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# IN IMMUNOLOGICALLY ENHANCED AND RESISTANT MICE

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FROM SARCOMA I

IN "IMMUNOLOGICALLY ENHANCED"

AND RESISTANT MICE


BY


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# INTRODUCTION

Tissue transplants between genetically dissimilar individuals or, more specifically, from one inbred strain to another unrelated inbred strain are known as "homografts". Transplants between genetically uniform animals of the same inbred strain are "isografts". Characteristically, homografts of mature tissue will not grow and isografts will. For exploration of these and related phenomena transplantable tumor grafts, because of the ease with which this tissue can be transplanted, are often the research tool of choice although normal tissue grafts may be preferred in some instances. Highly inbred strains of mice are convenient experimental animals because of the certainty with which genetic factors may be controlled and the relatively low cost per animal. The response of a host to a graft of tumor, or normal tissue, from a genetically dissimilar donor leading to ultimate graft rejection and destruction is known as the "homograft reaction". A host receiving two successive grafts from the same, or genetically similar, foreign hosts rejects the second graft more rapidly than the first. This accelerated reaction to a second graft is termed the "second set response".

Many investigators have found that the "second set response" under certain conditions will not take place. As early as 1907 Flexner and Jobling reported that a second

graft of a transplantable rat sarcoma grew progressively
in a large proportion of rats in which an initial graft
of the same tumor had previously regressed (1). The first
systematic studies on abrogation of the "second set response"
were done by Casey and his co-workers with the Brown-Pearce
tumor in rabbits and transplantable tumors in mice (2-8).
In animals which were pretreated with killed homologous tu-
mor tissues, grafts of tumor were found to grow and metas-
tasize more widely and rapidly. Further, this effect was
proved specific, in that it was only produced by killed
tissue of the tumor to be transplanted.

Kaliss demonstrated the essential unifying phenomenon
in these and similar studies pertaining to the induced sur-
vival of tumor homografts. He found that sera produced
in mice or rabbits by inoculating killed tissues, or using
live transplants, when injected into prospective hosts
prior to grafting the tumor would insure survival of the
grafts (9-11). By zone electrophoresis and salt fraction-
ation this effect was demonstrated to be due to an antibody
in the sera. The active fraction was in the globulin por-
tion, probably the gamma globulin (12). So the paradox
of enhanced growth of tumor grafts following prior treat-
ment intended to heighten resistance was the result of ex-
posure of the graft to antiserum produced against it.

This progressive growth of a homograft of a trans-
plantable mouse tumor in a foreign host strain of mice as

a result of the exposure of the graft to antiserum is
termed "immunological enhancement" (13,14). The anti-
serum may be produced by active immunization with lyo-
philized tumor or liver, kidney or spleen, from the strain
of mouse to which the tumor is native (15-19). Passive
immunization can be produced by injection of anti-tissue
sera (10-12).

Several hypotheses have been advanced as an explana-
tion of the action of antiserum in relation to enhanced
growth of the graft. A few are considered by Kaliss and
Bryant in their recent paper (20). A theory not discussed
by them has been suggested by Snell (21,22) and by Billing-
ham, Brent and Medawar (23). They propose that a "walling
off" of the tumor graft by the antiserum may occur which
will prevent the tumor cells from antigenically stimulat-
ing the host's lymphoid tissue and thus avoid the "cellu-
lar response" thought to be responsible for the destruc-
tion of the graft (24-26). As Snell says, "The sugges-
tion is that antiserum prevents or delays the antigens
of the homograft, or at least effective antigens, from
reaching the regional lymph nodes. The nodes are thus
unable to generate the cellular immune factor which is
the principal agent of graft destruction. Snell has called
this a 'walling off' of the graft, and Billingham et al.
an 'afferent inhibition' " (22).

It was the purpose of this experiment to test the
above hypothesis and, if possible, to shed further light

on the mechanism of "immunological enhancement". If tumor cells could be found in the lymph nodes of immunologically enhanced animals, and particularly if they could be found there within a short time after tumor grafting, then this would be direct proof that the "walling off" or "afferent inhibition" theory is fallacious. If viable tumor cells reach the lymph nodes, no more effective antigenic stimulus could be necessary or even possible. The design of this experiment is similar, with certain technical modifications, to work of Mitchison although he found no viable tumor cells in lymph nodes draining the area of regression of a tumor (25,26).

## MATERIALS AND METHODS

The transplantable mouse tumor used was Sarcoma I, which is indigenous to the inbred A strain of mice and grows in solid form when injected subcutaneously and in ascites form when injected intraperitoneally. The strains of mice used were: A/Ks, A/Jax and C57BL/Ks (a C57BL/6 subline). A/Ks and A/Jax are genetically similar, and grafts of Sarcoma I will grow and kill almost 100 per cent of those inoculated within five weeks. The C57BL/Ks strain is normally resistant to grafts of Sarcoma I. In C57BL/Ks hosts Sarcoma I grafts characteristically grow for about 12 days then rapidly regress. All the mice used in this experiment were 4 to 7 months old except for the A/Jax mice used for confirmation of tumors which were 2 to 3 months old.

