

GBK細胞でのイヌロタウイルスのブラック形成

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Plaque Formation of Canine Rotavirus in Cultures of GBK Bovine Cell Line

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The occurrence of rotaviral infection has been shown in the dog by the prevalence of rotavirus antibodies [7, 10, 11, 14–16], detection of rotaviral antigen or genome, or viral particles in the feces [1, 8, 10, 12] and virus isolation in cell cultures [2, 3, 5, 9], although the significance of rotaviruses as a cause of canine gastroenteritis has not been well documented. Recently, we reported a plaque assay of bovine rotavirus in cultures of GBK cells, a stable cell line derived from bovine kidney [4]. The assay was shown to be more sensitive than that in MA-104 cell cultures.

In the present study we applied this method for plaque assay of canine rotavirus and compared with that in MA-104 cell cultures.

Established cell lines, GBK [13] and MA-104 [6] cells were grown at 37°C in Eagle's minimum essential medium (MEM) containing 10% newborn calf serum, 10% tryptose phosphate broth (TPB) (Difco) and 0.06 mg/ml kanamycin.

The RS-15 strain of canine rotavirus [9] was kindly supplied by Dr. M. Mochizuki, Department of Veterinary Microbiology, Kagoshima University, Japan. The virus was stored at –80°C until use.

Infectivity assay was made in tube cultures of MA-104 and GBK cells. Cells grown in 13×100 mm test tubes were washed three times with MEM and inoculated with 0.2 ml of virus material. After virus adsorption at 37°C for 90 min the inoculated cultures were given the maintenance medium consisting of MEM, 10% TPB, 5 µg/ml trypsin (1:250, Difco) and 0.06 mg/ml kanamycin, and were incubated at 37°C for 5 days. Infectivity titer of the virus was estimated with tube cultures by the same method previously reported [4].

Plaque assay was carried out in GBK and MA-104 cell monolayers prepared in 60-mm plastic dishes (Terumo, Japan) by the same method previously described in our paper [4].

Established cell monolayers were washed three times with MEM and inoculated with 0.2 ml of virus material diluted with maintenance medium containing 50 µg/ml diethyl-aminoethyl dextran (DEAE-dextran). After virus adsorption at 37°C for 90 min, the cultures were overlaid with 5 ml of agar medium consisting of 0.8% Agar Noble (Difco), 10% TPB, 5 µg/ml trypsin and 50 µg/ml DEAE-dextran in MEM, and incubated at 37°C for 3 days in a CO₂ incubator. The cultures were further overlaid with agar medium containing 1:5000 neutral red for plaque count. The infectious titer was expressed in plaque forming units (PFU).

GBK cell cultures prepared in a 50-ml culture bottles were inoculated with 0.2 ml of the virus stock of the RS-15 strain of canine rotavirus. After virus adsorption at 37°C for 90 min, the cultures were fed with 5 ml of maintenance medium and incubated at 37°C. CPE was detected 24 hr after inoculation, and round cells detached from the glass surface 48 hr after inoculation. Passages of the virus in GBK cells were readily carried out at intervals of 2 days with culture fluid diluted tenfold in maintenance medium. After a few passages CPE developed within 12 hr after inoculation. CPE in GBK cells appeared earlier and more distinct than that in MA-104 cells, which were inoculated with the virus in parallel with GBK cells. These findings are the same as obtained previously in GBK cells infected with the Lincoln strain of bovine rotavirus [4].

The GBK- and MA-104-passage viruses were assayed for infectivity by tube cultures of GBK and MA-104 cells. The infectivity titer of both viruses reached 10 to 100 fold higher in GBK cells than in MA-104 cells.

