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Short Communication

A CONTINUOUS CELL LINE, GK, DERIVED FROM THE KIDNEY TISSUE OF MONGOLIAN GERBIL (*MERIONES UNGUICULATUS*) AND ITS VIROLOGICAL APPLICATION

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SUMMARY: A fibroblast-like continuous cell line was established from the kidney tissue of the female Mongolian gerbil (*Meriones unguiculatus*) and designated as GK cell line. The cells were susceptible to the infection with several DNA and RNA animal viruses, particularly with four prototype strains of dengue viruses and the HEP-Flury strain of rabies virus.

The mongolian gerbil has been used for primary tissue cultures. There are two reports of establishing its continuous cell lines; Tumilowicz and Cholon (1971) reported a cell line derived from gerbil fibroma designated as IMR-33 (ATCC CCL 146), and Macy and Schannon (1977) described a lung cell line, GeLu (ATCC CCL 100). The present author succeeded in developing a gerbil kidney cell line, GK. This paper describes the process of establishment, and characterization of the GK cell line and some studies on its susceptibility to viral infections.

The present cell line, GK, originated from the pooled kidney tissue of four healthy female Mongolian gerbils at 4 weeks of age. The kidneys were removed aseptically, and a cell suspension was prepared by the ordinary procedures of trypsinization for the primary culture preparation. The growth medium was Earle's balanced salt solution supplemented with 0.1% yeast extract, 0.5% lactalbumin hydrolysate, 0.05% $NaHCO_3$, 100 μg/ml gentamicin sulfate and 10% fetal calf serum (FCS). Primary gerbil kidney cells were cultured in stoppered 8 oz prescription glass bottles kept at 37 C. During the first 23 days, the cells were subcultured three times at a 1:2 split ratio at 8 day's intervals. Then during the following 34 days, the cells were maintained only by renewals of the culture medium at 7 days' intervals. On the 57th day after the primary culture, the majority of cells were floating in the medium but a few fibroblast-like cells remained attached. On this occasion, cells on the wall were trypsinized

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and seeded into a new bottle with Ham's F12 medium (Ham, 1965) supplemented with 5% FCS, 0.15% $NaHCO_3$ and 50 μg/ml gentamicin sulfate. One week later, i.e., 64 days after the primary culture, the culture formed a monolayer of fibroblast-like cells. This was subcultured at a 1:2 split ratio with 0.25% trypsin (Difco) to form a monolayer within a week, then the cell was serially subcultured for 155 generations at a 1:4 split ratio at 5 to 6 days' intervals. In the time course of subculturing of the GK cell line, about 50 days were required to attain 10 subcultures, and the interval between subcultures was usually about 5 days at 37 C. Figure 1 indicates the growth curve of GK cells at the 56th subculture; 7.5×10^4 viable cells increased to 30×10^4 within 5 to 6 days without renewal of the culture medium, and the population-doubling time was about 32.2 hr. At the 52nd subculture, there was no evidence for any mycoplasma contamination in GK cells in the Mycoplasma Stain Kit (lot 30100021; Flow Laboratories, USA). At the same time, GK cells were tested for the presence of latent viral agents by inoculation into other cell lines (VERO and RK-13), which were incubated for 14 days at 37 C. There was no cytopathic agent as judged by the microscopic observation of the cell cultures.

The cytotoxic antibody test described by Greene, Coriell and Charney (1964) was made to determine the origin of GK cells. Rabbit antiserum against the gerbil tissue destroyed 97% of cells as determined by the uptake of trypan blue, whereas only about 15% of cells were destroyed by the pre-immune serum.

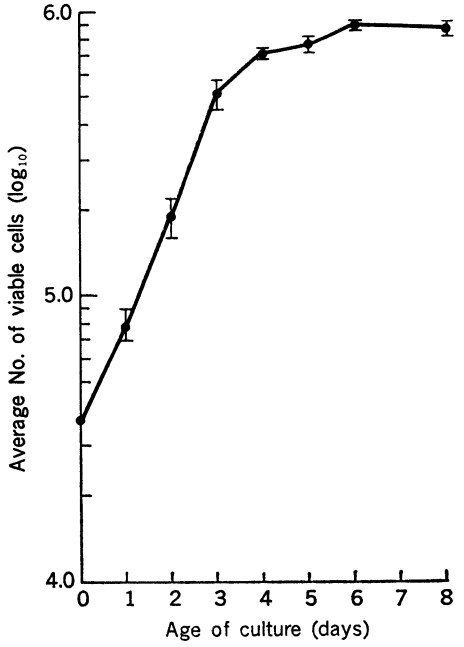

Fig. 1. Multiplication of GK cells at the 56th subculture. Points each represent mean of four experiments.

