Induced Host Resistance to a Transplantable Mouse Glioma*

LABE C. SCHEINBERG, MARK C. LEVINE, KUNIHIKO SUZUKI, AND ROBERT D. TERRY

(Albert Einstein College of Medicine, Yeshiva University, New York, N.Y.)

SUMMARY

A transplantable methylcholanthrene-induced glioma has been carried by subcutaneous transplantation in inbred C57/6 mice and grows rapidly within 3-4 weeks in almost all recipients. Immunization with glioma-adjuvant mixture resulted in inhibition or “rejection” of 70 per cent of tumor implants given later, whereas immunization with spleen-adjuvant, brain-adjuvant mixtures, or adjuvant alone resulted in inhibition or “rejection” of only 4 per cent of later tumor implants. The previous work on induced tumor immunity in inbred animals is reviewed.

This study was undertaken to investigate the effects of immunization with glioma and adjuvants on the growth of a transplantable glioma induced and carried in an inbred strain of mice. The effects of active and passive immunization upon the growth of experimental tumors have been studied by many investigators since the early part of this century. The entire field of the relationships of immunology to cancer has been recently reviewed by Southam (30). As he has pointed out, the studies in isologous systems are of greatest importance from the standpoint of possible therapeutic applications of immunology to neoplasia. In a truly isologous system those normal antigens present in tumor cells are not foreign to the host, and thus any immune response would be the result of antigens unique to the tumor cells.

In 1934 the first example of induced immunity in an inbred strain of mice to a transplantable tumor arising in that strain of mice was demonstrated by MacDowell, Taylor, and Potter (21). A spontaneous, lymphatic leukemia (line 1) which arose in C58 mice and had been carried for 441 transfer generations was employed. When 1/524,000 of the standard transfer dose was given, all the mice survived. Increasing doses were given intraperitoneally at 2- to 3-week intervals, and finally these mice survived the standard transfer dose of leukemia cells. These same workers later showed that an injection of unrelated StoLi or F₁ (C58 × StoLi) embryonic tissue immunized the C58 mice against line 1 leukemia, whereas C58 embryonic tissue did not (22). It was also shown that immunization with line 1 leukemic cells did not prevent the occurrence of spontaneous line 1 leukemic cells in C58 mice nor did the transfer of leukemic cells from recent spontaneous line 1 leukemias in C58 mice (19). MacDowell et al. (20) were able to confer partial immunity by one of three injections of a sediment of fragmented line 1 leukemia cells. It was concluded that the induced resistance to highly virulent transplantable leukemia depended on immunologic properties of the cells developed during the many transfers.

Marshak and Erf (23), using a transplantable lymphoma which had grown progressively in 100 per cent of untreated highly inbred Strong A mice, were able to immunize a small percentage (2.4-9.3) of mice by injecting fragmented lymphoma cells, lymphoma nuclei, or liver nuclei from Strong A mice. The highest percentage of immune animals occurred in those receiving fragmented lymphoma cells, whereas 50 per cent of these died during the course of immunization.

Gross (11) gave intradermal injections to C3H mice of a cell suspension of Gross’s sarcoma (S-1) arising in that strain. The tumor grew in all but then regressed in 18 per cent. These animals resisted a second inoculation. Later (12) he showed that the resistance to Gross’s sarcoma (S-1) did not
result following regression of another Gross sarcoma (S-2) or carcinoma arising in the same strain. He commented that the acquired tumor immunity was directed specifically against the tumor used for immunization and not caused by genetic differences in the cells of the host and those of the animal in which the tumor originated.

Goldfeder (7) used inbred rats 100 per cent susceptible to isotransplants of a reticulum-cell sarcoma. She showed that those animals which recovered from isotransplants of irradiated tumor tissue were later resistant to transplants of the same unirradiated tumor. Injections of embryo skin of the same species did not produce immunity (8).

Aptekman and his co-workers (2, 3, 17, 18), using rat sarcoma and inbred albino rats in which the tumor arose, prepared an alcoholic extract of the sarcoma which, given as repeated intratumoral injections, caused complete regression of 96 per cent of the sarcomas. Reinoculation demonstrated that 78 per cent were resistant. Extracts of human carcinoma resulted in regression of 90 per cent of the sarcomas. When the rats were given ten subcutaneous injections of tumor extract at 2- to 3-day intervals, 50 per cent became resistant to tumor implants given later. The rats which recovered were resistant over a period of 8 months, during which interval they were observed. Tumor tissue introduced into these immune rats was viable for about 3 days and inactivated after the 4th day.

