INCUBATION PERIOD FOR TUMOR INDUCTION BY
ADENOVIRUS TYPE 12

(Plate XXXVI)

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Synopsis

Adenovirus type 12 was inoculated intraperitoneally into newborn hamsters. At selected periods, the soft peritoneal tissues were excised and transplanted subcutaneously into 16- to 29-day-old hamsters. With the tissues taken at 6 hours or more after the virus inoculation, tumors developed at the transplanted site. The period from virus inoculation to excision and transplantation of the soft peritoneal tissues, and from transplantation to development of grossly recognizable tumors ranged from 24 to 38 days, which correspond to the average latent period for tumor development in hamsters intraperitoneally inoculated with the virus at birth. The virus was not isolated from the soft peritoneal tissues similarly excised from hamsters inoculated with the virus at birth. None of 10 young adult hamsters injected with the virus developed any visible tumors during 46 to 367 days of observation.

These results would imply that (1) the tumors developed at the site of tissue transplantation originate from the cells in the transplanted tissues, which would have been already neoplastically transformed or destined by the virus; and (2) transformation or transition of the target cells to malignant cells will be completed in 6 hours after the virus inoculation.

INTRODUCTION

The oncogenic effect of adenovirus type 12 in newborn hamsters is remarkable, and, among the various known oncogenic viruses or chemicals, this virus belongs to one of the groups whose incubation period for tumor induction is very short. It has been reported that when the virus is inoculated intraperitoneally or intrathoracically into newborn hamsters, animals usually die of tumor in 1-3 months after the inoculation.2,5,6,7) In our previous observation, when inoculated intraperitoneally, millet-size tumor nodules were macroscopically observed after about 18 days, and microscopically, tumor sprouts were observed about 10 days after the virus inoculation.5,4)

In the present work, we studied the earliest period, before development of microscopically recognizable tumor, of neoplastic change due to the virus, by transplanting virus-infected tissues into young adult hamsters.

MATERIALS AND METHODS

Solution and Medium for Tissue Culture Phosphate-buffered saline (PBS) and Earle’s solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolyzate (YLE) were used for the tissue culture. The bovine serum was sterilized by Seitz-filtration and inactivated by heating at 56° for 30 mins.

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Virus and Virus Titration  Adenovirus type 12, strain “Huie”, was cultured, prepared, and its 50% tissue culture infectious dose per 0.1 ml (TCID<sub>50</sub>/0.1 ml) was titrated as described previously.<sup>4</sup>

Animal  Syrian golden hamsters originally obtained commercially and bred in our laboratory were used.

Virus Inoculation  One-tenth milliliter of virus fluid of 10<sup>2.5</sup> TCID<sub>50</sub>/0.1 ml was inoculated intraperitoneally into hamsters within 24 hrs. after birth.

Transplantation of Infected Tissues into Young Hamsters  Two to 16 hamsters each were sacrificed 0.5, 3, 6, 12, and 24 hrs., and 2, 3, 5, 7, 14, and 16 days after the virus inoculation. Their soft peritoneal tissues (the peritoneum, the mesentery, and the omentum) were taken out and washed several times in physiological saline. A piece, about 2×2×2 mm, of the tissues was transplanted subcutaneously into 16- to 29-day-old hamsters with trocar. Transplanted hamsters were observed for 17 to 293 days. When a tumor nodule developed at the site of tissue transplantation and grew to a bean size, the nodule was taken out to study its nature; a part of it was cut for histology section and the rest was minced and transplanted into young hamsters successively.

Control Experiments  (1) One-tenth milliliter of the virus (10<sup>2.5</sup> TCID<sub>50</sub>/0.1 ml) was inoculated subcutaneously into 16- to 29-day-old hamsters. (2) Thirty minutes and 7 days after the intraperitoneal inoculation of 0.1 ml of the virus (10<sup>2.5</sup> TCID<sub>50</sub>/0.1 ml) into 17 newborn hamsters, the soft peritoneal tissues were taken out, minced finely, suspended in physiological saline to 10%, frozen and thawed 7 times, and its 0.1 ml was subcutaneously inoculated into 17 hamsters of 22 to 27 days of age. (3) Tumors developed in the peritoneal cavity of the animals inoculated with the same dose of the virus at birth were minced, and a piece measuring 2×2×2 mm was transplanted subcutaneously into young adult hamsters.

