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Calu-3/A-549 mixed cells as a replacement for primary rhesus monkey kidney cells for virus detection

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Abstract

Background: Primary kidney cells derived from rhesus macaques (pRhMK) are heavily relied upon for the detection and culture of a wide range of clinically relevant viruses. The use of these cultures is problematic due to the possible presence of endogenous viruses, the need to sacrifice a primate, and the inherent variance found in primary cultures.
Objective: To develop a continuous cell line or mixed cell co-culture that could replace dependence on pRhMK cells.
Study design: Cells from the Calu-3 and A-549 cell lines were used to prepare mixed cell monolayers that were compared to pRhMK cells for their ability to detect respiratory viruses, measles, mumps, enteroviruses, and herpes viruses. Clinically derived and laboratory virus strains were used for these comparisons in culture plates or 16 mm tubes.
Results: Calu-3/A-549 cells are more sensitive than pRhMK for the detection of adenovirus, enteroviruses and herpes simplex virus and are about equally sensitive for the detection of other respiratory viruses, measles, mumps and varicella-zoster virus.
Conclusions: Calu-3/A-549 cells are an equivalent or better alternative to pRhMK cells for the detection of many clinically relevant viruses.

Keywords: Cell culture; Primary rhesus monkey kidney; Calu-3; Viral detection; Respiratory; Enterovirus

1. Introduction

Clinical virology includes a wide range of diagnostic assays, including molecular detection methods, such as real-time PCR, and advanced culture systems, such as co-culture of mixed cells in shell vials (Espy et al., 2006; Leland and Ginocchio, 2007). Despite these improvements in diagnostic technology, millions of units of primary rhesus monkey kidney (pRhMK) cells continue to be used yearly for routine viral detection of respiratory, enterovirus and herpes viruses. The reliance on pRhMK cells is problematic for three reasons: cells may contain endogenous viruses (Clarke et al., 1969) use of these cultures requires sacrificing primates, and inherent lot-to-lot variations of primary cells. The availability of a continuous cell line or a co-culture of cell lines that could replace pRhMK cells in the clinical laboratory would be a desirable alternative.

We found Calu-3 cells, a human epithelial cell line derived from a pulmonary adenocarcinoma, to be broadly susceptible to influenza (A/H1N1; A/H3N2; B), which led us to generate cell monolayers by mixing Calu-3 cells with A-549 cells derived from a human alveolar epithelial lung carcinoma cell line, and compared this co-culture to pRhMK cells for

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the detection of clinically relevant viral pathogens that were chosen because they are present in a wide variety of specimens typically inoculated onto pRhMK cells in the clinical laboratory.

2. Methods

2.1. Influenza A and B growth on Calu-3 alone and pRhMK cells

Three A/H1N1, four A/H3N2 and four influenza B viruses (American Type Culture Collection (ATCC) (Manassas, VA)), grown in pRhMk cells, were compared in Calu-3 and pRhMK cells (Diagnostic Hybrids, Inc. (DHI), Athens, OH) prepared in 6-well tissue culture plates. Cells were re-fed with Opti-MEM (Invitrogen, Carlsbad, CA), with 2 μg/ml of TPCK-treated trypsin (T-8802, Sigma, USA), without fetal bovine serum (FBS) and a multiplicity of infection (m.o.i.) of 0.001 was inoculated. For 7 days, 100 μl aliquots of culture medium were collected daily and frozen at −80 °C. Influenza virus titers were determined by diluting the aliquots of supernatants in R-mix re-feed media (DHI), inoculating onto 48-well plates of Mv1Lu cells (DHI), centrifuging at 700 × *g* for 1 h and incubating the cells at 35 °C for 18 h before fixing, rinsing and staining with influenza A or B direct fluorescent antibody (DFA) (DHI). Infected cells were counted using a fluorescence microscope to determine titer and expressed as fluorescent focus units per milliliter (ffu/ml).

