Immunotherapeutic Effect of Allogeneic Tumor Cells on the Growth of Autochthonous 3-Methylcholanthrene-Induced Sarcomas in Rats

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Kudo, H., Suga, M., Ogasawara, M., Sato, T. and Usubuchi, I. Immunotherapeutic Effect of Allogeneic Tumor Cells on the Growth of Autochthonous 3-Methylcholanthrene-Induced Sarcomas in Rats. Tohoku J. exp. Med., 1979, 127 (4), 353-358 — The tumor-inhibitory effect of injections of allogeneic tumor cells was observed. Established autochthonous sarcomas induced in the subcutaneous tissue of rats by 3-methylcholanthrene (MCA) were treated with immunization by using allogeneic Hirosaki sarcoma cells. When MCA-induced sarcomas grew to approximately 1 cm in mean diameter, Hirosaki sarcoma cells were inoculated into various tissues of primary tumor-bearing rats. Immunizing procedures consisted of intraperitoneal and subcutaneous injections in one experimental group, and of intradermal and intraperitoneal injections in another. Significantly inhibitory effect on the growth of autochthonous sarcomas was observed in the initial stage up to 2 cm in diameter as compared with that of control sarcomas. No significant inhibition was seen in the course of the growth of sarcomas larger than 2 cm in diameter. This results may indicate that immunotherapy by using allogeneic tumor cells should be considered to be valuable for the treatment of human cancer. —— immunotherapy; allogeneic tumor; cross-immunity

On the basis of the following observations using allogeneic tumor cells: (1) existence of common antigenecity among syngenic tumors (Usubuchi et al. 1972b) and among allogeneic tumors (Usubuchi et al. 1972a), (2) the inhibitory effect of the autotransplantation of MCA-induced rat sarcomas by immunization with allogeneic tumor cells (Usubuchi et al. 1973), (3) induction of lymphoid cells into and around autochthonous MCA-induced sarcomas in rats by immunization with allogeneic tumor cells, and also negative takes of autotransplantation in close relation with the intensity of lymphoid cell infiltration (Kudo et al. 1975), immunotherapy against autochthonous MCA-induced sarcomas in rats was attempted. In the previous report, Usabuchi sarcoma cells were employed as immunizing allogeneic tumor cells (Usabuchi et al. 1976). This report describes the effect of immunization with Hirosaki sarcoma cells against autochthonous MCA-induced sarcomas in rats.

Received for publication, June 6, 1978.
MATERIALS AND METHODS

Tumor induction

Non-inbred male rats of the Gifu strain, weighing 120 to 150 g, were used. Autochthonous tumors were induced dorsally by subcutaneous implantation of pellets of paraffin that contained 5 per cent MCA.

Allogeneic tumor cells used for immunotherapy

Hirosaki sarcoma cells (diploid type) were employed. This sarcoma, having originated as a spontaneous lymphosarcoma of the cervical lymph node in non-inbred rat, was transplanted as an ascites tumor in non-inbred rats in this laboratory (Usubuchi and Abe 1956). When $10^7$ cells of this tumor were implanted into the peritoneal cavity of non-inbred rats, all of the animals died of proliferation of tumor cells.

Treatment

Hirosaki sarcoma cells were inoculated when MCA-induced sarcomas had grown to approximately 1 cm in mean diameter. In Experiment 1, immunotherapeutic procedures were carried out by 6 inoculations at intervals of one week. Three inoculations into the peritoneal cavity were done with gamma-irradiated (13,000 rad $^{60}$Co) Hirosaki sarcoma cells, followed by 3 inoculations with non-irradiated viable cells into the subcutaneous tissue of the back, avoiding the autochthonous MCA-induced sarcoma. In Experiment 2, the procedures were simultaneous intradermal inoculations of gamma-irradiated (13,000 rad $^{60}$Co) Hirosaki sarcoma cells into 10 different sites, avoiding the primary tumor, followed by intraperitoneal inoculations with non-irradiated viable sarcoma cells given twice. The intervals were also one week. One inoculum contained $10^6$ tumor cells.

Measurement

Measurement of tumors with a caliper was made at 3 to 4-day intervals. The inhibitory effect of the treatment was evaluated by the prolongation of the growth time of MCA-induced sarcomas of treated rats compared with that of control rats. The observation period lasted 43 weeks after the MCA implantation. Significance of the difference was determined by Student's $t$ test.

RESULTS

Tumor-growth curves of 9 treated cases in Experiment 1, 15 treated cases in Experiment 2 and 12 control non-treated cases are shown in Figs. 1, 2 and 3, respectively. The ratio of up-grades of the curves of treated cases in both experimental groups is more gentle than that of control cases in the initial stage. Finally, however, all the tumors in treated cases resulted in steep growth. None of the treated cases regressed or cured. The growth time was summarized in Table 1. As shown in the figures, the growth of tumors from 10 to 20 mm in diameter was significantly inhibited in treated cases ($16.0 \pm 1.6$ days, $19.8 \pm 2.0$ days versus $11.7 \pm 1.1$ days). However, when the mean diameter of the tumor was over 20 mm, the difference between treated and control cases was not significant.

