

Murine Norovirus Pathogenesis and Transmissibility

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BACKGROUND: Murine norovirus 1 causes a subclinical infection in immunocompetent mice without significant tissue pathology, and infection is limited to the intestines, liver, spleen, lymph nodes and lungs. Duration of infection ranges from less than 3 days to greater than 5 weeks. Other MNV isolates have been identified in the last few years and recently been shown to comprise a single genogroup and serotype. Two recent studies reported the presence of MNV antibodies in 22% of sera tested and MNV RNA in feces of 64% of mouse strains tested, making MNV the most prevalent virus in contemporary mouse colonies.

OBJECTIVES: To determine the pathogenesis and the transmissibility of several strains of MNV in adult and neonatal immunocompetent mice and to determine if fostering of neonatal mice from MNV-infected dams to MNV-naïve dams was an effective means of eliminating MNV from a colony of mice.

RESULTS: Thirteen isolates of MNV (A, B, C, D, E, F, G, H, J, K, L, M, and O) were identified in the feces of clinically normal, genetically engineered mice. Comparison of the predicted amino acid homologies of the capsid genes (147 amino acids) of the 13 new isolates and 21 previously reported isolates of MNV identified 5 groups of MNV strains where each member of the group had at least 96% homology with at least one other member of the group. MNV-C, -D, -G and -L were chosen for use in this study as representative members of groups 1-4.

The pathogenesis and the transmissibility of MNV-D and -L in 4 week-old female Swiss Webster (SW) mice following oral inoculation was investigated using MNV RT-PCR of feces and cage swabs as well as contact and bedding sentinels. All feces from mice inoculated orally with MNV-D were positive for MNV RNA on post-inoculation days (pid) 3-11 and 75% of feces were positive on pid 14-56. Most MNV inoculated mice seroconverted by pid 14 and all were seropositive at pid 28. MNV was detected on the surface of all cages housing MNV-D inoculated mice on pid 7-56. Sentinels in contact with MNV-D inoculated mice for 1 week starting on pid 7, 14 and 28 but not on pid 56 seroconverted. The majority of sentinels placed on soiled bedding for 1 week collected from cages on pid 7, 14, 28 and 56 seroconverted. Infected mice did not exhibit clinical signs and lesions were not evident in H&E stained tissues (spleen, liver, kidney and intestines). Oral inoculation of SW mice with MNV-L resulted in essentially identical results. In summary, MNV-D and -L cause chronic subclinical infections in SW mice, transmission to bedding and contact sentinel mice was highly efficient and RT-PCR of RNA extracted from feces and cage swabs was effective at detecting MNV infection.

The susceptibility of neonatal mice to MNV infection has not been previously reported. Several studies were performed to investigate the pathogenesis and transmission of MNV to neonatal mice. Intestinal homogenates from neonatal SW mice inoculated orally with MNV-L on postpartum days (ppd) 1-3 were negative for MNV RNA at pid 3 and 7. In

contrast, 69% of intestinal homogenates prepared on pid 3 and 7 from mice inoculated orally at ppd 5-8 were MNV RT-PCR positive. Infected neonatal mice did not exhibit clinical signs and lesions were not evident in H&E stained tissues. Oral infection of neonatal SW mice with MNV-D, confirmed that 2 and 4 day old mice are resistant to MNV infection and 6 and 8 day old mice are susceptible to MNV infection. Infection of 10, 14 and 18 day old mice by contact with dams inoculated orally with MNV-D was highly efficient as 33/45 intestines were positive for MNV RNA at pid 7-14. Neonatal/weanling mice infected by contact with infected dams did not exhibit clinical signs and lesions were not evident in H&E stained tissues. Because mice less than 10 days of age were not infected by contact with infected dams, studies was performed to determine whether fostering of neonatal mice from MNV-infected to MNV-naïve dams could be effective at preventing infection of neonatal mice. Four litters each of 1, 2, 4 or 6 day-old mice from MNV-L infected dams were transferred to naïve dams with similar aged litters and vice versa. On ppd 21, feces from all MNV infected dams and litters transferred to them were MNV RT-PCR positive. In contrast on ppd 21, feces from all MNV-naïve dams and litters transferred to them were MNV RT-PCR negative. Fostering of 2 day-old mice from 5/5 MNV-C, 5/6 MNV-D and 7/8 MNV-G infected dams onto MNV-naïve dams prevented MNV infection of the fostered mice. In the 2 litters where MNV was detected, dams were infected within 7 days of transfer suggesting that the neonatal mice had served as fomites. In summary, fostering was effective at preventing MNV infection in 33 of 35 litters of neonatal mice.

