Tumor-specific Immunity to Transplanted Dibenzo[a,h]-anthracene-induced Sarcomas*

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SUMMARY

Subcutaneous fibrosarcomas were induced in inbred mice by the implantation of glycerol-moistened crystals of dibenzo[a,h]anthracene. Eleven dibenzo[a,h]anthracene-induced sarcomas were investigated. The data demonstrate that the temporary growth of these tumors in other mice of the same inbred strain often produced a tumor-specific immunity to subsequent challenge inoculations of the same tumor. Normal tissues from the very mouse in which an antigenic tumor arose failed either to induce the immunity or to respond to it. The results are similar to previous findings with 3-methylcholanthrene-induced fibrosarcomas and therefore suggest that tumor-specific antigenicity may be a general property of fibrosarcomas induced by the aromatic hydrocarbons in mice.

Past work from this and other laboratories has demonstrated the fact that most sarcomas produced in inbred mice by 3-methylcholanthrene are antigenic within other mice of the same highly inbred strain (2, 5, 8, 9). These tumors are also antigenic within the mouse of origin (5). The several spontaneous tumors which have been examined have not shared this property (1, 2, 5, 8). The question, therefore, arose whether or not other chemical cancerogens likewise would produce tumors possessing tumor-specific antigenicity. Herein are reported the results obtained when subcutaneous sarcomas induced by dibenzo[a,h]anthracene were tested.

MATERIALS AND METHODS

All the animals employed were 2–4 months of age and were of the C3H/He or BALB/cAn strains. They had been born and raised in this laboratory and were in no case more than four generations removed from common ancestors. They were maintained in plastic cages on a diet of Purina Laboratory Chow and water ad libitum. Seven to nine animals were kept in each cage. Both sexes were employed, but in any single experiment the only sex utilized was that of the particular tumor donor.

The general plan of the experiments was similar to that previously carried out with 3-methylcholanthrene-induced fibrosarcomas (8). Tumors were induced in mice of the two inbred strains by the subcutaneous implantation of a few dibenzo[a,h]anthracene crystals which had been moistened with glycerol. With this method of administration the dibenzo[a,h]anthracene appeared to be somewhat less potent than had the 3-methylcholanthrene, the incidence of tumors being lower and the latent period longer. Many of the dibenzo[a,h]anthracene-induced tumors appeared to go through a long phase of gradual progression; often their growth appeared stationary for periods of several weeks or a month. Morphologically, the tumors were similar to those produced by 3-methylcholanthrene and were diagnosed as fibrosarcomas.

When a tumor reached a size of approximately 10 mm. in average diameter, it was excised and transplanted by the usual trocar technic to the dorsal skin of a number of other animals of the same strain and sex as the donor. In six cases the tumor was stored prior to use in a frozen tumor bank (3). A 13-gauge trocar was employed for transplantation. Despite this it was found that, by

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1 The term “antigenic” as used in this paper denotes the property of being able to produce a specific resistance to the growth of tumor implants. This tumor-specific resistance is termed “immunity” without any implication concerning the biologic nature of the process.

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directing the bevel upward beneath the skin of the recipient animal, the fragment of tumor tissue could often be lodged between the panniculus carnosus muscle and the epidermis. The transplants were made as superficially as possible in order to facilitate subsequent excision.

As has been the case with methylcholanthrene-induced tumors, the dibenz[a,h]anthracene-induced sarcomas grew poorly on primary transplantation. Whenever one of the tumor implants in a particular experiment reached a size of approximately 10 mm., the implants were excised under pentobarbital-alcohol anesthesia (7) from unimmunized, but in two series (10 and 11) they were “immunized” with spleen from the very same animal which had originally given rise to the particular tumor. In all cases, the control animals were subjected to sham procedures similar to those employed for the excision of the immunizing tumor transplants in the experimental mice. The control and experimental animals were challenged with tumor in an identical fashion, and inoculations were carefully alternated between the two groups.

The size of the challenge tumors was measured to the nearest millimeter at approximately weekly

### TABLE 1

**GROWTH OF “CHALLENGE” (SECOND INOCULATION) TUMORS**

<table>
<thead>
<tr>
<th>Tumor no.</th>
<th>Duration of growth (days)</th>
<th>Strain</th>
<th>Sex</th>
<th>Immunized group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. mice</td>
<td>No. tumors</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>CSH/He</td>
<td>♀</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>CSH/He</td>
<td>♀</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>BALB/cAn</td>
<td>♂</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>CSH/He</td>
<td>♂</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

* Tumors measuring 5 mm. and over in estimated average diameter.
† These animals had been immunized with spleen from the original tumor donor (see text).
‡ Ten of these immune animals were re-challenged from the original tumor donor with skin grafts, all of which were successful.

