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Original Research

A Comparison of Mouse Parvovirus 1 Infection in BALB/c and C57BL/6 Mice: Susceptibility, Replication, Shedding, and Seroconversion

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This study characterized the effects of challenge with a field isolate of mouse parvovirus 1 (MPV1e) in C57BL/6NCrl (B6) and BALB/cAnNCrl (C) mice. We found that C mice were more susceptible to MPV1e infection than were B6 mice; ID_{50} were 50 to 100 times higher after gavage and 10-fold higher after intraperitoneal injection in B6 as compared with C mice. To evaluate the host strain effect on the pathogenesis of MPV1e, B6 and C mice were inoculated by gavage. Feces and tissues, including mesenteric lymph nodes (MLN), ileum, spleen and blood, were collected for analysis by quantitative PCR (qPCR) to assess infection and fecal shedding and by RT-qPCR to evaluate replication. Peak levels of MPV1e shedding, infection, and replication were on average 3.4, 4.3, and 6.2 times higher, respectively, in C than in B6 mice. Peaks occurred between 3 and 10 d after inoculation in C mice but between 5 and 14 d in B6 mice. Multiplexed fluorometric immunoassays detected seroconversion in 2 of 3 C mice at 7 d after inoculation and in all 3 B6 mice at 10 d. By 56 d after inoculation, viral replication was no longer detectable, and fecal shedding was very low; infection persisted in ileum, spleen, and MLN, with levels higher in C than B6 mice and highest in MLN. Therefore, the lower susceptibility of B6 mice, as compared with C mice, to MPV1e infection was associated with lower levels of infection, replication, and shedding and delayed seroconversion.

Abbreviations: B6, C57BL/6; C, BALB/c; MFI, median fluorescence intensity; MFIA, multiplexed fluorometric immunoassay; MLN, mesenteric lymph node; MMV, mouse minute virus; MPV, mouse parvovirus; NS1, nonstructural protein 1; qPCR, quantitative PCR; r, recombinant; Rn, normalized reporter value; VP2, virus capsid protein 2.

Parvoviruses are small (20 to 28 nm), nonenveloped icosahedral single-stranded DNA viruses that infect a diverse range of vertebrate and arthropod species. Much of what is understood about the biology and pathogenesis of autonomous parvoviruses has been derived from studies of the original murine parvoviral isolates, particularly the prototypic and immunosuppressive strains of mouse minute virus (MMV).[9,13,32] Because autonomous parvoviruses have a requirement and predilection for proliferating cells to replicate, they are primarily teratogenic pathogens. In contrast, rodent parvovirus infections of older animals are usually asymptomatic, because the cells that divide in mature animals, such as enterocytes, lymphoreticular cells, and hematopoietic cells, are largely spared.[2,47,48] The most common parvovirus of laboratory mice, mouse parvovirus 1 (MPV1), was first isolated[29] from mouse T-lymphocyte cultures that had lost viability or the ability to proliferate when stimulated. In contrast to MMV,[10,27,40] MPV1 has not been shown to cause disease in newborn or immunodeficient mice[19,45] but nevertheless has been reported to modulate the immune response of infected mice.[30,31]

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Adventitious infections of laboratory mice with MPV1 and other parvoviruses continue to occur regularly, despite biosecurity improvements that have successfully excluded once-common pathogens such as Sendai virus.[22,37,39] One reason for the continued occurrence of these infections is that nonenveloped parvovirus virions are environmentally stable and resistant to disinfection.[18,49] Furthermore, related to their tendency to persist in host tissues even after seroconversion and their predilection for dividing cells, parvoviruses have been among the most frequent viral contaminants of transplantable tumor lines and other rodent-derived biologic reagents.[34,35] Inoculation of parvovirus-contaminated biologic reagents into experimental animals can contribute to the incidence of parvoviral outbreaks. Currently, mouse populations typically are housed in microisolation cages and are monitored for MPV1 infections through the use of soiled bedding sentinels. An MPV1 infection of the principal animals may not be transmitted to sentinels when the prevalence of infection is low, as is often the case after contamination, or when the sentinels are comparatively resistant to infection because of their genetic background or age.[7,16,17] However, a recent study found that sentinel age did not affect the likelihood of MPV1 infection.[17]

The C57BL/6 (B6) mouse strain is popular in biomedical research and is commonly used as the background strain for spon-

taneous and genetically engineered mutations. We and others have noted that B6 mice are less likely to be MPV1 seropositive than are mice of other strains and stocks, even in facilities where MPV1 is widespread.[44] There has been speculation that B6 mice might not seroconvert when infected with MPV1. However, data reported here and by others[7,15] indicate that B6 mice are less likely to seroconvert because they are comparatively resistant to MPV1 infection; when they become infected, they do seroconvert. The current study evaluated whether resistance of B6 mice to infection with MPV1, as compared with BALB/c (C) mice, varies with virus inoculation route and correlates with differences in the time course and levels of viral infection, replication, and shedding and of humoral immunity.

Most studies of MPV1 in mice have been performed with the cultivable MPV1a strain.[7,19,30,31,45] Cultivable murine parvoviruses are known to differ from wildtype strains genetically and in their cell tropisms, pathogenicity, and transmissibility in vivo. For example, MPV1d, a noncultivable field isolate, was more readily transmitted to sentinels than was MPV1a.[11] We therefore chose to perform the current experiments with MPV1e,[3,4] a representative field strain that we originally isolated from an adventitiously infected barrier colony[44] and that has been propagated only in mice.

Materials and Methods

Virus. MPV1e (NCBI Tax ID, 402802) was isolated from barrier-reared SPF mice adventitiously infected with MPV1 but no other pathogens.[44] The DNA sequence for the genome of MPV1e, which has been submitted to GenBank and assigned accession number DQ898166i, is most closely related to that of the MPV1c field strain.[3,5] To prepare the study inoculum, 4-wk-old naïve mice were orally and parenterally injected with the original MPV1e isolate that had been passaged only in mice; spleens and lymph nodes collected from the mice at 4 d after inoculation were homogenized in cell culture medium to a concentration of approximately10% w/v. Aliquots of the inoculum were stored in liquid nitrogen. The inoculum was shown to be sterile and to be free of extraneous rodent viruses and mycoplasma by PCR analysis and mouse antibody product (MAP) testing[8,42] and to contain $10^{7.6}$ MPV1 genome copies/mL according to qualitative PCR (qPCR) analysis.

Mice. Female BALB/cAnNCrl and C57BL/6NCrl mice (age, 3 to 5 wk) were obtained from Charles River colonies (Kingston, NY, and Raleigh, NC, respectively) demonstrated to be free of Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, mouse parvovirus, Theiler mouse encephalomyelitis virus, reovirus type 3, mouse adenovirus, polyoma virus, K virus, mouse cytomegalovirus, group A rotavirus (epizootic diarrhea of infant mice virus), mouse thymic virus, lymphocytic choriomeningitis virus, Hantaan virus, lactate dehydrogenase elevating virus, ectromelia, *Mycoplasma pulmonis*, *Helicobacter* spp., *Salmonella* spp., *Pasteurella* spp. (including *P. multocida* and *P. pneumotropica*), cilia-associated respiratory bacillus, *Corynebacterium kutscheri*, *Citrobacter rodentium*, *Clostridium piliforme*, *Streptobacillus moniliformis*, *Streptococcus pneumoniae*, and endo- and ectoparasites. Mice were housed in individually ventilated cages on woodchip bedding (Beta Chip, NEPCO, Warrensburg, NY). Cages were cleaned and sterilized once weekly. Environmental conditions were maintained at 70 ± 3 °F (21 ± 2 °C) with 50% ± 20% relative humidity and 15 room air-changes hourly. Animals were kept on a 12:12-h light:dark cycle and provided ad libitum access to water and feed (Lab Diet 5K52 or Lab Diet 5L79, Purina Mills, Richmond, IN). All procedures were approved by the Charles River IACUC, and all Charles River North American sites are AAALAC accredited.

