Combination of Active and Passive Immunization and Chemotherapy to Transplantation of Methylcholanthrene-induced Tumor in WKA Rats

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SUMMARY

The combined effect of active and passive immunization with chemotherapy was studied in the treatment of rapidly growing methylcholanthrene-induced fibrosarcoma KMT-17 in the WKA rat.

In active immunization, live Friend virus-infected KMT-17 tumor cells were inoculated s.c. into rats a few hr after KMT-17 transplantation. The survival rate with active immunization alone was only 19.0%. In passive immunization, syngeneic lymphoid cells from rats previously immunized with Friend virus-infected KMT-17 tumor cells were transferred i.v. a few hr after KMT-17 transplantation. The survival rate with passive immunization alone was only 18.2%. With chemotherapy, mitomycin C, 1 mg/kg, was inoculated i.v. 3 days after KMT-17 transplantation. The rate of survival was only 17.4%. However, the combination of active immunization and chemotherapy increased the inhibition of KMT-17 transplantation to 56.0%, while the combination of active and passive immunization brought the survival rate up to 60.9%. Furthermore, the combination of active and passive immunizations and chemotherapy resulted in a survival rate of 84.9%.

Also, the lymphoid cells used for passive immunization were not restricted to lymphoid cells obtained from donors immunized with the identical target tumor. When passive immunization was combined with active immunization and chemotherapy, the lymphoid cells for the passive immunization were allowed to be obtained from donors immunized with Friend virus-induced rat tumor WFT-13; this tumor possessed virus-related antigens identical to the newly acquired antigens on the Friend virus-infected KMT-17 tumor cells that were used as the immunizing material.

INTRODUCTION

Many attempts have been made to accelerate the effectiveness of cancer treatment by combining different means, and immunotherapy has played an important role (2, 5, 6, 14, 17). The technique used for active immunization was live tumor cells infected with murine leukemia virus. The virus-infected tumor cells grew and later regressed immunologically in syngeneic adult rats (9–11). This suggests that immunotherapy, using live tumor cells infected with murine leukemia virus as an immunogen, may be able to produce a strong effect in inhibiting the growth of tumor cells (7). However, the immunogen was not effective in curing tumors that were already growing in the host. Consequently, the authors attempted to accelerate the inhibitory effect by combining different treatments with active immunization.

MATERIALS AND METHODS

Rat. Male and female inbred WKA/Mk rats weighing 100 to 150 g were donated by the Experimental Animal Center, Faculty of Science, Hokkaido University, Sapporo, Japan (Dr. S. Makino and Dr. M. Sasaki). Skin transplantation succeeded in all cases.

Tumor. KMT-17 was a transplantable fibrosarcoma induced by 20-methylcholanthrene in WKA/Mk rats. This was donated by Dr. M. Aizawa, Department of Pathology, Hokkaido University School of Medicine, Sapporo, Japan. The tumor was maintained in ascites form, and the mean survival time was 20.7 days when transplanted s.c. in doses of 1 x 10⁴ cells. Minimal doses of this tumor killed all rats: using s.c. transplantation, it took 5 x 10³ cells, and i.p. transplantation required 1 x 10² cells.

The FV-KMT-17 tumor was the KMT-17 tumor artificially infected with Friend virus. For the virus infection, KMT-17 tumor cells were injected i.p. at 4-day intervals in WKA rats over 2 months old. These rats had been inoculated with Friend virus at birth. After passage of the tumor for 1 or 2 generations, the successful infection with the virus and the subsequent appearance of new antigens on the cell surface were borne out by electron microscopy (12), cytotoxicity test (16), and immunofluorescence (15). When FV-KMT-17 was transplanted s.c. into normal adult rats, it grew to reach a maximum of 30 mm on the 6th day and then regressed by 2 weeks after the transplantation.

The WFT-13 tumor was apparent in the enlarged spleen of the WKA/Mk rat 172 days after the neonatal injection of Friend virus, and it was maintained in Friend virus-tolerant rats. Friend virus-induced tumors grew only minimally in normal adult rats (8). This was due to the immunological mechanism, xenogenization (9).