There were 4 large groups of mice. Group I was composed of C57BL/Ks mice equally divided as to sex. This group received 4 intraperitoneal injections of lyophilized Sarcoma I over a period of 13 days. Each injection was composed of 4 mgm dry weight of the freeze dried tumor in 0.5 ml physiological saline. Ten days after the last injection of lyophilized Sarcoma I each mouse received in the flank a subcutaneous inoculation of 600,000 cells of Sarcoma I. This inoculum was prepared by taking Sarcoma I ascites fluid and counting the cells by the ordinary white cell count procedure and diluting the ascites fluid with physiological saline to the desired concentration of cells.

Group II was composed of C57BL/Ks mice equally divided as to sex. This group received an intraperitoneal injection of 0.5 ml of pooled anti-Sarcoma I sera. These sera were obtained by bleeding C57BL/Ks mice after primary immunization with a solid inoculum of Sarcoma I or bleeding after subsequent booster shots of 1 to 10 homogenates of frozen Sarcoma I. They were prepared by allowing them to remain for 2 hours at room temperature then spinning down the clot and pipetting off the serum which was frozen at -23°C. until used. On the same day as the injection of the anti-Sarcoma I serum these mice were inoculated subcutaneously with 600,000 cells of Sarcoma I prepared as previously described.

Group III was composed of C57BL/Ks mice and Group IV of A/Ks mice. These were equally divided as to sex.

They received no pretreatment, but were inoculated sub-
cutaneously with 600,000 Sarcoma I cells prepared as de-
scribed above.

Each of these large groups was subdivided into 5
smaller groups composed of 6 males and 6 females each.
One of these subgroups from each large group was sacri-
ficed 3,5,7, and 14 days after inoculation with Sarcoma I
tumor cells. When the animals were sacrificed, the axil-
lary, brachial and inguinal lymph nodes from the side of
implantation of the tumor cells were removed, trimmed free
of fat and minced by chopping the nodes finely with scissors
in a single drop of physiological saline on a sterile glass
slide. This mince was injected through a sterile trocar
intraperitoneally after a small transverse incision in the
abdominal skin had been made with sterile scissors. All
the lymph node inoculations were made into A/Ks mice, a
substrain of the A/Jax strain to which Sarcoma I is indi-
genous. The wound was closed with collodion to minimize
infection or possible leakage of the injected material.
Occasionally lymph nodes were found overgrown by tumor,
and these were discarded and not transferred. Two node
donor mice per host were used, and nodes were transferred
only from males to a male and from females to a female.
The contralateral lymph nodes were treated in the same
way as the ipsilateral nodes just described.

The fifth subgroups from each of the larger groups
were not sacrificed and the growth of the grafts was

followed by periodic palpation until the mice either died
with a progressively growing tumor or remained without an
evident sign of growth for a consecutive period of two
months at which time they were sacrificed and autopsied.
If no evidence of tumor was found, they were classified
as negative.

The A/Ks mice which received the lymph nodes were ob-
served daily for the appearance of ascites or solid tumors.
When a mouse developed ascites, it was sacrificed and 1 cc
of the ascitic fluid was injected subcutaneously into
A/Jax mice. Slide preparations were also made of the
ascitic cells, and an autopsy was performed on each mouse.
The A/Jax mice had to develop solid tumors which grew to
their death before the A/Ks mouse was considered to have
had tumor cells in the lymph nodes it received. All slides
were reviewed and confirmed as showing sheets of tumor cells.
The A/Ks mice which did not develop tumors were observed
for 3 months and then sacrificed and autopsied to search
for any evidence of tumors.

## RESULTS

The results of this experiment are given in Table I.
Twenty four animals developed tumorous ascites. Each
recipient group was composed of 6 males and 6 females
except in 3 groups, as noted in Table I, in which animals
died of unrelated causes. Of the 24 animals which demon-

strated that the nodes they received contained viable tumor
cells, 6 were hosts for node donors in Group I, the "ac-
tively enhanced" group; 3 were hosts for node donors in
Group II, the "passively enhanced" group; 8 were from Group
III, the hosts whose donor's nodes were supposedly resis-
tant; and 7 were from Group IV, the hosts whose donor's
nodes were considered maximally susceptible to invasion
by tumor cells. Viable tumor cells were present in the
contralateral nodes received by 5 animals. As to sex,
12 males and 12 females died with tumorous ascites. In
general it developed earlier in females, the first female
dying 22 days after receiving nodes, and 7 days later
8 females, as opposed to 3 males, had been killed by
the tumor.

