Host-induced Alterations in Strain Specificity of Sarcoma I in Mice: Effect of Active Immunization of the Host

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In earlier studies we have investigated host alterations by means of both active and passive immunization (4, 5) of the recipient hosts prior to cancer transplantation. Working with tumor antigens, Casey (1, 2) and Snell and co-workers (9) have reported enhancement of the growth of a tumor homograft. These workers, along with others who have observed this phenomenon, attribute the action to one or more enhancing substances (9) contained in the tumor tissue preparation. Our studies, on the other hand, are based on what we believe to be a fundamental immunologic concept: namely, that certain well known immune responses are elicited by injection of concentrated mixtures of multiple tumor antigens and that the stimulation of tumor growth is attributable to these responses in the host rather than to any enhancing action of special substances contained in the injected antigenic mixtures.

Following the technics of Snell and co-workers (9), we found in previous experiments (7, 8) that treatment with cortisone enhanced the rate of growth of the tumor homograft and induced metastatic spread. The primary grafts grew progressively and were followed by widespread intra-thoracic and intra-abdominal metastases. These induced metastatic tumors, when transplanted to normal adult mice of six genetically unrelated inbred strains, grew progressively and were subsequently transplanted for three additional passages in each of the six respective strains (8). In all instances, progressively enlarging solid tumors developed, and the host mice (except for those sacrificed for transplant)1 died. The original Sarcoma I growing in these unrelated host strains exhibited increasing anaplasia2 (10), observed histologically, with each subsequent passage.

The present study represents further investigation of this observed phenomenon.

METHODS AND RESULTS

The tumor used was the mouse Sarcoma I which originated at the University of California in 1947. It was obtained from the Jackson Laboratories in 1951 in its 152d transplant passage and is carried in A/He strain mice in our laboratories.3 The tumor grows in 100 per cent of A/He mice and F1 hybrids, both male and female, and is palpable in 2–3 days. Death occurs within 3 weeks. The tumor is composed of solid, dense sheets of spindle-shaped and polyhedral tumor cells, the latter predominating. Mitotic figures are numerous, and stroma is scant.

The recipient mouse strain was the C57BL/6Jax, which is unrelated to the A/He strain and in which a live Sarcoma I graft rarely, if ever, survives and grows progressively.

Twenty C57BL/6Jax mice were actively immunized by three injections of a homogenate prepared from lyophilized (nonviable) Sarcoma I tumor tissue obtained from A/He mice. Three injections of 15 mg/dose of lyophilized tissue, reconstituted in 0.5 ml. sterile 0.85 per cent NaCl, were given intraperitoneally at 5-day intervals. The viability of the Sarcoma I homogenate used for immunization of the C57BL/6Jax mice was tested by injection into twenty normal and twenty cortisone-treated A/He mice. In no instance did a tumor arise in these A/He control mice during 6 months of subsequent observation. Fifteen days after the last injection of the above-mentioned homogenate into the twenty A/He mice, a tumor was injected into twenty normal and twenty cortisone-treated A/He mice.

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1 Whenever reference is made to all mice of a passage dying with tumor, excepted are those mice sacrificed for serial transplants of the tumor.

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live Sarcoma I implant from an A/He donor mouse was implanted subcutaneously by trocar in the suprascapular region of each of the C57BL/6Jax recipients, under surgically aseptic conditions. At the time of tumor grafting, a pooled venous blood sample was obtained from the C57BL/6Jax mice, and the serum was tested for Sarcoma I antibody content by complement fixation (6), with the Sarcoma I tissue homogenate used for immunization of the mice as antigen. The antiserum titer was found to be 1:320.

The tumor grew progressively in all twenty of the C57BL/6Jax mice given injections of lyophilized whole Sarcoma I antigen. It became palpable between 8 and 11 days and grew progressively thereafter until the death of the animals—associated with unusually large tumor formation—occurred, between the 3d and 4th week following transplant.

Twenty untreated control C57BL/6Jax mice and twenty A/He mice received grafts, simultaneously, of the same viable minced tumor homogenate used in the case of the experimental group of C57BL/6Jax mice. There was progressive tumor growth followed by death in all twenty of the untreated A/He mice, and prompt regression or complete failure of the tumor to grow in all twenty of the untreated C57BL/6Jax mice.

