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PROPAGATION OF SHEEPPOX VIRUS IN CELL CULTURE[1])

F. A. JASSIM, *B. S. KESHAVAMURTHY

Veterinary Laboratories and Research Institute, Abu-Ghraib, Baghdad, Iraq; and *Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, India

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Summary. — The Ranipet strain of sheeppox virus (SPV) was adapted to secondary cultures of kid testes cells but not to BHK-21, Vero and chick embryo cells. The cytopathic effect (CPE) produced by SPV in the kid and lamb testes cells was almost similar except that conglomerates of rounded cells resembling to bunches of grapes appeared in the former.

Key words: sheeppox virus; testes cells

Introduction

Attempts were made to propagate the sheeppox virus (SPV) in different cell cultures like primary kid testes cells, BHK-21 cells, chick embryo cells and Vero cells (Ramyar and Hessami, 1967; Pandey and Singh, 1970; Mirchamsy and Ahouri, 1971; Edlenger and Ftimavici, 1973; Sundararajan *et al.*, 1973; Bhatnagar and Gupta, 1974; Davies, 1976; Subba Rao, 1978). The reports on the susceptibility of these cells to SPV were conflicting. Therefore, the ability of secondary kid testes cells, lamb testes cells, chick embryo cells, BHK-21 and Vero cells to support the growth of SPV was reinvestigated.

Materials and Methods

Virus. The virulent Ranipet strain of SPV obtained from the Institute of Veterinary Preventive Medicine, Ranipet, was used in the present study.

Cell culutres. Medium 199 was used to culture kid testes cells, chick embryo cells and Vero cells. BHK-21 cells were cultivated in Eagle's medium. Both media were supplemented with 10 per cent inactivated calf serum, except that five per cent goat serum was used for the Vero cell cultures. In addition, media used for culturing of kid testes and BHK-21 cells were supplemented with Tryptose phosphate broth (0.295 w/v). The maintenance medium contained one per cent calf serum.

Primary and secondary cultures of kid testes cells were prepared following an adopted method for preparation of lamb testes cells (Mateva *et al.*, 1974). The chick embryo cells were prepared from 9-10 days old chick embryos. BHK-21 and Vero cells were propagated by continuous culturing. All cultures were prepared in milk dilution bottles and on cover slips.

The completed monolayers were inoculated with 1 ml of 1 : 10 dilution of stock virus suspension. The bottles were gently shaked every 10 minutes to achieve even spread of inoculum. The excess of inoculum was discarded, monolayers were washed once with pre-warmed phosphate buffered saline (PBS), maintenance medium was added and then the cells were incubated at

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37 °C. Uninfected controls were incubated in parallel. The pH of the medium was corrected, if necessary, during the incubation period. The infected monolayers were daily observed.

The virus was harvested when maximum CPE appeared. The cultures were frozen at −20° C along with the medium and thawed once. The thawed material was used for further serial passages.

Both control and infected cultures grown on coverslips were stained with May-Grünwald-Giemsa at appropriate intervals post infection (p. i.).

Results and Discussion

In the secondary culture of kid testes cells SPV produced CPE in the first passage. The CPE was evident 48 hr p. i. and reached maximum by 96 hr. Single or clustered rounded cells formed bunches of grapes. The retraction of the affected cells left long gaps in the monolayer as the CPE progressed. The cytoplasm was granular in many cells. In the second passage, the CPE was observed as early as 24 hr p. i. and was complete by 72 hr. The cells were bulging and the aggregation of rounded cells was pronounced. The spindle shape of some cells was preserved. The control cultures did not show any degenerative changes.

In stained coverslip preparations large intracytoplasmic inclusions surrounded by a distinct halo were seen since 24 hr p. i. The syncytia were abundant. Both cytoplasmic vacuolation and degenerative changes of nuclei (pycnosis, karyorrhexis) were noticed at later stages of infection. No CPE was detected when the virus was put through six blind passages in chick embryo cells, BHK-21 or Vero cells.

CPE appeared early in secondary kid testes cell cultures infected with SPV. Clustering of rounded cells resembling to bunches of grapes was abundant. Pandey and Singh (1970) noticed only a few clusters of cells in primary kid testes cell cultures infected with three SPV strains (Rumania, Sirsa and Jaipur); the initiation of CPE was seen from 36—48 hr and 96—144 hr were required for its completion. These differences possibly reflect variations in susceptibility of the secondary and primary cultures of kid testes cells to SPV, but may be also attributed to strain differences. More comparative studies using different virus strains have to be done to understand this behaviour of the virus.

Subba Rao (1978) observed that the Ranipet strain of SPV could be grown in kid testes primary culture, but the onset of CPE was as late as 120 hr at the first passage as compared to 72 hr in subsequent passages. This observation is suggestive for lower susceptibility of the primary kid testes cells as compared to the secondary cultures used in this study. It also emphasizes that secondary cultures are better for growing SPV than the primary ones since both primary lamb and kind testes cells behaved alike. Cilli and Baldelli (1958) pointed out that fibroblast cells were more suceptible to SPV than epithelial cells.

The degenerative changes in kid testes secondary cultures infected at same multiplicity occurred earlier as compared to the lamb testes secondary cultures which remained healthy even up to 10-14 days. The view of early appearance of the CPE, the kid testes secondary culture seemed to be a more promissing alternative system for cultivation of SPV (Ranipet strain).

In our hands SPV failed to show evidence of propagation in chick embryo cells even after six blind passages. Regarding to the propagation of SPV in this system, different laboratories reported contraversal results. Sundarajan *et al.* (1973) and Bhatnagar and Gupta (1974), failed to propagate the virus in chick embryo fibroblasts; in contrast, Ramyar and Hessami (1967) claimed that these cells did support the growth of SPV. Subba Rao (1978) stated that the same strain of SPV as used by Sundarajan *et al.* (1973) and in the present study, was propagated in the chick embryo fibroblasts and that intracytoplasmic inclusion bodies were found in infected cells.

Vero cells were found resistant to SPV; similar findings were also observed by Mirchamsy and Ahouri (1971) and Davies (1976). Attempts to propagate SPV in BHK-21 cells were not successful. Ramyar and Hessami (1967) reported that BHK-21 cells were susceptible to SPV but Davies (1976) failed to propagate some strains in BHK-21 cells. The strain used in this study may belong to those that could not grow in BHK-21 cells.

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