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Temporal Transmission Studies of Mouse Parvovirus 1 in BALB/c and C.B-17/Icr-*Prkdc*^{scid} Mice

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Fecal shedding and transmission of mouse parvovirus 1 (MPV) to naïve sentinels, breeding mates, and progeny were assessed. Neonatal SCID and BALB/c mice inoculated with MPV were evaluated over 24 wk; several mice from each strain were mated once during this period. Fecal MPV loads for each cage were determined weekly by quantitative polymerase chain reaction (PCR) analysis, and all mice were evaluated by quantitative PCR analysis of lymphoid tissues and seroconversion to MPV antigens in immunocompetent mice. Results indicated persistently high fecal shedding of MPV in SCID mice throughout the evaluation period sufficient to allow transmission to sentinels, naïve breeding partners, and the progeny of infected male mice and naïve partners. Lymphoid tissue viral loads in the progeny of infected female SCID mice were high at weaning but low at 6 wk of age. Infected BALB/c mice shed high levels of MPV in feces for 3 wk postinoculation, with seroconversion only in sentinels exposed during the first 2 wk postinoculation. Thereafter the feces of infected BALB/c mice and the lymphoid tissues of sentinels, naïve breeding partners, and progeny intermittently contained extremely low levels of MPV DNA. Although pregnancy and lactation did not increase viral shedding in BALB/c mice, MPV exposure levels were sufficient to induce productive infection in some BALB/c progeny. These data indicate that the adaptive immune response suppresses, but does not eliminate, MPV shedding; this suppression is sufficient to inhibit infection of weanling and adult mice but allows productive infection of some progeny.

Abbreviations: ID_{50}, 50% infectious dose; MPV, mouse parvovirus; qPCR, quantitative polymerase chain reaction; rNS1, recombinant MVMp nonstructural protein 1; RT-PCR, reverse transcription polymerase chain reaction; rVP2, recombinant MPV1 viral capsid protein 2; SCID, severe combined immunodeficiency

Mouse parvovirus (MPV) is among the most prevalent infectious agents detected in contemporary laboratory mouse colonies,[5,11,15] and this virus can have deleterious effects on research due to in vitro and in vivo immunomodulatory effects, tumor suppression, and contamination of cell cultures and tissues originating from mice.[16-18] MPV is thought to have considerable potential for transmission among mice in research facilities, because of its high environmental stability,[9] its ability to induce persistent infection in mice and cell lines,[12] and the difficulties associated with eradicating this virus from infected laboratory mouse colonies. As a result, MPV infections comprise one of the most important infectious disease problems encountered in contemporary laboratory animal research facilities.

In 1993, MPV1a was isolated from cultures of L3 cytolytic T lymphocytes and splenocytes.[16] This isolate then was adapted to grow in CTLL2 cytolytic T lymphocytes. Subsequent sequencing of the original isolate and the adapted strain showed that several mutations had occurred during adaptation of the virus to the CTLL2 cells; therefore the adapted virus was designated MPV1b.[1,4] In addition, a field strain of MPV1 (designated MPV1c) that was sequenced directly from tissues collected from a naturally infected mouse displayed several nucleotide differences from the 2 cultivated strains of MPV1.[4] Initial genomic sequence analysis indicated that MPV1 was related genetically most closely to MVM and LuIII, a parvovirus species of unknown host origin, with approximately 88% nucleotide sequence homology. Hamster parvovirus subsequently was isolated, sequenced, and demonstrated to have 94.6% nucleotide sequence homology to MPV1.[4] The existence of 2 additional strains distinct from MPV1, designated MPV2 and MPV3, was recently reported, with sequence analyses showing MPV3 to be virtually identical to hamster parvovirus.[5,7] One of these reports[5] also indicated that MPV1 was the most common strain of MPV detected in contemporary mouse colonies.

Several studies have investigated the transmission of MPV among mice. MPV1a administered by a combined oral and intraperitoneal route to neonatal and weanling outbred mice was transmitted to naïve sentinels by direct contact and soiled bedding exposure, predominantly within the first 3 wk after inoculation, although 1 litter of neonatal mice intermittently transmitted virus by direct contact as long as 6 wk after inoculation.[23] BALB/c mice born in a colony enzootically infected with MPV transmitted virus by direct contact to sentinels by 1 mo old colony mice but not 2, 3, or 6 mo old colony mice.[22] Infectious virus was detected using the mouse antibody production test, which was performed on samples of small intestine of 1-mo-old mice, and small intestine, large intestine, and spleen in 2-, 3-, and 6-mo-old mice.[22] In addition, PCR detection of MPV DNA in feces was assessed in 10 female SENCAR mice from a colony with enzootic MPV and minute virus of mice. MPV DNA was detected in 90% to 100% of these mice from 5 to 11 wk of age, with the percentage positive decreasing to 20% by 17 wk of age and 0% by 19 wk of age.[2] Recent studies in our laboratory revealed persistently high tissue levels of MPV DNA in C.B-17/Icr-*Prkdc*^{scid} mice inoculated as neonates, suggesting these mice may persistently shed high levels of virus.

