Transplantation of Polyoma Virus-induced Tumors in Mice*

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SUMMARY

Fifty-two polyoma-induced primary mouse tumors were tested for transplantability in isologous recipients. Thirty-five tumors grew progressively in the subcutaneous tissue, and several lines have been established in serial transplantation. The remaining tumors did not grow out at all upon transfer.

Eleven among the transplantable tumors have been tested by inoculating known numbers of cells to the following three groups of adult isologous recipients: (a) untreated mice, maintained at a separate, polyoma-free colony, (b) mice preimmunized as adults against polyoma virus containing supernatant fluids from infected mouse embryo tissue cultures, and (c) mice pretreated with heavily irradiated cells of the same tumor. There was a clear and consistent difference between groups a and b for ten of eleven tumors, indicating a state of resistance against transplantation of the established polyoma tumors in the virus-immunized group. With the exception of two out of nine experiments, there was no evidence of resistance in group c, pretreated with irradiated tumor cells. Serum of virus-immunized mice had a certain inhibiting effect on viable polyoma tumor cells upon incubation in vitro with one of four tumors. The possible implications of these findings have been discussed.

Polyoma virus has been isolated from parotid tumors, induced in newborn mice given inoculations of cell-free extracts of spontaneous AKR and Ak-n leukemias, by adding whole cells or cell-free extracts of the tumor to tissue cultures of monkey kidney cells or mouse embryo cells (23). The virus could also be obtained from tissue cultures of the leukemic tissue itself (1) and from cultures of monkey kidney cells and mouse embryo cells to which leukemic cells of AKR and Ak-n origin or their cell-free extracts had been added (14, 23). This virus was found capable of inducing a great variety of different tumor types when inoculated into newborn mice (22), hamsters (5), rats (4), or rabbits (3). Upon inoculation into adult animals, however, no tumors were induced with the exception of hamsters (21), and antiviral antibodies could be demonstrated in the serum (2, 15). This fact tends to indicate that established tumor cells are not affected by these antibodies. This is also suggested by the findings of Habel (7), who found no difference in the transplantability of a polyoma-induced hamster sarcoma to untreated and virus-immunized recipients, respectively. The present study reports analogous transplantation experiments in mice which, however, led to entirely different results.

MATERIALS AND METHODS

Mice of the inbred strain A/Sn and its three cosogenic resistant (IR) sublines, A.SW, A.CA, and A.BY developed by Snell (18), were used, as well as F1 hybrids from the outcrosses A × A.SW and A × C3H. The sublines A.SW, A.CA, and A.BY all have an A strain background but carry different alleles at the histocompatibility-2 (H-2) locus. The A/Sn and C3H strains were bred by continuous single-line brother-to-sister matings in our laboratory. To maintain the common genetic background of the IR-sublines and the A/Sn strain as closely identical as possible, the

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interrupted after each three to five generations by a backcrossing to the A strain. F2 mice from these crosses are subsequently challenged with tumors of A/Sn origin, and the survivors are antigenically typed by the hemagglutination test of Gorer and Mikulska (6). Mice showing the H-2 isoantigenic type of the particular IR subline entering the cross are subsequently used to replace the breeding pairs of this line, and their progeny are bred by brother-sister crossings for another three to five generations.

The homozygosity of the strains used has recently been checked by skin and tumor transplantation (12). The inbred A and C3H strains tolerated intrastrain skin grafts for the entire observation period as expected. In contrast, evidence was obtained for the existence of considerable residual heterozygosis, manifested by skin incompatibility, within all three IR-lines. The cause of this was recently traced by Dr. Snell (20) to the use of various A/Sn sublines for the outcrosses during the early history of these strains. It has to be pointed out, however, that the remaining heterozygosis did not influence the fate of intrastrain tumor grafts in any of these lines, even if preimmunized hosts were used (12). The takes obtained upon intrastrain transplantation of primary carcinomas, sarcomas, and lymphomas within the three IR-lines were in no way inferior to the results obtained within standard inbred strains (12). Thus, the heterozygosis remaining in these lines, which does not involve the "strong" H-2 locus, although of great importance in influencing the fate of skin grafts, had no detectable effect on the fate of tumor grafts. It has been previously found that tumors tend to override minor histocompatibility differences, whereas skin is not likely to do so (10).

