Cross–Immunity among Allogeneic Tumors in Rats Immunized with Gamma–Irradiated Ascites Tumors

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It has been an almost universal opinion that cross-immunity exists among virus-induced tumors, but not among chemically induced tumors which have individually specific tumor antigen(s) (Klein et al. 1960). However, the establishment of cross-immunity among chemically induced tumors has been recently reported by not a few investigators (Jamash and Nettesheim 1977; Leffell and Coggin 1977; Burton and Warner 1978; Fritze et al. 1978; Hellström et al. 1978; Cleveland et al. 1979).

Usubuchi et al. (1975) observed, in studies employing 3-methylcholanthrene (MCA)-induced sarcomas in C3H/He mice, that cross-immunity was markedly demonstrated by immunization with living cells, but that it was hardly seen by immunization with gamma-irradiated tumor cells only. From these findings, it seemed to be suitable to employ living tumor cells as the immunizing cells in order to demonstrate cross-immunity by the transplantation technique. On the other hand, Kudo et al. (1980) observed cross-immunity between syngeneic ascites mammary carcinomas (MM46 and MM48) in C3H/He mice pretreated with gamma-
irradiated MM46 only. Ascites tumors seemed to have a higher resistance to gamma-irradiation than solid tumors. However, it is difficult to exclude the possibility of virus interaction in cross-immunity because the tumors employed were mammary carcinomas in C3H/He mice.

In the present study using allogeneic non-viral tumors of ascites type, we attempted to find out whether or not cross-immunity is observed among these tumors in rats immunized with gamma-irradiated tumors.

**Materials and Methods**

**Animals**

Non-inbred rats of the Gifu strain of both sexes, weighing from 120 to 150 g, were used. They were kept on Oriental MF (Oriental Yeast Ind. Co.) and watered freely.

**Tumors**

*Hirosaki sarcoma (diploid and tetraploid type)* ([Usubuchi and Abe 1956](#)). This tumor has been maintained in the form of a transplantable ascites tumor converted from a spontaneous lymphosarcoma of the cervical lymph node in a non-inbred rat of the Gifu strain in this laboratory. The original tumor was diploid. The tetraploid type was separated during serial intraperitoneal transplantation. When $10^7$ cells of both types of tumors are transplanted intraperitoneally, all of the rats die after a mean survival time of 7 days.

*Usubuchi sarcoma* ([Usubuchi 1955](#)). Usubuchi sarcoma has been maintained in the form of a transplantable ascites tumor converted from a subcutaneous MCA-induced sarcoma in a non-inbred rat of the Gifu strain in this laboratory. Intraperitoneal inoculation of $10^7$ cells of this tumor kills all of the rats after a mean survival time of 10 days.

*AH130* ([Yoshida 1957](#)). This transplantable ascites tumor was supplied by Dr. Satoh, The Sasaki Institute, Tokyo. AH130 was transformed from a 4-diethylaminoazobenzene-induced hepatoma into an ascites form in a non-inbred rat in the same institute. After a mean survival time of 9 to 14 days, all of the rats are killed by intraperitoneal inoculation of $10^7$ cells.

**Survival time of gamma-irradiated tumor cells**

In order to observe the effect of gamma-irradiation (13,000 rads $^{60}$Co) on ascites tumor cells, $10^7$ gamma-irradiated cells of each tumor were transplanted into the peritoneal cavity of rats. The examination of the tumor-ascites was carried out daily and the survival time was determined by the time up to the extinguishment of tumor cells.

**Immunization**

Immunization was carried out weekly by subcutaneous inoculations of $10^7$ gamma-irradiated cells of each of the tumors. The procedures were repeated 3 times.

**Challenge**

Four weeks after the final immunization, rats were intraperitoneally challenged with $10^5$ cells of the tetraploid type of Hirosaki sarcoma in 4 experimental groups and a non-treated control.

**Results**

*Mean survival time of gamma-irradiated ascites tumor cells in the peritoneal cavity*

The mean survival time of two cell lines (tetraploid and diploid type) of Hirosaki sarcoma, Usubuchi sarcoma and AH130 was 2.0, 1.4, 2.4 and 4.8 days,
respectively. The time of AH130 was remarkably longer than that of the other tumors (Table 1).

