Development of ELISA Using Recombinant Antigens for Specific Detection of Mouse Parvovirus Infection

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Abstract: Nucleotide sequences of mouse parvovirus (MPV) isolate, named MPV/UT, and mouse minute virus (MMV) were analyzed and used for expressing recombinant proteins in E. coli. ELISA tests using recombinant major capsid protein (rVP2) and recombinant major non-structural protein (rNS1) as antigens were developed and their performance in serologic detection of rodent parvovirus infection was assessed. MPV-rVP2 and MMV-rVP2 ELISAs reacted specifically with anti-MPV and anti-MMV mouse sera, respectively. MMV-rNS1 antigen had a wide reaction range with antisera to rodent parvoviruses including MPV, MMV, Kilham rat virus (KRV) and H-1 virus. All mice oronasally infected with MPV were seropositive at 4 weeks post-infection in screening by ELISAs using MPV-rVP2 and MMV-rNS1 antigens, but were negative by conventional ELISA using whole MMV antigen. A contact transmission experiment revealed that transmission of MPV occurred up to 4 weeks post-infection, and all cage mates were seropositive in screening with MPV-rVP2 and MMV-rNS1 ELISAs. These results indicate that MPV-rVP2 and MMV-rVP2 are specific ELISA antigens which distinguish between MPV and MVM infection, while MMV-rNS1 antigen can be used in generic ELISA for a variety of rodent parvoviruses. The higher sensitivity of MPV-rVP2 ELISA than conventional ELISA for detecting seroconversion to MPV in oronasally infected mice as well as in cage mates suggests the usefulness of MPV-rVP2 ELISA in quarantine and microbiological monitoring of MPV infection in laboratory mice.

Key words: ELISA, mouse, parvovirus, recombinant antigen

Introduction

Parvoviruses are one of the most prevalent infectious agents of laboratory rodents. In rodents, three prototypic parvovirus species have been extensively characterized: mouse minute virus (MMV; formerly minute virus of mice), which infects mice; and Kilham rat virus (KRV) and H-1 virus, which infect rats [6]. Mouse parvovirus (MPV) is a parvovirus species which infects mice but is biologically and antigenically distinct from the...
prototypic species including MMV, KRV and H-1 [1, 8, 10]. Initially, the isolated MPV strain was called mouse orphan parvovirus but it was renamed MPV-1. The original MPV-1 isolate is now designated MPV-1a, and two additional isolates of MPV-1 serotype have been named MPV-1b and MPV-1c [6]. MPV has a significant impact on research due to its immunomodulatory effects both in vivo and in vitro, although clinical symptoms and histopathologic lesions have not been reported in mice naturally or experimentally infected with MPV [5, 10]. Therefore, accurate monitoring of the MPV infection status is critical to prevent transmission within or between animal facilities and to minimize its interference in research.

Parvovirus is comprised of two structural proteins, the major capsid protein VP2 and the minor but larger VP1 protein. Two non-structural proteins, NS1 and NS2, are also synthesized and serve several functions for viral replication in parvovirus infected cells. The amino acid sequences of VP1 and VP2 vary among rodent parvoviruses and determine the serogroup for a particular strain. On the other hand, NS1 and NS2 are well conserved among rodent parvoviruses. Amino acid identities for NS1 are more than 90% among MPV, MMV, KRV, H-1 virus and rat minute virus (RMV; formerly rat orphan parvovirus) [1, 11].

Serologic testing is typically used to detect rodent parvovirus infection. The most common methods used for serologic diagnosis of MPV infection include the enzyme linked immunosorbent assay (ELISA), the indirect fluorescent antibody assay (IFA) and the hemagglutination inhibition (HI) test. Since growth of MPV in cell culture is difficult, MMV antigens prepared from infected cell lines have been used for screening MPV infection by detecting cross-reactive antibodies in ELISA and IFA format. Antigens used for conventional ELISA are MMV virions which mainly consist of capsid proteins, and those for IFA are MMV-infected cells containing non-structural proteins as well as capsid proteins. Because the cross-reactivity of anti-MPV antibodies to MMV is mainly concentrated on non-structural proteins based on the conserved amino acid sequences between MPV and MMV [9], and whole MMV antigens used for ELISA contain only a small amount of non-structural proteins, which are not incorporated into parvovirus virions, it has been reported that the sensitivity of conventional MMV ELISA for detecting MPV infection is lower than that of IFA [4]. In addition, it is difficult to distinguish whether mice are infected with MMV or MPV when the sera of mice are positive in conventional MMV ELISA or MMV IFA. Although the HI test is the principal method used to confirm serogroups of parvoviruses, it requires large amounts of purified viral antigens. A positive reaction in MMV ELISA or MMV IFA combined with a negative result in the HI test for MMV is interpreted as presumptive criteria of MPV infection.