MPV PCR. Two fluorogenic 5′endonuclease *Taq*Man PCR assays (Life Technologies, Carlsbad, CA) were used in this study, one targeting viral genomic DNA and the other viral mRNA. The template for the genomic assay was a region of the nonstructural NS1 gene conserved among parvoviruses of mice. The template for the RT–PCR analysis included an intron junction sequence unique to the MPV1 mRNA transcripts R1, R2, and R3, which are translated into NS1, NS2, and viral coat (VP) proteins, respectively.[25] The primers and probes for these assays were synthesized by Sigma–Aldrich (St Louis, MO) and Applied Biosystems (Foster City, CA), respectively. The forward and reverse primers for the MPV mRNA RT–PCR assay, designated MPVJ2236F and MPVJ2409R (Figure 1), are specific to MPV sequences before and after the intron junction. The MGB probe (5′ FAM–TCAGGCCT-TAGTCCAA–BHQ1 3′), designated MPVJ2386P, traverses the intron junction region and therefore does not anneal to intact viral genomic DNA.

In the infectious dose titrations of the MPV1e inoculum, the MPV NS1 PCR analysis was performed as an endpoint assay for infection of study mouse mesenteric lymph nodes (MLN). Total nucleic acid (that is, RNA and DNA), was isolated from MLN by using RNeasy 96 Kit (Qiagen, Valencia, CA) as follows. Approximately 15 mg MLN was placed in 650 µL of kit lysis buffer and homogenized by using a mixer mill (Laboratory Vibration Mill Type MM 300, Qiagen) at 25 Hz for 2 min. According to the RNeasy Kit instructions, ethanol was added to the homogenate, but to promote binding of DNA as well as RNA, 100% ethanol was substituted for the 70% ethanol specified in the instructions. Homogenates were applied to RNeasy 96 plate wells, which then were washed several times with the kit wash buffer to remove contaminants. Total nucleic acid was eluted from each well by using 100 µL molecular-grade water (VWR, Suwanee, GA) treated with diethylpyrocarbonate. Eluate containing total nucleic acid (5 µL) was added to each of 2 PCR plate wells prefilled with 25 µL of a 1× *Taq*Man Universal PCR Master mix (Applied Biosystems) supplemented with 0.01% Tween 20 (Sigma–Aldrich) and 0.05% gelatin (Sigma–Aldrich). The master mix in one well contained the MPV NS1 primers and probe; master mix in the other contained primers and a probe targeting the luciferase gene and 100 copies of a plasmid construct with a luciferase gene insertion (pGEM-luc Promega, Madison, WI). Amplification of the luciferase gene indicated that the sample did not contain PCR inhibitors and therefore was suitable for testing. Each PCR run included triplicate tests of a negative template control and a positive template control to verify assay performance and to demonstrate that the assay reagents were not contaminated with template. The negative template control was DNA extracted from uninfected Chinese hamster ovary cells; the positive template control was 100 copies of the MPV1a pV1 plasmid (obtained from Lisa Ball-Goodrich, Section of Comparative Medicine, School of Medicine, Yale University).

Reactions were thermocycled (model 9700, Applied Biosystems) by using the following parameters: 1 cycle of 50 °C for 2 min and 95 °C for 12 min; 5 cycles of 95 °C for 15 s and 64 °C for 1 min; and 55 cycles of 95 °C for 15 s and 58 °C for 1 min. The fluorescence signals in each test well for the reporter dye FAM

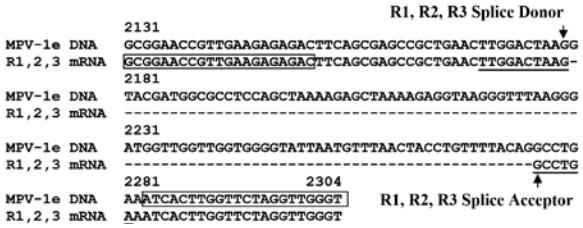

Figure 1. Location of MPV1 mRNA RT-qPCR assay targets within genomic DNA and mRNA. The sequences for the forward primer (MPVJ2236F) and reverse primer (MPVJ2409R), which are found in both viral DNA and mRNA, are outlined. The sequence (underlined) for the MGB probe (MPVJ2386P; 5′ FAM–TCA GGC CTT AGT CCA A–BHQ1 3′) location traverses the R1, R2, and R3 transcript splice donor and acceptor sites (arrows). The dashed line indicates the intron excised from the mRNA.

and reference dye ROX were read by using a fluorometer (Ascent-Fluoroskan, Thermo Fisher, Waltham, MA). The FAM signal was divided by the ROX signal to calculate a normalized reporter (Rn) value. A sample reaction was classified as positive when the sample net Rn (sample Rn – negative template control Rn) was greater than or equal to 20% of the difference between the positive and negative control values (positive template control Rn – negative template control Rn).

In the experiment comparing the courses of MPV1e infection in C and B6 mice, the viral genomic DNA and mRNA assays were performed as real-time qPCR assays to ascertain the levels of viral infection in tissues and shedding in feces and of virus replication, respectively. Homogenates of approximately 30 mg tissue in 1200 µL of kit lysis buffer were prepared as described for MLN. Each homogenate was divided into 2 equal aliquots. RNA was isolated from one aliquot by inclusion of a DNase digestion step in the isolation protocol. The second aliquot was processed without the DNase step, for isolation of total nucleic acid. After isolation, RNA from the DNase-treated nucleic acid was reverse-transcribed to cDNA by the addition of 5 µL RNA eluate to 25 µL of GeneAmp RT Master Mix (Applied Biosystems) prepared with 1mM mixed dNTP, according to the manufacturer's instructions for specific priming of the forward PCR primer. The RT incubation parameters were 25 °C for 10 min, 48 °C for 30 min, and 95 °C for 5 min.

DNA was extracted from feces by homogenizing approximately 75 mg feces in 250 µL PBS, pH 7.2 (Invitrogen, Grand Island, NY) and centrifuging the fecal slurry at 2400 × *g* for 2 min. DNA extracted from 100 µL of supernatant by using MagAttract reagents (Qiagen) on a Kingfisher96 processor (Thermo Scientific, Waltham, MA) was eluted in 200 µL of molecular-grade water. As in the endpoint MPV NS1 PCR assay protocol, controls for the qPCR analysis included assaying all samples for inhibitors by using the luciferase PCR assay and testing of positive and negative template controls in triplicate to verify that assay performance was satisfactory. In addition, a sample of RNA that had not undergone RT was assayed by the MPV NS1 qPCR protocol to demonstrate the effectiveness of the DNase treatment. The qPCR reaction components and thermocycler parameters were the same as those already described, except that the cycling was performed on an real-time PCR instrument (ABI 7300, Applied Biosystems). Ten-fold serial dilutions of plasmid standards, which contained the assay target sequences, were used in triplicate wells for analysis by the ABI 7300 software to determine template copy numbers, which were normalized to copies per mg of tissue or feces.

