Propagation of infectious bursal disease virus in continuous cell lines.

Abstract: The infectious bursal disease virus (IBDV) causes a immunosuppressive disease in young chickens. IBDV has been adapted to replicate and produce cytopathic effect (CPE) in primary chicken embryo fibroblast cultures (CEF) but replication also takes place in continuous cell lines. Various cell cultures systems were studied in order to choose the best susceptible cells to IBDV and be used in routine virus neutralization tests. For this, four serotype 1 commercial live-attenuated vaccines and four virus field samples of IBDV adapted in CEF were propagated in five continuous cell lines (CER, IB-RS-2, SK-6, RK-13 and Vero). All cell lines were susceptible to IBDV at least one virus sample however the RK-13 cell line supported the replication of all samples with CPE most pronounced and it occurring earlier.

Introduction

Infectious bursal disease (IBD) is an acute highly contagious viral infection, which results in immunosuppression and mortality in young chickens and in considerable economic losses in poultry industry worldwide (2, 9). IBD virus (IBDV) is a member of the Birnaviridae family (3) comprising non-enveloped virus particles with a genome consisting of two segments of double-stranded (ds) RNA (5, 10).

Chorioallantoic membrane (CAM) of 9 to 11 day old chicken embryos and or primary avian cell cultures are commonly used for isolation and propagation of avian viruses (9). Few researchers have reported growth of IBDV in cell lines which would have several advantages over the use of chicken embryos or primary cell culture (4, 6, 8). Continuous cell lines reported to be susceptible to IBDV including of mammalian origin are RK-13 (11) which is derived from rabbit kidney as well as, Vero, BGM-70 and MA-104 cells from monkey kidney (6, 8); and of avian origin: QT35 from quail fibroblast (4).

The broad antigenic diversity of IBDV makes continued isolation and antigenic analysis of field viruses imperative, although isolation of IBDV from field cases may be difficult (7, 9).

The purpose of the present study was to broaden the number of easily available cell culture systems that could support the replication of
CPE, four samples in RK-13 cells, two in Vero cell and five in IBRS-2 cells presented CPE during the first and second passage. However, two samples in RK-13 cells, six in Vero cells and one in SK-6 and CER cells presented CPE at the third and fourth passage. On the other hand, five passages were necessary before CPE was observed in three samples in SK-6 cells and in two samples in RK-13 cells.

After the virus was adapted to the given cell line, CPE occurred within 24 hours post-infection (pi) in CER cells, 24 to 48 hours in CEF and RK-13 cells, 24 and 72 hours in IBRS-2 cells and 72 hours in Vero and SK-6 cells. While CEF and CER cells presented up to 100% of the cells affected by virus infection, RK-13 and IBRS-2 cells presented approximately 50 to 75% and Vero and SK-6 cells presented only 25%.

The CPE induced by IBDV in CEF cells was similar to that seen in continuous cell lines, which was characterized by a marked cell rounding and detachment from the substrate.

Discussion

Although embryonated chicken eggs and primary avian cell cultures have been considered the most suitable systems for isolation and propagation of IBDV (4, 6, 8), the expenses, the time-consuming and laborious procedures, the limited access to specific-pathogen-free (SPF) embryos and possible contamination with extraneous avian viruses make them less desirable than continuous cell lines. Cell lines can be made easily and continuously available by simple procedures and from reliable and relatively inexpensive sources (4, 8).

Considering that the cell line used most in IBDV studies, BGM-70, is not easily available in Brazil, the study aimed to broaden the continuous cell lines that could be susceptible to IBDV, choosing to research cell lines that are usually available and employed in routine diagnosis and animal virology research. According to Kibenge et al. (6) the ability of IBDV to replicate in mammalian cells is an unusual cross-species biological properties since birnaviruses have not been isolated in higher animals. Thus, the use of such cells offers a valuable culture system of propagating IBDV. Consequently, most of cell lines used in the study were of mammalian origin and one of avian origin. Another objective of the study was to evaluate the real sensitivity of cell lines used in our laboratory.

The results of the studies showed a sensitivity of the RK-13 and Vero cells similar to that reported by Petek et al. (1) and Kibenge et al. (6). In both cells the CPE was similar to CEF cells, however more pronounced and it occurred earlier than in RK-13 cells. Kibenge et al. (6) have already reported that the replicative cycle of IBDV seems to be longer in Vero cells than in primary cultures.

Unfortunately, the cells of swine origin (SK-6 and IBRS-2) had only 50% of susceptibility for the samples. Furthermore, SK-6 cells required five passages to presented CPE while one or two passages were needed for the IBRS-2 cells, much less than for the Vero and RK-13 cells. It has not been described reports with IBDV in cells of swine origin. However, this sensitivity was too low to warrant further research.

The low sensitivity of CER cells of avian origin, for just one sample of IBDV may be due the number of cell passages since Arns et al. (1) reported successful propagation of IBDV in these cells only after the tenth passage. Another explanation could be the possible contamination this avian cell line during the development with the BHK-21 cell line, as reported by Smith et al. (12), that is not sensitive to IBDV (11).

Since RK-13 cells were shown to have the broadest spectrum for IBDV among the cells tested, they were selected for using in our VN tests.

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References

2- BECHT, H. Infectious bursal disease virus. Current Topics Microbiology Immunology, 90: 107-121, 1980
11- PETEK, M.; D'APRIILE, P.N.; CANCELLOTTI, F. Biological and phisico-chenical properties of the infectious bursal disease virus (IBDV). Avian Pathology, 2: 135-152, 1973