Modification of the Immune Response of Mice to Skin Homografts and Heterografts by Ehrlich Ascites Carcinoma*

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SUMMARY

The immunological response of mice given implants of Ehrlich ascites carcinoma was modified as evidenced by the failure of ascitic mice to reject primary or secondary skin homografts or rat skin heterografts. The site of tumor implantation influenced its effect on the host, since subcutaneously implanted Ehrlich ascites carcinoma did not alter the immune response.

The presence of the tumor in the ascites form resulted in the prolongation of survival of primary homografts by preventing the initial response of the host to the graft antigens. This inhibition of the initial interaction between the host and the primary homograft antigens was evidenced by the observation that lymph nodes obtained from mice following simultaneous implantation of tumor in the ascites form, and a primary homograft, had not been sensitized by the primary homograft antigens, since such lymph nodes were not capable of passively eliciting a secondary homograft response when transplanted into normal isologous hosts. Secondary homograft or heterograft survival time on ascitic hosts was prolonged as a result of an inhibition of the reaction between the previously sensitized host and the graft antigens. Possible mechanisms by which the ascitic tumor caused these inhibitions are discussed.

During studies of the antigenic structure of several normal and neoplastic mouse cell lines in continuous in vitro cultures, in which the response of a host to primary and secondary homografts was employed to detect the presence or absence of common antigens (6), it was apparent that factors independent of differences in antigenic structure were influencing the survival of skin homografts. It was observed that, following the development of an ascites tumor, the immune response of mice was modified so that skin homografts were not rejected. Brief preliminary reports of these observations, and of experiments with heterografts to ascitic hosts, have been presented elsewhere (13–15). The purpose of the present report is to describe further studies of the modified immune response of ascitic hosts to primary and secondary homografts and heterografts.

MATERIALS AND METHODS

Animals.—Mice of the A/J, C3H, C57BL/6, and DBA/2 inbred strains obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, and Swiss white mice of the CFW strain, obtained from Carworth Farms, New City, New York, were used. Male mice weighing 20–25 gm. were employed in all experiments. Inbred Lewis strain rats were obtained from Microbiological Associates, Bethesda, Maryland.

Skin grafting.—The graft technic employed was a modification of the procedure as outlined by Billingham and Medawar (3). Skin grafts were obtained from the ear of the donor and free-grafted to a bed on the dorsum of the recipient. Two or three grafts were obtained from the ear of a mouse and four to six from the ear of a rat. The operative site was covered by gauze and adhesive tape dressing. The dressing was removed after 7 days and the graft inspected daily thereafter. Rejection was determined by visual inspection of the graft in situ, according to the criteria used by Billingham and Silvers (4).

Primary and secondary grafts.—Two kinds of normal primary homografts were employed—mouse ear skin, grafted as described above, and a suspension of mouse liver and/or spleen cells obtained by gentle grinding of the minced tissue in a Potter-Elvehjem tissue grinder. The cells were suspended in Eagle's basal medium (5) without serum, and the inoculum used in all experiments was 1–2 × 10^6 cells contained in 0.1 ml., as determined by direct hemocytometer count. Primary rat heterografts were prepared in a similar manner. All secondary grafts were ear skin, grafted as previously described.

Ehrlich ascites carcinoma.—The strain of Ehrlich ascites carcinoma used in these experiments has been maintained in this laboratory for several years in CFW mice by intra-
peritoneal passage. A subline was established in C3H mice and maintained for several transplant generations prior to its use in those experiments in which C3H mice were used as graft recipients. The inoculum used in all experiments was \(10^6\) cells contained in 0.1 ml. of Eagle's basal medium without serum, as determined by direct hemocytometer count.

**Lymph node transplantation.**—Eight inguinal lymph nodes removed aseptically from four male C3H mice under light Nembutal anesthesia were minced in 1.0 ml. of Eagle's basal medium without serum and injected intraperitoneally into each recipient male C3H mouse. Lymph node donors that had been given injections of Ehrlich ascites carcinoma were retained for observation after removal of their nodes. These donor animals all died from the tumor.