MPV serology. Parvovirus recombinant viral protein antigens were developed and expressed in the Baculovirus Expression Vector System with Gateway Technology (Invitrogen, Carlsbad, CA). The recombinant (r) genes expressed included rVP2 from MMVp (VR663, ATCC, Bethesda, MD), rVP2 and rNS1 from the MPV1a plasmid clone pV1,[1] and rVP2 from MPV2a identified in the MLN of a naturally infected mouse. Seeds were prepared from plaque-purified recombinant baculovirus clones propagated in monolayer cultures of the *Spodoptera frugiperda* Sf9 insect cell line (Invitrogen). The orientations, sizes, and sequences of the recombinant genes were confirmed by agarose-gel electrophoresis of intact and restriction endonuclease-digested PCR products and by comparing gene sequences with those in GenBank. To produce a recombinant protein antigen, a suspension culture of the ExpresSF+ cell line (Protein Sciences, Meriden, CT) containing approximately 2×10^6 cells/mL was inoculated with 0.1 to 5 pfu per cell of recombinant baculovirus. SF+ cells were propagated in Insect-Xpress protein-free medium with L-glutamine (Cambrex, East Rutherford, NJ), 0.25 µg/mL amphotericin B (Fungizone, Invitrogen), and 50 µg/mL gentamicin sulfate (Cambrex). The inoculated cultures were harvested after incubation at 27 ± 2 °C with continuous mixing for 3 to 5 d, by which time SF+ cell viability had dropped from 90% or higher to 50% to 80%. Cell pellets were lysed with 1% (w/v) CHAPS (Sigma–Aldrich) to extract the recombinant viral protein. The lysate was clarified by centrifugation at approximately 3700 × *g*. The rVP2 proteins formed virus-like particles that were purified by differential or density gradient ultracentrifugation and further detergent treatments. The rNS1 was expressed as His_6-tagged fusion protein that was purified from the cell lysate by Ni-chelating affinity chromatography described elsewhere.[41] To verify antigenic purity and potency, recombinant proteins were used as antigens in ELISA or multiplexed fluorometric immunoassays (MFIA) of standard serum panels to demonstrate that they reacted positively only with the appropriate antisera (data not shown). A detergent extract of wild-type baculovirus-infected SF+ cells was used as a sample-suitability (that is, tissue) control to detect nonspecific binding in test serum antibodies.

ELISAs were performed in 96-well polystyrene microplates (Immulon 4, Thermo Scientific). To coat plates, 50 µL of antigen or tissue control diluted in 0.1 M sodium carbonate–bicarbonate buffer, pH 9.6, with 0.5% w/v ovalbumin was dispensed per well and the plates were incubated overnight; rVP2 plates were held at room temperature, whereas the rNS1 plates were refrigerated. After being washed several times, rVP2- and rNS1-coated plates were stored at –20 °C and –70 °C, respectively. Before being tested, serum samples were diluted 60-fold in PBS, pH 7.4 (Sigma-Aldrich) containing 5% w/v nonfat dry milk[24] and 15% v/v FBS; 50 µL of each diluted test or control serum was added to each of 2 adjacent wells, one coated with antigen and the other with the tissue control. After being incubated at 39 ± 2 °C for 40 min, plate wells were washed 4 to 5 times with a 0.9% NaCl solution in an automatic 96-well plate washer (Biotek, Winooski, VT). Then 50 µL of horseradish peroxidase-conjugated goat IgG antimouse and -rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted in a 15% v/v solution of FBS in PBS were added to every well. Plates were incubated and washed as just described and then incubated for 40 min at room temperature with 100 µL

per well of ABTS peroxidase substrate (KPL, Gaithersburg, MD). After 25 µL of 1% SDS stop solution was dispensed per well, the optical density of the substrate color development at 405 nm was determined by using a 96-well plate reader (Molecular Devices, Sunnyvale, CA). Absorbance values were converted to net scores by using the formula

$$\text{Score}_{\text{net}} = \left(\text{OD}_{\text{antigen}} - \text{OD}_{\text{tissue control}}\right) / 0.13$$

A net score of 2.5 or greater was classified as positive provided that the tissue control score (OD_{405} of tissue control well / 0.13) was less than 2; however, a positive net score was classified as nonspecific when the tissue control score was 2 or greater.

MFIA were developed by using xMAP suspension microarray technology (Luminex, Austin, TX). Approximately 200 µg of rVP2 or 10 µg of rNS1 protein antigen were covalently coupled per 50 million carboxylated polystyrene microspheres by using the 2-step carbodiimide method recommended by Luminex.[28] The selectivity and sensitivity of each coupled bead lot was qualified by testing a panel of standard mouse and rat immune and SPF sera. Coupled beads were stored individually or in panels at 5 ± 3 °C in PBS with 1% w/w BSA (Sigma-Aldrich), 0.02% v/v Tween 20, and 0.05% w/v NaN_3. MFIA were performed in 96-well 1.2 µm filter-bottom plates (Millipore, Bedford, MA); 50 µL of a suspension of assay and control microbead sets, containing 2500 microbeads per set, and an equal volume of serum diluted 25-fold in PBS containing 5% v/v FBS and 0.05% v/v Proclin 300 preservative (Sigma-Aldrich) were added to a plate well and incubated together for 60 min. This and subsequent incubations were completed at 27 ± 2 °C in darkness, with orbital mixing at approximately 500 rpm. After the serum incubation, microbeads were washed 2 times by the addition and evacuation (through the well filter bottom) of PBS containing 1% BSA and 0.05% v/v Proclin (that is, wash buffer). Mouse antibodies bound to the microbeads were detected by consecutive 30 min incubations with 100 µL per well of biotinylated goat IgG antimouse and antirat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) and of streptavidin–phycoerythrin conjugate (Invitrogen), with wash steps after each incubation. After the final wash was aspirated, 100 µL of wash buffer was added to each well, and the beads were resuspended by orbital mixing. A BioPlex Suspension Array Reader System (Bio-Rad Laboratories, Hercules, CA) reported the median fluorescence intensity (MFI) for each assay and control bead set. MFI readings were converted to scores using the following formulas:

$$\text{MFI}_{\text{net}} = \text{MFI}_{\text{antigen}} - \text{MFI}_{\text{tissue control}}$$

$$\text{If } \text{MFI}_{\text{net}} \le \text{MFI}_{\text{cutoff}}, \text{ then net score} = \left(\text{MFI}_{\text{net}} / \text{MFI}_{\text{cutoff}}\right) \times 3$$

$$\text{If } \text{MFI}_{\text{net}} > \text{cutoff}, \text{ then net score} = \left[\left(\text{MFI}_{\text{net}} - \text{MFI}_{\text{cutoff}}\right) / 1000\right] + 3$$

Thus, a net MFI equal to the cutoff MFI, which was 3000 for the assays reported in this study, always has a net score of 3. A net score of 3 or greater indicated a positive result, provided that the tissue control test for the sample did not exceed 2.

