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Prevalence of *Helicobacter hepaticus*, Murine Norovirus, and *Pneumocystis carinii* and Eradication Efficacy of Cross-Fostering in Genetically Engineered Mice

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Abstract: We investigated the prevalence of *Helicobacter hepaticus*, murine norovirus (MNV), and *Pneumocystis carinii* and the efficacy of cross-fostering for their eradication in 49 genetically engineered mouse (GEM) strains at our institute. Prevalences of *H. hepaticus*, MNV, and *P. carinii* were 33.9, 36.5, and 8.6%, respectively, and immunodeficient strains showed relatively higher prevalence of the 3 pathogens than immunocompetent strains. Additionally, the same immune phenotype strains showed similar prevalences. Furthermore, it was found that NKT cells might play a role in *H. hepaticus* resistance. Interestingly, there was a high incidence of *H. hepaticus* and MNV multiple infection. Strains with single or multiple infections of *H. hepaticus*, MNV, and/or *P. carinii* were selected, and cross-fostering was conducted. Cross-fosterings were successful at eradicating *P. carinii*, but there were some failures for *H. hepaticus* and MNV, and the efficacy of eradication was relatively low compared with previous studies. We thought that this low efficacy might have been due to persistent infection and the high suscepibility to *H. hepaticus* and MNV of immunodeficient GEM strains. Therefore, cross-fostering may be appropriate for *P. carinii* eradication, but be inappropriate for repopulation of a new breeding colony with *H. hepaticus* or MNV infected GEM strains. Our findings provide basic data on maintenance, strain susceptibility, and successful rederivation, especially for GEMs.

Key words: cross-fostering, *Helicobacter hepaticus*, murine norovirus, *Pneumocystis carinii*, prevalence

Introduction

The genetically engineered mouse (GEM) has been developed for biomedical research, and there have been many worldwide animal exchanges between researchers. The GEM is usually maintained under a qualified barrier with breeding. There are many immunodeficient GEMs, which may be more susceptible to infection and show more clinical signs than wild-type mice. For this reason, it might be hard to eradicate microbes from con-

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taminated GEMs. Thus, to minimize microbiological contamination, some laboratory animal institutes keep immunodeficient mice in an isolated area, or conduct Caesarean sections or perform embryo transfer for all newly introduced GEMs. There are some pathogens, such as *Pneumocystis carinii*, *Helicobacter hepaticus*, and Murine norovirus (MNV) which are pathogenic only to immunodeficient mice [25] and they have recently been added to a specific pathogen list [13, 25, 34]. *H. hepaticus* was isolated and characterized from an A/JCr mouse in 1994 [31]. It has been found to cause colitis in IL-10$^{-/-}$, TCR-$\alpha^{-/-}$, TCR-$\beta^{-/-}$, and TCR-$\alpha\beta^{-/-}$ mice [7, 16], and colitis and colon cancer in Tgfβ1$^{-/-}$ Rag2$^{-/-}$, Gpx$^{-/-}$, and BALB/c.Rag2$^{-/-}$ Apc$^{Min/+}$ mice [6, 24]. Moreover, this pathogen may cause intestinal disease and gallstones in immunocompetent mice such as C57BL/6 and A/JCr [19, 21, 23, 35]. Mice are usually infected with *H. hepaticus* through fecal-oral contact, and transplacental infection can occur in immunodeficient mice [18]. In 2003, MNV was first isolated in a Rag2$^{-/-}$ Stat1$^{-/-}$ mouse shows genetic defects in the innate immune system [13]. MNV is thought to be harmless to normal innate immune mice, but it can induce clinical disease with high mortality in innate immunodeficient mice [22]. However, MNV does not induce disease in adaptive immunodeficient mice such as Rag1$^{-/-}$ and Rag2$^{-/-}$ [32]. *P. carinii* is a well defined pathogen that induces fatal exudative alveolitis in a severe combined immunodeficient strains. CD4^{+} T lymphocytes are important for host defense against *P. carinii* infection [2], and CD40L$^{-/-}$ mice are known to have high susceptibility to it [9].