**Cell-free ascitic fluid.**—Ascitic fluid was obtained from several C3H mice by aseptic aspiration, pooled, and allowed to clot. The clotted fluid was homogenized for 1 minute in a sterile Waring Blendor, centrifuged, and the clear supernatant removed. The supernatant was frozen and thawed 3 times, to rupture any remaining cells, and stored at 4°C. until used.

### RESULTS

**Effect of Ehrlich ascites carcinoma on primary grafts.**—The experiments summarized in Table 1 indicate that mice given injections intraperitoneally of Ehrlich ascites carcinoma on the same day they received a skin homograft, or 5 days before a rat skin heterograft, tolerated these grafts for periods of time significantly longer than nonascitic control mice. Since all tumor-bearing hosts died of their tumor with their grafts in good condition, mean survival times of grafts could not be determined. These donor animals all died from the tumor.

<table>
<thead>
<tr>
<th>Host strain</th>
<th>Skin graft donor strain</th>
<th>No. hosts</th>
<th>Graft survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>DBA/2</td>
<td>8</td>
<td>15-22</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>DBA/2</td>
<td>8</td>
<td>9.6 ± 0.3</td>
</tr>
<tr>
<td>C3H</td>
<td>A/J</td>
<td>7†</td>
<td>13-16</td>
</tr>
<tr>
<td>C3H</td>
<td>A/J</td>
<td>16</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td>CFW</td>
<td>Lewis rat</td>
<td>7§</td>
<td>9-12</td>
</tr>
<tr>
<td>CFW</td>
<td>Lewis rat</td>
<td>10</td>
<td>7.9 ± 0.8</td>
</tr>
</tbody>
</table>

* Numbers indicate range of time in which graft was observed to be in good condition prior to death of host. Since all tumor-bearing hosts died with graft in good condition, mean survival times of grafts could not be determined.
† Mean survival time ± S.D. is given for grafts on normal hosts.
‡ Ehrlich ascites carcinoma injected intraperitoneally on day of grafting.
§ Ehrlich ascites carcinoma injected intraperitoneally 5 days before grafting.

### EFFECTS PROLONGATION OF SURVIVAL TIME OF SECONDARY SKIN HOMOGRAFTS AND HETEROGRAFTS ON CFW MICE BEARING EHRILICH ASCITES CARCINOMA*

<table>
<thead>
<tr>
<th>Skin graft donor strain</th>
<th>No. of hosts</th>
<th>Graft survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetic hosts†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal hosts†</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>14</td>
<td>9-14†</td>
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<tr>
<td>C57BL/6</td>
<td>24</td>
<td>8.0§</td>
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<tr>
<td>Lewis rat</td>
<td>7</td>
<td>9-13§</td>
</tr>
<tr>
<td>Lewis rat</td>
<td>10</td>
<td>7.0§</td>
</tr>
</tbody>
</table>

* Primary graft 15-20 days before secondary graft.
† Numbers indicate range of time in which graft was observed to be in good condition prior to death of host. Since all tumor-bearing hosts died with graft in good condition, mean survival times of grafts could not be determined.
‡ Ehrlich ascites carcinoma injected intraperitoneally 5 days before second grafting.
§ Ehrlich ascites carcinoma injected intraperitoneally on day of second grafting.
* All grafts in this group rejected on same day.
† All grafts in this group rejected on or before day of first examination.

**Effect of Ehrlich ascites carcinoma on survival of secondary grafts.**—The data summarized in Table 2 indicate that mice actively immunized by recent exposure to primary homografts are incapable of rejecting a secondary homograft when bearing an Ehrlich ascites tumor. Similar results were obtained with secondary rat skin homografts. It is evident that the usually vigorous rejection of a heterograft by a sensitized host was effectively blocked, even when the Ehrlich ascites cells were injected on the same day that the second graft was made. Since all tumor-bearing hosts died of their tumor with their grafts in good condition, the maximum survival time of these grafts was determined by the lifetime of the hosts, not by graft rejection.