2.2. Respiratory virus, measles and mumps culture on Calu-3/A-549 and pRhMK cells

Calu-3/A-549 mixed cells (DHI) and pRhMK cells prepared in 48-well culture plates were re-fed with Opti-MEM and inoculated in duplicate wells with 100 μl of supernatants from frozen clinical respiratory samples previously shown to be positive by DFA staining. Thirty-two samples each of parainfluenza 1 and 3, adenovirus, and respiratory syncytial virus (RSV) were tested along with 20 samples of both influenza A and B. Inoculated plates were centrifuged at 700 × *g* for 1 h and incubated at 35 °C. At day 1 or 3, cells were fixed and stained by DFA with appropriate virus specific antibodies (DHI).

Laboratory virus stocks of measles and mumps (ATCC), previously grown in pRhMK cells, were individually prepared in serial 10-fold dilutions and 100 μl of each dilution was inoculated in duplicate wells of Calu-3/A-549 and pRhMk cells before being centrifuged at 700 × *g* for 1 h and incubated at 37 °C. Cells were observed for 7 days for the presence of CPE. Infection was confirmed with fluorescent antibodies against each virus (Mumps MAb, Light Diagnostics, Temecula, CA; Measles MAb kindly provided by Ewa Bjorling (Karolinska Institutet, Stockholm, Sweden)).

2.3. Influenza viruses hemadsorption (HAD) on Calu-3/A-549 and pRhMK cells

Glass tube (16 mm) cultures of Calu-3/A-549 and pRhMK cells were rinsed once with HBSS (Mediatech, Herndon, VA), re-fed with Opti-MEM and inoculated in triplicate with frozen clinical influenza A and B specimens (DFA positive). Ten influenza A and eight influenza B samples were tested. After inoculation, tubes were incubated 1–3 days in stationary racks at 35 °C. One tube from each specimen was tested daily for HAD using guinea pig red blood cells (Hsiung, 1982).

2.4. Enterovirus culture on Calu-3/A-549 and pRhMK cells

Calu-3/A-549 and pRhMK cells in 48-well culture plates were re-fed with EMEM (Mediatech) containing 0.1% FBS (HyClone, Logan, UT) before inoculation. The 28 different enteroviruses (ATCC) were passaged once in Super BGMK cells (DHI) and stored at −80 °C. These frozen samples were arbitrarily diluted 1:1000 and inoculated at 100 μl per well onto duplicate wells of each cell preparation. Plates were centrifuged at 700 × *g* for 1 h and incubated for 6 days at 37 °C. Cells were monitored daily for CPE. All cells positive for CPE were confirmed with enterovirus antibody staining (Enterovirus Clone 5-D8/1 antibody, Dako Corp., Carpinteria, CA).

2.5. HSV-1 and 2 culture on Calu-3/A-549 and pRhMK cells

Glass tube cultures as in Section 2.3 were infected in duplicate with 200 μl of frozen clinical HSV-1 HSV-2 positive specimens. These clinical specimens were freshly diluted 1:100 or 1:1000 depending on the extent of CPE previously detected by culture in H&V mix cells (CV-1/MRC-5) (DHI). Fourteen HSV-1 and 23 HSV-2 samples were used. The inoculated tubes were incubated 21 days in stationary racks at 37 °C and were monitored daily for CPE. The original isolates were typed as HSV-1 or HSV-2 by fluorescent antibody staining (Syva, Trinity Biotech Inc., Wicklow, Ireland).

2.6. VZV culture on Calu-3/A-549 and pRhMK cells

Duplicate wells of 48-well culture plates were inoculated with 100 μl each of 13 frozen clinical samples previously found to be positive in H&V Mix cells. Plates were processed as in Section 2.4. Cells were monitored for CPE daily for 7 days and positives were confirmed by VZV DFA (DHI).

2.7. Statistical analysis

Significant differences ($p < 0.05$) between results for each evaluation were determined using dependent, two-tailed *t*-tests.