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Effect of Immunodeficiency on MPV Shedding and Transmission

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C57BL/6 (B6) mice briefly shed low levels of MPV, and transmission is inefficient. To determine whether deficits in B or T cells or in interferon γ on a B6 background increased the duration of MPV shedding or transmission, B-cell–deficient (Igh), interferon-γ–deficient (Ifnγ), B- and T-cell–deficient (Rag), and B6 mice were inoculated with MPV. At 1 and 2 wk postinoculation (wpi), 11% to 94% of mice shed MPV. From 4 to 18 wpi, 80% to 100% of Rag mice and 0% of B6 and Ifnγ mice shed MPV; Igh mice sporadically shed MPV through 20 wpi. MPV was transmitted from B6 mice and Ifnγ mice at 2 to 4 wpi. Rag and Igh mice transmitted MPV to sentinels at all or most time points, respectively, between 2 to 16 wpi. Once transmission ceased from B6, Ifnγ, and Igh mice, breeding trios were setup and showed that MPV was transmitted to offspring in only one cage of Igh mice. In another experiment, MPV shedding ceased from B6, CD8-deficient (CD8), CD4-deficient (CD4), and T-cell–receptor-deficient (TCR) mice by 2, 6, 8, and 8 wpi, respectively. MPV was transmitted to sentinels only at 1 to 4 wpi. Mesenteric lymph nodes collected from 61% to 100% of B6, Ifnγ, TCR, CD4, CD8, and Rag mice were MPV DNA-positive. In conclusion, MPV transmission did not differ between mice deficient in T cell functions or Ifnγ and B6 mice. In contrast, B-cell deficiency posed an increased risk for MPV transmission in mice.

Abbreviations: B6, C57BL/6J mice; CD4, B6.129S2-*Cd4*^{tm1Mak}/J mice; CD8, B6.129S2-*Cd8a*^{tm1Mak}/J mice; Igh, B6.129S2-*Ighm*^{tm1Cgn}/J mice; Ifnγ, B6.129S7-*Ifng*^{tm1Ts}/J mice; MLN, mesenteric lymph nodes; MPV, mouse parvovirus; Rag, B6.129S7-*Rag1*^{tm1Mom}/J mice; TCR, B6.129P2-*Tcrb*^{tm1Mom}/J mice; wpi, weeks post-inoculation.

Mouse parvovirus (MPV) was isolated initially from cloned CD8$^+$ T cells, and the primary cell type infected in vitro and in vivo is lymphocytes.[20] MPV infects the intestine and several lymphoid tissues, including the mesenteric and peripheral lymph nodes, spleen, and thymus.[13] Although MPV infections do not directly result in clinical disease, they have the ability to disrupt mouse-based research through the production of aberrant T cell proliferative responses and the acceleration of T-cell–mediated rejection of tumors, skin allografts, and syngeneic skin grafts.[21,22] The acute phase of MPV infection occurs 3 to 14 d after infection, and MPV can be detected in the intestine and several lymphoid tissues including the mesenteric lymph nodes (MLN), Peyer patches, spleen, and thymus, and transmission via contact or soiled bedding is reliable.[6,13,30,35,36] All ages and strains of mice can be infected with MPV, but the ease with which a productive infection is induced and its duration depend on many factors, including the strain and age of the mouse, the dose and strain of MPV and its passage history in cultured cells, and the type of caging in which the mice are housed.[2,6,36] During the persistent phase of MPV infection—from 4 to 24 wk postinfection (wpi) in immunocompetent mice—transmission of MPV to sentinels occurs infrequently, and low levels of viral DNA frequently are detected in the feces and MLN.[13,35] Several studies have suggested that the MPV DNA detected in the feces during the persistent phase of the infection is noninfectious or below the threshold required to initiate a productive infection.[30,36] Although infectious MPV can be detected in the MLN of most mouse strains for far longer than in the feces or intestines,[36] attempts to reactivate transmission of MPV by using dexamethasone once fecal shedding had ceased have been unsuccessful,[19] suggesting that the risk of transmission during the persistent phase of infection is lower than first thought.

MPV infection of C57BL/6 (B6) mice is less reliable than that of BALB/c, C3H/HeN, DBA/2, and Swiss Webster mice.[2,7,18] For example, 10- to 100-fold more MPV was needed to infect B6 mice than BALB/c mice, and MPV DNA levels at 1 wpi in the feces of Swiss Webster mice were 300-fold higher than those of B6 mice.[7,28] In addition, compared with BALB/c and Swiss Webster mice, B6 mice seroconverted later, MPV DNA levels in the small intestine and feces were lower, and MPV did not disseminate to the MLN or spleen.[7] These studies suggest that the likelihood of MPV transmission from MPV-infected B6 mice, the most common background of genetically engineered mice, is low given the very brief acute phase of infection and apparent lack of a persistent phase of infection. However, because genetic engineering may result in some level of immunodeficiency, either directly or indirectly, understanding the risk of specific immune defects in the context of the B6 background could be useful to better define broad categories of risk. It is known that immunodeficient C.B-17-scid mice infected as neonates showed extended MPV shedding. These profoundly immunodeficient mice sustained high levels of MPV in the intestine, spleen, lymph nodes, thymus, and feces as adults and reliably transmitted MPV to sentinels for several months.[1] However, it is unclear whether age at time of infection, genetic background, and immunodeficiency contribute equally to persistent transmission or whether there is synergism between factors that allows prolonged transmission to occur. To provide a better framework for risk analysis of large cohorts of adult B6 mice, we examined whether immune defects in one or more arms of the immune response (humoral, cell-mediated, or cytokine dysfunction affecting both innate and adaptive [Ifnγ deficiency]

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immune responses) increased the duration of shedding and transmission of MPV.