Preliminary summary of enrichment density/studies conducted at UC Davis

Joy Mench

Methods

Female mice (4 per cage) of three strains (BALBc, C57Bl and CD-1) were given amounts of space ranging from 5-32 square inches per mouse. They were provided either with no enrichment, or given varying levels of enrichment (minimal enrichment consisting of a tunnel and nestlet, maximal (“super enriched”) consisting of tunnel, hut, nestlet, Shepherd Shack, gummabone, and running wheel). The mice were housed under these conditions for three months. Measures taken included immune response, body weight, body condition score, food consumption, blood cell counts, pathology, organ weights, corticosterone levels, abnormal behaviors, aggression, and enrichment use.

Results

- There were significant and marked strain differences in almost every measure taken
- On the other hand, housing density had little effect on most of the variables, and the few effects found were inconsistent. Corticosterone levels were higher in mice of all strains housed at intermediate densities (current *Guide* standard) than at high or low densities. There were fewer white blood cells in CD-1 mice housed in cages at intermediate density, and fewer WBC in BALBc housed at low and medium than at high densities. There were also a few strain x density interactions for specific cell types. CD-1 mice at the highest density consumed more food than those at intermediate or low density. There were no other density effects.
- Enrichment had the most significant effects on the measures taken in this study. These effects were mainly on behavioral measures, although enrichment did affect the corticosterone response of all mice over time, and enriched BALBc mice had heavier adrenal glands than non-enriched BALB's. CD-1 mice given the greatest level of enrichment weighed less (probably because they ran so much on the running wheel). Mice in unenriched cages had more hair loss. Super-enriched mice consumed more food, especially CD-1 mice. Mice of all strains were less aggressive when enriched overall, and aggression was further decreased in super-enriched cages. Enrichment also decreased stereotypic behavior, but only in CD-1 mice unless cages were super-enriched, in which case stereotypy was decreased in all mice.

Proposed Experiment

Our study, in combination with previous published studies, shows that female mice are relatively insensitive to stocking density, whether achieved by changing the size of the cage while keeping group size constant, or by increasing the group size in standard cages. However, there were significant effects of enrichment, especially on abnormal behavior but also to some extent on physiology. This, however, raises questions about what kind of enrichment mice actually require. Many enrichments are too space-consuming to put in conventional cages if stocking densities are increased, and there could also be competition for resources at higher densities if the enrichments are highly valued but insufficient in number for the mice to share them.

Because the mice in our study were given all enrichments simultaneously, it is impossible to determine which enrichments are most important in affecting stress and reducing abnormal behavior. It is obvious from our data that there are strain differences – in fact, our data suggest that perhaps only some mouse strains (e.g. more active strains like CD-1 mice) actually benefit from enrichment, but this is not clear without further analysis of interactions between strain and particular enrichment types. We do have data showing that there are different patterns of enrichment use among strains – for example, CD1 mice, unlike BALBc and C57Bl mice, rarely used a tunnel when other enrichments were present. However, patterns of enrichment use when multiple enrichments are present can only provide tentative information about what types of enrichment are most important. We therefore propose to study how specific enrichments or enrichment combinations affect the behavior and physiology of CD-1, C57Bl, and BALBc female mice. The mice will be housed 4 per cage in standard shoebox cages. The cages will be either unenriched, provided with a single enrichment (chosen based on frequency of use in the study above): nestlet, running wheel/igloo, Shepherd Shack, Mouse Hut; or provided with two enrichments that associated with different types of behaviors (nestlet or Shack and Hut; nestlet or Shack and wheel) . All of the measures outlined in our originally proposed project will be taken.

Developing a metric for comparing analgesic efficacy in algesiometric testing with postsurgical pain relief