Isolation of Virus from Infected Tissues  From hamsters inoculated intraperitoneally with adenovirus type 12 at birth, the soft peritoneal tissues were taken out 6 and 24 hrs., and 3, 7, and 14 days after the virus inoculation. These tissues were washed thoroughly in PBS, minced, and suspended to 10 and 1% in YLE with 10% bovine serum (medium A). One-tenth milliliter of each suspension was inoculated into HeLa cell tubes containing 0.9 ml of medium A and maintained at 36.5<sup>°</sup>. The medium was changed every 3 to 4 days, and after the 15th day, it was changed to YLE with 2% bovine serum (medium B). On day 35, these tubes were frozen and thawed twice, and 1 ml of the fluid was inoculated into HeLa culture tubes and maintained for 14 days, refeeding the cultures with medium B. Throughout these 49 days of maintenance, viral cytopathogenic effect was examined every 2 to 3 days. With the peritoneal tissues taken 6 and 24 hrs. after the virus inoculation, the virus was isolated also from the tissues without washing.

Preparation of Tissue Sections  Tissues were fixed in 20% Formalin and stained with Hematoxylin-Eosin.

RESULTS

Transplantation Test of Virus-infected Tissues  Table I shows the result of transplantation of virus-infected tissues. Tumors appeared in 1 in 2, 2 in 3, 4 in 7, 2 in 6, 2 in 10, 4 in 9, 2 in 16, none in 3, and 2 in 10 hamsters transplanted with the infected
tissues taken at 16, 14, 7, 5, 3, and 2 days, and 24, 12, and 6 hours after the virus inoculation, respectively. No tumors developed in 18 hamsters transplanted with the tissues taken 3 hours or 30 minutes after the virus inoculation. The incubation period from tissue transplantation to development of grossly recognizable tumors became shorter with the increase of the period from virus inoculation to excision of infected tissues:

Table I. Tumor Development by Transplantation of Adenovirus-12-infected Tissues

<table>
<thead>
<tr>
<th>Periods from virus inoculation to excision of infected tissues</th>
<th>No. of tumorous hamsters/ no. of transplanted hamsters</th>
<th>Days from tissue transplantation to tumor development$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min.</td>
<td>0/10</td>
<td>24, 38</td>
</tr>
<tr>
<td>3 hrs.</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>6 $^{a)}$</td>
<td>2/10</td>
<td>53</td>
</tr>
<tr>
<td>12 $^{a)}$</td>
<td>0/3</td>
<td>36</td>
</tr>
<tr>
<td>24 $^{a)}$</td>
<td>1/10</td>
<td>60</td>
</tr>
<tr>
<td>2 days</td>
<td>1/6</td>
<td>36</td>
</tr>
<tr>
<td>3 $^{a)}$</td>
<td>2/10</td>
<td>23, 25</td>
</tr>
<tr>
<td>5 $^{a)}$</td>
<td>2/6</td>
<td>23, 53</td>
</tr>
<tr>
<td>7 $^{a)}$</td>
<td>4/7</td>
<td>17, 20, 25, 30</td>
</tr>
<tr>
<td>14 $^{a)}$</td>
<td>2/3</td>
<td>17, 22</td>
</tr>
<tr>
<td>16 $^{a)}$</td>
<td>1/2</td>
<td>17</td>
</tr>
</tbody>
</table>

$^a)$ Transplanted with tissues excised from hamsters inoculated with 0.1 ml of virus (10$^{3.5}$ TCID$_{50}$/0.1 ml) at birth. Except for this one, tissues were taken out from hamsters inoculated with 0.1 ml of 10$^{2.5}$ TCID$_{50}$/0.1 ml.

$^b)$ All hamsters were observed for 17 to 293 days.

Fig. 1. Tumor development by transplantation of adenovirus-12-infected tissues
17 days by the 16-day tissue, 17 to 22 days by the 14-day one, 17 to 30 days by the 7-day one, 23 to 53 days by the 5-day one, 33 to 60 days by the 2-day one, 36 to 53 days by the 24-hour one, and 24 to 38 days by the 6-hour tissue.

**Histomorphology of Tumors** The tumors which developed at the site of tissue transplantation were macroscopically and microscopically similar to those developing in hamsters injected with adenovirus type 12 at birth (Photos 1, 2a, 2b, and 2c).

