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Isolation of Feline Parvovirus from Peripheral Blood Mononuclear Cells of Cats in Northern Vietnam

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***Abstract*: Feline parvovirus (FPV) was isolated rather frequently from the peripheral blood mononuclear cells (PBMCs) of cats in northern Vietnam by coculturing with MYA-1 cells (an interleukin-2-dependent feline T lymphoblastoid cell line) or Crandell feline kidney (CRFK) cells (a feline renal cell line). Efficiency of virus isolation was higher in MYA-1 cells than in CRFK cells. Interestingly, among the 17 cats from which FPV was isolated, 9 cats were positive for virus neutralizing (VN) antibody against FPV, indicating that FPV infected PBMCs and was not eliminated from PBMCs even in the presence of VN antibodies in the cats.**

***Key words*: CPV, FPV, Cats, Vietnam**

Feline panleukopenia (FPL) is a highly contagious viral disease of domestic cats characterized by acute depression, anorexia, gastroenteric symptoms such as diarrhea and vomiting, and leukopenia, with a high mortality rate among nonimmune kittens (18). The causative agent, FPL virus (FPLV), belongs to the family *Parvoviridae*. Along with canine parvovirus (CPV) and mink enteritis virus, FPLV is classified in the feline parvovirus (FPV) subgroup (19). Dogs and cats affected by CPV or FPLV, respectively, suffer similar enteric diseases. The first clinical sign is pyrexia between 3 and 5 days after virus infection, and shortly thereafter virus is shed in high titers in the feces and is transmitted via the fecal-oral route (18).

Previously, Mochizuki et al (16) detected three CPV-like viruses from healthy cats and one FPLV-like virus from a dog clinically diagnosed with CPV infection, and pointed out the possibility that FPVs have been mutually transmitted between cats and dogs in nature. In addition, CPV-like virus was also isolated from a cat which manifested clinical signs of FPL, indicating that the CPV-like virus may cause diseases in cats (17).

For isolation of FPV, the feces of infected cats showing clinical signs have been commonly used as virus sources, and fecal samples are usually inoculated onto feline epithelial or fibroblast cell lines such as Crandell feline kidney (CRFK) cells (4, 9, 13). It is considered that virus shedding stops once the immune response develops, and surviving animals recover without apparent long-term sequelae (18). However, some FPV strains were isolated from the normal feces of healthy cats (15), indicating that virus is shed into the feces in some recovered cats.

On the other hand, lymphoid cells are a major target for CPV and FPLV replication in dogs and cats (1, 3). CPV replicates efficiently in both feline and canine peripheral blood lymphocytes (PBLs), whereas FPLV replicates only in feline PBLs *in vitro* (21). However, it is unknown whether the FPVs can be isolated from peripheral blood mononuclear cells (PBMCs) of naturally

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Abbreviations: Con-A, concanavalin A; CPEs, cytopathic effects; CPV, canine parvovirus; CRFK, Crandell feline kidney; FCS, fetal calf serum; FCV, feline calicivirus; FHV-1, feline herpesvirus type 1; FIV, feline immunodeficiency virus; FPL, feline panleukopenia; FPLV, feline panleukopenia virus; FPV, feline parvovirus; FSV, feline syncytial virus; IL-2, interleukin-2; PBLs, peripheral blood lymphocytes; PBMCs, peripheral blood mononuclear cells; VI, virus isolation; VN, virus-neutralizing.

infected cats. Recently, we found that feline T lymphoblastoid cell lines such as FL74 and MYA-1 cells are quite susceptible to FPV infection (6, 7). In this study, we attempted to isolate FPV from PBMCs using MYA-1 cells and found that FPV can be isolated rather frequently even in the presence of high virus-neutralizing (VN) antibodies in cats.

FL74 cells (20), a feline lymphoid cell line, were maintained in RPMI 1640 growth medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 μM 2-mercaptoethanol and antibiotics. MYA-1 cells (11), an interleukin-2 (IL-2)-dependent feline T lymphoblastoid cell line, were cultured in RPMI 1640 growth medium supplemented with 10% FCS, 50 μM 2-mercaptoethanol, 2 μg/ml of polybrene, 100 units/ml of recombinant human IL-2 and antibiotics. CRFK cells (2), a feline epithelial cell line, were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS and antibiotics.

A total of 69 blood samples of domestic cats (*Felis catus*) were collected in the Hanoi district in Vietnam in August and December 1997. Most of the domestic cats at various ages, mainly less than 4 years old, were bought from animal markets. The cats were immobilized by an intramuscular injection with 20 mg/kg of Ketamine HCl (Sankyo Inc., Tokyo) before sampling. The blood samples were collected by adding heparin for anticoagulation. All the cats used in this study were unvaccinated.

