Recovery of a Canine Herpesvirus from Primary Kidney Cultures Derived from a Closed Dog Colony

R. F. SMITH, H. M. YAMASHIROYA, AND J. M. MAGIS
Life Sciences Division, IIT Research Institute, Chicago, Illinois 60616

Received for publication 13 April 1970

The recovery of a canine herpesvirus from one of eight lots of primary canine kidney cultures derived from a closed dog colony is reported.

The problem of endogenous viral contamination of simian cell cultures is well-recognized and was the subject of a recent review (7). Only sporadic reports have appeared in the literature on viral contamination of canine kidney cell cultures. Among viruses recovered in kidney cultures derived from apparently normal dogs are canine hepatitis (12) and canine distemper virus (10). Spertzel et al. (13) reported on the recovery and characterization of a canine herpesvirus from primary dog kidney cultures showing a spontaneous cytopathic effect (CPE). This communication extends the findings of Spertzel et al. (13) on recovery of a similar agent from an uninoculated batch of primary canine kidney cell cultures.

The herpes-like virus was recovered from one of eight lots of primary canine kidney cultures (obtained through the Division of Biologics Standards, National Institutes of Health) which were being examined for adventitious agents. Puppies serving as donors of kidney tissues for the cell cultures were derived from a closed colony of apparently healthy beagle dogs. The primary canine kidney cells were placed on maintenance medium consisting of Eagle's minimal essential medium plus 2% fetal calf serum on the day after receipt of the monolayer cultures. Foci of CPE consisting of rounded cells and plaques (Fig. 1A) appeared at 14 days after initiation of the cultures. The CPE progressed to involve 50 to 75% of the cell sheet by the end of the third week of cultivation. The cultures were harvested at this time, and the viral isolate was designated CK-5.

Inoculation of the CK-5 isolate in Madin-Darby canine kidney (MDCK) monolayers produced cytopathology similar to that observed in the primary cultures (Fig. 1B). Hematoxylin and eosin-stained preparations of infected MDCK cells showed typical herpesvirus type A intranuclear inclusions (Fig. 1C, D). Acridine orange staining revealed the presence of deoxyribonucleic acid (DNA)-containing intranuclear inclusions. The identification of the isolate as a herpesvirus was further corroborated by electron microscopy (Fig. 2). The CK-5 isolate did not produce CPE in established cultures of rabbit kidney (IITRI) and African green monkey kidney (BSC-1) cells or in human diploid embryonic lung (WI-38) cultures during an observation period of approximately 3 weeks.

The physical and chemical properties of the CK-5 isolate assayed in MDCK cells are summarized in Table 1. Sensitivity to lipid solvents was determined by the method of Feldman and Wang (6). The effect of 5-ido-2'-deoxyuridine (IUDR) treatment (13) on the isolate was assessed with reference canine herpesvirus (D004 strain; reference 2) and canine distemper virus (Rockborn strain) as DNA and ribonucleic acid virus controls, respectively. The various treatments indicated the presence of essential structural lipids, heat lability, and IUDR sensitivity of the agent. These properties are consistent with those of the herpesvirus group.

The results of neutralization tests conducted in MDCK cells are shown in Table 2. Serum-virus mixtures were incubated for 1 hr at room temperature before inoculation of stationary tube cultures. Viral concentrations of 18 to 560 TCID₅₀ determined by simultaneous back titrations of the test dose, were employed in the neutralization test. The CK-5 isolate was neutralized by 16 antibody units of canine herpesvirus (D004 strain) antiserum provided by L. Binn of Walter Reed Army Institute of Research. The cross-neutralization of infectious canine hepatitis virus by Toronto A26/61 antiserum can be attributed to the serologic relationship between these two adenoviruses (5) and the high concentration of antiserum employed in the test. The herpes simplex antiserum did not cross-react with canine herpesvirus in the tube neutralization
Fig. 1. Cytopathic effects (CPE) of CK-5 isolate. (A) Uninoculated primary canine kidney culture, focal CPE at 15 days; unstained. X 60. (B) Madin-Darby canine kidney (MDCK) culture 6 days after inoculation; hematoxylin and eosin stain. X 100. (C) Uninoculated MDCK control, 3 days; hematoxylin and eosin stain. X 970. (D) Infected MDCK cells showing type A intranuclear inclusions, 3 days; hematoxylin and eosin stain. X 970.