To eradicate these pathogens, various methods have been used such as antibiotic therapy, embryo transfer, Caesarean section, and cross-fostering. In most studies, embryo transfer has shown the highest efficacy for rederivation [30], and Caesarean section can be successful for eradication of pathogens if there is no transplacental transmission. However, these surgical methods are expensive and time-consuming. Thus, simple methods like antibiotic therapy and cross-fostering have been investigated as an alternative. Antibiotic therapy and cross-fostering have been conducted to eradicate *H. hepaticus* [5, 14, 15, 28, 33], and intranasal immunization and cross-fostering have been studied for preventing or eliminating MNV [4, 11]. In addition, antibiotic therapy and Caesarean section was successful for *P. carinii* eradication [20].

Although antibiotic therapy is one of the simplest methods for eradicating *H. hepaticus* and *P. carinii*, there may be individual or strain variations in treatment efficacy. It may also induce antibiotic-resistant microorganisms and it cannot be used for MNV eradication. Thus, we studied cross-fostering as a rederivation method. We assumed that: 1) because we used a filter top cage and not an individual ventilation cage system (IVCS) for 2 years (2004–2005), microbiological contamination may have spread in our facility; and 2) if mice were unable to naturally eradicate the pathogens, contaminated mice would transmit them while being bred. Based on these assumptions, we monitored *H. hepaticus*, MNV, and *P. carinii* in 49 GEM strains, investigated the prevalence of each strain and assessed the efficacy of cross-fostering of infected mice in order to provide valuable data on maintenance and clearance of pathogens from GEM breeding colonies.

Materials and Methods

GEM and health monitoring

The Center for Animal Resource and Development, Bio-max Institute, Seoul National University, which was opened in 2002 and has maintained about 250 GEMs, is one of the core research facilities for GEMs in Korea. All mice housed at this facility were kept in filter top cages for the first 2 years (2004–2005) of breeding, and in IVCS (Thoren casing systems, PA, USA) since 2006, on corn cob bedding (Bed-O' Cob, Anderson's Inc., Maumee, OH, USA). The mice used in this study, 4 to 32 weeks of age, were conditioned in the same animal room at 24 ± 2°C and 50 ± 5% humidity with a 12/12 (light/dark) cycle. Mice were given *ad libitum* access to irradiated mouse feed (Purina Korea, Seoul, Korea) and 2 ppm chloride added reverse osmosis water.

GEMs were introduced to our facility from worldwide sources such as commercial vendors and research institutes. In cases of microbiological contamination, hysterectomy was conducted before their introduction. *H. hepaticus*, MNV, and *P. carinii* have been monitored since 2006 using quarantined mice, random selected

wild-type siblings, retired breeder, and sick mice, since there might be many GEMs positive for these 3 pathogens. In addition, if a mouse was found to be contaminated by specific pathogens, rederivation was conducted using hysterectomy, though *H. hepaticus*, MNV, and *P. carinii* have not yet been designated as specific pathogens at our institute. ICR mice have been maintained in the isolated breeding area using IVCS, free from specific pathogens including *H. hepaticus*, MNV, and *P. carinii*. This study was approved as an animal use protocol by the Institutional Animal Care and Use Committee of our university.

Monitoring was conducted using 236 mice representing 46 strains of GEM for *H. hepaticus*, 192 mice representing 46 strains for MNV, and 268 mice representing 48 strains for *P. carinii*. Based on previously reported data, brief immune phenotype information of each strain is presented in Table 1.

Screening of Helicobacter hepaticus, MNV, and Pneumocystis carinii

To investigate the prevalence of *H. hepaticus* and *P. carinii*, PCRs were conducted. DNA was extracted from the cecum and lung tissues by a DNA extraction kit (Bioneer, Daejeon, Korea). Genes of *H. hepaticus* and *P. carinii* were amplified with premixture taq (Takara, Otsu, Shiga, Japan) according to the manufacturer's instructions. The specific primers were B38 (5'-GCATTTGAAACTGTTACTCTG-3') and B39 (5'-CTGTTTTCAAGCTCCCC-3') for *H. hepaticus* [27], and pAZ102-E (5'-GATGGCTGTTTCCAAGCCCA-3') and pAZ102-H (5'-GTGTACGTTGCAAAGTACTC-3') for *P. carinii* [1].

