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# A Comparison Between Replication of Rinderpest Virus-Saudi 1/81 Virulent Strain in B95a and MoMo Cell Lines

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**Abstract:** *In vitro* cultivation of rinderpest virus is a routine procedure to isolate the virus in B95a cell lines. Monocyte modified human cell lines (MoMo) were not known to be used commonly for cultivation of Rinderpest Virus (RPV). In this study, the ability of both cell lines to support replication of RPV was tested. B95a and MoMo cell lines sustained replication of RPV-Saudi 1/81 virulent strain. Classical RPV Cytopathic Effect (CPE) was observed in both cell lines starting at day 5-6 of co- cultivation with Peripheral Mononuclear Cells (PBMC) of the infected calves. RPV P-protein antigen was detected in both infected cell lines on day 4 of inoculation.

**Key words:** B95a cell lines, MoMo cell lines, rinderpest virus, PBMC, RPV, BCE

## INTRODUCTION

The infectivity of rinderpest as a viral disease to even-toed animals was known very early (Barrett, 1999) Pathogenicity of the virus was known to be due to involvement of the lymphoid and epithelial tissues (Rossiter and Wardly, 1985; Wohlsein *et al*., 1995; Tatsuo *et al*., 2001). Many in vivo and in vitro studies were conducted to investigate the ability of Rinderpest Virus (RPV) field isolates to infect different lymphoid subsets and myeloid or epitheloid cells. The virus grew readily in mitogen-stimulated bovine lymphoblastoid cell lines and better growth was observed in populations of predominantly T-lymphocytes than in T-depleted cultures (Rossiter and Wardly, 1985; Rossiter *et al*., 1988). Furthermore, ready growth was also observed in lymphoid B-cells, $CD4^+$ and $CD8^+$ alphal/beta and gamma/delta T-cells producing new infectivity, viral antigens, Cytopathic Effect (CPE) and total cell death (Rossiter *et al*., 1993). None of the lymphoblastoid phenotypes was found to be a particular site of preference to the virus. All known RPV strains were capable to infect B95a, a derivative of B95-8 Epestein-Barr virus transformed marmoset B-lymphoid cell lines (Kobune *et al*., 1991). However, infectivity to animal macrophoge monolayers was observed to be greater than that of lymphocytes (Rossiter *et al*., 1988).

Aiming to further study the ability of RPV-Saudi 1/81 virulent strain to reproduce in target cells, two cell lines, B95a and modified monocyte human cell lines (MoMo) were used for this purpose.

## MATERIALS AND METHODS

**Preparation of the lymphoid cell lines:** B95a and MoMo cell lines, a gift from Dr. Kobune F., Department of Measles Virus, National Institute of Health, Tokyo, to Pirbright Laborotary, IAH, UK in 1998, were maintained by continuous culturing using RPMI-1640 medium containing L-glutamine and 25 mM HEPES (Life Technologies) and supplemented with 5% foetal calf serum. Confluent monolayers of B95a in 75 $cm^3$ tissue culture flasks (Falcon) were passaged in a ratio of 1:3 using 15 mL fresh RPMI-1640 medium. MoMo cells were passaged in the same ratio of the B95a cell lines after washing the confluent cells twice with 10 mL versine-trypsin solution and detachment of cells with 5 mL

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versine-trypsin solution. All flasks were incubated at 37°C in presence of 5% $CO_2$ tension and 95% atmospheric pressure.

**Isolation of the Peripheral Blood Mononuclear Cells (PBMC):** Heparinized venous blood from two 5-month-old RPV-Saudi 1/81 experimentally-infected Angus calves were collected on days 0, 2, 5, 7 and 9, of infection following the local Animal Ethics Committee for experimental animals. Initially, about 20-30 mL of the blood from each calf was centrifuged at 500-600× for 10 min at 18-20°C. Collecting the buffy coat at the plasma-RBCs interface, the volume of each was brought up to 20 mL with sterile Hank's-Balanced Salt Solution (HBSS) or Phosphate-Buffered Saline (PBS) making an approximate dilution of 10%. PBMC were purified from the buffy coats by centrifugation over 10 mL sucrose (histopaque) 1083 solution (Sigma) at 800×g at 18-20°C for 30 min. Cells harvested at the interface were washed 3 times in HBSS or PBS at 400×g at 4°C for 10 min using 8 times the harvested PBMC volume. Prior to use, each PBMC pellet was dissolved into 2 mL RPMI-1640 medium containing L-glutamate, 25 mM HEPES and 5% fetal calf serum or were stored at -196°C.

**Cytopathic effect of rinderpest virus-Saudi 1/81 virulent strain in cell lines:** Twenty four hours before infection, B95a and MoMo cell lines were passaged either in 22 mm diameter cell culture plates (Corning) or on coverslips contained into these plates using the same procedure of passage described previously. Two hundred microliter/well of the freshly harvested and/or stored PBMC were co-cultivated at a rate of $4\times10^6$ cells $mL^{-1}$ RPM1-1640 medium with the prepared cell lines after decanting their growth medium. Allowing incubation period of 1h, fresh RPMI-1640 medium was added at a volume of 800 μL $well^{-1}$. 100 μL of 10 μg $mL^{-1}$ phytohaemaggulatinin-P was added to each stored and recovered PBMC to assist tissue differentiation. All plates were incubated at 37°C 70 and observed for CPE.