**Effect of sequence and site of implantation of Ehrlich carcinoma and first homograft on response of hosts to homografts.**—Table 3 summarizes the effect of the time and site of implantation of tumor and first homograft on the response of mice to additional homografts. Groups A1 and A2 are controls which establish that the sites and doses of primary homografts employed were capable of sensitizing the host. Groups B1 and B2 illustrate the failure of subcutaneously implanted Ehrlich cells to block the response of CFW mice to primary homografts and further indicate that the tumor in this form did not prevent the host from rejecting a second homograft at a typically accelerated rate. In contrast, Groups C1 and C2 illustrate that, when implanted intraperitoneally, Ehrlich ascites cells prevented the rejection of secondary skin homografts, irrespective of the site of the first graft of normal tissue. However, since there was no direct means of determining whether the host responded to its primary graft, it is not clear from these data whether the Ehrlich ascites cells prevented an initial host response to the primary graft, in which case the second graft would be ex-
EFFECT OF SEQUENCE AND SITE OF IMPLANTATION OF EHRlich CARCINOMA AND FIRST HOMOGRAFT ON THE RESPONSE OF CFW Mice TO SECOND AND THIRD SKIN HOMOGRAFTS

As evidenced by the experiments summarized in Group D, hosts proved to have been sensitized by primary homografts of homogenized tissue. These data do not conclusively resolve the original question.

Inhibition of the primary immune response.—The experiments outlined in Chart 1 and Table 4 were undertaken to determine the effect of Ehrlich ascites carcinoma on the sequence of events during the response of a host to a primary homograft but in the absence of further prolonged influence of the ascites on the immunological response. C3H hosts received an intraperitoneal implant of Ehrlich ascites cells and a primary homograft of A/J liver and spleen cells, either intraperitoneally or subcutaneously. After an interval of 4 days, lymph nodes from these mice were transplanted into normal isologous C3H hosts. The immunological state of the transplanted lymph nodes was then determined by observing the rejection time of A/J skin homografts by the lymph node recipients. An accelerated rejection of a primary skin homograft by the lymph node recipients would indicate that an immunological response had been elicited in the lymph node donors, whereas rejection of the homograft at the usual slow rate would mean that no passive immunization had occurred and that, therefore, the lymph node donors had not responded to the liver and spleen homografts. As indicated in Table 4, the rejection time of an A/J skin homograft by recipients of lymph nodes from such homografted, ascitic donors was typical of a primary homograft, indicating that the initial immune response of the lymph node donors to a primary homograft had been prevented by the Ehrlich ascites carcinoma, since lymph nodes taken from these animals were not capable of passively sensitizing an isologous host. It should be noted that these lymph node recipients actively rejected skin homografts in a manner characteristic of a primary homograft rejection. None of the lymph node recipients considered here developed ascites tumors during a period of 30 days post-transplantation. The occasional node recipients that did develop ascites tumors retained their grafts until the death of the hosts, as would be expected from the data summarized in Tables 1, 2, and 3 (Group C).

Experiments similar to those described by Billingham, Brent, and Medawar (2), in which implantation of Ehrlich ascites cells was omitted, established that the experimental plan outlined in Chart 1 was suitable for demonstrating passive sensitization with the test system employed.

Effect of nonspecific stress and sterile ascites on the homograft response.—The effect of cell-free ascitic fluid and the nonspecific effects of stress associated with either fluid in the peritoneal cavity or inflammation resulting from the injection of foreign material have been studied extensively. The intraperitoneal injection of an irritant, such as glycerol (0.25 and 0.50 mg. daily, beginning 3 weeks before test-grafting), physiological saline, or Earle's salts solution, was not well tolerated. None of the lymph node recipients considered here developed ascites tumors during a period of 30 days post-transplantation. The occasional node recipients that did develop ascites tumors retained their grafts until the death of the hosts, as would be expected from the data summarized in Tables 1, 2, and 3 (Group C).
and secondary homografts and heterografts is modified effect on the rejection of homografts. Produced no toxic effects in the recipients and had no fluid, containing subcellular elements but no intact cells, extrave, although not well, rejected primary or secondary examination of the grafts. Those animals that did sur

Skin homografts.

