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CHANNEL CATFISH VIRUS: COMPARATIVE REPLICATION AND SENSITIVITY OF CELL LINES FROM CHANNEL CATFISH OVARY AND THE BROWN BULLHEAD[1]

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Abstract: A cell line derived from channel catfish ovary tissue was compared with the brown bullhead (BB) cell line for their respective abilities to replicate and detect channel catfish virus (CCV). The channel catfish ovary cell line (CCO) produced cytopathic effects (CPE) more rapidly and detected CCV at higher dilutions than did the BB cell line. Production of CCV was more rapid in CCO cells than in BB cells, but the peak titers of the two lines were not significantly different. The CCO cell line was shown to be the more sensitive cell line for CCV research and diagnostics.

INTRODUCTION

Channel catfish virus disease (CCVD) is a highly contagious herpesvirus infection of young cultured channel catfish, *Ictalurus punctatus*. The original isolation of CCV by Fijan[2] was in primary cultures of channel catfish ovary. Fijan *et al.*[3] investigated the suitability of four established fish cell lines for use in their investigations of CCV. The RTG-2 (ATCC CCL 55),[10] FHM (ATCC CCL 42),[4] the brown bullhead (BB) (ATCC CCL 59), and the bluegill (BF-2) (ATCC CCL 91) cell lines were challenged with CCV. The BB cell line was the only one susceptible to CCV. In addition to the above cell lines, Wolf and Darlington[9] found cell lines from bullfrog tongue (FT), *Rana pipiens* (3 AKRP), primary chick embryo, the established human lines HEp-2, WI-38, HeLa and the hamster line BHK-21 to be refractory to CCV infection, whereas only BB cell cultures were susceptible. Two cell lines from the walking catfish *(Clarias batrachus)* have recently been developed and shown to be susceptible to CCV.[6]

Fijan *et al.*[3] noted that primary channel catfish cultures seemed more sensitive to CCV than were BB cells; however, the BB cells were a recognized standard and at that time, the only susceptible cell line.[5] A permanent cell line has been established from the ovaries of a healthy juvenile channel catfish.[1] In this paper we report a comparison of the channel catfish ovary (CCO) cell line with the BB cell line for viral replication and sensitivity of detection of CCV.

MATERIALS AND METHODS

A stock of channel catfish virus, CCV_{1a} (Auburn strain) was serially diluted in 10-fold steps in Hanks' balanced salt solution (HBSS) from 10^{-1} to 10^{-6}. Microculture plates were seeded with CCO (passages 79-83) or BB (passages 134-137) cells in Eagle's minimal essential medium with 10% fetal bovine serum (MEM-10) and antibiotics (penicillin - 100 IU/ml, streptomycin - 100 μg/ml, gentamicin - 50 μg/ml). Titra-

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tion employed three replicate wells for each virus dilution. Virus was titrated 10 times in CCO cells and 11 times in BB cells. Cultures at pH 7.2-7.4 were incubated at 30 C for 5 days then examined for cytopathic effects (CPE). The tissue culture infective dose - 50% endpoint ($TCID_{50}$) was calculated for each titration.[7]

Comparisons were made of CCV replication by CCO (passage 103) and BB (passage 157) cells. Monolayer culture were grown in MEM-10 in 60mm plastic plates. For the comparison, the medium was aspirated from the plates, the cultures were inoculated with $10^{4.8}$ $TCID_{50}$ of virus and allowed to adsorb for 1 hr at 25C. Cultures were washed three times with 5 ml of HBSS and fed with 5 ml of MEM-10 and incubated at 30C. Duplicate cultures of CCO and BB cells were collected at four 12-hr intervals after inoculation. The cultures were observed and scored for CPE. The cells were sonicated with a Branson sonifier (20kHz at 60w) for 3 s. Each cell sonicate was titrated for infectivity.

A comparison of sensitivity of CCO and BB cells to CCV at dilutions of 10^{-6} 10^{-7} and 10^{-8} was performed by the plaque assay method described by Wolf and Quimby.[11] Cultures were incubated at 30 C for 2 days, then fixed, stained and the plaques counted.

RESULTS

The mean of 10 titrations of stock CCV and CCO cell cultures was 10^{5} $TCID_{50}$/ml. The mean of 11 titrations of the same stock of CCV and BB cells was $10^{4.4}$ $TCID_{50}$/ml. A one-way analysis of variance showed this difference to be significant ($p = 0.01$, $df = 1,9$, calc $F = 8.664$).

CCV replicated more rapidly in CCO cells than in BB cells (Fig. 1). At 12 hr the combined cell-associated and cell-free virus titer in the BB cells was $10^{3.75}$ $TCID_{50}$/ml compared to $10^{7.25}$ $TCID_{50}$/ml in the CCO cells. Titers at 24 hr from the two cell systems were less than 1 $\log_{10}$ different. A one-way analysis of variance showed virus production to be significantly different only at 12 hr post-infection ($p = 0.01$, $df = 1,4$, calc $F = 64.33$). All CCO cells showed CPE at 12 hr post-infection, while no CPE was seen in the BB cells at that time. All BB cells showed CPE at 24 hr post-infection.

