of naive mice following exposure to infected populations. Furthermore, MHV is so highly contagious that it is very likely to re-contaminate mouse populations that have become rid of the agent by re- derivation, re-population or quarantine, particularly if identical husbandry practices prevail. The best means of MHV control is preventing its entry into a facility. This can be achieved by purchase of mice from MHV-free sources and shipment in filtered boxes. Also, transplantable tumors and other mouse biological products must be MHV-free. Since most institutions are unable to achieve this goal, MHV-free mice can be maintained in properly controlled barrier facilities, plastic film isolators or filtered cage systems. Filter-top cages offer a relatively inexpensive means of preventing MHV infections, but handling and service of the cages must be strictly aseptic (13,18,19,120).

Vaccination has been shown to effectively reduce severity of challenge MHV infection, but not totally prevent it (60,108). Since vaccination immunity is produced against virus peplomeric antigens, it is likely to be strain-specific (108). The number of antigenic MHV biotypes therefore makes vaccination impractical. Furthermore, MHV usually causes subclinical infections, particularly when the

virus is enzootic in a colony. It is likely that vaccination will cause greater effects on the host than natural infection. If exacerbation of MHV disease is a problem in an experimental protocol, newly arrived mice can be allowed to become infected and recover prior to use, or virus-free mice under appropriate preventive husbandry conditions can be used.

X. FUTURE RESEARCH

A number of aspects of MHV biology are worthy of future research pursuit. Rather than generating a list of such topics, the author has attempted to indicate throughout the text where deficiencies in knowledge exist. Since scientific knowledge evolves best from a multi-disciplinary approach, it is hoped that scientists in various fields will seek ways to better understand this agent from their own perspectives.

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