ID_{50} titration of the MPV1e standard inoculum in B6 and C mice. Five serial 0.5 or 1.0 $\log_{10}$ dilutions of the standard of MPV1e inoculum were made in HBSS, and 100 µL of each inoculum dilution was administered to each of 4 mice by gavage or intraperitoneal injection (with the exception that only 3 mice were inoculated intraperitoneally with the 10^{-1} dilution); 4 control mice were inoculated per strain with HBSS. Four weeks postinoculation, animals were euthanized by CO_2 inhalation. Sera derived from blood samples collected by cardiocentesis were diluted 5-fold in PBS and stored at –20 °C or lower until they were tested for MPV antibodies by ELISA. MLN were stored at –70 °C or lower; the MPV NS1 PCR was performed on DNA extracted from MLNs. By using either the ELISA or PCR results, the ID_{50} for each mouse strain was calculated according to the Reed–Muench method.[38]

Course of MPV1e infection and humoral immunity in B6 and C mice. Each of 27 mice per strain was given approximately 100 µL (B6, approximately 1000 C-mouse ID_{50}; C, approximately 100 C-mouse ID_{50}) of the MPV1e inoculum by oral gavage. Five additional mice of each strain were gavaged with HBSS diluent to serve as negative controls. Feces were collected antemortem from 6 MPV1e-inoculated mice of each strain at 1 and 2 d after inoculation. Three mice of each strain were euthanized by CO_2 inhalation at 3, 5, 7, 10, 14, 21, 28, 42, and 56 d after inoculation. The mice sampled antemortem on days 1 and 2 after inoculation were again sampled postmortem on days 42 and 56 after inoculation. Blood, fecal pellets, MLN, spleen, and ileum were collected aseptically from each euthanized mouse. Serum harvested from the clotted blood was diluted 5-fold in PBS. Fecal pellets, MLN, spleen, and, ileum were aseptically transferred to individual tubes containing RNALater (Life Technologies). All samples were stored at –20 °C until analysis.

Statistical analysis Corresponding qPCR and RT-qPCR results for the B6 and C mouse tissues were compared by ANOVA; comparison of B6 and C fecal qPCR results during the acute phase of infection was done by using the Student *t* test. Multiple comparisons of specimen effects were done by using the Tukey test. All calculations were performed in R software (http://www.r-project.org/). A *P* value less than 0.05 was considered to be statistically significant.

Results

ID_{50} titrations of an MPV1e inoculum in C and B6 mice. Mice of each strain received serial dilutions of a standard MPV1e inoculum by gavage (to simulate natural infection) or by intraperitoneal injection to determine the effect of inoculation route on susceptibility to infection. MPV ELISA and PCR analyses were performed on serum and MLN specimens collected at 28 d after inoculation. After oral gavage, the MPV ELISA- and PCR-determined ID_{50} were both $10^{3.2}$ in C mice and were $10^{1.2}$ and $10^{1.5}$, respectively, in B6 mice (Table 1). The viral DNA copy number was determined by qPCR assay to be approximately 10^8 copies/mL, corresponding to 3000 DNA copies/ID_{50} by gavage in C mice and 190,000 copies/ID_{50} by gavage in B6 mice.

Compared with oral gavage ID_{50}, those after intraperitoneal injection were approximately 70% higher in C mice and slightly lower in B6 mice. Therefore, the inoculum ID_{50} were 50- to 100-fold higher after gavage and 10-fold higher after intraperitoneal injection in C compared with B6 mice. Control mice inoculated

Table 1. Effect of inoculation route on ID_{50} of MPV1e inoculum in C and B6 mice

		Log_{10} ID_{50}/100 µL	
		ELISA	PCR
Gavage	C mice	3.2	3.2
	B6 mice	1.2	1.5
	Difference	2.0	1.7
Intraperitoneal	C mice	2.7	2.7
	B6 mice	1.7	1.7
	Difference	1.0	1.0

MPV ELISA and PCR were performed on serum and mesenteric lymph node specimens collected 4 wk after inoculation; control mice inoculated with HBSS were virus-negative by ELISA and PCR analysis (data not shown).

Five doses were tested per strain and inoculation route with 4 mice per dose ($n = 20$), except for the B6 intraperitoneal titration, for which the 10^1 dose was injected into 3 mice ($n = 19$). In each titration, 4 control mice of each strain were inoculated with HBSS; these mice remained negative by PCR analysis and ELISA (data not shown).

Table 2. Efficacy of DNAse digestion of total nucleic acid extracted from MPV1e-infected tissues prior to viral mRNA RT-qPCR

			Average log_{10} copies/mg[a]		
	Tissue	*n*	Before DNAse	After DNAse	% Reduction ± CV
C mice	Ileum	12	5.9	3.0	99.9% ± 0.1%
	MLN	12	6.1	3.3	100.0% ± 1.2%
	Spleen	12	5.5	3.7	99.6% ± 1.5%
	All	36	5.8	3.3	99.8% ± 1.2%
B6 mice	Ileum	12	5.2	2.2	99.9% ± 0.2%
	MLN	12	5.5	2.6	99.9% ± 0.4%
	Spleen	12	4.8	2.5	99.4% ± 8.5%
	All	36	5.2	2.4	99.7% ± 5.2%
All	All	72	5.5	2.9	99.8% ± 3.8%

The probe used is complementary to a sequence that traverses the splice junction region and therefore is found only in viral mRNA. The assay primers, however, anneal to sequences found in viral DNA as well as mRNA. DNAse digestion was used to reduce competition for the primers by viral DNA.

[a]For specimens collected from 3 mice per strain at 7, 10, 14, and 21 d after inoculation.

with HBSS were MPV-negative by ELISA and PCR analysis (data not shown).

In these titrations, various doses of MPV1e were administered to a total of 79 mice (C, 40; B6, 39). Results from ELISA and PCR analysis agreed for 78 (99%) of the 79 mice, including 45 that were MPV-positive and 33 that were MPV-negative by both tests. The single discrepancy was for a gavage-inoculated B6 mouse that was virus-positive by PCR but ELISA-negative. This nearly perfect agreement demonstrates that MPV1e infection consistently elicited a humoral immune response in B6 as well as C mice.

Course of MPV1e infection and humoral immunity in B6 and C mice. To investigate the correlation between susceptibility to the virus and the course of infection, B6 and C mice were inoculated by gavage with approximately 1000 and 100 C-mouse ID_{50} MPV1e, respectively. Feces were collected antemortem from 6 mice per strain at 1 and 2 d after inoculation. MLN, ileum, spleen, feces, and blood were collected postmortem from 3 mice per strain at each of 9 time points between 3 and 56 d after inoculation. qPCR analysis was used to determine the amount of MPV1e genomic DNA in tissues and feces, to demonstrate the levels of viral infection and shedding, respectively. RT-qPCR was used to measure MPV1e mRNA in tissues to evaluate the amount of viral replication. All results were normalized to number of nucleic acid copies per gram of tissue or feces. To minimize competition from viral DNA for the primers used to amplify viral mRNA-derived cDNA, a DNase digestion step was included in the total nucleic isolation protocol, prior to RT-PCR analysis. DNase treatment of total nucleic acid extracted from 72 infected tissue specimens collected from B6 and C mice between 7 and 21 d after inoculation reduced the average number of MPV DNA copies per gram of specimen by 99.8% ± 3.8%, from $10^{5.5}$ to $10^{2.9}$ (Table 2). The average reduction for every mouse strain–tissue combination exceeded 99%.

