Properties in Cell Culture of a Hamster Brain-adapted Subacute Sclerosing Panencephalitis Virus

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Several viruses antigenically indistinguishable from measles virus have been isolated from the brains of patients with subacute sclerosing panencephalitis (SSPE), a progressive neuropathy of children, by co-cultivation of brain cells with other cell types (Horta-Barbosa et al. 1969a; Payne, Baublis & Itabashi, 1969). The SSPE-1 virus isolated by Horta-Barbosa et al. (1969b) was obtained as second passage fluids from Dr Wolfgang Zeman, Indiana University Medical Center. In our laboratory, SSPE-1 virus (MANTOOTH strain) was adapted to the brains of newborn hamsters by four pairs of alternate passages in hamster brain and monkey cell tissue culture (Byington, Castro & Burnstein, 1970). This communication presents some of the characteristics of the hamster-brain-adapted (HBS) virus in cell culture.

Stock virus was the 10th passage from the original brain isolate. It was prepared by intracerebral inoculation of neonatal hamsters with 0.02 ml. of HBS virus; affected brains were harvested at 96 hr, when pronounced neurological signs were evident. The virus-infected brains were homogenized as a 10% (w/v) suspension in Eagle's minimal essential medium (MEM) supplemented with 2% foetal bovine serum (FBS) and antibiotics. The brain suspension was centrifuged at 1200 g at 4 ° for 30 min. and the supernatant fluids, which represented stock virus were collected. The virus stock had a titre of $10^{4.5}$ LD₅₀/0.02 ml in neonatal hamsters. HBS virus was identified as measles by in vivo (Byington et al. 1970) and in vitro virus neutralization tests using National Institutes of Health and other measles reference antisera.

The BSC-I monkey kidney cell line (Hopps et al. 1963) was grown in test tube cultures in MEM + 10% FBS + antibiotics and used for HBS virus growth studies at 36°. A total of three cell culture tubes/harvest interval each received 0.1 ml. of stock HBS virus for an input multiplicity of 1 LD₅₀/cell and infected cultures were observed, harvested and pooled at the intervals indicated (Fig. 1). The cell fluids were then assayed for cell-associated and extracellular virus by the plaque assay described by Rapp (1964).

Haemagglutination and haemadsorption tests were done with a 0.5% suspension (v/v) of cercopithecus monkey erythrocytes according to the method of Rosanoff (1961). The haemagglutinin titre was expressed as the reciprocal of the highest virus dilution with complete haemagglutination. The haemadsorbed cultures were scored by counting and represent the mean number of foci (cluster of at least 5 erythrocytes) observed in three tubes (Fig. 1).

The assay for interferon was performed on the extracellular supernatant fluids which were acidified, then centrifuged and the pH adjusted according to the method described by Mirchamsy & Rapp (1969). A challenge dose of 50 p.f.u. of vesicular stomatitis virus (VSV) was inoculated on to cultures which had been pre-treated for 24 hr with test fluids. A 50% reduction in VSV plaques as compared to untreated cultures was used as an indicator for the presence of interferon. For comparison, extracellular fluids from BSC-1 cell cultures infected with the PHILADELPHIA 26 strain of measles virus (Burnstein, Jensen & Waksman, 1964) were harvested at the same growth intervals described for HBS virus and tested for interferon.

In conjunction with the growth study, virus antigen formation was followed by immunofluorescence. Cultures which had been tested for haemadsorption (24, 48 and 72 hr) were...
FIG. 1. HBS virus growth at 36° in roller tube cultures. Production of cell-associated (●—●) and extracellular (○—○) virus and cell-associated (▲—▲) and extracellular (△—△) virus haemagglutinin. Development of virus haemadsorption (HAD) and cytopathic effects (c.p.e.) is shown by stippling with progressively darkened areas representing an increased reaction. Each value represents average of two tests.

Fig. 2. Comparison of plaques formed in BSC-1 cell cultures by SSPE-1 and HBS viruses under agar at 6 days post-inoculation. Note larger HBS virus plaques and ‘comet’ effects in SSPE-1 plaques × 0.9.

Cell cultures infected with HBS virus were observed for cytopathic effects (c.p.e.) and showed virus damage by 24 hr post-inoculation which consisted primarily of formation of syncytia. By 48 hr the cell monolayers were extensively involved with massive syncytia and the beginning formation of clear plaques. Complete cellular destruction occurred by 96 hr. Intracytoplasmic and intranuclear inclusions were observed by 72 and 120 hr, respectively.
Fig. 3. A BSC-1 cell culture at 24 hr following infection with HBS virus. Specific virus immunofluorescence and haemadsorption localized in same focus. Arrow points to particulate fluorescence; circled area indicates adsorbed erythrocytes.

The virus inclusions observed were smaller that those usually produced by measles virus in BSC-1 cells.

Virus plaques formed under agar were visible within 4 days and were round and irregular in appearance. The diameter of HBS virus plaques ranged from 0.5 to 2.5 mm. HBS virus plaques were larger than those produced by the non-hamster adapted SSPE-1 virus (Fig. 2). ‘Comet’ plaques (Rapp, 1964) were produced by SSPE-1 virus and were seen by 6 days but were not observed in HBS preparations.

In the growth studies (Fig. 1) progeny virus was detected intracellularly and extracellularly by 18 and 36 hr after infection respectively and maximal virus production occurred by 36 hr. Haemadsorption was observed on infected cultures at 18 hr after infection, and increased in intensity until by 48 hr the entire monolayer was involved. Supernatant virus haemagglutinin was produced by 36 hr. There was more intracellular than extracellular haemagglutinin. Interferon, which is not generally produced by virulent measles strains (Enders, 1962), was
Table I. Sensitivity of various cell cultures to HBS virus

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cytopathic effects</th>
<th>Haemadsorption</th>
<th>Haemagglutination</th>
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</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
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<tr>
<td>Dog kidney</td>
<td>±</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Rhesus monkey kidney</td>
<td>+</td>
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<tr>
<td>Bovine kidney</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Chicken embryo fibroblast</td>
<td>±</td>
<td>+</td>
<td>0</td>
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<tr>
<td><strong>Established</strong></td>
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<tr>
<td>Rat kidney</td>
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</tr>
<tr>
<td>Madin-Darby canine kidney</td>
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* Reaction or effects, 0 = none; ± = slight; + = positive.

not detected in any of the extracellular fluids tested. However, interferon was found in extracellular fluids of cells infected with PHILADELPHIA 26 virus at 48, 72, and 96 hr.

Immunofluorescence studies on cells infected with HBS virus revealed the presence of particulate fluorescing antigen in the cytoplasm by 24 hr. Homogeneous masses of cytoplasmic antigen were found by 48 and 72 hr after infection. Nuclear fluorescence was not found, however, in areas where virus-specific cytoplasmic fluorescence was observed haemadsorption was localized (Fig. 3).

Various cell culture types were tested for their ability to support replication of HBS virus (Table 1). C.p.e., haemadsorption and haemagglutination, indicated that virus growth occurred in all primary cell cultures tested except for bovine kidney. The virus had no measurable effect on the two established cell lines tested. It was of interest that the established canine cells did not support growth of this virus although they were generally susceptible to most strains of measles (Burnstein et al. 1964).

The results presented show that this virus propagated in rodent brain retained certain characteristics of classical measles with respect to: its ability to replicate productively in cell culture; formation of plaques; typical measles c.p.e. and inclusion bodies; haemadsorption and haemagglutination; and immunofluorescence.

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