Materials and Methods

Mice. Male and female 5-wk-old B-cell deficient B6.129S2-*Ighm*^{tm1Cgn}/J (Igh), Ifnγ-deficient B6.129S7-*Ifng*^{tm1Ts}/J (Ifnγ), B- and T-cell–deficient B6.129S7-*Rag1*^{tm1Mom}/J (Rag), CD4-deficient B6.129S2-*Cd4*^{tm1Mak}/J (CD4), CD8-deficient B6.129S2-*Cd8a*^{tm1Mak}/J (CD8), T-cell–receptor-deficient B6.129P2-*Tcrb*^{tm1Mom}/J (TCR), and C57BL/6J (B6) mice were obtained from Jackson Laboratory (Bar Harbor, ME). Female Swiss Webster mice (Crl:CFW [SW]; age, 4 to 6 wk) were obtained from Charles River Laboratories (Wilmington, MA). Vendor reports indicated that mice were seronegative for ectromelia virus, murine rotavirus, lymphocytic choriomeningitis virus, mouse hepatitis virus, MPV, minute virus of mice, murine norovirus, pneumonia virus of mice, reovirus, Sendai virus, and *Mycoplasma pulmonis* and were free of bacterial and parasitic infections at the time of shipment. Mice were housed in individually ventilated cages (ACE MicroVent, Allentown, NJ) containing corncob bedding (Harlan Teklad, Indianapolis, IN), rodent chow (Global 2018S, Harlan Teklad), and nesting material (Nestlets, Ancare, Bellmore, NY) that had been autoclaved (8 min at 225 °F [107 °C]) as a unit. Mice drank hyperchlorinated (4 to 6 ppm) water ad libitum, and the animal room had a negative pressure differential relative to the corridor, a 12:12-h light:dark cycle, 10 to 15 air changes hourly, room temperature of 22.2 ± 1.1 °C, and room humidity of 50 ± 10%. All animal care and experimental procedures were approved by the Yale IACUC and were conducted in accordance with Yale's Office of Laboratory Animal Welfare Assurance.

Mouse parvovirus inoculation and detection. Mice were inoculated orally with 300 infectious doses (ID_{50}; 20 µL of a 10% spleen stock in DMEM) of MPV1d.[33] At the end of the study, blood was collected from all mice by cardiocentesis after CO_2 overdose, and sera were tested for antibodies to mouse parvovirus in an indirect immunofluorescent assay using MPV1d-infected L3 cells as previously described.[35] Fecal samples were collected from the anus of mice and were frozen at −70 °C prior to PCR analysis to evaluate MPV shedding. Fecal pellets were homogenized in 400 to 800 µL PBS, and 10% (w/v) homogenates of MLN were made in DMEM (Life Technologies, Grand Island, NY). DNA was purified by using DNeasy Tissue kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. PCR analysis was performed by using 3 µL DNA, DyNAmo SYBR Green qPCR kit (Thermo Scientific, Waltham, MA), and primers specific for the MPV nonstructural gene.[18] The reaction conditions were 2 min at 94 °C; 35 cycles of 30 s at 92 °C, 30 s at 56 °C, and 60 s at 72 °C; and 5 min at 72 °C. All PCR assays included positive and negative controls.

MPV infection of Igh, Ifnγ, Rag, and B6 mice to determine the duration of MPV shedding and transmission in B-cell, cytokine-, and B-T-cell–deficient genotypes on a B6 background. Groups of 12 female and 6 male Igh, Ifnγ, Rag, and B6 mice (index mice) were inoculated at 6 wk of age with MPV1d and were housed as 2 female or 3 male mice per cage. Feces were collected from each index mouse at 1 wpi and then biweekly starting at 2 wpi for MPV PCR analysis. One Swiss Webster mouse (contact sentinel) was added to each cage of mice (8 cages per mouse genotype) at 2 d after inoculation to allow for direct contact with the infected index mice for 12 d. Biweekly starting at 2 wpi, all cages were changed, the contact sentinels were removed, and one Swiss Webster mouse was added to each index cage to serve as a new contact sentinel. After exposure to the index mice, contact sentinels were housed singly in clean cages for an additional 2 wk to allow for seroconversion and then were euthanized by CO_2 overdose, and blood was collected for MPV serology.

Mating of Igh, Ifnγ, and B6 mice after MPV infection to determine whether MPV is transmitted to offspring once MPV shedding and transmission have ceased. At 8 wpi for B6, 10 wpi for Ifnγ, and 16 wpi for Igh mice (that is, at 4 wk after the initial time point when all contact sentinels for a given mouse genotype were seronegative for MPV), one male mouse was added to each cage of 2 female mice to initiate mating. Male mice were removed from each cage of breeder female mice when pups were 24 to 48 h old and were housed singly until all female mice of the given genotype had delivered litters. These male mice were placed into cages in which the female mice did not become pregnant within 4 wk of the pairing with the original male mouse. When all female mice of a given genotype had given birth, male mice were euthanized by CO_2 overdose, blood was collected for MPV serology, and feces and MLN were collected for MPV PCR analysis. When pups were 1 wk old, cages were changed, and a Swiss Webster mouse (litter contact sentinel) was added to each cage for a 2-wk exposure to the dams and pups; these sentinels then were housed singly in clean cages for 2 additional weeks to allow for seroconversion. Pups were euthanized at 3 wk of age, feces were collected and pooled for MPV PCR, and blood was collected and pooled for MPV serology. When pups were 3 wk of age, dams were euthanized by CO_2 overdose, blood was collected for MPV serology, and feces and MLN were collected for MPV PCR. Because contact sentinels in all cages of Rag mice were seropositive at all time points, breeding trios of Rag mice were not established, and Rag mice were euthanized by CO_2 overdose at 16 wpi, blood was collected for MPV serology, and feces and MLN were collected for MPV PCR.