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Doses and time courses of analgesics are derived from either of two sources: (a) algesiometric tests and (b) measures of postsurgical wellbeing or pain relief. Unfortunately, these two methods are not cross-indexed, *i.e.*, we don't know how well correlated the results are. Often, doses derived from one source seem inappropriate in the other context. As a step toward determining an index for translating doses obtained in one context to utility in the other context, we investigated the minimum effective dose of buprenorphine, an opioid analgesic that we have extensively studied in algesiometric tests, in a postsurgical model. We used multiple criteria to determine the clinical effectiveness of the treatment: acute analgesia; secondary (rebound) hyperalgesia after 4, 24 and 72 hr; and proximal and distal allodynia at various times after surgery. A single, postsurgical SC injection (0, .005; .01; .05 mg/kg) of buprenorphine was given to rats receiving a hind paw incision + isoflurane anesthesia, rats receiving no surgery + isoflurane anesthesia, or rats receiving no surgery and no anesthesia. The .05 mg/kg dose elevated pain threshold to normal levels (isoalgesia) at 1 and 4 hr after surgery. The .01 dose produced slight but significant elevations of pain threshold at the same intervals. However, allodynia measurements indicated that 24 hr after surgery, rats that had surgery and received .05 mg/kg buprenorphine were showing sensitivity to touch (secondary hyperalgesia) whereas those that received .01 mg/kg were not. Clearly, a single criterion (e.g., acute elevation of pain threshold) is insufficient to determine the most appropriate dose of opioid analgesic. The subjects in the current study were also used in a follow up study to determine whether the single dose of buprenorphine they received sensitized them to the subsequent effects of morphine administered 2 weeks later. This is an important issue to opioid researchers who find it necessary to administer opioid analgesics for surgery. Both nociceptive and locomotor sensitivity were assessed. The data indicate that the single experience with buprenorphine at therapeutic doses did not sensitize the rats to a subsequent 5 mg/kg cumulative regimen of morphine sulfate.

Strategies for MPV Screening

J. Macy

MPV is one of the most difficult infections to address as it is asymptomatic, persistent for up to nine weeks, may occur in small “pockets” of mice, and is a non-enveloped DNA virus that is relatively stable in the environment. A key component to cost-effective containment and proactive elimination of MPV is the quick and accurate detection of infection in colonies. Current strategies often require labor intensive individual testing of mice to identify infected mice/cages within a room and are not amenable to large scale testing. The goal of this project was to assess whether PCR testing of cage swabs is an effective and feasible large-scale, real-time MPV screening strategy to augment current soiled bedding sentinel programs.

Methods and Results

Initially we determined the cage components with the most consistent contamination by infecting fifteen Swiss Webster (SW) mice in five cages (3 mice per cage; index mice) with MPV. On post infection day (PID) 3, 5, 7 (cage change), 10, 12, 14 (cage change), 17, 19, 21 (cage change), feces were collected from mice, and cages were swabbed in the following locations: the wire bars; the cage wall above the bedding; and cage wall below bedding line and in corners. To confirm if detected virus was infectious, 2 sentinel mice were placed in each soiled cage (after removal of index animals) at cage changes (PID 7, 14, & 21) and remained there for 1 week. MPV fecal shedding by SW was detected in all five cages until day 33 and in one cage until day 56. MPV DNA was detected in all three locations at days 7 and 14. At later time points, cage swabbing detected MPV DNA only below the bedding in 5/5 cages at day 21, 4/5 cages (with fecal shedding) at day 28, 3/4 cages at day 35, and 2/3 cages day 42. Sentinels exposed to soiled cages at day 7 and 14 seroconverted; day 21 sentinels remained negative. The results indicated that swabbing the cage bottom was most effective.

Next, we determined if genotype (BALB/c, C57BL/6) influences MPV detection using the optimal swabbing method (cage bottom). Five cages containing 3 mice of each genotype were infected. MPV fecal shedding by BALB/c occurred in 5/5 cages until day 12, 3/5 until day 14, and 0/5 by day 17. Cage swabbing detected MPV in 4/5 BALB/c mice on day 5, 5/5 on day 7, 4/5 on days 10 and 14. Sentinels exposed to 3/5 BALB/c cages soiled cages were positive on day 7 and 5/5 on day 14. C57BL/6 mice in 5/5 cages shed MPV on day 7, 4/5 on day 10, 3/5 until day 12, 1/5 by day 14, and 0/5 by day 15. Cages swabs detected MPV in 4/5 C57BL/6 cages on day 5, 5/5 on day 7, 0/5 on day 10. Sentinels exposed to 3/5 C57BL/6 cages were positive on day 7 and 1/5 was positive on day 14. These results indicate that the sensitivity of cage bottom swabbing PCR in BALB/c and C57BL/6 mice is slightly less than fecal PCR but greater than soiled bedding sentinels during the first week after infection (PID 7). However, two weeks after infection (PID 14) in BALB/c mice, soiled bedding was the most accurate method, cage swabbing was intermediate, and fecal PCR was the lowest. C57BL/6 feces and sentinels were positive in only one cage and in no swabbed cages at PID 14.

