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Transmission of Enterotropic Mouse Hepatitis Virus from Immunocompetent and Immunodeficient Mice

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Mouse hepatitis virus (MHV) is the most prevalent virus that infects mice, and most MHV strains are enterotropic. Experiments were performed to elucidate the duration of enterotropic MHV-Y shedding by immunocompetent BALB/c and C57BL/6 mice and immunocompromised B and T cell-deficient mice. Although the use of molecular diagnostics to detect MHV infection is increasing, it is unclear whether the viral RNA detected is always infectious. The ability to detect MHV-Y transmission to sentinel mice exposed directly to infected mice or to soil bedding from infected mice was compared with reverse transcriptase–polymerase chain reaction-based detection of viral RNA in the feces. The BALB/c mice developed subclinical intestinal infection, and transmitted MHV-Y for four weeks. The C57BL/6 mice also developed subclinical intestinal infection, but only transmitted virus for two weeks. The T cell-deficient mice developed severe disseminated disease by two weeks and transmitted virus for four weeks. The B cell-deficient mice developed subclinical intestinal infection and transmitted virus for longer than three months, although virus RNA was not detected in feces late in the infection. Viral RNA detected in the feces of infected mice was almost always infectious. Non-infectious RNA was detected in a few mice for several days after transmission had ceased. In addition, constant exposure of naive mice to infected mice, via the use of serial sentinels, prolonged viral transmission.

Mouse hepatitis virus (MHV) is the most prevalent virus that infects laboratory mice (31), and is a major concern in laboratory animal facilities because of its potential to disrupt mouse-based immunologic and oncologic research by interfering with biological responses and through clinical illness and mortality. Infection with MHV has been documented to alter tumor growth, invasion patterns, regression, and oncolysis (10, 25, 37). Disruption of immunologic research is linked to the ability of MHV to alter normative responses through viral replication in T and B lymphocytes and macrophages (3, 20, 34). Infection with MHV can alter peritoneal macrophage function, impair dendritic and/or T-cell function in Peyer's patches, suppress splenic $CD8^+$ T-cell functions, alter cytokine production, impair antigen processing, and decrease rejection of skin allografts (9, 13, 18-22, 33, 45, 48).

Mouse hepatitis virus is the collective name for a diverse group of murine coronaviruses that cause a wide spectrum of clinical outcomes. Polytropic MHV strains, which infect multiple organs, cause acute and chronic infections in adult immunocompetent mice that vary from subclinical infections to chronic demyelinating disease, hepatitis or encephalitis (16). Polytropic MHV strains initially replicate in the respiratory epithelia of the nose and spread to other organs via the olfactory nerve, lymphatic system, and/or viremia; infection of immunodeficient mice is frequently lethal. Enterotropic MHV strains cause acute subclinical infection of adult immunocompetent mice. Their primary sites of replication are the distal portion of the small intestine, cecum, and ascending colon (4). In contrast, infection of immunodeficient mice with enterotropic MHV results in viral dissemination that causes a multi-systemic, chronic infection (6). Natural MHV infections are due principally to enterotropic strains and are highly contagious (29).

The duration of MHV shedding after oral inoculation of immunocompetent mice with enterotropic strains is unclear. Studies performed in the early 1990s indicated that low amounts of infectious enterotropic MHV were detectable in the ascending colon at postinoculation day (PID) 30 in a small number of immunocompetent mice (4). Infectious polytropic MHV-JHM was detected in the central nervous system of immunocompetent mice at four weeks after intranasal inoculation, though transmission of the virus was not investigated (5). A recent MHV transmission study in a colony of transgenic, knockout, and wild-type mice indicated that soiled bedding from naturally infected MHV seropositive mice could transmit virus for at least 10 weeks (49). Another study, using transgenic mice with subtle immunodeficiency, indicated that MHV seropositive mice could transmit virus to direct contact sentinels for more than a year (43).