MPV infection of CD4, CD8, TCR, and B6 mice to determine the duration of MPV shedding and transmission in T-cell–deficient genotypes on a B6 background. Groups of 10 female CD4, CD8, TCR, and B6 mice (index mice) were inoculated at 6 wk of age with MPV1d and were housed at 2 mice per cage (5 cages per mouse genotype). Feces were collected from each index mouse at 1 wpi and pooled by cage. After which, individual fecal pellets for MPV PCR analysis were collected biweekly starting at 2 wpi. One Swiss Webster mouse (contact sentinel) was added to each cage of mice (5 cages per mouse genotype) at 1 wpi for contact with the index mice for 1 wk. Biweekly starting at 2 wpi, all cages were changed, the contact sentinels were removed, and one Swiss Webster mouse was added to each index cage to serve as a new contact sentinel. After exposure to the index mice, contact sentinels were housed singly in clean cages for 2 additional weeks to allow for seroconversion and then were euthanized by CO_2 overdose, and blood was collected for MPV serology. At 10 wpi for CD8, TCR, and B6 mice and 12 wpi for CD4 mice (that is, at 4 wk after all contact sentinels for a given mouse genotype were seronegative for MPV), index mice were euthanized by CO_2 overdose, blood was collected for MPV serology, and feces and MLN were collected for MPV PCR analysis.

Statistical analysis. Two-tailed Fisher exact probability tests were performed by using an online statistics application (vassarstats.net). Statistical significance was set at a P value of less than or equal to 0.05.

Results

To determine the effect of immunodeficiency on MPV shedding and duration, groups of 12 female and 6 male 4-wk-old B-cell–deficient Igh, γ-interferon–deficient Ifnγ mice, B- and T-cell–deficient Rag, and B6 mice were inoculated with MPV1d. PCR analysis of feces collected at 1 wk wpi indicated that 44%

to 94% of B6, Igh, Ifnγ, and Rag mice became infected with and were shedding MPV, with Rag mice having the fewest mice that shed MPV (Figure 1). MPV DNA in feces was highest at 1 wpi for B6, Igh, and Ifnγ mice, whereas levels were lowest at 1 wpi and highest later (8 wpi) in Rag mice. The median levels of MPV DNA in the immunodeficient genotypes were at least 6-fold higher than those in B6 mice. At 4 wpi, all of the B6 and Ifnγ mice had ceased shedding MPV, whereas feces from most of the Igh and increasing numbers of Rag mice were positive for MPV DNA (Figure 1). From 6 to 18 wpi, feces from all Rag mice were positive for MPV DNA. In contrast to that in Rag mice, MPV infection in Igh mice was initially high (94%) at 1 wpi and then persisted, intermittently and at low levels. Feces from 4 or 6 Igh mice in 2 cages were positive for MPV DNA at 6 and 8 wpi. Although infection in Igh mice appeared to be cleared by 10 wpi (MPV DNA was not detected in any Igh mice at either 10 or 12 wpi), shedding resumed in 1 or 2 mice in 3 different cages at 14, 16, and 18 wpi. Therefore, MPV shedding had ceased completely for 6, 12, or 14 wk before resuming again, or low levels of virus were maintained in the mice but were below the level of detection at several time points before rising to detectable levels again (Figure 1). For each of the 4 mouse genotypes, the percentage of cages containing mice that were shedding MPV was similar to the percentage of individual mice with feces positive for MPV DNA.

MPV was transmitted to 25% of the sentinels in contact with B6 mice through 2 wpi but not at any later time points (Figure 2). MPV was transmitted to the majority (75%) of the sentinels in contact with Ifnγ mice through 2 wpi, but transmission dropped rapidly, with transmission to only a single sentinel in contact with Ifnγ mice from 2 to 4 wpi (Figure 2). Although transmission from B6 and Ifnγ mice appeared to differ between 2 and 4 wpi (Figure 2), this difference was not statistically significant. In addition, MPV was transmitted to the majority of the sentinels in contact with Igh mice through 2 wpi and transmission levels declined slowly (Figure 2). By 12 wpi, transmission appeared to have ceased, but transmission was detected again in a single cage at 14 and 16 wpi (Figure 2). Significantly ($P \leq 0.05$) more Igh mice had MPV-positive feces than did B6 mice at 2, 4, and 8 wpi, and significantly ($P \leq 0.05$) more Igh mice transmitted MPV to a contact sentinel than did B6 mice at 4 wpi. There were no significant differences in the ability of Igh and Ifnγ mice to transmit infection to contact sentinels at any time point (Figure 2), despite significantly ($P \leq 0.05$) increased shedding of MPV in feces by Igh mice as compared with Ifnγ mice at 2, 4, and 8 wpi (Figure 1). Transmission to contact sentinels from Rag mice was at its lowest early (2 wpi) but then increased to maximal levels (100%) from 4 to 16 wpi, in contrast to the decline in transmission that occurred over time in the other 3 mouse genotypes (Figure 2). To illustrate the contrast in MPV DNA shedding patterns between B6 and Rag mice, the percentage of Rag mice shedding MPV was significantly ($P = 0.03$) less than that of B6 mice at 2 wpi and significantly ($P \leq 0.00004$) higher than that of B6 mice at 4 to 14 wpi. In addition, the percentage of sentinels that had been in contact with Rag mice and that became MPV-seropositive was significantly ($P \leq 0.0002$) higher than that of B6 mice starting at 4, 6, and 8 wpi (when the breeding trios of B6 mice were established), higher ($P \leq 0.002$) than that of Ifnγ mice starting at 4 wpi until 10 wpi (when the breeding trios of Ifnγ mice were established), and higher ($P \leq 0.03$) than that of Igh mice starting at 6 wpi until 16 wpi (when the breeding trios of Igh mice were set up).

