Quantitative Studies on the Transplantability of Murine and Human Tumors into the Brain and Subcutaneous Tissues of NCr/Sed Nude Mice


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ABSTRACT

The transplantability of experimental tumors into the brain (i.e.) and s.c. tissues of C3Hf/Sed and athymic NCr/Sed nude mice was examined using quantitative cell transplantation assays. Studies using the immune-competent C3H animals showed that brain is a more favorable site for the transplantation of syngeneic tumor than s.c. tissue and that this is true for nonimmunogenic as well as immunogenic tumors. The capacity of the brain to act as an immunological sanctuary can be overwhelmed by a strong, systemic, secondary immune response such as that evoked by the methylcholanthrene-induced sarcoma FSal. In studies performed using NCr/Sed nude mice, the allogeneic tumor MCalV was found not to be demonstrably immunogenic. The cell dose required to transplant the tumor into 50% of recipients (TD50) could neither be increased by immunization procedures nor decreased by six Gy whole-body irradiation (WBI) prior to transplantation. Delayed-type hypersensitivity to this tumor was not expressed by nude mice after rechallenge with tumor antigen. The TD50 was again lower for i.c. than s.c. transplantation and the ratio s.c./i.c. was comparable to that found in syngeneic C3Hf/Sed hosts. Three human tumors have been similarly tested. They were: FaDu, a pharyngeal squamous carcinoma; HFSal, a fibrosarcoma, and U87, a malignant glioma. s.c. TD50 values were in all cases significantly higher than those obtained i.c. The ratios TD50 s.c./i.c. ranged from 6.4 to >50 in five studies, substantially higher than those found for transplantation of murine tumors into either the syngeneic or the allogeneic recipients. Six Gy WBI reduced the s.c. TD50 for these tumors, but in each case the value remained significantly higher than that obtained i.c. 19.4 Gy WBI given in 10 equal fractions and followed by i.v. bone marrow rescue reduced further the s.c. TD50 for FaDu. NCr/Sed nude mice demonstrated cross-reacting delayed-type hypersensitivity against FaDu and HFSal. A small proportion of FaDu tumors (<2%) displayed a spontaneous halt in growth or even regression. When the host cell infiltrate of these tumors was analyzed, an increase was seen in the proportion of Thy 1.2 and asialo-GM1-positive cells as compared with progressively growing tumors. These data strongly suggest that a residual low level of immune reactivity exists in nude mice against xenotransplanted human tumors. This resistance to s.c. transplantation may be diminished by WBI and is less for intracerebral implantation.

INTRODUCTION

The ability of athymic nude mice to accept allo- and xenotransplants of a variety of normal and neoplastic tissues has been well documented since their development in the 1960s (1, 2). However, not all transplanted human neoplasms will take and grow progressively in nude mice and only a few develop metastatic tumor (3). Numerous explanations, including local nutritional, vascular, endocrine, and individual tumor-specific requirements, have been proposed for the growth failures. That immunological factors are important in the xenografting of human tumors into nude rodents has been demonstrated by an increased take rate: following immunosuppression by such methods as antilymphocyte serum (4, 5), whole-body irradiation (5, 6), and cyclophosphamide (7); with use of immunologically immature young animals (8–10); or with transplantation into immunologically privileged sites such as the brain (11–13). Nude mice, though athymic, do possess small numbers of T-cells (14, 15), which have been shown to be capable of cytotoxic allo- and xenoreactivity (16, 17). In addition, their numbers of splenic and nodal B-cells have been reported to be normal (9) or higher (15) than that found in heterozygotes. The activity of NK cells and peritoneal macrophages may also be higher (15, 18, 19).

In this study, we employed quantitative cell transplantation, TD50, assays (the TD50 is the cell dose required, on the average, to transplant tumor into 50% of recipients) to assess the importance of residual immunity in the nude mouse and the efficacy of strategies to either suppress (whole-body irradiation) or circumvent (intracerebral implantation) that immunity.

