212. Regression of Methylcholanthrene-Induced Sarcoma Transplanted to Neonatally Thymectomized Allogeneic Mice by the Restoration of Immunologic Capacity of Hosts

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It has been recognized that the capacity for transplantation immunity is depressed markedly in neonatally thymectomized mice and strain-specific allogeneic tumors of various origins could be accepted by these animals. We reported that methylcholanthrene-induced autochtonous tumors developed earlier and isogenic transplantable methylcholanthrene-induced sarcoma (MC-induced sarcoma) grew more rapidly in neonatally thymectomized mice than in sham-thymectomized mice.

From these facts, it is apparent that the growth of tumors is affected by the immunologic response of the hosts. On the other hand, the restoration of immunologic capacity of neonatally thymectomized mice by means of thymus grafting or lymphoid cell transfusion has been reported by several authors. This work was undertaken to know whether or not the strain-specific allogeneic tumors accepted by neonatally thymectomized mice could be affected by the restoration of immunologic capacity of the hosts.

Experimentals. Sarcoma. MC-induced sarcoma (BL 12) of a C57BL male mouse was serially transferred in male mice of C57BL strain and used for the experiment at second or third transfer generation. Tumor cell suspensions were prepared by ordinary trypsinization technique. Cell suspensions of $5 \times 10^5$ cells were inoculated subcutaneously into the back of recipients.

Recipient animals. The recipients were the inbred mice of SL and AKR strains, and the outbred mice of CF-1 strain. Thymectomy was performed at birth (0-TE), one day (1-TE) or two days (2-TE) after birth. Sham-thymectomized mice were used as control. Both groups of mice were used for the experiment at 3 to 7 weeks of age.

Thymus grafting. The thymus grafts of three or four intact lobes taken from new born mice of the same strain were transplanted into the anterior and superior mediastinum of thymectomized mice.

Lymphoid cell transfusion. The spleens and thymuses of adult
mice of the same strain were minced with razor blades and suspended in Hanks' solution. The suspensions were left standing in Kahn tubes for 10 minutes and the lymphoid cells in the supernatant were counted after staining with trypan blue. The lymphoid cell suspensions of $1 \times 10^8$ cells were injected into the intraperitoneal cavities of recipients.

**Skin grafting.** Skin grafting was performed following the fitted pinch graft method described by Billingham et al.7)

Table I. Survival time of allogeneic skin grafts in neonatally thymectomized mice and the restoration of transplantation immunity after grafting thymus of the same strain (C57BL→SL)

<table>
<thead>
<tr>
<th>Recipients (SL)</th>
<th>Donor</th>
<th>Survival time of skin grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td>thymectomized mice</td>
<td>C57BL</td>
<td>23*, &gt;45, &gt;45, &gt;45, &gt;45</td>
</tr>
<tr>
<td>thymectomized mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grafted with thymus</td>
<td></td>
<td>14, 15, 21, 22, 31</td>
</tr>
<tr>
<td>sham-thymectomized mice</td>
<td></td>
<td>9, 10, 10, 10, 10</td>
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* Died with intact skin graft.

Table I shows the degree of restoration of transplantation immunity after thymus grafting in inbred SL mice. The mice of 6 weeks of age were used as recipients. Allogeneic skin grafts of C57BL female mice transplanted to sham-thymectomized mice were rejected 9 to 10 days after skin grafting. Allogeneic skin grafts survived over 45 days in the neonatally thymectomized mice. Allogeneic skin grafts were rejected 14 to 31 days after grafting in neonatally thymectomized mice grafted with thymus at 4 weeks of age. Table II shows the restoration of transplantation immunity after thymus grafting in outbred CF #1 mice. The survival times of skin grafts of C57 BL mice in sham-thymectomized and neonatally thymectomized CF #1 mice were almost equal to those in corresponding groups of the above-stated inbred SL mice. However, the survival times of skin grafts in neonatally thymectomized CF #1 mice grafted with thymus at 4 weeks of age were longer than those in the SL mice treated in the same way.

