Inhibitory Effect of Cross-immunity on Autotransplantation of Methylcholanthrene-induced Rat Sarcomas

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Usubuchi, I., Kudo, H., Sobajima, Y., Sato, T., Kakisaka, Y. and Nishimura, S. Inhibitory Effect of Cross-immunity on Autotransplantation of Methylcholanthrene-induced Rat Sarcomas. Tohoku J. exp. Med., 1973, 110 (2), 155-160 — The autologous transplantation of methylcholanthrene (MCA)-induced sarcomas in non-inbred rats, which had been immunized intensively with various allogeneic tumors, was inhibited in 33 out of 65 cases tested. The difference of tissues from which allogeneic tumors originated did not influence the inhibitory effect on autotransplantation. In 10 out of 69 non-immunized controls, the autotransplantation of MCA-induced sarcomas proved to be negative. In addition, remarkable lymphoid cell infiltration was demonstrated in most of tumors induced by MCA in non-inbred rats which were immunized intensively with allogeneic tumors. The autologous transplantation was more markedly inhibited in the tumor infiltrated with massive lymphoid cells than in that not infiltrated. —— autologous transplantation; cross-immunity; lymphoid cell infiltration

The final proof of the immunological response of the organism to the tumor itself, which had been a focus of discussion for a long time, was obtained by Klein et al. (1960), who demonstrated that resistance could be induced in the autochthonous mouse immunized against its own MCA-induced tumor. Additionally, Usubuchi et al. (1962) found resistance to autologous transplantation of MCA-induced sarcomas in a few non-inbred rats by means of repeating the surgical removal and the autotransplantation. Then, Takeda et al. (1966) succeeded in the inhibition of autotransplantation of MCA-induced sarcomas in inbred rats after inducing the immunity due to the ligation-and-release procedure.

But, if cross-immunity among syngeneic or allogeneic tumors cannot be established, the theoretical rationale for practical application of tumor immunity does not exist. Prehn and Main (1957) had already reported some cross-immunity among syngeneic tumors induced by MCA. So Prehn (1961) attempted to inhibit MCA-carcinogenesis by using the cross-immunity, but the results were completely negative against his expectation. Failure of immunization against tumorigenesis
was also reported in the experiments of Klein et al. (1960). As a result of these failures, it has become a common opinion that chemically induced tumors do not cross-react. On the other hand, cross-immunity was noted by Stern (1960), Koldovsky (1961), and Reiner and Southam (1967, 1969). Further, common antigens among syngeneic guinea pig tumors were reported by Zbar et al. (1969) and Holmes et al. (1971). Usubuchi et al. (1972a) revealed cross-immunity between mammary carcinoma and MCA-induced sarcoma of C3H/He mouse, and also demonstrated cross-immunity among mammary carcinomas of C3H/He mice. Moreover, autotransplantation of mammary carcinomas of C3H/He mice was inhibited by using MCA-induced tumors as antigens. On the other hand, Usubuchi et al. (1972b) demonstrated cross-immunity among non-inbred rat tumors. Taking advantage of the cross-immunity, Usubuchi (1956) succeeded in delaying the induction of MCA-carcinogenesis in non-inbred rats. In the previous experiments (Usubuchi et al. 1969), autologous transplantation of MCA-induced sarcomas in non-inbred rats was inhibited to some degree by immunization with subcutaneous inoculation of allogeneic tumors. This paper reports more effective inhibition of autotransplantation of MCA-induced sarcomas in non-inbred rats by intense immunization with subcutaneous and intraperitoneal inoculation of various allogeneic tumors.

**MATERIALS and METHODS**

**Animals**

Non-inbred male and female rats, weighing about 150 g at the beginning of the experiments, were used. They were housed 3 per cage and fed Oriental MF (Oriental Yeast Ind. Co.) and watered ad libitum.

