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Comparison of Low- and High-Passage Bovine Turbinate Cells for Assay of Bovine Viral Diarrhea Virus[1]

By

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With 2 Figures

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Summary

The preparation and maintenance of a culture of bovine turbinate cells is described. The cell line is free of bovine viral diarrhea, parainfluenza-3, and infectious bovine rhinotracheitis viruses; and although the modal chromosome number changed significantly between the 9th passage and the 81st passage, no definite morphological changes were observed. Low- and high-passage bovine turbinate cells were equally susceptible to bovine viral diarrhea virus.

1. Introduction

Bovine viral diarrhea (BVD) virus can be readily propagated in primary bovine kidney or testis cell cultures (3, 4, 7, 8). However, because the virus is widespread in the cattle population and readily crosses the placental barrier, it is difficult to consistently prepare from calves or fetuses primary cell cultures that are not already infected (1). The degree of susceptibility to BVD may vary with individual primary cell cultures, and they are unacceptable for either diagnostic work or experimental propagation of virus isolates until they are proven to be free of BVD virus.

Previous investigations with turbinate cells prepared from a 2-year-old Hereford indicated that these cells were uniformly susceptible to BVD and were readily passed in continuous culture (1). However, the cell line decreased in susceptibility to BVD beyond the 50th passage and did not grow well beyond the 70th passage. Recently, a new continuous line of bovine turbinate (BT) cells has been established and is the subject of this paper.

2. Materials and Methods

2.1. Cell Culture

The turbinates from a colostrum-deprived "caught calf" (2) from a BVD antibody-negative dam were removed aseptically. The turbinate tissue was cut into small pieces

[1] Mention of commercial products does not constitute endorsement.

and trypsinized in 0.25 per cent trypsin for 10 minutes at room temperature. The cells were allowed to settle, the trypsin was decanted, and then fresh trypsin was added; the process was continued for 30 minutes. The dispersed cells were decanted and saved, and the trypsinization procedure was repeated on the remaining tissue. The dispersed cells were pooled and washed twice in Dulbecco's phosphate buffered saline (PBS), diluted at a ratio of 1 ml of packed cells per 200 ml of medium, and seeded 25 ml per flask in 75 cm^2 plastic flasks (Falcon Plastics, Oxnard, CA. 93030).

The medium consisted of F15 (Grand Island Biological Co., Grand Island, New York) to which was added 0.11 mg sodium pyruvate per ml of reconstituted medium 0.5 per cent LAH and 10 per cent bovine serum. The bovine serum was known to be free of BVD, parainfluenza (PI-3), infectious bovine rhinotracheitis (IBR), and bovine adenoviruses, as well as free of antibodies to these viruses. Fetal calf serum was not used because it was difficult to get such serum free of BVD virus.

The cells grew well at 35° C and were divided and passed at a ratio of 1 to 3 as frequently as twice a week or at intervals of several weeks to several months. When the cultures were held for more than 1 month, the medium was changed at monthly intervals. Low-passage cells were frozen in liquid nitrogen for future reference. The growth medium could either be left on the cells for certain types of routine work such as virus propagation and virus neutralization or the growth medium could be removed and the cells exposed with viral inoculum. Medium without serum could be used for maintenance.

2.2. Determination of Modal Chromosome Number

To correlate a likely change in the modal chromosome number of the BT cell line with possible change in susceptibility to BVD, the modal chromosome number for the 9th and 81st cell passage was determined. Chromosomal variation in the BT cell line was evaluated as previously described (5).

2.3. BVD Viruses

Oregon C24V (3), NADL (4), and Singer (isolated from an outbreak of BVD in Maryland), all cytopathogenic isolates, were used in the comparison of susceptibility of BT cell cultures. The Oregon C24V and Singer isolates were used at the 10^{-5} dilution, and the NADL isolate was used at the 10^{-4} dilution. For each experiment, adequate volumes of the stated dilutions were made in a single tube to inoculate both low- and high-passage cell cultures.

2.4. Virus Plaque Assays

For plaque studies, the cells from 75 cm^2 plastic flasks were suspended at a ratio of 1 to 50 ml of medium, and 8 ml of cell suspension was seeded into 60-mm plastic Petri dishes (Falcon Plastics, Oxnard, CA.). Cultures were incubated at 35° C in a humidified atmosphere containing 3 per cent carbon dioxide (CO_2). A confluent sheet formed in approximately 2 days, and the cells were used 3 to 4 days after seeding.

At the time of inoculation, the medium was removed, and 0.1 ml of a predetermined virus dilution that would give countable plaques was added to each plate. Viral inoculum was allowed to adsorb for 1 hour at room temperature. Agar overlay was then added, the agar was allowed to solidify, and the plates were inverted and returned to the CO_2 incubator. The agar overlay consisted of equal parts of 1.5 per cent Ionagar No. 2S and 2× Eagle's essential amino acids, vitamins, and glutamine, with 0.5 per cent LAH in Earle's balanced salts solutions, and without serum. Ten plates were used for each virus dilution, and the plaque-forming units per 0.1 ml of virus suspension were determined. Five ml of 1 per cent Noble agar containing 0.015 per cent neutral red was added to the plates after 4 days' incubation, and the plaques were counted 1 to 2 days later.

