免疫酵素抗体を用いた猫伝染性腹膜炎ウイルスの力価検定法

誌名	Japanese journal of veterinary science
ISSN	00215295
著者	甲斐, 一成 黒木, 雅彦 金田, 佳枝
巻/号	50巻1号
掲載ページ	p. 247-249
発行年月	1988年2月

A Titration Method of Feline Infectious Peritonitis Virus Using Immunoperoxidase Antibody

Kazushige KAI, Masahiko KUROKI, Yoshie KANEDA, Hiroshi TSURUOKA, Masamitsu KANOE, and Makoto INOUE[1)]

Departments of Veterinary Microbiology, and Veterinary Pathology[1)], Faculty of Agriculture, University of Yamaguchi, Yamaguchi 753, Japan

(Received 18 May 1987/Accepted 10 September 1987)

Jpn. J. Vet. Sci. 50(1): 247–249, 1988

KEY WORDS: FIPV, IPA

Monitering of the infection of feline infectious peritonitis virus (FIPV) has depended on the direct observation of viral cytopathic effect (CPE) developed on various cells, rather than on the detection of foci of viral antigens. Considering that the persistent infection of the virus probably occurs, the immunological method seems to be more suitable. The immunological method which is generally prevailed at present to detect the antigens of FIPV is the one using fluorescent antibody. However, because of a restricted view area of fluorescent microscope, it is less practical to screen the viral antigen foci disseminated in a dish by the fluorescent antibody method. Thus, in this report, we intended to establish an immunological method to detect the foci of viral antigens using immunoperoxidase antibody (IPA) and applied it to some FIPV-cell combinations.

UCD1 strain of FIPV and fcwf-4 cells [10] were kind gifts from Dr. K. Fujiwara (Dept. Veterinary Pathology, Faculty of Agriculture, University of Tokyo). S+L-CCC cells [4] were provided by Dr. H. Hoshino (School of Medicine, University of Gumma). CRFK cells [3] were purchased from Dainihon Pharmaceutical Co. Ltd. (Osaka, Japan). FMA cells were established from a spontaneously developed mammary adenocarcinoma of a household Japanese cat. Above four cell lines were all feline origin. Vero cells were provided by Mr. T. Yamashita (Yamaguchi Prefectural Laboratory of Animal Hygiene, Yamaguchi). LBC cells were established from a mammary tumor of an LEW/Jms rat. All cells were maintained at 37°C in Eagle's minimal essential medium (MEM) supplemented with 5% newborn bovine serum.

Cells were plated on 24 well-multidishes (Sumitomo-Bakelite Co. Ltd., Tokyo, Japan) at a concentration of 2.5×10^5 cells per well, except Vero (2×10^5 cells) and FMA (3×10^5 cells), and cultivated overnight at 37°C in a CO_2-incubator. Cells were treated with polybrene (5 μg/ml; 1 ml/well) for 1 hr before infection with the virus preparation (0.1 ml/well) appropriately diluted in culture medium. After 1 hr adsorption of virus to cells at 37°C, the cultures were overlaid with 1% methylcellulose in MEM supplemented with 2% fetal bovine serum and 0.001% trypsin, because we observed that UCD1 induced secondary plaques on fcwf-4 cells. After 48 hr incubation at 37°C, the cultures were fixed by adding methanol to a final concentration of 50% and incubating overnight at 4°C, washed thoroughly with water, dried, and then stored at −85°C until used. Fixed cultures were rinsed once with 0.1% (v/v) Tween-20 in phosphate-buffered saline (PBST; 1 ml/well), then treated with antibody which was obtained from ascitic fluid of a cat suffering from feline infectious peritonitis (FIP), reacted with FIPV antigens (1:1,000 dilution in PBST; 0.1 ml/well), and incubated at 37°C for 1 hr. The plates were washed three times with PBST (2 ml/well). Peroxidase-conjugated goat anti-cat IgG antibody (Jackson Laboratory, USA) was added to each well (1:1,000 dilution in PBST; 0.1 ml/well), and the plates were incubated at 37°C for 1 hr. The plates were washed three times with PBST, then substrate mixture modified from the one of Towbin *et al.* [12] (100 μg/ml of dianisidine, 0.0175% H_2O_2 in 0.01 M tris-HCl, pH 7.4) was added to each well (1 ml/well). The plates were incubated at 37°C for 30 min, and washed with distilled water.