To determine whether MPV infection could be transmitted to offspring from mice previously infected but no longer shedding, we set up breeding trios at 4 wk after transmission was not detected from B6, Ifnγ, and Igh mice (that is, at 8, 10, and 16 wpi, respectively). Pooled sera from each litter of 21-d-old mice from these pairings indicated that most B6 and all litters of Ifnγ mice contained mice that were seropositive for MPV (Table 1). MPV PCR testing of feces and contact sentinel serology indicated that none of the B6 or Ifnγ litters had detectable levels of MPV DNA in their feces nor did they transmit infection to sentinels that were in contact with the litters and their dam from 7 to 21 d postpartum (Table 1). Taken together, these data suggest that the pups in the B6 and Ifnγ litters were seropositive due to transfer of maternal antibody rather than silently infected and without MPV shedding and transmission. As expected, none of the litters of B-cell–deficient Igh mice seroconverted to MPV. Pups in 2 of the Igh litters had MPV DNA in their feces, and MPV was transmitted to one contact sentinel (Table 1), indicating a productive MPV infection. This finding is not surprising, because contact sentinel serology at 16 wpi, when the breeding trios of Igh mice were set up, indicated transmission had recommenced from mice in a single cage of Igh mice. However, the fact that the Igh mice were still shedding MPV in levels sufficient to infect contact sentinels and potentially the neonatal mice was not known at the time of breeding. This situation arose because the turnaround time for the results of the sentinel testing was approximately 3 wk, given that contact sentinels were held in clean cages for 2 wk after the last contact exposure to ensure sufficient time for MPV seroconversion, and serologic testing can take as long as 1 wk. Breeding of Igh mice was initiated at 16 wpi in light of the negative MPV serology for the 12-wpi contact sentinels. Rag mice were not bred because transmission of MPV did not cease and remained at 100% from 4 through 16 wpi.

Mice were necropsied at the end of the study, that is, at 14 wpi for B6, 16 wpi for Ifnγ, 18 wpi for Rag, and 22 wpi for Igh mice (Table 2). At the time of necropsy, most B6 mice and Ifnγ mice were seropositive. As expected, none of the B-cell–deficient Igh or B- and T-cell–deficient Rag mice seroconverted to MPV, due to their immune deficits. At this time, MPV DNA was detected in the feces from a minority of Igh and all of the Rag mice but not in feces from B6 or Ifnγ mice (Table 2). In comparison, MPV DNA was detected in the MLN from a majority of Ifnγ and all of the Rag mice but not in the MLN from B6 or Igh mice (Table 2). Therefore, MPV was cleared from both the intestine and MLN of all B6 mice by 14 wpi, from the intestine but not the MLN of Ifnγ mice by 16 wpi, and from all the MLN and most of the intestine of Igh mice by 22 wpi. However, MPV was not cleared from either the intestine or MLN of Rag mice by 18 wpi.

To determine the duration of MPV shedding and transmission from T cell deficient mice, groups of 10 female 4-wk-old CD4-deficient, CD8-deficient, T-cell–receptor-deficient, and B6 mice were inoculated with MPV1d. PCR analysis of feces collected at 1 wpi revealed 60% to 100% of B6, CD4, CD8, and TCR mice were shedding MPV (Figure 3). MPV DNA was undetectable in the feces of B6 mice by 2 wpi, of CD8 mice by 6 wpi, and of CD4 and TCR mice by 8 wpi. The only significant differences in MPV shedding occurred at 2 wpi, with more CD4 and TCR mice than B6 mice with MPV DNA in their feces ($P < 0.01$) and more CD4 mice than CD8 mice with MPV-positive feces ($P = 0.005$; Figure 3). However, there were no statistically significant differences in the numbers of mice shedding among all genotypes at the other time points (1, 4, 6, 8, and 10 wpi; Figure 3). For each of the 4 mouse genotypes, the percentage of cages containing mice that were shedding MPV was similar to the percentage of individual mice with feces positive for MPV DNA. MPV was