The breeding of the mice was carried out in a building where no outside animals were allowed to be introduced and where no polyoma inoculations have ever been carried out. In this colony no evidence of polyoma virus contamination could be found upon routine checkups, in the course of these experiments testing the sera of randomly selected mice for the possible presence of antibodies inhibiting polyoma virus hemagglutination.

All mice were bred and kept in this building until used for the present experiments. Before use they were transported to another building about 1½ miles away, where all polyoma experiments were carried out, including the tissue culture work.

Polyoma virus of the strain 1956-11C1 was originally obtained from Drs. Sarah E. Stewart and Bernice Eddy. It was propagated in mouse embryo tissue cultures by the following procedure: Embryos of different inbred mouse strains, 14–18 days old, randomly chosen among the available strains, were minced, treated with 0.25 per cent trypsin, and the liberated cells were suspended in nutrient medium in 6-oz. bottles. The nutrient medium consisted of 0.5 per cent lactalbumin hydrolysate in Earle's salt solution supplemented with 20 per cent horse serum, heat-inactivated at 56°C, for 30 minutes. For maintenance Parker 199, supplemented with trypsin in an amount of 25 mg/100 ml medium and heat-inactivated horse serum to a concentration of 2 per cent, was used; 100 I.U. penicillin and 100 µg streptomycin/ml medium were added both to the initial and to the maintenance medium before use. The cultures were incubated at 37°C, and the nutrient medium was changed once weekly. Four to 5 days after the explantation, when a dense continuous monolayer had formed, the cultures were infected with 1.0 ml. of undiluted tissue culture fluid from virus-infected cultures having a hemagglutinating (HA) titer of 1:128 or 1:256. Fourteen to 21 days after infection the medium was harvested and tested for HA activity. Fluids with titers of 1:256 or more were stored at −20°C and used in this experiment.

Virus-containing fluids were inoculated subcutaneously into newborn mice. The frequency and histological type of the tumors obtained will be reported in detail elsewhere; for the purposes of the present paper it may be sufficient to state that there was good agreement with the results of other workers on other mouse strains (1, 22) with one conspicuous exception: the parotid tumors found so regularly in other strains were extremely rare in our material. Osteogenic sarcoma was the dominating tumor type.

All tumors of adequate size (4 mm. or more in diameter) were transplanted subcutaneously into groups of isologous recipients. These consisted of two to five untreated, 2- to 5-month-old mice and/or one litter of newborn, less than 4-day-old mice (three to eight animals) receiving 300–400 r total-body irradiation immediately before transplantation. In most cases the transplantations were carried out with concentrated tumor cell suspensions, prepared by forcing the tissue through a 60-mesh stainless steel screen and diluting it 1:5 on a volume basis with Ringers solution containing 100 I.U. penicillin and 100 µg streptomycin/ml. With some osteogenic sarcomas and fibrosarcomas, which did not readily lend themselves to the preparation of cell suspensions by

1. S. E. Stewart and B. E. Eddy, personal communication.
this technic small tumor pieces, 2–3 mm. in diam-
eter, were implanted subcutaneously through a
little incision in the skin of the right flank which
was afterwards sutured. All tumors that grew
out after transplantation were carried serially by
cell transplantation, two or more untreated isolo-
gous adult mice being inoculated upon each trans-
fer.

