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SUSCEPTIBILITY OF CELL LINES TO AVIAN VIRUSES

Isabela Cristina Simoni[1*]; Maria Judite Bittencourt Fernandes[1]; Renata Marconi Custódio[1]; Alda Maria Backx Noronha Madeira[2]; Clarice Weis Arns[3]

[1]Instituto Biológico, São Paulo, SP, Brasil; [2]Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brasil; [3]Departamento de Microbiologia, Universidade Estadual de Campinas - UNICAMP, Campinas, SP, Brasil

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ABSTRACT

The susceptibility of the five cell lines – IB-RS-2, RK-13, Vero, BHK-21, CER - to reovirus S1133 and infectious bursal disease virus (IBDV vaccine GBV-8 strain) was studied to better define satisfactory and sensitive cell culture systems. Cultures were compared for presence of CPE, virus titers and detection of viral RNA. CPE and viral RNA were detected in CER and BHK-21 cells after reovirus inoculation and in RK-13 cell line after IBDV inoculation and with high virus titers. Virus replication by production of low virus titers occurred in IB-RS-2 and Vero cells with reovirus and in BHK-21 cell line with IBDV.

Key words: reovirus, infectious bursal disease virus, cell lines, susceptibility

INTRODUCTION

Avian reoviruses and infectious bursal disease virus (IBDV) are usually isolated and grown in embryonated eggs and/or in primary avian cell cultures (5, 13, 17, 18). However the use of a continuous cell line has several advantages over the use of primary cell cultures (1, 2, 9). Various authors have evaluated numerous cell lines for the isolation of these viruses. Of many mammalian established cell lines tested, reovirus has been grown in Vero, BHK-21, GBK, PK, RK and CRFK (1, 17, 18). Cells susceptible to the IBDV include mammalian cell lines such as RK-13, Vero, MA-104 and BGM-70 (9, 13) and the avian cell line-QT35 (2).

This report describes a comparative study of the susceptibility of one avian and four mammalian cell lines to avian reovirus S1133 and IBDV vaccine GBV-8 strain (Biovet Laboratory) through of presence of cytopathic effect (CPE), virus titration and detection of viral RNA by polyacrylamide gel electrophoresis (PAGE) to better define satisfactory and sensitive cell culture systems for their isolation.

MATERIALS AND METHODS

Cell cultures. Chicken embryo fibroblast (CEF) cultures were prepared from 9-to-11 day old embryos of specific-pathogen-free (SPF) chicken eggs (Biovet Laboratory) by standard procedures. Baby hamster kidney (BHK-21), african green monkey kidney (Vero), rabbit kidney (RK-13) and porcine kidney (IBRS-2 clone D-10) mammalian cell lines obtained from our own laboratory and a chicken fibroblast (CER) avian cell line obtained from the Microbiology Department of the State University of Campinas were

* Corresponding author. Mailing address: Av. Conselheiro Rodrigues Alves, 1252, Caixa Postal 12898, CEP 01064-970, São Paulo, SP, Brasil, E-mail: simoni@biológico.br

used in the study. Cells were grown in Eagle's minimum essential medium (MEM) containing 8% fetal calf serum (FCS).

Viruses. IBDV vaccine GBV-8 strain (Biovet Laboratory) and avian reovirus S1133.

Virus propagation. 25cm^2 cultures flasks were inoculated with 0.1 ml of each virus and observed for 3-4 days for production of cytopathic effect (CPE) and for three blind passages. The monolayers were frozen and thawed on time and the supernatants fluids were collected for virus titer assay and electrophoresis.

Assay for virus yield. Virus yields from CEF and cell lines infection with both viruses was determined in the CEF culture. The serial 10-fold dilutions of each cell cultures-virus supernatants were prepared in growth medium and 50 µl of each dilution were transferred to each of the four wells of a 96-well microtiter plate that contained the same volume of fresh CEF suspensions (3.0 x 10^5 cells/ml). Plates were incubated at 37°C in 5% CO_2 atmosphere for 7 days and the virus titer was determined by the Reed and Muench method (16).

Polyacrylamide gel electrophoresis (PAGE) of viral RNA. The viral RNAs from infected cultures were isolated by phenol: chloroform extraction followed by ethanol precipitation (14). RNA was analyzed on 3.5% stacking gel and 7.5% separating gel using the discontinuous SDS-gel system of Laemmli (11). Electrophoresis was carried out for 18 h at 4°C at a constant current of 10 mA. The RNA bands were visualized by the silver staining method described by Herring *et al.* (6).

RESULTS

Primary culture-CEF was susceptible to both viruses. The RK-13 cell line was the only one that presented CPE after IBDV inoculation, while the CER and BHK-21 cells were sensible to the reovirus (Table 1).

CPE of reovirus infection was characterized by formation of syncytia followed by degeneration leaving holes in the monolayer and giant cells floating in the medium. The IBDV infection produced a CPE characterized by marked cell rounding and detachment from the substrate.