Though use of molecular diagnostics is increasing as a method of monitoring MHV shedding in feces, it is unclear whether the viral RNA detected in feces by use of reverse-transcriptase-polymerase chain reaction (RT-PCR) analysis is part of an infectious virus particle. Enterotropic MHV-UAB RNA was detected in feces of 80% of experimentally infected BALB/c mice at PID 21, and 10% of mice at PID 24 (12). Polytropic MHV-A59 RNA was detected by use of fluorogenic RT-PCR analysis in a low propor-

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tion of intestinal and lung tissue specimens from outbred mice four weeks after intranasal inoculation (8). Thus, the ability to detect MHV-RNA for up to one month in sites amenable to viral shedding agrees with the duration of shedding observed during natural infections. However, evidence from research using polytropic MHV strains indicates that the presence of MHV RNA does not always correlate with the presence of infectious virus. Low amounts of MHV RNA, believed to be non-infectious, were detected in the central nervous system and liver of mice inoculated with polytropic MHV-A59 for up to 10 months (35). Additionally, MHV-JHM RNA was detected in the central nervous system through day 787 after intracerebral inoculation, whereas infectious virus was detected in brain homogenates only through day 13 after inoculation (1, 23, 44).

Although lactogenic immunity to enterotropic MHV-Y protects neonatal mice from infection (28, 30), it is unclear which components of the immune response are necessary to resolve enterotropic MHV infections in adult mice. The objectives of the study reported here were to determine the duration of MHV-Y shedding by immunocompetent and immunodeficient mice and whether detection of MHV RNA in feces of MHV-inoculated mice by use of RT-PCR analysis correlated with transmission of infection to sentinel mice.

Materials and Methods

Mice. Four- to six-week-old female outbred Swiss Webster mice were obtained from Taconic (Germantown, N.Y.), and four- to six-week-old female inbred BALB/cJ, C57BL/6J, B6.129S2-Igh-6^{tm1Cgn} (μMT) and B6.129P2-Tcrb^{tm1Mom} Tcrd^{tm1Mom} (Tcrβδ-) mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). On arrival, mice were free of bacterial and parasitic infections and were seronegative for MHV, ectromelia virus, lymphocytic choriomeningitis virus, minute virus of mice, mouse parvovirus, murine rotavirus, pneumonia virus of mice, reovirus, Sendai virus and *Mycoplasma pulmonis*. All fecal specimens collected from index mice on the day prior to inoculation and at the conclusion of each experiment were negative for *Helicobacter* DNA on the basis of results of PCR analysis (data not shown). All animal procedures were approved by the Yale Animal Care and Use Committee, and animal care was in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Mice were housed in a quarantine facility, and room conditions included a negative pressure differential relative to the corridor, a 12:12-h light:dark cycle, and 10 to 15 air changes/h. Mice were housed on sterilized corncob bedding (Harlan, Indianapolis, Ind.) in sterilized cages (Polysulfone Standard Mouse Cage, ACE, Allentown, Pa.) equipped with stainless steel wire bar tops and filtered cage tops. Mice were fed sterilized standard rodent chow (Purina Mills, 5010, PMI, St. Louis, Mo.), and hyperchlorinated water was available ad libitum in water bottles. Cages were husbanded every seven days in a class-II biological safety cabinet in the animal room.

Virus stock. To generate a *Helicobacter*-free stock of MHV-Y, 24-day-old BALB/c mice, which had been fed food pellets containing anti-*Helicobacter* antibiotics (600 mg of amoxicillin, 138 mg of metronidazole, and 37 mg of bismuth/kg of food; Bio-serve Inc., Frenchtown, N.J.) starting at day 15 postpartum (24), were inoculated orally with 20 μl of an infant mouse intestinal stock containing *Helicobacter hepaticus* and MHV-Y. Mice were euthanized at PID 2, and the ileum, cecum, and colon from each mouse were collected and frozen at –70°C. Ten percent intestinal stocks with titer of 1.5×10^6 median infectious doses (ID_{50})/ml were generated by homogenization of tissues in 90% Dulbecco's minimal essential medium with 10% fetal bovine sera (Invitrogen Life Technologies, Carlsbad, Calif.). By use of PCR analysis, this stock was determined to be free of *Helicobacter* sp.

