Minor Research Project

On

In Vitro Replication and Serial Passaging of BmNPV In A Homologous Cell Line

By

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Summary of Findings:

- DZNU-Bm-12, a homologous cell line for BmNPV was obtained from Insect Cell Culture laboratory, P.G.T.D. Zoology, RTM, Nagpur University, Nagpur. It was maintained in a MGM-448 medium. The cells were grown in 25 cm^2 polystyrene and glass tissue culture flasks and incubated at 25 ± 1^oC. They were subcultured at appropriate intervals depending on cell density. This cell line was then used for inoculation of BmNPV. The ovarian cell line, DZNU-Bm-12 was found highly susceptible to BmNPV showing 84-90% infection and about 4.6-8.5 x 10^5 infected cells/ml yielding 0.9 x 10^7 to 1.3 x 10^7 OBs/ml.
- BmNPV inoculum was obtained from diseased larvae of the silkworm, *B. mori.* Each larva was inoculated individually via ingestion of 2-cm^2 piece of mulberry leaf coated with 10 µl suspension of OBs of BmNPV. Turbid haemolymph of fifth instar larvae infected with BmNPV was collected through an incision on proleg. After centrifugation (1000g for10 min) the supernatant was diluted with equal volume of medium, passed through 0.45 µm membrane filter and used as inoculum to infect the cultures. Budded virus of passage one was harvested and cell suspension was centrifuged at 1000g for 5 min at room temperature. The cell pellet was discarded and the supernatant was stored in the refrigerator at 4^oC.
- Duplicate 25cm^2 cultures were seeded at 3x 10^5 cells /ml in MGM-448 medium and allowed to grow 1x10^6 cells /ml before being diluted to 5 x 10^5 cells/ml with 10 ml fresh medium. These cultures were infected at m. o. i. of 10 infectious units (IU). The infected cultures were maintained at 25 ±1^oC. Addition of inoculum of BmNPV at m. o. i. of 10 to the healthy cells did not immediately affect the integrity of the cells. However within an hour the cells exhibited aggregation and formed cell aggregates of varying size indicating the initiation of infection process. Subsequently at 16-18 hours post

inoculation (h. p. i.), enlargement of cells due to nuclear hypertrophy could be seen in the cultures

- Cells were examined in culture flasks with a Olympus inverted phase contrast microscope. The criterion of BmNPV infection was presence of polyhedral inclusion bodies (PIBs) in a cell. A small number of cells was removed from the infected cultures and transferred to microscopic slides. The cells were then spread out by placing a cover glass over the slide. Differential counts of healthy and infected cells were made. Percentage infection and number of OBs per cell were determined.
- The cells from infected cultures were harvested at different intervals post infection, pelleted and washed in 0.1 M phosphate buffer saline (PBS) with a pH of 7.2. They were fixed for 4 h at 4°C in modified Karnovsky fixative (David *et al.*, 1973) buffered with 0.1M Sodium Phosphate Buffer at pH 7.4. The pellet was washed in fresh buffer, and post fixed for two hours in 1% Osmium Tetraoxide in the same buffer at 4°C. After several washes in 0.1M Phosphate Buffer, the cells were dehydrated in graded acetone solutions and embedded in CY 212 araldite. Ultrathin sections of 60-80 nm thickness were cut using an Ultracut E (Reichert Jung) microtome and the sections were stained in alcoholic uranyl acetate (10 min) and lead citrate (10 min). The grids were made and viewed under a transmission electron microscope.
- The free nucleocapsids present in the inoculum were adsorbed to the surface of cells and attached to the plasma membrane within 1-2 h. p. i. The nucleocapsids were engulfed in the invaginations of plasma membrane. Subsequently, the nucleocapsids appeared bounded by a membrane that was presumably acquired from invaginated plasma membrane.
- In most of the cells small aggregates of fine electron opaque granules appeared throughout the nucleus by 24 h. p. i. These structures are probably the precursors of virogenic stroma. About 32 h.p.i. the aggregates of numerous small particles increased in size and density. Nucleoplasmic matrices became considerably electron lucent 32 h.p.i., whereas the virogenic stroma increased in size and electron opacity and formed a mass in the nucleus. No distinct virus rods were detected in nuclei at this time.
- Bm-12 cells showed progressive and characteristic changes like clumping of cells followed by enlargement of nuclei and displacement of chromatin along the nuclear envelope up to 20-24 h.p.i. From 40-44 h.p.i. several unenveloped virus rods appeared

and were closely associated with virogenic stroma. At this stage several viral rods were seen in the areas between the virogenic stroma and the nuclear envelope (peristromal area) . At 52 h.p.i several virus rods were observed in the cytoplasm that appeared to be enveloped. These enveloped virus rods traveled through the cytoplasmic matrix towards the plasma membrane. They budded from the host cell surface, acquired the plasma membrane and subsequently were released from the host cell surface.

- A few developing OBs were seen in infected cells after 52 h.p.i. During the late period of infection ECV release was abrogated in favour of OBs formation as a result number of OBs were observed in the nuclei. The breakdown of nuclear membrane and lysis of plasma membrane in the OBs-loaded cells was not evidenced in the present study.