Replication of Bovine Herpesvirus Type 4 in Human Cells In Vitro

LAŠZLÓ EGYED*
Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest, Hungary

Received 7 November 1997/Returned for modification 4 March 1998/Accepted 7 April 1998

A reference strain (Movár 33/63) of bovine herpesvirus type 4 (BHV-4) was inoculated into 14 different human cell lines and five primary cell cultures representing various human tissues. BHV-4 replicated in two embryonic lung cell lines, MRC-5 and Wistar-38, and in a giant-cell glioblastoma cell culture. Cytopathic effect and intranuclear inclusion bodies were observed in these cells. PCR detected a 10,000-times-higher level of BHV-4 DNA. Titration of the supernatant indicated a 100-fold increase of infectious particles. Since this is the first bovine (human herpesvirus 8 and Epstein-Barr virus related) herpesvirus which replicates on human cells in vitro, the danger of possible human BHV-4 infection should not be ignored.

In contrast to other beta- and gammaherpesviruses, bovine herpesvirus type 4 (BHV-4) replicates in a wide variety of cell cultures, i.e., established primary cell cultures of cattle, sheep, goats, dogs, cats, rabbits, pigs, and primary chicken kidneys (3). Various cell lines of these species are susceptible to BHV-4. The American reference strain (DN-599) was found to grow to high titers in mink lung and ferret kidney cells (14). Crandell feline kidney (CRFK) cells also support replication of BHV-4 (8). Several species are susceptible to BHV-4 infection. The virus was previously isolated from cattle (3), American bison (Bison bison) (18), African buffalo (Syncerus caffer) (15), goats (10), and nonruminant species such as lions (2a) and a cat suffering from ulorhinosiasis (6). Simian herpesvirus aotus type 2, isolated from the kidney of an apparently normal owl monkey (Aotus trivirgatus), was proven to be a BHV-4 strain (4). This monkey isolate replicated in four monkey cell lines (owl monkey kidney [OMK]; squirrel monkey kidney, intestines, and lung [SMC]; cebus monkey kidney [CMK]; and African green monkey kidney [Vero]), rabbit kidney (RK) cells, and goat cells (GC), where cytopathic effect (CPE) and inclusion bodies were observed. No CPE was seen in a primary culture of whole human embryo cells (2).

To examine the susceptibility of various human cell lines to BHV-4, 105 cells from each type were added to wells of 24-well tissue culture plates (Greiner, Frickenhausen, Germany). The cells were maintained in minimal essential medium (Serva, Heidelberg, Germany) containing NaHCO3, supplemented with 10% fetal calf serum, 0.34 g of L-glutamine (Sigma, St. Louis, Mo.) per liter, 500,000 IU of penicillin per liter, and 0.5 g of streptomycin sulfate per liter. Two milliliters of cell culture fluid was added to each well, and the cells were inoculated with 50 µl of tissue culture fluid containing 105 PFU of the European reference strain of BHV-4 (Movár 33/63).

One hundred microliters from one well without cells was immediately titrated. The rest of the liquid was left to serve as the negative control for PCR studies, to determine the number of the inoculated virus particles. The plates were incubated in a humidified 5% CO2 atmosphere at 37°C for a week, and then the cell-free supernatant was assayed by PCR. The cell cultures were monitored daily for CPE.

For the 14 human cell lines, the five primary cell cultures, and the positive control Madin-Darby bovine kidney (MDBK) cell line, see the data in Table 1.

To titrate the supernatant of the virus-infected cells, after the 7-day-long incubation period 10-fold dilutions of the supernatant were inoculated into dividing MDBK cells and were inoculated as described above.

Replication of BHV-4 in human cells was examined by a thymidine kinase nested PCR (5). Limiting (10-fold) dilutions were examined in the PCR to detect the highest dilution which contained at least 1 to 10 particles. The dilutions yielding negative results were retested three times in order to confirm the absence of viral DNA. The plates were frozen and thawed three times, and the DNA was extracted by the phenol-chloroform method (16) with proteinase K (Sigma) digestion (100 µl of the suspension was incubated with 40 µg of proteinase K enzyme at 55°C for 1 h). Supernatants of all types of cell lines, primary cell cultures, and the cell-free control were examined by PCR.

Inoculation of cells was performed twice with all cell lines and cultures, even if the virus did not show any signs of replication, to verify that the cells were not permissive for BHV-4 infection. Where CPE was seen, the inoculation, titration of the supernatant, and PCR studies were repeated three times to confirm the positive result and prove its reproducibility.

CPE was detected on three cell lines, i.e., WI-38, MRC-5, and giant-cell glioblastoma cells (12). CPE appeared on postinoculation day (PID) 3, when scattered round, enlarged cells were observed. By PID 5, the round cells spread to all parts of the cell sheet, and at PID 7, the cells were floating in the supernatant and were not attached to the bottom of the flask.

BHV-4-infected WI-38, MRC-5, and giant-cell glioblastoma cells, where CPE was observed, were stained with hematoxylin-eosin to detect BHV-4-specific intranuclear inclusion bodies (1) (Fig. 1).

