



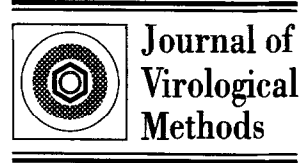
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Short communication

Enhancement of feline infectious peritonitis virus Type I infection in cell cultures using low-speed centrifugation

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Abstract

The effects of centrifugation on the ability of feline infectious peritonitis virus (FIPV) to infect cells in culture was investigated. The infectivity titer was the highest when the plates were centrifuged at $400 \times g$ (1500 rpm) for 2 h. All five strains classified as FIPV Type I showed infectivity titers enhanced 10–100-fold by centrifugation at $400 \times g$ for 2 h. The centrifugal enhancement of infection was obtained only by centrifugation immediately after inoculation of the virus, suggesting that the enhancement occurs during attachment or adsorption of viruses to the cells. This method may be useful for the culture of FIPV Type I strains.

Keywords: Feline infectious peritonitis virus; Centrifugal inoculation; Enhancement

Feline infectious peritonitis virus (FIPV), family *Coronaviridae*, genus *Coronavirus*, produces a chronic, progressive, immunologically mediated disease in domestic and exotic cats. FIPV is classified as Type I and Type II (Pedersen et al., 1984; Hohdatsu et al., 1991a,b). The FIPV Type I virus group shows poor proliferative activity in cell culture, and a neutralization test for this virus group is also difficult. On the other hand, the FIPV Type II virus group proliferates well in cell culture, and is widely used for studying FIPV. In the case of strain 79-1146 in the Type II group, all of the base sequences have been identified (Jacobs et al., 1987; Vennema et al., 1991). Both Types I and II cause infectious peritonitis in cats, but the pathogenicity of Type II FIPV is

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greater than that of Type I FIPV (Pedersen, 1987). However, in the field, the prevalence of FIPV Type I is high, and about 70% of feline cases of FIP are due to infection with Type I (Hohdatsu et al., 1992). To help the study of FIPV Type I it is necessary to improve the method of *in vitro* culture. Some studies have shown that infectivity is enhanced by centrifugation of cells following viral inoculation onto cell cultures for influenza virus (Woods and Johnson, 1989; Seno et al., 1990), herpesvirus (Oefinger et al., 1990; Tenser, 1978), human immunodeficiency virus (Pietroboni et al., 1989), and blue tongue virus (Sundin and Mecham, 1989). Centrifugal inoculation of FIPV Type I was investigated as a technique to improve the *in vitro* infectivity of this virus.

FIPV strains UCD-1, UCD-2, NW-1, Black, KU-2 and 79-1146 were used in this study. Among these virus strains, UCD-1, UCD-2, NW-1, Black and KU-2 show cell-associated growth, and are therefore regarded as Type I virus strains in the classification of Pedersen et al. (1984). Strain UCD-1 was kindly supplied by Dr. N.C. Pedersen (University of California, Davis, CA, USA) and strains NW-1, Black and UCD-2 were kindly supplied by Dr. J.K. Yamamoto (University of Florida, USA). Strain KU-2 was isolated from peritoneal cells of an adult cat with the effusive form of FIP in this laboratory. Type II FIPV strain 79-1146, showing non-cell associated growth, was kindly supplied by Dr. H.C. Horzinek, The state University, Utrecht, The Netherlands. These viruses were grown in feline whole fetus cells (fcwf-4). The fcwf-4 cells were grown in Eagle's minimum essential medium containing 50% Leibovitz L-15 medium, 10% fetal calf serum, 100 units of penicillin per ml and 100 μ g of streptomycin per ml.