The daily administration of 0.1 ml. of mouse ascites carcinoma. Lymph node recipient exhibits typical primary response to A/J homograft—i.e., rejection in 14.0 ± 1.0 days. The immunological response of mice to both primary and secondary grafts, however, must be due to another effect of the tumor. Since the host has already responded to a primary graft, as evidenced by its rejection, the only way in which the ascites tumor can effect prolongation of the survival of the second graft is by blocking the reaction between the sensitized host and the graft antigens. Recent reports (10, 18) of the demonstration of the importance of humoral antibodies in the homograft reaction suggests the possibility that the prolonged survival of secondary grafts on ascitic hosts could have resulted from an "inactivation" or "blocking" of these antibodies, rather than from a direct effect (e.g., immunological paralysis) of the tumor on the sensitized cells of the host. Experiments involving secondary graft rejection, designed to determine whether the antibody or the antibody-producing cell is the primary locus of the inhibitory effect of the ascites tumor, are in progress.

It has been reported that mice with advanced mammary carcinoma will tolerate skin homografts (12) and that humans with various forms of advanced cancer will tolerate skin homografts (9) and heterografts (16). The explanation suggested for such observations was that there had been a depression of the homograft reaction following extensive tumor development and the resulting general debilitation of the graft recipients. Inhibition of the immune response observed in the present experiments, however, does not appear to be the result of such an effect of the tumor on the host. In the case of primary grafts, results of experiments (Table 4, Chart 1) in which lymph nodes were removed from ascites-bearing hosts only 4 days after implantation of the tumor cells indicate that the tumor exerted its inhibitory effect on the host’s immune response mechanism during that time. Although neither extensive tumor growth nor debilitation of the lymph node donors occurred during that brief period, it is evident that the tumor-bearing hosts had not responded to the antigens of their primary grafts.

The relative speed with which the ascites tumor affected secondary as well as primary grafts also indicates that the prolongation of graft survival was not dependent upon suppression of the immune response subsequent to exten-
sive tumor development but was due to an inhibition of a previously sensitized host defense mechanism. It is possible that this inhibition may be effected by an interaction between the tumor and humoral antibodies as previously mentioned (10, 18). There are, however, several reports in the literature which indicate that antibodies induced in mice against several types of antigen retained their specific immunological properties (i.e., were not inactivated) when the mice were subsequently made ascitic by one of several agents. When crystallized egg albumin or bovine serum albumin was used as antigen and ascites was induced by intraperitoneal injection of Freund’s adjuvant, the presence of precipitating and hemagglutinating antibodies could be demonstrated in both the serum and in the ascitic fluid (17). Viral antibodies also have been demonstrated in ascitic fluid in other studies in which several viruses and ascites-inducing agents were employed. In each instance, the mice were first immunized by injection of the viral antigen, and the preformed antibody was demonstrated in both the serum and in the ascitic fluid, as well as in the serum. The ascites-inducing agents used included Freund’s adjuvants (11), Sarcoma 180 (7, 8), and Ehrlich ascites carcinoma (1). It is apparent from these reports that preformed antibodies against some types of antigens are not adversely affected when the host becomes ascitic, even when the ascites-inducing agent is Ehrlich carcinoma.

It would appear that inhibition of the homograft reaction by Ehrlich ascites carcinoma may be unique and specific for tissue antigens and antibodies. Elucidation of the precise biological mechanisms concerned with this inhibition may prove to be of significance, not only with respect to the effect of a tumor upon its host but also to the problem of abrogation of the homograft reaction.

REFERENCES

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Robert E. McCarthy

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