Fink and her co-workers (5) were apparently the first to use adjuvants with the tumor for immunization. Using BALB/c and a methylcholanthrene fibrosarcoma arising in this strain, they were able to produce 39 per cent survival by immunizing with lyophilized tumor and Freund's adjuvant, 22 per cent survival by using sonicated tumor cells with adjuvant, 15 per cent survival by using whole tumor implanted in the ear followed by tumor alone, and no survival in the unimmunized mice.

Gorer and Amos (9) prepared antiserum against C57BL leukemia E.L.4 which, if given before or simultaneously with implantation, prevented tumor growth. If given afterward, the tumor developed. The antibody was adsorbed by tumor tissue but not by fetal or liver tissue.

Aptekman and Day (1) prepared antibody by hyperimmunizing mice of a resistant strain to leukemia and lymphoma, and this antibody protected both susceptible and resistant mice to tumor implants. This was not adsorbed by liver or fetal tissue, and its effectiveness was reduced by adsorption with E.L.4 tumor tissue. The antibody protected if given 3 days before or 1 day after injection of tumor cells.

Mitchison (25), using a mouse lymphoma, prepared both isoantibody and antibody in another strain of mice. When the isoantibody was incubated with the tumor cells prior to injection, there was no tumor growth, whereas there was some inhibition with the other antibody and none with normal mouse serum. He points out that the cytotoxic effect of isoantiserum is slight and may be easily missed.

Martinez et al. (24), using isotransplants of 100 per cent transplantable mammary tumor in inbred mice, implanted the tumor in each ear and then amputated these successively after 10 days. The tumor was then implanted in the tail for 10 days and then amputated. Tumor implanted in the groin region was rejected by 89 per cent of these mice. However, using a similar technic in another strain of inbred mice, Hirsch and his co-workers (18) concluded that the inbred mouse could not be immunized against its own tumor.

Lewis and Aptekman (16) showed that a transplantable carcinoma or sarcoma damaged by strangulation was absorbed in a few days. This resulted in immunity to subsequent implantation of viable tumor tissue of the same type. Foley (6), using a similar technic, showed that destruction by ligation resulted in destruction of a spontaneous mammary carcinoma with no immunity developing to subsequent tumor implantation. However, when the same technic was applied to methylcholanthrene-induced sarcoma, there was immunity demonstrated to subsequent implantation.

Prehn (27) extended his observations in a very ingenious experiment using inbred strains of mice and dibenzanthracene-induced tumors from the same strain which had not been transplanted previously, and he was able to show 45 per cent tumor “takes” in immunized mice as compared with 81 per cent in unimmunized mice. The method of “immunization” was to excise recently induced tumors.

Schoenberg and Moore (29) were unable to affect the growth of Sarcoma 180 (a nonisologous tumor) in mice by injecting Freund's adjuvant before or after tumor implantation. Old and co-workers (26) were able to cause a rejection of tumor implants in mice by prior injections of Bacillus Calmette-Guérin, apparently stimulating the reticuloendothelial system.

In humans, Graham and Graham (10) showed some antineoplastic effect by using a vaccine of autologous tumor and adjuvants in metastatic carcinoma. Bloom et al. (4) implanted autologous malignant glioma tissue subcutaneously in humans without affecting the natural course of the primary glioma.
In summary, there have been a few excellent studies showing induced immunity in an inbred strain of animals against a tumor arising in that strain. Most of the success has been with long-transplanted or carcinogen-induced tumors, however. Also, other studies with other tumors and hosts have been unable to show this. In addition, a few studies have been undertaken in humans with autologous tumors or vaccines.

MATERIALS AND METHODS

The inbred mice used in this experiment are highly inbred C57/6, supplied by Millerton Research Farm, Millerton, N.Y. These mice appear to be genetically uniform in that they accept skin grafts from one to the other for periods of 3 months or longer without rejection.