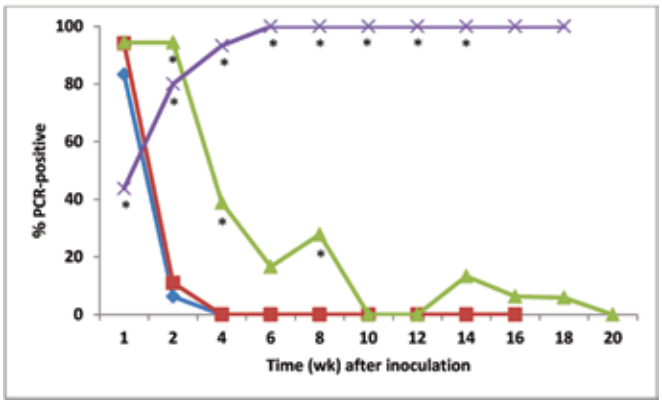

Figure 1. Percentages of MPV-inoculated mice testing positive for MPV DNA in their feces. B6 (blue diamonds), Ifnγ (red squares), Igh (green triangles), or Rag (purple crosses) mice. *, Significant ($P < 0.05$) difference between values for B6 and the immunodeficient mice.

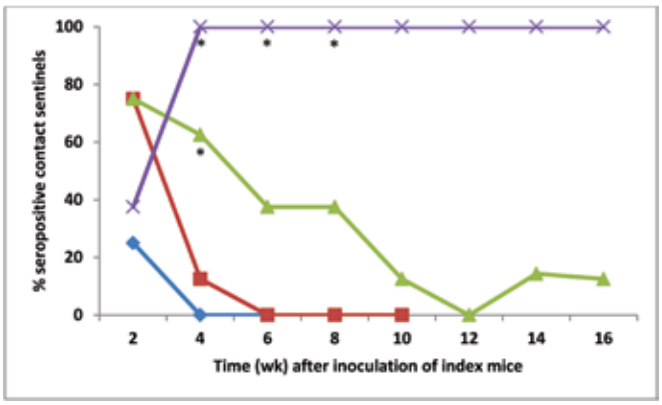

Figure 2. Percentages of sentinel mice testing seropositive for MPV that were in contact with B6 (blue diamonds), Ifnγ (red squares), Igh (green triangles), or Rag (purple crosses) mice. *, Significant ($P < 0.05$) difference between values for B6 and the immunodeficient mice.

transmitted to 20% to 60% of contact sentinels during the first week of the infection (Figure 4). Transmission ceased by 4 wpi in all genotypes except CD4 mice, which transmitted MPV until 6 wpi (Figure 4). MPV was transmitted from TCR mice to only a single sentinel mouse during the first week of the infection and to none of the sentinels thereafter (Figure 4). Although TCR mice appeared to transmit MPV to fewer sentinel mice and for a shorter duration, there were no statistically significant differences in transmission among the 4 genotypes at any time point (Figure 4). B6, CD8, and TCR mice were necropsied at 10 wpi and CD4 mice were necropsied 12 wpi (8 to 10 wk after transmission ceased; Table 2). At the time of necropsy, 44% to 70% of B6, CD4, and CD8 mice and none of the TCR mice had seroconverted to MPV (Table 2). MLN from the majority of B6, CD4, CD8, and TCR mice were positive for MPV DNA at 10 to 12 wpi, whereas none of the feces from any of these mice was positive for MPV DNA, indicating clearance of the virus from the intestines but persistence in the MLN. As in the experiment with Rag, Igh, and Ifnγ mice, neither shedding nor transmission correlated with the presence of MPV DNA in the MLN of the T-cell–deficient mice.

Discussion

Of the 7 genotypes of mice investigated, B- and T-cell deficient Rag mice were clearly the most susceptible to MPV infection, with all Rag mice shedding MPV in their feces from 6 through

Table 1. Transmission of MPV from breeding mice to their offspring

	Bred (wpi)	Serology of 21-d-old pups	Fecal PCR of 21-d-old pups	Serology of litter sentinels
B6	8	5/6[a]	0/6	0/6
Ifnγ	10	6/6	0/6	0/6
Igh	16	0/6	2/6	1/6

[a]No. of mice positive for MPV/no. of mice tested

Table 2. MPV infection of adult immunodeficient and C57BL/6 mice

	Necropsy (wpi)	Index mouse		
		Serology	Fecal PCR	MLN PCR
Study 1				
B6	14	13/17[a]	0/17	0/17
Ifnγ	16	17/18	0/18	11/18
Igh	22	0/16	2/16	0/16
Rag	18	0/10	10/10	10/10
Study 2				
B6	10	7/10	0/10	7/10
CD4	12	4/9	0/10	8/10
CD8	10	6/9	0/10	7/10
TCR	10	0/10	0/10	6/10

[a]No. of mice positive for MPV/ no. of mice tested

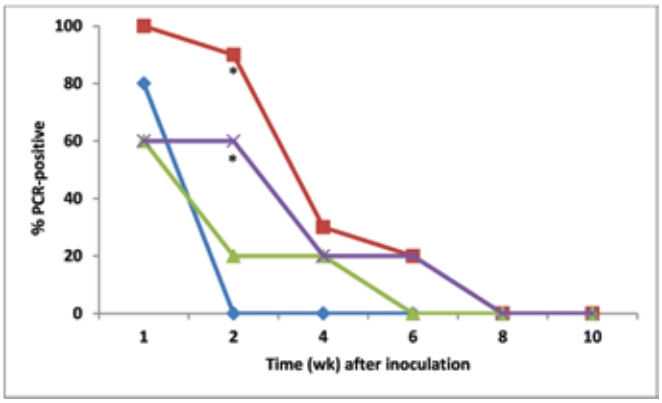

Figure 3. Percentages of MPV-inoculated mice testing positive for MPV DNA in their feces. B6 (blue diamonds), CD4 (red squares), CD8 (green triangles), and TCR (purple crosses) mice. *, Significant ($P < 0.05$) difference between values for B6 and the immunodeficient mice.

