IN VITRO
Volume 12, No. 10, 1976

THE ORIGIN AND CHARACTERISTICS OF A PIG KIDNEY CELL STRAIN, LLC-PK$_1$

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SUMMARY

A stable epithelial-like pig kidney cell strain has been established. This strain has been carried through more than 300 serial passages, has remained free of microbial and viral contaminants, and has retained a near diploid number of chromosomes. Attempts to produce tumors with these cells in immunosuppressed laboratory animals have been uniformly negative. The cells have grown rapidly in monolayer cultures with a split ratio of 1 to 15 at weekly intervals, but have failed to proliferate in suspension cultures. A subline adapted to growth on serum-free medium 199 has been carried through 145 passages on this medium. Several unusual morphologic features have been observed in these cultures including three-dimensional "domelike" structures. These cells have been found susceptible to some viruses and have been especially useful for viruses of domestic animals. LLC-PK$_1$ cells have produced significant levels of plasminogen activator.

Key words: pig kidney cells; dome-like structures; chemically defined media; plasminogen activator; virus susceptibility.

INTRODUCTION

Trypsin-dispersed cells prepared from the kidneys of domestic pigs grow well in tissue culture and can readily be established as permanent cell strains. At least six such strains have been described, or mentioned, in the literature (1-6). Most of these reports deal with virus susceptibility. Several pig kidney strains have been developed in our laboratory, and one of these, LLC-PK$_1$, is described in detail in this report. This strain has some unique characteristics which may be of interest to investigators in several areas of research.

MATERIALS AND METHODS

Cell culture preparation and origin of LLC-PK$_1$. The kidneys from a 17-lb juvenile male Hampshire pig were aseptically removed after exsanguination of the donor animal. Both kidneys were minced, pooled together, and trypsinized by the procedure described by Younger (7). A 1-to-400 suspension was made of the final cell pack in medium 199 containing 10% horse serum (HS) and 100 units penicillin and 100 μg streptomycin per ml. This was used as the inoculum for 16-oz bottle cultures. By 4 days of incubation at 37°C, the cultures were essentially confluent, and were refed with medium 199 containing 5% HS and antibiotics. The first subculture was made on the 6th day by stripping the cells from the glass with 0.1% Armour's crystalline trypsin prepared in Earle's balanced salt solution. Antibiotics were discontinued at this time. Passages were made at approximately weekly intervals, and the split ratio was gradually increased until it became routine at 1 to 15. A total of 88 consecutive passages were made before the cell strain was frozen and stored in liquid nitrogen. It has been in and out of our frozen cell bank on numerous occasions over the 17 years since its origin in 1958. The highest passage to which these cells have been advanced is 318.

Media, sera and cell strains. Medium 199 (M199) was prepared in our laboratories, essentially according to the formula given by Morton in her review article (8). Hanks' balanced salt solution was used, and the following slight modifications were made: Niacin 0/0.75, inositol 0.05/0.50, Tween 80, 50/20.0 and ATP 2.0/10.0 (Lilly/Morton, mg/l). Other media were purchased from commercial sources. HS was obtained from our own horses; fetal bovine serum (FBS) was purchased. The Y-15 cells were obtained from Dr. Benjamin Sweet, and PK$_{15}$ was

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TABLE 1

TUMORIGENICITY TESTS WITH LLC-PK$_1$ CELLS

Cell Passage	Animals (Nos.)	Cell Inoculum	Inoc. Site	Immuno-suppression
176	Rats[a] (14)	3×10^5	Brain	X-ray and Cortisone
80	Hamsters[b] (10)	10^6	Pouches	Cortisone
146	Hamsters[b] (9)	10^6	Pouches	Cortisone
176	Hamsters[b] (9)	3×10^5	Pouches	Cortisone
199	Hamsters[b] (6)	9.75×10^5	Pouches	Antilymphocyte Serum
176	Hamsters[c] (24)	3×10^5	S. C.	None

[a]Twenty-one days old.
[b]Weanlings.
[c]Neonates.

purchased from the ATCC. LLC-PK$_2$ and LLC-PK$_3$ were developed by the same procedure described for LLC-PK$_1$. LLC-PK$_2$ was initiated from the kidney cortex tissue from one donor, while LLC-PK$_3$ was from the medulla of the kidney of another donor. Both donors were 14-day-old male Hampshire pigs. A modified or altered subline of LLC-PK$_1$, LLC-PK$_{1A}$, was obtained from Mr. Paul Kelley.

Quantitative growth studies. Replicate cultures were planted in T15 flasks as described by Evans and co-workers (9), and nuclear counts were performed by the method of Sanford and co-workers (10). Procedures for growth of cells in suspension cultures were published previously (11).

Karyology. Chromosome preparations were made either by the method of Tjio and Puck (12) or by that of Moorhead and Nowell (13). Direct counts were made under oil immersion at a magnification of ×1125 from cells which were still intact. More precise counts were obtained from photomicrographs.

Tumorgenicity studies. These tests were performed as indicated in Table 1. The tests on 21-day-old white rats were done by the method of Sigel and co-workers (14). In the hamster cheek-pouch studies the animals were treated with 5.0 mg cortisone at the time of cell inoculation, and 5 additional doses were given during the next 24 days. The cells contained in 0.1 ml of medium were inoculated into both cheek pouches. Hamsters immunosuppressed with rabbit antihamster lymphocyte serum (Microbiological Associates) were given 0.25 ml on 2 consecutive days prior to cell inoculation, on the day of inoculation, and at 3- to 4-day intervals during 24-days post cell inoculation. Neonatal hamsters were inoculated subcutaneously in the nape of the neck.

Virology. An influenza A strain (1134) and a B strain (1760), isolated in human cell cultures, were obtained from Dr. William G. Mogabgab. These strains were passed 8 and 12 times in primary rhesus monkey kidney cells (pRMK) prior to passage in LLC-PK$_1$ cells. All other influenza viruses were originally egg-passaged strains, carried in our laboratories, which had been adapted to growth in pRMK cells. Swine parvovirus was obtained from Dr. D. L. Croghan. Hemagglutination (HA) assays for parvovirus were done in tubes with 0.5 ml of virus dilution, and 0.5 ml of 0.4% guinea-pig red blood cells. M199 was used as the diluent in these tests. The tubes were incubated for 2 to 4 hr at room temperature before the tests were read out. $TCID_{50}$ (Tissue culture infective dose) determinations and HA assays were done 7 days after virus inoculation.

Plasminogen activator. For production of plasminogen activator (PA) cultures were grown to confluency with serum-containing medium, and then refed with M199 only. Samples of the medium were taken periodically for PA assay. The assays were done by the fibrin clot lysis method of Astrup (15) and expressed as CTA units per ml (Committee on Thrombolytic Agents).

Liquid nitrogen preservation and tests for contamination. Cultures in the advanced logarithmic growth phase were used for freeze-preservation. The monolayer was washed with M199, and then stripped from the flask by treatment with 0.2% crystalline trypsin solution. Following two rinses with M199, 1 to 3×10^6 cells were put into glass-sealed ampoules in 1.0 ml M199 containing 3% FBS and 10% filtered glycerol. The ampoules were held in the vapor phase of liquid nitrogen for 30 min, and then submerged for storage. The subline adapted to serum-free M199 was handled in like manner, except that the serum was omitted. For recovery, ampoules were thawed quickly in a 37°C water bath, and the cells were transferred to 10 ml M199 with 3% FBS and 2% filtered