Viral Interference in TO-2 Cells Infected with IPN Virus Isolated from Clam, *Meretrix lusoria*

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The viral interference exhibited by HB-1 virus, a strain of infectious pancreatic necrosis virus (IPNV) isolated from hard clam, *Meretrix lusoria*, was studied. The TO-2 cells infected with serial passage HB-1 virus at 10^0 to 10^{-4} dilutions showed that viral interference was related to the degree of dilution of virus inoculum. The specific viral antigens were detected with immunofluorescent antibody stain technique in TO-2 cells which survived from high multiplicity infection of HB-1 virus. The virus yield in culture fluid from a series of 20 serial undiluted or diluted passages indicated that serial undiluted or low-diluted (10^{-1} to 10^{-2}) passaging of HB-1 virus could induce autointerference in TO-2 cells. The defective interfering (DI) particles in the virus samples were considered to be responsible for the interference.

The SDS-PAGE analyses for the polypeptide composition of virions from serial diluted and undiluted passaging showed that there were differences in β group. The truncated β polypeptides were regarded to be specific polypeptides produced by DI particles generated by serial undiluted passaging. We, thus, provided new information concerning IPN DI particles by comparing the properties of the virions produced by serial diluted and undiluted passaging of HB-1 virus, a strain of IPNV.

Materials and Methods

Cells and Viruses

The continuous cell line, TO-2, derived from tilapia hybrid (*Tilapia mossambica* × *T. nilotica*) ovary (Chen et al., 1983) was used in this study. TO-2 cells were cultured at 28°C in 25 cm² plastic flasks in L-15 medium supplemented with 5% fetal calf serum. The cells were routinely subcultured at 3 or 5 day intervals. HB-1 clam virus, a strain of infectious pancreatic necrosis virus (AB strain), isolated from the gills of *Meretrix lusoria* (Lo et al., 1988) was used in the present studies. Virus stock was prepared by five low-multiplicity (0.01 TCID₅₀ unit/cell) rounds of amplification in TO-2 cells.

Cytotoxic Effect (CPE) in TO-2 Cell Cultures Infected with Diluted and Undiluted HB-1 Virus

The infected cells inoculated with 10^0 to 10^{-4} dilutions of virus stock were observed under Olympus inverted microscope. For electron
microscopy, the cells infected with HB-1 virus for 7 days were fixed at 4°C in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer to pH 7.2 for 2 h. Postfixation was carried out in 1% osmium tetroxide in 0.05 M phosphate buffer, pH 7.2 for 1 h. The cell blocks were made by pelleting the double-fixed cells at 1500 rpm for 20 min. The cell blocks were dehydrated in a graded ethanol series, followed by an absolute acetone, acetone/Spurr epon series and then embedded in pure Spurr epon. The thin sections were made by Reichart U3 ultramicrotome and stained with uranyl acetate and lead citrate. The stained sections were observed by JEOL 100S electron microscope.

Detection of viral antigens in TO-2 cells survived from HB-1 virus infection at high multiplicity of infection was also carried out by immunofluorescent antibody stain technique with procedures similar to that of KENNEDY and MACDONALD (1982).

Virus Yield in Culture Fluid from Undiluted and Diluted Passages

Serial undiluted passaging of HB-1 virus was initiated by infecting TO-2 cells (1 × 10^7 cells) with 1 ml of undiluted virus stock. The passages were made at 7 day intervals. For the subsequent passages, 1 ml membrane (0.22 μm) filtered culture fluid from previous passage was used as virus inoculum. For every passage, the infection was done by absorbing the virus inoculum to the cells for 1 h at 25°C. The monolayer was washed three times to remove the unabsorbed virus, and then 5 ml of fresh L-15 medium plus 2.5% FBS was added per culture. Cultures were incubated at 20°C for 7 days. Virus titers in the culture fluids of infected cells were determined by TCID_{50}/0.1 ml/analysis at 20°C. All the results were shown by the geometric mean of TCID_{50}/0.1 ml/analysis values for the culture fluids from two flasks which were assayed separately. Serial diluted passaging of HB-1 virus was also done at the same time. Instead of undiluted virus, the virus inoculum for the absorption of the cells was at a dilution from 10^{-3} to 10^{-4}. The virus titer of each passage was analyzed in the same manner as those of serial undiluted passaging for comparison.

Polypeptides of Purified Virus Particles Produced by Serial Undiluted and Diluted Passaging

Passage 50 virus particles of diluted (10^{-4}) or undiluted virus preparation were purified using CsCl gradient centrifugation with a procedure described by CHANG et al., (1978) with some modifications. Virus bands at densities of 1.30 g/mL and 1.34 g/mL in CsCl were collected for the analysis of viral polypeptides using 10% SDS polyacrylamide gel electrophoresis and stained with silver stain.

Results

Cytopathic Effect (CPE) in TO-2 Cell Cultures Infected with Diluted and Undiluted HB-1 Virus

In TO-2 cells, inoculated with diluted HB-1 virus, lysis usually commenced at 3 day post-infection and resulted in lytic infection (Fig. 1B). In contrast, the TO-2 cells infected with a undiluted virus failed to show any serious CPE. The survival of infected cells was also observed (Fig. 1E). The cells infected with serial pas-
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sage 15 HB-1 virus at 10\(^{-6}\) to 10\(^{-4}\) dilutions showed that viral interference was related to the degree of dilution of virus inoculum (Fig. 1 B-E). The specific viral antigens were detected in immunofluorescent antibody stain technique TO-2 cells survived from high multiplicity infection of HB-1 virus (Fig. 2). The results suggested that autointerference occurred in the cells inoculated with HB-1 virus at high multiplicity input.