ELISA was conducted to examine MNV using an ELISA kit for MNV (Charles River Laboratories, Wilmington, MA, USA) according to the manufacturer's instructions.

Cross-fostering

Strains single or dually infected with *H. hepaticus*, MNV, and/or *P. carinii* were selected as shown in Table 2, and mating was conducted. Before parturition, dirty bedding was changed and males were removed from the cage. Within 24 h of parturition, all pups were transferred with disinfected forceps to a pathogen-free ICR foster mother in a bio-safety cabinet, and the ICR dam was moved to a separate area. The ICR foster mother was monitored every month, and was maintained *H. hepaticus*, MNV, and *P. carinii* negative. The ICR foster mother had delivered pups within 2 days prior to fostering, and all these pups were removed before cross-fostering. Microbiological monitoring was conducted to evaluate the successful rederivation using 1 or 2 GEM pups per litter and the ICR foster mother, 4 to 5 weeks after the transfer day.

Results

Prevalence of H. hepaticus, MNV, and P. carinii

Prevalences of the 3 pathogens were calculated by the percentage of infected mice relative to the total number of examined mice (Table 1). Prevalences of *H. hepaticus*, MNV, and *P. carinii* were 33.9 (80/236), 36.5 (70/192), and 8.6% (23/268), respectively. Although, the number of immunocompetent strains might be too small to compare the prevalence, immunodeficient strains showed a relatively higher prevalence of the 3 pathogens than immunocompetent strains: 35.4 (80/226) vs 0.0% (0/10) for *H. hepaticus*; 37.5 (69/184) vs 12.5% (1/8) for MNV; and 8.9 (23/258) vs 0.0% (0/10) for *P. carinii*. Since MNV was monitored using the ELISA technique which detects antibody to MNV, B cell immune null strains such as NOD/SCID and NOD/SCID.IL-2rg$^{-/-}$ might show false negatives, and the actual prevalence of MNV would be higher than the ELISA results suggest.

IL-10 deficient phenotype strains, such as B6.IL-10$^{-/-}$ and B6.LDLR$^{-/-}$, showed a high prevalence of *H. hepaticus* as reported in a previous study [16]. Interestingly, almost all strains related to NKT cells (B6.gulo$^{-/-}$, BALB/c.CD1d$^{-/-}$, B6.CD1d$^{-/-}$, and B6.Jα281$^{-/-}$) showed a high prevalence of *H. hepaticus*. Some of the *H. hepaticus* infected mice, such as B6.OT-2, B6.IL-10$^{-/-}$, B6.IL-10$^{-/-}$ CD40$^{-/-}$, and B6.MHCII$^{-/-}$ β2m$^{-/-}$, showed rectal prolapse, but MNV infected mice showed no significant clinical symptoms. Some of the *P. carinii* infected mice showed emaciation and exudative alveolitis.

If results with *P. carinii* infection are excluded, 20 strains had dual infection of *H. hepaticus* and MNV, 5 strains were singly infected with *H. hepaticus* and 6

Table 1. Prevalence of *Helicobacter hepaticus*, MNV, and *Pneumocystis carinii* in various mouse lines