MPV1e infection, replication, and fecal shedding reached their peaks in C mice between 3 and 10 d after inoculation and in B6 mice between 5 and 14 d after inoculation (Figure 2). Table 3 contains the averages of the 9 highest qPCR and RT-qPCR template concentrations per specimen and mouse strain between 3 and 14 d after inoculation. Peak values were chosen by rank rather than time after inoculation to accommodate variations by measurement, specimen-type, and mouse strain. Peaks of MPV1e shedding, infection, and replication were on average 3.4 ($P = 0.6$, Student t test), 4.3 ($P < 0.001$, ANOVA), and 6.2 ($P < 0.001$, ANOVA) times higher in C than in B6 mice, respectively. C:B6 ratios of infection and replication were highest in spleen and lowest in MLN ($P < 0.001$, Tukey). The peaks of shedding, infection, and replication occurred at a mean of 5.8, 7.8, and 5.3 days after inoculation, respectively, in C mice and 2 to 3 d later in B6 mice.

Changes in the levels of MPV1e replication and fecal shedding followed one another (Figure 2). When the study concluded at 56 d after inoculation, RT-qPCR analysis no longer detected replication (that is, viral mRNA transcripts) in tissues from either mouse strain; viral shedding in feces as determined by qPCR assay was undetectable in B6 mice and had dropped in C mice by 3 logs from a peak of approximately 10^6 genome copies/mg feces (Figure 2).

The qPCR plots for tissue specimens in Figure 2 show that MPV1e infection levels had dropped substantially from their peaks by 28 d after inoculation, but remained stable through the study conclusion at 56 d after inoculation. In comparison to the peak levels of infection (Table 3), the persistent levels of infection (Table 4) were 80% lower in C mice and 88% lower in B6 mice. MPV1 copy number data (Table 4) show that infections persisted in the tissues of C mice at concentrations that were significantly ($P < 0.001$, ANOVA) higher than those in B6 mice, with the host-strain differences being most dramatic for ileum. The highest and lowest levels of infection occurred in the MLN and spleen, respectively ($P < 0.001$, Tukey).

Antibodies to MPV1 capsid proteins (that is, VP2) were first detected at 7 d after inoculation in 2 of 3 C mice and 3 d later in

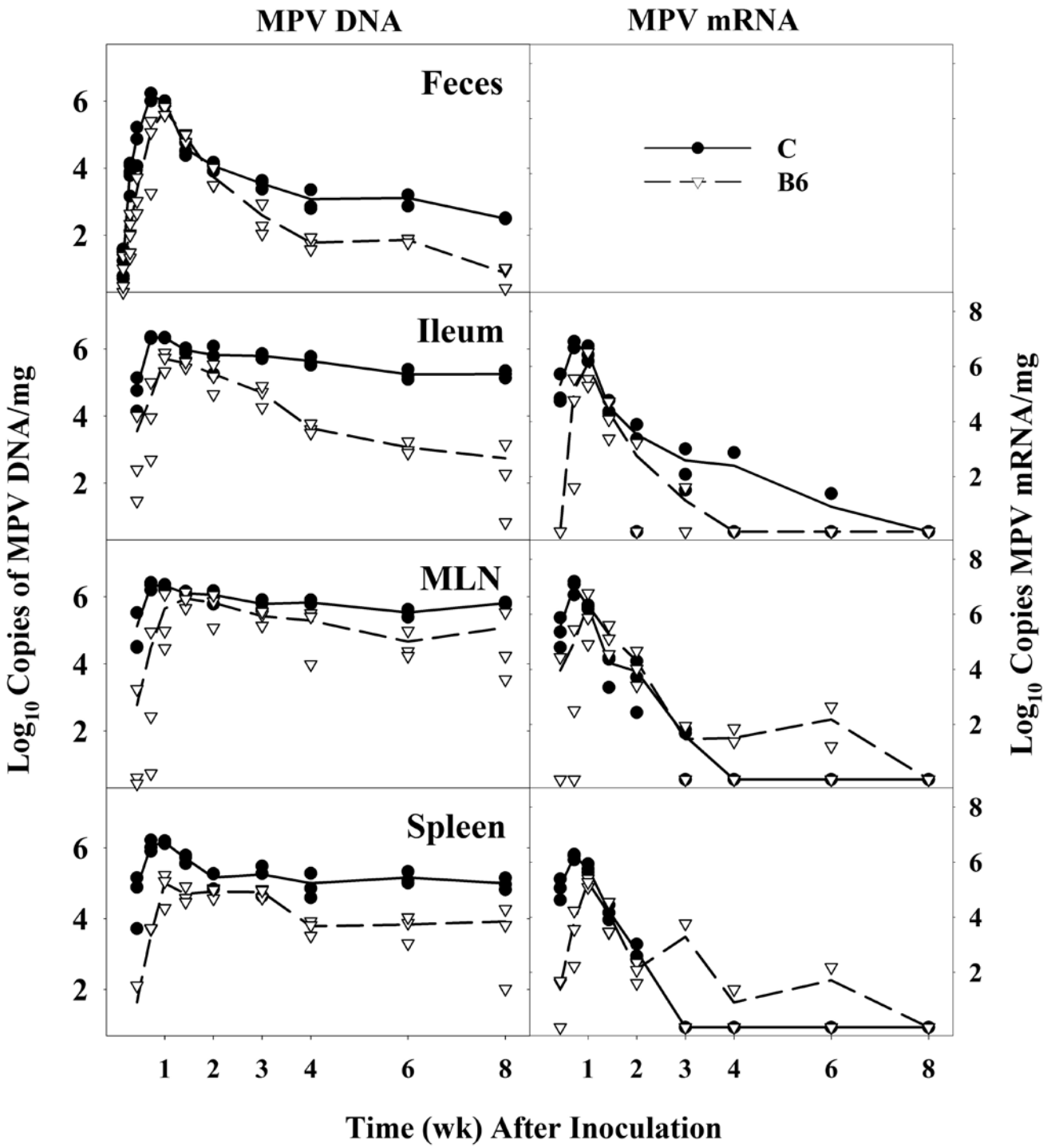

Figure 2. Time course of MPV1e infection, replication, and fecal shedding in C and B6 mice. Infection and fecal shedding were measure by viral genomic DNA qPCR; replication was determined by viral mRNA RT–qPCR. The solid and dashed lines connect the time-point averages for C and B6 mice, respectively.

all 3 B6 mice (Figure 3). By 14 d after inoculation, all mice of both strains were seropositive for MPV1. In comparison, seroconversion to the highly conserved nonstructural NS1 protein did not occur until 10 d after inoculation in C mice and 21 d after inoculation in B6 mice. However, once B6 mice seroconverted to NS1, their rNS1 MFIA scores between 3 and 8 wk averaged 18, compared with the average score of 12 for C mice during the same period. MPV1 rVP2 and rNS1 MFIA-positive sera were completely negative in the MMV rVP2 MFIA; of the 9 sera tested from each strain between 28 and 56 d after inoculation, a single C sample and 4 B6 sera gave borderline MPV2 rVP2 MFIA results, with an average score of 4.6.