Results of virus titers obtained in CEF culture from infected supernatants of different cell lines with both virus are shown in Table 2. The RK-13 cell line and the CEF culture presented high virus titers (6.9 and 6.7 $\log_{10}$ $TCDI_{50}$ respectively) to IBDV. The reovirus caused similar virus titers in the BHK-21 and CER cells (6.4 and 6.15 $\log_{10}$ $TCDI_{50}$) however these were lower than the virus titers obtained from CEF culture (7.5 $\log_{10}$ $TCDI_{50}$). Low virus titers were detected only in IBRS-2 and Vero cells for reovirus infection and BHK-21 cells to IBDV infection without visible CPE manifestation (Table 1).

The detection of viral RNA can be seen in Table 3. The viral RNA bands from infected CEF cultures were always visible, while in infected cell lines it was only possible to observe them in cells which presented CPE. The electrophoretic profile of ds-

Table 1. Susceptibility of cell cultures to reovirus and IBDV.

Cell Culture	IBDV (Strain GBV–8)	Reovirus S1133
CEF	+	+
IB-RS-2	-	-
RK_{13}	+	-
Vero	-	-
BHK – 21	-	+
CER	-	+

+ ECP positive
- ECP negative

Table 2. Infectivity titers of cell cultures-adapted Reovirus and IBDV in CEF cells.

	Virus titre[a]	
Propagation System	IBDV	Reovirus
CEF	6.70	7.50
IB-RS-2	0	2.50
RK_{13}	6.90	0
Vero	0	1.12
BHK – 21	2.00	6.40
CER	0	6.15

a: values expressed as $\log_{10}$ $TCDI_{50}$/ml

Table 3. Detection of viral RNA from cultures infected with IBDV and reovirus by PAGE

	IBDV (GBV-8)	Reovirus S1133
CEF	+	+
IB-RS-2	-	-
RK-13	+	-
Vero	-	-
BHK-21	-	+
CER	-	+

RNA of both cell culture-passaged viruses strains was indistinguishable from the ds-RNA of original strain.

DISCUSSION

This study was carried out with the aim of better defining satisfactory and sensitive cell culture systems for isolation the reovirus and IBDV. In order to find the most susceptible cells, the results obtained were evaluated and correlated: observation of CPE, high virus titers and presence of viral RNA.

IBDV replicated and caused CPE in RK-13 cells as reported by Petek *et al.* (15) and presented virus titers similar to that in CEF cells. It did not cause CPE in BHK-21 cells, however there was some virus replication determined by the production of low virus titer. This is opposite of reported by Petek *et al.* (15). The Vero, CER and IBRS-2 cells presented neither CPE nor virus titer. Vero cells have been used to propagate this virus including for virus-neutralization test (4) as described by others authors (8, 9, 12) however initial passages can not produce visible CPE (7). On the other hand, viral RNAs were not detected in any of these cells, except RK-13. Since RK-13 cell line presented CPE, high virus titer besides the presence of viral RNA, it is the best to IBDV propagation.

Avian reovirus propagated in IBRS-2, Vero, CER and BHK-21 cell lines, where it caused a visible CPE, however it produced high virus titers, similar to that in CEF cultures, only in the two last cell lines. In other cells the only alteration observed was production of low virus titers. The RK-13 cell line was not susceptible (15). The presence of viral RNA was detectable only in those cells with CPE (CER and BHK-21 cells), which were therefore selected as the most sensitive to reovirus replication.

Passage of both viruses strains in cell lines did not result in detectable change in the electrophoretic profile of the ds RNA genome segments in PAGE.

The difference of ours results and those of others authors may be due, among others reasons, to cell-culture passage levels of virus strains used or variation in sensitivity of different strains of cell lines (3, 7,10, 19).

Further studies are needed to better determine whether CPE will occur and virus titers will increase after additional passages in all cases with negative CPEs and low virus titer. Moreover, the amount of the virus in the cells in these cases was also not high enough to detect the viral RNA by PAGE, which would also succeed after more passage and viral adaptation. However, it was a priority in this study, to obtain a cell line producing CPE as soon as possible, that would provide the greatest chance of recovery of viruses.

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RESUMO

Suscetibilidade de linhagens celulares a vírus aviários

Estudou-se a suscetibilidade de cinco linhagens celulares (IB-RS-2, RK-13, Vero, BHK-21, CER) ao reovírus S1133 e ao virus vacinal (GBV-8) da doença infecciosa bursal de galinha (IBDV). As culturas foram comparadas quanto a presença de efeito citopático (ECP), título viral e detecção de RNA viral. Nas linhagens CER e BHK-21 detectou-se ECP e RNA viral após inoculação com reovírus e na linhagem RK-13 após inoculação com IBDV, com produção de altos títulos virais. Replicação viral com produção de baixos títulos ocorreu nas linhagens IB-RS-2 e Vero inoculadas com o reovírus e na linhagem BHK-21 com IBDV.

Palavras-chave: reovírus, vírus da doença infecciosa bursal de galinha, linhagens celulares, suscetibilidade.

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