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Mouse hepatitis virus and host determinants of vertical transmission and maternally-derived passive immunity in mice

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Summary. Transmission of mouse hepatitis virus (MHV) in utero following oronasal inoculation of pregnant mice was found to depend upon MHV strain and host genotype. Virulent, polytropic MHV-JHM was recovered from multiple maternal tissues, including liver and uterus, as well as placenta and fetus in susceptible BALB/cByJ mice. Fetuses were infected during all 3 trimesters of pregnancy. Low virulence, polytropic MHV-S infected fetuses in a low percentage of susceptible BALB/cByJ dams. Infection of resistant CD-1 mice with MHV-JHM was limited, with no fetal infection. Enterotropic MHV-Y was largely restricted to intestine of BALB/cByJ and CD-1 dams, with minimal dissemination and no fetal infection. Maternally-derived MHV IgG antibody was detectable in pup sera through 4 weeks of age. Antibody titers were generally lower in second litters of the same dam. Cross-fostering experiments showed that antibody was transferred via colostrum and not in utero, and that pups were capable of absorption through 2 weeks of age. Pups nursing immune dams were protected against MHV challenge at 1 and 2 weeks of age, compared to pups nursing naive dams. Immunity to MHV challenge was cross-protective against both antigenically homotypic and heterotypic strains of MHV.

Introduction

Mouse hepatitis virus (MHV) is a common and highly contagious coronavirus of laboratory mice. Like other coronaviruses, MHV exists as numerous, constantly changing strains that vary in primary tropism for upper respiratory or enteric mucosa and secondary tropism for several other target organs. Strains of MHV vary considerably in virulence, but most cause brief, clinically silent infections in adult mice. Disease expression is also significantly influenced by host age, genotype and immune status [1]. Difficulty in understanding the biology of MHV clearly emanates from the interaction of all of these variables on outcome of infection. The significance of maternally-derived passive im-

munity and the dynamics of MHV infection during pregnancy have not been adequately explored. Anecdotal natural observations and experimental data using artificial routes of MHV immunization and challenge suggest that MHV infection of neonatal mice can be influenced by maternally-derived passive immunity [11, 14, 16, 18, 19, 21, 22]. It is unclear if MHV is transmitted in utero. Experimental studies, using artificial routes of inoculation, have both supported and negated vertical transmission of MHV [10, 15, 20]. Clearly, the dynamics of MHV following natural routes of infection in pregnant and post-parturient mice needs careful evaluation, so that controlled, experimental data can be extrapolated to the natural situation. This paper examines these questions in an effort to understand this aspect of MHV biology.

Materials and methods

Mice

Inbred BALB/cByJ and outbred CD-1 [Cr-1 : CD-1(ICR)BR outbred albino] mice were purchased from The Jackson Laboratory, Bar Harbor, ME, and Charles River Breeding Laboratories, Portage, MI, respectively. They are shipped in filtered containers. Mice from both commercial sources were MHV-free. Mice were transferred upon arrival into autoclaved Micro-isolator (Lab Products, Maywood, NJ) containment cages containing pine shavings, food and water. Cage handling and changing were performed aseptically as previously described [5]. Mice were killed with carbon dioxide gas, followed by exsanguination by cardiac puncture. Neonatal mice were killed by hypothermia and decapitation. Interim blood samples were drawn by aspiration of blood from the peri-orbital sinus with a Pasteur pipette under light ether anesthesia. Mice were twice inoculated intranasally (i.n.) or per os with 10 µl MHV-JHM, MHV-S or MHV-Y stocks containing approximately 10^3 median tissue culture infectious doses ($TCID_{50}$), or dilutions thereof. Previous studies have shown that with oronasal inoculation (unlike parenteral routes of inoculation), MHV disease severity is not affected by higher doses of virus beyond the infectius dose [4]. Tissues collected for virus isolation and sera were stored at —70 °C until tested.