A preliminary experiment determined whether or not the centrifugal inoculation method would be effective for culture of FIPV as well. Strain KU-2, which was serially diluted 10-fold, was inoculated at 100 μ l/well onto fcwf-4 cells cultured in a 96-well microplate, and then centrifuged at 25°C and 400 \times *g* (1500 rpm) for 2 h by means of a TS-9 rotor for plates (Tomy Seiko Co., Ltd., Japan). After centrifugation, the cells were incubated in a CO₂ incubator at 37°C for 5 days. The infectious titers obtained with centrifuged cells and the control cells without centrifugation were compared. The centrifuged cells had infectious titers about 30 times as high as that in the control group, suggesting that the centrifugal inoculation method is effective for culturing FIPV as well.

The most efficient time for centrifugation at 400 \times *g* was determined by plaque assay. 1 ml of strain KU-2 was inoculated onto fcwf-4 cells cultured in a 24-well multiplate, and then the cells were centrifuged at 25°C for 0 min, 30 min, 1 h, 2 h and 3 h. After centrifugation, the cells were incubated at 37°C for 1 h, and washed with Hanks' balanced salt solution (HBSS). 1 ml/well of the medium containing 1.5% carboxymethyl cellulose was added. The cells were incubated in a CO₂ incubator at 37°C for 5 days, fixed in 10% buffered formalin, and stained with 1% crystal violet. The numbers of plaques obtained after each centrifugation time are shown in Fig. 1. The number of plaques after centrifugation for 2 h was about 20 times as large as that in the absence of centrifugation. It was also found that the number of plaques was decreased by centrifugation for 2 h or longer. The reason for the decrease in infectious titer after a certain centrifugation time is not clear, but prolonged centrifugation may affect the cells adversely. The centrifugation time may be shortened and the infectious titer may be

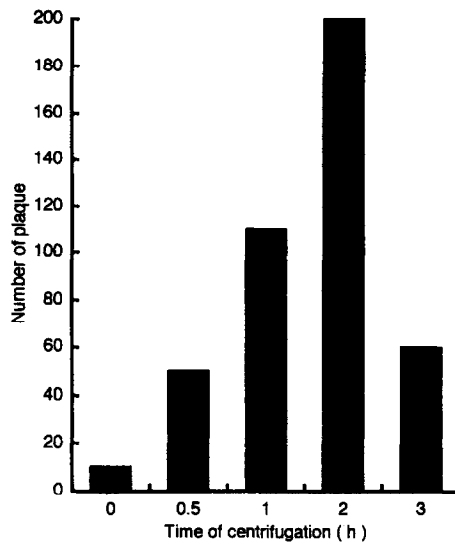


Fig. 1. Effect of the length of centrifugation time on the infectivity of FIPV KU-2 strain.

increased by centrifugation at $400 \times g$ or greater. Since the maximum frequency of rotation of the TS-9 rotor, which was used, is 1500 rpm ($400 \times g$), however, these results could not be confirmed.

An investigation was carried out to determine whether or not the centrifugal inoculation method would be effective for FIPV Type II strain 79-1146 and FIPV Type I strains other than strain KU-2. Each virus strain was inoculated onto fcwf-4 cells cultured in a 96-well microplate, and then centrifuged at $400 \times g$ for 2 h. Table 1 shows the comparison of infectious titers in the centrifuged cells with those without centrifuga-

Table 1

The effect of centrifugal inoculation on the replication of FIPV in fcwf-4 cell cultures

Virus strain	Centrifugation	Virus dilution								
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
UCD-1	with	4/4 ^a	1/4	0/4						
	without	1/4	0/4							
UCD-2	with	4/4	4/4	3/4	0/4					
	without	2/4	0/4							
NW-1	with	4/4	4/4	2/4	0/4					
	without	4/4	3/4	0/4						
Black	with			4/4	2/4	0/4				
	without		4/4	1/4	0/4					
KU-2	with		4/4	4/4	0/4					
	without	4/4	2/4	0/4						
79-1146	with							4/4	1/4	0/4
	without						4/4	3/4	0/4	

^a Number of CPE positive wells/number of tested wells.

tion. The titer was increased in all of the strains, especially Type I virus strains, suggesting that this method is effective for culturing FIPV.