Concentrated cell suspensions from a number
of the transplantable, polyoma-induced tumors
were inoculated into adult isologous mice, preim-
munized against the polyoma virus and untreated
controls. The virus immunization was carried out
by treating 2- to 4-month-old mice with three sub-
cutaneous, bilateral injections of 0.1 ml. of thawed
tissue culture fluid, not centrifuged or filtered, hav-
ing an HA titer of 1:256–1:1024. The injections
were given at 1- to 2-week intervals. The mice
were used for the experiments 10–30 days after the
last injection. The antibody titers obtained, as
measured by the hemagglutination (HI) test (15),
varied between 1:2560 and 1:10240. When pilot
experiments indicated that the virus-immunized
animals may be more resistant to the trans-
plantation of the tumor cells than untreated mice
of the same age, sex, and genetic constitution,
other experiments were carried out with in-
ocula containing known numbers of tumor cells.
As a preliminary, the approximate minimal cell
number was determined that was necessary to
give rise to tumors in 100 per cent of untreated
isologous mice. The cell suspensions used for these
experiments were prepared in the following way:
Starting with concentrated cell suspensions pre-
pared as described above, macroscopic tissue frag-
ments were allowed to sediment for 5–10 minutes
at 0°C. Subsequently the supernatant was re-
moved, and the number of eosin-unstained cells
was counted (17). In most cases, only isolated
cells were seen, although small clusters containing
about eight to ten cells were seen in a few in-
stances. These clusters did not interfere with the
cell counting to any appreciable extent. Aliquots
containing 10³, 10⁴, 10⁵, and 10⁶ unstained cells
were inoculated into three isologous recipients
each. The minimal cell number that grew in
all three mice varied between 10³ and 5 × 10⁵
for eleven different tumors. This cell number was
selected for each tumor and inoculated into the
following experimental groups: (a) 3- to 5-month-
old, untreated, isologous mice; (b) 3- to 5-month-
old, isologous mice preimmunized against polyoma
virus as described above; (c) 3- to 5-month-old,
isologous mice pretreated with heavily irradiated
cells of the same tumor. This latter pretreatment
was carried out in the following way. Small, solid
tumor pieces were introduced into small plastic ir-
radiation chambers submerged in an ice-bath and
irradiated with x-rays generated at 185 kv., 15 ma.,
and filtered by 1 mm. Al. They received a total
dose of 15,000 r. Inoculations of 0.1 ml. of a con-
centrated suspension of irradiated tumor cells pre-
pared in the same way as described above for the
viable concentrated suspension were given sub-
cutaneously, bilaterally to 2- to 4-month-old
animals. Four such inoculations were given, at in-
tervals of 7–14 days, the last 7–30 days before the
tumor transplantation. All three recipient groups
were checked for tumor development at appropri-
ate regular intervals. Developing tumors were
measured by caliper. Three different diameters
were measured, and the geometric mean was calcu-
lated.

The possible effect of serum of virus-immunized
mice upon polyoma tumor cells in vitro has been
studied by a combined in vitro-in vivo technic
(9). Counted numbers of tumor cells were incu-
bated at 37°C for 1 hour with immune serum and
control serum, respectively, in the presence of
guinea pig serum (complement). After incubation
the mixtures were inoculated subcutaneously into
isologous mice preirradiated with 300–400 r, each
mouse receiving the previously estimated approxi-
mate minimum of tumor cells. The irradiation of
the recipients was a precaution introduced on the
basis of the experience obtained with other sys-
tems in which host resistance was shown to exist
against the tumor used, and in which preirradia-
tion of the recipients was found suitable to elimi-
nate the experimental complications depending
on the reactivity of the host (11).

RESULTS

The transplantability of 52 polyoma virus-
induced primary tumors was tested in untreated
adult and preirradiated newborn, isologous mice.
There was no difference in the number of takes in
adult and newborn recipients. The results are
shown in Table 1. Thirty-five out of the 52 tumors
grew progressively at the site of inoculation, and
they were carried for one to twelve passages until
the time of the present writing. In no instance was
temporary growth observed followed by regression.