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1 FV-KMT-17, Friend virus-infected KMT-17; MMC, mitomycin C.
Active Immunizations. FV-KMT-17 cells (1 x 10^7) were inoculated s.c. into the left flank of WKA rats.

Passive Immunization. Syngeneic WKA rats were immunized s.c. with a large amount of FV-KMT-17 tumor cells about 5 times, and the thymus, spleen, and submandibular and peritoneal lymph nodes were removed aseptically from the rats 1 to 2 weeks after the final immunization. The excised thymus, spleen, and lymph nodes were separated into single cells with a loosely fitting glass homogenizer, and the cells were suspended in Eagle's minimal essential medium. Rats were given i.v. injections of 5 x 10^7 cells through the tail vein after the viable cells were counted by the trypan blue dye exclusion method. Normal lymphoid cells or lymphoid cells from rats immunized with WFT-13 were both prepared and transferred by the same procedures as described above.

Chemotherapy. MMC, supplied by the Kyowa Hakko Kogyo Co., Tokyo, Japan, was dissolved with Eagle's minimal essential medium just before the injection. MMC was injected i.v. 1.0 mg/kg body weight, through the tail vein. No rats died from the toxicity of the drug.

Time Schedule of Treatments. As shown in Chart 1, 1 x 10^4 KMT-17 tumor cells were transplanted s.c. into the right flank of the rat on Day 0. Active or passive immunization was carried out on Day 0 just a few hr after transplantation of the KMT-17 tumor. In the case of chemotherapy, MMC was injected on Day 3. The effectiveness of the treatment was measured by the number of rats that survived more than 3 months. They are referred to as “survivors” in the results.

RESULTS

Immunizing Effect of FV-KMT-17 Tumor Cells with Reference to Its Time and Dose Response. As previously reported, rats that had already rejected the murine leukemia virus-infected tumor acquired complete resistance to the identical non-virus-infected tumor (11), but it was not known when rats immunized with virus-infected tumor cells acquired transplantation resistance to non-virus-infected tumors of the same line. One of the most important problems related to active immunization is determining at what time after immunization the effect of an immunogen appears in hosts. Therefore, the immunizing effect of FV-KMT-17 tumor cells in the transplantation of the KMT-17 tumor was examined with special reference to its time and dose response (Table 1).

FV-KMT-17 (1 x 10^7) was inoculated into the left flanks of rats, and graded doses of KMT-17 tumor cells were then transplanted into the right flank of rats either on the same day or 3, 6, 9, or 50 days afterwards. As the results show in Table 1, all rats died when they received 1 x 10^4 KMT-17 tumor cells a few hr after immunization with FV-KMT-17. One of 4 rats that received 1 x 10^7 KMT-17 3 days after immunization survived. When KMT-17 was transplanted 6 days after immunization, all rats receiving a dosage of 1 x 10^4 KMT-17 survived, but 1 of 2 rats given injections of 1 x 10^6 cells and all rats receiving 1 x 10^8 cells died of tumor growth. All rats survived with an inoculation of 10^4 to 10^7 cells 9 days after immunization but no rats could survive at a dosage of 1 x 10^7 tumor cells. However, all survived when KMT-17 was transplanted in graded doses 50 days after immunization with FV-KMT-17. These results indicate that at least 6 days were required after the FV-KMT-17 immunization for the induction of sufficient inhibitory effect to a KMT-17 challenge. Afterward, the effect became stronger as the FV-KMT-17 tumor regressed and it persisted until 50 days after immunization. The FV-KMT-17 tumor regressed completely even in cases where the KMT-17 tumor grew lethally.