No.	Strain	Brief immune phenotype[a)]	*H. hepaticus*	MNV	*P. carinii*
1	NOD/SCID (NOD.CB17-*Prkdc*^{scid}/J)	↓T, B, NK, granulocyte and macrophage	**10/17**[b)]	0/12	**6/12**
2	NOD/SCID.*IL-2rg*$^{-/-}$ (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ)	↓T, B, NK and immunoglobulin	**3/4**	0/3	0/4
3	B6.Rag1$^{-/-}$ (B6.129S7-*Rag1*^{tm1Mom}/J)	↓T, B cell	**2/3**[c)]	**1/1**	**1/4**
4	B6.CD80$^{-/-}$ (B6.129S4-*Cd80*^{tm1Shr}/J)	abnormal immunoglobulin, ↓B cell			**2/2**
5	B6.IL-4$^{-/-}$ (B6.129P2-*Il4*^{tm1Cgn}/J)	abnormal Th2 physiology and humoral response	**4/6**	**4/6**	0/5
6	B6. CD80.CD86$^{-/-}$ (B6.129S4-*Cd80*^{tm1Shr} *Cd86*^{tm2Shr}/J)	abnormal T and spleen morphology	0/9	**3/8**	**6/11**
7	B6.bm1 (B6.C-*H2*^{bm1}/ByJ)	abnormal T cell physiology	**4/4**	**3/3**	0/2
8	B6.bm12 (B6(C)-*H2-Ab1*^{bm12}/KhEgJ)	abnormal T cell physiology	**2/6**	**6/6**	0/9
9	B6.CD40L$^{-/-}$ (B6.129S2-*Cd40lg*^{tm1Imx}/J)	abnormal T cell clonal deletion	**1/11**	**3/11**	**1/11**
10	B6.OT-II (C57BL/6-*Tg(TcraTcrb)425Cbn*/J)	abnormal positive T cell selection, ↑CD4$^+$ T	**7/12**	**5/7**	0/11
11	B6.OT-I (C57BL/6-Tg(TcraTcrb)1100Mjb/J)	abnormal CD8$^+$ T morphology, CTL and T cell clonal deletion	**2/9**	**1/5**	0/7
12	B6.TCRα$^{-/-}$ (B6;129S2-*Tcra*^{tm1Mom}/J)	↓CD4$^+$ T, ↓CD8$^+$ T, colitis	0/5	**1/4**	**1/10**
13	B6.TAP1$^{-/-}$ (B6.129S2-*Tap1*^{tm1Arp}/J))	lack CD8$^+$ T, Th1 ↑	**5/7**	**4/6**	0/6
14	B6.MHCII$^{-/-}$ (B6.129S2-*H2*$^{dlAb1-Ea}$/J)	lack CD4$^+$ T, bacterial infection↑	**5/7**	0/5	0/9
15	Do11 (C.Cg-*Tg(DO11.10)10Dlo*/J)	↓CD4$^+$ and CD4$^+$ CD8$^+$ in OVA	0/4	**1/5**	**1/5**
16	B6.b2m$^{-/-}$ (B6.129P2-*B2m*^{tm1Unc}/J)	abnormal IL, TNF, ↓CD8$^+$ T, ↑CD4$^+$, ↑Th1	**2/9**	**1/8**	0/5
17	B6.IL-10$^{-/-}$ (B6.129P2-*Il10*^{tm1Cgn}/J)	abnormal regulatory T cell physiology	**2/3**	**1/2**	0/8
18	B6.IFNg$^{-/-}$ (B6.129S7-*Ifng*^{tm1Ts}/J)	↓IFNg and IL-10, ↑NKT and IL-4	**2/2**	**2/3**	
19	B6.LDLR$^{-/-}$ (B6.129S7-*Ldlr*^{tm1Her}/J)	decrease IL-10	**6/7**	**7/7**	0/5