**Tumors used for immunization**

Non-inbred rat ascites tumors such as Usubuchi sarcoma, AH130, AH7974, AH13, Yoshida sarcoma, Hiroasaki sarcoma (tetraploid type), Hiroasaki sarcoma and Takeda sarcoma, were employed. These tumors are described in detail in the preceding report (Usubuchi et al. 1972). Cell number of approximately \(10^7\) as one transplantation dose were implanted respectively into the peritoneal cavity or into the subcutaneous tissue.

**Immunization**

The courses for immunization were as follows:

Experiment 1. After 5 subcutaneous transplantations of Usubuchi sarcoma at intervals of 4 to 5 days, Usubuchi sarcoma, AH130, AH7974 and AH13 were successively implanted at intervals of a few days into the peritoneal cavity.

Experiment 2. After 5 subcutaneous transplantations of Usubuchi sarcoma as in Exp. 1, Usubuchi sarcoma, Yoshida sarcoma, Hiroasaki sarcoma (tetraploid type) and Hiroasaki sarcoma were successively implanted intraperitoneally at intervals of a few days.

Experiment 3. After 3 intraperitoneal transplantations of \(^{60}\)Co-irradiated (13,000 R) AH130, AH130, AH7974 and AH13 were successively transplanted into the peritoneal cavity at intervals of a few days.

Experiment 4. After 3 intraperitoneal transplantations of \(^{60}\)Co-irradiated (13,000 R) Yoshida sarcoma, Yoshida sarcoma, Hiroasaki sarcoma (tetraploid type) and Hiroasaki sarcoma were successively transplanted into the peritoneal cavity at intervals of a few days.
Induction of MCA-induced sarcoma in the immunized rats and its autotransplantation

Rats immunized with various allogeneic tumors as mentioned above were injected subcutaneously in the back with 5 mg of 3-methylcholanthrene dissolved in 0.1 ml olive oil. The tumor, grown to about 1 cm in diameter, was removed surgically. At the same time, a piece of the excised tumor containing about $10^8$ cells was autografted in the subcutaneous tissue of the back and of the flank by trocar, and the other piece was investigated histologically.

Induction of MCA-induced sarcoma in the non-inbred rats and its autotransplantation

Non-inbred rats, weighing about 200 g, were injected with MCA under the skin as in the immunized rats. The MCA-induced tumor was excised surgically. At the same time, a piece of the tumor was transplanted in the subcutaneous tissue of the back and of the flank, and the other piece was examined histologically.

Determination of negative autotransplantation

The transplantations mentioned above were determined to be negative in cases where both implants of the back and of the flank showed no growth 16 weeks after the autotransplantation.

RESULTS

The results of experiments are recorded in Table 1. Fourteen out of 28 animals in Exp. 1, 8 out of 19 in Exp. 2, 4 out of 7 in Exp. 3, and 7 out of 11 in Exp. 4 developed no palpable tumor during the course of the experiment of autotransplantation. Specifically, negative autotransplantation totaled 33, or 51 per cent, out of 65 animals in the immunized groups. In the non-inbred controls, the autotransplantation of MCA-induced sarcoma was negative in 10 cases, or 15 per cent, out of 69 animals.

Histological examination of tumors induced by MCA in rats immunized with allogeneic tumors revealed intensive lymphoid cell infiltration in 31 out of 65 MCA-induced tumors (Fig. 1). In most of the other tumors considerable infiltration of lymphoid cells was also observed. It was more manifest at the margin of the

<p>| Table 1. Inhibitory effect of cross-immunity on autotransplantation of MCA-induced rat sarcomas |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Allogeneic tumors used for immunization</th>
<th>No. of negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Usubuchi sarcoma, AH130, AH7974, AH13</td>
<td>14/28</td>
</tr>
<tr>
<td>2</td>
<td>Usubuchi sarcoma, Yoshida sarcoma, Hirosaki sarcoma (tetraploid type), Hirosaki sarcoma</td>
<td>8/19</td>
</tr>
<tr>
<td>3</td>
<td>AH130, AH7974, AH13</td>
<td>4/7</td>
</tr>
<tr>
<td>4</td>
<td>Yoshida sarcoma, Hirosaki sarcoma (tetraploid type), Hirosaki sarcoma</td>
<td>7/11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>33/65 (51%)</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>10/69 (15%)</td>
</tr>
</tbody>
</table>
tracing the relationship between the degrees of lymphoid cell infiltration and autologous transplantation, inhibition of autotransplantation was remarkable in tumors showing marked lymphoid cell infiltration.