Viral infection. The BALB/c, C57BL/6, μMT or Tcrβδ- index mice were inoculated orally with 20 μl of MHV-Y intestinal stock and were observed daily for clinical signs of infection. To test for *Helicobacter* sp. by use of PCR analysis, fecal specimens were collected from mice prior to inoculation and at the conclusion of each experiment to test for *Helicobacter* sp. using PCR analysis. In the first experiment, 16 BALB/c mice were inoculated orally with MHV-Y. On PID 1, groups of four index mice were moved to clean cages housing a single sentinel Swiss Webster mouse. After two days of contact exposure, fecal specimens collected from index mice were tested by use of RT-PCR analysis for the presence of MHV RNA and index mice were moved to clean cages housing new sentinel mice.

For the first three weeks, contact sentinels were housed with index mice for two days; from three to six weeks, contact sentinels were housed with index mice for three days; and from six to nine weeks, contact sentinels were housed with index mice for five days. Sentinel mice remained housed in soiled cages for one week and were housed in a clean cage for an additional week. Two weeks after contact exposure, sentinel mice were euthanized by inhalation of carbon dioxide and blood was collected for MHV serologic testing.

In the second experiment, three groups of four BALB/c index mice inoculated orally with MHV-Y were transferred to clean cages containing a single Swiss Webster sentinel mouse on PID 7 and at weekly intervals through PID 49 for a one-day contact exposure. After a single day of contact between index and sentinel mice, fecal specimens were collected from index mice; then they were moved to clean cages until the next contact exposure. Sentinel mice remained on the soiled bedding for one week, then were housed on clean bedding for an additional week. Two weeks after contact exposure, sentinel mice were euthanized by carbon dioxide inhalation and blood was collected for MHV serologic testing.

In the third experiment, three groups of four BALB/c and three groups of four C57BL/6 index mice inoculated orally with MHV-Y were transferred to clean cages on PID 4 and at weekly intervals through PID 39. Three days later (on PID 7, and at weekly intervals through PID 42), a single fecal specimen was collected from each index mouse for MHV RT-PCR analysis; then index mice were moved to clean cages, and a single sentinel mouse was housed on the soiled bedding from four index mice for a week. After soiled bedding exposure, mice were housed on clean bedding for two weeks prior to carbon dioxide-induced euthanasia and testing for MHV antibodies.

In the last experiment, seven groups of four μMT index mice were inoculated orally with MHV-Y. Mice were transferred to clean cages at weekly intervals from PID 5–89 and on PID 103 and 125. Three days later (at weekly intervals from PID 8–92 and on PID 106 and 128), a single fecal specimen was collected from each index mouse for MHV RT-PCR analysis and index mice were moved to clean cages. A single sentinel mouse was housed on the soiled bedding from each group of four index mice for a week and on clean bedding for two weeks. Two weeks after

soiled bedding exposure, sentinel mice were euthanized by inhalation of carbon dioxide and blood was collected for serologic testing. To confirm they were MHV seronegative, sentinels used after PID 78 were bled from the retro-orbital sinus prior to placement on soiled bedding.

Molecular diagnostics. RNA was extracted from feces using the RNeasy kit (Qiagen, Chatsworth, Calif.). MHV RT-PCR analyses were performed, using the Superscript One-Step RT-PCR System (Invitrogen, Carlsbad, Calif.). The MHV N gene primers were MN512: GTCATGAGGCTATTCCTACTA and MN1027: ATACACATCTTTGGTGGG. The reaction cycles were as follows: 30 min at 50°C; two minutes at 94°C; 40 cycles of 15 sec at 94°C, 30 sec at 50°C, 90 sec at 90°C; and 10 min at 72°C. The RT-PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide; then the 533-basepair amplicon was visualized by use of ultraviolet illumination (15).

