SUCCESSIVELY TRANSPLANTED CANINE TRANSMISSIBLE SARCOMA

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Canine transmissible sarcoma has been maintained since 1967 as an allogeneic tumor cell line. The tumor originated from a naturally occurring venereal sarcoma in a 7-year-old female dog, Hokkaido-ken. The animals used for the serial transfer from 1st to 50th passages were 1- to 8-month-old puppies. Histological features and karyological characteristics of the tumor were not modified by the passive transfer. In most of the dogs, the palpable growths in the subcutaneous tissues were present at approximately the same time. These findings suggest that the sarcoma is an only established tumor cell line in dogs.

A number of transmissible tumors maintained as many allogeneic or syngeneic established cell lines in small experimental animals have been used in tumor studies. In the dogs used as large experimental animals and spontaneous tumors frequently observed by many workers, however, long-term maintained tumors as allogeneic established cell lines were scarcely reported.

We have maintained a canine transmissible sarcoma from the external genitalia of a female dog, and established as an allogeneic tumor cell line. The tumor was employed for the transplantation and immunological studies. Observations from 1st to 20th generation of the experimental transfer have already been reported. This paper describes the observations from 21st to 50th generation of the transfer.

Materials and Methods
Origin of Tumor: On September 27, 1967, a naturally occurring canine transmissible sarcoma was obtained from a 7-year-old female dog, Hokkaido-ken, which visited Hokkaido University Veterinary Hospital. The tumor was the source of canine transmissible sarcoma cells used in our serial transmission studies. The original neoplasm was located in the vagina and appeared as a nodular lump of hen egg size. The dog made a favorable progress after surgical removal of the tumor and the disease did not recur.

Transplantation and Experimental Animals: The tumor was removed and minced to a brei with scissors. The brei was filtered once through a guaze and suspended in Ringer's solution containing 2000 units/ml of penicillin. Viable tumor cell counts of the cell suspension were checked by Eosin dye exclusion tests. The cell suspension was injected subcutaneously into the hypogastric region of random mongrel puppies, which were routinely dewormed, immunized against hepatitis and distemper, and observed for a period of 3 weeks before use. The dogs were fed on a proprietary diet with water freely. The same methods were used for maintenance of the established tumors.

Results and Discussion
Transplanted Dogs and Duration of Serial Transfer: The results on the serial transfer from 21st to 50th generation are shown in Table I, in which the total number was 170 dogs (79 males and 91 females) and the ages were during 1 to 8 months (mostly 2 to 4 months).

The same sex matchings from donor to recipient were made in 88 cases (male to male 30 and female to female 58) and re-
Table I. Age in Dogs Transplanted with Canine Transmissible Sarcoma

<table>
<thead>
<tr>
<th></th>
<th>Age (months) of dogs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Males</td>
<td>2</td>
<td>11</td>
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<tr>
<td>Females</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>31</td>
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</table>

verse sex matchings were 82 cases (male to female 32 and female to male 50).

A period of 7 months and 4 years from March 15, 1973, to October 15, 1977, was needed to maintain the established tumor cell line from 21st to 50th generation, respectively, and a period of 9 years had also been needed to maintain the tumor cell line from 1st to 50th generation. The period was a little longer than the period reported by Karlson and Mann, which required 7 years to the 40th generation. The intervals of inoculation from 21st to 50th generation ranged from 42 to 98 days (mean, 68 days). The interval was a little shorter than the period from 1st to 20th generation (mean, 86 days).

Rate of Tumor Appearance: The tumor from 1st to 20th generation developed in 98.8% of the dogs and the tumor was detected at the site of implantation in 94.4%. It developed in 100% of the dogs from 21st to 50th generation and 100% of the tumor was detected at the site. Other workers reported lower rates of tumor appearance than our results. The tumor developed in 50%, 45.3%, 36.3%, and 68% in experimental dogs. We have employed puppies, up to 8 months old, for the purpose of transplantation but other investigators used adult dogs. The difference seemed to be due to the age of experimental dogs. After this, to test this possibility, the tumor cells from 48th generation were transplanted into 17 beagles between the ages of 1 and 3 years and the tumor was detected at the site of implantation in 100%. This result revealed feature of our tumor cell line that both puppies and adult dogs could take the transplanted tumor.