**DISCUSSION**

The establishment of immunity against the tumor itself was demonstrated definitely in the primary autochthonous host by Klein *et al.* (1960). Moreover, in tumors induced by chemical carcinogens, individual specificity of the tumor antigen(s) has been reported by the majority of investigators in this field. There are a few reports suggesting the existence of cross-immunity among chemically induced tumors as described above. If cross-immunity among syngeneic or allogeneic tumors does not exist, however, the therapeutics, diagnosis and prevention of cancer by using tumor immunity may be practically impossible. Usubuchi *et al.* (1972a) demonstrated the cross-immunity between MCA-induced sarcomas and mammary carcinomas of C3H/He mice, and succeeded in the inhibition of autotransplantation of mammary carcinomas by taking advantage of the cross-immunity. Then, Usubuchi *et al.* (1969, 1972b) attempted to inhibit the autotransplantation of allogeneic tumors by using the cross-immunity shown among allogeneic tumors. After MCA-induced sarcomas in non-inbred rats were removed surgically, the hosts were immunized subcutaneously 3 times with Usubuchi sarcoma. The primary MCA-induced sarcoma, which was maintained subcutaneously in other non-inbred rats, was inoculated into the primary animal. The result was that 5 out of 16 animals proved to be negative in the autologous transplantation. In the non-immunized controls, the autologous transplantation was negative in 3 out of 29 animals (Usubuchi *et al.* 1969).
From the results mentioned above, the present experiments were designed in expectation of more marked inhibitory effect by the method of intraperitoneal inoculation of various allogeneic tumors. As recorded in Table 1, the autologous transplantation was inhibited more effectively in the present experiments than in those of subcutaneous immunization previously reported. For the purpose of detecting the differences of inhibitory effect on autotransplantation, the experiments were divided into 4 groups. In Exp. 1, Usabuchi sarcoma and ascites liver carcinomas, i.e., AH130, AH7974 and AH13, were employed as antigens. In Exp. 2, non-epithelial tumors, i.e., Usabuchi sarcoma, Yoshida sarcoma and Hirotsuki sarcoma, were used for immunization. In Exp. 3, liver carcinoma series of AH130, AH7974 and AH13 were employed as immunizing tumors. In Exp. 4, Yoshida sarcoma and Hirotsuki sarcoma, both of which were considered to have originated from the same tissues, were employed as antigens. As a result, no obvious difference was demonstrated among these 4 experimental groups as shown in Table 1. This result suggests that, in possible practical application, the type of tumor employed as antigen needs not be taken into serious consideration.

Lymphoid cell infiltration, which was observed in MCA-induced sarcomas of intensively immunized non-inbred rats, is worthy of notice. Autologous transplantation was more markedly inhibited in the tumors infiltrated with massive lymphoid cells than in those not infiltrated. Apart from the significance of cellular immunity, it is an undeniable fact that the findings of lymphoid cell infiltration in MCA-induced sarcomas were related to the inhibition of autologous transplantation.

A series of studies by Usabuchi et al. (1956, 1969, 1972b) made it clear that the existence of cross-immunity was observed among non-inbred rat tumors which occurred spontaneously or which were induced by chemical carcinogens. In the present studies, autologous transplantation of the primary tumor could be inhibited remarkably by using this cross-immunity. The data seem to indicate that there are some hopes of success in the immunological prophylaxis or therapeutics of cancer in human beings which can not be classified as inbred animals.

References