2.5. Determination of 50 Per Cent End-Point

Low- and high-passage cell cultures were seeded in roller tubes held in stationary racks; the viruses were titrated when the cell sheet became confluent. Cytopathogenic BVD viruses in BT cells cause vacuole formation within 3 to 5 days of inoculation

and complete destruction of the cell sheet within 5 to 6 days. When the cytopathic effect (CPE) was complete, the 50 per cent endpoint was calculated by the method of REED and MUENCH (6).

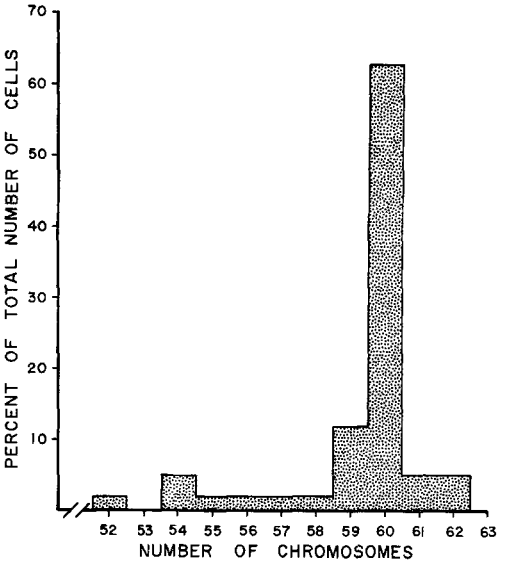

Fig. 1. Histogram of 9th passage of bovine turbinate cells

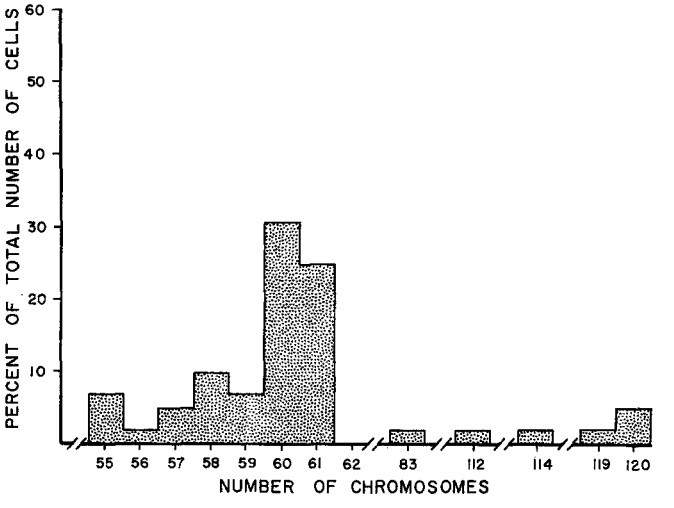

Fig. 2. Histogram of 81st passage of bovine turbinate cells

3. Results

3.1. Modal Chromosome Number for the Low- and High-Passage BT Cells

Figure 1 shows the modal number of chromosomes for the 9th passage. Approximately 65 per cent of the cells had a diploid chromosome number of 60,

whereas 12 per cent had 59, and 5 per cent had 61 and 62 chromosomes per cell, suggesting that changes from the diploid chromosome number of 60 had already begun.

Figure 2 shows the modal number of chromosomes for the 81st passage. Changes in numbers of chromosomes per cell were significant; only 30 per cent of the cells had 60, 25 per cent had 61, and 5 per cent had 120 chromosomes per cell.

Table 1. *Plaque Production of Selected Isolates of BVD Virus on Low- and High-Passage Bovine Turbinate Cells*

Experiment No.	Cell passage No.	Virus isolate	PFU/0.1 ml[a]
1	8	Singer	6.4
1	83	Singer	6.5
2	12	NADL	5.6
2	85	NADL	6.0
2	12	C24V	6.8
2	85	C24V	6.9
3	16	NADL	5.5
3	87	NADL	5.7
3	16	C24V	6.8
3	87	C24V	7.0
3	16	Singer	6.6
3	87	Singer	6.7
4	19	NADL	5.4
4	89	NADL	5.5
4	19	C24V	6.8
4	89	C24V	6.8
4	19	Singer	6.5
4	89	Singer	6.5

[a] Expressed as log base 10.

Table 2. *Fifty Percent End-Points of Selected Isolates of BVD Virus on Low- and High-Passage Bovine Turbinate Cells*

Cell passage No.	Virus isolate	Fifty percent end-point[a]
19	NADL	5.5
89	NADL	5.6
19	C24V	6.6
89	C24V	7.3
19	Singer	6.5
89	Singer	6.6

[a] Expressed as the log base 10.

3.2. Comparison of Low- and High-Passage Bovine Turbinate Cells in Their Ability to Propagate Selected Isolates of BVD Virus

Table 1 compares the titers of plaque-forming virus from stocks of NADL, C24V, and Singer isolates on low- and high-passage BT cells. Table 2 compares the 50 per cent end-points of these viruses titrated on low- and high-passage cells.

There appears to be no significant difference in susceptibility to BVD between the low- and high-passage cells. Results of virus assay by enumeration of plaque-forming units or by the 50 per cent end-point method were comparable.

4. Discussion

Although there is a definite change in the modal chromosome number as the BT cell line is passed continuously, there is no observable change in cellular morphology or in growth characteristics, nor is there a trend toward resistance to replicating BVD viruses. The cells readily supported IBR, PI-3 viruses, and bovine adenoviruses types 1, 3, 5, and 6 and an untyped adenovirus isolated from sick calves. However, sensitivity of the BT cells of these viruses at low- and high-passage levels has not been compared.

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