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An *in-vitro* study on the alterations induced by *Mycoplasma arginini* on cell cultures and virus titres

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ABSTRACT

After 4 days of post-infection (dpi) of Madin Darby bovine kidney (MDBK) cell monolayers with *Mycoplasma arginini*, rounding of cells, vacuolation in the cytoplasm and lysis of cells in the monolayer thereby creating empty space, lysis and condensation of karyon along with perinuclear hallowing were observed. In VERO cell monolayers. *Mycoplasma arginini*, induced enlargement, granulation and vacuolation in the cytoplasm, empty spaces in the monolayers and presence of clumped masses in the karyon. At a dilution of 10^{-7}, and above and 10^{-8} and above of *Mycoplasma arginini* produced no observable alterations in MDBK and VERO cell monolayers respectively. *Mycoplasma arginini* decreased the titre of infectious bovine rhinotracheitis virus (IBRV) 10 fold when it was cultivated in MDBK cell line.

Key words : Cell cultures, *Mycoplasma arginini*

Mycoplasmas the most common contaminants of cell cultures, alter the virus titres significantly and threat in the propagation of stable cell lines useful for cultivation of animal viruses and production of viral vaccines. *Mycoplasma arginini*; *Mycoplasma hyorhinis, Mycoplasma orale* and *Acholeplasma laidlawlii* are the most common cell culture contaminating mycoplasmas.

Mycoplasmas mimic the *in vitro* effects of viruses. In cell culture systems, they bring about cell death, alter cell growth, affect the virus titres, induce interferon, cause chromosomal aberrations, induce transformation and cytopathic effects, alter phenotypic expression and metabolic pathways and products of cells. Hence such contaminated cell lines are no longer useful for the cultivation of viruses and production of vaccines.

In this investigation ,attempts were made to study the alterations induced by *Mycoplasma arginini* specifically on MDBK and VERO cell culture monolayers. Attempts were also made to study the effects on IBRV titres on experimentally mycoplasma infected MDBK cell line.

MATERIALS AND METHODS

Mycoplasma arginini was obtained from Division of Bacteriology and Mycology, IVRI, Izatnagar. Mycoplasma free Madin Darby bovine kidney (MDBK) and VERO cell lines maintained in the Department of Veterinary Microbiology and Public Health, Veterinary College, Bangalore, were used. Infectious bovine rhinotracheitis (IBR) virus, strain OCS 814 maintained in this department was used.

Revival and maintenance of M. arginini *culture*

Mycoplasma arginini from the freeze-dried ampoule was inoculated into 5 ml MBHS-L medium and incubated at 37° C for 5 days. After incubation, organisms were spread onto petriplates containing MBHS-A medium and incubated for 7 to 10 days at 37°C. Plates were observed for the presence of fried egg like colonies and stored at 4°C for further use. Culture was revived at monthly intervals.

Growth and maintenance of Madin Darby bovine kidney (MDBK) cells

The MDBK monolayer in milk dilution bottle was washed thrice in warm calcium -magnesium free PBS (CMF-PBS), flooded with 10 ml of warm trypsin versene glucose saline (TVGS) solution and left at 37°C for 5 min. The bottle was gently shaken to dislodge the cells from the glass surface. After adding 10 ml of growth medium, forceful pipetting was done to separate the individual cells. The volume was made up to 50 ml and about 5 ml quantities were distributed into each of the test tubes with coverslips. The tubes were sealed with rubber corks and incubated at 37°C till a confluent layer was formed. The monolayer was washed with warm CMF-PBS and was maintained using maintenance medium containing 2% neonatal calf serum (NCS).

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Growth and maintenance of VERO cells

The procedure adopted to grow MDBK cell line was followed to maintain the VERO cells also.