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Vertical transmission of MPV has not yet been demonstrated, but potential for vertical transmission is suggested by the detection of MPV DNA in the sperm of infected mice.[8]

Since the discovery of MPV, multiple diagnostic methods have been developed to detect and distinguish murine parvoviruses.[14,19,20] Despite the availability of these highly sensitive and specific diagnostic assays, detection of MPV infections in contemporary laboratory mouse colonies remains problematic, with intermittent detection even in enzootically infected colonies. The widespread use of sentinel mice exposed to soiled bedding as the primary detection system, a relatively short period of viral transmission postinfection in immunocompetent mice, and the need for a fairly high viral dose to induce productive infection are likely key factors that result in intermittent detection of MPV. In addition, although MPV can be detected in tissues for several months after infection[12] and until 6 mo of age in tissues from naturally infected mice,[22] whether physiologic stress can induce increased MPV shedding in persistently infected immunocompetent mice is unknown. The objectives of the present study were to quantitatively and temporally assess MPV shedding in feces from immunocompetent BALB/c and immunodeficient C.B-17/Icr-*Prkdc*^{scid} mice, the effect of pregnancy and lactation on fecal viral loads and transmission, and biologic transmission of MPV to naïve mice and the progeny of MPV-infected mice.

Materials and Methods

Virus. MPV1e was isolated from naturally infected mice during an epizootic in a commercial barrier facility. The isolate is maintained by oral inoculation of naïve mice with 0.2-μM filter-sterilized tissue homogenate (liver, spleen, mesenteric lymph node resuspended in 50 mM Tris, 10 mM ethylenediaminetetraacetic acid [pH 8.7]) obtained from infected mice. The 50% infectious dose (ID_{50}) was determined in weanling BALB/c mice by oral inoculation of 10-fold serial dilutions of the filtered tissue homogenate. The DNA sequence for the genome of this isolate excluding terminal hairpins was determined and is most closely related to MPV1c (99.5% homology at the nucleotide level). The genomic sequence of the isolate was submitted to GenBank and was assigned accession number DQ898166.

Mice. Untimed pregnant BALB/cAnNCrl (BALB/c) and C.B-17/Icr-*Prkdc*^{scid}/Crl (SCID) female mice and weanling C3H/HeNCrl (C3H) mice were obtained from Charles River Laboratories (Wilmington, MA). All mice were specified to be free of murine viruses, pathogenic bacteria, and endo- and ectoparasites by the supplier. All mice were housed in static microisolator cages on aspen-chip bedding, NIH-31 diet (Harlan Teklad, Madison, WI) and hyperchlorinated water were provided ad libitum, and all animal manipulations were performed in a class-IIA biological safety cabinet under standard microisolator technique. Animals were housed in a biocontainment facility at a temperature of 22 to 24 °C, humidity of 40% to 60%, 12 to 15 air exchanges hourly, and a 12:12-h light:dark cycle. The University of Arizona Institutional Animal Care and Use Committee approved all animal procedures.

Animal infections. We oronasally inoculated 1-d-old BALB/c (n = 13) and SCID (n = 15) mouse pups with 100 ID_{50} MPV1e; pups were weaned at 4 wk of age into age- and sex-matched groups of 3 to 4 mice per cage and were maintained for 24 wk. At 13 wk of age, 3 mice from each strain and sex were moved into separate cages, and naïve 8-wk-old strain-matched mice of the opposite sex were introduced to form 6 breeding pairs per strain. These breeding pairs each were allowed to produce 1 litter, with male mice separated from females during late pregnancy. Weekly throughout the entire 24-wk study, 10 fecal pellets were collected from the floor of each cage of mice and stored frozen at –80 °C until fecal viral load could be determined by quantitative polymerase chain reaction (qPCR). In addition, biologic transmission was monitored on a biweekly basis (that is, sentinel exposure periods of 2 wks) by placing a single naïve weanling C3H sentinel into each soiled cage that had housed MPV-infected mice the previous week. After 2 cage changes (2 wk of exposure), exposed sentinels were placed into clean cages, maintained individually for an additional 3 wk to allow time for serconversion, and then euthanized by carbon dioxide inhalation. Blood samples were collected by cardiocentesis; the resulting sera were diluted 1:5 in phosphate-buffered saline and stored at –80 °C until use. Approximately 20 mg each of spleen and mesenteric lymph node were harvested from each animal and stored frozen at –80 °C until tissue DNA was extracted. Tissue DNA was subsequently stored at –20 °C until evaluated by qPCR. Similar samples were collected from MPV-infected mice and their progeny and naïve breeding partners at various time points to evaluate tissue viral loads and seroconversion. Some tissue specimens were placed in RNAlater (Qiagen, Valencia, CA) and frozen until RNA was extracted for MPV reverse transcription (RT)-PCR.

