New Quantitative Methods for Detection of Feline Parvovirus (FPV) and Virus Neutralizing Antibody against FPV Using a Feline T Lymphoid Cell Line

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ABSTRACT. Previously, we reported that a feline T lymphoid cell line, FL74 cells, was very sensitive to feline parvovirus (FPV) infection. In the present study, we developed new quantitative methods for detection of FPV and virus neutralizing antibody against FPV using FL74 cells. The methods presented here were very simple and applicable to both canine parvovirus and feline panleukopenia virus. — KEY WORDS: CPV, FPLV, neutralization test.

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Feline panleukopenia virus (FPLV) and canine parvovirus (CPV) are classified as host range variants of the feline parvovirus (FPV). FPLV causes acute depression, gastroenteric symptoms such as diarrhea and vomiting, and lymphopenia, with a high mortality among nonimmune kittens [11]. In CPV-infected dogs, acute myocarditis and hemorrhagic enteritis are generally observed [11].

The induction of virus neutralizing (VN) antibody is one of the most effective host defense mechanisms against virus infections. Thus, monitoring VN antibody against FPV is important to evaluate immune responses and vaccine efficacy against FPV infection in cats. In previous studies on FPV [1, 3–5, 7, 13], adherent cells, such as Crandell feline kidney (CRFK) cells [2], were used for titration of FPV and VN antibody against FPV. However, since FPV-infected CRFK cells did not generally show remarkable cytopathic effect (CPE), May-Grünwald-Giemsa stainings, immune-staining methods or hemagglutination (HA) tests were needed to detect the FPV antigens. Recently, we reported that FPV rapidly induced apoptosis to the infected feline or canine lymphoid cells [5]. A feline lymphoid cell line, FL74 cells [12], showed rapid and profound CPE after FPLV or CPV infection [5]. A canine lymphoid cell line, CL-1 cells [10], showed CPE by CPV but not FPLV infections [5]. Thus, the two lymphoid cell lines might be applicable to a quantitative method for titration of FPV and VN antibody against FPV. In the present study, we compared the sensitivity of CRFK, FL74 and CL-1 cells against FPLV and CPV, and showed that FL74 cells were most sensitive to both FPLV and CPV infection. By means of FL74 cells, we developed a new sensitive quantitative method for detection of FPV and a new simple VN assay against FPV.

Feline and canine lymphoid cell lines, FL74 and CL-1, respectively, were maintained in RPMI 1640 growth medium supplemented with 10% fetal calf serum (FCS) and antibiotics [10, 12]. CRFK cells [2] were grown in Dulbecco's modified Eagle's medium supplemented with 8% FCS. TU1 strain of FPLV [6] and Cp49 strain of CPV type 2 [1, 8] were used in this study. Stock viruses of TU1 or Cp49 strain were derived from the infected CRFK cells as described previously [5]. The 50% tissue culture infective dose (TCID50) was calculated by the method of Behrens-Kärber. For detection of FPLV or CPV antigen in CRFK cells, the indirect immunofluorescence (IF) assay was performed as reported previously using an anti-VP2 monoclonal antibody 2D9, which can react with both FPLV and CPV [5, 9].

Firstly, to compare the sensitivity of CRFK, FL74 and CL-1 cells against FPLV and CPV, we titrated TU1 or Cp49 strain in the three cell lines. The assay was carried out in a 96-well flat bottom microplate. Ten-fold serially diluted viruses (1:5 to $1:5 \times 10^7$) were distributed at 100 μl/well into 12 wells of a 96-well microplate. Then 100 μl of CRFK, FL74 or CL-1 cells (2×10^4 cells/ml) were added to the wells in quadruplicates. Consequently, the total volume was 200 μl/well and each well contained the diluted viruses ranged from 1:10 to $1:10^8$. The inoculated CRFK cells were harvested by trypsinization 7 days post inoculation (p.i.) and FPLV or CPV infection was examined by the indirect IF assay. On the other hand, in FL74 and CL-1 cells, the virus infection was determined by appearance of their CPE.