DNA was extracted from feces using the DNeasy Tissue kit (Qiagen, Chatsworth, Calif.). PCR analyses were performed using *Taq* polymerase (Roche Molecular Biochemicals, Indianapolis Ind.) and primers specific for the *Helicobacter species* 16S rRNA gene (H380:CGTGGAGGATGAAGGTTTTAG and H1372: CCGACTTAAGGCGAATACAAC). The reaction cycles for PCR reactions were as follows: two minutes at 94°C; 35 cycles of 30 sec at 94°C, 30 sec at 50°C, 60 sec at 72°C; and 5 min at 72°C. The PCR products were electrophoresed on 1% agarose gels and stained with ethidium bromide; then the 1,013-basepair amplicon was visualized by use of ultraviolet illumination (15).

In situ hybridization. In situ hybridization to detect MHV RNA used a gel-purified cDNA of the complete MHV-JHM N gene to generate a random-primed ^{32}P-labeled DNA probe. Stomach, small intestine, cecum, colon, mesenteric and cervical lymph nodes, spleen, brain, liver, kidney, pancreas, lung, heart, and uterine tissues were hybridized, washed, and visualized, using described conditions (2).

Serologic testing. All sera were tested for MHV antibodies by use of immunofluorescence as described (47). The seropositivity of sera with low immunofluorescence signal was confirmed by use of an enzyme-linked immunosorbent assay and 75 ng of bacterial expressed MHV-1 N protein/well (15).

Results

Transmission of MHV-Y from immunocompetent mice to contact sentinels. Swiss Webster mice were exposed via co-housing to MHV-Y-inoculated BALB/c index mice to determine the duration of enterotropic MHV transmission and to correlate infectivity with RT-PCR-based detection of MHV RNA in feces of index mice. Sentinel mice were co-housed with index mice for an interval ranging from two to 20 days, remained housed on soiled bedding for an additional week, then were housed in a clean cage for an additional week before blood samples were collected for MHV serologic testing at two weeks after contact exposure to the index mice. Contact sentinel infection was indicated by production of anti-MHV antibodies (seroconversion). Virus shedding from index mice was assessed by MHV RT-PCR analysis of fecal specimens collected from index mice (Table 1). Infectious MHV was transmitted to all sentinel mice in contact with index mice between PID 1 and 30. Index mice in a single cage did not transmit MHV to the contact sentinel housed with them between PID 30 and 33, and MHV RNA was not detected in the feces of this group of index mice at PID 33 or later. However, between PID 30 and 39, virus was transmitted to contact sentinels in the other three cages and MHV RNA was detected in feces from index mice housed in the same three cages. Results of MHV RT-PCR analyses of fecal specimens were negative after PID 39 in a second group of index mice and on PID 57 in the third group of index mice. Therefore, index mice stopped transmitting virus to sentinels prior to PID 42 and 52 in the second and third groups of mice, respectively. Index mice in the last group shed infectious virus for more than 62 days, but fewer than 77 days, and viral RNA was detected in the feces of index mice in this group on PID 62 but not on PID 97.

Table 1. Transmission of mouse hepatitis virus (MHV) from MHV-Y-inoculated BALB/c mice to contact sentinels

Contact exposure* (PID interval)	Sentinel serology†	Fecal MHV RT-PCR‡ of index mice
1–3	4/4	4/4
3–5	4/4	4/4
5–7	4/4	4/4
7–9	4/4	4/4
9–11	4/4	4/4
11–13	4/4	4/4
13–15	4/4	4/4
15–17	4/4	4/4
17–19	4/4	4/4
19–21	4/4	4/4
21–24	4/4	4/4
24–27	4/4	4/4
27–30	4/4	4/4
30–33	3/4	3/4
33–36	3/4	3/4
36–39	3/4	3/4
39–42	3/4	2/4
42–47	2/4	2/4
47–52	2/4	2/4
52–57	1/4	2/4
57–62	1/4	1/4
62–77	1/4	ND
77–97	0/4	0/4

*Postinoculation days (PID) that sentinel mouse was in contact with four index mice.
†No. of MHV seropositive sentinel mice (two weeks after end of exposure to index mice and one week after exposure to soiled bedding)/total sentinel mice tested.
‡No. of cages of index mice that had positive results of MHV reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of feces at end of contact exposure/total cages of index mice tested.
ND = Not done.