qPCR. DNA was extracted from tissues and feces and screened by an MPV-specific qPCR assay as previously described.[5,19] Briefly, DNA was extracted using a MagneSil KF Genomic DNA extraction kit (Promega, Madison, WI) and a KingFisher robotic extraction station (Thermo Labsystems, Franklin, MA) per the manufacturer's recommendations. qPCR reactions were performed with an Mx3000P QPCR System (Stratagene, Cedar Creek, TX), and products were analyzed by use of the accompanying software. Each 20 µl reaction consisted of 1× TaqMan buffer (50 mM KCl, 10 µM EDTA, 10 mM Tris-HCl [pH 8.3] and 60 nM passive reference dye; PE Applied Biosystems, Foster City, CA), 5.5 mM $MgCl_2$, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 400 µM dUTP, 300 nM each primer, 125 nM probe, 0.2 U AmpErase uracil-N-glycosylase (PE Applied Biosystems), 0.5 U Ampli*Taq* Gold polymerase (PE Applied Biosystems), and 2 µl template DNA (approximately 50 ng tissue DNA or 20 ng fecal DNA). Thermal cycling conditions consisted of 50 °C for 2 min for uracil-N-glycosylase incubation, polymerase activation at 95 °C for 10 min, and then 45 cycles of 95 °C for 15 s followed by 60 °C for 1 min. Samples were considered positive if they exhibited a mean fluorescence that was greater than 0.1 and a cycle threshold that was less than 45. We used 10-fold serial dilutions of cloned amplicon DNA (range of 10^8 to 10^0 template copies) to generate a standard curve, with linear regression of this standard curve ($R^2 = 0.993$) then used to estimate the viral genome copy number detected in each DNA sample by qPCR. Absolute viral copy number per fecal pellet was calculated by multiplying this estimate by the dilution factors used during DNA extraction and qPCR set-up.

RT-PCR. Total RNA was isolated from approximately 15 mg of tissues into 50 µL elution buffer using the RNeasy Mini Kit (Qiagen). RNA was reverse-transcribed to cDNA in a 30-µl reaction containing 5.5 mM $MgCl_2$, 2.0 mM each dNTP, 2.5 µM random hexamers, 1× PCR buffer 2, 20 U RNase inhibitor, 37.5 U MultiScribe reverse transcriptase (Applied Biosystems, Foster City, CA), and 5 µl RNA template. The reaction was incubated at 25 °C for 10 min, 48 °C for 30 min, and then heat-inactivated at 95 °C for 5 min. PCR primers were designed to flank splice junction 2, which is

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Table 1. qPCR and serology results for MPV1-infected SCID and BALB/c mice and their naïve breeding cagemates

Strain	Sex	Age at necropsy (wk)	MPV1e status	MLN+spleen qPCR	MLN+spleen mean MPV copies (log10)	rNS1 and MPV rVP2 serology	Mean rNS1 (mfi)	Mean rVP2 (mfi)
SCID	Male	25–32	Infected	6/6[a]	6.4	ND	ND	ND
	Female	13–27	Infected	6/6	6.6	ND	ND	ND
	Male	27	Naïve	3/3	7.2	ND	ND	ND
	Female	27	Naïve	3/3	5.1	ND	ND	ND
BALB/c	Male	26	Infected	5/6	1.1	6/6[a]	1361[b]	14305[b]
	Female	26	Infected	6/6	0.5	6/6	6356	17158
	Male	21	Naïve	2/3	–0.1	0/3	32	25
	Female	21	Naïve	1/3	0.1	0/3	39	36

mfi, median fluorescence intensity; MLN, mesenteric lymph node; ND, not done.
[a]No. of mice positive/no. of mice tested.
[b]Multiplex fluorescent immunoassay values > 125 mfi were considered positive for both the rNS1 and MPV rVP2 serology assays.

found in MPV mRNA transcripts R1, R2, and R3, and splice junction 3, which occurs in MPV mRNA transcript R3.[13] Each 50-µl PCR reaction contained 0.2 mM of each dNTP, 0.5 mM $MgCl_2$, 150 nM each of forward primers PV-NS1FA (5' GAG CCC AAC TTG GTC AGA GAT 3') and PV-NS1FB (5' GAG CCC AAC YTG GTC CGA GAT 3'), 300 nM of reverse primer PV-VP1RA (5' CAG SAG AGA AGT ACA GGT AAG GAT 3'), 1 × PCR buffer, 2.5 U HotStar *Taq* polymerase (Qiagen), and 5.0 µl cDNA template. Thermal cycling conditions consisted of 15 min 94 °C; 5 cycles of 94 °C for 40 s, 62 °C for 50 s, and 72 °C for 30 s; and 40 cycles of 94 °C for 40 s, 57 °C for 50 s, and 72 °C for 30 s. PCR products were evaluated on a 1% agarose gel to detect amplified mRNA transcripts containing splice junctions 2 (276 basepairs) and 3 (290 basepairs). Amplification of genomic MPV DNA yields a 372-basepair PCR product.

Serology. The multiplex fluorescent immunoassay format was used to evaluate sera for the presence of virus-specific antibodies. Baculovirus-expressed recombinant MVMp nonstructural protein 1 (rNS1) and MPV1 viral capsid protein 2 (rVP2) were prepared essentially as previously described.[14,20] Purified rNS1 and rVP2 were covalently coupled to carboxylated polystyrene microspheres (Luminex, Austin, TX) at a coupling concentration of 25 µg of protein per 5×10^6 microspheres according to the manufacturer's recommended protocols. Bovine serum albumin, $A9_{2L}$ mouse fibroblast cell lysates, and Hi-five insect cell (Invitrogen, Carlsbad, CA) lysates were similarly used to coat microspheres to serve as control antigens. Microspheres were stored at 4 °C in the dark until use.

