Survival of Skin Homografts in Methylcholanthrene-treated Mice and in Mice with Spontaneous Mammary Cancers*

OWE E. A. LINDER

(Institute for Tumor Biology, Karolinska Institutet Medical School, Stockholm, Sweden)

SUMMARY

The effect of methylcholanthrene (MC) on the survival of skin homografts was studied in various donor/host combinations. In systems where there was a strong antigenic difference involving the histocompatibility-2 (H-2) locus, MC did not influence the survival of the skin grafts. In weaker (non-H-2) antigenic systems the survival of the skin was prolonged in animals grafted shortly before, at the same time as, or shortly after the appearance of MC-induced sarcomas. In animals grafted during the earlier part of the latent period of tumor development, graft survival was not affected.

The effect of the spontaneous mammary cancers was more variable, and most of the tumor-bearing animals did not show a depressed homograft response; the others, being in a highly advanced stage of the disease, showed moderate prolongation of graft survival.

It has been shown that sarcomas induced by methylcholanthrene (MC) can be antigenic in genetically compatible, isologous hosts (1, 6, 15, 16) and even in their own primary, autochthonous hosts (10). It seems puzzling that a tumor can be shown, in appropriate test systems, to be antigenic for the organism in which it originally arose; and yet the host was unable to prevent the development of the tumor in the first place. To explain this, Prehn and Main (15) infer that a depression of antibody production may occur during the MC-induced carcinogenesis. The findings of Malmgren et al. (13) and of Davidsohn et al. (5) were quoted to support this assumption. These workers showed that all carcinogens they tested, including MC, produced a significant depression of humoral antibody formation, although the doses they used were well in excess of the amounts required for carcinogenic action.

Since host response against antigenically foreign grafts of living cells fall into the general category of homograft reactions which are akin to delayed hypersensitivity (4, 11) and are mediated by cellular rather than humoral factors, it appeared to be of interest to test the possible effect of MC on a bona fide homograft reaction. This was done in the present work by using the sensitive method of skin grafting and varying the time intervals between MC administration and transplantation. A similar approach has been used by Rubin (17), who reported a marked increase of graft survival in methylcholanthrene-pretreated DBA recipients given transplants of (DBA × C3H)F1 skin.

In a preliminary attempt to distinguish between the action of MC per se on a homograft reaction and the secondary consequences following upon the presence of a neoplastic growth in an animal, mice bearing spontaneous mammary cancers were also included as controls.

MATERIALS AND METHODS

Mice.—Mice of the CBA/Ki, DBA/2/Ki, C57BL/Ki, C3H/Ki, and C3H/Ki strains were used, together with F1 hybrids, obtained by mating females of the CBA/Ki and of the DBA/2/Ki strains with C3H/Ki males. The C3H strain shows a high incidence of spontaneous mammary car-
cinomas in all females but not in the males (3). The C3Hf mice were obtained from C3H by cesarean section of pregnant females and by nursing of the baby mice by foster-mothers from a factor-free strain (C57BL) which has a low mammary cancer incidence. Donors and recipients were of the same sex; they were 3-5 months old at the beginning of the experiments and fully compatible with respect to intrastrain skin isografts (9, 12).

Methylcholanthrene treatment.—The methylcholanthrene-treated groups received 0.1 ml. of a 0.5 per cent MC solution in trioctanoin, injected intramuscularly into the right hind leg. The controls were given injections of 0.1 ml. trioctanoin alone.

Skin grafting.—Skin fragments about 1 sq. cm. in area and in the resting stage of the hair cycle were grafted according to the technic recommended by Billingham and Medawar (2). On the 10th day after operation the bandages were removed, and the grafts were inspected once daily. The day when only scar tissue was left at the transplantation site (as a rule this occurred 2-3 days after the first ulceration of the graft) was taken as the day of rejection.

RESULTS

Tables 1 and 2 show the results. In the MC experiments the intervals between carcinogen injection and skin grafting were varied as follows: C3H to CBA: 20, 27, 37, 48, 62, and 80 days; (C3H×CBA)F1 to CBA: 30, 35, 56, 72, and 78 days; (C3H×DBA)F1 to DBA: 20, 50, 60, and 75 days; C57BL to DBA: 40, 80, and 100 days. Each group consisted of four to eight animals. Palpable tumors were registered in all recipients within 80-116 days. No tumor regression was observed.

It was a striking finding in all combinations that, provided no tumor was present, the skin homografts were rejected as promptly in the recipients pretreated with MC at different times prior to skin grafting as in the untreated mice (Table 1). Animals bearing progressively growing primary tumors showed a more variable behavior. In the combination C57BL to DBA, which involved a strong H-2 difference, no graft prolongation could be obtained, whereas an increased survival was apparent in 23 out of 23 cases in the other combinations. These latter mice died frequently from their tumors before any grossly detectable signs of a homograft reaction could be seen.