UCD1 strain of FIPV induced apparent CPE on fcwf-4 and CRFK cells. The CPE formed on fcwf-4 cells showed the same characteristics and plaque-type as described previously [1], while that formed on CRFK cells resembled the focus induced by sarcoma viruses rather than a plaque. The plaquing efficiency of UCD1 on CRFK was about 20-fold lower than that on fcwf-4 cells. When the methanol-fixed cultures of UCD1-infected fcwf-4 or CRFK were tested by IPA, only plaques were stained and no other stained foci were found. This indicates that UCD1 strain

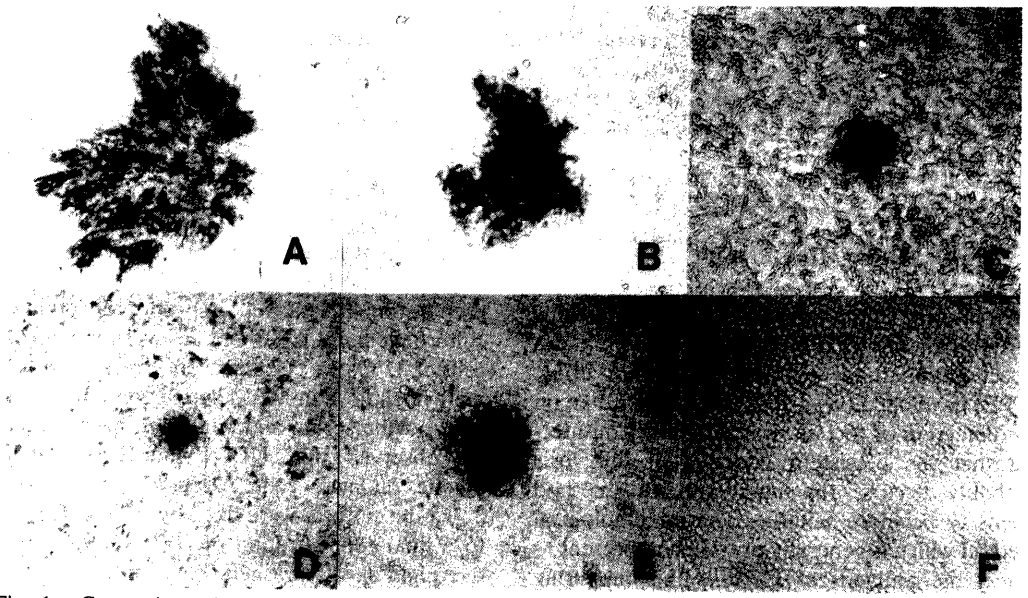

Fig. 1. Comparison of IPA-foci developed on various cell lines infected with UCD1. Cell lines (A; fcwf-4, B; CRFK, C; FMA, D; S+L−CCC, E; Vero, and F; LBC) were infected with UCD1 and treated as described in the text. (Magnification, ×80).

Table 1. IPA-focus-forming efficiency of UCD1 on various cell lines

Cell line	Virus dilution			
	10^0	10^{-1}	10^{-2}	10^{-3}
fcwf-4	ND[a)]	TMC[b)]	10.1[c)](3–18)	0.8(0–3)
CRFK	46.0(27–79)	5.9(0–11)	0.8 (0– 2)	ND
FMA	27.8(1–39)	2.0(0– 8)	0.1 (0– 1)	ND
S+L−CCC	3.5(0–10)	0	0	ND
Vero	ND	6.3(2–10)	0.5 (0– 2)	0
LBC	0	0	0	ND

a) Not done.
b) Too much to count.
c) Mean of 8 wells after 4 experiments with a range in parenthesis.

of FIPV is composed of a homogeneous virus population as described previously [1], and that the used ascitic fluid of an FIP-diseased cat recognizes no other antigens than those of FIPV.

On the other hand, UCD1 did not induce any CPE on FMA, S+L-CCC, Vero or LBC cells, at least within the first 48 hr of culture. When these cultures were tested by IPA, stained foci were found on FMA, S+L-CCC and Vero cultures but not on LBC culture. As seen in Fig. 1, the foci stained by IPA (IPA-focus or -foci) showed various sizes on respective cells, and the size decreased in the order of fcwf-4, CRFK, Vero, FMA and S+L-CCC. After four independent tests, the numbers of IPA-foci counted at various virus dilutions on respective cell lines were summarized in Table 1. Cell lines were more susceptible in the order of fcwf-4, Vero=CRFK, FMA and S+L-CCC, and LBC cells seemed unsusceptible to FIPV infection. The mode of

FIPV-infection showed almost one-hit pattern in all cases. However, the susceptibilities of CRFK, FMA and S+L-CCC cells were somewhat unstable, and the titers of IPA-foci formed on these cells varied from test to test.

