Differences in Growth of Transplantable Ascites Hepatoma among Various Lines of Donryu Rat

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A total of 48 male Donryu rats aged 4-5 weeks from 8 colonies of 5 lines were used (Fig 1). Rats of the lines C and E were used for re-conventionalization. Some of the lines of Donryu rat have been kept under SPF conditions in barrier facilities after performing a Caesarean section and foster-nursing, and some of the established SPF colonies have been re-conventionalized.

Recently, it has been pointed out by several cancer researchers that experimentally implanted tumor cells such as Yoshida sarcoma and Ehrlich ascites carcinoma can not grow in SPF animals in contrast to conventional ones in which the tumor cells have been shown to grow (personal communication). The above observations pose an important question, i.e. whether genetical variations have occurred in Donryu rats during the establishment of new SPF colonies or the growth of implanted tumor cells is influenced by the microflora of the animal. Therefore, the tumor cells of the ascites hepatoma AH 66 were intravenously injected into various lines of conventional, re-conventionalized ex-SPF and SPF Donryu rat in order to examine the in vivo growth of the tumor cells.

A total of 48 male Donryu rats aged 4-5 weeks from 8 colonies of 5 lines A, B, C, D and E were used (Fig 1). Rats of the lines C and E...
were inbred, and the lines A, B and D randomly bred. The lines D-1 and D-2 were reared under conventional conditions, and the lines A-1, B-1, C and E under SPF conditions. All the SPF rats were housed in barrier rooms using sterilized cages, beddings, diet, drinking water and other materials. Ex-SPF rats of the lines A-2 and B-2 were reared outside barrier rooms after the establishment of SPF colonies. Microbiological checking data on the animals will be described on the following report.

The rats used in the present study were housed in an SPF animal room of Sasaki Institute. The animals were kept individually in autoclaved Makrolon-type cages with filter-top caps and woodchip bedding. The standard rodent diet CE-2 (CLEA Japan Inc.) and chlorinated tap water were available ad libitum. The room temperature was maintained at 22-25°C and the relative humidity at 50-60%. The air was renewed with 100% fresh air 15 times per hour. The lighting cycle was 12-12 hr (07:00-19:00 light).

The ascites hepatoma strain AH 66 established by Odashima in 1954 [3] was used in the present experiments. The tumor cells have been passaged by H. Satoh, one of the authors, at Sasaki Institute, and the passaged tumor cells have reached the 1,020th generation at the beginning of the present experiments (as of July 14, 1986). The tumor cells were injected into the peritoneal cavity of SPF Donryu rats. The ascitic fluid was collected from the rats one week after inoculation. The number of tumor cells in the ascitic fluid was counted using a hemocytometer, and the ascitic fluid was diluted with sterile saline. One ml of the diluted ascitic fluid containing 10⁷ tumor cells was inoculated into the tail vein of each rat.

The rats were observed for 60 days after inoculation of the tumor cells. The animals which died during the 60-day observation period were examined pathologically, and the animals which survived for 60 days were euthanized using ethyl ether and also examined in the same way. The survival of the inoculated tumor cells was examined by Odashima's method [3]: apparent growth of the implanted tumor cells in the liver, lungs and other organs (score 1); incomplete regression of the tumor (score 2); complete regression of the tumor (score 3); no growth of the tumor cells (score 4). The scores 1 and 2 were regarded as positive tumor growth, the scores 3 and 4 negative.