Once a group of mice became negative for virus transmission, it did not become positive again, indicating that intermittent shedding of MHV-Y was not occurring. The time when index mice stopped transmitting MHV to contact sentinels correlated well with the time that fecal MHV-RNA became undetectable in index mice. In a single group, MHV RNA, assumed to be non-infectious, was detected in feces after transmission to contact sentinels had ceased (transmission ceased by PID 52, and RNA was present at PID 57 but not at PID 62; Table 1). These results indicate that in most of the mice, MHV-RNA detected by use of the RT-PCR analysis was infectious.

The extended shedding of MHV for more than two months by some immunocompetent BALB/c mice and the divergent times of MHV clearance were somewhat unexpected. Serial sentinels were used in this experiment to detect low-level intermittent shedding if it existed, but the constant presence of newly infected contact sentinels could have been a factor in perpetuating shedding from the index mice. For example, if naive sentinel mice became infected the first day after contact with index mice,

Table 2. Transmission of MHV from MHV-Y-inoculated BALB/c mice to sentinels via one-day contact with inoculated mice and one-week contact with soiled bedding

Contact exposure* (PID)	Sentinel serology†	MHV fecal RT-PCR‡ of index mice
7	3/3	3/3
14	3/3	3/3
21	3/3	3/3
28	2/3	3/3
35	1/3	3/3
42	0/3	0/3
49	0/3	0/3

*,†*See* Table 1 for key.
‡No. of cages of index mice that had positive results of fecal MHV RT-PCR analysis on the day that sentinel mice were exposed to index mice/total cages of index mice tested.

they could shed high doses of virus for several days. This could have resulted in the ingestion and subsequent excretion of high doses of virus by index mice or contamination of the pelage of index mice. Thus, index mice may have served as fomites and transferred virus to the next naive sentinel mouse. Therefore, a second experiment was designed to investigate the possible role of fomite-based MHV transmission, by limiting the time of contact between index and sentinel mice to a single day.

Transmission of MHV-Y to sentinel mice by one-day contact with BALB/c index mice. Sentinel mice were exposed to index mice for one day, followed by exposure to soiled bedding for one week. All index mice had detectable MHV RNA in the feces through PID 35, but only 80% of sentinel mice exposed to index mice on or before PID 35 seroconverted (Table 2). This indicates that MHV RNA detected in feces from index mice in one of the three groups tested on PID 28 and two of three groups tested on PID 35 was non-infectious or below the amount required to initiate infection (Table 2).

Transmission of MHV-Y to sentinel mice by one-week exposure to soiled bedding from cages housing MHV-Y infected immunocompetent mice. A third experiment investigated whether mouse genotype affected the duration of MHV transmission via soiled bedding contact. Sentinel mice were housed on three days worth of soiled bedding collected from groups of BALB/c or C57BL/6 index mice for one week. All BALB/c index mice had detectable MHV RNA in the feces through PID 28, and 11 of 12 sentinel mice exposed to soiled bedding from the BALB/c index mice on or before PID 28 seroconverted (Table 3). In contrast, all C57BL/6 index mice had detectable MHV RNA in the feces only through PID 14, and five of six sentinel mice exposed to soiled bedding from the C57BL/6 index mice on or before PID 14 seroconverted (Table 3). These results indicated that MHV-Y is shed from BALB/c mice for at least four weeks and from C57BL/6 mice for only two weeks. Also, in most of the mice, MHV-RNA detected in the feces was infectious, as MHV RT-PCR results correlated with sentinel mouse infection and seroconversion.