Lymphocytic thymoma group showed a lower
degree of transplantability than did subcutaneous
sarcomas and osteogenic sarcomas. The fact that
some tumors did not grow after transplantation
cannot be attributed to the residual heterozygosis
demonstrated in the A.SW, A.BY, and A.CA
lines, since tumors of the skin-compatible A mice
were least transplantable in the whole material.

Five tumors, originating in A × A.SW F₁ hy-
brid mice, were tested by transplantation to the parental strains to investigate to what extent variant sublines, compatible with the parental strains, can be isolated from polyoma tumors, in analogy with a variety of other neoplasms (10). From two tumors, an undifferentiated subcutaneous sarcoma and a subcutaneous fibrosarcoma, it was possible to isolate variant sublines growing progressively in one or the other parental strain; variants appeared in high frequency already in the first test, and each variant was specific for the parental strain in which it was selected, even if the recipients were immunized against tissues of the other parental strain and it refused to grow progressively in the other parental strain or in unrelated foreign genotypes. The three remaining tumors did not yield any variants in the tests that have been performed until the time of the present writing. It has been found previously on other types of tumors that variant formation was an individual and variable characteristic of different tumors of the same type (10).

Fifteen of the transplantable polyoma-induced tumors and two spontaneous mammary carcinomas were tested by transplantation into isologous, adult recipients preimmunized as adults against the polyoma virus or pretreated with heavily irradiated (HR) cells of the same tumors. The latter had been pretreated with 300–400 r total-body x-radiation. There was no difference between the results obtained in these two groups of recipients. The mice were kept under observation for possible tumor development for a minimum of 90 days.

As mentioned above, the resistance of virus-immunized mice but in none of 21 virus-immunized animals. With an osteogenic sarcoma (SEWA) 10^5 cells grew in 10/14 untreated mice, in 1/1 animal pretreated with irradiated tumor cells, but in none of thirteen virus-immunized recipients. Pretreatment with irradiated tumor cells did not induce any detectable resistance against the tumor in seven cases whereas with two tumors (SEY1 and SENSE) a certain resistance may have been induced. With two other tumors (SESI and SEBCO), however, there may have been some slight enhancing effect, since the inocula grew out more rapidly in the mice pretreated with the irradiated cells than in the untreated control groups.

As mentioned above, the resistance of virus-immunized animals. Three tumors gave only occasional takes in virus-immunized animals, and one tumor (SENSM) grew as well in virus-immunized mice as in untreated controls. Three tumors have been tested in fairly large groups of mice. With a subcutaneous undifferentiated sarcoma (SESQ) 10^6 cells grew out in 9/9 untreated mice and in 1/1 animal pretreated with irradiated tumor cells, but in none of ten virus-immunized recipients. With a thymoma (SECBT), 5 × 10^5 cells grew progressively in 21/21 untreated

### Table 1

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Fraction of Tumors Successfully Transplanted*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/Sn A BY A CA A SW A × A SW F1 hybrid</td>
<td></td>
</tr>
<tr>
<td>Osteogenic sarcoma</td>
<td>1/3 5/3 1/1 1/1 4/6</td>
<td>10/14</td>
</tr>
<tr>
<td>Subcut. fibrosarcoma</td>
<td>2/2 4/4 2/2 1/1 3/5</td>
<td>12/15</td>
</tr>
<tr>
<td>Subcut. undiff. sarcoma</td>
<td>2/2 4/4 1/1 1/1 2/2</td>
<td>5/5</td>
</tr>
<tr>
<td>Epithelial thymoma</td>
<td>1/1 0/1 2/2 1/3 1/3</td>
<td>1/1</td>
</tr>
<tr>
<td>Lymphoect. thymoma</td>
<td>0/2 0/1 2/2 1/3 1/3</td>
<td></td>
</tr>
<tr>
<td>Kidney sarcoma</td>
<td>2/2 2/2 1/3 1/3 2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>Hemangiomata</td>
<td>1/1 1/1 1/1 1/1 1/1</td>
<td></td>
</tr>
<tr>
<td>Malignant epithelial tumor</td>
<td>2/2 2/2 2/2 2/2 2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>of unknown origin</td>
<td>1/1 1/1 1/1 1/1 1/1</td>
<td></td>
</tr>
<tr>
<td>Total:</td>
<td>8/15 8/10 5/5 4/4 10/18</td>
<td>35/52</td>
</tr>
</tbody>
</table>

