Sensitivity of Seven Different Types of Cell Cultures to Three Serotypes of Foot-and-Mouth Disease Virus

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ABSTRACT

The ability of bovine tongue origin foot-and-mouth disease virus serotypes A, O and C to replicate in seven different types of cell cultures was studied. Primary and secondary calf thyroid cells were equivalent in susceptibility to bovine kidney cell cultures passaged up to five times. Calf thyroid cells lost their susceptibility after two passages. Cryopreserved bovine kidney cell cultures passaged three and four times were equivalent in susceptibility to sensitive calf thyroid and bovine kidney cells. Susceptibility to foot-and-mouth disease virus serotype C was most variable among the cells tested.

Lamb testicle and porcine kidney cells were susceptible to foot-and-mouth disease virus while goat and calf testicle and calf lung cells were refractory.

RÉSUMÉ

Cette expérience consistait à vérifier si des échantillons des sérotypes A, O et C du virus de la fièvre aphteuse, obtenus de lésions linguales bovines, pouvaient se multiplier dans sept variétés de cultures cellulaires. Les cultures primaires et secondaires de cellules thyroïdiennes de veau affichèrent une susceptiblité équivalente à celle de cultures de cellules rénales de veau qui avaient subi jusqu'à cinq passages successifs. Les cellules thyroïdiennes de veau perdirent leur susceptibilité, après deux passages. Des cultures surgelées de cellules rénales de veau, auxquelles on fit subir de trois à quatre passages successifs, affichèrent une susceptibilité équivalente à celle de cellules thyroïdiennes et rénales susceptibles de veau. La susceptibilité des diverses cultures cellulaires du sérotype C s'avéra très irrégulière.

Les cultures de cellules testiculaires d'agneau et de cellules rénales de porcelet se révélèrent susceptibles au virus de la fièvre aphteuse, tandis que celles de cellules testiculaires de veau et de chevreau, ainsi que celles de cellules pulmonaires de veau, s'y avérèrent réfractaires.

INTRODUCTION

The laboratory diagnosis of acute foot-and-mouth disease (FMD) is by virus isolation using inoculation of samples into cattle, swine or cell cultures (2, 7) and by complement fixation tests (10) on sample tissues or laboratory isolates.

Infected animals develop type specific neutralizing antibody (3), and antibodies to FMD virus (FMDV) infection associated antigen (VIA) (2, 4); however, it is not always possible to isolate the virus (9). Virus isolation is important to confirm the diagnosis, allow viral subtyping, permit production of vaccines effective against the specific subtype and aid in epizootiological studies.

Various cell cultures have been used to isolate FMDV including ones prepared from calf thyroid (CTh) (8), bovine kidney (BK) (1,2), lamb testicle (LT) (6) and porcine kidney (PK) (8). Cottral *et al* (2) showed that BK cells were at least as sensitive to FMDV as cattle and that BK cells and cattle were at least as sensitive to FMDV as mice. Snowdon (8) noted that CTh cells detected more FMDV than cattle, mice, secondary PK cells, and a baby hamster kidney (BHK) cell line. Literature was not found which directly compared the sensitivity of CTh and BK cells to FMDV. The work presented in this paper examined the relative susceptibility of seven cell cultures and specifically focuses on the ability of FMDV to replicate in CTh and BK cells.

MATERIALS AND METHODS

CELL CULTURES

Cell cultures were prepared from three pairs of calf testicles (CT), nine pairs of calf thyroids, one lot of calf lungs (CL), eight pairs of bovine kidneys and one pair of porcine kidneys from neonatal animals (up to three weeks of age). One pair each of lamb testicles and goat testicles (GT) were tissue cultured from animals one to two months of age. Testicles and lungs were teased and minced, then trypsinized at 37°C for 30

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Submitted August 13, 1981.

minutes in a fluted flask using a magnetic stirrer. Kidneys and thyroids were sequentially trypsinized four to five times at 30 to 45 minute intervals. Trypsin at 0.25% in Hank's balanced salt solution was used for all organs except that 0.5% trypsin in Hank's balanced salt solution with 0.25% lactalbumin hydrolysate (HLH) was used for thyroid cell culture preparation. Eagle's minimal essential medium (MEM) in Earl's balanced salt solutions[1] was used for growth of all cell cultures except CTh cell cultures for which a mixture of two parts of MEM with one part of HLH was used. All cell cultures were grown using 5 to 10% bovine serum as enrichment. Gentamicin sulfate[2] at 30 mcg/mL and fungizone[3] at 2.5 mcg/mL were used in all medium. Cell cultures were passaged using 0.06% trypsin and 0.025% disodium ethylene-diaminetetraacetate in a saline solution (STV) to remove cells from culture vessels.