Virology

MHV-JHM was obtained from the American Type Culture Collection, Bethesda, MD, passaged twice in NCTC 1469 cells, once in adult BALB/cByJ brain, once in 17 Cl 1 cells and frozen in aliquots at —70 °C until used. Inoculum contained approximately 10^3 $TCID_{50}$/10 µl. This virus strain is a moderately virulent, polytropic virus [6, 7]. MHV-S was obtained from the American Type Culture Collection and passaged twice in NCTC 1496 cells and frozen in aliquots at —70 °C until used. Inoculum contained approximately 10^4 $TCID_{50}$/10 µl. This virus strain is a low virulence, polytropic virus [5, 6, 12]. MHV-Y was isolated from infant mice with typhlocolitis [8], passaged in NCTC 1469 cells and infant CD-1 mice. Inoculum consisted of unquantified, clarified homogenate of infected mouse intestine, which was aliquoted and frozen at —70 °C. This virus strain is highly enterotropic, regardless of host age or genotype [2, 6 8]. Virus in suspect tissues was detected and titrated using infant mouse infectivity assays. MHV-JHM and MHV-S were assayed by intracerebral inoculation of tissue homogenates, and establishing the $\log_{10}LD_{50}$ per gram of tissue, as previously described [7]. MHV-Y was assayed using similar methods, but infectivity was established by oral inoculation of infant mice. These mice were then killed at 48 hours after inoculation and intestines were processed and examined for histopathology.

Serology

Sera were tested for MHV antibody by enzyme immunoassay (EIA) with formalin-fixed MHV-JHM infected 17 Cl 1 cells as antigen [7, 23].

Histology/immunohistochemistry

Tissues were fixed in 10% neutral buffered formalin (pH 7.2) paraffin embedded, sectioned at 5–7 μm and stained with hematoxylin and eosin or processed for MHV antigen by immunoperoxidase [7].

Statistical analysis

Virus titers were compared between groups with the student's paired or unpaired t tests and proportions were compared between groups by Chi square analysis.

Vertical transmission experiments

Since outcome of maternal infection is likely to depend on virus virulence, virus tropism, host genotype and stage of pregnancy, a series of experiments were performed to determine if MHV can be transmitted in utero to the fetus. In utero transmission would most likely occur during infection of a genetically susceptible dam with a polytropic MHV strain with sufficient virulence to induce disseminated infection of multiple target tissues. Previous studies [7] have demonstrated that BALB/cByJ mice, at 5 days after i.n. MHV-JHM inoculation, would be a suitable model system to test this hypothesis. Groups of timed-pregnant and non-pregnant female BALB/cByJ mice were inoculated i.n. with MHV-JHM on days 3, 9, and 15 of pregnancy, then necropsied on day 5 after inoculation (days 8, 14, and 20 of pregnancy). Vertical transmission of a low virulence MHV strain in genetically susceptible mice was tested with MHV-S in BALB/cByJ dams. Like MHV-JHM, MHV-S is polytropic [6], but is considerably less virulent [5, 12]. Mice were inoculated i.n. with MHV-S on day 15 of pregnancy and necropsied on day 5 after inoculation (day 20 of pregnancy). Vertical transmission of MHV-JHM in genetically resistant mice was next tested in outbred CD-1 mice. Mice were inoculated i.n. with MHV-JHM on day 15 of pregnancy and necropsied on day 5 after inoculation (day 20 of pregnancy). Since enterotropic MHV strains tend to be highly restrictive in intestinal mucosa, with little dissemination to other organs, vertical transmission of enterotropic MHV-Y in genetically susceptible BALB/cByJ and resistant CD-1 mice [2] was examined. Mice were inoculated per os with MHV-Y on day 15 of pregnancy and necropsied on day 5 after inoculation (day 20 of pregnancy).

Effect of pregnancy and sex on MHV infection

The physiological state of pregnancy and sex may influence severity of infection. In concert with the above experiment, male mice and non-pregnant female mice were compared to pregnant mice.

Transfer of maternal MHV antibody from dam to pups

Six week old CD-1 female mice were inoculated i.n. with MHV-JHM to initiate an immunizing infection, or sham-inoculated (controls). Two weeks later, recovered mice were mated with virus-free CD-1 males. At the time of parturition, females were bled for serum antibody and litters were culled or combined to form groups of 12–14 pups/litter. At 2, 4, 6, 8, and 10 weeks post-partem, 2 pups from each litter were randomly selected, removed, and bled for antibody determination. Dams were bled again when litters were weaned. After weaning, 3 immune dams and 1 control dam were allowed to rest 5 weeks, then

remated to determine if the same levels of antibody would be transferred to subsequent litters. All serum samples were collected and frozen, then run simultaneously for MHV IgG serum antibody.