* The figures denote the number of tumors growing progressively at the site of inoculation in one or more recipients for one or more passages, as related to the total number of different primary tumors tested. The "unsuccessful" tumors did not grow out at all. In no instance were tumors seen to grow out temporarily and then regress. All tumors were tested in at least two adult untreated animals and/or one litter (three to eight animals) of newborn mice (less than 4 days old). The latter had been pretreated with 300–400 r total-body x-radiation. There was no difference between the results obtained in these two groups of recipients. The mice were kept under observation for possible tumor development for a minimum of 90 days.

† This column denotes the number of passages with the different tumors until the time of the present writing. For the serial transfers at least two untreated adult isologous mice were used on each passage, and the recipients were observed for possible tumor development for a minimum of 60 days.
<table>
<thead>
<tr>
<th>Designation</th>
<th>Tumors</th>
<th>Strain of origin</th>
<th>n</th>
<th>Untreated isologous mice</th>
<th>Isologous mice pre-immunized against virus</th>
<th>Isologous mice pretreated with HR tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SESF</td>
<td>Subcut. undiff. sarcoma</td>
<td>A/Sn</td>
<td>$10^5$</td>
<td>6/6</td>
<td>0/6</td>
<td>2/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$</td>
<td>4/4</td>
<td>2/4</td>
<td>1/1</td>
</tr>
<tr>
<td>SESO</td>
<td>Subcut. undiff. sarcoma</td>
<td>A/Sn</td>
<td>$10^5$</td>
<td>9/9</td>
<td>0/10</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
</tr>
<tr>
<td>SESP</td>
<td>Subcut. fibrosarcoma</td>
<td>A/Sn</td>
<td>$10^6$</td>
<td>2/2</td>
<td>0/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Sesi</td>
<td>Epithelial thymoma</td>
<td>A/Sn</td>
<td>$10^6$</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
</tr>
<tr>
<td>SECBT</td>
<td>Lymphocytic thymoma</td>
<td>A.CA</td>
<td>$10^6$</td>
<td>9/10</td>
<td>1/8</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$5 \times 10^5$</td>
<td>21/21</td>
<td>0/21</td>
<td>7/7</td>
</tr>
<tr>
<td>SECBO</td>
<td>Osteogenic sarcoma</td>
<td>A.CA</td>
<td>$10^6$</td>
<td>0/2</td>
<td>0/2</td>
<td>1/2</td>
</tr>
<tr>
<td>SEYB</td>
<td>Osteogen. sarcoma</td>
<td>A.BY</td>
<td>$10^6$</td>
<td>1/1</td>
<td>2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>SEYF</td>
<td>Subcut. fibrosarcoma</td>
<td>A.BY</td>
<td>$10^6$</td>
<td>2/3</td>
<td>1/3</td>
<td>12/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$</td>
<td>2/2</td>
<td>0/2</td>
<td>11/12</td>
</tr>
<tr>
<td>SEYI</td>
<td>Subcut. fibrosarcoma</td>
<td>A.BY</td>
<td>$10^6$</td>
<td>3/4</td>
<td>1/4</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$</td>
<td>2/2</td>
<td>1/2</td>
<td>15/15</td>
</tr>
<tr>
<td>SEWA</td>
<td>Osteogen. sarcoma</td>
<td>A.SW</td>
<td>$10^4$</td>
<td>10</td>
<td>0/10</td>
<td>2/2</td>
</tr>
<tr>
<td>SENA</td>
<td>Subcut. fibrosarcoma</td>
<td>A X A.SW F_1</td>
<td>$10^6$</td>
<td>10/12</td>
<td>0/13</td>
<td>2/2</td>
</tr>
<tr>
<td>SENSE</td>
<td>Subcut. undiff. sarcoma</td>
<td>A X A.SW F_1</td>
<td>$10^4$</td>
<td>4/4</td>
<td>0/6</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$</td>
<td>6/6</td>
<td>0/6</td>
<td>4/4</td>
</tr>
<tr>
<td>SENSP</td>
<td>Osteogen. sarcoma</td>
<td>A X A.SW F_1</td>
<td>$10^4$</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
</tr>
<tr>
<td>SENSV</td>
<td>Osteogen. sarcoma</td>
<td>A X A.SW F_1</td>
<td>$10^4$</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
</tr>
<tr>
<td>SENSMA</td>
<td>Lymphocytic thymoma</td>
<td>A X A.SW F_1</td>
<td>$10^4$</td>
<td>10/10</td>
<td>14/14</td>
<td>10/10</td>
</tr>
<tr>
<td>S13S</td>
<td>Spont. mammary cancer</td>
<td>A/Sn</td>
<td>$10^4$</td>
<td>1/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>S14S</td>
<td>Spont. mammary cancer</td>
<td>A/Sn</td>
<td>$10^4$</td>
<td>2/4</td>
<td>3/4</td>
<td>2/2</td>
</tr>
</tbody>
</table>