Fig. 2. Electron micrographs of CK-5 isolate stained with potassium phosphotungstate. (A) Complete particles, measuring approximately 150 nm in diameter. (B) Icosahedral core devoid of envelope. X 200,000.
test, thus confirming the results of other investigators (11, 13). However, canine herpesvirus and herpes simplex virus have been shown to be immunologically related but not identical by the plaque reduction (multiplicity analysis) technique (1).

In summary, a canine herpesvirus has been isolated from primary kidney cell cultures derived from a closed colony of apparently healthy beagle dogs. The association of a canine herpesvirus with a fatal neonatal infection of dogs was first reported independently in 1965 by Carmichael et al. (3) and Stewart et al. (14). The acute neonatal infection by canine herpesvirus has been substantiated by several other investigators as reviewed in a recent paper (4). Canine herpesvirus has also been isolated from natural cases of respiratory disease in adult dogs (2, 9) and from a dog with a spontaneous malignant lymphoma (8). Because of the potential latency of canine herpesvirus in dogs (14), primary consideration should be given to this agent as an adventitious contaminant which can be encountered in cell cultures employed in biological studies.

Reference viral reagents were obtained through the courtesy of L. N. Binn, Walter Reed Army Institute of Research, and L. E. Carmichael of Cornell University or were supplied by the Research Reference Reagents Branch of the National Institutes of Health.

We thank W. Richter of the University of Chicago for the excellent electron microscopic work.

This investigation was supported by contract no. PH-43-67-1106 from the Division of Biologics Standards, National Institutes of Health.

### ADDENDUM IN PROOF

Subsequent to preparation of this manuscript, we have recovered an infectious canine hepatitis virus from a lot of primary canine kidney cells obtained from a second supplier. The infectious canine hepatitis isolate was differentiated from the Toronto strain on the basis of cross-serological reactions in the hemagglutination-inhibition test (L. J. Swango, G. A. Eddy, and L. N. Binn, Amer. J. Vet. Res. 30: 1381–1387, 1969).

### LITERATURE CITED


### TABLE 1. Physical and chemical properties of canine kidney isolate CK-5

<table>
<thead>
<tr>
<th>Virus treatment</th>
<th>Virus titer* a in MDCK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Chloroform, 4.8% (4 C, 10 min)</td>
<td>3.5</td>
</tr>
<tr>
<td>Heat (56 C, 30 min)</td>
<td>3.8</td>
</tr>
<tr>
<td>IUDR, b 50 µg/ml</td>
<td>2.5</td>
</tr>
<tr>
<td>CK-5 isolate</td>
<td>2.3</td>
</tr>
<tr>
<td>Canine herpesvirus</td>
<td>3.5</td>
</tr>
<tr>
<td>Canine distemper virus</td>
<td>3.2</td>
</tr>
</tbody>
</table>

a Log10 TCID50/1.0 ml at 6 to 9 days.

b 5-Iodo-2'-deoxyuridine.

### TABLE 2. Neutralization tests with CK-5 isolate and reference canine viruses

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Serum neutralization titer</th>
<th>Test dilution</th>
<th>Cytopathogenic effect in MDCK cells at 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine herpesvirus (D004 strain)</td>
<td>1:64/0.1 ml</td>
<td>1:4</td>
<td>Canine kidney isolate CK-5</td>
</tr>
<tr>
<td>Infectious canine hepatitis virus (Lederle avirulent)</td>
<td>1:1,280/0.1 ml</td>
<td>1:64</td>
<td>Canine herpesvirus (D004 strain)</td>
</tr>
<tr>
<td>Canine adenovirus (Toronto A26/61)</td>
<td>1:1,024/0.1 ml</td>
<td>1:4</td>
<td>Infectious canine hepatitis virus</td>
</tr>
<tr>
<td>Herpes Simplex virus (Mayo 1814)</td>
<td>1:64/0.5 ml</td>
<td>1:4</td>
<td>Canine adenovirus (Toronto A26/61)</td>
</tr>
<tr>
<td>Pseudorabies virus (Au- jeszyki strain)</td>
<td>1:32/0.5 ml</td>
<td>1:2</td>
<td></td>
</tr>
</tbody>
</table>

a Not done.