In utero vs. colostral MHV antibody

Six week old CD-1 female mice were immunized with MHV-JHM or sham-inoculated, as above. Dams were bled at whelping and weaning (3 weeks post-partum). Litters were exchanged between immune and naive dams at the time of birth. Pups were randomly selected, removed, and bled at 1, 2, or 3 weeks of age. In addition, litters were exchanged at 2 weeks post-partem.

Resistance of passively-immune pups to MHV challenge

The resistance of mice at 1, 2, or 3 weeks of age to challenge with MHV-JHM was compared between litters from immune and naive (control) dams. Six week old CD-1 dams were immunized and mated as described above. Pups were challenged i.n. with 100-fold dilutions of MHV-JHM and mortality was recorded. The specificity of resistance of 1 week old infant mice to challenge with MHV-JHM or MHV-S was compared between litters from naive dams or dams that were immunized with the homologous or heterologous virus strains. MHV-S and MHV-JHM are antigenically heterotypic by serum cross neutralization [6].

Results

Vertical transmission

Virulent, polytropic MHV-JHM caused disseminated infection in genetically susceptible BALB/cByJ mice, resulting in in utero infection of fetuses during all 3 trimesters of pregnancy (Fig. 1). Stage of pregnancy did not affect either the prevalence of infection or virus titers in dam liver and uterus. Virus titers in liver of pregnant mice ($7.9 \pm 1.0 \log_{10}LD_{50}$/gram) tended to be higher than in non-pregnant females ($6.3 \pm 1.0 \log_{10}LD_{50}$/gram), and were significantly higher than in males ($5.9 \pm 0.3 \log_{10}LD_{50}$/gram; unpaired t, $p \leqslant 0.05$). Uterine MHV titers among all pregnant mice combined ($3.7 \pm 1.9 \log_{10}LD_{50}$/gram) were consistently lower than MHV titers in liver ($7.4 \pm 1.0 \log_{10}LD_{50}$/gram; paired t, $P \leqslant 0.001$). Virus antigen was found in endometrial stroma and epithelium. Only 4 of 10 dams that were inoculated on day 3 of pregnancy were pregnant at necropsy, in contrast to an over 80% pregnancy rate among dams inoculated on day 9 and 15 of pregnancy, suggesting fetal resorption or abortion. Additional replicates were not sought, since vertical transmission was verified in 1 of the mice. Virus was demonstrable in both placenta and fetus of this mouse (Fig. 1). Virus titers in placentas of mice inoculated on days 9 and 15 of pregnancy ($6.7 \pm 1.2 \log_{10}LD_{50}$/gram) were significantly higher than uterine virus titers ($3.7 \pm 1.9 \log_{10}LD_{50}$/gram; paired t, $P \leqslant 0.001$) and nearly equivalent to liver virus titers ($7.4 \pm 1.0 \log_{10}LD_{50}$/gram; Fig. 1), suggesting a preferential site of viral tropism. This could not have been due to viremia alone, since although all mice inoculated on days 9 and 15 of pregnancy were viremic, blood virus titers ($3.9 \pm 0.7 \log_{10}LD_{50}$/gram) were lower than placental virus titers