The time when the effect (centrifugal enhancement) would have occurred, at the time of attachment/adsorption of viruses to the cells or thereafter, was experimentally determined in the FIPV system. The enhancing effect was studied with strain Black by plaque assay. After viral inoculation, the cells were divided into four groups according to the following methods: those that were centrifuged at $400 \times g$ for 2 h immediately after the inoculation (Method A); those that were washed with HBSS after adsorption at 37°C for 1 h, followed by centrifugation (Method B), those onto which viruses were inoculated after centrifugation of the cells alone for 2 h (Method C), and those that were not centrifuged as the controls (Method D). Infectious titers of these groups were

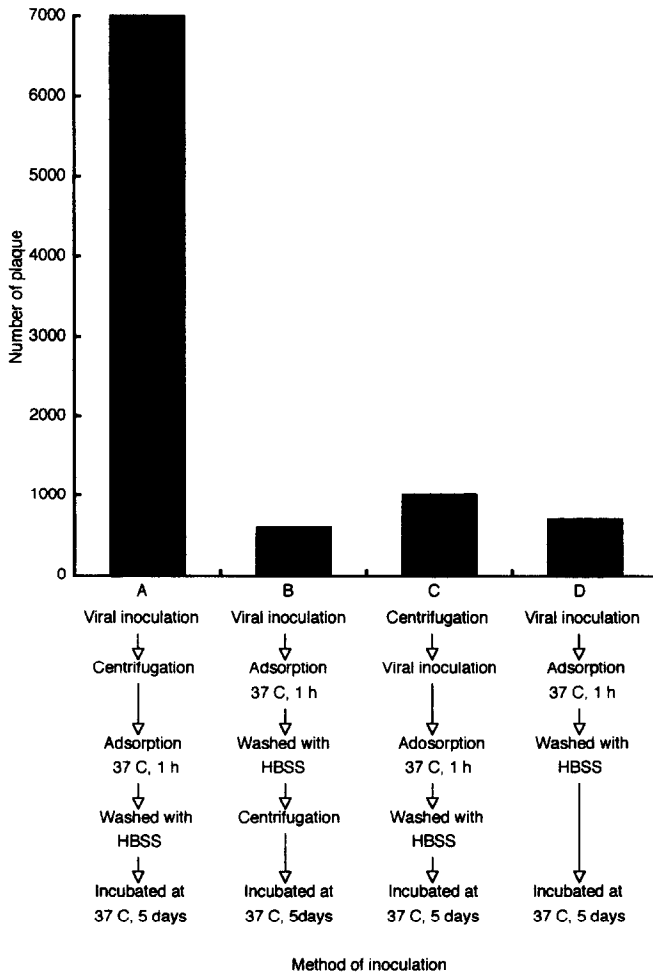


Fig. 2. Study on the mechanism of centrifugal enhancement of FIPV Black strain infectivity.

compared. The results are shown in Fig. 2. The titer was increased only when the cells were centrifuged immediately after viral inoculation. This result suggests that the enhancement of infection occurs at the time of attachment/adsorption of viruses to the cells. Even when viruses were inoculated onto cells that were preliminarily centrifuged, infection was not enhanced. Simultaneous centrifugation of the viruses and the cells was necessary for the enhancement of infection. It is impossible that centrifugation at $400 \times g$ would have directly caused an increase in the efficiency of attachment/adsorption of the viruses to the cells. Centrifugation of cells may induce changes in the cell's virus receptor, and if a virus is present there, it will become easy for it to be attached or adsorbed to the cell, possibly leading to enhancement of infection.

Prior to this study, centrifugal enhancement of infection had not been reported for any coronavirus. We have shown that the centrifugal inoculation method allowed an increase in infectivity titer of FIPV, especially Type I virus strains. This method may also be effective for the isolation of other coronaviruses whose culture is difficult.

Acknowledgement

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