**TABLE 1. Mean survival time of gamma-irradiated (13,000 rads) ascites tumor cells transplanted into the peritoneal cavity of allogeneic rats (days)**

<table>
<thead>
<tr>
<th>Transplanted tumors (n=5)</th>
<th>Hirosaki sarcoma (tetraploid type)</th>
<th>Hirosaki sarcoma (diploid type)</th>
<th>Usubuchi sarcoma</th>
<th>AH130</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean survival time</td>
<td>2.0±0.28*</td>
<td>1.4±0.45</td>
<td>2.4±0.88</td>
<td>4.8±0.87</td>
</tr>
</tbody>
</table>

* Mean±S.E.

Challenges of Hirosaki sarcoma (tetraploid type) into the peritoneal cavity of rats immunized with gamma-irradiated allogeneic tumor

The inhibition of the growth of the tetraploid type of Hirosaki sarcoma in rats immunized with one of the tumors different from the immunizing tumor, such as the diploid type of Hirosaki sarcoma, Usubuchi sarcoma or AH130, was observed as well as in rats immunized with the tetraploid type of Hirosaki sarcoma which was the same tumor as the immunizing tumor. In rats immunized with AH130, the inhibition of the growth was markedly observed (Table 2).

**TABLE 2. Challenges of Hirosaki sarcoma (tetraploid type) into the peritoneal cavity of rats immunized with gamma-irradiated allogeneic ascites tumors**

<table>
<thead>
<tr>
<th>Immunizing tumor</th>
<th>Hirosaki sarcoma (tetraploid type)</th>
<th>Hirosaki sarcoma (diploid type)</th>
<th>Usubuchi sarcoma</th>
<th>AH130</th>
<th>None*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tumor deaths</td>
<td>4/10</td>
<td>5/10</td>
<td>5/10</td>
<td>1/8</td>
<td>10/10</td>
</tr>
<tr>
<td>Number of challenges</td>
<td>0.0054</td>
<td>0.0163</td>
<td>0.0163</td>
<td>&lt;0.0003</td>
<td>N.A;</td>
</tr>
</tbody>
</table>

* Untreated control rats.
† Calculated using Fisher's exact probability test for Chi-square for differences in tumor-death incidence between immunized animals and untreated controls.
‡ N.A, not applicable.

**DISCUSSION**

The data obtained in the present study show clearly cross-immunity among non-viral allogeneic tumors by immunization with gamma-irradiated ascites tumors (Table 2). In our previous work on cross-immunity between mammary carcinomas in C3H/He mice by using gamma-irradiated syngeneic tumor, it was difficult to exclude completely the participation of the virus (Kudo et al. 1980). Non-viral tumors in rats being used in this study, no participation of the virus is considered.

Usubuchi et al. (1972) reported previously that cross-immunity was demonstrated among all of 5 allogeneic rat tumors employed without exception and that
the cross-immunity can be explained, therefore, only by the common antigens among allogeneic tumors and not by the interaction of the histocompatibility antigens. The findings in this study showing marked cross-immunity among all of 4 allogeneic tumors definitely confirmed that conclusion.

How can it be reasonably explained that cross-immunity between allogeneic ascites tumors was remarkably shown by immunization with gamma-irradiated ascites tumors? In spite of a high dose of gamma-irradiation (13,000 rads 60Co), the tumor cells transplanted into the peritoneal cavity were alive for 1.4 to 4.8 days showing degenerative changes (Table 1). From this observation, it seems that the ascites type of tumor is more resistant to irradiation than the solid type and, so, causes cross-immunity by the remaining common antigen(s) during the survival time. The dominant cross-immunity observed in rats pretreated with AH130 is considered to be the expression of a high resistance of AH130 to irradiation. On the other hand, in a study employing the solid type of tumors, it was difficult to demonstrate the common antigenicity in animals pretreated with gamma-irradiated solid tumors (Usubuchi et al. 1975). As compared with the present study, the solid tumors seem to have a low resistance to irradiation, and, therefore, their common antigenicity may be destroyed easily.

Based on the results obtained in this laboratory, how shall we make practical use of the cross-immunity among allogeneic tumors? Some attempts at immunotherapy on the growth of autochthonous MCA-induced sarcomas in rats by using allogeneic tumors were carried out in this laboratory (Usubuchi et al. 1976; Kudo et al. 1979). In immunotherapy of human cancer, it is not easy to get the ascites type of human cancer which has a high resistance to irradiation. However, by immunization first with irradiated solid tumors, it may then be possible to safely use viable cells of the same tumor which induces cross-immunity to the primary autochthonous tumor.

References
6) Klein, G., Sjögren, H.O., Klein, E. & Hellström, K.E. (1960) Demonstration of
Cross-Immunity with Irradiated Ascites Tumors