20	B6.IL-12b$^{-/-}$ (B6.129S1-*Il12b*^{tm1Jm}/J)	abnormal granulocyte, lymphocyte, macrophage, ↓IFNg, IL-17, ↑bacterial infection	0/1	**3/3**	0/4
21	B6.IL-12Rβ2$^{-/-}$ (B6.129S1-*Il12rb1*^{tm1Jm}/J)	↓IFNg	0/2	0/1	0/4
22	B6.Apoe$^{-/-}$ (B6.129P2-*Apoe*^{tm1Unc}/J)	abnormal B cell activation, ↓IFNg, ↑Ig	0/7	**1/6**	0/8
23	B6.Rag2$^{-/-}$ (C57BL/6-*Rag2*^{tm1Cgn}/J)	abnormal B cell activation	0/10	0/5	0/7
24	B6.CD14$^{-/-}$ (B6.129S-*Cd14*^{tm1Frm}/J)	↓IL-6, TNFa, ↑viral infection	**1/6**	**1/5**	0/9
25	NOD (NOD/ShiLtJ)	Autoimmune (Type 1 Diabetes)	**2/7**	**3/4**	0/11
26	B6.CD40$^{-/-}$ (B6.129P2-*Cd40*^{tm1Kik}/J)	abnormal DC, bacterial infection ↑	0/3	0/2	**1/5**
27	B6.IL-2Rg$^{-/-}$ (B6.129S4-*Il2rg*^{tm1Wjl}/J)	abnormal DC cell physiology	0/5	0/1	
28	B6.cd28$^{-/-}$ (B6.129S2-*Cd28*^{tm1Mak}/J)	abnormal NKT, ↓IFNg, IL-4	0/1	0/1	0/4
29	B6.TLR2$^{-/-}$	↓APC	**1/5**	**3/4**	0/5
30	B6.TLR4$^{-/-}$	↓APC	**1/5**	**4/6**	
31	myD88$^{-/-}$	↓APC	0/2	0/1	0/5
32	B6.TLR2-/- B6.TLR4$^{-/-}$	↓APC	0/2	0/1	0/9
33	B6. δTCR$^{-/-}$	↓NK	0/1	0/1	0/1
34	B6.gulo$^{-/-}$ (B6.129p2-*Gulo*^{tmlunc}/Mmod)	↓NKT	**5/9**	**1/4**	0/5
35	BALB/c.CD1d$^{-/-}$ (C.129S2-*Cd1*^{tm1Gru}/J)	absent NKT	**4/11**	**2/10**	0/7
36	B6.CD1d$^{-/-}$	absent NKT	**4/7**	**6/7**	0/5
37	B6.Jα281$^{-/-}$	absent NKT	**3/4**	**2/2**	0/5
38	B6.IL-10$^{-/-}$ CD40$^{-/-}$	Combined	**1/1**	0/2	
39	B6.Rag1$^{-/-}$ H2$^{-/-}$	Combined	0/3	0/1	0/8
40	B6.MHCII$^{-/-}$ b2m$^{-/-}$	Combined	**1/2**	0/3	0/5
41	B6.IL-2rg$^{-/-}$ H2$^{-/-}$ Rag1$^{-/-}$	Combined	0/2	0/5	**1/1**
42	B6.Rag2$^{-/-}$ cd14$^{-/-}$	Combined	0/2	0/2	0/6
43	B6.Rag2$^{-/-}$ TLR2$^{-/-}$ TLR4$^{-/-}$	Combined	0/3	0/5	0/8
44	B6.IL-2rg$^{-/-}$ H2$^{-/-}$	Combined			**1/4**
45	B6.IL2rg$^{-/-}$ Rag1$^{-/-}$	Combined			**2/6**
46	B6.Gal$^{-/-}$		0/2	0/1	0/2
47	B6.Muc1 Tg		0/2	0/1	0/3
48	B6.GFP Tg		0/2	**1/2**	0/4
49	B6.MMTV Tg		0/4	0/4	0/1
50	C57BL/6[d)]		0/8	0/16	0/30
51	ICR (foster mother)[d)]		0/30	0/30	0/16