Evaluation of mouse sera for rNS1- and MPV rVP2-specific antibodies was performed with a LiquiChip Workstation (Qiagen) as previously described.[10] Briefly, antigen-coated microspheres were resuspended by vortexing and added to 96-well filter-bottom plates (MSBV N12 50; Millipore, Bedford, MA); test sera were added at a final dilution of 1:500 in 100 µl PBS-1% bovine serum albumin diluent (Sigma-Aldrich, St Louis, MO). Plates were covered and incubated for 60 min on an orbital shaker at 400 revolutions per min at room temperature in the dark. Fluid was removed using a plate vacuum manifold, each well was washed twice with diluent, the plate was shaken at 900 revolutions per min for 2 min, and then fluid was removed with the vacuum manifold. Microspheres were resuspended in 100 µl of diluent, and 10 µl of a 1:20 dilution of phycoerythrin-conjugated $F(ab')_2$ fragment goat anti-mouse immunoglobulin G (heavy plus light chains) secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was added to each well. To remove trapped beads, the plate was shaken another 2 min at 900 revolutions per min, covered, and then incubated for 60 min on an orbital shaker at 400 revolutions per min at room temperature in the dark. Plates were washed twice as described earlier, and microspheres were resuspended in 100 µl fresh diluent and 10 µl of a stop solution containing 2% (v/v) formalin. The microplate was shaken at 900 revolutions per min for 5 min and analyzed on the LiquiChip Workstation. Baseline values (rNS1, 125; MPV rVP2, 125) to discriminate negative and positive samples were determined as the mean plus 2 standard deviations of results obtained for 70 sera from mice known to be negative for murine parvovirus infection. Results are reported as the median fluorescence intensity of 100 antigen-coated microspheres. Sera were to be excluded if they exceeded baseline values for 2 of the 3 control antigens, although none of the sera evaluated exceeded the median fluorescence intensity baseline for any of the control antigens.

Results

MPV1 infections in SCID and BALB/c mice. A total of 15 SCID and 13 BALB/c mice were inoculated oronasally with 100 ID_{50} MPV1 within the first 24 h of birth. Mice were weaned at 4 wk of age into same-sex groups of 3 to 4 mice per cage (2 cages of each sex per strain, for a total of 8 cages). At 12 wk of age, 3 SCID mice and 1 BALB/c mouse were euthanized to confirm infection. High levels of MPV1 DNA were detected in the lymphoid tissues of all 3 SCID mice, suggesting that their infection was still productive (Table 1). The BALB/c mouse showed seroconversion to both rNS1 and MPV rVP2 antigen and had low levels of viral DNA in lymphoid tissues (mesenteric lymph node and spleen).

The remaining mice were separated into same sex non-breeding control groups (3 mice per cage, 1 cage per strain/sex) or paired with age-matched naïve breeding partners (1 infected mouse per cage, 3 cages of each sex per strain). All 6 infected female SCID mice died (mean age at death, 20 wk; range, 13 to 27 wk), with high levels of viral DNA detected in lymphoid tissues of 4 mice that were minimally autolyzed and necropsied. One of these mice was preparturient, with dystocia considered to be the likely cause of death. Two of the infected male SCID mice were euthanized at 25 and 31 wk of age due to poor clinical condition, with thymic lymphoma detected in both mice at necropsy. The remaining 4 infected male SCID mice were euthanized at 32 wk of age. High levels of MPV were detected in the lymphoid tissues of all infected male SCID mice. Productive infection in SCID mice was

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Table 2. Detection of MPV mRNA in tissues from SCID mice by RT-PCR

Tissue	12 wk PI	32 wk PI
Colon	3/3[a]	3/3
Mesenteric lymph node	3/3	3/3
Peripheral lymph node	3/3	3/3
Spleen	2/3	3/3
Thymus	3/3	3/3

PI, postinoculation with MPV1e.
[a]No. of mice positive/no. of mice tested.

corroborated by RT-PCR detection of MPV mRNA transcripts in multiple tissues harvested from several SCID mice at 12 and 32 wk of age (Table 2). We euthanized 12 infected BALB/c mice at 26 wk of age. Seroconversion to rNS1 and MPVrVP2 antigen was detected in all 12 mice by multiplex fluorescent immunoassay, and low levels of MPV DNA were detected in lymphoid tissue in 11 of the mice (Table 1).

Temporal analysis of fecal viral loads in MPV1-infected SCID and BALB/c mice. Fecal pellets were collected from each cage of infected mice weekly at cage change for 24 wk postinoculation, and fecal DNA extracts were analyzed by qPCR. qPCR results were translated into absolute DNA template copy number per reaction on the basis of a standard curve generated by linear regression analysis of 10-fold serial dilutions of cloned amplicon DNA. These results then were normalized to viral copy number per fecal pellet by multiplying by the dilution factors used during extraction and qPCR reaction set-up. During the first 4 wk of fecal pellet collection, the dam of the infected sucklings was also present in each cage.

Fecal viral loads in infected SCID mice were approximately 10^3 to 10^4 viral copies per fecal pellet at 1 wk postinoculation, increased to approximately 10^5 viral copies per fecal pellet during weeks 2 to 4, then remained steady after weaning at approximately 10^6 to 10^7 viral copies per fecal pellet until termination of the study at 24 wk (Figure 1). In contrast, the highest levels of fecal viral load were detected during the first 3 wk postinoculation in infected BALB/c mice (approximately 10^5 to 10^6 viral copies per fecal pellet), and then dropped to 10^3 viral copies per fecal pellet or less for the remainder of the study period.