Transmission of MHV-Y to sentinel mice by one-week exposure to soiled bedding from cages housing MHV-Y infected immunodeficient mice. A preliminary experiment was performed to determine the possible roles of B and T cells in the resolution of MHV-Y infection. Mice deficient in the *Igh6* gene (μMT mice), which renders them B cell deficient, did not develop signs of clinical illness after MHV-Y inoculation, and MHV RNA was detected in the feces for a month (data not shown). In contrast, mice deficient in α, β, γ, and δ T-cell receptors (Tcrβδ-) in-

Table 3. Transmission of MHV from MHV-Y-inoculated BALB/c and C57BL/6 mice to sentinels via contact with soiled bedding for one week

Bedding exposure (PID)*	BALB/c contact serology†	BALB/c index mice fecal RT-PCR‡	C57BL/6 contact serology†	C57BL/6 index mice fecal RT-PCR‡
7	3/3	3/3	3/3	3/3
14	3/3	3/3	2/3	3/3
21	3/3	3/3	0/3	0/3
28	2/3	3/3	0/3	0/3
35	0/3	0/3	0/3	0/3
42	0/3	0/3	0/3	0/3

*PID that three days worth of soiled bedding was collected from cages housing index mice.
†No. of MHV seropositive sentinel mice (two weeks after soiled bedding exposure)/total sentinel mice tested.
‡No. of cages of index mice that had positive results of fecal MHV RT-PCR analysis on day of soiled bedding exposure/total cages of index mice tested.

Table 4. Transmission of MHV from MHV-Y-inoculated B cell-deficient mice to sentinels via contact with soiled bedding for one week

Bedding exposure* (PID)	Sentinel serology†	Fecal MHV RT-PCR‡ for index mice
8	7/7	7/7
15	7/7	7/7
22	5/6	6/6
29	5/5	5/5
36	4/4	4/4
43	3/3	3/3
50	2/2	2/2
57	1/1	1/1
64	1/1	0/1
71	1/1	0/1
78	1/1	0/1
85	1/1	0/1
92	1/1	0/1
106	1/1	0/1
122	0/1	ND
128	ND	0/1

See Table 3 for key.

oculated with MHV-Y developed disseminated disease between PID 8 and 15 and were euthanized on PID 29 when hunched posture, wasting, and dehydration were observed (data not shown). MHV RNA was detected in the feces of Tcrβδ- mice for a month. Since they developed progressive severe clinical disease that required euthanasia, they are unlikely to pose a long-term MHV transmission risk.

To determine how long B cell-deficient mice were capable of transmitting MHV-Y via soiled bedding, sentinel mice were exposed to soiled bedding from groups of μMT index mice over a four-month period. All μMT index mice had detectable MHV RNA in the feces through PID 57, and all but one sentinel mouse (39/40) exposed to soiled bedding on or before PID 106 seroconverted (Table 4). Virus was eventually cleared as the sentinel mouse exposed to soiled bedding from μMT index mice on PID 122 did not seroconvert. In the absence of mature B cells and a humoral immune response, mice became chronically infected with MHV and transmitted virus for more than three months. Necropsy was performed on one group of index mice at PID 15, 22, 29, 36, 43, 50, and 128 to determine the site of MHV persistence.

Gross and histologic lesions were not observed in the intestine, liver, brain, lymph nodes, spleen, kidneys, or lungs at any time, and MHV RNA was localized, by use of in situ hybridization, to scattered enterocytes within the colon on PID 15–50 (data not shown). From PID 57–106, the amount of virus shed by

MHV-Y infected μMT mice were below the limits of RT-PCR analysis detection but were sufficient to infect soiled bedding sentinels.