Finally, we determined the efficacy of swabbing multiple cages in a row when only one of the cages was infected. The location of the infected cage varied within the row to determine if the location of MPV infected cage impacted the results (see Figure 1). Twenty-one SW mice in 7 cages and 9 C57BL/6 mice in 3 cages were infected. Cages with MPV infected index mice were located on one side of an individually ventilated cage rack. The remaining 60 cages contained 3 uninoculated SW (rows 1-7) or C57BL/6 (rows 8-10) mice each. On PID 6, feces were collected from all MPV-inoculated mice to confirm infection. On PID 7, 14 and 21 one set of swabs was used to sequentially (left to right) sample the cage bottoms of all 7 cages in each row. A second set of swabs was used on PID 7 and 14 to sample the cage bottom of each of the index cages containing the MPV-infected mice. Soiled bedding sentinels were set up on PID 7 and 14 by placing 25 ml of soiled bedding from each cage in a cage containing 2 SW mice. Two SW mice were also placed in each soiled index mouse cage. Sentinel mice were housed on soiled bedding for 1 week and then were transferred to clean cages for an additional two weeks before being tested for MPV seroconversion.

Figure 1

row 1	MPV						
row 2		MPV					
row 3			MPV				
row 4				MPV			
row 5					MPV		
row 6						MPV	
row 7							MPV
row 8	MPV						
row 9				MPV			
row 10							MPV

Yellow boxes represent cages housing SW mice

Green boxes represent cages housing C57BL/6 mice

Red MPV represents cages housing MPV-inoculated mice

Index mice became infected in rows 1-9 (SW 1-7; C57 8, 9) but not row 10 as determined by fecal PCR or by any indices measured. At day 7, infected SW mice (7/7 cages) could be detected in 100 % of cages using any of the methods (index sentinel, row sentinel, or index swab) except row swabbing (3/7 cages). For the two C57 cages that became infected (rows 8 and 9), a combination of row sentinel and index sentinel or row swab and row sentinel were required to detect infection in both cages.

At Day 14, all SW cages containing infected SW mice and 1/2 C57BL mice could be detected using a combination of index sentinels, row sentinels, and index cage swabs. However, 6/7 of the SW cages infection could be detected by using only index and row sentinels. However, index cage swabbing was required to detect infection in SW-7.

Summary and Conclusions

The results indicate that swabbing the cage bottom was the optimal swabbing location within the cage. With the exception of one cage in study 3, swabbing individual cages housing MPV infected SW mice was 100 % sensitive during the initial 14 days after infection. It was less sensitive in BALB/c and C57BL/6 mice; 80-100 % during the initial 10 days in BALB/c mice, and either 80-100 % (study 2) or 0 % (study 3) sensitive during the initial 7 days after infection in C57BL/6 mice. The sensitivity of individual cage swabbing, as compared to seroconversion of soiled bedding sentinels, was equally sensitive in SW mice, more sensitive early in the infection of BALB mice (first 10 days) and highly variable in C57 mice after PID 7. The low sensitivity of soiled bedding in BALB/c and C57BL/6 mice suggests that the “viral load” within soiled bedding of cages housing BALB/c mice must reach a threshold to induce seroconversion in sentinel mice and requires 10 days of shedding to do so. In contrast, cages housing infected SW mice do not require 7-10 days to reach a threshold suggesting the amount of virus shed and/or viral infectivity is higher in SW mice. The SW mice also shed MPV significantly longer (56 days) compared to BALB or C57 mice (14 days) in Study 2.

Swabbing individual cages is easier to perform compared to collection of feces from individual mice because cages can be swabbed quickly at the time of cage change. Although not as effective overall as fecal PCR, individual cage swabbing increases the sensitivity of row soiled bedding sentinels at day 14 in SW mice (43% to 100 % in study 3) in C57BL/6 mice (50 to 100 % in study 3) if the two techniques are combined. However, cage swabbing at PID 7, but not PID 14, increased the sensitivity of index cage sentinels in C57BL/6 mice or BALB/c mice in Study 2. These results indicate that individual cage swabbing can be used as adjunct to increase sensitivity of bedding sentinels during the first 2 weeks of infection in a genotype dependent manner.

Swabbing multiple cages (row swabbing) is an efficient technique. Unfortunately, its sensitivity is low (40%) during the first week of infection and ineffective at 14 and 21 PID. Location of infected cage within the row did not impact the ability to detect MPV. Our results indicate that row swabbing is not worthwhile because of its low sensitivity and does not increase the chance of detection when combined with soiled bedding sentinels. We also noted that in Study 3 only one of the two SW mice within the same cage seroconverted in 18 % of the cages. The results support the use of two sentinel mice per cage for accurate MPV detection using soiled bedding.