[a)]Among 49 GEMs 45 strains (No. 1~45) were immnodeficient, and 4 strains (No. 46~49) were immunocompetent. [b)]Data are expressed as number of infected mice/number of total examined mice, and bold type indicates infection with each pathogen. [c)]Gray boxes indicate dual infection with *H. hepaticus* and MNV. [d)]Results of C57BL/6 and ICR dam were not included in the prevalence analysis. "↓" indicate decrease and "↑" indicate increase.

Table 2. Transmission of *H. hepaticus*, MNV, and *P. carinii* after cross-fostering of selected GEMs

No.	Strain	*H. hepaticus*		MNV		*P. carinii*	
		Original[a)]	Pup[b)]	Original	Pup	Original	Pup
1	B6.IL-4$^{-/-}$ (B6.129P2-*Il4*^{tm1Cgn}/J)	4/6[c)]	2/4[d)]	4/6	4/4		
2	B6.TAP1$^{-/-}$ (B6.129S2-*Tap1*^{tm1Arp}/J))	5/7	2/2	4/6	2/4		
3	NOD/SCID (NOD.CB17-*Prkdc*^{scid}/J)	10/17	2/6			6/12	0/6
4	B6.bm12 (B6(C)-*H2-Ab1*^{bm12}/KhEgJ)	2/6	1/4	6/6	4/4		
5	B6.Rag1$^{-/-}$ (B6.129S7-*Rag1*^{tm1Mom}/J)	2/3	1/1	1/1	0/1	1/4	0/2
6	B6.LDLR$^{-/-}$ (B6.129S7-*Ldlr*^{tm1Her}/J)	6/7	0/2	7/7	2/2		
7	B6. CD80.CD86$^{-/-}$ (B6.129S4-*Cd80*^{tm1Shr} *Cd86*^{tm2Shr}/J)			3/8	0/3	6/11	0/3
8	B6.OT-II (C57BL/6-*Tg(TcraTcrb)425Cbn*/J)	7/12	0/1	5/7	0/1		
9	B6.TCRα$^{-/-}$ (B6;129S2-*Tcra*^{tm1Mom}/J)			1/4	0/17	1/10	0/7
10	Do11 (C.Cg-*Tg(DO11.10)10Dlo*/J)			1/5	0/5	1/5	0/5
11	B6.b2m$^{-/-}$ (B6.129P2-*B2m*^{tm1Unc}/J)	2/9	0/5	1/8	0/3		
12	B6.IFNg$^{-/-}$ (B6.129S7-*Ifng*^{tm1Ts}/J)	2/2	0/1	2/3	0/1		
13	B6.Apoe$^{-/-}$ (B6.129P2-*Apoe*^{tm1Unc}/J)			1/6	0/3		
14	NOD (NOD/ShiLtJ)	2/7	0/1	3/4	0/1		
15	B6.TLR4$^{-/-}$	1/5	0/4	4/6	0/4		
16	BALB/c.CD1d$^{-/-}$ (C.129S2-*Cd1*^{tm1Gru}/J)	4/11	0/4	2/10	0/2		

[a)]Strain prevalence before rederivation. [b)]4~6 weeks old pup after rederivation using cross fostering. [c)]Number of infected mice/number of total examined mice. [d)]Gray box indicates existence of infected pup.

Table 3. Efficacy of rederivation by cross-fostering in GEMs for *Helicobacter hepaticus*, MNV, and *Pneumocystis carinii*

	Helicobacter hetaticus	MNV	*Pneumocystis carinii*
Prevalence % in infected original strains	48.8% (80/164)[a)]	51.9% (70/135)	32.4% (23/71)
Prevalence % in rederived pups	22.9% (8/35)	21.8% (12/55)	0% (0/23)

[a)]Data is expressed as percentage (number of infected mice/number of examined mice), and data of foster mothers are excluded.

strains were singly infected with MNV. The percentage of strains dually infected with *H. hepaticus* and MNV relative to the total number of H. hepaticus and/or MNV infected strains was about 65% (20/31). Additionally, one strain showed dual infection with *H. hepaticus* and *P. carinii*, and MNV and *P. carinii* dual infection was shown by 3 strains. There were also 2 triply infected strains, B6.Rag1$^{-/-}$ and B6.CD40L$^{-/-}$.