Time to Appearance of Palpable Mass: From 1st to 20th generation, appearance of a palpable tumor was detected within 4 and 20 days, respectively, after the inoculation of tumor cells, but could be detected within 4 and 5 days from 21st to 50th generation. The induction, representing time to the appearance of a palpable mass after the tumor cell inoculation, was shorter than other reports, indicating 60 days and between 2 and 9 weeks. Karlson and Mann also detected the tumor in 88.7% of dogs within 60 days after the inoculation, and half of the tumor mass developed within 40 days.

Progress after Appearance of Tumor: The spontaneous regression of tumor induced by subcutaneous inoculation was observed by Sticker in 15% of his cases and by Karlson and Mann in 50% of the inoculated site within 80 days. In our previous work, by 20th generation, the developed growth completely regressed in 31 of 85 animals. Tumors in 16 of them regressed within 4 months after the inoculation. Of the remaining 15 dogs, 14 tumors regressed between 5 and 8 months but only one tumor was present over 1 year. Metastasis developed in a few animals. Surgical excision of the tumor mass of the remaining dogs was carried out, and other dogs were lost by accident. From 21st to 50th generation, the tumor regression was observed in 26 animals. Tumors in 17 of them regressed within 5 months but 2 tumors were present over 1 year. However, metastasis developed in 22 of 170 animals from 21st to 50th generation. The metastasis occurred in dogs in-
CANINE TRANSMISSIBLE SARCOMA

Table II. Age of Dogs Inoculated Tumor Cells in Metastasis

<table>
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<th>Age (months)</th>
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<tr>
<td>3</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>1</td>
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<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No. of dogs</td>
<td>22</td>
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</table>

Oncology transplanted the tumor cells between ages of 2 and 6 months (Table II). In 15 of them, the metastatic tumor mass was observed in the liver and subcutaneous tissue of the whole body. Surgical excision of the tumor mass of the remaining 112 of 170 dogs was carried out and remainder of the dogs was lost by accident. Of 17 adult beagles inoculated the tumor cells, 9 tumors regressed between 60 and 145 days. Remaining 8 dogs were lost by accident.

Inoculated Tumor Cell Counts: Cell concentration of the tumor cell suspension from 21st to 50th generation averaged $8.28 \times 10^7$ (range, $2.28 \times 10^7$ to $19.8 \times 10^7$) cells/ml, and injected fluid volume averaged 4.7 (range, 2.0 to 5.0) ml/site. Adams and Chineme injected 5 ml of $2.5 \times 10^9$ tumor cells/ml into each site of the recipient. Prier suggested that a count of the minimum cells required for a tumor mass was $3.0 \times 10^7$ tumor cells. However, these authors did not discuss the count of inoculated viable tumor cells. We proved that the tumor in a recipient arose from multiplication of the inoculated viable tumor cells. Therefore, positive result of the tumor implantation depends on the number of viable cells. From 21st to 50th generation, the number of inoculated viable tumor cells averaged $16.3 \times 10^7$ (range, $3.0 \times 10^7$ to $51.3 \times 10^7$) cells. The minimum of viable tumor cells from 1st to 20th generation was $1.19 \times 10^6$, and for the purpose of successive transplantation, it was $3.0 \times 10^7$ cells from 21st to 50th generation.

Karyological Characteristics of Tumor Cells: Using a method previously reported, chromosome counts in transplanted tumor cells were examined in samples from 19th and 39th passages, and the results are summarized in Table III. In metaphases, frequent distribution of the chromosome number ranged from 52 to 62, with 59 as a modal number. Well-spread metaphases contained 59 chromosomes which consisted of 17 metacentrics or submetacentrics and 42 acrocentrics.

Histological Features: Histological features from 1st to 50th passages of the tumor were essentially similar to those in specimens from Japan, U.S.A., France, and Jamaica.

Histological features and karyological characteristics of the successively transplanted sarcoma studied by us were consistently identical with those reported by many investigators in the analogous tumors and were not modified by the passive transfer.

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REFERENCES


Table III. Chromosome Counts in Tumor Cells of Experimentally Transplanted Sarcoma from the 19th and 39th Passages

<table>
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<th>No. of transplant passage</th>
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<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>39th</td>
<td></td>
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<td>1</td>
<td>2</td>
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