DISCUSSION

Needless to say, the research on tumor immunity aims at diagnostic or therapeutic availability. Most of tumors possess tumor-specific antigen(s), and it
Fig. 1. Growth curves of autochthonous MCA-induced sarcomas in Experiment 1, in which therapeutic immunization is composed of each 3 i.p. injections of gamma-irradiated and non-irradiated living Hirosaki sarcoma cells.

Fig. 2. Growth curves of autochthonous MCA-induced sarcomas in Experiment 2, in which therapeutic immunization is composed of simultaneous intradermal injections of gamma-irradiated Hirosaki sarcoma cells into 10 different sites and 2 i.p. injections of non-irradiated living cells.

Fig. 3. Growth curves of autochthonous MCA-induced sarcomas without any treatment.
TABLE 1. The growth-time of autochthonous MCA-induced sarcomas in rats (days)

<table>
<thead>
<tr>
<th>Growth-time of sarcomas from</th>
<th>11 to 20 mm</th>
<th>21 to 30 mm</th>
<th>31 to 40 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1* (n=9)</td>
<td>16.0±1.6a,b</td>
<td>7.1±0.8</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>Exp. 2* (n=15)</td>
<td>19.8±2.9b</td>
<td>9.4±0.7</td>
<td>7.5±0.5</td>
</tr>
<tr>
<td>Control (n=12)</td>
<td>11.7±1.1</td>
<td>7.5±0.6</td>
<td>7.3±1.1</td>
</tr>
</tbody>
</table>

* Immunotherapeutic procedures are as follows: i.p. injections of irradiated (× 3) and non-irradiated living cells (× 3) of allogeneic Hirosaki sarcoma in Exp. 1, and intradermal injections (× 1) of irradiated Hirosaki sarcoma cells into 10 different sites at one time and i.p. injections of non-irradiated living cells (× 2) in Exp. 2.

a Mean±S.E.
b Significantly different from control (Student’s t test, p<0.05).

is important whether common antigenicity exists or not. In general, tumors induced by a chemical carcinogen have been reported to have individually specific tumor-specific transplantation antigens which do not show cross-immunity. However, there are some reports indicating the common antigenicity among chemically induced tumors (Prehn and Main 1957; Koldovsky and Svoboda 1963; Reiner and Southam 1967, 1969; Zbar et al. 1969; Holmes et al. 1971; Taranger et al. 1972; Steele and Sjögren 1974). More recently, data have been presented indicating the existence of common antigen(s) in chemically induced tumors both in vivo (Economou et al. 1977; Jamasbi and Nettesheim 1977; Leffell and Coggin 1977) and in vitro (Hellström and Hellström 1975; Fritzke et al. 1976).

Usubuchi et al. (1972a, b), by using various transplantable ascites and solid tumors, demonstrated cross-immunity among allogeneic tumors as well as among syngeneic tumors, and concluded that tumors possess common antigen as well as individually unique antigen. It was also concluded that transitory viability of tumor cells in the living body plays an important role in the appearance of tumor immunity (Usubuchi et al. 1975). By immunization with allogeneic tumor cells, autotransplantation of MCA-induced rat sarcomas was markedly inhibited as compared with that in non-immunized controls (Usubuchi et al. 1973). Moreover, immunization of MCA-induced sarcoma bearing rats by using allogeneic tumor cells was effective in inducing mononuclear lymphoid cell infiltration in and around the autochthonous sarcomas (Kudo et al. 1975). On the basis of these findings obtained in our laboratory, the immunotherapy with allogeneic tumor cells was undertaken against established autochthonous MCA-induced sarcomas in rats. In the experiment reported previously, immunotherapy against autochthonous MCA-induced sarcomas in rats was carried out, using Usubuchi sarcoma cells as immunizing allogeneic tumor cells. Usubuchi sarcoma is a transplantable ascites tumor derived from MCA-induced subcutaneous sarcoma. The result showed the
inhibitory effect of tumor-growth in the initial stage (Usubuchi et al. 1976).

In this study, Hirosaki sarcoma cells were employed as immunizing allogeneic tumor cells. In both experimental groups, the ratio of tumor-growth in rats treated with allogeneic Hirosaki sarcoma cells fell significantly in the initial stage where tumors were smaller than 20 mm in mean diameter. Growth over 20 mm in mean diameter was not inhibited by immunization with allogeneic tumor cells, and the growth curves were as steep as those of non-treated cases. As the immunotherapeutic effect by allogeneic tumor cells against autochthonous tumors was observed at least in the initial stage of growth, the immunotherapy using allogeneic tumor cells is considered to be valuable for the treatment of human cancer in the early stage or in the post-operative stage.

References