Discussion

Our findings indicate that *Helicobacter*-free adult immunocompetent BALB/c mice inoculated with MHV-Y shed virus in the feces and are capable of transmitting virus infection to sentinel mice by short-term contact or by soiled bedding exposure for approximately one month. These data are consistent with those of a previous study performed in BALB/c and SJL mice infected with MHV-Y, in which MHV was detected in the ascending colon of a few mice of both strains at PID 30 (4). Until recently, most academic and commercial mouse colonies were endemically infected with *H. hepaticus* or other *Helicobacter* species and early MHV-Y pathogenesis studies were performed using intestinal stocks of MHV-Y that were PCR positive for *H. hepaticus* DNA. Therefore, it is likely that the mice used in the early studies were co-infected with MHV-Y and *Helicobacter* sp. (4, 14, 31, 46). Even though results of a previous study indicated that *H. hepaticus* influenced the duration of MHV-G shedding from immunocompromised gamma interferon-deficient mice (15), it did not substantially alter the duration of MHV-Y transmission by BALB/c mice.

This study also documented that duration of transmission was influenced by mouse genotype, in that C57BL/6 mice inoculated with MHV-Y transmitted virus to sentinel mice via soiled bedding for only two weeks, whereas BALB/c mice transmitted virus for at least four weeks. MHV infection in BALB/c and C57BL/6 mice has been previously reported to differ. C57BL/6N mice produced higher titer antisera than did BALB/c mice in response to intranasal infection with enterotropic MHV-NuU (42). Also, in vivo infection of BALB/c hepatocytes with neurotropic MHV-JHM resulted in significantly higher viral titers than did infection of C57BL/6 hepatocytes (32). The production of higher antibody titers and lower viral titers in C57BL/6, compared with BALB/c mice, could account for the accelerated clearance of MHV-Y infection from C57BL/6 mice.

The extended shedding of MHV from BALB/c index mice housed with naive sentinel mice (Table 1) appears to be the result of persistent high amounts of virus in the cages housing these index mice. These results indicate that, in breeding colonies, where MHV infected mice may be serially transferred to cages containing naive mice, MHV infection could be maintained for several months. Our results agree with those of two recent reports involving endemic MHV infection in breeding colonies containing transgenic, gene-targeted, and wild-type mice, on several genetic backgrounds. The investigators concluded that extended shedding and transmission of MHV occurred for a period of several months to over a year (43, 49).

The ability to detect MHV shedding in the feces of immunocompetent mice by use of RT-PCR analysis correlated well with the ability of mice to transmit virus to sentinels early in infection (PID 4–21) when viral titers would be expected to be their highest. A similar duration of fecal excretion was also reported in BALB/c mice inoculated with enterotropic MHV-UAB, where fecal excretion was consistently detected by use of the MHV RT-PCR assay for 21 days (12). However, late in infection at PID 28 and 35 (Table 2), use of RT-PCR analysis detected viral RNA in feces from all mice, whereas transmission to sentinel mice exposed to index mice for one day and soiled bedding for seven days had ceased in some groups. Rat coronaviruses have been documented to remain infectious in the environment for only two days, whereas rat coronavirus RNA is detectable by RT-PCR analysis for over a week (17, 26). Therefore, it is likely that MHV RNA detected in some feces on PID 28 and all feces on PID 35 may have been non-infectious RNA in the form of non-enveloped, non-infectious, environmentally stable, nucleoprotein complexes.

The B cell-deficient μMT mice transmitted MHV-Y via soiled bedding to sentinels for 106 days. The lack of B cells resulted in a chronic infection that was localized to the intestine and resulted in extended transmission and low amount of viral shedding. Although infectious virus was transmitted to sentinel mice exposed to a week's worth of soiled bedding from four μMT mice at six times between PID 64 and 106, RNA was not detected by RT-PCR analysis in pooled feces from the four index mice and was, therefore, below our limit of detection. Alternatively, if virus was shed sporadically, collection of feces from mice only once a week could have been an ineffective means of sampling for viral shedding. Analysis of feces from individual mice or use of more sensitive molecular methods, such as nested or fluorogenic nuclease RT-PCR analysis, might have increased our ability to detect low amounts of viral RNA late in infection (8, 38).