In general, consistency of infection and infection detection efficacy varies from study to study. We show that individual cage swabbing can be a worthwhile adjunct to row soiled bedding sentinels and as a labor saving alternative to PCR of feces from individual mice, especially since every mouse within the cage should be tested based on the varying attack rate of mice that can occur within a single cage. These results also underscore how routine testing using row sentinel testing can provide false negative results and that “outbreaks” may be a threshold or timing effect originating from an existing low level of infection, rather than an “acute” contamination of the colony. These issues further underscore the need to better understand all of the factors that influence MPV susceptibility and detection within and among mouse genotypes.

“Mouse Parvovirus: Effects of Immune Status and Pregnancy on Duration and Potential Reactivation of Viral Shedding”

Peter C. Smith

Previous studies have shown that MPV is enterotropic during early infection, and lymphocytotropic during persistent infection, especially in mesenteric lymph nodes (MLN). It is assumed that fecal shedding facilitates mouse-to-mouse transmission, but it is not clear what risks prolonged infection of the MLN pose for the duration of shedding, including the possibility of episodic or reactivated shedding as occurs during infection with Theiler's mouse encephalomyelitis virus. Preliminary molecular and serological results from our group indicate that while virus resides in the MLN of experimentally infected immunocompetent mice for as long as 18 weeks, viral shedding in the feces ends between 2-5 weeks. Little is known about the course of MPV in immunodeficient mice. While there is initial evidence that severe combined immunodeficient mice can be infected, that report suggested that they are not at higher risk for prolonged transmission of infection than immunocompetent mice. This result could reflect the lack of target cells (lymphocytes) for virus replication in such mice. However, those studies did not establish the long-term sequelae of MPV infection in immunologically dysfunctional mice.

In order to determine how the immune system impacts MPV infection shedding, transmission, and immune cell tropism 10 SCID (CB-17/Icr-Prkdc^{scid}/Crl), 10 nude (CBy.Cg-Foxn1^{nu}/J) 5 SCID-BG (CB-17/Icr.Cg-Prkdc^{scid} Lyst^{bg}/Crl), 5 Igh (STOCK-Igh-J^{tm1Dhu} N[?]+N2), 5 CB17 (C.B-Igh1^b/IcrTac) and 5 BALB (BALB/cAnNTac) mice were infected with MPV. Fecal samples were collected weekly from each mouse for PCR. One SW sentinel was added to each cage at post-infection day (PID) 0, replaced at PID 14, and PID 28. Sentinel mice were held for an additional two weeks and tested for seroconversion and fecal shedding. Index mice were euthanized on PID 14 (5 SCID) or PID 42 (all other mice) and mesenteric lymph nodes (MLN) were harvested. PCR and FACS were performed on MLNs to detect the presence of MPV in CD4, CD8, B220, and NK cells. The results showed that all BALB, nude, CB17 and Igh, 8/10 SCID and 1/5 SCID-BG mice were shedding MPV in the feces on PID 7. At PID 14, feces from SCID, SCID-BG, Igh mice averaged 4.2 pg/ul of MPV DNA whereas CB17 and BALB feces contained 1.6 and 2.8 pg/ul of MPV DNA, respectively. 4/10 nude mice were shedding MPV at PID 14 based on PCR results, but the amount was not quantified. MLNs from SCID and CB17 mice contained 66.5 and 7.4 pg/ul of MPV DNA, respectively. Seroconversion and fecal shedding occurred in contact sentinels placed with SCID-BG or Igh mice on PID 14, but not with SCID, nude, CB17 or BALB mice. By PID 21, CB17, BALB, and nude mice ceased shedding MPV whereas the other immunodeficient mice were still shedding on PID 28, 35, and 42. Sentinels placed on PID 28 with SCID-BG mice but not with other mouse strains seroconverted and shed virus. At PID 42, PCR detected 0.77 (Igh) to 6.7 (SCID-BG) pg/ul of MPV DNA in feces and detected from 1.9 (CB17) to 86.4 (SCID) pg/ul of MPV DNA in MLNs. FACS analysis indicated low number of B cells and NK cells contained MPV antigen on PID 42 (SCID > SCID-BG > Igh). These results indicate that fecal shedding is delayed in SCID and SCID-BG but virus levels in the feces and MLNs were high at PID 42. Although SCID and SCID-BG

had high levels of virus in the feces and MLN, only the SCID-BG transmitted infection after PID28. This suggests a role for NK cells in controlling infection.

The original scope of this proposal included an investigation of MPV in pregnant mice, however this work was not completed. Rather, we decided to do a more comprehensive investigation of MPV in immunodeficient mouse strains. Investigating the effect of pregnancy on MPV infection will be examined in future studies.