The transplantable glioma used in this experiment is an ependymoblastoma induced in the C57/6 mouse by intracerebral implantation of a methylcholanganthrene pellet at the laboratories of Dr. H. M. Zimmerman, Montefiore Hospital, New York (31). This tumor was carried through 85 subcutaneous transfers and is accepted by almost all the C57/6 hosts. The tumor still retains the original histological characteristics of an ependymoblastoma, being made up of small nests and cords of medium-sized, pleomorphic, polyhedral cells with poorly defined plasma membranes and moderate quantities of cosinophilic cytoplasm. The nuclei are oval or round with prominent mitotic figures. In some areas the cells occur in perivascular masses arranged in a somewhat radial fashion approximating the wall of the vessels (30). In all generations the morphology is retained.

A fragment of the glioma from the subcutaneous tissues was analyzed for sodium/potassium ratios by Dr. Robert Katzman. This revealed a Na/K ratio of 1.4, as compared with a top value of 0.8 for other nonglial neoplasms and compares with the high values seen in glial neoplasms (15). This would indicate that the glial tumor had retained one of its biochemical characteristics in addition to morphologic characteristics.

When the glioma is implanted subcutaneously with a trocar in unimmunized C57/6 mice, it grows to a massive size in 3–4 weeks, and the mouse dies in about 8 weeks. The tumor was rejected by less than 3 per cent of the unimmunized mice. This failure to grow in these few mice was attributed to technical failure. When the tumor grows to a large size, there are areas of necrosis, and occasionally some necrotic tumor tissue may be implanted and fail to grow. The few unimmunized mice which “rejected” implanted tumor tissue were not challenged and were discarded.

The immunization mixtures were prepared by homogenizing in a glass homogenizer equal parts of tumor by wet weight and physiological saline with twice the total volume of Freund’s complete adjuvant (Difco®). For control immunizations normal C57/6 spleen or brain tissue was substituted for tumor in the mixtures.

The immunizations were given intradermally via a 25-gauge, 1-inch needle. For each inoculation 0.1 ml. of tumor or normal tissue-adjuvant mixtures was given in each of two sites. There were three immunization schedules employed. In some mice one inoculation was given (0.2 ml. total), and in others three inoculations were given at weekly intervals (0.6 ml. total). In a third group the mice received 0.5 ml. H. Pertussis vaccine (Lederle) diluted 1:5 with physiological saline given intraperitoneally 4 days prior to the first intradermal immunization with tumor-adjuvant mixtures. Ten days after the first tumor-adjuvant immunization, a second intradermal injection was given (0.4 ml. total).

The tumor fragment was implanted subcutaneously about a week after completion of immunization. The implantation site was inspected daily; and if after 1 month no tumor was obvious, it was recorded as inhibited. If no tumor was obvious after 2 months, it was recorded as negative or “no growth.”

In one small group of mice, an effort was made to test the effects of prior skin isotransplantation on tumor growth. Skin grafts were taken from each donor member of ten pairs of mice and given to the host member of the pair. It was not accepted by three hosts for purely technical reasons and was accepted by the remainder. Tumor was then implanted in each donor member of the pair; when it was growing well, it was transferred to its host member of the pair and its growth observed.

RESULTS

The results of immunization upon tumor growth are shown in Table 1. Multiple inoculations with tumor-adjuvant homogenate were effective (70 per cent) in delaying and in most cases rejecting the growth of a tumor which in unimmunized animals was rejected by 3 per cent or less of the animals. This rejection in 8 per cent of the unimmunized mice was thought to be due to technical reasons, as has been previously discussed. A single inoculation appeared to be much less effective (43 per cent) in inhibiting growth. Immunization by means of intraperitoneal H. Pertussis vaccine followed by intradermal injections of tumor-adjuvant homogenate was less effective (20 per cent) than were the other methods of immunization.
Immunization with tumor homogenate alone was only partially effective (25 per cent). Skin iso-transplantation prior to tumor implantation resulted in delayed growth in one tumor implant and rejection of another (28 per cent).

The tumor was inhibited or rejected in only 3/71 (4 per cent) of the total control series comprising the mice receiving three inoculations of spleen-adjuvant, brain-adjuvant, or adjuvant alone. Immunization after implanting tumor tissue was not effective in inhibiting growth. At no time was growth of tumor tissue noted at the immunization site, and histological examination of these sites revealed no viable tumor cells.