Inhibition of the KMT-17 Tumor in WKA Rats by Different Treatments. The inhibition of the growth of the KMT-17 tumor by respective treatments with active immunization, passive immunization, and chemotherapy was examined (Table 2). Rats not receiving treatment succumbed in all cases. In active immunization, FV-KMT-17 was inoculated s.c. a few hr after the transplantation of KMT-17 because of the slow rate at which inhibition developed (see Table 1). With active immunization alone, 4 of 21 rats survived (19.0%). After passive immunization with lymphoid cells from donors immunized with FV-KMT-17, 4 of 22 rats survived (18.2%), even when the rats were treated on the same day. Only 4 of 23 rats survived (17.4%) when treated by chemotherapy 3 days after the transplantation of KMT-17.

Inhibition of the KMT-17 Tumor in WKA Rats by the Combination of 2 Treatments. Since none of the treatments singly was effective against KMT-17, the effects of combination treatments were examined (Table 3). Either chemotherapy or passive immunization with lymphoid cells from donors immunized with FV-KMT-17 was combined with active immunization following the time schedule of treat-

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**Table 1**

Inhibitory effect to KMT-17 tumor in WKA rats by single treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survivors/no. of rats used</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active immunization</td>
<td>4/21</td>
<td>19.0</td>
</tr>
<tr>
<td>Passive immunization*</td>
<td>4/22</td>
<td>18.2</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>4/23</td>
<td>17.4</td>
</tr>
<tr>
<td>None</td>
<td>0/11</td>
<td>0</td>
</tr>
</tbody>
</table>

*Carried out with lymphoid cells from WKA rats immunized with FV-KMT-17 tumor.
ments in Chart 1. Untreated rats died in all cases. With a combination of active immunization and chemotherapy, 14 of 25 rats survived, bringing the survival rate up to 56.0%. With the combination of active immunization and passive immunization with lymphoid cells from donors immunized with FV-KMT-17, 14 of 23 rats survived (60.9%).

**Inhibition of the KMT-17 Tumor in WKA Rats by the Combination of 3 Treatments.** The effect of triple combination treatment (active and passive immunization and chemotherapy) was examined to determine whether a synergistically enhanced effect could be obtained (Table 4). All rats receiving no treatment died of tumor growth. When, as a control, normal (rather than immunized) lymphoid cells from syngeneic rats were transferred to rats being treated with active immunization and chemotherapy a few hr after the transplantation of KMT-17, 11 out of 20 rats survived (55.0%). This survival rate closely corresponded to the result of the combined treatment of active immunization and chemotherapy cited above (56.0%). However, when active and passive immunization and chemotherapy were combined, 28 of 33 rats survived, and the survival rate was enhanced synergistically up to 84.9%.

**Effect of Passive Immunization with Lymphoid Cells from Donors Immunized with a Different Tumor.** Synergistically enhanced resistance to the transplantation of the KMT-17 tumor was obtained when passive immunization (with lymphoid cells from donors immunized with FV-KMT-17) was combined with active immunization and/or chemotherapy. As FV-KMT-17 tumor cells possessed not only KMT-17 tumor-specific antigen but also Friend virus-related antigen on the cell surface as a result of virus infection (12, 13), the transferred, immune lymphoid cells would have acted on both KMT-17 and FV-KMT-17 as an immunogen. Therefore, the immune lymphoid cells that acted only on the FV-KMT-17 tumors were transferred into rats to see whether this kind of passive immunization would also heighten inhibition of the KMT-17 tumor when combined with active immunization and/or chemotherapy. Lymphoid cells were obtained from rats immunized with the WFT-13 tumor, the cells of which possessed Friend virus-related antigen, but not KMT-17 tumor-specific antigen. The results (Table 5) show that, by itself, passive immunization with lymphoid cells from donors immunized with WFT-13 was as ineffective in resisting KMT-17 as no treatment at all. It was slightly more effective when passive immunization was combined with FV-KMT-17 as an immunogen, i.e., 4 of 13 rats survived (30.7%). Moreover, when chemotherapy was added, the results became much more impressive, with 10 of 13 rats surviving (76.9%).

