Tumour Specific Transplantation Antigen in Hamster Tumour Cells Induced with BK Virus

(Accepted 9 May 1978)

SUMMARY

Immunization of hamsters with purified BK virus (BKV) followed by transplantation of BKV-induced hamster tumour cells revealed a tumour specific transplantation antigen (TSTA) in these cells. The antigen did not cross-react with the TSTA of SV40 since immunization with BKV did not protect against challenge of SV40 tumour cells.

BKV virus (BKV), a human polyoma virus, transforms rodent cells in vitro (Major & Di Mayorca, 1973; Portolani et al. 1975) and induces tumours in hamsters (Näse et al. 1975; Shah et al. 1975). It shares minor virion antigen components with other viruses of the polyoma-SV40 subgroup of papovaviruses (Penney & Narayan, 1973). In addition, T antigen present in cells infected or transformed with BKV cross-reacts strongly with the T antigen of SV40 and JC virus, by immunofluorescent staining. The latter virus is another human polyoma virus which also induces tumours in hamsters (Walker et al. 1973). Cells transformed by SV40 or by JC virus contain a tumour-specific transplantation antigen (TSTA), and it has been shown that the TSTA of these two viruses are mutually distinct (Padgett et al. 1977). Furthermore, immunization of hamsters with BKV before transplantation of SV40 or JC virus-transformed cells did not confer protection against tumour growth (Padgett et al. 1977).

We have previously reported induction of tumours in hamsters with BKV, and described some characteristics of cell lines derived from these tumours (Sten et al. 1976). Since it has not been previously shown, we were interested in finding out whether BKV-induced hamster tumour cells also contained a TSTA, and whether immunity against BKV TSTA would give any protection against SV40 tumour cells.

BKV was purified from a medium of infected Vero cells (Mäntyjärvi et al. 1972). Virus-containing medium was concentrated tenfold by vacuum dialysis (Biofiber; Bio-Rad, California). Virus was pelleted through 20 % (w/v) sucrose solution in tris-buffered saline, pH 7-5, by ultracentrifugation (100 000 g for 90 min), treated with receptor destroying enzyme (Cholera Filtrate, N.V. Philips Duphar, Holland) and banded in a CsCl gradient (100 000 g for 18 h). Fractions containing full virus particles were pooled, dialysed, and used for immunization. The virus concentration of the pools was 2.0 to 6.4 × 10^4 haemagglutinating units/ml.

The inbred strain of hamsters used for immunization was the same as that from which the BKV tumour cells were obtained (LSH; Lake View Hamster Colony, New Jersey). After varying immunization schedules, hamsters were challenged by subcutaneous injections of BKV or SV40 tumour cells. The origin of the BKV tumour cells (BKT cell lines) and of the SV40 tumour cells (H-50/LSF2 cells) has been described previously (Sten et al. 1976). Three to five different doses of these cells were injected into groups of three to eight hamsters. Hamsters were examined twice weekly for 12 weeks. The median tumour-producing dose of transplanted cells (TPD_{50}) was calculated (Reed & Muench, 1938).


### Table 1. Effect of immunization with BKV on transplantation of BKV tumour cells in hamsters

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Immunogen</th>
<th>Age when immunogen (weeks)</th>
<th>Challenge tumour cells</th>
<th>TPD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Resistance index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BKV</td>
<td>0, 4 and 8</td>
<td>BKT-2C</td>
<td>2.5 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>BKT-2C</td>
<td>4.2 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BKV</td>
<td>4, 5 and 6</td>
<td>BKT-2A</td>
<td>5.9 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BKT-2A</td>
<td>7.6 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BKV</td>
<td>0, 2 and 4</td>
<td>BKT-2A</td>
<td>5.0 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BKT-2A</td>
<td>6.0 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BKV</td>
<td>0, 2 and 4</td>
<td>BKT-2C</td>
<td>5.9 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BKT-2C</td>
<td>5.1 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

The resistance index (RI) expressed as a ratio of TPD<sub>50</sub> of immunized animals to TPD<sub>50</sub> of non-immunized animals. The tumour growth index was calculated according to Barra et al. (1977).