Alternative ELISAs using recombinant NS1 (rNS1) antigen of MMV and recombinant VP2 (rVP2) antigens of MPV-1a, MPV-1b and MMV have been developed in several laboratories [2, 7, 9]. The rNS1 ELISA appears to detect infection with rodent parvoviruses broadly, therefore it is not serogroup specific. Furthermore, the rNS1 ELISA is less sensitive than rVP2 ELISA because not all mice infected with MPV seroconvert to NS1 protein [2, 7]. Meanwhile MPV-rVP2 ELISA is believed to detect anti-MPV antibodies with high sensitivity and specificity [2, 7]. Here we report on the gene cloning and expression of the VP2 protein of MPV/UT, which we newly isolated, as well as VP2 and NS1 proteins of MMV in E. coli, followed by the evaluation of ELISAs using these recombinant antigens to detect antibodies against rodent parvoviruses. MPV/UT used for producing the rVP2 protein in this study like most wild type MPV does not replicate well in currently available cell lines. Whereas, the prototype MPV strains, MPV-1a and MPV-1b, used for generating rVP2 proteins in previous studies [2, 7] were initially isolated as cell culture contaminants and adapted for growth in cell culture. In this context, MPV/UT is biologically distinct from prototype MPV strains although their VP2 amino acid sequences are highly identical. The expression system of the recombinant proteins utilized in this study is also different from those previously reported. Livingstone et al. [7] produced rVP2 as virus-like particles in a baculovirus-infected insect cell system, which needed purification by CsCl gradient ultracentrifugation, a disadvantage in large scale production. Ball-Goodrich et al. [2] expressed and extracted histidine-tagged rVP2 in an insoluble fraction of E. coli and purified it under denatured conditions. Aiming at mild and simple procedures for antigen preparation, we produced rVP2 as a GST-fusion protein which allowed us to use soluble and
undenatured conditions for extraction and purification of the recombinant proteins. The goal of this study was to develop ELISAs using recombinant antigens that can be applied to the highly specific and sensitive detection of MPV and MMV infections in mice.

**Materials and Methods**

**Viruses**

MPV/UT was isolated at the University of Tsukuba from a clinically healthy mouse introduced from a MPV-infected mouse colony in the U.S.A. The virus was propagated in ICR mice by intraperitoneal (i.p.) inoculation of liver and spleen homogenates. One week after inoculation, liver and spleen were collected and homogenized in Eagle’s minimum essential medium to prepare 10% (w/v) homogenates. The supernatant was collected after centrifugation at 2,000 rpm for 5 min at 4°C and used as inoculum for experimental MPV infection and as a source for isolating MPV DNA.

The prototype strain of MMV [MMV(p); ATCC VR-1346] was obtained from ATCC, propagated in the A9 mouse fibroblast cell line (ATCC CCL-1.4), and was used for experimental infection of mice to generate anti-MMV serum. KRV (ATCC VR-235) and H-1 virus (ATCC VR-356) were obtained from ATCC and propagated in the C6 rat glial cell line (ATCC CCL-107), then used for experimental infection of rats to generate anti-KRV and anti-H-1 sera.

**Cloning of MPV VP2 and MMV VP2 genes**

The MPV/UT DNA was isolated from liver and spleen homogenates of mice inoculated with MPV/UT. After treatment with proteinase K and SDS, DNA was extracted by phenol, precipitated by isopropanol and used as a template for PCR amplification of the VP2 gene. The primer pair was designed to generate the entire MPV-VP2 sequence: forward primer, 5′-ctagaattcATGAGTGATGGCGAG-3′, and reverse primer, 5′-gcgctcgagTTAGTAAGTATTTCTAGC-3′. To amplify the MMV-VP2 gene, the same reverse primer as that for MPV-VP2 gene was used with the MMV specific forward primer, 5′-ctagaattcATGAGTGATGGCACCAGC-3′. The amplified VP2 genes of MPV and MMV were isolated by 1% agarose gel electrophoresis and purified from the excised agarose band by using a QIAquick gel extraction kit (Qiagen, Valencia, U.S.A.). Purified VP2 genes were cloned into a plasmid vector pGEM-T Easy (Promega, Madison, U.S.A.) and the sequences were determined by using a DYEEnamic ET terminator cycle sequencing kit (Amersham Biosciences, Piscataway, U.S.A.) and an ABI PRISM 3100-Avant genetic analyzer (Applied Biosystems Japan, Tokyo).