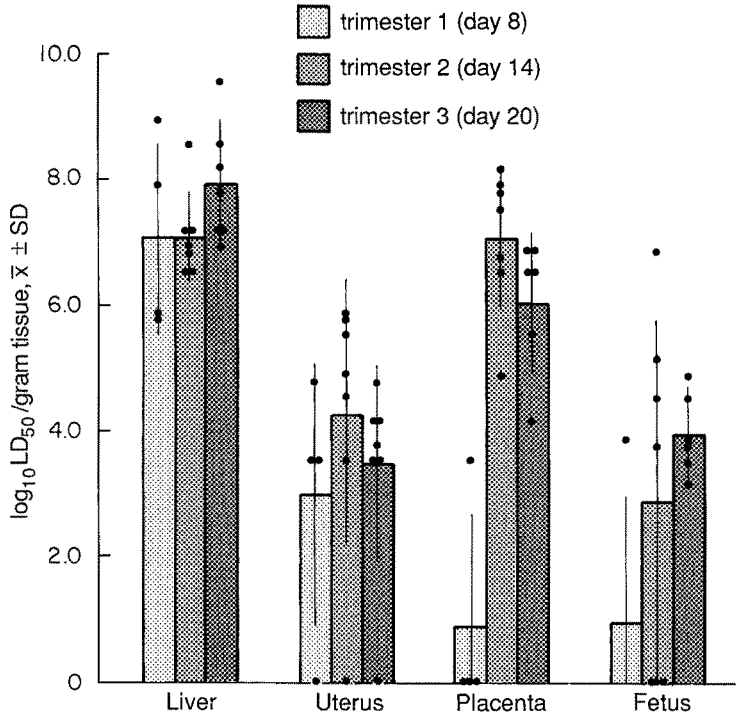

Fig. 1. MHV titers in maternal liver and uterus, placenta and fetal liver in BALB/cByJ mice inoculated intranasally with MHV-JHM on days 3, 9, and 15 of pregnancy and necropsied on days 8, 14, and 20 of pregnancy

($6.7 \pm 1.2 \log_{10}LD_{50}$/gram; paired t, $P \leqslant 0.001$). Viral antigen was found throughout placenta, but particularly at the maternal-fetal junction.

Vertical transmission of MHV was found to be dependent upon MHV strain and host genotype when genetically susceptible and resistant dams were inoculated with different virus strains at 15 days of pregnancy (Table 1). As noted above, virulent MHV-JHM caused disseminated infection in susceptible BALB/cByJ mice, as shown by a high prevalence of detectable virus in maternal liver and uterus, as well as placenta and fetus (Table 1). In contrast, avirulent MHV-S infection of susceptible BALB/cByJ mice resulted in a significantly lower prevalence of fetal infection (Chi-square, $P \leqslant 0.001$), as well as a lower prevalence of infection in maternal liver and no detectable virus in uterus or placenta (Table 1). Virus titers in livers with detectable virus were significantly lower than MHV-JHM titers in liver of BALB/cByJ mice (Fig. 2; unpaired t, $P \leqslant 0.001$). Compared to MHV-JHM in susceptible BALB/cByJ mice, MHV-JHM infection of resistant CD-1 mice resulted in no fetal infection (Chi-square $P \leqslant 0.001$) and a lower prevalence of infection in maternal liver, no detectable virus in uterus and only 1 mouse with virus in placenta (Table 1). Virus titers in liver with detectable virus were significantly lower than MHV-JHM titers in livers of BALB/cByJ mice (unpaired t, $P \leqslant 0.001$), and equivalent to titers observed in MHV-S infected BALB/cByJ livers (Fig. 2). Enterotropic MHV-Y

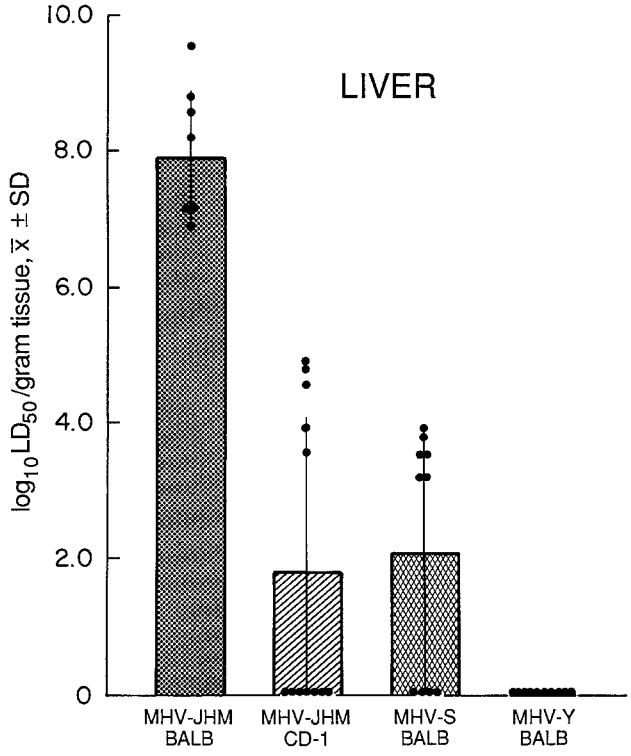

Fig. 2. MHV titers in maternal liver of susceptible BALB/cByJ or resistant CD-1 mice infected with virulent MHV-JHM, low virulence MHV-S or enterotropic MHV-Y

Table 1. Virus detection in tissues from genetically susceptible BALB/cByJ and resistant CD-1 mice, 5 days after inoculation with different MHV strains at 15 days of pregnancy