18 wpi. The profound susceptibility of the Rag mice, despite the natural resistance afforded by the B6 background, was not unexpected and is similar to previously reported findings in SCID mice on a C.B-17 background.[1] Combined B- and T-cell deficiency on either the of these backgrounds greatly increased the duration and robustness of MPV infection. However it took longer—more than 4 wk—for MPV to become established in Rag mice than in B6 and the other 5 immunodeficient genotypes of mice, in which the highest number of mice shedding MPV occurred at 1 to 2 wpi. Similarly, the highest level of transmission from Rag mice to sentinels was detected later (at 4 to 16 wpi) rather than at 1 to 2 wpi, as in the other genotypes of mice. Although the time required for all mice in cages of B- and T-cell–deficient mice to become infected has not been reported, a previous study showed that the level of MPV DNA in pooled fecal samples from SCID mice inoculated at 1 d of age rose 10,000-fold between 1 and 5 wpi and then remained

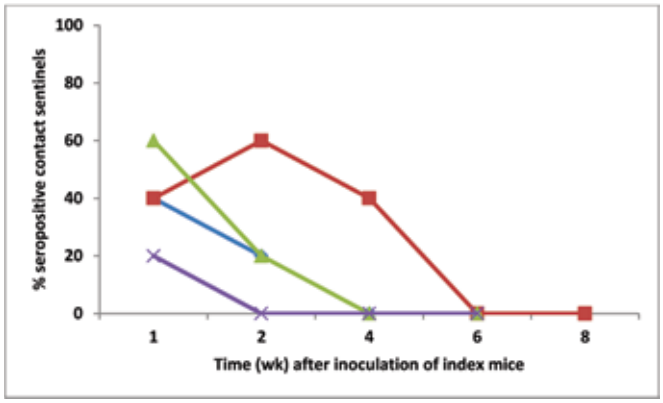

Figure 4. Percentages of sentinel mice testing seropositive for MPV that were in contact with B6 (blue diamonds), CD4 (red squares), CD8 (green triangles), or TCR (purple crosses) mice.

high through 24 wpi.[1] The delayed onset of shedding and transmission from Rag mice in our current study may reflect the presence of fewer target cells within the intestine for MPV to infect initially. However, as MPV infection was established, target cells may have been recruited to the intestine and become infected, and the infection then progressed without interference from the adaptive immune response. The high percentage of Rag mice that had MPV DNA in their feces at 18 wpi was mirrored by the data from the MLN, indicating efficient dissemination of the virus to lymphoid tissues. This pattern is consistent with the documented dissemination of MPV to the mesenteric and peripheral lymph nodes, spleen, and thymus in SCID mice.[1] Although we did not breed Rag mice in the current study, previous results in SCID mice suggest that MPV would have been transmitted to neonatal Rag mice from the infected adult mice.[1]

Like the Rag mice, B-cell–deficient Igh mice, which have only rudimentary B cells and cannot make antibodies, shed and transmitted MPV longer than did B6, Ifnγ, and T-cell–deficient mice. The difference between Rag and Igh mice was that peak MPV shedding in Igh mice occurred earlier (during the first 2 wk) and was not sustained at high levels. Rather, shedding from Igh mice tapered, with sporadic shedding from only a few mice after 6 wpi, and shedding appeared to cease from all mice at several time points. Specifically, we showed that MPV DNA was undetectable in the feces of Igh mice in 3 cages at 10, 12, and 20 wpi but then MPV DNA was detected again at 14, 16, 18, and 22 wpi. It is important to note that in 2 cages, MPV DNA was not detected in feces for 12 to 14 wk before it was detected again. Although the tissues in which MPV persisted during these times when shedding was not detected is unknown, MPV DNA was not detected in the MLN of Igh mice at 22 wpi, suggesting that MPV may not persist in the lymphoid tissues of Igh mice. It also cautions against using the presence or absence of MPV DNA in MLN to predict transmission risks.

In both Rag and Igh mice, the pattern of MPV transmission to sentinels correlated with shedding, indicating that the level of virus required to detect MPV DNA in feces by PCR was similar to that required to initiate infection. Igh mice were bred at 16 wpi, given the negative serology of contact sentinels at 12 wpi. However, retrospective analysis of serology from sentinels exposed to Igh mice at 14 and 16 wpi revealed that a few mice had resumed MPV shedding at levels sufficient to infect the sentinel mice and 2 litters of Igh mice. Taken together, these data highlight the risk of Igh mice, and possibly other strains of B-cell– or antibody-deficient mice, as a covert source of seemingly sporadic MPV infections in laboratory mouse colonies.