The VP2 genes of MPV and MMV were digested from the vector with EcoRI and XhoI, whose sites were present in the primer sequences used for PCR amplification. The pGEX-5X-1 plasmid (Amersham), a bacterial expression vector was also digested with EcoRI and XhoI. Digested VP2 genes and pGEX-5X-1 plasmid were gel purified as described above, ligated by Ligation High (Toyobo, Osaka), and transformed into E.coli BL21. MMV NS1 gene was cleaved from pCMV-NS1, kindly provided by Dr. Astell (University of British Columbia), and ligated into the pGEX-4T-1 plasmid using SalI and NotI sites.

**Expression and purification of recombinant proteins**

To produce MPV-rVP2, MMV-rVP2 and MMV-rNS1 for use as ELISA antigens, each transformed E. coli clone was cultured in 1 L of Luria-Bertani (LB) medium containing 100 µg/ml ampicillin at room temperature. When the culture media had reached OD600 of 0.6-0.8, isopropyl β-D-thiogalactopyranoside (IPTG) was added to it at a final concentration of 0.4 mM and incubated for an additional 4 h at room temperature. Cultures were placed on ice for 10 min and centrifuged at 2,000 × g for 10 min. Next, the supernatant was removed, and the pellet was suspended in 50 µl of Dulbecco’s phosphate-buffered saline (PBS); then, Triton X-100 at a final concentration of 1% and dithiothreitol at a final concentration of 1 mM were added and the mix was stored at −80°C until purification. The bacterial suspension was thawed and sonicated on ice with 10 second pulses for 10 times. The soluble fraction was collected after centrifugation at 8,000 × g for 20 min, then 1 µl of 50% glutathione sepharose 4B equilibrated with PBS was added. After incubation with gentle inversion at room temperature for 30 min, the matrix was transferred to a disposable column and washed with 15 µl of PBS. Protein was eluted 4 times by incubation with 500 µl of 15 mM glutathione, 50 mM Tris-HCl (pH 8.0) at room temperature for 20 min. Usually, only fractions -1 to -3 were pooled for further use because they showed signifi-
Significantly higher OD\textsubscript{280} values than the elution buffer and fraction -4. The protein concentration of the pooled sample was determined by using the Bradford protein assay (BioRad Laboratories, Hercules, U.S.A.). To assess quality of the purified protein, 1 μg of the pooled sample was electrophoresed on 6% SDS-PAGE gel and stained with BioSafe Coomassie (BioRad). The immunological reactivity of the purified protein was confirmed by immunoblot analysis, using mouse antisera to MPV and MMV.

**Mice and experimental infections**

Four- and 10-week-old male and female Jcl:ICR mice were purchased from CLEA Japan Inc. (Tokyo) and used for infection after 2 weeks acclimation. The source colonies were specified to be free of mouse hepatitis virus, Sendai virus, ectromelia virus, lymphocytic choriomeningitis virus, Hantavirus, epizootic diarrhea of infant mice virus, pneumonia virus of mice, mouse minute virus, mouse parvovirus, mouse adenovirus, mouse cytomegalovirus, mouse encephalomyelitis virus, reovirus type3, *Mycoplasma pulmonis*, other known pathogenic bacteria, and endo- and ectoparasites.

In experiment 1, 6-week-old and 12-week-old male and female ICR mice were inoculated oronasally with 10 ID\textsubscript{50} of MPV/UT. At 4 weeks post-infection (wpi), the mice were euthanized by ether inhalation and blood was collected for sampling serum. In experiment 2, ICR female mice (index mice) of 12-week-old were inoculated oronasally with 10 ID\textsubscript{50} of MPV/UT. At 1 wpi, 4 wpi, and 8 wpi, index mice were placed in a new cage with naive 12-week-old female ICR mice. One week later, contact-exposed mice were transferred to a clean cage, retained for 3 additional weeks, then exsanguinated under ether anesthesia to collect their sera. Fresh fecal samples were collected from the index mice at 1 wpi, 4 wpi, 8 wpi, 12 wpi, and 16 wpi, and MPV in feces was detected by PCR assay. The experiment protocol was approved by the Animal Experiment Committee, University of Tsukuba and all animal experiments were performed according to the University of Tsukuba’s Regulation of Animal Experiment.