Table II. Survival time of allogeneic skin grafts in neonatally thymectomized mice and the restoration of transplantation immunity after grafting thymus of the same strain (C57BL→CF #1)

<table>
<thead>
<tr>
<th>Recipients (CF #1)</th>
<th>Donor</th>
<th>Survival time of skin grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td>thymectomized mice</td>
<td>C57BL</td>
<td>30*, 34*, &gt;45, &gt;45, &gt;45</td>
</tr>
<tr>
<td>thymectomized mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grafted with thymus</td>
<td></td>
<td>22, 24, 31, 34, &gt;45</td>
</tr>
<tr>
<td>sham-thymectomized mice</td>
<td></td>
<td>9, 9, 10, 10, 11</td>
</tr>
</tbody>
</table>

* Died with intact skin grafts.
Table III shows the frequency of regression, after thymus grafting, of allogeneic tumors transplanted to neonatally thymectomized mice. In this group, thymus grafting was performed immediately after tumor transplantation at 4 weeks of age. The regression began 14 to 24 days after transplantation and at this stage tumors reached the size of 1 to 2 cm in diameter. The frequency of regression of tumors was higher in inbred SL and AKR mice than in outbred CF #1 mice. Fig. 1 and 2 shows the growth and regression curves of tumors transplanted to SL mice.

All the neonatally thymectomized mice, which were grafted with tumor cells at 7 weeks of age, showed wasting syndrome and died within 16 days after grafting. On the other hand, neonatally thymectomized mice, grafted with tumor cells at 7 weeks of age and...
immediately thereafter transfused with non-immunized lymphoid cells, survived longer. In these mice, 2 of the developed tumors regressed but the rest of them grew progressively (Fig. 3). When tumor cells were transplanted at 3 weeks of age, and lymphoid cells were transfused 12 days thereafter, 2 tumors of 6 regressed completely (Fig. 4). When lymphoid cells derived from the mice immunized with spleen cells of C57 BL and with the tumor cells were injected, regression was induced much earlier and in higher percentage (Fig. 5).

Discussion. Immunologic regression of tumor is an interesting problem, not only because of its significance in immunobiology of tumor, but also the future possibility of clinical application. It has been recognized that a part of skin papillomas of rabbits, induced by shope papilloma virus,⁸ and also of mice induced by chemical carcinogen,⁹ regress spontaneously. The mechanism of this phenomenon appears to be related to the immunologic response of hosts. However, little has been known about the regression of autochthonous
and transplantable isogeneic malignant tumors. This experiment was undertaken to approach the clue to the mechanisms of immunologic regression of malignant tumors.

Regression of allogeneic tumors occurred both in the neonatally thymectomized SL and AKR mice, in which thymus grafting was performed immediately after tumor transplantation, and also in the neonatally thymectomized SL mice, in which transfusion of non-immunized lymphoid cells were performed immediately after, or 12 days after tumor transplantation. Acceleration of the regression of tumors by thymus grafting did not occur in CF #1 mice. However, the rate of growth was relatively slow in the neonatally thymectomized CF #1 mice grafted with thymus. The difference in the frequency of regression of tumors between inbred mice and outbred mice is in accordance with the fact that the restoration of transplantation immunity, by thymus grafting in CF #1 mice, is rather insufficient compared to that of inbred SL mice (Table II). When immunized lymphoid cells against spleen cells of C57 BL mice and the tumor were used for transfusion, the regression was induced much earlier and in higher percentage than in the cases transfused with non-immunized lymphoid cells. This finding will be interpreted as to be due to second set reaction caused by immunized lymphoid cells.

Since the immune reaction would be directed not only to tumor specific antigens but also to normal histocompatibility antigens, this experiment did not show the exact aspects of immunologic regression of malignant tumors. However, from the results described above, it is apparent that tumors which have already reached to considerable size could be regressed by immunologic response of the hosts.

Summary. Thymus grafting or transfusion of lymphoid cells to neonatally thymectomized mice was performed immediately after, or 5 to 12 days after, tumor transplantation. A part of tumors regressed 14 to 24 days after thymus grafting or lymphoid cell transfusion. These results indicate that tumors which have already grown to considerable size would be regressed by immunologic response of the hosts.

References