* This was the minimal number capable of giving rise to progressively growing tumors in untreated mice regularly.
† Every mouse was given inoculations of 0.10 ml. of a tumor cell suspension obtained by forcing the tissue through a 60-mesh stainless steel screen and then diluted 1:5 on a volume basis with Ringer's solution containing 100 I.U. penicillin and 100 µg streptomycin/ml.
‡ The tumors have been designated according to the following principles: the first two letters S and E refer to the induction of the tumor by the S E polyoma virus. The next letter indicates the strain of origin, S, Y, C, and W standing for A/Sn, A.BY, A.CA, and A.SW, respectively, whereas NS symbolizes the A X A.SW F_1 hybrid. The last letter is the serial designation of the individual tumors, applied in alphabetic order according to their chronological appearance. SECBT and SECBO represent two different primary tumors originating in the same mouse. The letters T and O denote thymoma and osteogenic sarcoma, respectively. In the designation of the spontaneous mammary carcinomas the first S indicates that it is a spontaneous tumor, the second S stands for A/Sn, the strain of origin. The figures 13 and 14 are the serial number according to the chronological appearance of the tumors.
§ The figures denote the fraction of mice with progressively growing tumors. All animals were observed for a minimum of 60 days.
immunized mice was also apparent in certain cases when concentrated cell suspensions were inoculated. With some tumors the resistance was strong, wholly inhibiting the outgrowth or causing the tumors to regress after an initial outgrowth (Chart 1). With one tumor (SESO) the resistance came to expression in the form of a prolonged latency period (Chart 2).

To summarize, the virus-immunized mice turned out to be wholly or partially resistant to ten out of eleven tumors tested with the minimal cell number. Mice pretreated with irradiated tumor cells appeared to be resistant against two of nine tested tumors, but with two other tumors there was a certain enhancing effect. Virus-immunized animals showed a variable degree of resistance against concentrated cell suspensions with eight out of fourteen tested tumors.

The first transfer generation of two spontaneous mammary carcinomas which had developed in two A/Sn females, not exposed to polyoma virus, were also treated in a similar way. Minimal cell numbers and concentrated cell suspensions were inoculated into untreated and virus-immunized mice, respectively. There was no difference with regard to tumor growth or latency. Other tests of this type are presently being carried out.