**ELISA tests**

Costar 96-well EIA plates (Corning Inc., Corning, U.S.A.) were coated with 200 μl/well of MPV-rVP2, MMV-rVP2 and MMV-rNS1 diluted to 10 μg/ml in 0.1 M carbonate-bicarbonate buffer (pH 9.8). After overnight incubation at 4°C, the plates were washed three times with 0.05% Tween 20 in PBS and 250 μl/well of 1% bovine serum albumin (BSA) in PBS was added. Plates were incubated for 1 h at 37°C, then the liquid was removed from each well, and 200 μl/well of mouse or rat sera diluted with 0.1% BSA-PBS was added, and the plates were incubated for 1 h at 37°C. After washing as before, 200 μl/well of horse-radish peroxidase (HRP)-conjugates in 1% BSA-PBS was added and the plates were incubated for 1 h at 37°C. HRP-conjugated protein A and HRP-conjugated anti-rat IgG (originally prepared for ELISA testing at ICLAS Monitoring Center Japan) were used for detecting mouse and rat antibodies, respectively. After washing, 200 μl/well of 1.5 mg/ml o-phenylenediamine dichloride containing 0.01% H\textsubscript{2}O\textsubscript{2} was added to each well and plates were incubated for 10 min at 37°C in dark. The substrate reaction was stopped by adding 50 μl/well of 3.5N H\textsubscript{2}SO\textsubscript{4} and colorimetric reactivities were read at 490 nm on a microplate reader (BioRad).

**PCR assay**

Feces collected from mice were suspended in lysis buffer (20 mM Tris-HCl [pH 8.0], 400 mM NaCl, 5 mM EDTA, 1% SDS, 180 μg/ml proteinase K) and heated at 55°C overnight. DNA was extracted from the lysate using phenol-chloroform and precipitated by isopropanol. Fecal DNA of 100–400 ng were subjected to a MPV-specific PCR assay using Pyrobest DNA polymerase (Takara, Otsu). Primers used for the PCR were 3759f 5'-GCAGCAATGATGTAACTGAAGCT-3' and 4018r 5'-CCATCTGCCTGAATCATAGCTAA-3' as described by Besselsen et al. [3]. The reaction cycles for the PCR assay were: 2 min at 94°C; 35 cycles of 5 sec at 94°C, 5 sec at 55°C, 30 sec at 72°C; and 2 min at 72°C. The PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide and visualized by UV illumination.

**Results**

**Sequence analysis of cloned DNA**

The DNA fragment amplified from MPV/UT genomic DNA using PCR primers covering the entire VP2 gene (1764-bp) had 98% and 69% nucleotide identity
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with VP2 genes of MPV-1a and MMV(p), respectively. The deduced amino acid sequence of the VP2 protein revealed that MPV/UT was more closely related to MPV-1a (98% amino acid identity) than to MMV(p) (73% amino acid identity). These results suggest that MPV/UT is a new MPV isolate closely related to MPV-1a and that the cloned DNA encodes VP2 protein. The nucleotide sequence of MPV/UT determined in this study was submitted to DDBJ/EMBL/GenBank under accession No. AB234205.

SDS-PAGE and Immunoblot analysis of recombinant proteins

Recombinant proteins expressed and purified from E. coli were analyzed by SDS-PAGE. As shown in Fig. 1, GST-fusion proteins of MPV-rVP2 and MMV-rVP2 were detected as protein bands at approximately 93 kDa and 91 kDa, respectively. The estimated molecular weight for GST-fusion protein of MMV-rNS1 was approximately 108 kDa. In immunoblot analysis, MPV-rVP2 was strongly detected with anti-VP2 serum, but very weakly with anti-MMV serum. In contrast, MMV-rVP2 showed definite reactivity with anti-MMV serum, but only faint reactivity with anti-VP2 serum. Immunoblot analysis of MMV-rNS1 revealed that this protein possesses similar reactivities with antisera to MPV and MMV.