Temporal analysis of MPV1 transmission to naïve weanling C3H sentinels. Biologic transmission of MPV1 was monitored by exposure of sentinel mice to undiluted soiled bedding. One naïve weanling C3H sentinel mouse was placed into each soiled cage on which infected SCID and BALB/c mice had been maintained the previous week. After 2 consecutive weeks of exposure, sentinels were placed onto clean bedding and maintained individually in cages for an additional 3 wk to allow time for seroconversion. New sentinel mice were placed every 2 wk. Infected SCID mice were monitored by sentinels for 18 wk postinoculation, whereas infected BALB/c mice were monitored for 22 wk postinoculation. Sentinel mice exposed to soiled bedding from infected male and female SCID mice showed seroconversion to both rNS1 and MPV rVP2 and high levels of MPV DNA in lymphoid tissues throughout the 18 wk of monitoring (Figure 2 A through C). In contrast, sentinel mice exposed to soiled bedding from infected BALB/c mice showed seroconversion to rNS1 and MPV rVP2 antigen and high levels of MPV DNA in lymphoid tissues only during the first 2 wk postinoculation. Although low levels of viral DNA could be intermittently detected in lymphoid tissues of sentinel mice

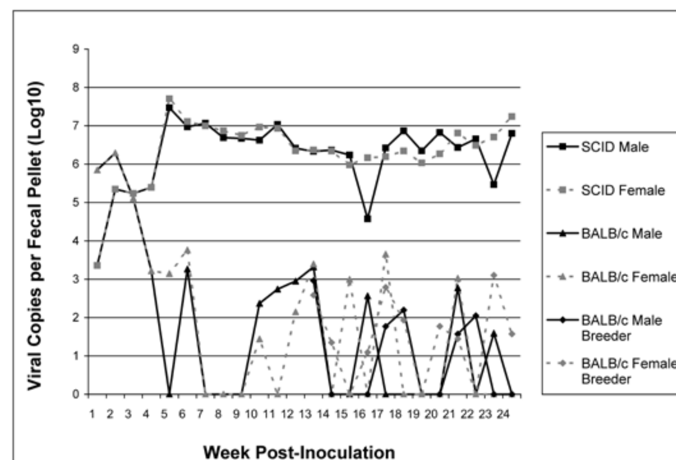

Figure 1. Mean MPV DNA copies per fecal pellet (expressed in log10 scale) over 24 wk postinoculation for SCID and BALB/c mice infected as neonates, as determined by qPCR.

exposed to infected BALB/c mouse bedding during weeks 3 to 22 postinoculation, seroconversion to rNS1 or MPV rVP2 was not detected in any of these sentinel mice.

Effect of pregnancy and lactation in infected BALB/c mice on MPV1 fecal viral load and transmission to naïve sentinel mice. Infected male and female BALB/c mice (n = 3 cages per sex) were paired with naïve 8-wk-old strain-matched mice of the opposite gender at 13 wk postinoculation to assess the effects of pregnancy and lactation on viral shedding and transmission. Male mice were separated from females once pregnancy was visually confirmed (approximately 2 wk into gestation) to prevent postpartum pregnancies that would complicate data interpretation. All mice delivered litters during weeks 16 and 17 postinoculation, with lactation occurring until 20 wk postinoculation. Fecal viral loads were not different among fecal pellets collected from cages of pregnant or lactating BALB/c mice as compared with control cages containing same sex groups of infected male and female BALB/c mice (Figure 1). In addition, seroconversion to rNS1 or MPV rVP2 was not detected in any of the sentinel mice maintained on soiled bedding obtained from the infected breeding mice throughout the time period of pregnancy and lactation. However, low levels of viral DNA were intermittently detected in lymphoid tissues obtained from sentinel mice exposed to the breeding mouse cages, as they were for sentinels exposed to control cages of sex-matched nonbreeding mice (Figure 2 A through C).

MPV1 transmission from infected mice to naïve breeding partners. Breeding pairs of infected SCID mice and naïve strain-matched breeding partners were established as described for BALB/c mice. At 26 wk postinoculation, the naïve breeding partners of all SCID and BALB/c mice were euthanized. High levels of viral DNA were detected in the lymphoid tissues of all naïve SCID mice paired with infected SCID mice (Table 1). In contrast, low levels of MPV1 DNA were detected in lymphoid tissues of few of the naïve BALB/c mice paired with infected BALB/c mice. Seroconversion to rNS1 and MPV rVP2 antigen did not occur in the naïve BALB/c breeding partners.

MPV1 transmission from infected mice to progeny. Five litters were produced from the 6 infected SCID mice, and 6 litters were produced from the 6 infected BALB/c mice. One infected pregnant female SCID mouse died due to dystocia during the immediate preparturient period. Average litter size was 5.8 pups for SCID