Antibodies against feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV) and FPV were detected by IFA using glass slides smeared with CRFK cells infected with each of the viruses as described previously (10). For detection of FPV antigens in cultures, an IFA using anti-FPV VP2 monoclonal antibody 2D9 (14) was performed as reported previously (6). For detection of feline syncytial virus (FSV), which belongs to the genus *Spumavirus* family *Retroviridae*, a reference anti-FSV cat serum kindly provided by Dr. T. Ishida (Nippon Veterinary and Zootechnical College, Tokyo) was used for the IFA as described previously (10).

PBMCs of cats were isolated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) and stimulated with 10 μg/ml of concanavalin A (Con-A) for 72 hr. After washing PBMCs with RPMI 1640 medium to remove Con-A, half of the culture was cocultured with CRFK cells and the other half was maintained in the RPMI 1640 growth medium under the presence of 100 units/ml of rhIL-2. The cultures of PBMCs which showed cytopathic effects (CPEs) were cocultured with MYA-1 cells, and maintained in the same medium for several more days. To determine whether the cats were viremic with FPV, 50 μl of plasma samples, which were stored at −80 C, were inoculated to FL74 cells. Two weeks after inoculation, FPV isolation was determined by the appearance of CPE and IFA against FPV.

VN antibody titer against FPV in the plasma samples of cats was measured as described previously (6).

By IFA, we demonstrated that 27 (39%), 1 (1.4%) and 36 (52%) cats among the 69 specimen cats were seropositive for FCV, FHV-1 and FPV, respectively (Fig. 1). There was no significant difference in the infection rates of the viruses between male and female cats. We have reported that the 69 cats were neither infected with feline immunodeficiency virus (FIV) nor feline leukemia virus, although 20 cats (29%) were infected with FSV (12).

Some of the cultures of PBMCs showed severe CPEs, such as cell rounding and nuclear disintegration, three to nine days after cultivation (data not shown). The observed CPEs were similar to those observed in FPLV-infected PBMCs (7). The cultures showing CPEs were cocultured with MYA-1 cells to isolate the viruses. The cocultured MYA-1 cells showed similar CPEs one to three days after cocultivation. By IFA, the MYA-1 cells cocultured with PBMCs from 15 cats were positive for FPV-specific antigens, thus confirming the isolation of FPV; however, no FPV-specific antigen was detected in the other cultures (Table 1). On the other hand, in the CRFK cells cocultured with PBMCs, several cultures showed mild and severe CPE characteristics of FPV and FSV infections, respectively. Virus isolations of FPV and FSV were confirmed by IFA. Among the 44 samples, FPV was isolated from 12 samples in the cocul-

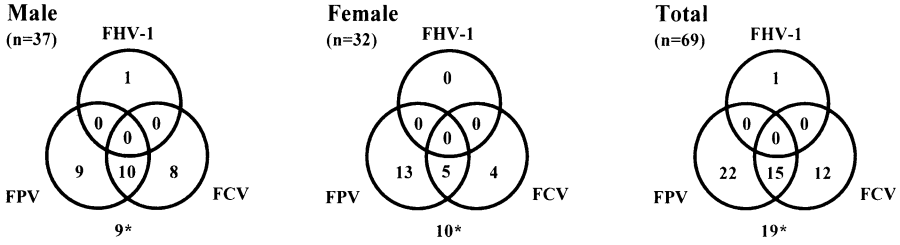

Fig. 1. Detection of antibodies against FHV-1, FPV and FCV in domestic cats in northern Vietnam. Numbers of the positive cats are shown by Venn diagrams. Number in each set represents number of cats infected with the indicated virus(es). *Number of cats which are not infected with any of the indicated viruses.

Table 1. Results of IFA and neutralizing antibody titer against FPV, and FPV isolation from PBMCs or plasma of cats