Reactivity of recombinant proteins in ELISA with antisera to rodent paroviruses

The optimal protein concentration used for ELISA antigen was chosen on the basis of preliminary ELISA experiments using homologous antisera. All the antigens including MPV-rVP2, MMV-rVP2 and MMV-rNS1 were used at 10 µg/ml. To determine cut-off OD values for positive sera, uninfected mouse sera (1:40 dilution) obtained from ICR mice older than 6 months (n=30) were tested against each recombinant antigen. The OD value (the mean ± standard deviations) for the MPV-rVP2 ELISA was 0.084 ± 0.018, that for MMV-rVP2 was 0.072 ± 0.013, and that for MMV-rNS1 was 0.079 ± 0.031. From these results, the baseline OD values for positive sera were set at 0.2 against all recombinant antigens by calculating the mean plus three standard deviations. The specificity of each ELISA antigen was evaluated by the immunoreactivities of sera from mice experimentally infected with MPV/UT and MMV(p), and sera from rats experimentally infected with KRV and H-1. The MPV-rVP2 ELISA showed specific immunoreactivity with anti-VP2 serum and showed no reactivity with antisera to MMV, KRV and H-1. Conversely, the MMV-rVP2 ELISA specifically reacted with anti-MMV serum but not with antisera to MPV, KRV and H-1. MMV-rNS1 ELISA showed a broad reactivity with all antisera to rodent paroviruses tested. Conventional MMV ELISA using whole MMV antigen specifically detected anti-MMV serum and showed no cross-reaction with antibodies against MPV, KRV and H-1.

Detection of MPV antibodies in experimentally infected mice by ELISAs

To compare the performances of MPV-rVP2 ELISA and MMV-rNS1 ELISA to that of conventional MMV ELISA, sera from mice oronasally infected with MPV/UT were tested. No antibody was detected from sera at 2 wpi by all ELISAs (data not shown). At 4 wpi, MPV-rVP2 ELISA and MMV-rNS1 ELISA detected anti-VP2 antibodies in the sera from all mice infected at 12 weeks of age, but conventional MMV ELISA did not (Fig. 3). It was also found that only one of twelve mice infected at 6 weeks of age showed seroconversion.
Detection of MPV infection by seroconversion of cage mates and MPV-specific PCR

The results of the contact transmission experiment are summarized in Table 1. After oronasal MPV inoculation, MPV was detected in fecal samples from 2 of 5 index mice at 1 wpi and from 4 of 5 index mice at 4 wpi by MPV-specific PCR. All index mice were found to be PCR-positive at 8 wpi, but they all reverted to PCR-negative at 16 wpi. Seroconversion to MPV-rVP2 was evident in cage mates placed with the index mice at 1 wpi (5 of 5 mice) and those placed with the index mice at 4 wpi (5 of 5 mice). At 8 wpi, none of 5 cage mates showed seroconversion by MPV-rVP2 ELISA. All the cage mate sera which showed positive results in the MPV-rVP2 ELISA were also positive in the MMV-rNS1 ELISA.

Table 1. Evaluation of ELISAs for detecting contact transmission of MPV

<table>
<thead>
<tr>
<th>Tests</th>
<th>Samples</th>
<th>Weeks after inoculation of index mice</th>
<th>1</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
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<tbody>
<tr>
<td>PCR for MPV</td>
<td>Feces of index mice</td>
<td>2/5&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>4/5</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
<td>0/5</td>
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<tr>
<td>MPV-rVP2 ELISA</td>
<td>Sera of cage mate mice</td>
<td>5/5&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>5/5</td>
<td>5/5</td>
<td>0/5</td>
<td>NT&lt;sup&gt;c)&lt;/sup&gt;</td>
<td>NT</td>
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<tr>
<td>MMV-rNS1 ELISA</td>
<td>Sera of cage mate mice</td>
<td>5/5&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>5/5</td>
<td>5/5</td>
<td>0/5</td>
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<sup>a)</sup> Index mice (n=5) were oronasally inoculated with MPV. From 1 to 16 weeks after inoculation, shedding of MPV in feces of index mice were tested by PCR. Cage mate mice (n=5) were in contact with index mice in a single cage for 1 week. Sera of cage mates were collected 4 weeks after initial contact and tested by ELISAs. <sup>b)</sup> Number positive/number tested. <sup>c)</sup> Not tested.
In this report, we described the development of ELISAs using recombinant parvoviral proteins expressed in *E. coli* as antigens. The MPV-rVP2 ELISA and the MMV-rVP2 ELISA were shown to be useful for serotype-specific detection of mouse antibodies against MPV and MMV, respectively. The conventional MMV ELISA using whole virus antigen showed MMV specific reactivity similar to the MMV-rVP2 ELISA, but it could not detect anti-VP2 antibody effectively, possibly because the antigen preparation might contain only a small amount of NS protein. As expected from conserved NS sequences, MMV-rNS1 ELISA showed broad reactivity with antisera to all rodent parvoviruses tested, including MMV, MPV, KRV and H-1.