The inefficiency of μMT mice in clearing MHV from the intestine is probably related to the absence of a humoral response to the virus, although the remaining immune components were sufficient to prevent the viral dissemination and resulting disease observed in Tcrβδ- mice. The inability of μMT mice to control infections has been reported for several viral systems, including neurotropic MHV-JHM and hepatotropic MHV-A59. Lymphocytic choriomeningitis virus infection of μMT mice results in a transient decrease in viral titer after the initial acute phase of the infection, but by three months after inoculation, virus titer has increased to values equivalent to those observed initially (11, 50). Epidemic diarrhea of infant mice (EDIM) virus was shed sporadically in the feces from μMT mice for longer than 93 days, whereas immunocompetent C57BL/6 mice shed virus for only 10 days (40). The titer of MHV-JHM was initially reduced in the central nervous system of B6-μMT mice, but then increased again after PID 10, with death in these mice from PID 30–45 (36). Recruitment of T cells to the central nervous system of MHV-JHM-infected B6-μMT mice was equal to that in control C57BL/6 mice, but virus-specific $CD4^+$ and $CD8^+$ responses were decreased in μMT mice (7). However, passive transfer of anti-MHV antibodies to MHV-JHM- infected B6-μMT mice protected them from viral reactivation and death, indicating a role for antibodies in the prevention of viral reactivation (36). Intracerebral or intrahepatic inoculation of MHV-A59 in B6-μMT mice resulted in acute infection of the liver. Infectious virus was cleared from liver by PID 10, but the central nervous system was chronically infected, with high virus titers in the spinal cord and brain at PID 148 (39). Similar to MHV-JHM serotherapy, antibody transfer protected B6-μMT mice from MHV-A59-induced death and resulted in a substantial decrease in virus titers in the central nervous system. Antibody-deficient JhD and mIgM-Tg mice also had cleared MHV-A59 from liver but not the brain, indicating that antibody, not other B-cell functions, is needed in the central nervous system to prevent viral reactivation (39). This organ-specific pattern of clearance was not restricted to a single genetic background of mice and was postulated to be related to

the regenerative capacity of the organ (39). Although the intestine is an organ that has high regenerative capacity, we did not observe rapid clearance of MHV-Y from the intestine of μMT mice. The absence of B cells in μMT mice results in the absence of mucosal antibody production and other perturbations of the mucosal immune system, including decreased size and number of Peyer's patches, with abnormal follicle-associated epithelium overlying the Peyer's patches and decreased follicular dendritic cell networks within the Peyer's patches (27). The absence of B cells in μMT mice could also lead to an inability to undergo B cell-mediated cytolysis, an antibody-independent, natural killer cell-like, immune process in which B cells lyse MHV infected cells after interaction of the MHV receptor on B cells, with MHV S protein expressed on infected cells (41, 51). The combination of Peyer's patch abnormalities, lack of B cell-mediated cytolysis, and absence of an anti-MHV antibody response most likely resulted in the ineffective clearance of MHV infection within the intestine of MHV-Y infected μMT mice.

In conclusion, the duration of enterotropic MHV transmission was affected by the genetic background of the mouse strain as well as the immunocompetence of the mouse. The B cell-deficient mice infected with enterotropic MHV strains pose a substantial risk for transmission of MHV. Infection is clinically silent, yet mice shed virus for more than three months, frequently at amounts below the sensitivity of routine RT-PCR methods. Other immunodeficient mouse strains that develop subclinical MHV infections may also silently transmit MHV. The frequent movement of mice between cages, as demonstrated by the use of serial sentinel mice in our first experiment, may lead to prolonged viral shedding. The RT-PCR-based detection of MHV RNA in the feces was, in most instances, a good indicator of infectious virus.

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