The growth rates of the subcutaneous glioma in a tumor-adjuvant immunized group of mice can be compared with that in a group of spleen-adjuvant immunized mice in a typical experiment (Chart 1). It would appear that some of the smaller tumor nodules had undergone regression in

| Table 1 Effect of Immunization on Growth of Transplantable Glioma |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Immunisation                | No. inoculations | No. mice | Normal growth | Delayed growth | No. growth |
| None                        | 0               | 113        | 110           | 0              | 3              |
| Adjuvant only               | 3               | 35         | 35            | 0              | 1              |
| Spleen and adjuvant         | 3               | 25         | 25            | 1              | 8              |
| Brain and adjuvant          | 3               | 10         | 10            | 0              | 0              |
| Prior skin graft            | 3               | 7          | 5             | 1              | 1              |
| Spleen and adjuvant with H. Pertussis | 2         | 9          | 9             | 0              | 0              |
| Tumor and adjuvant with H. Pertussis | 2         | 10         | 8             | 1              | 2              |
| Tumor only                  | 3               | 12         | 9             | 2              | 1              |
| Tumor and adjuvant          | 1               | 28         | 16            | 6              | 6              |
| Tumor and adjuvant          | 3               | 27         | 8             | 4              | 15             |

* No obvious growth after 4 weeks.
† No obvious growth after 8 weeks.

The growth of transplanted glioma in mice immunized with tumor + adjuvants and spleen + adjuvants can be compared in Chart 1. Growth of a transplantable glioma in C37/6 mice implanted after triple immunization with tumor-adjuvant or spleen-adjuvant (S) mixtures. Bars show number of animals at 17, 33, 49, and 64 days following implantation. The white areas indicate no growth, light gray areas represent questionable or minimal tumors, dark gray represents moderate-size tumors, and black represents massive tumors. The differences in the total numbers at 17 and 64 days represent animals dying because of tumor. None died before the 17th day. This is one experiment of total represented in Table 1.

**DISCUSSION**

It is possible to obtain more effective inhibition of growth of a transplantable tumor by immunization of the hosts with tumor-adjuvant mixture than by using tumor homogenate alone. The use of antigen-adjuvant mixtures has been shown—e.g., in experimental allergic encephalomyelitis—to be more effective in production of antibodies than injections of homologous tissues alone (14). It is felt, perhaps, that optimal tumor inhibition has yet to be achieved in our experiments and that, by altering the tumor-adjuvant proportions or the immunization schedule, better inhibition might be attained. However, imitating the effective method of producing experimental allergic encephalo-
myelitis in mice of Schneider et al. (28) by giving
intraperitoneal H. Pertussis vaccine followed by
intradermal antigen-adjuvant inoculation did not
give as good results in tumor inhibition as did
triple intradermal inoculation.

The question that is raised in this experiment is
whether the tumor inhibition is related to non-
specific stimulation of the reticuloendothelial sys-
tem as is accomplished by Bacillus Calmette-
Guérin infection prior to tumor implantation (29).
It is not possible to answer this with present
observations, since the commercial adjuvant em-
ployed does not contain Mycobacterium Tubercu-
losis. Infection with Bacillus Calmette-Guérin
must be used prior to implantation of the glioma
to answer this question.

If uniformly inbred C57/6 mice are used as in
this experiment, prior skin isotransplantation
should have no effect upon the transplantable
glioma. However, two tumors of seven were either
inhibited or rejected by mice receiving skin iso-
transplants from the same two mice which later
acted as tumor donors. This is a very small group
of animals, and the rejection and inhibition could
conceivably have been a result of technical errors.
This will have to be studied again in a larger series.
However, if “homograft immunity” were the basis
for the tumor inhibition and rejection, it should
occur equally in the “tumor-immunization” and
“normal tissue-immunization” groups.

The one defect in the experimental design that
is not easily corrected is the use of tumor which
had undergone many subcutaneous transfers fol-
lowing its induction. There is always the possi-
bility of a divergence of the cell lines of the
induced tumor and the inbred strain of mice with
mutations in the tumor or inbred strain and
genetic differences. Attempts are being made to
correct this, if possible. Such experiments would
attempt to prevent spontaneous tumors or induc-
tion of tumors by prior immunization with tumor
and adjuvants. In addition, studies on “crossed
immunizations” to other tumors are currently
under way.

This experimental model would seem to provide
a useful tool for the investigation of mechanisms
of tumor immunization.

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