Infection of MDBK and VERO cell lines with M. arginini

Both the cell lines were washed thrice with warm CMF-PBS (pH 7.40). Different dilutions of *M. arginini*, ranging from 10^{-1} to 10^{-8} were made in the mycoplasma liquid medium from each dilution was inoculated into a set of 2 tubes each of the cell lines, respectively, and incubated at 37° C for 1 hr with an intermittent shaking to enable the organisms to adsorb to the cells. At the end of the incubation, the inoculum was discarded, the monolayers were washed with warm PBS and maintained in 1.5ml of the maintenance medium. Uninoculated monolayers served as controls . The tubes were incubated at 37°C for 8 days and were observed for the cytopathic changes daily under inverted microscope.

Staining of infected monolayers

May -Grunwald Giemsa's staining technique was applied to stain the infected monolayers. The monolayers were cleared by rinsing 3 times in acetone - xylol (2:1), 3 times in acetone -xylol (1:2) and 10 min in fresh xylol. The coverslips were finally mounted using DPX and observed under microscope.

Virus titration

Infectious bovine rhinotracheitis virus revived in MDBK cell line was titrated for the infectivity titres following the procedure of Rovozzo and Burke (1973). The titres were calculated (Reed and Muench 1938) and expressed as log10 TCID50/0.1ml.

Titration of IBR virus with monolayers infected with M. arginini

The procedure described by Rovozzo and Burke (1973) was followed. The titre of virus was calculated (Reed and Muench1938) and expressed as log10 TCID50/0.1ml.

RESULTS AND DISCUSSION

Changes due to M.arginini *in cell culture monolayers*

Generally, mycoplasmas have deleterious effects on cell culture monolayers. In the present study MDBK and VERO cell culture monolayers were infected with different dilutions of *M. arginini*.The cytopathic changes in MDBK cell lines when infected with *M. arginini* were , rounding of cells , vacuoles in the cytoplasm, presence of empty spaces in the monolayer due to desquamation of cells, condensation and hallowing of the nucleus and karyolytic changes. In addition to the above changes, in VERO cell lines, 'leopard' clumped masses in the karyon, granules and vacuoles in the cytoplasm were also observed. In both the cell lines, the intensity of alterations decreased with the increase in dilution of the organism. However, the changes were more intense in VERO cells than MDBK cells. This indicates the increased susceptibility of VERO cells to *M.arginini* than MDBK cells. This may be due to variation in the cell line sources. The eighth dilution of *M.arginini* did not show any observable changes either in MDBK or VERO cell lines and the MDBK cell lines remained normal when infected with *M. arginini* at seventh dilution. Similar results were also reported by Grumbles *et al*.(1964), and Ahuja and Chandiramani (1975) when avian mycoplasmas infected the avian cell cultures. Srivastava *et al.* (1987) also observed the breaking of chromatin materials inside the nucleus when BHK21 cells were infected with bovine mycoplasmas.

Boatman *et al.* (1976) observed an increase in the size of the infected cells when different cell lines were infected with the mycoplasmas. The results of this experiment were closely parallel to the results obtained by Aldridge (1975). He noticed that the changes caused by *M.synoviae* in chick embryo cell cultures included cytopathic granularity and mild vacuolation followed by complete degeneration of the cytoplasm and nuclear degeneration of most of the cells.

The above studies clearly indicate that the changes are strain dependent. Highly diluted inoculum (10^{-8}) did not produce any observable changes, this may be due to the low concentration of the organisms.

Effect of M.arginini *on the titre of IBR virus*

In this study, prior contamination of MDBK cell lines with *M.arginini* at 10^{-8} dilution caused a decline in the titre of IBR virus by 10.17 units. But contamination by coinfection of MDBK cells with *M. arginini* (10^{-9}) and IBRV had no effect on the titre of the virus. The decrease in the titre of herpes simplex virus was also reported by Manischewitz *et al.* (1975) in VERO cells contaminated with *M.arginini*. However, the above inhibitions were partly reversed by the addition of arginine to the growth medium.

The reduction in the titre of IBR virus in the presence of *M.arginini* in this study, was most probably because of the depletion of arginine resulted by the competition for the same by both IBRV and *M.arginini* in the medium.

This study indicated the types of cytopathic changes produced in MDBK and VERO cell lines by various concentrations of *M.arginini* and also highlights the effect on IBRV titre in mycoplasma infected cell cultures.

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