Studies on the Mechanism of Prolonged Survival of Allografts from Tumor-bearing Donors

E. Robinson, V. K. Golakai, and M. Schlesinger

Department of Oncology, Hadassah University Hospital, and Department of Experimental Medicine and Cancer Research, Hebrew University-Hadassah Medical School, Jerusalem, Israel

SUMMARY

The H-2 isoantigenicity of murine tissues was unaffected by the intraperitoneal growth of the Ehrlich ascites tumor, as shown by their normal capacity to absorb H-2 isohemagglutinins. Skin grafts from Ehrlich ascites tumor-bearing C57BL mice had a longer survival in RIII mice than grafts from normal donors. First-set allografts from tumor-bearing donors effectively immunized the hosts against second-set allografts from normal donors. Second-set allografts from tumor-bearing mice showed a prolonged survival in mice presensitized by first-set allografts from either normal or tumor-bearing donors. It therefore seems that the prolonged survival of skin allografts from tumor-bearing donors is not due to their decreased antigenicity or deficient immunogenicity, but rather to their increased resistance to the allograft reaction of the host.

INTRODUCTION

Skin grafts from cancer patients survive longer than grafts from normal donors in both cancer patients (9) and in normal recipients (2). Similarly, skin grafts obtained from mice bearing ascites tumors (4) or other types of tumors (7) show a prolonged survival in allogeneic hosts.

The aim of the present study was to elucidate the mechanism underlying this phenomenon. The antigenicity of the tissues of tumor-bearing animals was assayed by determining their capacity to absorb isohemagglutinins in vitro. First- and second-set skin allografts from normal and tumor-bearing animals were examined to determine whether the skin of tumor-bearing animals showed either decreased immunogenicity or increased resistance to the allograft reaction.

MATERIALS AND METHODS

Mice

All mice belonged to inbred strains bred at the Department of Experimental Medicine and Cancer Research. Male mice of the C57BL/6 strain served as donors of skin grafts, while RIII/Jem mice of the same sex served as recipients. For some of the serologic tests mice of the C3H/An and A strains were used.

Tumor

Five million Ehrlich ascites tumor cells were injected intraperitoneally into C57BL/6 mice one week before their skin was used for grafting or their tissues were analyzed serologically.

Skin Grafts

Full-thickness skin grafts were sutured with 4-0 silk sutures and dressed with a sterile layer of vaseline-gauze, followed by pressure dressing. Each recipient was kept in a separate cage. The dressings were removed on the 5th postoperative day, and the grafts examined daily by two observers. Second-set grafts were applied two to three weeks after the first grafts.

The criteria for graft rejection were scale formation, drying and hardening of the grafts, or black discoloration and necrosis of 80–100% of the surface of the graft.

Serologic Technics

Isoantisera were prepared by repeated intraperitoneal injections of C57BL spleen cells to C3H mice and of A spleen cells into C57BL mice. One week after the last injection, mice were bled from the retroorbital sinus through a glass capillary. Sera were stored at −20°C.

Isohemagglutinins were determined according to the modification by Amos (1) of the dextran-human serum method of Gorer and Mikulska (6).

Tissues used for absorption were homogenized with cold normal saline, 1.0 gm tissue per 1.0 ml saline; the tissue-mince was stored at −20°C until used.

Method of Absorption

The H-2 isoantigenicity of murine tissues was assayed by determining their capacity to absorb isohemagglutinins according to a technic described previously (10).

Serial dilutions of 0.025 ml antiserum were prepared in 6 x 50 mm test tubes, and an equal volume of tissue-mince was added to each tube. The test tubes were left at room temperature for 1 hour, shaken from time to time, and then centrifuged at 3,000 rpm for 5 minutes. The supernatant was tested for the presence of hemagglutinins. In each absorption experiment the lowest antiserum dilution that was still absorbed completely by the tissue preparation was determined.
tumor-bearing C57BL donors in RIII recipients. N, Normal
MARCH 1968

Results

AC57BL used animals had an immunizing capacity similar to that of grafts seems that the first-set graft derived from tumor-bearing an

mice (Table 1), but the difference was statistically significant

of antigen.

tissue-mince that absorbed completely a 1:16 serum dilution

using an antiserum with a hemagglutination titer of 1024, a

the absorption procedure, one unit of antigen was defined as

agglutination of red blood cells. Due to the 2-fold dilution in

in a volume of 0.025 ml that would just cause macroscopic

Survival of first- and second-set skin grafts from normal and
tumor-bearing C57BL donors in RIII recipients. N, Normal
C57BL mouse; T, Ehrlich ascites tumor-bearing C57BL mouse.

Table 1

<table>
<thead>
<tr>
<th>Type of allograft</th>
<th>No. of experimental group</th>
<th>Donor of 1st-set graft</th>
<th>Donor of 2nd-set graft</th>
<th>No. of mice</th>
<th>Mean survival time (days ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-set</td>
<td>1</td>
<td>N</td>
<td></td>
<td>27</td>
<td>9.7 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>T</td>
<td></td>
<td>27</td>
<td>10.9 ± 0.35</td>
</tr>
<tr>
<td>Second-set</td>
<td>3</td>
<td>N</td>
<td>T</td>
<td>15</td>
<td>8.4 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>N</td>
<td>N</td>
<td>8</td>
<td>6.4 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>T</td>
<td>T</td>
<td>12</td>
<td>9.1 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>T</td>
<td>N</td>
<td>8</td>
<td>7.9 ± 0.36</td>
</tr>
</tbody>
</table>

Survival of first- and second-set skin grafts from normal and
tumor-bearing C57BL donors in RIII recipients. N, Normal
C57BL mouse; T, Ehrlich ascites tumor-bearing C57BL mouse.