Four tumors (SESF, SESO, SESI, and SEWA) have been tested by incubating their cells in vitro with serum from virus-immunized animals or untreated controls in the presence of complement, and then inoculating the mixture into adult, preirradiated isologous hosts. With one tumor (SESO) there was an inhibitory effect of the immune serum as compared with the effect of control serum, as manifested by a prolongation of the latency period. This could be confirmed in a repeat experiment, as illustrated by Chart 3. With two other tumors (SESF and SEWA) there was no detectable retardation in the immune serum-treated group in any of the two experiments performed with each tumor. Neither was there any inhibition with the fourth tumor (SESI). There was possibly some enhancement (Chart 4), as
manifested by a more rapid growth rate in four of four recipients in the immune serum-treated group as compared with three of four mice in the control group. In addition, one of the four mice in the immune-serum group showed an unusually short latency period.

**DISCUSSION**

The finding that polyoma virus-immunized adult isologous recipients exhibit a certain resistance against the transplantation of established polyoma-induced tumors, as compared with untreated isologous controls, was entirely unexpected. It could be demonstrated with ten out of eleven tested neoplasms. It is at variance with the results of Habel (7), who showed that there was no difference in the transplantability of a polyoma-induced hamster sarcoma to untreated and virus-immunized adult hamsters. This discrepancy may be related to a possible difference in the reactivity of hamsters and mice or, alternatively, to differences in the virus-cell-host relationship in polyoma-induced hamster as compared with mouse tumors. In fact, there are many differences in the details of the oncogenic process in hamsters and mice, both in vivo and in vitro (25). Also, since Habel reports only data on one hamster tumor, transplanted with an unknown, probably large dose of cells, it cannot be excluded that a similar difference may be demonstrable even in the hamster system, provided that small inocula are used.

A discussion of the possible mechanism of the effect in mice must remain largely speculative at present. First, the question may be raised whether the experiments represent true cellular transplantation of established tumors or viral induction of new neoplasms in the grafted recipients. If the latter would be the case, resistance of virus-immunized mice against new viral induction of tumors would be only natural. This possibility can be excluded, however, and cellular transplantation can be proved by the following arguments:

1. Tumors arising after implantation of cells in doses suitable to demonstrate the difference between untreated and virus-immunized adult isologous mice appeared after a comparatively short time, which could be as low as 4–5 days in the case of some undifferentiated sarcomas. They always appeared at the site of inoculation. They had the same morphology as the donor tumors. An osteogenic sarcoma (SEWA) revealed altered stemlines and the presence of marker chromosomes (3). The karyotype of each tumor was breeding true during serial transfer. This finding finally excludes the possibility of viral induction in these experiments.

2. Although some F1 tumors gave rise to variants in the parental strains, not all of them did so. For instance, an osteogenic sarcoma (SENSV) gave no variants, and, nevertheless, in the F1 hybrid mice, it clearly showed the difference between virus-immunized and untreated mice, even if concentrated cell suspensions were used for the test. This would strongly speak for cell transplantation rather than viral induction. Those tumors that did give rise to variants in the parental strains did not take in a third cosogenic resistant strain. In the event of viral induction, there is no reason why the parental types and other IR-lines should not be equally susceptible as the F1 type of origin.

3. Cytological studies on two tumors (SEWA and SENSE) revealed altered stemlines and the presence of marker chromosomes (3). The karyotype of each tumor was breeding true during serial transfer. This finding finally excludes the possibility of viral induction in these experiments.

If it is accepted that the experiments repre-

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*3* H. O. Sjögren and N. Ringertz, unpublished.
sent true cell transplantation, the question arises whether the difference between the virus-immunized and the untreated mice is really due to the effect of antiviral antibodies on polyoma tumor cells. Since there was some residual heterozygosis in the three IR-lines used (A.SW, A.BY, A.CA) as demonstrated by skin grafting (12), the possibility must be considered that immunization with virus-containing tissue culture fluid may have led to an increased resistance of the recipients against the grafts, caused by a homograft reaction based on differences in histocompatibility factors. The mouse embryo cells used for propagating the polyoma virus in tissue culture had been derived from randomly chosen mouse strains. It might be argued that the supernatant fluids used for immunization may have contained soluble antigens and/or cell debris containing the histocompatibility factors of the right kind. This possibility can be excluded, however, since the resistance of virus-immunized mice was present and equally clear-cut when using the A strain itself and its polyoma-induced tumors. The homozygosity of this strain was proved by critical skin grafting tests (12). Furthermore, even though the IR-lines reveal residual heterozygosis when tested with skin grafts, tumor grafts usually grow in 100 per cent, and it is extremely difficult to reveal the existence of genetic heterogeneity by transplantation of neoplastic tissue even if preimmunized hosts are used (12). Resistance of the virus-immunized mice against polyoma tumors was regularly demonstrable, however, with all tested tumors except one.