**Results of Control Experiments** Table II shows the results of control experiments. None of 10 hamsters subcutaneously inoculated with 0.1 ml of adenovirus type 12 at 19 to 22 days of age developed tumors in 37 to 367 days of observation.

<table>
<thead>
<tr>
<th>Preparations inoculated</th>
<th>No. of tumorous hamsters/ no. of inoculated hamsters[a) (days observed)</th>
<th>Days from inoculation to tumor development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus 12[b)</td>
<td>0/10 (46~367)</td>
<td></td>
</tr>
<tr>
<td>Frozen-thawed tissues taken after 30 mins.</td>
<td>0/7 (79~285)</td>
<td></td>
</tr>
<tr>
<td>Frozen-thawed tissues taken after 7 days</td>
<td>0/10 (79~120)</td>
<td></td>
</tr>
<tr>
<td>Tumor tissue induced by adenovirus 12</td>
<td>11/13 (7~100)</td>
<td>7~10</td>
</tr>
</tbody>
</table>

[a) Hamsters of 16 to 29 days of age were used.
b) 0.1 ml of $10^{2.5}$ TCID$_{50}$/0.1 ml was inoculated subcutaneously.

Seventeen young hamsters were subcutaneously inoculated with the frozen-thawed preparation of the soft peritoneal tissues taken at 30 minutes and 7 days after the inoculation from hamsters inoculated with the virus at birth. None of them developed tumors in 37 to 285 days of observation.

Tumors induced by inoculation of newborn hamsters with adenovirus type 12 were similarly transplanted into 13 young hamsters. Eleven of them developed tumors, and tumors became grossly recognizable in 7 to 10 days after the transplantation.

**Isolation of Virus from Soft Peritoneal Tissues of Virus-inoculated Hamsters** Isolation of the virus was made with the soft peritoneal tissues of virus-inoculated newborn hamsters taken at 6 and 24 hours, and 3, 7, and 14 days after the inoculation, but the results were all negative.

**DISCUSSION**

The fact that, when adenovirus type 12 is inoculated into newborn hamsters, the complete transformation or the transformation process to cancer cells is induced in the target cells within 6 hours after the inoculation is suggested by the following evidences: (1) Tumors developed in young adult hamsters by transplantation of the tissues taken as early as 6 hours after the virus inoculation; (2) no tumor developed in young adult hamsters by inoculation of the virus itself; (3) virus was not isolated from the tissues taken at 6 hours to 14 days after the virus inoculation; (4) no tumor developed in hamsters similarly transplanted with the frozen-thawed preparation of the tissues taken at 3 hours and 7 days after the virus inoculation. The data of the present experiment
are not enough to analyze if the complete neoplastic transformation occurs in the target cells of virus-inoculated newborn hamsters within 6 hours after the inoculation or it occurs after transplantation of virus-infected tissues into young adult hamsters. Dales has reported that adenovirus type 7 is adsorbed to the HeLa cell membrane 1 hour after the inoculation and appears in the cytoplasm in 2 hours.\textsuperscript{1} This fact suggests that adenovirus type 12 enters into cells within a few hours after the inoculation and completes neoplastic transformation of the target cells within 6 hours. As for the negative results with the tissues taken within 3 hours after the virus inoculation, following possibilities are considered: (1) Transformation to the neoplastic cell is not completed in 3 hours, (2) transformation to the neoplastic cells is completed but, when transplanted into new hosts, their growth as a cancer tissue is inhibited, and (3) the number of animals used for this experiment was too small and possible positive results might have been overlooked. It is presently not known which is the true reason.

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**References**


**Explanation of Plate XXXVI**

Photo 1. Subcutaneous tumor which developed 43 days after the transplantation of soft peritoneal tissues taken 24 hrs. after the intraperitoneal inoculation of adenovirus type 12 at birth. (Hamster No. 49) \( \times 1 \)

Photo 2a. Histological figure of subcutaneous tumor which developed from transplanted soft peritoneal tissues. Rosette-like structure (pseudo-rosette) can be observed. (Hamster No. 72) \( \times 600 \).

Photo 2b. Irregular arrangement of small spindle-shaped tumor cells. (Hamster No. 49) \( \times 1,000 \).

Photo 2c. Irregular arrangement of large polygonal tumor cells and giant cells. (Hamster No. 70) \( \times 1,000 \).