Viral antigens specifically reacting with guinea pig antiserum against the RS-15 strain, supplied kindly by Dr. M. Mochizuki, were demonstrated by indirect immunofluorescent staining of coverslip cultures of GBK cells infected 12 hr earlier with the virus at the 8th passage level. Specific fluorescence was observed in the peri-

Table 1. Comparison of plaque numbers produced by canine rotavirus in GBK and MA-104 cells

Assayed in	Addition ^{a)} of		(Diluted)	Plaque number ^{b)}
	Trypsin	DEAE-dextran		
GKB	+	+	(10 ⁻⁷)	53, 50, 55
MA 104	+	+	(10 ⁻⁶)	62, 55, 43
GKB	+	-	(10 ⁻⁷)	17, 13, 14
MA 104	+	-	(10 ⁻⁶)	34, 40, 45

a) Trypsin (5 μ g/ml) or DEAE-dextran (50 μ g/ml) was added to the virus diluent and agar overlay medium.

b) 8th-passaged virus in GKB cells.

nuclear cytoplasm of infected GBK cells. Non-infected cells were negative.

The virus readily formed plaques in GBK cell monolayers, when trypsin (5 μ g/ml) and DEAE-dextran (50 μ g/ml) were added to the agar overlay medium. As shown in Table 1, plaques were formed when trypsin was added to overlay medium, but the number of plaques increased by the additional incorporation of DEAE-dextran in the overlay medium. No plaques were formed under overlay medium without trypsin.

Similar findings had been obtained with the Lincoln strain of bovine rotavirus [4]. Matsuno *et al.* [6] reported that the Lincoln strain formed plaques in MA-104 cell monolayers, when DEAE-dextran, trypsin or both were included in the overlay medium. However, in our studies bovine and canine rotaviruses formed no plaques in GBK cell monolayers under overlay medium containing DEAE-dextran but no trypsin. The reason for this discrepancy between GBK and MA-104 cells is unknown.

After 48 hr of incubation small plaques became visible without staining with neutral red. At 72 hr of incubation plaques increased in size and number. Plaque size was 1.0 to 1.5 mm in diameter and its number reached a plateau (Fig. 1). The plaque number obtained was directly proportional to the virus concentration, indicating that one infectious particle was sufficient to produce one plaque. Plaques produced in GBK cells were more distinct than those in MA-104 cells. Even with inoculation of the MA-104-passage virus the plaque assay in GBK cells was

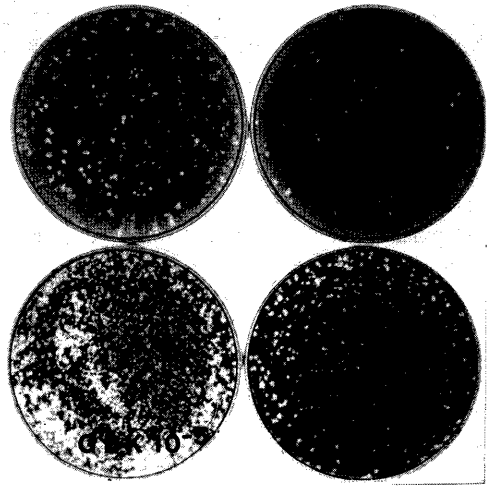


Fig. 1. Comparison of plaque size and numbers produced in MA-104 (upper) and GBK (lower) cells.

shown to be about 10 times more sensitive than that in MA-104 cells.

The plaque formation of the RS-15 strain in GBK cell cultures was specifically inhibited by guinea pig antiserum against the RS-15 strain.

The plaque assay and neutralization test in GBK cell cultures developed for canine rotavirus in the present study could find a wide application in the study of canine rotavirus infection.

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要 約

GBK 細胞でのイヌロタウイルスのブラック形成 (短報): 平野紀夫・佐々木敦子・小野勝彦・村上敏明・松本 稔¹⁾ (岩手大学農学部家畜微生物学教室, ¹⁾北里研究所ウイルス部) —— イヌロタウイルス RS-15株は明瞭な細胞変性効果を示して GBK 細胞で増殖した。トリブシンと DEAE デキストランを加えた重層寒天によるブラック法では, GBK 細胞でのブラック形成は MA104 細胞にくらべて約10倍高かった。