On the 16th day following implantation in the immunized C57BL/6Jax mice, four mice were sacrificed. The tumor from each mouse was removed separately under aseptic conditions, minced and transplanted to normal mice of the following strains: C3H, C57BL/6Jax, and BALB/c mice. Each of the four tumors used was handled separately, and each was implanted into one-fourth of the recipients of each respective strain. In subsequent passages, in each instance, one to three donor mice were sacrificed, the tumors handled separately, and each was implanted into separate subgroups of the recipients. The fate of each donor tumor was determined separately. In each of the

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CHART 1.—Host passages of Sarcoma I in unrelated strains of normal adult mice

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Starting with the twelfth passage in the C3H strain and the seventh passage in the BALB/c and C57BL/6Jax strains, three animals were sacrificed from each passage for subsequent transplantation.
has grown progressively in all normal adult C3H mice grafted, and in all instances\(^4\) the mice have died with progressively growing large tumors. In the normal C57BL/6Jax mice, the tumors grew progressively in one mouse and regressed in nine; in the second passage, the tumor regressed in all ten mice. In normal BALB/c mice, the tumor grew progressively on initial passage in all the seven mice injected; in the second passage, it regressed in three and grew progressively in six; in the third passage, it grew progressively in all; and in the fourth passage it regressed in all. We believe that the variable results of successive passages in normal BALB/c mice may have been due to a technical error, since in some of the mice the tumor site was found to be abscessed and a bacterial contamination was found.

At the sixth transplant passage in normal C3H mice, this Sarcoma I subline was again transplanted to normal C57BL/6Jax mice and normal BALB/c mice. The tumor grew progressively in both of these latter strains, and at the present time is in the fifteenth passage in the BALB/c mice and the thirteenth passage in the C57BL/6Jax mice. It is growing progressively in all instances, and the hosts die with large tumors.\(^5\)

Control transplants of Sarcoma I directly from A/He mice to six normal mice of the BALB/c, C57BL/6Jax, and C3H strains, respectively, failed to grow. These control passages were transplanted at the same time, with the same minced tumor as for the subline passages, and, as far as possible, litter-mates were used.

Histologic examination of the tumors removed from each successive transplant in the sacrificed mice confirmed the existence of tumor tissue which is readily identifiable morphologically as Sarcoma I when compared with the original control from the A/He mice. This identity has been maintained in the adapted tumor sublines in C3H, C57BL/6Jax, and BALB/c mice. Certain histologic differences have been observed which might be best stated in general terms as evidencing increasing anaplasia through successive transplants in each of the experimental strains of animals. This anaplasia was associated with a gradual loss of the spindle-type cells. Even where spindling still persisted, the cells tended to be larger and their nuclear detail appeared less regular. Random mitotic figure counts emphasized the fact that mitoses were very much more numerous in the later transplants (ninth to eleventh passage in C3H mice). Similarly, it was apparent that, with the more rapid growth of the tumor, breakdown of the tumor

\(^4\) The Sarcoma I subline in each of the unrelated mouse strains is being carried routinely in each respective strain.


cells with areas of necrosis were more prominent in these later transplants.

The Sarcoma I tumor sublines from the normal C3H, C57BL/6Jax, and BALB/c passages were transplanted to normal adult A/He mice (strain of origin), in addition to being maintained in each respective strain. The transplants were made to ten normal A/He mice per passage from the tenth passage in C57BL/6Jax and the ninth passage in BALB/c, and in both instances were carried for two passages in A/He mice, respectively. The tumor was palpable in 6–8 days in these latter A/He mice, grew progressively, and all the mice died with large tumors. At both the sixth and the fifteenth passages of the tumor subline in C3H mice, the tumor was transplanted to twelve normal A/He mice. The graft was palpable in 5–6 days, grew progressively, and caused death of ten of the twelve A/He mice. Two of these A/He mice were sacrificed for tumor transplantation to twelve normal adult C3H mice. These latter tumor grafts were palpable in 6–9 days, and all the C3H mice died with large tumors.

It is also interesting to note that, with the transplantation of the tumor sublines from the normal C3H, C57BL/6Jax, and BALB/c mice back to the original A/He strain hosts, there was a tendency for the tumor tissue to revert toward the original morphologic pattern of Sarcoma I in the initial transplant. This was seen by more differentiation of the cells to the spindle type, less necrosis, and, in some samples at least, a lower mitotic figure count.

Additional experiments are under way to determine: whether continuous passage in normal A/He mice will result in a reversal to strain specificity of the Sarcoma I graft; and whether the alterations observed in the Sarcoma I tumor will also occur with other transplantable tumors.