Titration of the supernatant of these cells showed a 100-fold increase in the number of infectious particles, since the titer rose from 102 to 104 in MRC-5, WI-38, and giant-cell glioblastoma cell lines and to 103 in MDBK cell culture.

The PCR revealed a 104-fold increase in the number of viral genomes after a weeklong incubation (1 µl of the cell-free control fluid gave a positive result in the PCR assay, while 104-
The data on BHV-4 replication in vitro on human cell lines may be interesting since only some primate herpesviruses, e.g., herpesvirus simiae (B virus), herpesvirus saimiri, and herpesvirus aotus, are known to replicate in human cells (HEp-2 and human foreskin fibroblasts) in vitro (7, 11). Replication of BHV-4 in lung cells is not unexpected, since the lungs play a key role in BHV-4 infections, the virus replicates in vivo and in vitro in tissues and cell lines of the respiratory tract (bovine lung and fetal calf turbinate (10, 13)), and the virus has often been isolated from animals with respiratory illnesses (3, 9).

Heparin-like moieties of the cell surface serve as the initial receptors for BHV-4 (19). Such receptors must be present on human embryonic lung cells, but the receptor may be missing or changed on cell lines originating from adult individuals. The giant-cell glioblastoma culture consisted of mostly undifferentiated cells.

Another factor which is in accordance with virus replication on embryonic tissues is the dependence of BHV-4 replication on S phase of the cell cycle (20). BHV-4 needs dividing cells for effective DNA replication, and all three BHV-4-permissive cell lines were fast growing. Even though this paper contains in vitro data on BHV-4 replication, the danger of possible human infection (especially by consuming raw bovine milk or beef) cannot be ignored.

Since BHV-4 is in the same herpesvirus subgroup as Kaposi’s sarcoma herpesvirus (human herpesvirus 8), identification of cells permissive and nonpermissive for BHV-4 infection may provide additional insight into the cell surface receptors utilized by this related human herpesvirus.

This work was supported by the Hungarian National Research Grant OTKA T 21183.

The cells were kindly provided by János Minárovits and György Berencsi, from the Johan Bélá Institution for Public Health, Budapest; József Ongrádi, from the Virology Department of Semmelweis Medical University, Budapest; Ivett Mandy, from the Virology Department of Semmelweis Medical University, Budapest; and Ilona Katona, from the National Institute of Neurosurgery, Budapest, Hungary. I am grateful to technician Szilvia Pap for her skilled and reliable assistance throughout the experiment.

REFERENCES


---

**TABLE 1. Data on human cell lines and primary cultures included in the experiments**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>ATCC no. or reference</th>
<th>Cell type</th>
<th>BHV-4 growth at PID 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC-5</td>
<td>CCL 171</td>
<td>Embryonic diploid lungs</td>
<td>10⁴</td>
</tr>
<tr>
<td>WI-38</td>
<td>CCL 75</td>
<td>Embryonic lung</td>
<td>10⁴</td>
</tr>
<tr>
<td>U937</td>
<td>CRL 1593</td>
<td>Histiocytic lymphoma</td>
<td>10⁴</td>
</tr>
<tr>
<td>THP-1</td>
<td>TIB 202</td>
<td>Monocyctic leukemia</td>
<td>10⁴</td>
</tr>
<tr>
<td>CEM-CM3</td>
<td>TIB 195</td>
<td>Lymphoblastic leukemia</td>
<td>10⁴</td>
</tr>
<tr>
<td>UAC</td>
<td>17</td>
<td>Amniotic cell</td>
<td>+</td>
</tr>
<tr>
<td>Jurkat</td>
<td>21</td>
<td>T-cell leukemia</td>
<td>+</td>
</tr>
<tr>
<td>293</td>
<td>CRL 1573</td>
<td>Brain endothelium</td>
<td>10⁴</td>
</tr>
<tr>
<td>CACO-2</td>
<td>HTB 37</td>
<td>Colonic adenocarcinoma</td>
<td>+</td>
</tr>
<tr>
<td>HeLa</td>
<td>CCL 2</td>
<td>Cervical epithelioid carcinoma</td>
<td>10⁴</td>
</tr>
<tr>
<td>HEP-2</td>
<td>CCL 23</td>
<td>Epidermoid carcinoma, larynx</td>
<td>+</td>
</tr>
<tr>
<td>Namalwa</td>
<td>CRL 1432</td>
<td>Burkitt’s lymphoma, lymphoblastoid B cell</td>
<td>10⁴</td>
</tr>
<tr>
<td>SK-N-SH</td>
<td>HTB 11</td>
<td>Neuroblastoma</td>
<td>+</td>
</tr>
<tr>
<td>McCoya</td>
<td>CRL 1696</td>
<td>Fibroblast</td>
<td>10⁴</td>
</tr>
</tbody>
</table>

a Mixed McCoy A (human) and B (mouse) cells.

b Primary cell cultures.
extracellular mineral crystal formation induced by viral infection of cell cultures. Infect. Immun. 3:416–419.