Table 3. Peak levels of MPV1e infection, replication, and fecal shedding in C and B6 mice

Assay	Process evaluated	Specimen	No. of template copies (×10³) /mg (no. of days after inoculation)[a]			
			C	B6	C:B6[b]	B6 – C[c]
qPCR for viral DNA	Shedding	Feces	852 (5.8)	253 (8.3)	3.4[d]	2.6
	Infection	Ileum	1801 (7.8)	360 (9.3)	5.0	1.6
		MLN	1907 (8.2)	674 (10.1)	2.8	1.9
		Spleen	1039 (7.3)	70 (10.3)	14.8	3.0
		All tissues	1582 (7.8)	368 (9.9)	4.3[e]	2.1
RT-qPCR for viral mRNA	Replication	Ileum	3433 (5.8)	473 (8.3)	7.3	2.6
		MLN	4371 (5.0)	837 (8.1)	5.2	3.1
		Spleen	782 (5.0)	69 (8.3)	11.3	3.3
		All tissues	2862 (5.3)	460 (8.3)	6.2[e]	3.0

[a]The peak no. of template copies/mg specimen are the average of the 9 highest values between 3 and 14 d after inoculation; the values were selected by rank rather than from specific days to adjust for variation by measurement, specimen type, and host strain. The numbers in parentheses are the average d after inoculation at which the 9 peak template copies/mg specimen.
[b]The ratio of C to B6 number of template copies/mg of specimen
[c]The difference in the average d after inoculation at which peak template copies/mg of specimen were found in B6 and C mice
[d]$P = 0.06$ (Student t test)
[e]$P < 0.001$ (ANOVA)

Table 4. Persistence of MPV1e infection in the tissues of seropositive C and B6 mice

	Average no. viral DNA copies/mg (× 10³)		Ratio
	C	B6	C:B6
Ileum	64	2	131.8
MLN	556	121	4.6
Spleen	116	7	16.3
All tissues	312	43	7.2

The average number of viral DNA copies/mg of tissue was evaluated by qPCR analysis. Tissues were collected from 3 mice per strain at 4, 6, and 8 wk after inoculation (n = 9 per strain), when all mice of both strains were seropositive.

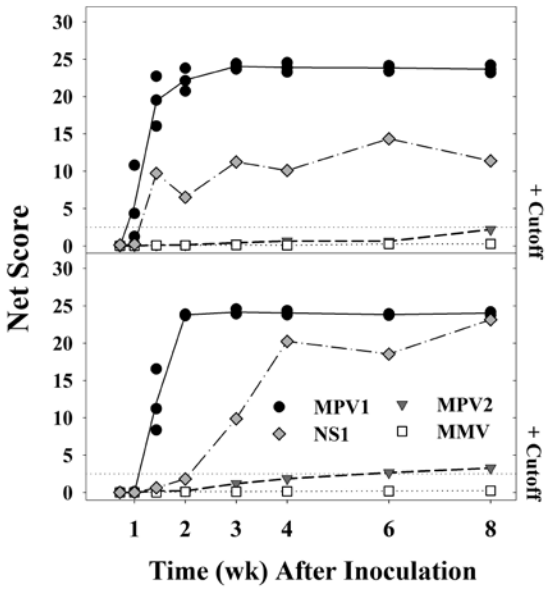

Figure 3. Antibody response to MPV1e infection in C (upper panel) and B6 (lower panel) mice measured by MFIA with recombinant VP2 viral capsid protein antigens for MPV1, MPV2, and MMV and the recombinant NS1nonstructural protein antigen. The MPV1 MFIA data points denote individual sample results; the other symbols represent the average for 3 samples per strain.

Discussion

MPV1 is one of the more common contaminants of laboratory mouse colonies,[22,37,39] largely because parvoviruses are environmentally stable and resistant to disinfection.[12,23] However, even in animal facilities where MPV1 is endemic, serology and PCR rarely detect the infection in B6 mice.[44] B6 mice were thought to lack seroconversion when infected with a low dose of MPV1, but data reported by others[7,15,16] and the nearly perfect correspondence between the MPV ELISA and PCR assay results from the MPV1e ID_{50} titrations we present here provide clear evidence that, once infected, B6 mice consistently seroconvert to MPV1.

The main goals of our current experiments were to: 1) corroborate and quantify the difference between the susceptibilities of B6 and C mice to MPV1 infection; 2) evaluate whether the route of inoculation altered susceptibility; and 3) determine whether the difference in susceptibility to MPV1 between C and B6 mice was associated with variations in the timecourse and levels of MPV1 infection, replication, and fecal excretion (that is, shedding) and humoral immunity. Rather than the cell-culture-adapted MPV1a strain used in many earlier investigations,[7,19,30,31,45] our experiments used a standard inoculum of MPV1e, a representative field strain propagated only in mice,[3,6] To simulate a natural route of exposure, we wanted to inoculate mice by oral gavage, but results of attempts to thus use MPV1a were inconsistent. We compared B6 with C mice because in our experience and in published reports,[7,43] C mice are among the strains most susceptible to MPV1

infection. Moreover, the T lymphocyte subsets of C and B6 have been characterized extensively and shown to differ substantially. For example, regulatory T lymphocytes in the blood and spleen of C mice are mainly $CD4^+$ T helper lymphocytes, whereas $CD8^+$ cytotoxic T lymphocytes predominate in B6 mice.[36] Helper T lymphocytes differentiate into Th1 and Th2 cells, which regulate distinct immune responses.[14] B6 and C mice are considered the prototypical Th1 and Th2 responder strains,[36] respectively. Because lymphoid tissues[3,19] and T lymphocytes[29] have been shown to be targets of MPV1 infection, these established differences between the percentages of T lymphocyte subsets and the T-cell–dependent immune responses of C and B6 mice provide an avenue for investigating the mechanism of genetically based variation in susceptibility to MPV1.

The ID_{50} titration in the current study confirmed that C mice were more susceptible than were B6 mice to MPV1e infection, regardless of whether the virus was administered by gavage or intraperitoneal injection. The ID_{50} by intraperitoneal injection differed by 0.5 $\log_{10}$ or less from those by oral gavage, but because the titer after intraperitoneal injection was slightly higher in B6 mice and lower in C mice, the C:B6 ID_{50} ratio decreased from 50 to 100 for gavage to 10 for intraperitoneal injection. Therefore, the minimal dose to infect a B6 mouse was 10 to 100 times greater than that needed to infect a C mouse.

In the time course study, C and B6 mice were inoculated with MPV1e by gavage to simulate natural infection. Tissue and fecal specimens collected at various times after inoculation were tested by qPCR assay to assess levels of viral infection and excretion; tissues also were assayed by RT-qPCR to assess viral replication. Ours is not the first study in which molecular genetics techniques have been used to monitor MPV1 replication. An early study[19] identified sites of MPV1a replication in mouse tissues based on detection of mRNA and replicative-form DNA by in situ hybridization with a minus-sense RNA probe; however, the authors noted that the hybridization labeling was "sparse" even during the acute infection and suggested that a more sensitive technique such as PCR analysis might be preferable to in situ hybridization. Other authors[3] used a qualitative, gel-based RT-PCR approach to demonstrate that MPV1e replication continued in SCID mouse tissues for at least 32 wk after inoculation. The RT-qPCR assay that we used is more specific than is the qualitative gel-based RT-PCR test it supplanted, because the assay we used incorporates a *Taq*Man probe that targets an intron junction sequence unique to MPV1e RNA. The high sensitivity of this test allowed us to detect and track MPV1 replication during the persistent as well as the acute phases of infection. The close correlation between the concentrations of virus being shed in feces and of viral mRNA transcripts in tissues supports the value of the RT-qPCR assay results for measuring viral replication in tissues.