The TO-2 cells infected with diluted HB-1 virus for 7 days exhibited CPE as follows. The cytoplasm appeared to be highly vacuolated; the swollen mitochondria and the secondary lysosome containing many degenerated organelles were readily seen; the nuclei exhibited some chromatin reduction and usually possessed more than one large mass of about the same electron density; the mature virions were released from the cell (Fig. 3A). Filamental capsids and virus crystals were infrequently observed.

The TO-2 cells infected with an undiluted inoculum appeared as normal cells except for the swelling of the mitochondria and Golgi apparatus in the cytoplasm, as well as the presence of a large amount of heterochromation in the nucleus. The virus particles were aggregated and limited to several areas of the cytoplasm. The filamentous capsids or virus crystals were seen frequently (Fig. 3B).

Virus Yield in Culture Fluid from Serial Undiluted and Diluted Passaging

A series of 20 serial undiluted or diluted passages of HB-1 virus was studied. The titer at each passage was shown in Fig. 4. The reduction of virus yield was observed in serial undiluted passaging. The virus titer fluctuated from 10\(^{6.5}\) to 10\(^{9.2}\) TCID\(_{50}\)/0.1 ml. The virus yield in culture fluids of serial passaging at low dilution (10\(^{-1}\) to 10\(^{-3}\)) was also reduced. Passaging at higher dilutions (10\(^{-3}\) to 10\(^{-4}\)) induced little viral interference. The virus titer at each passage fluctuated from 10\(^{6.3}\) to 10\(^{8.4}\) TCID\(_{50}\)/0.1 ml. These fluctuation approximated the yields obtained routinely under optimal conditions. The results indicated that serial undiluted or low-diluted (10\(^{-1}\) to 10\(^{-3}\)) passaging of HB-1 virus could induce autointerference in TO-2 cells.

Polypeptides of Purified Virus Particles Produced by Serial Undiluted and Diluted Passaging

The structural polypeptides of virus particles were analyzed using polyacrylamide gel electro-
Fig. 4. Comparison of virus yield in culture fluid from undiluted and diluted passages. Culture fluids at A, $10^6$ (▼▼) and $10^{-4}$ (▼▼); B, $10^{-1}$ (▼▼) and $10^{-4}$. 
Fig. 4. C, $10^{-2}$ (▼▼) and $10^{-4}$; D, $10^{-3}$ (▼▼) and $10^{-4}$ dilutions.
phoresis. Fig. 5 shows the relative mobilities of the viral structural polypeptides produced by undiluted and diluted passages. Three size classes of viral polypeptides were observed. In the α and γ groups of viral polypeptides, no differences were observed in virus particles produced by serial undiluted and diluted passing. In contrast, differences were observed in the β group. The β polypeptides, including β dimer, β1, β2, and β3 were smaller than that of the virions produced by serial diluted passing. The polypeptide profile of mixed virus particles produced by serial undiluted and diluted passing confirmed that in the β group, the virions of serial undiluted passing have smaller viral polypeptides than those of serial diluted passing (Fig. 5).

Discussion

HB-1 virus was isolated from hard clam, *Meretrix lusoria*. The serological and biochemical studies indicated that this virus shared the characteristics of AB IPNV (Lo et al., 1988). The present studies revealed that with serial passaging at high multiplicity input, HB-1 virus exhibited a viral interference in TO-2 cells. The TO-2 cells have been assayed in our laboratory for interferon production by poly I, poly C induction experiments, and the results indicated that TO-2 cells did not form an antiviral state in response to poly I, poly C treatment (data not shown). Thus the viral interference of HB-1 virus in TO-2 cells did not appear to involve the interferon system. Instead, the DI particles in the virus samples were considered to be responsible for the interference.

According to the data shown in Fig. 4, the virus yield in culture fluids from serial undiluted or low-diluted (10⁻¹ to 10⁻²) passaging was gradually decreased after passage 3 or 4. These results suggested that HB-1 virus DI particles were constantly generated at low levels by infectious virus and only amplify to interfering levels when virus was passaged at high multiplicity of infection. The fluctuations of virus titer implied that the multiplication of HB-1 virus DI particles depended on the presence of a standard virus.

The SDS-PAGE analyses for the polypeptide composition of virions from serial diluted and undiluted passaging showed that differences in the β group were present. The decrease in the size of β polypeptides in virions suggested that serial undiluted passaging induced the generation of DI particles, in which the large genomic RNA segment is probably shorter than normal.

The electron microscopic observations revealed that the filamental capsids and virus crystals were frequently seen in the TO-2 cells infected with HB-1 virus at high multiplicity input. The present results also indicated that the major capsid protein, the β polypeptide,
changed in virions produced by serial undiluted passaging. It is considered here that the changes of β polypeptide favoured the formation of filamental capsids and virus crystals.

Further studies on the viral polypeptides and RNA analyses would be required to elucidate the origin of truncated β polypeptides and their role in viral interference as well as the formation of viral crystals, of HB-1 virus.

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References