### DISCUSSION

As indicated in the results, the effectiveness of active and passive immunization and chemotherapy was extremely low when each treatment was used by itself. However, the KMT-17 tumor used in our experiment was an exceptionally rapidly growing tumor, which killed the untreated host only 4 days after i.p. inoculation and 20 days after s.c. inoculation of $10^4$ cells. Our previous experiment, using slowly growing methylcholanthrene-induced KMT-68 tumor, indicated a high percentage of survivors, even when the treatment was begun 3 days after tumor transplantation (7). Therefore, the low rate of KMT-17 tumor survival in the present experiments should be based on the characteristics of the growth of the tumor used.

The Friend virus-infected tumor cells were used for active immunization. Both specific and nonspecific immunizing effects would have been beneficial to our combination treatments. We previously reported that hosts immunized with Friend virus-infected tumor cells completely rejected the transplantation of non-virus-infected tumor cells of the same line, but not that of other lines (11). Therefore, specific immunization was shown to play an important role in
rejection of the identical, non-virus-infected, tumor cells. On the other hand, it is not known whether the Friend virus-infected cells really acted as a nonspecific stimulant. The growth of the non-virus-infected tumor was not inhibited by previous administration of Friend virus itself (10). However, immunologically significant results have been obtained by infecting tumor cells with Friend virus. The virus-infected cells have been shown to become more immunosensitive through cytotoxicity tests in vitro (16) and more immunogenic in vivo (11) than the non-virus-infected tumor cells. The mechanism behind increased immunogenicity of tumor cells infected with Friend virus is still unknown, but many reports indicate that weak antigens, such as tumor-specific antigens, become stronger by the addition of some substances such as the influenza virus or by chemicals (3, 4, 13).

In passive immunization, thymus cells, which were almost totally ineffective in rejecting tumor cells, were transferred with spleen and lymph node cells in our experiments. It was because of this that thymus cells, in addition to spleen and lymph node cells, improved the positive immune responses in tumor-bearing hosts, which were often in an immunodepressive state or tolerant to tumor-specific antigens.

As a chemotherapeutic agent MMC was shown to have powerful immunosuppressive effects in mice, as was demonstrated by allogeneic skin grafting (1). However, no marked immunosuppression was observed in WKA rats, which received i.v. injections of MMC at a dosage of 1.0 mg/kg body weight 3 days before or after the allogeneic skin grafting (E. Gotohda, unpublished results). Then cytoreductive effect (rather than immunosuppression, which would not have been detected in our experiments) of the drug would have enhanced the cure rate in combination of immunotherapy.

An interesting result was a synergistic, as opposed to a cumulative, increase in the inhibition of tumor growth when the treatments were combined with each other. The dosage of each treatment was possibly the maximum level that could be used in the experiment. In reference to the mechanism, a synergistic increase of the inhibitory effect may be the result of certain mechanisms in one treatment serving to counterbalance the deficiencies in the mechanisms of the other treatments. Therefore, in combination, the treatments assisted each other and a synergistic increase was observed in the survival rate of the host. A possible explanation in the case of the combination of active and passive immunization is that lymphoid cells from Friend virus-infected KMT-17-immune rats may not only have acted on the target KMT-17 tumor cells specifically but also on the tumor cells of the immunogen, which possessed both KMT-17 tumor-specific antigen and Friend virus-related antigen. The latter may possibly accelerate the early development of the immunizing effect, which may lead to a synergistic increase in the effect.

Lymphoid cells used for the passive immunization need not be restricted to lymphoid cells from donors immunized with the target tumor cells. In other words, a similar level of effect was observed when the immune lymphoid cells came from donors immunized with the WFT-13 tumor, which possessed Friend virus-related antigens acquired by xenogenization of the KMT-17 tumor used for the immunogen. This is because the transferred immune lymphoid cells may not act directly on the target tumor cells, but only on the immunogen in this case. Thus, the immune lymphoid cells obtained from donors immunized with FV-KMT-17 or WFT-13 would have acted mainly on the immunogen, which would have evoked early development of the immunizing effect.

REFERENCES

Specific Immunotherapy and Chemotherapy in Rats


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