At the 52nd subculture, GK cells were analyzed for chromosome constitution by the method described by Kuchler (1977). The distribution of chromosomal complements were assayed on the cells from 60 plates. GK cell showed a heteroploid chromosome constitution with a modal number of 75, whereas the diploid constitution of normal gerbil cells is 44 chromosomes (Altman and Dittmer, 1972).

At the 92nd subculture, GK cells were assayed for malignant potential in hamsters. A dose of 1.0×10^6 viable cells or a homogenate of the same number of cells was injected subcutaneously into 15 hamsters. Progressive tumors were formed in 100% (15/15) of the animals injected with the viable cells within 37 days after injection, whereas no animal (0/15) injected with the homogenate developed tumors by 55 days after injection.

To check the susceptibility to virus infections as revealed by plaque formation, cytopathic effect (CPE) and virus yield, the following viruses were employed and their plaque titers in GK cell line were compared with those in the reference cell lines: herpes simplex virus, type 1 (HSV-1), HF strain; vaccinia virus, Dairen 1 strain; adenovirus type 1; poliovirus type 1; Sendai virus, Z strain; Sindbis virus, HR strain; Japanese encephalitis virus (JEV),

TABLE I

Susceptibility of GK cells to virus

Virus	Source of inoculum	CPE	Plaque[2]	Virus yield ($\log_{10}$ pfu/ml)[1]	
				in GK cells	in reference cells
HSV-1	VERO	+	+	6.3	7.0 (VERO)
Vaccinia	RK-13	+	+	6.8	7.8 (RK-13)
Adeno type 1	Hep 2	−	−	ND[3]	ND
Polio type 1	LLC-MK_2	−	−	ND	ND
Sendai	E[4]	+	ND	ND	ND
Sindbis	CE[5]	+	+	7.5	7.8 (CE)
JEV	CE	+	+	7.3	7.0 (CE)
Rubella	VERO	−	−	ND	ND
Dengue type 1	SMB[6]	±	+	5.3	4.0 (C6/36)
type 2	SMB	+	+	5.9	3.5 (C6/36)
type 3	SMB	−	+	5.7	4.8 (C6/36)
type 4	SMB	−	+	6.1	4.6 (C6/36)
Rabies CVS	SMB	−	−	ND	ND
HEP-Flury	CE	−	−	ND	ND
	GK♯3[7]	−	+	5.5	6.5 (BHK)
	GK♯5	±	+	6.0	6.5 (BHK)
	GK♯10	+	+	6.5	5.7 (BHK)

1) Virus infectivity titer was obtained by the plaque method in GK cells.
2) Plaques were stained with Hucker's crystalviolet.
3) Not done.
4) Fertile eggs.
5) Primary chick embryo cells.
6) 10% homogenate of the suckling mouse brain.
7) Virus preparation at the 3rd passages in GK cells.

Nakayama NIH strain; rubella virus, Baylor strain; dengue virus type 1; Hawaiian strain, type 2, New Guinea C strain, type 3, H87 strain, type 4, H241 strain; and rabies virus, HEP-Flury and CVS strains. After the virus infection, GK cells were maintained in Ham's F12 medium supplemented with 2% FCS, and were incubated at 35.5 C for 7 days. For plaque assay, the cells were overlayed with the maintenance medium supplemented with 1% methylcellulose, then incubated in the air with 5% CO_2 at 35.5 C for 7 days. Table I shows the results on the susceptibilities of GK cells to various viruses. The GK cell line was susceptible to HSV-1, vaccinia virus, Sendai virus, Sindbis virus and JEV, and particularly susceptible to four strains of dengue and rabies virus. These viruses formed plaques and developed CPE, while adenovirus, poliovirus and rubella virus did not form plaques nor develop CPE even after three passages in GK cells.

With regard to the virus susceptibility, the GK cell line seems to have a wide spectrum against various DNA and RNA animal viruses. Particularly in rabies virus, GK cell-adapted HEP-Flury strain formed small but distinct plaques on the 7th day of infection, leading to the possibility of the plaque titration of the rabies virus infectivity. Another remarkable point may be the fact that all four prototype dengue viruses formed plaques in GK cells by the 7th day of infection at 35.5 C. The specificity of plaque formation was confirmed by neutralization with specific mouse sera against four types of dengue virus (data not shown). The infectivity assay of dengue virus is notoriously difficult compared with that of other flaviviruses. From the results of the present study, GK cells seem to be a useful cell line for infectivity titration and other virological analysis with all four types of dengue virus by the plaque method. A more detailed study to define the conditions of the plaque formation by dengue viruses is now under way.

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