The titers of TU1 and Cp49 strains in CRFK cells reached $10^{4.25}$ and $10^{4.5}$ $TCID_{50}$/100 μl, respectively. In FL74 cells, the ten-fold diluted FPLV or CPV showed the typical CPE as early as 1 day p.i. and the end point dilution of FPLV or CPV showed the CPE 3 or 4 days p.i., respectively. The titers of TU1 and Cp49 strains in FL74 cells reached $10^{4.5}$ and $10^{5.25}$ $TCID_{50}$/100 μl, respectively. In CL-1 cells, the ten-fold diluted CPV showed the typical CPE as early as 2 days p.i. and the end point dilution of CPV showed the CPE 9 days p.i. The titer of Cp49 strain in CL-1 cells reached $10^{2.0}$ $TCID_{50}$/100 μl. In addition, although TU1 strain did not show any CPE in the CL-1 cells in the present study, it was repeatedly confirmed that TU1 strain could grow and cause CPE in CL-1 cells when the cells were infected with the virus at much higher multiplicity of infection. From these results, it was clearly demonstrated that the system using FL74 cells was most sensitive to FPLV and CPV infections among the three cell lines. By using FL74 cells, we could determine the titer of FPLV or CPV within 4 days. Mochizuki *et al.* [8] previously indicated the possibility that transmission of parvovirus occurred between

cats and dogs more frequently than generally believed. Some FPV strains isolated from cats are reported to grow efficiently in a canine cell line and possess very similar properties to a new antigenic variant of CPV type 2 [7, 8]. These observations suggest that growth characteristics of FPV in canine cells reflect an antigenic variabilities and that the infectivity of FPV in canine cells is significant for determination of biological phenotype of the isolate. In the previous reports [7, 8], Madin-Darby canine kidney (MDCK) cells and HA tests were used to estimate the growth of FPVs in canine cells. Here, we demonstrated that CL-1 cells could be used for titration of CPV. When CL-1 cells are used in titration of newly isolated FPVs, we might easily assess the infectivity of the isolates to canine cells by CPE and show the infectivity as a titer. Thus, the titration system using CL-1 cells might be one of the useful methods to examine growth characteristics of FPVs in canine cells.

Next, we applied the system using FL74 cells to VN assay. For VN assay, TU1 strain was titrated by a newly established system using FL74 cells. Plasma samples designated as FN and CN from a specific pathogen-free (SPF) cat and an SPF dog, respectively, were used as negative controls. Two plasma samples designated as FP and CP from a cat and a dog, respectively, which had been vaccinated with commercial vaccines for prophylaxis of parvovirus infections, were used as positive controls. In addition, unvaccinated field plasma samples designated as F1 to F5 from 5 homeless cats in Vietnam were used. The VN assay was carried out in a 96-well flat bottom microplate in quadruplicate. Plasma samples were heat-inactivated at 56°C for 30 min prior to the assay. Fifty μl of serially diluted plasma samples (1:5 to 1:2,560) were mixed with 10^2 $TCID_{50}$ of TU1 strain (50 μl). A total volume was 100 μl/well and each well contained the diluted plasmas ranged from 1:10 to 1:5,120. These mixtures were incubated at 37°C for 1 hr. Then, 50 μl of uninfected FL74 cells (5×10^4 cells/m*l*) were added to the mixture and incubated at 37°C for 5 days. VN antibody titers were defined as the reciprocal of the highest plasma dilution that protected the cells from showing CPE. In addition, for detection of antibodies against FPV, the indirect IF assay was also performed using the 100-fold diluted plasma samples and TU1-infected CRFK cells.

As shown in Table 1, we could not detect any VN activity in both FN and CN. In contrast, both FP and CP had VN antibody titers of 1:640. Four plasma samples (F1, F2, F3 and F5) from Vietnamese homeless cats were shown to have high VN antibodies. On the other hand, one plasma sample (F4) did not contain detectable VN antibody. The results of the indirect IF assay were in accord with those of the VN assays (Table 1). From these results, it was clearly demonstrated that FL74 cells were applicable to the VN assay. Since we can determine the infection of FPV by the CPE in FL74 cells, the present VN assay system using FL74 cells seems to be a better method than that using CRFK and MDCK cells.

Table 1. Results of the VN assay using feline and canine plasma samples

Sample	Status	IFA	VN titer
FN	SPF	–	<10
CN	SPF	–	<10
FP	Vaccinated	+	640
CP	Vaccinated	+	640
F1	Homeless	+	2560
F2	Homeless	+	5120
F3	Homeless	+	>5120
F4	Homeless	–	<10
F5	Homeless	+	2560

In the present study, we developed a new quantitative method for detection of FPV and a new virus neutralizing assay against FPV using FL74 cells. These methods are very simple and applicable to both CPV and FPLV. Additionally, this newly established VN assay may be useful for monitoring vaccination efficacy or for screening the FPV-specific antibodies of wild carnivores.

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