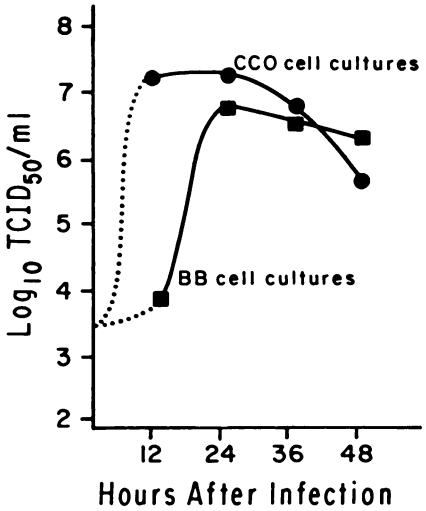

FIGURE 1. Comparative production of CCV by CCO and BB cells incubated at 30C. (Data points indicate the sum of cell-associated plus released virus.)

In comparative plaque assay, CCO cells detected CCV at higher dilution than did BB cells. CCO cells detected a mean of 16 infective units at the 10^{-7} dilution (Table 1). At the 10^{-6} dilution, the plaques formed in CCO cultures were too numerous to count. Using the same stock virus dilutions, BB cells did not detect CCV at any of the dilutions tested (10^{-6} to 10^{-8}).

DISCUSSION

Our study showed that the CCO cell line was more sensitive for the detection

TABLE 1. Comparative plaque assays of CCV in CCO and BB cells[a].

Virus dilution	Cells	
	BB	CCO
10^6	0	TNC[b]
	0	TNC
	0	TNC
10^7	0	1.8×10^8
	0	1.5×10^8
	0	1.6×10^8

[a]Values given in plaque-forming units per ml

[b]Too numerous to count

of CCV than was the BB cell line. The CCO cells produced higher titers of CCV and could detect CCV at higher dilutions than could the BB cell line. However, the peak CCV titers in this study were somewhat lower than those found by other authors.[8,9] These lower titers may have been due to an attenuation effect on the stock CCV brought about by numerous *in vitro* passages or to a variation of the density of the cell cultures.

The peak replication of CCV by CCO and BB cells was not significantly different. There was, however, a difference in speed of replication. The CCO cells replicated virus more rapidly than did the BB cells (Fig. 1).

The microculture and plaque assays detected different amounts of CCV in this study. This may be due in part to the use of different stocks of CCV in each portion of the study. The stock of CCV used in the microculture titration had been stored frozen for a longer period of time during which infectivity had been lost.

A desired objective of developing the CCO cell line was to have a more sensitive cell system for use in channel catfish virus disease (CCVD) research and diagnosis. Preliminary evaluations show it to be slightly more sensitive in detecting CCV from infected catfish than is the BB cell line which could be related to the fact that the cell line was derived from the host species for CCV, the channel catfish, but the CCO cells did not detect CCV from adult channel catfish injected with CCV (Bowser, unpubl.). The inability of anyone to break latency of CCV in adult fish continues to be a problem in pathogenesis of CCVD. A more thorough evaluation of the CCO cell line in the area of adult channel catfish and channel catfish virus disease may prove valuable.

LITERATURE CITED

1. BOWSER, P.R. 1978. Development and evaluation of a new cell line from the channel catfish, *Ictalurus punctatus*. Ph.D. Thesis, Auburn University, Auburn, Ala., 48 pp.
2. FIJAN, N. 1968. Report on acute mortality of channel catfish fingerlings caused by a virus. Bull. Off. Int. Epizoot. 69: 1167-1168.
3. ——, T.L. WELLBORN, Jr. and J.P. NAFTEL. 1970. An acute viral disease of channel catfish. Bur. Sport Fish Wildl. Tech. Pap. 43, 11 pp.
4. GRAVELL, M. and R.G. MALSBERGER. 1965. A permanent cell line from the fathead minnow *(Pimephales promelas)*. Ann. N.Y. Acad. Sci. 126: 555-565.
5. McCRAREN, J.P. 1972. Channel catfish virus disease (CCVD): a current review. Proc. Ann. Conf. West. Ass. Game Fish Comm. 52: 528-537.
6. NOGA, E.J. and J.X. HARTMANN. 1977. Establishment of cell lines from the walking catfish *(Clarias batrachus)* and their infection with channel catfish virus. In Vitro 13: 160 (Abstr.).
7. REED, L.J. and H. MUENCH. 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27: 493-497.

8. ROBIN, J. and A. RODRIGUE. 1978. Purification of channel catfish virus: a fish herpesvirus. Can. J. Microbiol. 24: 1335-1338.

9. WOLF, K.E. and R.W. DARLINGTON. 1971. Channel catfish virus: a new herpesvirus of Ictalurid fish. J. Virol. 8: 525-533.

10. —— and M.C. QUIMBY. 1962. Established eurythermic line of fish cells *in vitro*. Science 135: 1065-1066.

11. —— and ——. 1973. Fish viruses: buffers for plaquing 8 agents under normal atmosphere. Appl. Microbiol. 25: 659-664.

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