Table 1 shows the results of four separate experiments. Immunized and non-immunized control hamsters were challenged with tumour cells 2 to 3 weeks after the last immunization. The resistance index calculated 5 to 9 weeks after challenge was only 6 in experiment 1. In subsequent experiments, however, RI values above 10 were obtained, which is considered to be the limit of significance (Tevethia et al. 1971). In a further experiment hamsters were again immunized with BKV and challenged 1 week after the last immunization with BKT-1B as well as with H-50/LSH<sub>2</sub> cells. The immunization schedule was the same as in experiment 3. Tumour growth indices at various times after challenge are shown in Table 2. Immunization with BKV did not give any protection against H-50/LSH<sub>2</sub> cells, whereas homologous immunity against BKV tumour cells was, again, present. In the groups receiving 10<sup>6</sup> BKT-1B challenge cells in which the number of tumours produced was high enough for a reliable comparison, the difference between growth indices of immunized and non-

### Table 2. Growth of SV40 and BKV-induced tumour cells in hamsters immunized with BKV

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunogen</th>
<th>Challenge tumour cells</th>
<th>Number of tumour cells injected</th>
<th>Weeks after challenge</th>
<th>Tumour growth index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BKV</td>
<td>H-50/LSH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8 18 31 32 41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 4 7 13 19</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 0 2 10 15</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BKV</td>
<td>H-50/LSH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8 18 27 33 38</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 3 4 9 11</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0 2 2 2 6</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BKV</td>
<td>BKT-1B</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0 0 3 8 10</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0 0 0 2 2</td>
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<td>0 0 0 0 0</td>
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<td></td>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0 0 0 0 0</td>
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<tr>
<td>4</td>
<td>BKV</td>
<td>BKT-1B</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0 8 11 13 18</td>
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<td></td>
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<td></td>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0 0 0 0 0</td>
<td></td>
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</tbody>
</table>
immunized hamsters was significant at a level of $0.01 > P > 0.001$ (Student’s $t$ test for paired observations).

Our results indicate that transformation by BKV induces an antigen which can be detected by a transplantation rejection test. The variation in RI is probably an indication of a different immunosensitivity of the tumour cell lines used. The transplantation antigen of BKV is comparable to the TSTA of SV40 and JC virus. Like the TSTA of JC virus, the TSTA of BKV did not cross-react with the TSTA of SV40. Therefore our results together with those reported by others (Takemoto & Mullarkey, 1973; Shah et al. 1975; Padgett et al. 1977) show that each of the three viruses, SV40, JC and BKV has a distinct TSTA. On the other hand, immunization with JCV or BKV-transformed hamster cells protected mice against a challenge of SV40-induced mouse tumour cells (Law et al. 1977). It was also reported by Molinaro et al. (1977) that, by using an in vitro technique, common surface antigens were found in cells transformed by SV40 and BKV. In both of these cases the immunization was performed in a heterologous species which might recognize antigens not immunogenic in a syngeneic system. The distinctiveness of the TSTA of SV40, JC and BKV is a very interesting phenomenon because of the immunological similarity of the T antigens of the same viruses (Takemoto & Mullarkey, 1973; Walker et al. 1973). TSTA and T antigen are both coded for by the same, early region of virus DNA. Differences in tryptic peptides of T antigens of SV40 and BKV may explain the presence of specific TSTA if both antigenic determinants are located in the same polypeptide (Rundell et al. 1977; Simmons et al. 1977). Post-translational modification of the primary early protein (A protein) or transcription of two mRNAs in different phases from early DNA region are two possible explanations for having T and TSTA determinants on separate molecules. The latter possibility would be comparable to the relationship between VP1 and VP2/VP3 of SV40 (Contreras et al. 1977).

This work was supported by the Academy of Finland and by Sigrid Jusélius Foundation.

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REFERENCES


(Received 5 January 1978)