The peaks of infection and viral shedding and of replication occurred in C mice between 3 and 10 d after inoculation. In B6 mice, these peaks occurred 2 to 3 d later and were significantly lower than those in C mice. Similarly, the MFIA detected seroconversion to MPV1 capsid at 7 d after inoculation in C mice and several days later in B6 mice. All mice of both strains were MPV1 seropositive from 14 d after inoculation onward. MFIA detected seroconversion to the nonstructural NS1 protein in C and B6 mice at 10 and 21 d after inoculation, respectively. The delay in the NS1 antibody response compared with the capsid response is consistent with other published data showing that NS1 antibodies may be absent from the MPV1 humoral immune response or be detected later than are viral capsid antibodies.[7,16] It is interesting that rNS1 MFIA scores from 21 to 56 d after inoculation were nearly twice as high in B6 as in C mice. Of the 48 sera collected from MPV1e-inoculated mice over the course of the experiment, 39 (81%) were MPV1 MFIA positive; none were MMV MFIA positive, and only 5 (10%) were MPV2 MFIA positive albeit with borderline positive scores (mean, 5). The serotype specificity of ELISA and MFIA using purified capsid protein antigens (whether virions or recombinant virus-like particles) and the delayed or sometimes absent antibody response to the conserved NS1 protein antigen underscore the importance of including capsid antigens representing several parvovirus strains in serosurveillance panels.

In the weeks after seroconversion, viral replication and fecal shedding decreased rapidly and in concert with one another. By the time the last group of mice was sampled at 56 d after inoculation, virus replication was no longer detectable in tissues from mice of either strain; virus fecal shedding had dropped to low concentrations in C mice and was undetectable in B6 mice. By 28 d after inoculation, MPV1e infection levels determined by qPCR analysis had dropped from their peaks by 74% to greater than 99%, but they remained stable thereafter for the duration of the experiment. As was the case during the acute infection, the levels of persistent infection were significantly higher in C than B6 mice and (irrespective of mouse strain) were highest in MLN and lowest in spleen. Persistent infection with little or no discernible replication or cytolysis has been demonstrated for parvovirus infections in other host species,[20,21] including people,[26] and in vitro. The mechanisms by which MPV1 silently persists in mouse lymphoid tissues have not been completely defined but one possibility is that encapsidated viral genome is sequestered in mitotically inactive cells.[12] The adaptive immune response appears to be a key factor limiting MPV1 replication and shedding because, as suggested in a previously cited study,[3] viral mRNA transcripts and therefore replication persisted in the tissues of SCID mice for as long as 32 wk after inoculation; this point is well beyond the time when viral mRNA transcripts were no longer detectable in immunocompetent C and B6 mice in this study.

MPV1 transmission studies demonstrated that the period during which immunocompetent mice were contagious was limited to the first couple of weeks after natural or experimental infection.[3,43] In contrast, neonatally infected immunocompetent mice transmit infection intermittently for as long as 6 wk.[45] Personnel responsible for maintaining SPF mouse colonies have understandably been concerned that replicating and shedding of MPV1 can be reactivated after stress or other factors, such as an antigenic stimulus eliciting the mitosis of lymphocytes that sequester virus, thereby converting the latent infection of those cells to a productive one.[12,19] However, a case of such conversion has not yet been reported. The stress of pregnancy and lactation on persistently infected immunocompetent female mice did not lead to an increase in MPV1 shedding or transmission to naïve cagemates or soiled bedding sentinels.[3]

In summary, our data indicate that B6 mice are less susceptible to MPV1e infection than are C mice, regardless of the inoculation route, and that the lower susceptibility is associated with lower levels of viral infection, replication, fecal shedding, and seroconversion. Taken together, our findings suggest that B6 lymphocytes are less permissive for MPV1 replication than are C lymphocytes and that susceptible lymphocyte subsets (for example, $CD4^+$ Th

cells) are more numerous in C than in B6 mice.[36] Infection of cells by autonomous parvoviruses is dependent on the attachment of the viral capsid to host receptors, with subsequent transport of the capsid across the cytoplasmic membrane and into the nucleus and the release of the viral genome for replication, which requires mitotically active host cells[2,47,48] and perhaps other host cell factors expressed during differentiation.[2,33,46] Differences in the virus–host interaction at any of these points could account for disparity between the susceptibilities of C and B6 mice to MPV1 infection.