All but this one animal. This was done even though some of the implants might not have grown at all. The single remaining tumor-bearing animal was then used as a source of tumor with which to administer by trocar a second, or challenge, tumor inoculum. This was given subcutaneously into the lower abdominal wall. Because of the change of sites there was no possibility of confusing the growths of the second tumor implants with possible recurrences of the excised primary transplants. The second inoculation was in most cases carried out immediately after the excision of the first or immunizing implant; in two cases the challenge was delayed for 3 days.

Control groups of animals were, as far as possible, litter-mates of those employed in the experimental series. The control animals were usually intervals, and an average diameter was recorded for each implant.

In one experiment, a special control procedure was performed. The original mouse in which the tumor (11) had been induced by the cancerogen was kept alive, and subsequently, after evaluation of the challenge inoculations, ten animals of this particular tumor-immune series were re-challenged with small skin grafts obtained from this original tumor donor.

**RESULTS**

Eleven dibenz[a,h]anthracene-induced tumors were studied. The data are presented in Table 1. It can be seen that in most cases the challenge tumors grew distinctly better in the control than in the immunized animals. The immunity pro-
duced by prior immunization with tumor was
marked in five experiments and slight or non-
existent in three. In all of the eleven cases the dif-
ference between the experimental and the control
groups was in the direction of immunity. It is
probable that at least some of the apparently non-
antigenic tumors would have shown immunizing
capacity if the challenge doses of tumor had been
smaller (5). In some cases the immunity was mani-
fested only by slower growth of challenge tumors
in the immune animals, the tumor incidence even-
tually becoming quite comparable in the two
groups. In other cases the tumor incidence in the
two groups remained permanently different.
Most animals “immunized” with tumor itself
were immune, but those control animals which had
been “immunized” with spleen from the original
tumor donor did not show immunity to the chal-
lenge tumor inoculations. Although they were im-
mune to tumor growth, the mice of the tumor-
immunized group which were challenged with
small skin grafts obtained from the original tu-
mor-bearing animal grew these grafts fully as well
as did previously unimmunized animals.

DISCUSSION

For a general discussion of the role of immunol-
ogy in oncology, the reader is referred to the recent
reviews by Southam (10) and by Klein (4).

The data presented here demonstrate that many
(and possibly all) tumors induced by the action of
dibenz[a,h]anthracene are capable of producing an
immunity against subsequent tumor inoculation
within mice of the inbred strain of origin. In this
respect the dibenz[a,h]anthracene-induced sarco-
mas behaved in a manner similar to that previously
demonstrated by fibrosarcomas induced with
3-methylcholanthrene (8). The failure of normal
tissue grafts, from the very same animal in which
the tumor had been originally induced, either to
initiate or respond to the immunity suggests
strongly that the resistance was tumor-specific
and that the antigens in the tumor responsible
for the immunity were not shared with the other
tissues of the mouse. The fact that the various tu-
mors differed in the degree of immunity produced
recalls the fact that in the previous studies with
3-methylcholanthrene-induced tumors, the anti-
genicity was found to be due to a number of dif-
ferent antigens which varied from tumor to tumor
(8). The questions of possible cross-reactions
among the various tumors requires further study
(5, 8).

The failure of Lewis (8) to find immunity in ex-
periments in which the tumors had also been in-
duced with dibenz[a,h]anthracene requires com-
ment. The explanation is not clear but may lie in
one or both of the following factors: the tumors
which she used were specially selected for ability
to grow across a strain barrier; they were therefore
not typical of most dibenz[a,h]anthracene-induced
tumors. They also probably had been transplanted
a number of times before testing, and the previous
work from this laboratory has shown that the anti-
genic property may be lost thereby (8).

The fact that both 3-methylcholanthrene and
dibenz[a,h]anthracene produce tumors with the
capacity to immunize mice of the strain of origin
suggests that this may be a general property of
the aromatic hydrocarbon cancerogens. Whether or
not other types of chemical cancerogens will also
produce antigenic tumors awaits investigation;
nor has it yet been determined that aromatic hy-
drocarbon-induced tumors of morphologic types
other than fibrosarcoma will behave in this fashion.

Previous work which indicated that the anti-
genicity might persist through a number of tumor
transplant generations has been construed to indi-
cate that the cancerogen itself does not constitute
a portion of the antigen, since it would presumably
have been eliminated by dilution (8). The mode of
action of the cancerogen in producing the anti-
genicity remains a most important area for future
investigation.

The results obtained make it almost certain that
these dibenz[a,h]anthracene-induced sarcomas are
antigenic in the animal as well as in the strain of
origin, but this has not been tested directly. Klein
has recently presented direct data in the case of
the methylcholanthrene-induced sarcomas (5).

Data concerning the fibrosarcomas induced by
the aromatic hydrocarbon cancerogens constitute
the only compelling evidence currently available
which demonstrate that a cancer may be antigenic
in its host. The success in this case should offer en-
couragement to the growing belief that the study
of tumor immunology may ultimately provide the
knowledge necessary for cancer control.

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