The Sarcoma I C3H subline is currently being carried in serial passage as a routine tumor in our colony.\(^6\)

DISCUSSION

These data indicate that the active immunization of an unrelated mouse strain results in increased susceptibility of the immunized host to a tumor graft which does not grow in the untreated host. Moreover, the tumor is histologically changed when growing in the altered host's en-
vironment. In our previous studies, we thought
that the use of cortisone was a necessary precondi-
tion for the suppression of the inflammatory re-
ponses. On the basis of these experiments, it ap-
pears that this is not entirely true. On the other
hand, several workers, who have reported the suc-
cessful transplantation of tumor homografts and
heterografts to animals which had been pretreated
with cortisone, have found in each instance that
the recipient host required cortisone treatment.
Recently, Handler (3) reported the establish-
mnt of a leukemia heterograft in cortisone-treated
hamsters and subsequent serial passage in normal
hamsters. It is apparent that the alteration of a
tumor graft is not limited to Sarcoma I.

These data indicate that the alteration of the
host by injection of whole nonviable tumor ho-
menogates results both in (a) survival of a sub-
sequent live graft and (b) alteration of the graft it-
self. When the relationship between two living bio-
logic systems—cancer graft and host animal—is
altered, both are affected. The alterations may be
temporary and reversible, or permanent. The ob-
servation that the Sarcoma I subline in C3H mice
reverts to its original morphologic character when
passaged through A/He mice (strain of origin) in-
dicates that the morphologic alterations induced
in the homograft specificity of Sarcoma I are re-
versible. It is significant that the tumor sublines
from the normal C3H mice grow progressively
when passaged through normal A/He mice (strain
of origin) and back to normal C3H mice. Appar-
etly, the biologic alterations induced in the strain
specificity of the Sarcoma I graft are retained even
when passaged through the A/He mice. It is ap-
parent that the host's environment influences the
immunogenic, biological, and biochemical char-
acter of the graft and modifies the graft's ability to
elicit the "rejection" response when homografted,
thus permitting survival of the graft in unrelated
mouse strains. This phenomenon of alteration of a
homograft may provide an experimental approach
to the mechanism by which a cancer graft acquires
"invasiveness" in a host. The abnormal cells which
survive continue to adapt to the new environment
and to participate in further altering the host, and
the host-altered mechanisms continue to change,
leading to progressive growth of the tumor cells
now made compatible with the host's environ-
ment.

The concept that an immune state represents
"protection" needs further examination in the
light of the total physiologic complex of the ani-
mal's responses in a homograft situation.

In the above-cited experiments there was no altera-
tion of the several mouse strains used for tu-
mor homografting. Presumably, therefore, their
potential "immune," "foreign body," or "rejec-
tion" responses are unimpaired. The failure to so
respond is due to experimentally induced changes
in the "immunogenic" and other characteristics of
the graft.

These findings may be related to the phenom-
non recently reported by Rhoads (11) that a can-
er homograft in a "normal" (noncancerous) pa-
tient is rejected, while a similar homograft in a
cancer-bearing patient does not elicit the "im-
une" responses and survives.

Current experiments by us are based on the
hypothesis that a cancer graft surviving in a host
whose immunologic responses have been modified
may influence the cells of the graft growing in its
environment by inclusion of host tissue factors in
the cell progeny of the graft, thus imparting an
altered immunogenic complex. If this is true, it
may be a basis for understanding the biologic
mechanisms by which a tumor graft proceeds from
a latent dependent state to a rapidly growing inva-
sive state within the same individual.

SUMMARY

Passage of a mouse tumor graft, Sarcoma I from
A/He mice, through C57BL/6Jax mice, actively
immunized with whole nonviable Sarcoma I tumor
antigen, resulted in a biologic modification of the
tumor graft.

The modification of the tumor was evidenced by
progressive growth of the altered tumor in serial
passages in normal adult mice of three genetically
unrelated inbred strains.

Sarcoma I tissue from immunized C57BL/6Jax
mice showed progressive evidence of anaplasia on
being transplanted from the immunized C57BL/
6Jax mice to normal C3H, C57BL/6Jax, and
BALB/c mice. The tumor tended to revert to its
original morphology when transplanted back to
A/He mice from the subline passages in the three
unrelated mouse strains.

The sarcoma graft established in C3H mice,
when transplanted in serial passage to normal
A/He mice and then back to normal C3H mice,
grew progressively, and all mice of both strains
died with large tumors.

A variant of Sarcoma I, designated as Sarcoma
Mo, has been described.

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Tumor Strain of XYZ Treatment of the Host. Results
Host-induced Alterations in Strain Specificity of Sarcoma I in Mice: Effect of Active Immunization of the Host

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