Infectivity, Oncogenicity and Transforming Ability of BK Virus and BK Virus DNA

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SUMMARY

The human papovavirus, BK, appeared weakly oncogenic in newborn hamsters and was able to induce in vitro transformation of rat kidney cells. The infectivity of BK virus DNA was determined by employing the DEAE-dextran method. In human embryonic cells the infectivity was approx. 10^5 p.f.u./μg of DNA. The transforming ability of BK virus in primary rat kidney cells was measured by employing the calcium method and appeared to vary from 1 to 10 foci per μg of DNA.

The isolation of the human papovavirus BK from the urine of an immunosuppressed renal transplant recipient has been described by Gardner et al. (1971). The BK virus appeared to be oncogenic in newborn hamsters (Shah, Daniel & Strandberg, 1975) and was able to cause transformation of hamster cells (Mayor & di Mayorca, 1973). Further aspects of the biological activity of this virus and its DNA are reported here.

The BK virus (Gardner et al. 1971) was kindly supplied by Dr Takomoto (N.I.H., Bethesda, U.S.A.) and was propagated in our laboratory in primary cultures of human embryonic cells. The cells were cultured in roller bottles in Eagle’s basal medium supplemented with 10% calf serum and antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml). The cultures were inoculated at subconfluency at a multiplicity of infection of 0.001 p.f.u./cell and the virus was harvested after 10 to 14 days when about 75% of the cells showed c.p.e. Plaque titrations of BK virus were performed on monolayer cultures of human embryonic cells in 5 cm Falcon Petri dishes. An overlay of 0.9% agar in medium was added one day after inoculation and after 24 days the plaques were stained by neutral red (0.01% in final agar overlay). The titre of the crude lysate used as a stock virus was 5 × 10^6 p.f.u./ml.

To test the oncogenicity of BK virus, 155 newborn hamsters were inoculated subcutaneously with approx. 10^6 p.f.u./animal. The animals were kept for an observation period of one year and at that time 8 animals had developed tumours at the site of inoculation. One of the tumours was studied histologically and appeared to be a fibrosarcoma. This tumour was also grown in tissue culture and the cultured cells were investigated by immunofluorescence for the presence of antigens which reacted with sera from tumorous animals. In most of the nuclei (95%) an antigen which in its appearance strongly resembled SV40T antigen was present. Staining of the cells with SV40 anti T-serum gave the same pattern. No infective virus could be rescued from the cultured cells either by co-cultivation or by Sendai virus-mediated cell fusion.

To investigate the transforming ability of BK virus, subconfluent tube cultures of primary rat kidney cells derived from 5 days old Wistar rats were inoculated with 0.1 ml of undiluted virus (5 × 10^5 p.f.u.). No cytopathology was noted in the inoculated cultures indicating that rat kidney cells are non-permissive to BK virus. In two subsequent experiments transformation was observed 4 to 6 weeks after inoculation. In the first experiment transformation had
Short communications

Table 1. Infectivity of BK virus DNA (p.f.u./μg)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Type of cells</th>
<th>Treatment of cells</th>
<th>None</th>
<th>DEAE-dextran</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Primary human embryonic cells</td>
<td>N.T.*</td>
<td>1·8 x 10⁵</td>
<td>6·4 x 10⁹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diploid human embryonic lung cells</td>
<td>&lt; 1·0 x 10²</td>
<td>1·1 x 10⁵</td>
<td>&lt; 1·0 x 10²</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Diploid human embryonic lung cells</td>
<td>N.T.</td>
<td>3·0 x 10⁶</td>
<td>1·0 x 10³</td>
<td></td>
</tr>
</tbody>
</table>

* Not tested.

Table 2. Transformation efficiency of BK virus DNA (foci/μg)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Amount of DNA per dish (μg)</th>
<th>Number of foci per dish</th>
<th>Average/μg of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>1, 1, 3, 2, 3, 0, 1</td>
<td>1·3</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>10, 11, 9, 5, 11</td>
<td>9·2</td>
</tr>
</tbody>
</table>

occurred in 3 of 8 cultures and in the second in 1 out of 12 cultures, indicating a low transformation efficiency. The foci of transformed cells consisted of many dividing cells which piled up and the cells from these foci could easily be subcultured. The clonal lines derived from these foci showed differences in cell morphology which varied from fibroblastic to epithelial. The transformed cells were tested for BK neo-antigen by employing the BK anti-T-serum. Approx. 95% of the nuclei of the cells from 4 clonal lines showed granular fluorescence. By employing cell fusion BK virus could be rescued from 3 of the 4 clonal lines of transformed rat kidney cells.

For extraction of the viral DNA the infected cells were detached from the glass by freeze-thawing and cells and virus were spun down in the SW 27.1 rotor (Beckman) for 2 h at 25,000 rev/min at 4 °C. The viral DNA was extracted from the pellet by the Hirt procedure (Hirt, 1967) and was further purified by equilibrium centrifugation in caesium chloride containing ethidium bromide (Radloff, Bauer & Vinograd, 1967) and chromatography on methylated albumin kieselguhr. The DNA was analysed by agarose gel electrophoresis (2·5% agarose) according to the procedure described by Aay & Borst (1972).

Only one band of viral DNA could be seen in the electropherogram and therefore the presence of incomplete BK DNA was considered to be unlikely. The infectivity of BK virus DNA was determined by two different methods, the DEAE-dextran (McCutchan & Pagano, 1968) and the Calcium method (Graham & Van der Eb, 1973) and in two types of cells, diploid human embryonic lung cells and primary human embryonic cells.

For the DEAE-dextran method the cells were first washed with phosphate-buffered saline (PBS) and then with tris-buffered minimal essential medium (MEM-tris, pH 7·4) without serum and bicarbonate. The cells were incubated for 30 min at room temperature with 1 ml of MEM-tris buffer containing 500 μg of DEAE-dextran (Pharmacia). After removal of the DEAE-dextran the viral DNA was added in 0·2 ml samples. After 15 min the cells were washed with MEM-tris and the medium was supplied. The agar overlay was added the next day. The results of the plaque counts are shown in Table 1. The DEAE-dextran method resulted in the highest infectivity (1 to 3 x 10⁵ p.f.u./μg).

The transforming ability of the viral DNA was only determined by employing the calcium method, because the DEAE-dextran method gave negative results for transformation of
rat kidney cells by SV40 DNA (Abrahams & Van der Eb, 1975). In one preliminary experiment no transformation by BK DNA could be obtained by employing the DEAE-dextran method. Furthermore it was repeatedly found in our laboratory that DEAE-dextran was too toxic for rat kidney cells. The DNA was diluted in hepes buffer (pH 7.05), calcium chloride was added to a final concentration of 125 mM and calf thymus DNA was added as carrier. This mixture was kept at room temperature for 30 min and then 0.5 ml of the diluted DNA was added to subconfluent 5 cm Petri dish cultures of primary rat kidney cells. The medium was changed after 4 h. The final concentration of calf thymus DNA was 5 μg per dish. After 4 weeks the cultures were fixed and stained with Giemsa and the number of transformed foci were counted. The results are shown in Table 2. In two subsequent experiments an average of respectively 1.3 and 9.2 foci/μg of DNA was found. Control cultures which were mock infected with or without calf thymus DNA were not found to contain foci of transformed cells.

From our findings on BK virus DNA and those of Abrahams & Van der Eb (1975) on SV40 DNA the calcium method appeared suitable for the in vitro transformation of primary rat kidney cells.

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REFERENCES


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