Although we did not note any clinical signs in MPV-infected Igh mice on a B6 background, Igh mice on a NOD background that were coinfected with MPV and minute virus of mice presented with runting, reduced fecundity, and death.[26] The prolonged shedding and transmission in Igh mice points to the important role of B cells in the control and elimination of infections. The essential role of B cells and antibodies in the resolution of intestinal infections in mice is well documented. Igh (μMT) mice transmitted mouse hepatitis virus for 15 wk, 3 times longer than transmission from B6 mice,[4] and Igh mice transmitted murine rotavirus for 13 wk, 9 times longer than transmission from B6 mice.[23] Antibody was shown to be essential for the clearance of murine norovirus infections, because Igh mice became persistently infected with murine norovirus, and adoptive transfer of immune splenocytes from antibody-deficient mice into Rag mice did not result in clearance of persistent infection. However, adoptive transfer of polyclonal antimurine norovirus antisera limited infection in Rag mice.[3] In addition, Igh mice have various intestinal abnormalities that could affect, directly or indirectly, MPV shedding from these mice. For example, B-cell–deficient mice have substantially altered immunity and metabolism gene expression in the jejunum, Igh mice have increased intraepithelial $CD8^+$ T cells, and Igh, SCID, and Rag mice have accelerated intestinal epithelia cell turnover.[27,34]

That none of the Rag and Igh mice seroconverted to MPV eliminates the use of direct serologic testing of individual mice as a diagnostic tool in these strains. In particular, Igh mice represent a considerable challenge, because there is a substantial risk that detection of MPV infection can be missed if only a single fecal sample is tested. Unlike Rag mice, in which MPV shedding is consistent after 4 wpi, shedding in Igh mice is sporadic and underscores the need to collect multiple fecal samples over several weeks to ensure that the infection has been cleared. Because infection of Rag mice takes several weeks to become established, it also is important to collect samples from Rag mice over several weeks, because early infection could otherwise be missed.

Ifnγ mice have increased susceptibility to many infections, because interferon γ (type II interferon) is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections and for tumor control.[32] In particular, Ifnγ is produced predominantly by natural killer and natural-killer T cells as part of the innate immune response and by activated T cells (CD4+ Th1 and CD8+ cytotoxic effector T lymphocytes) once antigen-specific immunity develops. Thus, Ifnγ is an important component of the host response to viral infections and plays a key role in the regulation of the innate and adaptive immune responses.[32] Surprisingly, Ifnγ deficiency in mice did not lead to prolonged MPV shedding or transmission, compared with that in B6 mice. This finding is in sharp contrast to the effect Ifnγ deficiency had on mouse hepatitis virus infection, which resulted in severe disease in Ifnγ mice that was characterized by a lethal multisystemic infection that included hepatic necrosis, necrotizing gastrointestinal tract lesions, and acute peritonitis and pleuritis with adhesions on the serosal surface of the viscera.[5,10,16] In addition, Ifnγ receptor-deficient mice maintained higher loads of murine polyomavirus in the kidney and had higher tumor incidence than did B6 mice.[37] As we illustrate here, not all viruses are uniformly susceptible to the actions of interferons—some have developed strategies to evade interferon responses. Precisely how interferons control viral infections—the interplay between the interferon response and other host defense mechanisms—is not fully understood,

and it is therefore difficult to draw conclusions about why MPV infection in Ifnγ-deficient mice did not differ from that in the B6 mice we tested.[15,29]

The percentages of B6 mice that were seropositive for MPV were similar (70% to 76%) in our 2 experiments. Likewise, the percentages of B6 mice that were positive for MPV DNA in their feces at 1 wpi (80% to 83%) were similar. The fact that 70% of the MLN from the second group of B6 mice was positive for MPV DNA at 10 wpi but none of the MLN from the first group of B6 mice was positive at 14 wpi suggests resolution of MPV infection from the MLN during this 4-wk time period. MPV was detected in the majority of MLN from Ifnγ mice at 16 wpi, similar to the percentage of B6 mice in which MPV DNA was detected in MLN at 10 wpi. Therefore, perhaps MPV persists for longer in the MLN of Ifnγ mice than of B6 mice. Breeding of Ifnγ mice did not result in transmission of MPV to litters, despite the detection of MPV in the MLN of many of the Ifnγ mice at the times the litters were weaned.

Our second experiment focused on the effect of T-cell deficiency on MPV shedding and transmission. The only significant difference in shedding occurred at 2 wpi, when more CD4 and TCR mice shed MPV than did B6 mice and more CD4 than CD8 mice shed MPV. However, these differences did not translate into differences in transmission. MPV disseminated to MLN in all genotypes of T-cell–deficient mice, with the percentage of mice with MPV DNA present in the MLN at the conclusion of the study similar to that of B6 mice. In general, both T and B cells and their associated cytokine production are required for optimal function of adaptive immunity to viral infection. For MPV infections, when B cell function is normal, the lack of T cell functions apparently has minimal effect on MPV shedding or transmission. MPV shedding and transmission was not affected in CD8 mice, although the dose of MPV used was not sufficient to infect both mice in one cage of CD8 mice. TCR mice similarly did not show increased susceptibility to MPV infection, and the dose of MPV used again was not sufficient to infect both mice in one cage of TCR mice, possibly suggesting that the dose of virus needed to infect TCR and CD8 mice is higher than that for B6 mice. Somewhat unexpectedly, none of the TCR mice seroconverted to MPV, and therefore direct serologic testing of TCR mice should not be used as a diagnostic tool for detecting MPV infection. In fact, TCR mice infected with murine rotavirus manifest impaired antibody responses.[11] Comparable levels of antirotavirus IgM were detected in the serum of TCR and B6 mice, but levels antirotavirus IgG in the TCR sera were reduced by more than 2500-fold and levels of antirotavirus IgA in the sera and intestine were reduced 175- to 250-fold compared with those in B6 mice.[11] Given the effect that the lack of antiMPV antibody production had in Igh mice and the fact that the TCR mice did not seroconvert, it is surprising that MPV shedding and transmission in TCR mice was no different than what occurred in B6 mice. In addition, it is interesting to note that in TCR mice, the time courses for detection of MPV by fecal PCR and by contact sentinels were not well correlated, given that detection by contact sentinels occurred only at 1 wpi whereas detection by fecal PCR analysis occurred through 6 wpi. T cells are targets for MPV infection and because the maturation of αβ T cells in the thymus is blocked in TCR mice, these mice have only 8% of the normal number of T cells.[24] Therefore perhaps only low levels of virus (below those necessary to transmit infection) are produced in the intestine, because there are fewer target cells for MPV to infect. However, TCR mice have normal numbers of mature γδ T cells, which might serve as targets for MPV replication or facilitate viral clearance.[20] Furthermore, MPV may infect B cells in TCR mice, because H1 parvovirus has recently been shown to infect human B cells.[25]