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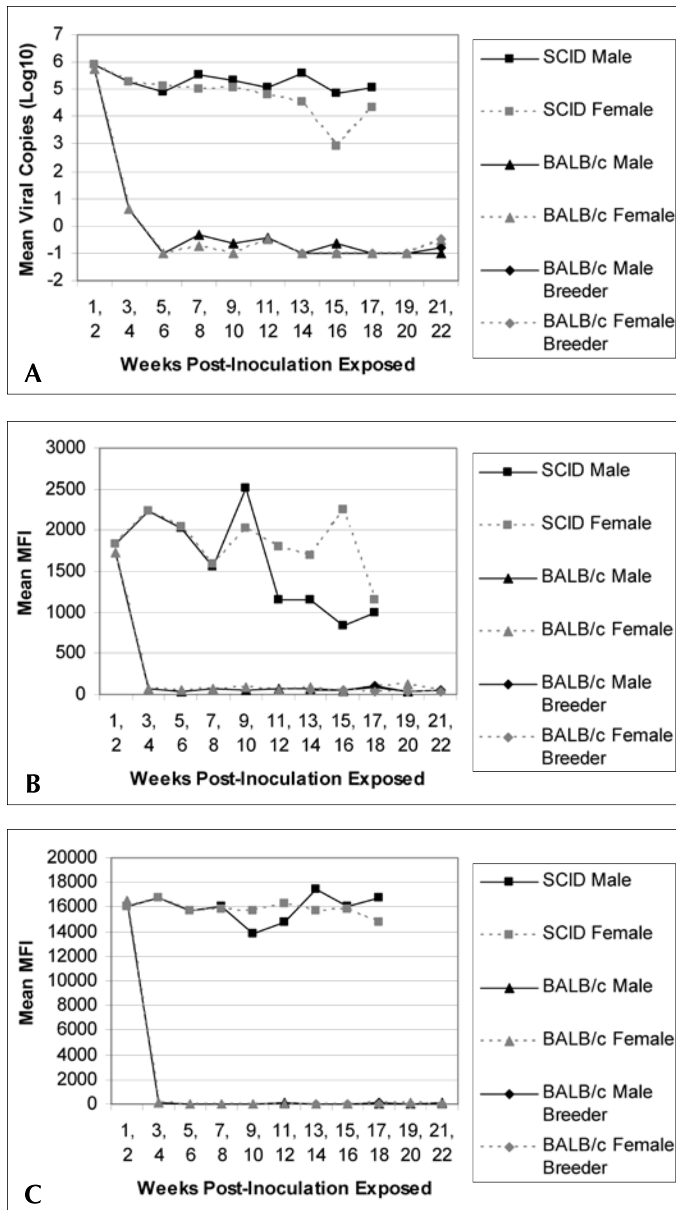

Figure 2. (A) Mean MPV DNA copies per 2 µl DNA extract (expressed in log10 scale) detected in lymphoid tissues (mesenteric lymph node and spleen) of C3H sentinels exposed for 2-wk periods to soiled bedding from MPV-infected SCID and BALB/c mice, as determined by qPCR. (B) Mean rNS1 serology results for C3H sentinels exposed for 2-wk periods to soiled bedding from MPV-infected SCID and BALB/c mice, as determined by multiplex fluorescent immunoassay. Positive multiplex fluorescent immunoassay results for rNS1 are those above 125 mfi. (C) Mean MPV rVP2 serology results for C3H sentinels exposed for 2-wk periods to soiled bedding from MPV-infected SCID and BALB/c mice, as determined by multiplex fluorescent immunoassay. Positive multiplex fluorescent immunoassay results for rVP2 are those above 125 mfi.

mice and 6.8 pups for BALB/c mice. Progeny of SCID mice were euthanized at 1, 3, and 6 wk of age. Among progeny of infected male SCID mice, intermediate levels of MPV DNA were detected in the lymphoid tissues of most progeny evaluated at 1 and 3 wk of age, whereas high levels of MPV DNA were detected in all progeny evaluated at 6 wk of age (Table 3). In contrast, high levels of MPV DNA were detected in the lymphoid tissues of all progeny of infected female SCID mice evaluated at 1 and 3 wk of age, whereas low levels of MPV DNA were detected in only 3 of 5 progeny at 6 wk of age. Only low levels of MPV DNA were detected in lymphoid tissues of the 3- and 6-wk-old progeny of infected BALB/c mice, with MPV DNA less likely to be detected in 6-wk-old progeny versus 3-wk-old progeny. All 3-wk-old progeny of infected female BALB/c mice were strongly seropositive to both rNS1 and MPV rVP2 antigen, whereas few of the 6-wk-old progeny were seropositive and in general displayed lower serologic signal for both antigens consistent with lower antibody levels. In contrast, all 3-wk-old progeny born from infected male BALB/c mice were seronegative for both rNS1 and MPV rVP2 antigen, whereas the majority of the 6-wk-old progeny were seropositive to both rNS1 and rVP2 antigen.

Discussion

Our studies use an MPV1 strain that was isolated recently from naturally infected mice and that has been propagated only by inoculation of mice. Genomic sequencing of the entire coding region of this isolate revealed 99.5% nucleotide sequence identity with MPV1c, a strain also sequenced directly from tissues of a naturally infected mouse from a different source. This recent isolate has been designated MPV1e, to maintain compliance with previously established taxonomy. Because MPV1e has been propagated only in mice and has not undergone genetic adaptation to cell culture, the strain is considered to be representative of naturally occurring ‘field strains’ and is an ideal isolate for studying the natural biology of transmission of this virus. In addition, recent molecular epidemiologic studies have demonstrated MPV1 is the most common murine parvovirus strain detected in laboratory mouse strains in the United States.[5] Therefore investigations with MPV1e apply to the vast majority of mouse colonies naturally infected with MPV.