**Indirect immunfluorescent antibody technique:** A monoclonal antibody-based Indirect Immuno Fluorescence (IIF) was used to detect viral antigens in 4-day-old RPV-infected B95a and MoMo cell lines. Coverslips showing confluent monolayers or very early signs of CPE were removed, fixed at room temperature through washing 3 times with 2 mL $Ca^{2+}$ and $Mg2^{+}$ free sterile Phosphate-Buffered Saline (PBSA) and incubated for 20 min in 2 mL 3% formaldehdye. All coverslips were again washed with 2 mL PBSA before quenched with 2 mL 50mM $NH_4Cl$ in PBS for 10 min. Then, the specimens were washed with 2 mL 3 times PBSA followed by 2 mL 0.2% PBSA-gelatin for 5 min. Using parafilm strips in moist chamber, the coverslips were incubated at room temperature on top of about 30 μL of 1:1000 PBSA-gelatin-diluted first Ab (RPV- anti-P Mab) for 20-30 min. Following this, the specimens were washed twice with fresh 2 mL PBSA-gelatin buffer followed by two washings with 2 mL PBSA, 5 min each step. Then, the cells were stained with 1:1000 PBSA-gelatin-diluted second Ab (marinablue rabbit anti-mouse conjugate), for 20-30 min at room temperature. Finally, the cells were washed with 2 mL PBSA-gelatin and PBSA, respectively, for 5 min before rinsing the coverslips in distilled water, drying and mounting them in about 30 μL Mowoil. All slides were examined by fluorescent microscopy after drying for not less than 45 min at room temperature.

## RESULTS

**Recovery of RPV-Saudi 1/81 virulent strain from PBMC of the experimental calves:** RPV-Saudi 1/81 virulent strain was successfully recovered in the cell lines from PBMC of the infected calves from day two postinfection till euthanize on day eight. Classical RP viral CPE indicative of viral recovery were shown in B95a and MoMo cell lines at days 5-6 of infection. Multinucleated cell syncytiaformation preceded by cell aggregation was observed (Fig. 1). Stellate cells were also seen in some slides.

**Detection of viral antigens in infected tissues by indirect immunofluorescence:** Positive IIF was observed in

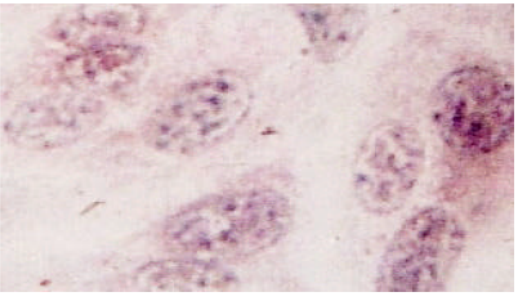

Fig. 1: Syncetial cell formation in 8-day-infected B95a lymphoblastoid cell lines passaged at a ratio of 1:3 and co-cultured in a volume of 200 μL/well with $4\times10^6$ PBMC/mL RPM1-1640 medium isolated from control infected Angus calf No. TQ96 (H and E staining, 100X)

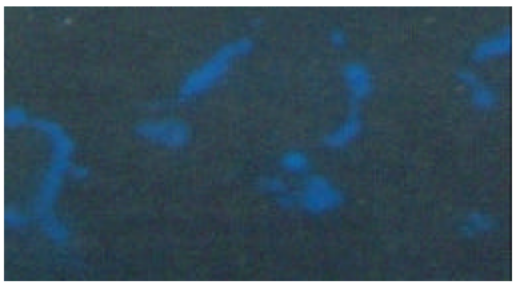

Fig. 2: Indirect immunofluorescence microscopy of cell cytoplasm and cytoplasmic membrane of 4 days RPV-Saudi 1/81-infected MoMo cell lines after staining with marinablue conjugated anti-RPV P Mab (100X)

MoMo cell lines co-cultured with PBMC obtained from RPV-Saudi 1/81-infected Angus calves (Fig. 2). Immunofluorescence was observed from day 2 of infection till euthanize on day 9.

## DISCUSSION

In this study, the infectivity of the highly virulent RPV- Saudi 1/81 strain was studied using two cell lines. Both B95a and MoMo cell lines showed characteristic RPV CPE. However, the time of detection of the CPE and its pattern was observed to be earlier and more pronounced in B95a than in MoMo cell lines. This could be due to species difference of the origin of the two cell lines. The variations in mitotic and metabolic rates of the individual cell line should also be considered (Rossiter *et al*., 1993). Compared to other cell lines, a relatively slow but productive infection by RPV strains was observed on using bovine monocytes and monocyte-derived macrophages (Rey *et al*., 1995). On the other hand, it was found that measles virus, antigenically close to RPV, replicated in and damaged B-lymphocytes, T-lymphocytes and monocytes leading to immunosupression (Hyypia *et al*., 1985; Mcchesney and Oldstone, 1989; Salonem *et al*., 1988). Contrary to this, monocytes but not the lymphocytes were found to be the main leucocyte targets during natural RPV and measles virus infection (Rossiter and Warrdley, 1985; Esolen *et al*., 1993).

The production of rinderpest characteristic CPE with expression of H and N proteins (Rey *et al*., 1995) simulate the findings in this study. Convenient expression of P-protein in B95a and MoMo cell lines using anti-P protein Mab and IIF staining technique was demonstrated as early as day four of infection. Since the P-protein is claimed to be involved in RNA transcription and replication (Alkatib *et al*., 1988), successful study of the sequential events in viral replication could be attained. A fact that could be initiated by the major role played by macrophages as antigen presenting cells. Since viruses and viral RNA were retrieved from PBMC of RPV-infected calves from day 2 till death on day 9 (Haroum *et al*., 2007) the techinque could be useful for diagnosis compared to CPE.

## CONCLUSION

The technique could be used alone or in conjunction with CPE. However, the type of the Mab used is very crutial. Further studies to investigate the time and rate of experession of different RPV- protiens in relation to the CPE in both infected-B95a and MoMo cell lines are needed.

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