When mice experimentally infected with MPV were screened for their anti-VP2 antibodies, MMV-rNS1 ELISA showed a performance almost equal to MPV-rVP2 ELISA in sensitivity, which was more than sufficient for detecting seroconversion in most ICR mice infected with MPV/UT at 12 weeks of age. In contrast, a previous work by Besselsen *et al.* [4] indicated that ICR and several inbred mice experimentally infected with MPV-1b at 12 weeks of age generated anticapsid antibodies which were detected by the MPV HI test, but they rarely developed anti-NS1 antibodies as measured by the rNS1 ELISA of MMV. Livingstone *et al.* [7] also reported that the sensitivity of the MMV-rNS1 ELISA was only 26% of that of the MPV-rVP2 ELISA for detection of mice experimentally infected with MPV-1b. One of the reasons for the inconsistency with our findings may be the difference in MPV strains used for experimental infection. A second possibility is that MPV does not productively infect 12-week-old mice as inferred by Besselsen *et al.* [4]. However, in our study, propagation of MPV in 12-week-old mice was un questionable according to the positive PCR results in feces as shown in Table 1, and the mesenteric lymph node examined at 4 wpi in Besselsen’s study [4]. A third possibility is the difference in the expression system used for generating recombinant proteins. However, this possibility can be rejected, because the absence of anti-NS1 antibodies in mice infected with MPV-1b at 12 weeks of age was confirmed by the MVM IFA test [4]. Although rNS1 ELISA is expected to be able to detect new divergent strains of parvovirus because of conserved NS1 homology, further studies using naturally infected sera are necessary for assessing its reliability. Ball-Goodrich *et al.* [2] have reported that rNS1 ELISA is unsuitable for screening mice naturally infected with MPV due to a high rate of false-negative results.

Our data indicate that ICR mice infected with MPV/UT at 12 weeks of age showed seroconversion to VP2 and NS1, but those infected at 6 weeks of age rarely developed antibodies to both VP2 and NS1. Most of the mice infected with MPV/UT at 6 weeks of age also showed negative in the PCR assay for detecting MPV (data not shown). On the other hand, Besselsen *et al.* [4] reported that most ICR mice inoculated with MPV-1b at 4 and 8 weeks of age developed anti-VP2 and anti-NS1 antibodies efficiently. Another *in vivo* transmission study using MPV-1a showed that the MPV transmission in adult mice was equally efficient as that in infant mice [9]. Such conflicting results may be attributed to the difference in growth-permissive cell types or replication speed of the MPV strains used for experimental infection, and the difference in turnover rate of primary target cells for MPV infection such as enterocytes which depends on the age of the mice. Although MPV-1a and MPV-1b, prototype strains of MPV, are virus strains adapted for *in vitro* replication in mouse T cell lines, this phenotype is not common for most wild-type MPV isolates, including MPV/UT. Therefore caution must be taken in generalizing the findings obtained from such laboratory strains to natural infections.

In the contact transmission experiment, index mice started shedding MPV from 1 wpi, and continued shedding MPV until 12 wpi. Seroconversion of cage mates by direct contact with index mice appeared during 1 wpi to 4 wpi but no transmission was detected at 8 wpi. These results suggest that MPV-rVP2 ELISA can detect seroconversion to MPV in mice infected by direct contact, a process that resembles natural infection. Furthermore, quarantine and microbiological monitoring using direct contact sentinels are presumed to be effective for detecting MPV-infected animals only at the early stage of infection. After the early stage of infection, serological tests on the infected animals themselves may be recommended. We have presented here the evaluation data of our newly developed ELISAs which
measure the reactivity of sera obtained from mice and rats experimentally infected with rodent parvoviruses. Diagnostic serological analysis using naturally infected mouse sera are necessary for further evaluation of the specificity and sensitivity of the ELISA antigens developed in this study.

In conclusion, MPV-rVP2 and MMV-rVP2 ELISAs were more specific than the rNS1 ELISA, and the MMV-rNS1 ELISA was more sensitive than the conventional MMV ELISA for generic detection of rodent parvoviruses. Therefore, the MMV ELISA and MMV IFA test traditionally employed for serological testing of rodent parvovirus infection may have missed MPV positive sera. A recent serological survey of 2,473 mice in 83 research institutions throughout North America indicated that the prevalence of sera positive for parvoviruses (MMV or MPV) was nearly 10%, with the majority of these sera being positive for MPV [7]. A serological survey for MPV infection among laboratory mouse colonies in Japan is now taking place using our MPV-specific ELISA.

Acknowledgments

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