MHV strain	Mouse genotype	Liver	Colon	Uterus	Placenta	Fetus[a]
MHV-JHM	BALB/cByJ	7/7[b]	—[c]	6/7	6/6	6/6
	CD-1	5/12	—	0/12	1/9	0/12
MHV-S	BALB/cByJ	6/10	—	0/10	0/7	2/10
MHV-Y	BALB/cByJ	0/10	10/10	1/10	2/10	0/10
	CD-1	0/10	10/10	0/10	0/10	0/10

[a] Fetal liver
[b] 7 positive/7 tested
[c] Not tested

was detected primarily in colons of BALB/cByJ and CD-1 dams, and not fetuses. Other tissues were negative, excepting the placenta in 2 and uterus in 1 BALB/cByJ dams (Table 1, Fig. 2).

Maternal antibody

MHV IgG antibody was detected in serum of pups suckling MHV-JHM immune dams at 2 and 4 weeks post-partem, but not at 6 or more weeks (Table 2).

Table 2. Serum MHV IgG antibody titers (reciprocal dilutions) in immunized and naive dams and their pups at intervals after parturition, with comparison between first and second litters

Dam	Antibody titer, weeks postpartem												
	First litter							Second litter[a]					
	Dam		Pups					Dam		Pups			
	0	3	2	4	6	8	10	0	3	2	4	6	8
Immune A	3,200[b]	25,600	2,400	800	—[c]	—	—	6,400	6,400	6,400	250	—	—
Immune B	400	25,600	400	100	—	—	—						
Immune C	25,600	51,200	25,600	6,400	—	—	—	12,800	12,800	6,400	600	—	—
Immune D	3,200	6,400	3,200	300	—	—	—						
Immune E	12,800	25,600	25,600	6,400	—	—	—	3,200	6,400	1,600	300	—	—
Control	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] Dams remated 5 weeks after weaning first litter. Interval between whelping of first and second litters approximately 8–10 weeks
[b] Reciprocal of geometric mean, MHV IgG titer, 2 replicates
[c] — $\leqslant 50$

Antibody titers at 2 weeks were nearly equivalent to antibody titers in the corresponding dams at whelping. Curiously, antibody titers rose in all dams between whelping and weaning of the first litter, but remained static while nursing their second litters. Antibody titers were generally lower in second litter pups compared to first litter pups of the same dams.

Based on cross-foster nursing results, maternally derived MHV antibody in suckling mice was colostral, and not placental in origin (Fig. 3). Pups born to an immune dam but foster-nursed at birth on a naive dam had no detectable serum antibody to MHV (Fig. 3A). Pups from a naive dam, but foster-nursed at birth on an immune dam had serum antibody to MHV which peaked at 2 weeks and was declining at 3 weeks of age (Fig. 3B). When pups were transferred at 2 weeks of age, pups from an immune dam that were foster-nursed on a naive dam had antibody titers at 2 weeks which diminished to undetectable levels by 4 weeks of age (Fig. 3C). In contrast, pups from a naive dam that were foster-nursed on an immune dam starting at 2 weeks of age had no MHV antibody at the time of transfer, but had absorbed colostral antibody by 3 and 4 weeks of age (Fig. 3D). Thus, maternally-derived MHV antibody is acquired via colostrum and significant absorption can still occur at 2 weeks of age.