A total of 15 neonatal SCID and 13 neonatal BALB/c mice were inoculated oronasally with MPV1e. High levels of MPV DNA that greatly surpassed the estimated number of virions present in the inoculum were detected in the mesenteric lymph node and spleen of all inoculated SCID mice. In addition, MPV transcripts were detected in the vast majority of tissues evaluated in multiple SCID mice euthanized at 12 and 32 wk postinoculation. Together these findings indicate that viral transcription and viral replication occurred and demonstrate that the inoculated SCID mice were productively infected. Strong antibody responses to both rNS1 and MPV rVP2 were detected in all BALB/c mice that were inoculated. A strong antibody response to rNS1 suggests productive infection because the response develops after virus entry and transcription in susceptible cell populations.[21] Altogether these results indicate that all inoculated SCID and BALB/c mice were productively infected and validate results from subsequent arms of this study that investigate transmission of MPV1 from inoculated mice.

Horizontal transmission was assessed temporally by quantitative analysis of feces of inoculated mice, biologic transmission of infectious virus via exposure of naïve weanling C3H sentinel mice to soiled bedding, and direct contact exposure of naïve strain-matched breeding partners. Fecal MPV loads in SCID mice were modest during the first week postinoculation, yet were high from week 2 postinoculation throughout the 24 wk study period. Biologic transmission through soiled bedding to naïve C3H sentinels correlated well with the fecal viral loads through 18 wk postinoculation. All sentinels exposed for 2 wk to soiled

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Table 3. qPCR and serology results for progeny of MPV1-infected SCID and BALB/c mice

Strain	Infected parent	Week PI	MLN+spleen qPCR	MLN+spleen mean MPV copies (log10)	rNS1 and MPV rVP2 serology	Mean rNS1 (mfi)	Mean rVP2 (mfi)
SCID	Male	1	11/11 [a]	2.9	ND	ND	ND
	Male	3	7/8	3.9	ND	ND	ND
	Male	6	10/10	7.5	ND	ND	ND
	Female	1	9/9	6.9	ND	ND	ND
	Female	3	6/6	6.9	ND	ND	ND
	Female	6	3/5	1.0	ND	ND	ND
BALB/c	Male	3	7/9	1.3	0/9 [a]	52 [c]	42 [c]
	Male	6	5/11	0.7	8/11[b]	137	1834
	Female	3	7/9	0.9	9/9	1049	9633
	Female	6	5/12	0.9	5/12	97	643

MLN, mesenteric lymph node; mfi, median fluorescence intensity; ND, not done; PI, postinoculation.
[a]No. of mice positive/No. of mice tested.
[b]Only 5 of 11 mice were seropositive to rNS1 antigen.
[c]Multiplex fluorescent immunoassay values > 125 mfi were considered positive for both the rNS1 and MPV rVP2 serology assays.

bedding from SCID mice developed strong antibody responses to both rNS1 and MPV rVP2, and high levels of MPV DNA were detected in the lymphoid tissues of these sentinels, regardless of when they were exposed postinoculation. Sentinel exposure to soiled bedding from inoculated SCID mice was stopped at 18 wk postinoculation due to the consistently high levels of fecal MPV DNA detected in inoculated SCID mice, consistent transmission from SCID mice to naïve sentinel mice, and the loss of multiple inoculated SCID female mice due to premature death. Successful transmission of MPV1 to all naïve breeding partners exposed for 2 to 3 wk by direct contact was evidenced by detection of high levels of MPV DNA in the lymphoid tissues of the naïve breeding partners. Altogether these data indicate that SCID mice inoculated as neonates persistently shed high levels of infectious virus that is transmitted well by both direct contact and by soiled bedding exposure.

In contrast, high levels of fecal MPV DNA were detected only during the first 3 wk after inoculation in inoculated neonatal BALB/c mice, followed by an approximate 3-log reduction in fecal MPV loads, with fluctuations between barely detectable and nondetectable fecal MPV DNA thereafter. Results in naïve C3H sentinel mice correspond well, with seroconversion to rNS1 and MPV rVP2 and high levels of MPV DNA detected in lymphoid tissues only in sentinel mice exposed during the first 2 wk postinoculation, when fecal MPV DNA loads were high in the inoculated BALB/c mice. These data are also consistent with data previously reported in which transmission to naïve sentinels from soiled bedding of infected outbred mice occurred predominantly within the first 3 wk postinoculation.[23] Naïve BALB/c breeding partners exposed by direct contact to inoculated mice during weeks 13 through 16 also did not seroconvert to rNS1 or MPV rVP2, although low levels of MPV DNA were detected in the lymphoid tissues of 3 of these 6 mice. Altogether these data indicate that MPV1 is acutely shed at high levels that are sufficient to productively infect naïve sentinel mice by soiled bedding exposure. Thereafter fecal MPV loads in inoculated BALB/c mice dropped to levels that are incapable of inducing productive infection in sentinel mice exposed by soiled bedding or to naïve adult mice exposed by direct contact for several weeks. However, low levels of MPV DNA were intermittently detected in lymphoid tissues obtained from naïve mice exposed to the infected BALB/c mice or their soiled bedding. Therefore, the adaptive immune response in immunocompetent mice appears to substantially reduce fecal shedding of MPV to levels that do not induce productive infection in naïve immunocompetent mice exposed by direct contact or soiled bedding, at least with regard to exposure periods of 2 to 3 wk.