MPV infections are unpredictable, likely a result of the many factors that modulate infection. In reality, we have only begun to understand the factors that modulate MPV infection. Because B6 mice are, by far, the most common background for genetically engineered mice and because MPV-infected B6 mice have a very short transmission window, we assessed the transmission risks in a manner that defines categorical transmission risks. This information is especially important when dealing with MPV in large cohorts of genetically engineered mice, for which only general categories of immunocompetencies are known or can be presumed, depending on the nature of the genetic engineering. It is not surprising that an absolute deficiency in both T and B cells resulted in high, long-term shedding and transmission risks, given that both the humoral and cell-mediated arms of the immune system were compromised. However, B-cell–deficient, but not T-cell–deficient, mice represent an increased risk for MPV transmission. In addition to understanding the risk associated with immunodeficiencies, other factors such as age and mouse genotype and strain of MPV need to be considered when designing strategies to address control and elimination of MPV.[14,17,31] For example, pregnant outbred Swiss Webster mothers exposed to an MPV-infected mouse around the time of parturition do not transmit MPV to their pups. However, when Swiss Webster dams with litters were exposed to an MPV-infected mouse at 7 or 14 d postpartum, the pups became infected and transmitted infection to sentinel mice.[8] Thus, exposure of pregnant mice until parturition to MPV did not result in transmission to pups and suggests that removal of pregnant mice from cohorts of MPV-infected or potentially infected mice could be used as a measure to control the spread MPV in mouse colonies. There also has been a concern that using older mice as sentinels may compromise MPV detection, as demonstrated in a study that showed that MPV infection was more difficult to detect in 12-wk-old ICR mice than in 4- or 8-wk-old ICR mice by using a MPV NS1 ELISA.[2] However we have shown recently that the age of outbred soiled bedding sentinel mice (4 to 44 wk old) does not affect the ability to detect MPV infection in experimentally and naturally infected Swiss Webster mice.[2,12] In addition to age and pregnancy factors that need to be considered, some genotypes of mice are more susceptible to MPV. For example, BALB, C3H, DBA/2, and outbred mice are more susceptible to MPV than are B6 mice.[2,7,18]

The use of PCR screening of MLN has emerged as a screening strategy for MPV. However, MPV can frequently be detected in the feces or MLN of mice at levels that are below that necessary to infect mice in contact with these MPV-infected mice. Because we sampled the MLN from different genotypes at different time points, we were unable to determine the contribution of the immune deficit to the absence or presence of MPV DNA in MLN. However, the presence of DNA in the MLN did not appear to increase the risk of MPV transmission to offspring and was not predictive of the past or future shedding or transmission level. PCR screening of feces frequently is used to detect real-time shedding of MPV. The percentage of cages containing mice that were shedding MPV was similar to the percentage of individual mice with MPV DNA-positive feces for each of the 7 mouse genotypes we evaluated, suggesting that either pooled or individual feces can be used to determine the status of a 'cage' when managing an outbreak. Another diagnostic option is to survey the environment for MPV. We have shown that MPV can be readily detected on cage components that have housed MPV-infected mice[18] and that all cage components can readily

transmit MPV infection to mice that come in contact with them.[9] In addition, we have shown that the cages and cage components can be decontaminated by washing alone, making it possible to stop cage-based spread without the labor, time, and expense of autoclaving of the cages.[9]

Collectively, the studies presented here and those published previously will aid laboratory animal professionals in better stratifying transmission risks of immunodeficient mice that are often intermixed within colonies of genetically engineered mice. Our current study showed that T-cell–deficient and Ifnγ–deficient mice on a B6 background likely do not present an increased risk for spreading MPV infection, and elimination of infection from these mice likely will be amenable to test-and-cull strategies.[17] However, mice with deficiencies in B cell function or in antibodies pose an increased risk for transmitting MPV infection to other mice with the colony, given their prolonged and intermittent shedding. Not surprisingly, mice with combined B and T deficiencies (for example, Rag and SCID mice) pose the highest risk. Attempts to eliminate MPV from Igh and Rag mice in endemically infected colonies by using embryo rederivation should be approached with caution, because this method has been shown to be ineffective in C.B-17scid mice.[1] Because B6 mice are naturally more resistant to MPV infection than are BALB, C3H, and DBA/2 mice, B-cell deficiency or combined T- and B-cell deficiencies on these background strains of mice likely pose at least the same or greater risk for spreading MPV infection as that associated with mice on a B6 background.

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