In this report, we could demonstrate following points. 1) The monitering of FIPV-infection by IPA is a simple and facile method for comparing the sizes or titers of IPA-foci formed on the cells. 2) FIPV could grow in some cell lines without inducing any CPE as in the case of leukemia viruses of various animals, at least within the first 48 hr of culture. 3) FIPV could grow in a monkey cell line.

All of the feline cell lines used were susceptible to FIPV. This indicates that all cell lines derived from animals of felidae may support the propagation of FIPV though their susceptibilities differs from each other. Interestingly, though S+L-CCC line has the same root as CRFK [2], its susceptibility was fairly lower than that of CRFK, and it developed no CPE by FIPV. It is unknown whether the introduction of S+L-sarcoma virus genome into cat cells affected these phenomena. We expected FMA cells to be suitable for FIPV-propagation, because they are derived from a mammary tumor as is LBC line which sustains good propagation of rat coronavirus [7]. However the results are as shown in this paper.

Surprisingly, monkey-derived Vero cells were fairly susceptible to FIPV. This suggests that some xenogeneic cell lines other than those of felidae, are possibly susceptible to FIPV infection, and that a certain monkey may propagate FIPV *in vivo*.

The IPA method has been elucidated to be useful in titrating antibody to some viruses [5, 6, 9, 11] or to FIPV [8]. As shown in this report, it is also useful in titrating the viruses that may not induce any apparent CPE on infected cells. This fact implies that the IPA method is applicable not only to host range studies but also to seeking for susceptible cell lines to some viruses which neither induce any apparent CPE, nor are adapted *in vitro* cultivation.

REFERENCES

1. Boyle, J. F., Pedersen, N. C., Evermann, J. F., McKeirnan, A. J., Ott, R. L., and Black, J. W. 1984. *Adv. Exp. Med. Biol.* 173: 133–147.
2. Crandell, R. A., Fabricant, C. G., and Nelson-Rees, W. A. 1973. *In Vitro* 9: 176–185.
3. Evermann, J. F., Baumgartner, L., Ott, R. L., Davis, E. V., and McKeirnan, A. J. 1981. *Vet. Pathol.* 18: 256–265.
4. Fischinger, P. J., Peebles, P. T., Nomura, S. and Haapala, D. K. 1973. *J. Virol.* 11: 978–985.
5. Gerna, G., and Chambers, W. 1977. *Intervirology* 8: 257–271.
6. Gerna, G., McCloud, C. J., and Chambers, W. 1976. *J. Clin. Microbiol.* 3: 364–372.
7. Hirano, N., Ono, K., Sada, Y., Inoue, A., Murakami, T. and Takamaru, H. 1985. *Arch. Virol.* 85: 301–304.
8. Ishida, T., Washizu, T., Fukuoka, J., Toriyabe, K., Uchino, T., and Motoyoshi, S. 1987. *Jpn. J. Vet. Sci.* 49: 145–149.
9. Kodama, Y., Ogata, M., and Shimizu, Y. 1980. *Am. J. Vet. Res.* 41: 133–135.
10. Pedersen, N. C., Boyle, J. F. and Floyd, K. 1981. *Am. J. Vet. Res.* 42: 363–367.
11. Saunders, G. C. 1977. *Am. J. Vet. Res.* 38: 21–25.
12. Towbin, H., Staehelin, T. and Gordon, J. 1979. *Proc. Natl. Acad. Sci. USA* 76: 4350–4354.

要　約

免疫酵素抗体を用いた猫伝染性腹膜炎ウイルスの力価検定法（短報）：甲斐一成・黒木雅彦・金田佳枝・鶴岡浩志・鹿江雅光・井上　誠[1]（山口大学農学部家畜微生物学教室，家畜病理学教室[1]）—— 猫伝染性腹膜炎ウイルス UCD1 株を感染させた猫由来細胞 4 株のうち 2 株に細胞変性がみられたが，他の 2 株および Vero 細胞あるいはラット乳癌由来細胞では変性は観察されなかった．これらの感染細胞に抗ウイルス抗体と免疫酵素抗体を作用させると，ラット由来細胞を除くすべての細胞でフォーカス状のウイルス抗原が検出された．