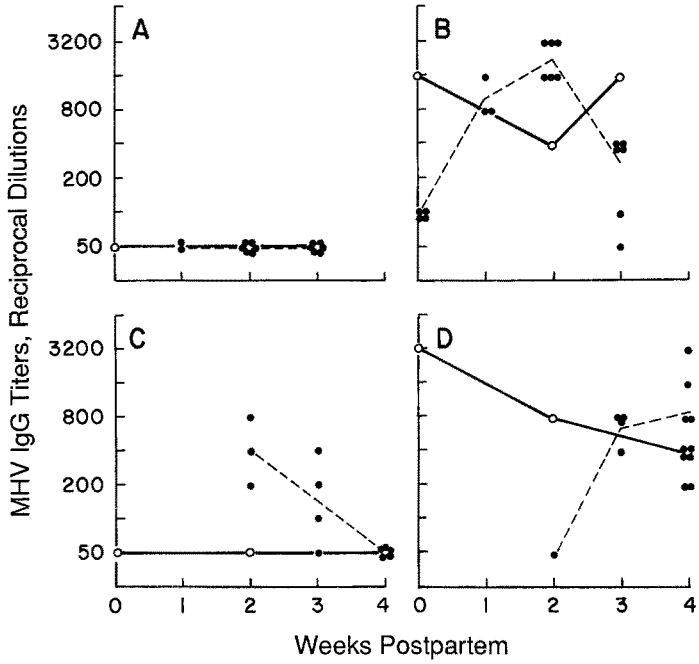

Fig. 3. Serum IgG MHV antibody titers in immune and non-immune CD-1 dams foster nursing pups transferred from immune and non-immune dams. Solid lines and open circles represent dam sera. Dashed lines and solid circles represent pup sera. **A** Non-immune dam foster-nursing pups transferred at birth from an immune dam. **B** Immune dam foster-nursing pups transferred at birth from a non-immune dam. **C** Non-immune dam foster-nursing pups transferred at 2 weeks of age from an immune dam. **D** Immune dam foster-nursing pups transferred at 2 weeks of age from a non-immune dam

Resistance of passively-immune pups to MHV challenge

Pups challenged with 2 different doses of MHV-JHM at 1 or 2 weeks or age were significantly protected against mortality if their dams were immune, compared to pups nursing naive dams (Table 3; Chi-square, $P \leqslant 0.001$). None of the pups that were 3 weeks of age and nursing naive dams died, indicative of age-dependent resistance. Therefore, 3 week-old pups from immune dams were not tested. In a second trial with MHV-S and MHV-JHM challenge of 1 week old pups, similar results were shown with MHV-JHM (Table 4). Compared to pups nursing naive dams, pups nursing MHV-S immune dams were protected against MHV-S (Chi-square, $P \leqslant 0.001$), but not MHV-JHM challenge. Pups nursing MHV-JHM-immune dams were protected against both MHV-JHM

Table 3. Mortality among 1, 2, or 3 week old CD-1 pups nursing immune or naive dams, following intranasal challenge with different doses of MHV-JHM

	Infant mortality rate, age at inoculation				
	1 week		2 weeks		3 weeks
Virus dose	Naive dam	Immune dam	Naive dam	Immune dam	Naive dam
10^4 $TCID_{50}$[a]	25/25[b]	26/37 (<0.001)[c]	17/21	5/25 (<0.001)	0/12
10^2 $TCID_{50}$	10/35	0/25 (<0.001)	6/22	1/25 (<0.001)	0/20

[a] *$TCID_{50}$* Median tissue culture infections dose
[b] 25 dead/25 inoculated
[c] In brackets, Chi-square probability, immune vs. naive dam

Table 4. Mortality among 1 week old CD-1 mice nursing immune and naive dams, following intranasal challenge with different doses of homologous and heterologous MHV

		Infant mortality rate			
Challenge virus	Virus dose	Naive dam	MHV-JHM-immune dam	MHV-S-immune dam	Chi-square JHM vs S
MHV-JHM	10^3TCID$_{50}$[a]	27/29[b]	10/53 (<0.01)[c]	11/11 (n.s.d.)[d]	<0.001
	10^2TCID$_{50}$	15/15	—[e]	9/9 (n.s.d.)	—
	10^1TCID$_{50}$	2/29	—	1/11 (n.s.d.)	—
MHV-S	10^4TCID$_{50}$	11/12	5/12 (<0.001)	17/18 (n.s.d.)	<0.001
	10^3TCID$_{50}$	12/12	—	0/13 (<0.001)	—
	10^2TCID$_{50}$	3/13	—	1/10 (n.s.d)	—

[a] *$TCID_{50}$* Median tissue culture infectious doses
[b] 27 dead/29 inoculated
[c] In brackets, Chi-square probability, immune vs. naive dams
[d] *n.s.d.* No significant difference
[e] Not tested

and MHV-S challenge (Chi-square, $P \leqslant 0.01$ and $P \leqslant 0.001$, respectively). Thus, passive immunity to MHV challenge is effectively conferred to 1 and 2 week old pups following infection of the dam by a natural route of inoculation and resistance does not appear to be virus strain-specific. At 3 weeks of age, passive immunity was irrelevant, since age-related resistance had evolved.