Cross-fostering

Rederivation using cross-fostering was conducted with 16 infected strains. It was successful for 10 strains, but 6 strains still remained infected with *H. hepaticus* or MNV (Table 2). Three strains, B6.IL-4$^{-/-}$, B6.TAP1$^{-/-}$, and B6.bm12, showed dual failure to eradicate *H. hepaticus* and MNV. In the case of B6.LDLR$^{-/-}$, eradication was successful only for *H. hepaticus*. *P. carinii* was completely eradicated by cross-fostering. Even though ICR dams used for the rederivation were negative for *H. hepaticus* and MNV, some of the fostered pups remained infected with *H. hepaticus* and/or MNV. The overall prevalence dropped after cross-fostering from 48.8 to 22.9% in the case of *H. hepaticus*, from 51.9 to 21.8% in the case of MNV, and from 32.4 to 0.0% in the case of *P. carinii* (Table 3). This result demonstrates that cross-fostering may be a useful method for *P. carinii* eradication and that it may decrease the prevalence of *H. hepaticus* and MNV.

Discussion

We investigated the prevalence of 3 pathogens (*H. hepaticus*, MNV, and *P. carinii*) using mice, especially focusing on GEM mice, housed in our facility. We also studied the effect of cross-fostering on the eradication of the pathogens. Previous studies of the prevalence of *H. hepaticus* showed high variation [3, 7, 10, 26], and there have been few studies of the prevalences of MNV and *P. carinii*. Thus, it is difficult to confirm whether the prevalences of the infected mice in our facility are

high or low compared to those of previous studies. The prevalence of MNV in our facility was about 36.5%, which was similar to that reported by Henderson (32.4%) [12].

MNV monitoring was conducted using a serological test, which might produce false negatives due to null B cell immunity, failure of seroconversion or an immature immune system in young mice. Thus, RT-PCR for the MNV virus may be an alternative method for measuring its prevalence. We do not know how many mice showed false negatives for MNV in the present study, however, we thought serological MNV screening might be of value for in GEMs colonies.

Pathogen prevalence may be affected by many factors such as exposure to the pathogen, cross-contamination and the sensitivity of the diagnostic assay rather than the immune phenotype. Despite the lack of controls for these factors, there were some interesting results. The importance roles of IL-10 in resistance to *H. hepaticus* and $CD4^+$ T cells in resistance to *P. carinii* were confirmed in this study [2, 16]. Additionally, NKT cell immunity might play a role in *H. hepaticus* resistance. Furthermore, there was a high incidence of *H. hepaticus* and MNV dual infection. It is uncertain whether this dual infection is due to opportunistic infection, one pathogen triggering infection by the other pathogen, and/or the GEM is susceptible independently to each of the 2 pathogens. However, since *H. hepaticus* and MNV have digestive organ pathogenicity [7, 17], simultaneous infections with *H. hepaticus* and MNV may induce high susceptibility and/or clinical signs [8].

The efficacy of previously reported cross-fostering for *H. hepaticus* was about 95–100% in wild-type mice and some immunocompetent mice [28, 29, 33], and that for MNV was 94% in Swiss Webster mice [4]. In this study, the efficacy of cross-fostering was relatively low compared with these previous studies. We thought that the low efficacy seen in our study might have been due to the high susceptibility of the pups and the persistent infection of *H. hepaticus* and MNV in immunodeficient GEM strains. In immunodeficient GEMs, persistent infection might induce bacteremia and viremia, increasing the number of shedding organisms and the possibility of infection during and just after delivery. Even though, rederivation for *H. hepaticus* and MNV using cross-fostering was successful in 10 out of 16 strains (Table 2), there might have been unexamined infected pups. Therefore, cross-fostering, screening and selection may be inappropriate for the repopulation of a new breeding colony with *H. hepaticus* or MNV infected strains. Nevertheless, cross-fostering did eradicate *P. carinii* in this study, and might be appropriate for repopulation of infected strains.

In conclusion, prevalences of *H. hepaticus*, MNV, and *P. carinii* at our institute were 33.9, 36.5, and 8.6%, respectively. Relationships between immune phenotypes and the prevalence of these pathogens were unclear, and we thought that further study, such as controlled infection experiment of immunodeficient GEMs, might give valuable results. In addition, we thought that embryo transfer might be better than cross-fostering for *H. hepaticus* and MNV eradication in GEMs.

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