CRYOPRESERVATION OF CELLS

First passage BK cells from one pair of kidneys were removed from culture vessels with STV, suspended in a solution of MEM with 10% fetal bovine serum, then slowly mixed with an equal volume of MEM containing 15% dimethylsulfoxide and 10% fetal bovine serum. The final count was approximately 4×10^6 cells per mL. One and one half mL of cell suspension was aseptically placed in sterile glass ampules which were flame sealed and tested for microcannulae by submersion for approximately 1/2 hour in cold (4°C) 95% ethanol containing 1% crystal violet. Intact vials were submerged for at least 18 hours in 95% ethanol at -70°C then stored submerged in liquid nitrogen. The cells were designated cryopreserved BK (C-BK).

VIRUSES

Foot-and-mouth disease virus serotypes A Venceslau, O_1 Campos and C_3 Resende passaged one or two times by intradermalingual inoculation of bovine tongue were used in the study. The bovine tongue tissue was stored at -50°C or colder until ground into a homogeneous suspension in MEM with 10% bovine serum and clarified by centrifugation at 1000 g for ten minutes. Stock virus suspensions were then stored in aliquots at -50°C or colder until used.

PLAQUE ASSAY

Plaque assays of the aliquots of the stock virus suspension were done several times to determine the viral titer. Two dilutions of each stock virus suspensions which contained approximately 50 and 500 plaque forming units (PFU) per 0.1 mL were used to inoculate cell cultures.

Cell cultures in 25 cm^2 plastic culture vessels[4] were tested for susceptibility by adsorbing 0.1 mL of virus suspension onto culture monolayers for one hour at 37°C then overlaying with 5 mL of MEM containing 0.8% gum tragacanth and 5% fetal bovine serum. Inoculated cell cultures were incubated for 20 to 24 hours at 37°C prior to stain-inactivation for 30 minutes in a solution of 1% crystal violet and 5% ethanol in 25% formalin. Four cell cultures were inoculated with each virus dilution and the geometric mean PFU per mL were calculated.

STATISTICAL ANALYSIS

Data were analyzed for significant differences using the analysis of variance employing the program available for a Tektronix™[5] 4051 computer.

RESULTS

Efficiency of replication of FMDV in BK cells was examined by combining the data from FMDV serotypes A, O and C (Table I, Fig. 1). Plaque titers were significantly lower in cell culture passages 1, 2, 6 and 7 than in passages 3, 4 and 5 ($p < 0.003$). Types

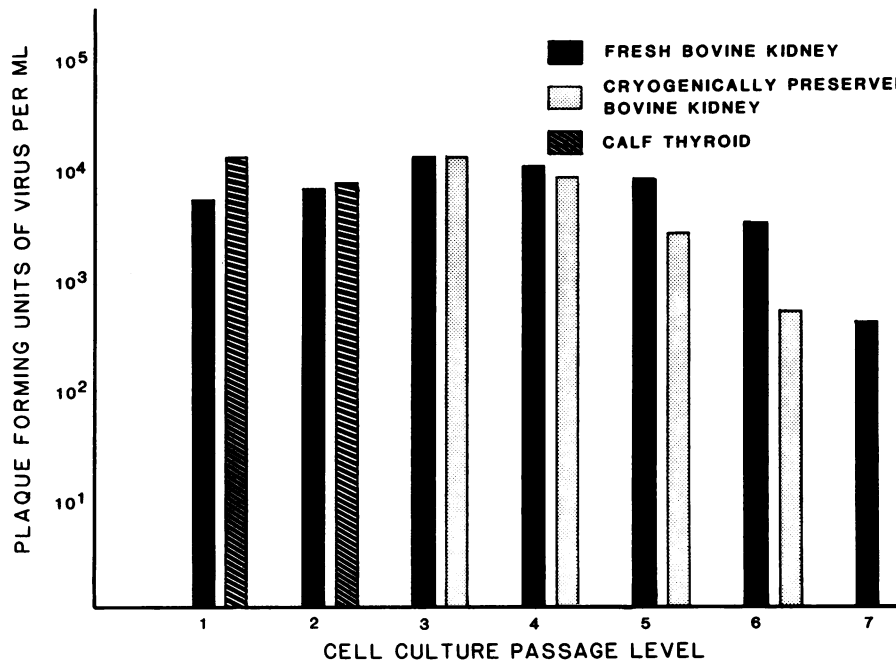

Fig. 1. Means of plaque forming units of three serotypes of foot-and-mouth disease virus in three cell cultures.