We also evaluated the effects of pregnancy and lactation on shedding of MPV1 in infected BALB/c mice to determine whether the associated hormonal and physiologic changes increased viral shedding and subsequently enhanced productive infection in progeny. We did not evaluate SCID mice specifically for the effects of pregnancy and lactation because they consistently shed high levels of MPV1 that induced productive infection in cage mates and sentinel mice, but we did set up breeding pairs of SCID mice to evaluate the efficiency of MPV transmission to progeny. For both mouse strains, infected males were paired with naïve strain-matched females and infected females were paired with naïve strain-matched males to assess gender-specific tendencies. BALB/c mice showed no apparent differences in the fecal viral loads obtained during pregnancy and lactation, and biologic transmission to naïve weanling C3H sentinel mice did not occur during these time periods, irrespective of whether the male or female mouse was infected with MPV1. These results suggest that physiologic and hormonal changes associated with pregnancy and lactation do not alter fecal shedding of MPV1 in immunocompetent BALB/c mice.

Studies of generational transmission from infected sires and dams to progeny revealed that MPV DNA levels in progeny of infected male SCID mice increased from 1 through 6 wk of age, suggesting progressive MPV infection in these mice. In contrast, progeny of infected female SCID mice had high levels of MPV DNA in lymphoid tissues at 1 and 3 wk of age but markedly reduced levels at 6 wk of age. The low MPV levels detected at 6 wk of age were unexpected because the progeny were exposed continually to high levels of MPV1 from the persistently shedding infected female SCID mice throughout lactation until the pups were weaned at 4 wk of age. Other studies performed in our laboratory demonstrate high and progressively increasing levels of viral DNA in lymphoid tissues collected from MPV1b-inoculated neonatal SCID mice at 1, 2, 4, and 8 wk postinoculation.[3] We therefore cannot explain why we did not obtain similar results for 6-wk-old progeny exposed

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to high levels of MPV1e from infected female SCID mice. Further studies of this phenomenon will need to be pursued.

Progeny of infected BALB/c male mice were all seronegative at 3 wk of age, whereas 8 of 11 progeny were seropositive by 6 wk of age. Low levels of DNA were detected in lymphoid tissues of approximately half of these progeny. These findings indicate that detectable maternal antibody was not present at 3 wk of age, consistent with lack of seroconversion of the naïve BALB/c dams, despite viral exposure, as evidenced by low levels of MPV DNA in the lymphoid tissues of 7 of the 9 progeny. Because the infected BALB/c males were removed from the cages prior to parturition, the presence of virus that resulted in exposure could have been residual virus left in the cage from the infected male or virus that was 'recycled' through ingestion and subsequent excretion by the naïve female or due to low-level virus replication in the naïve female that was insufficient to induce seroconversion. Regardless, seroconversion of multiple 6-wk-old progeny to rNS1 and MPV rVP2 suggests that productive infection occurred in some (but not all) of the progeny of infected male mice. In contrast, all progeny of infected female BALB/c mice displayed high antibody levels to both rNS1 and MPV rVP2 at 3 wk of age, consistent with transfer of maternal antibody from the infected BALB/c dams, which were all strongly seropositive. By 6 wk of age, slightly less than half of the progeny were seropositive, with low to modest levels of antibody detected to both rNS1 and MPV rVP2. All of the 6-wk-old seropositive progeny were from the same litter, whereas the 6-wk-old mice evaluated from the other 2 litters were all seronegative. Low MPV DNA levels in lymphoid tissues were detected intermittently in all progeny of infected BALB/c mice and did not correlate well with seropositivity. In other words, MPV DNA was detected in some (but not all) seropositive progeny and in some seronegative progeny. The high levels of maternal antibody present in the progeny of infected BALB/c females at 3 wk of age complicate the determination of whether the antibodies detected in some of the progeny at 6 wk of age reflects residual maternal antibody or antibodies produced by the progeny secondary to virus replication.

In conclusion, these studies illustrate some of the complexities associated with MPV1 transmission to sentinels, naïve cagemates, and progeny that make detection of MPV in enzootically infected colonies problematic. In particular, the adaptive immune response appears to substantially suppress (but not completely eliminate) MPV1 viral shedding in the feces of immunocompetent BALB/c mice. This results in biologic transmission to weanling sentinel mice only during the acute stages of infection in BALB/c mice when fecal viral loads exceed the exposure dose required for productive infection. In contrast, immunodeficient SCID mice persistently shed high levels of virus that consistently result in biologic transmission to cage mates and immunocompetent sentinels. Pregnancy and lactation do not appear to influence viral shedding markedly in latently infected immunocompetent BALB/c mice. However, levels of virus sufficient to induce productive infection in at least some of the BALB/c progeny indicates generational transmission does occur horizontally in this immunocompetent strain, perhaps because younger mice display higher susceptibility to MPV1 infection.[6] Transmission to BALB/c progeny likely was complicated by transfer of maternal antibodies and exposure to MPV1 levels that were at best barely sufficient to induce productive infection and seroconversion. As a result, although the diagnostics used for MPV detection have improved substantially during the past decade and have improved identification of infected mice, aspects of transmission that reflect age susceptibility and adequacy of viral dose exposure will remain problematic with regard to sentinel mouse detection systems. This drawback is of particular importance if MPV exposure via soiled bedding is diluted considerably by mixing soiled bedding from cages containing uninfected mice or MPV-seropositive mice that are not shedding high levels of MPV. Further studies are warranted to elucidate other potential triggers that might increase viral shedding in MPV-infected immunocompetent mice, in particular immunosuppression, given the persistently high levels of MPV shedding observed in the immunodeficient SCID mice in these studies.

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