As another possibility, it has been suggested to us by Prof. S. Gard that polyoma-infected cells may contain a new antigen, possibly a surface antigen, not identical with any viral antigen. Models for such an event are available, e.g., in the phenomena of lysogenic conversion in bacteria (13), and Zilber (26) has recently demonstrated the presence of a specific cellular antigen in Rous sarcoma cells, not identical with any viral antigen. In view of the fact that polyoma virus may induce neoplastic change in embryonic cells cultivated in vitro, such as used for virus production in the present work, it is conceivable that the postulated new antigen already appears at this stage. Such an antigen might be present in the tissue culture fluids used for immunizing the mice in our experiments. The animals would thus develop immunity to the virus and to the cellular antigen as well. Presently, this hypothesis is being tested by purifying the virus material used for preimmunization of the recipients. Meanwhile, it may be pointed out that the experiments with heavily irradiated cells, included in the present work for the purpose of demonstrating the possible occurrence of such cellular antigens, have not given any positive results, with the possible exception of two experiments. Such heavily irradiated cells were previously found to be perfectly adequate for building up immunity against weak cellular antigens of the histocompatibility system (8) or against the antigens contained in the cells of methylocholanthrene-induced sarcomas, demonstrable in the primary autogenous host (11). Nevertheless, it cannot be concluded that the absence of demonstrable resistance after pretreatment with HR cells in the present study excludes the possible presence of cellular antigens differentiating the polyoma-infected tumor cell from the normal host, since this particular antigen might have been more radiosensitive than the antigens previously studied with this experimental design.

Another, somewhat different possibility, also based on the appearance of new cellular antigens after polyoma infection, has been suggested to us by Prof. S. E. Luria. It might be speculated that the polyoma virus "converts" some cells of the host in the course of the immunization process so that they develop a new antigenic specificity which they share with the tumor cells, and, although they do not themselves become neoplastic in the adult host, they manage to immunize the host against the later challenge of viable polyoma tumor cells. This possibility will be tested by using inactivated, purified virus for the preimmunization which can be expected to retain its viral antigenicity but not the postulated "converting" ability, since the latter property would require cellular infection.

Should these experiments exclude the possibility that the effect observed is due to some antibody directed against antigenic specificities peculiar to polyoma-infected cells, the possible action of the antiviral antibodies proper upon polyoma tumor cells will have to be considered. This question is intimately linked with problems relating to the nature of the virus-cell relationship in the polyoma tumor cell. Before the above-mentioned control experiments have clarified this question, however, it may be most suitable to refrain from a discussion that would have to remain entirely speculative.

Another, very paradoxical problem is connected with the fact that, whereas mice inoculated with polyoma virus as newborns develop high titers of antiviral antibodies, they cannot prevent the development of their own, polyoma-induced primary tumors, although animals immunized as adults prove to be resistant against the transplantation of established polyoma tumor cells. Either it must be assumed that the nature of the anti-
bodies is different in mice inoculated as newborns and in those inoculated as adults, or the vulnerability of transplanted tumor cells must be greater than that of neoplastic cells transformed in situ. At present the former possibility is tested by repeating the transplantation experiments with mice that have been inoculated with polyoma virus as newborns.

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Transplantation of Polyoma Virus-induced Tumors in Mice

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