[1]Grand Island Biological Co., Grand Island, New York.
[2]GRS Garamycin (Gentamicin sulfate), Schering Corp., Kenilworth, New Jersey 17033.
[3]Fungizone (amphoteracin B), E.R. Squibb and Sons Inc., Princeton, New Jersey 08540.
[4]Product Code 3013, Falcon Division, Becton Dickenson and Co., Oxford, California 93030.
[5]Tektronix Inc., P.O. Box 500 Beaverton, Oregon 97077.

A and O had significantly lower titers in BK cell culture passages 1 and 2 than in passages 3 and 4 (respectively p 0.01 and 0.006). There was no significant difference in the growth of serotype C in BK cell culture passages 1, 2, 3 and 4.

When data for FMDV serotypes A, O and C were combined, there was no significant difference ($p > 0.05$) in virus plaque titers in BK and C-BK cell culture passages 3, 4 and 5 (Table I, Fig. 1). Titers of serotypes A and O did not differ significantly in BK or C-BK cell culture passages 3, 4 and 5. Serotype C had a significantly lower titer in the 5th passage of C-BK cell cultures (p 0.05) compared to passages 3 and 4.

In CTh cells serotype C had a significantly higher titer in the first passage than in second ($p < 0.0001$). However, no significant difference was detected in the titers of FMDV serotypes A and O when tested in first and second passage CTh cell cultures.

There was a rapid and uniform decrease in susceptibility of CTh cells after two passages since plaques developed in only two of six lots of third passage CTh cell cultures using up to 500 PFU of FMDV serotype A, O and C as inoculum per cell culture (Table I).

Comparison of combined data for serotypes A, O and C did not reveal any significant differences for growth in CTh cell culture passages 1 and 2 compared with BK cell culture passages 1 through 5. Examination of combined data for the growth of serotypes A, O and C in each of BK cell culture passages 1 and 2, C-BK cell culture passages 3 and 4, and CTh cell culture passages 1 and 2 did not reveal any significant differences ($p > 0.05$).

One lot of PK cells tested for susceptibility to FMDV showed that the first passage was as susceptible as sensitive BK and CTh cells. However, the second passage PK cells lost sensitivity to FMDV as shown by lower titers of serotypes A and O and the lack of plaques with serotype C.

One lot of second passage LT cells was similar in sensitivity to BK cell culture passages 3, 4 and 5 and CTh cell culture passages 1 and 2 when inoculated with FMDV serotypes A, O and C.

Plaques did not develop in three lots of primary CT, one lot of primary GT and one lot of primary CL cells when inoculated with up to 500 plaque forming units per mL of FMDV serotypes A, O and C.

DISCUSSION

Seven different cell cultures showed widely differing susceptibility to bovine tongue origin FMDV serotypes A, O and C.

Passages 3, 4 and 5 of BK cell cultures were significantly more susceptible to FMDV infection than passages 1, 2, 6 and 7. First passage BK cell cultures contained epitheloid "nests" and fibroblastic cells and became more uniform and fibroblastic with passage, but no explanation is available for the greater sensitivity of passages 3, 4 and 5.

The titers of FMDV in BK cell culture passages 1 through 5 were not significantly different from titers in CTh cell culture passages 1 and 2. The variability (standard error 0.46) of titers in passages 1 and 2 of CTh cell culture was higher than that of BK cells (0.28). The more uniform sensitivity of BK compared to CTh cells is a distinct advantage. The standard error of assays done in C-BK cells

TABLE I. Plaque Forming Units Detected in Passages of Three Types of Cell Cultures Inoculated with Three Serotypes of Foot-and-Mouth Disease Virus