Table 2

<table>
<thead>
<tr>
<th>Tissue used for absorption</th>
<th>Antigenic activity (units/0.025 ml tissue mince) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated mice</td>
</tr>
<tr>
<td>C57BL spleen</td>
<td>256, 256, 128, 128</td>
</tr>
<tr>
<td>A spleen</td>
<td>256, 128</td>
</tr>
<tr>
<td>C57BL liver</td>
<td>128, 128, 64</td>
</tr>
<tr>
<td>A liver</td>
<td>128, 128, 64</td>
</tr>
<tr>
<td>C57BL thymus</td>
<td>32, 32, 32</td>
</tr>
<tr>
<td>A thymus</td>
<td>64, 32, 32</td>
</tr>
<tr>
<td>C57BL kidney</td>
<td>64, 32, 32</td>
</tr>
</tbody>
</table>

Distribution of H-2 isoantigenic activity in tissues of normal and
tumor-bearing C57BL and A mice.
* Each value represents the result of one absorption experiment.

Antigen Units

One unit of antibody was defined as that amount of antibody in a volume of 0.025 ml that would just cause macroscopic agglutination of red blood cells. Due to the 2-fold dilution in the absorption procedure, one unit of antigen was defined as the antigenic activity of 0.025 ml of tissue-mince that would absorb completely two units of antibody (cf. 10). Accordingly, using an antiserum with a hemagglutination titer of 1024, a tissue-mince that absorbed completely a 1:16 serum dilution (containing 64 antibody units) was defined as having 32 units of antigen.

Results

Survival of Skin Allografts from Normal and Tumor-bearing Donors

The survival time of first-set allografts from tumor-bearing donors was only slightly longer than that of grafts from normal mice (Table 1), but the difference was statistically significant (P < 0.02).

Second-set skin allografts from normal donors showed a similar accelerated rejection in animals having received first-set grafts from either normal or tumor-bearing donors (Table 1, Groups 4 and 6). Since there was no significant difference between the survival of second-set grafts in these two groups, it seems that the first-set graft derived from tumor-bearing animals had an immunizing capacity similar to that of grafts from normal donors.

In mice immunized by first-set allografts from normal donors, the survival of second-set grafts from tumor-bearing animals was significantly greater than that of second-set grafts from normal donors (Table 1, Groups 3 and 4) (P < 0.001). A significantly prolonged survival of second-set skin grafts from tumor-bearing donors, as compared to second-set grafts from normal donors, was also evident in recipients who had received first-set grafts from tumor-bearing donors (Table 1, Groups 5 and 6) (P < 0.005).

Serologic Absorption Experiments

The intraperitoneal growth of the Ehrlich ascites tumor produced no detectable change in the capacity of various murine tissues to absorb H-2 isoagglutinins (Table 2). The antigenicity of the tissues examined in the present study was similar to that previously found in normal mice (3, 10).

Discussion

It was found previously that, when skin from normal and from cancer patients was grafted simultaneously to other cancer patients, the grafts derived from cancer patients showed a prolonged survival (9). Amos et al. (2) noted a prolonged survival of skin allografts derived from cancer patients in normal recipients. Similar to the findings in man, skin grafts from mice bearing various types of tumors had a prolonged survival in allogeneic hosts (4, 7, 8).

Several factors could account for the prolonged survival of grafts from tumor-bearing donors: (a) decreased antigenicity, i.e., decreased concentration of antigens in the tissue grafted; (b) decreased immunogenicity, i.e., deficient capacity of the grafts to elicit an immune response; and (c) increased resistance of the grafts to the allograft reaction of the host.

In the present study, serologic absorption experiments failed to show any decrease of the H-2 isoantigenicity in various tissues of tumor-bearing mice. Similarly, cytotoxicity tests did not reveal any decrease in the concentration of the H-2 isoantigenicity of the spleen and thymus cells of tumor-bearing mice (12). Since in the present study skin of tumor-bearing animals was not analyzed serologically, it could be argued that, in contrast to other tissues, skin might have undergone antigenic changes. However, this possibility seems unlikely, in view of the fact that the skin of tumor-bearing mice showed no evidence of decreased immunogenicity. Skin allografts from tumor-bearing animals curtailed the survival of second-set allografts to the same extent as skin grafts from normal mice.

There was thus no evidence for decreased antigenicity or for decreased immunogenicity of tissues of tumor-bearing animals. There was, however, evidence that skin grafts obtained from tumor-bearing donors had an increased resistance to the allograft reaction. This was clearly shown by the prolonged survival of second-set grafts from tumor-bearing donors in hosts preimmunized by first-set allografts from either normal or tumor-bearing donors.

Various physiologic alterations in the tumor-bearing organism could render its tissues less susceptible to the allograft reaction (cf. 11). The increased secretion of corticosteroid hormones in tumor-bearing hosts could lead to stabilization of cell and lyso-
E. Robinson, V. K. Golakai, and M. Schlesinger

some membranes (13) and thus increase the resistance of the tissues. Alternatively, the anemia of tumor-bearing organisms could lead to acclimatization of the tissues to hypoxia, and render them less susceptible to the adverse effects of the allograft reaction (cf. 5).

ACKNOWLEDGMENTS

We are grateful to Miss Lea Keren for her skilled technical assistance. One of us (V. K. G.) acknowledges support by a W.H.O. Fellowship.

REFERENCES

Studies on the Mechanism of Prolonged Survival of Allografts from Tumor-bearing Donors

E. Robinson, V. K. Golakai and M. Schlesinger