The results are shown in Table 1 and Figure 2. All the conventional rats of the lines D-1 and D-2 died between 9 and 15 days after intravenous inoculation of the ascites hepatoma with the mean survival days of 11.0 and 12.5, respectively. Remarkable growth of the tumor cells was observed in the liver, lungs, kidneys and other organs of all the conventional rats on autopsy. These findings are similar to the previous observations on experimental transplantation of the ascites hepatoma strain AH 66 using conventional Donryu rats [3]. In contrast, all the SPF rats of the lines A-1, B-2, C and E survived for 60 days with no clinical symptoms and the tumor growth was shown to be negative on autopsy. A half of the ex-SPF rats of the lines A-2 and B-2 died between 18 and 23 days after inoculation of the tumor cells, with positive tumor growth. The ex-SPF rats which died showed a prolonged survival period as compared with conventional rats, with the mean survival days of 21.1 (line A-2) and 19.0 (line B-2).
Table 1. Implantation of Ascites Hepatoma and Survival Days

<table>
<thead>
<tr>
<th>Lines</th>
<th>Microbiological Conditions</th>
<th>Genetical Conditions</th>
<th>Positive Tumor Growth /Inoculated</th>
<th>Mean Survival Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>S P F</td>
<td>Random-bred</td>
<td>0/6 (0%)*</td>
<td>—</td>
</tr>
<tr>
<td>A-2</td>
<td>ex-S P F</td>
<td>Random-bred</td>
<td>3/6 (50%)</td>
<td>21.1</td>
</tr>
<tr>
<td>B-1</td>
<td>S P F</td>
<td>Random-bred</td>
<td>0/6 (0%)</td>
<td>—</td>
</tr>
<tr>
<td>B-2</td>
<td>ex-S P F</td>
<td>Random-bred</td>
<td>3/6 (50%)</td>
<td>19.0</td>
</tr>
<tr>
<td>C</td>
<td>S P F</td>
<td>Inbred</td>
<td>0/6 (0%)</td>
<td>—</td>
</tr>
<tr>
<td>D-1</td>
<td>Conventional</td>
<td>Random-bred</td>
<td>6/6 (100%)</td>
<td>11.0</td>
</tr>
<tr>
<td>D-2</td>
<td>Conventional</td>
<td>Random-bred</td>
<td>6/6 (100%)</td>
<td>12.5</td>
</tr>
<tr>
<td>E</td>
<td>S P F</td>
<td>Inbred</td>
<td>0/6 (0%)</td>
<td>—</td>
</tr>
</tbody>
</table>

*: No. of rats and %

Supposing the line difference is negligible, the take rates of the implanted tumor in conventional, ex-SPF and SPF rats were 12/12 = 100%, 6/12 = 50% and 0/24 = 0%, respectively. The differences in take rate of the tumor between conventional and ex-SPF rats (Yates' $\chi^2 = 5.556*$), and between ex-SPF and SPF rats (Yates' $\chi^2 = 11.025**$) were statistically significant. Yokokura et al. [6] described that a little growth inhibition of Sarcoma 180 was seen in ICR mice after oral inoculation of Lactobacillus casei. In addition, there were several literatures which indicated that the growth of implanted tumor cells was influenced by the microflora of the animals [1, 2].

The above reports and the present results seem to support the previous observations by cancer researchers that the take rate of implanted tumor was decreased in SPF animals as compared with conventional ones. There is little evidence that genetical variations which occurred in Donryu rats during the establishment of SPF colonies are responsible for the growth inhibition of the tumor. We could not, however, draw a definitive conclusion, because in the present experiments neither conventional inbred rats of the lines C and E nor SPF random-bred rats of the line D were utilized (Table 1). Further studies should be carried out to investigate the modification of tumor growth by the microbial conditions of the animals.

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References

可移植腹水肝癌の生着性に関するドンリュウラット家系間の差異

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5家系8コロニーの計48匹のドンリュウラットの静脈内に腹水肝癌細胞Aetr66を10^7個ずつ移植した。家系Dに属する2コロニーの普通ラット（D-1, D-2）ではすべての個体に移植細胞が生着し、9〜15日に死亡した。一方、家系A-1, B-1, C, Eの4コロニーのSPFラットはすべて60日間生残し、移植された細胞は完全に排除された。また、SPFコロニーとして確立された後に普通化された家系A-2, B-2の2コロニーのex-SPFラットでは、半数の個体で移植細胞が生着した。今回の一覧において、移植腹水肝癌細胞の生着性に微生物的要因、たとえばSPFであるか否かが重要な役割を果たしているものと推察された。