sample name	sex	IFA	VN titer	VI from PBMCs		VI from plasma
				MYA-1	CRFK	
VN113	F	−	<20	−	−	NT[a]
VN114	M	−	<20	−	−	NT
VN118	F	+	5,120	−	−	NT
VN119	M	−	<20	−	−	NT
VN120	M	+	5,120	+	−	NT
VN121	F	+	5,120	−	−	NT
VN123	F	+	>5,120	+	+	NT
VN124	M	−	<20	−	−	NT
VN125	M	−	<20	−	−	NT
VN128	F	−	<20	−	−	NT
VN129	F	−	<20	+	−	NT
VN130	F	+	>5,120	−	−	NT
VN131	F	+	2,560	−	−	NT
VN132	M	−	<20	−	−	NT
VN134	F	+	5,120	−	−	NT
VN142	M	−	<20	+	+	NT
VN145	M	−	<20	−	−	NT
VN146	M	−	<20	−	−	NT
VN148	M	+	5,120	−	−	NT
VN149	M	+	5,120	−	−	NT
VN150	M	−	<20	−	−	NT
VN153	F	+	5,120	−	−	NT
VN154	F	+	>5,120	+	−	NT
VN155	M	+	2,560	−	−	−
VN206	M	+	640	−	−	−
VN207	M	+	>5,120	+	−	−
VN208	M	−	<20	+	+	+
VN209	M	+	5,120	+	−	−
VN210	M	−	<20	+	+	−
VN211	M	−	<20	+	+	−
VN212	M	+	5,120	+	+	−
VN213	F	−	<20	+	+	+
VN214	F	+	5,120	−	−	−
VN215	F	+	5,120	−	+	−
VN216	M	+	2,560	−	−	−
VN217	M	+	5,120	+	+	−
VN218	F	−	<20	+	+	−
VN219	F	+	>5,120	−	−	−
VN220	M	+	>5,120	+	+	−
VN221	F	−	<20	−	−	−
VN222	F	−	<20	−	+	−
VN223	F	+	5,120	−	−	−
VN224	F	−	<20	−	−	−
VN225	M	+	320	−	−	−

[a] NT, not tested.

tured CRFK cells (Table 1) and FSV was isolated from 6 samples (data not shown). Without coculturing with CRFK cells, we could not isolate FSV from PBMCs. From plasma samples, we isolated FPV from only two out of 21 cats, and both the cats were seronegative for FPV (Table 1).

We conducted a VN assay to measure the VN antibody activity against FPV in the plasma samples. All the IFA-positive plasma samples have VN antibodies against FPV, ranging from $\times$320 to $>\times$5,120 (Table 1). Surprisingly, we could not find any significant difference between the results of virus isolation (VI) and VN titer (Fig. 2).

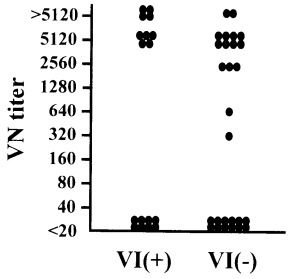

Fig. 2. Comparison of the results between virus isolation (VI) on either MYA-1 or CRFK cells and virus-neutralizing (VN) titer determined on FL74 cells. The data are taken from Table 1 and each dot represents a single sample.

In this study, we isolated FPV from the PBMCs of cats irrespective of the presence of VN antibody. These data indicate that FPV infects feline PBMCs *in vivo*. The seronegative cats from which FPV was isolated were considered to be at the early stage of infection before eliciting VN antibodies. On the other hand, the fact that FPV could be isolated from cats with high VN antibody titers indicated that FPV was not eliminated from the PBMCs of cats even under the presence of VN antibodies. These data may suggest the possibility that some FPVs are present in the PBMCs under high VN antibody activity in cats. Some lymphotropic viruses such as FIV and FSV can be isolated from cats with neutralizing antibodies (10). However, to our knowledge, this is the first report on the isolation of FPV from the PBMCs of cats with high VN antibody activity.

Epithelial or fibroblast cell lines such as CRFK cells have been used for the isolation and propagation of FPV. Recently, we reported that feline T lymphoblastoid cell lines such as FL74 and MYA-1 cells are more sensitive to FPV than CRFK cells and show prominent CPEs after FPV infection (6). Although FL74 cells are more sensitive to FPV than MYA-1 cells (6), FL74 cells produce feline leukemia viruses. Therefore, it was expected that MYA-1 cells were suitable for the isolation and propagation of FPV excluding the FeLV in the FPV isolates. In this study, the frequency of FPV isolation was higher in MYA-1 cells (15/44) than CRFK cells (12/44), suggesting the usefulness of MYA-1 cells for the isolation of FPV. However, we failed to isolate FPV using MYA-1 cells in two samples in which FPV was isolated using CRFK cells. Since both the isolates can infect and kill the MYA-1 cells (data not shown), the reason for the failure of the FPV isolation was that quite low

numbers of PBMCs were infected with FPV and the infected cells were lost during the passage of PBMCs.

Previously, we reported that both FHV-1 and FCV can infect PBMCs and feline T lymphoblastoid cell lines and induce severe CPEs rapidly in the cells (5, 8). In the domestic cats in northern Vietnam, the infection rate of FHV-1 was quite low while that of FCV was relatively high. In this study, we could not isolate FCV from PBMCs using MYA-1 and CRFK cells, suggesting that FCV was not present in the PBMCs.

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