	Bovine Kidney[a] (BK)			Cryopreserved Bovine Kidney (C-BK)			Calf Thyroid (CTh)		
Cell Passage	Type A	Type O_1	Type C_3	Type A	Type O_1	Type C_3	Type A	Type O_1	Type C_3
1	3.1[b] (8)[c]	4.6 (8)	3.4 (8)	ND[d]	ND	ND	3.3 (8)	4.9 (8)	4.3 (8)
2	3.4 (7)	4.8 (7)	3.2 (7)	ND	ND	ND	3.4 (9)	4.8 (9)	3.4 (7)
3	3.6 (5)	5.1 (5)	3.7 (5)	3.7 (2)[e]	5.2 (2)	3.7 (2)	f	f	f
4	3.6 (5)	5.1 (5)	3.4 (5)	3.5 (2)	5.0 (2)	3.2 (2)	ND	ND	ND
5	3.6 (4)	5.0 (4)	3.3 (4)	3.3 (2)	4.8 (2)	2.1 (2)	ND	ND	ND
6	3.3 (4)	4.7 (4)	2.2 (4)	3.1 (2)	4.4 (2)	$\leq .8$ (2)	ND	ND	ND
7	2.8 (2)	4.1 (2)	$\leq .8$ (2)	ND	ND	ND	ND	ND	ND

[a]Standard error for data sets: BK was 0.28, C-BK was 0.05, CTh was 0.46
[b]Geometric means ($\log_{10}$) of plaque forming units per mL
[c]Number of lots of BK and CTh cell cultures tested once
[d]Not done
[e]Number of titrations using one lot of C-BK cells
[f]Four of six lots failed to show any plaques at the dilutions of virus used for testing other cultures

(0.05) was even smaller than for BK cells.

Passages 3, 4 and 5 of BK and C-BK cell cultures were equally sensitive to FMDV serotypes A and O. Passage 5 of C-BK cell cultures was significantly less sensitive to serotype C. This decrease in sensitivity may have resulted from more cell divisions in C-BK cells following retrieval from liquid nitrogen storage or a loss of sensitive cells from cryopreservation. Because of the lower sensitivity to serotype C, only third and fourth passage C-BK cell cultures should be used for primary isolation of FMDV. Indeed C-BK cell culture passages 3 and 4 were as sensitive to FMDV serotypes A, O and C as CTh cell culture passages 1 and 2 and BK cell culture passages 1 through 5.

Several advantages of C-BK over BK and CTh cell cultures are: 1) once prepared and frozen, they are readily available, 2) the precision of assays in them is very high, 3) their growth after retrieval from liquid nitrogen is consistent, 4) they may be pretested for sterility and 5) pretested for the ability of viruses to replicate in them. Although, CTh cells have been traditionally used for FMDV isolation, susceptibility between lots is quite variable, they are laborious to prepare and lose their susceptibility to FMDV rapidly (after two passages).

It is difficult to generalize about the behavior of all strains of FMDV in cell cultures because growth characteristics may vary considerably (2, 6, 8). Before exclusive use of a given type of cell culture could be made, careful assessment of its sensitivity to various FMDV strains should be done. However, there is a clear pattern in the susceptibility behavior of BK and CTh cells which may be consistent among different virus subtypes; i.e. BK cells peak in susceptibility at 3 to 5 passages while CTh cells lose their susceptibility after two passages.

The use of PK cells for the isolation of FMDV was not fully explored, since the lot studied did not show consistent sensitivity and PK cells are often difficult to propagate and passage for routine usage.

The susceptibility of LT cells to FMDV was reported by Hess *et al* (6) and confirmed in the present study. They were able to successfully passage FMDV serotypes A, O, C and Southern African Territories (SAT) serotype 2 in LT cell cultures passaged up to 23 times. It is difficult to obtain LT from young animals throughout the year to prepare cell cultures at will. The propagation and cryopreservation of LT cells at a low passage could circumvent the temporal limitations imposed by lambing seasons. An apparent advantage of LT cells over CTh and BK cells is their sustained susceptibility to FMDV through numerous culture passages.

No explanation can be made as to why CT or GT cell cultures were refractory to FMDV since calves and kids are at least as susceptible to FMDV as lambs.

ACKNOWLEDGMENTS

The authors greatly appreciate the excellent technical help of David Perkins, Marjory Llewellyn, Catherine Roy, the Research Services group for preparation of primary BK cell cultures and the guidance on statistical analyses by Dr. John H. Graves.

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