References

1. **Ball-Goodrich LJ, Hansen G, Dhawan R, Paturzo FX, Vivas-Gonzalez BE.** 2002. Validation of an enzyme-linked immunosorbent assay for detection of mouse parvovirus infection in laboratory mice. Comp Med **52:**160–166.
2. **Berns KI.** 1990. Parvovirus replication. Microbiol Rev **54:**316–329.
3. **Besselsen DG, Becker MD, Henderson KS, Wagner AM, Banu LA, Shek WR.** 2007. Temporal transmission studies of mouse parvovirus 1 in BALB/c and C.B-17/Icr-Prkdc^{scid} mice. Comp Med **57:**66–73.
4. **Besselsen DG, Myers EL, Franklin CL, Korte SW, Wagner AM, Henderson KS, Weigler BJ.** 2008. Transmission probabilities of mouse parvovirus 1 to sentinel mice chronically exposed to serial dilutions of contaminated bedding. Comp Med **58:**140–144.
5. **Besselsen DG, Pintel DJ, Purdy GA, Besch-Williford CL, Franklin CL, Hook RR Jr, Riley LK.** 1996. Molecular characterization of newly recognized rodent parvoviruses. J Gen Virol **77:**899–911.
6. **Besselsen DG, Romero-Aleshire MJ, Munger SJ, Marcus EC, Henderson KS, Wagner AM.** 2008. Embryo transfer rederivation of C.B-17/Icr-Prkdc^{scid} mice experimentally infected with mouse parvovirus 1. Comp Med **58:**353–359.
7. **Besselsen DG, Wagner AM, Loganbill JK.** 2000. Effect of mouse strain and age on detection of mouse parvovirus 1 by use of serologic testing and polymerase chain reaction analysis. Comp Med **50:**498–502.
8. **Blank WA, Henderson KS, White LA.** 2004. Virus PCR assay panels: an alternative to the mouse antibody production test. Lab Anim (NY) **33:**26–32.
9. **Bonnard GD, Manders EK, Campbell DA Jr, Herberman RB, Collins MJ Jr.** 1976. Immunosuppressive activity of a subline of the mouse EL4 lymphoma. Evidence for minute virus of mice causing the inhibition. J Exp Med **143:**187–205.
10. **Brownstein DG, Smith AL, Jacoby RO, Johnson EA, Hansen G, Tattersall P.** 1991. Pathogenesis of infection with a virulent allotropic variant of minute virus of mice and regulation by host genotype. Lab Invest **65:**357–364.
11. **Compton SR, Homberger FR, Paturzo FX, Clark JM.** 2004. Efficacy of 3 microbiological monitoring methods in a ventilated cage rack. Comp Med **54:**382–392.
12. **Cotmore SF, Tattersall P.** 2007. Parvoviral host range and cell entry mechanisms. Adv Virus Res **70:**183–232.
13. **Crawford LV.** 1966. A minute virus of mice. Virology **29:**605–612.
14. **Dong C, Flavell RA.** 2001. Th1 and Th2 cells. Curr Opin Hematol **8:**47–51.
15. **Filipovska-Naumovska E, Abubakar SM, Thompson MJ, Hopwood D, Pass DA, Wilcox GE.** 2010. Serologic prevalence of MPV1 in mouse strains in a commercial laboratory mouse colony determined by using VP1 antigen. J Am Assoc Lab Anim Sci **49:**437–442.
16. **Filipovska-Naumovska E, Thompson MJ, Hopwood D, Pass DA, Wilcox GE.** 2010. Strain- and age-associated variation in viral persistence and antibody response to mouse parvovirus 1 in experimentally infected mice. J Am Assoc Lab Anim Sci **49:**443–447.
17. **Grove KA, Smith PC, Booth CJ, Compton SR.** 2012. Age-associated variability in susceptibility of Swiss Webster mice to MPV and other excluded murine pathogens. J Am Assoc Lab Anim Sci **51:**789–796.
18. **Harris RE, Coleman PH, Morahan PS.** 1974. Stability of minute virus of mice to chemical and physical agents. Appl Microbiol **28:**351–354.
19. **Jacoby RO, Johnson EA, Ball-Goodrich L, Smith AL, McKisic MD.** 1995. Characterization of mouse parvovirus infection by in situ hybridization. J Virol **69:**3915–3919.
20. **Jacoby RO, Johnson EA, Paturzo FX, Ball-Goodrich L.** 2000. Persistent rat virus infection in smooth muscle of euthymic and athymic rats. J Virol **74:**11841–11848.
21. **Jacoby RO, Johnson EA, Paturzo FX, Gaertner DJ, Brandsma JL, Smith AL.** 1991. Persistent rat parvovirus infection in individually housed rats. Arch Virol **117:**193–205.
22. **Jacoby RO, Lindsey JR.** 1998. Risks of infection among laboratory rats and mice at major biomedical research institutions. ILAR J **39:**266–271.
23. **Jacoby RO, Smith AL.** 2003. Mouse parvovirus: survival of the fittest. Comp Med **53:**470–471.
24. **Johnson DA, Gautsch JW, Sportsman JHE Jr.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal Tech **1:**3–8.
25. **Jongeneel CV, Sahli R, McMaster GK, Hirt B.** 1986. A precise map of splice junctions in the mRNAs of minute virus of mice, an autonomous parvovirus. J Virol **59:**564–573.
26. **Kerr JR.** 2005. Pathogenesis of parvovirus B19 infection: host gene variability and possible means and effects of virus persistence. J Vet Med B Infect Dis Vet Public Health **52:**335–339.
27. **Kimsey PB, Engers HD, Hirt B, Jongeneel CV.** 1986. Pathogenicity of fibroblast- and lymphocyte-specific variants of minute virus of mice. J Virol **59:**8–13.
28. **Luminex.** 2004. Two-step carbodiidmide coupling of protein to Luminex carboxylated microspheres. Luminex 100 system training manual, document no. 00371 rev. B, p. 29–33. Austin (TX): Luminex.
29. **McKisic MD, Lancki DW, Otto G, Padrid P, Snook S, Cronin DC 2nd, Lohmar PD, Wong T, Fitch FW.** 1993. Identification and propagation of a putative immunosuppressive orphan parvovirus in cloned T cells. J Immunol **150:**419–428.
30. **McKisic MD, Macy JD Jr, Delano ML, Jacoby RO, Paturzo FX, Smith AL.** 1998. Mouse parvovirus infection potentiates allogeneic skin graft rejection and induces syngeneic graft rejection. Transplantation **65:**1436–1446.
31. **McKisic MD, Paturzo FX, Smith AL.** 1996. Mouse parvovirus infection potentiates rejection of tumor allografts and modulates T cell effector functions. Transplantation **61:**292–299.
32. **McMaster GK, Beard P, Engers HD, Hirt B.** 1981. Characterization of an immunosuppressive parvovirus related to the minute virus of mice. J Virol **38:**317–326.
33. **Miller RA, Ward DC, Ruddle FH.** 1977. Embryonal carcinoma cells (and their somatic cell hybrids) are resistant to infection by the murine parvovirus MVM, which does infect other teratocarcinoma-derived cell lines. J Cell Physiol **91:**393–401.
34. **Nicklas W, Kraft V, Meyer B.** 1993. Contamination of transplantable tumors, cell lines, and monoclonal antibodies with rodent viruses. Lab Anim Sci **43:**296–300.
35. **Parker JC, Collins MJ Jr, Cross SS, Rowe WP.** 1970. Minute virus of mice. II. Prevalence, epidemiology, and occurrence as a contaminant of transplanted tumors. J Natl Cancer Inst **45:**305–310.
36. **Pinchuk LM, Filipov NM.** 2008. Differential effects of age on circulating and splenic leukocyte populations in C57BL/6 and BALB/c male mice. Immun Ageing **5:**1.
37. **Pritchett-Corning KR, Cosentino J, Clifford CB.** 2009. Contemporary prevalence of infectious agents in laboratory mice and rats. Lab Anim **43:**165–173.
38. **Reed LJ, Muench H.** 1938. A simple method of estimating 50% endpoints. Am J Hyg **27:**493–497.
39. **Schoondermark-van De Ven EM, Philipse-Bergmann IM, Van Der Logt JT.** 2006. Prevalence of naturally occurring viral infections, *Mycoplasma pulmonis* and *Clostridium piliforme*, in laboratory rodents in Western Europe screened from 2000 to 2003. Lab Anim **40:**137–143.
40. **Segovia JC, Gallego JM, Bueren JA, Almendral JM.** 1999. Severe leukopenia and dysregulated erythropoiesis in SCID mice persistently

infected with the parvovirus minute virus of mice. J Virol **73:**1774–1784.

41. **Seletsakia EM, Cowley JP, Wunderlich ML, Jennings SJ, Henderson KS, Shek WR, Dhawan RK**. 2004. Development of a parvovirus assay using rNS1 His-tagged antigen. Bioprocessing J **3:**35–39.
42. **Shek WR.** 1987. Detection of murine viruses in biological materials by the mouse antibody production test. In: Seaver SS, editor. Commercial production of monoclonal antibodies: a guide for scale-up. New York (NY): Marcel Dekker.
43. **Shek WR, Paturzo FX, Johnson EA, Hansen GM, Smith AL.** 1998. Characterization of mouse parvovirus infection among BALB/c mice from an enzootically infected colony. Lab Anim Sci **48:**294–297.
44. **Shek WR, Pritchett KR, Clifford CB, White WJ.** 2005. Large-scale rodent production methods make vendor barrier rooms unlikely to have persistent low-prevalence parvoviral infections. Contemp Top Lab Anim Sci **44:**37–42.
45. **Smith AL, Jacoby RO, Johnson EA, Paturzo F, Bhatt PN.** 1993. In vivo studies with an 'orphan' parvovirus of mice. Lab Anim Sci **43:**175–182.
46. **Spalholz BA, Tattersall P.** 1983. Interaction of minute virus of mice with differentiated cells: strain-dependent target cell specificity is mediated by intracellular factors. J Virol **46:**937–943.
47. **Tattersall P.** 1972. Replication of the parvovirus MVM. I. Dependence of virus multiplication and plaque formation on cell growth. J Virol **10:**586–590.
48. **Tattersall P, Bratton J.** 1983. Reciprocal productive and restrictive virus-cell interactions of immunosuppressive and prototype strains of minute virus of mice. J Virol **46:**944–955.
49. **Tattersall P, Cotmore J.** 1986. The rodent parvoviruses. In: Bhatt PN, Jacoby RO, Morse HC, New AE, editors. Viral and mycoplasmal infections of rodents: effects on research. Orlando (FL): Academic Press.