Discussion

This study has attempted to control virus strain, route of inoculation, and host variables in answering several questions about MHV biology, using a natural route of inoculation or immunization, and selected model virus strains or host genotypes. Extrapolation of experimental data to the natural situation cannot be made with certainty. However, this study shows that MHV has the potential for in utero transmission, infection of neonates can be modified by colostral antibody from immune dams, and that passively acquired antibody, which can be measured for up to 4 weeks postpartem, is protective against both homotypic and heterotypic strains of MHV.

In utero transmission of MHV to the fetus following oronasal inoculation of the dam depended upon a combination of virus and host factors that favored disseminated, multisystemic infection in the dam. Relatively virulent, polytropic MHV-JHM could be transmitted in utero in genetically susceptible (BALB/cByJ), but not resistant (CD-1) mice. Low virulence, polytropic MHV-S could also be transmitted in utero in BALB mice, but not as efficiently as MHV-JHM. The restrictive enterotropism of MHV-Y precluded multisystemic infection of the dams, and thus fetal infection, regardless of genotype. The present study suggests that the physiological state of pregnancy may also increase host susceptibility to MHV, based on MHV-JHM titers in maternal livers. Vertical transmission occurred during all 3 trimesters of pregnancy, which is in agreement with others when ICR mice were inoculated intravenously with MHV-JHM [15]. On the other hand, in utero transmission was not found in NMRI mice inoculated intraperitoneally with MHV-3 [10, 20].

In the mouse, a small amount of antibody is transferred from dam to fetus through the placenta, but most is transferred via colostrum. Absorption of antibody through bowel mucosa continues for up to 16 days of age [9]. Using dams that had recovered from an immunizing MHV infection and cross-fostering, MHV-specific IgG was shown to be transferred postnatally, but not in utero, and pups were capable to active MHV antibody absorption until at least 2 weeks of age. We did not attempt to measure other immunoglobulin types, since IgG is absorbed selectively through infant mouse intestine [13]. Passively acquired serum antibody to MHV was detectable in pups for up to 4 weeks of age if pups nursed immune dams continuously, but declined within 2 weeks if seropositive pups were transferred to a non-immune dam. These observations suggest that once access to colostral antibody ceases, by virtue of closure of gut transfer mechanisms or blocking access, serum antibody to MHV declines

to undetectable levels within 2 weeks. Similar results were found in pups nursing dams immunized by intraperitoneal inoculation of MHV-JHM [22].

Maternally-derived passive immunity appears to protect neonatal mice from MHV under natural conditions [11] and previous experimental studies have confirmed this observation, but with dams that were immunized by intraperitoneal injection of inactivated or live MHV [11, 14, 16, 18, 19, 21, 22]. The data presented in this paper show that dams, immunized by a more natural means (recovery from oronasal inoculation of MHV), also confer significant protection to their young against MHV challenge. Furthermore, we have shown that colostrally-derived immunity is cross-protective against antigenically heterotypic strains of MHV. Although all MHV strains are antigenically cross-reactive, active immunity is directed against strain-specific components of the virion [3]. Stronger challenge resistance was conferred by dams immunized with MHV-JHM compared to MHV-S, presumably because the more severe infection of the dam by MHV-JHM stimulated a stronger immune response. Passive immunity was effective in pups at both 1 and 2 weeks of age, intervals when they are highly susceptible to MHV. Age-related resistance to MHV evolves between 2 and 3 weeks of age [1].

These findings allow a rational approach to control and detection of MHV in breeding populations of mice. Natural infections with MHV are likely to be short-term and mild, and spread rapidly through a population [1]. The likelihood of active infection during pregnancy in an MHV-naive mouse is therefore statistically remote. The appropriate combination of host genotype and virus strain for successful in utero infection is likewise remote. Thus, although MHV can potentially be vertically transmitted, it is unlikely. Concern over in utero transmission is valid during cesarean rederivation, but this can be circumvented by breeding MHV seropositive, recovered mice, and protecting such mice from exposure to another strain of MHV during pregnancy. Based on our current findings, progeny that have been rederived by hysterotomy and foster nursed on MHV-naive dams should be seronegative to MHV at birth, and certainly at weaning. If seropositive dams are bred and allowed to whelp, their progeny should be seronegative by 6 weeks of age. If pups are foster-nursed on naive dams, they should be seronegative at or before weaning. Rederivation of MHV-free progeny without hysterotomy has been recently demonstrated with enzootically infected stock, using foster dams or by breeding recovered dams [17, 24].

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