TABLE I

Key:

    Column 1: Subgroups
    Column 2: Pretreatment of node doners
    Column 3: Lymph node doner strain
    Column 4: Days from donor tumor inoculation to sacri-
             fice and node transfer
    Column 5: Number of recipient mice dying with tumor /
             number inoculated with nodes
    Column 6: Number of males dying / number of females dying
    Column 7: Mice dying that received contralateral nodes

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| **Group I:** | | | | | | |
| A | Fr. dr. SaI | C57BL/Ks | 3 | 1/12 | 1M/0F | 0 |
| B | " | " | 5 | 5/12 | 3M/2F | 0 |
| C | " | " | 7 | 0/12 | 0 | 0 |
| D | " | " | 14 | 0/11 | 0 | 0 |
| E | " | " | not sacrificed (control group) | | | |
| **Group II:** | | | | | | |
| F | Anti-SaI serum | C57BL/Ks | 3 | 1/12 | 1M/0F | 0 |
| G | " | " | 5 | 0/12 | 0 | 0 |
| H | " | " | 7 | 2/12 | 2M/0F | 0 |
| I | " | " | 14 | 0/12 | 0 | 0 |
| J | " | " | not sacrificed (control group) | | | |
| **Group III:** | | | | | | |
| K | Nothing | C57BL/Ks | 3 | 3/12 | 2M/1F | 0 |
| L | " | " | 5 | 4/12 | 3M/1F | 1F |
| M | " | " | 7 | 1/11 | 1M/0F | 0 |
| N | " | " | 14 | 0/12 | 0 | 0 |
| O | " | " | not sacrificed (control group) | | | |
| **Group IV:** | | | | | | |
| P | Nothing | A/Ks | 3 | 2/12 | 2M/CF | 0 |
| Q | " | " | 5 | 4/12 | 1M/3F | 1M/3F |
| R | " | " | 7 | 0/12 | 0 | 0 |
| S | " | " | 14 | 1/12 | 1M/0F | 0 |
| T | " | " | not sacrificed (control group) | | | |

DISCUSSION

The limits of this tumor-donor-host combination should be defined by the groups composed of A/Ks donors (Group IV, Table I) since this is the strain to which Sarcoma I is native. These maximally susceptible donor animals should have produced the greatest possible number of lymph nodes containing viable tumor cells. As an approximate indication of the sensitivity of the method used in this experiment, Kaliss found that 200 to 500 Sarcoma I cells injected intraperitoneally into A/Ks mice will kill 60 per cent of those injected in 22 to 25 days (Kaliss, unpublished data).

The presence of viable tumor cells in lymph nodes excised from mice in Groups I and II is evidence that tumor grafts in "immunologically enhanced" mice are not "walled off". The finding of metastases as early as 3 days (Subgroups A and F) after tumor inoculation indicates that "afferent inhibition" plays no important role in enhancement. These results make improbable the hypothesis that antiserum prevents or delays effective antigens of the homograft from reaching the regional lymph nodes.

Viable tumor cells being present in the nodes of 8 Group III mice is at variance with the findings of Mitchison who "repeatedly implanted in mice susceptible to the tumor lymph nodes draining the area of regression of a tumor in order to test for the presence of viable tumor

cells" and found "the transferred nodes never gave rise
to tumors" (26). Perhaps an explanation of this discrepancy lies in the different methods used to inject lymph
nodes. Use of a trocar made possible the injection of the
entire inoculum of minced nodes, while enough of the mince
might have remained in the 19 gauge needle and the bored
out nozzle of the 0.25 ml syringe used by Mitchison so
that a critical concentration of tumor cells was not injected. Mitchison does not indicate how many nodes composed an inoculum for a single mouse.

The fact that all of the nodes containing viable tumor cells which were from the contralateral side of the
donor were excised on the fifth day after tumor inoculation suggests that there may be a critical time during
which hematogenous metastases occur. That lymphatic metastases continue to occur is demonstrated by the animals
which received nodes on the seventh and twelfth post inoculation days (Subgroups H, M, and S). These factors probably vary with the host-tumor combination under study.

## SUMMARY

Sarcoma I was inoculated into 4 groups of mice:
(1) actively enhanced mice, (2) passively enhanced mice,
(3) unenhanced mice of a strain normally resistant to
grafts of this tumor and (4) mice of the strain in which
this tumor is propogated. The lymph nodes from these mice
were excised at 3,5,7, and 14 day intervals after implan-
tation and inoculated into mice to which the tumor is in-
digenous. Twenty four animals developed tumors, demon-
strating the lymph nodes they received contained viable
tumor cells. Nine of these mice received nodes from ac-
tively or passively enhanced donors proving that sufficient
antigenic stimulus is not lacking or delayed in immunologi-
cally enhanced animals. This makes it seem unlikely that
"walling off" or "afferent inhibition" can be a valid ex-
planation of the mechanism of action of antisera in this
phenomenon. Finding viable tumor cells in lymph nodes
draining the area of regression of a tumor in eight animals
of Group III conflicts with Mitchison's reported results.

An A/Ks mouse with tumorous ascites.

A C57BL/Ks mouse with a subcutaneous tumor.

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