In a similar series of experiments (Table 2) C3H mice bearing spontaneous mammary carcinomas were grafted with CBA skin. The controls consisted of mice which did not bear a detectable tumor. The tumor-bearing mice gave somewhat

<table>
<thead>
<tr>
<th>Donor-recipient combination</th>
<th>C3H→CBA</th>
<th>(C3H×CBA)F1→CBA</th>
<th>(C3H×DBA)F1→DBA</th>
<th>C57BL→DBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated mice</td>
<td>15.8±0.3</td>
<td>20.5±0.3</td>
<td>16.1±0.3</td>
<td>11.1±0.2</td>
</tr>
<tr>
<td>Pretreated mice. Tumors appeared after the skin grafts were rejected</td>
<td>15.7±0.2</td>
<td>19.0±0.4</td>
<td>16.2±0.3</td>
<td>11.0±0.1</td>
</tr>
</tbody>
</table>

* Individual data are given for the tumor-bearing mice, while the mean survival times ± S.E. are indicated for all other groups.
† Mice died on the day indicated + with healthy skin grafts.

Table 2

<table>
<thead>
<tr>
<th>Recipients*</th>
<th>Mean survival times±S.E.</th>
<th>Range</th>
<th>No. mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3Hf male</td>
<td>15.8±0.4</td>
<td>14-18</td>
<td>10</td>
</tr>
<tr>
<td>C3Hf female</td>
<td>16.0±0.3</td>
<td>14-17</td>
<td>9</td>
</tr>
<tr>
<td>C57BL male</td>
<td>16.3±0.5</td>
<td>14-19</td>
<td>10</td>
</tr>
<tr>
<td>C57BL female†</td>
<td>16.1±0.5</td>
<td>14-17</td>
<td>8</td>
</tr>
<tr>
<td>C57BL female‡</td>
<td>17.9±0.9</td>
<td>14-24</td>
<td>10</td>
</tr>
</tbody>
</table>

* The CBA donors were always the same sex as the recipients.
† No tumor appeared during the time of the experiment.
‡ The recipients were carrying mammary carcinomas of different sizes at the time of skin grafting.

Four animals died from the tumors 25, 37, 27, and 35 days after skin grafting and were at the time still carrying healthy grafts.
ambiguous results. In the majority of cases there was no clear prolongation as compared with that in the controls, whereas six out of fourteen mice showed some prolongation, which, however, was not so pronounced as that in the MC groups. In particular, animals in a bad general condition and bearing advanced tumors showed a decreased reactivity against foreign tissue.

DISCUSSION

The dose of MC used in these experiments, the solvent, and the mode of administration were the same as in the study that has demonstrated the antigenicity of established, MC-induced sarcomas in their own primary hosts. Nevertheless, MC-treated mice showed no unequivocal depression of the homograft reaction in the present work, as far as the major part of the latency period preceding the appearance of palpable tumors is concerned. In the donor-host system involving a difference at the "strong" histocompatibility-2 (H-2) locus, there was no prolongation of skin survival time at all, not even in mice bearing growing sarcomas. On the other hand, MC-treated recipients which had tumors at the time of skin grafting or which developed sarcomas shortly thereafter displayed a clear prolongation of skin graft survival, provided that the antigenic donor-host difference involved was of the weaker (non-H-2) type. Two alternative explanations may be considered. The first possibility is a breakdown of the antibody-forming capacity occurring in parallel with, and causally related to, the appearance of antigenic tumors. On this assumption, MC would depress the host response, but only after a latency period of several months. Once this occurs, neoplastic and antigenic cell clones, already present in the host either as a result of MC action or, under more extreme hypothesis, as a result of spontaneous cellular changes, would be able to grow progressively into frank malignancies. The other explanation would interpret the prolonged graft survival in the MC-treated, tumor-bearing hosts not as a cause but as a consequence of tumor development. It has been shown that cancerous patients tolerate the survival of homografted skin (7, 8, 14) and homografted tumors (18, 19) better than do healthy controls. In view of the varying nature of the human tumors involved and the probability that they were not related to the action of polycyclic hydrocarbons, the interpretation of the depressed homograft reaction as a consequence of tumor development appears to be more plausible. The experiments involving skin grafting to mice bearing spontaneous mammary cancers which showed some prolongation of skin survival (but only in animals in a highly advanced stage of the disease and of a less pronounced extent than in the MC groups) would tend to weaken this conclusion somewhat, although more extensive experiments are desirable on this point. Another suitable experiment to clarify this issue, suggested to us by Dr. R. T. Frehn, would be to study the status of the immune mechanism in the presence of a serially transplanted MC-free but originally MC-induced tumor. Such experiments are now being started at our laboratory.

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REFERENCES

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Owe E. A. Linder


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