Initial studies were performed on a syngeneic murine system to compare the receptiveness of the cerebral and s.c. tissues of immune-competent C3Hf/Sed mice to tumor transplantation. Thereafter, the transplantability of one allogeneic murine, and three xenogenic human tumors into either the cerebral or s.c. tissues of control, whole-body irradiated, or preimmunized athymic NCr/Sed nude mice was examined. Evidence is presented that nude mice can mount a delayed-type hypersensitivity reaction on rechallenge with human tumor and that this correlates with the TD50 findings.

MATERIALS AND METHODS

Experimental Animals

NCR/Sed (nu/nu) mice were used as recipients of tumor allo- and xenotransplants. This strain was derived at the National Cancer Institute by backcross of a BALB/c nude gene into an NIH Swiss mouse background. Dr. Carl Hansen generously provided the initial breeding stock of Ncr (±) and Ncr (nu/nu) mice. We have maintained these mice for more than 25 generations. C3H/Sed mice were used as recipients of tumor isotransplants. All mice were maintained in an ammonia-free environment in our defined flora and pathogen-free colony (20). They were maintained in microisolators and fed sterile laboratory pellets and acidified water ad libitum. Mice were put into experiments when 8 weeks old and approximately equal numbers of males and females were used in each assay.

Tumors

FSal. A fibrosarcoma induced in a young C3H female mouse by injection of 1 mg of methylcholanthrene (in peanut oil) into the s.c. tissue of the flank (21). Tumor tissue has been maintained in liquid nitrogen. For these experiments FSal was used as a 7th generation isotransplant.

MCalV. A mammary carcinoma which spontaneously arose in a C3H female mouse. Tumor tissue has been maintained in liquid nitrogen and was used as a fifth generation transplant. Its histological characteristics have been previously described (22).

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2 To whom requests for reprints should be addressed.
FaDu. A human squamous carcinoma which arose in the hypopharyngeal region. This tumor cell line has been passaged over one hundred times in culture before being supplied to us by the American Type Culture Collection, Rockville, MD. FaDu grows in nude mice as a poorly differentiated carcinoma. In our studies FaDu was used as a third generation xenotransplant.

U87. A human high grade malignant glioma also obtained from the ATCC, Rockville, MD. This cell line has been passaged over one hundred times in culture. U87 grows in nude mice as a highly anaplastic tumor. In our studies U87 was used as third generation xenotransplant.

HFSal. A human fibrosarcoma of bone obtained from the surgical resection specimen from a patient at the Massachusetts General Hospital. HFSal grows in nude mice as a spindle cell sarcoma. This tumor was transplanted directly from the surgical specimen into nude mice. We have used HFSal as a third generation xenotransplant.

For each experiment, source tumors were excised and single cell suspensions prepared. These were made by mincing tumors with scissors, repeated passing through needles of decreasing bore, and finally filtering to remove cell clumps. HFSal was particularly fibrous and, after mincing, required enzymatic digestion with 0.5% collagenase (Sigma Laboratories) at 37°C for 1 h. Viability of cells was assessed using trypan blue exclusion, and only viable tumor cells were counted.

**TD50 Assay.** Quantitative transplantation assays are described in terms of the TD50 or the number of cells which would be expected, on the average, to transplant the tumor into half of the recipients. The TD50 represents the number of tumor-forming units in the prepared suspension; this depends upon the immunological condition of the recipient animal and upon the anatomic site.

For a TD50 assay, serial twofold dilutions of the tumor cell suspension were made in Hanks’ balanced salt solution (GIBCO). The TD50 assays using s.c. tissue and cerebral tissue (i.e.) as the transplant site were performed concurrently using one cell suspension. For the assays using s.c. tissue, the needle was passed through the triceps muscle and 0.1 ml inocula deposited into the right axilla. The i.c. assays were performed by injecting 20 µl into the right parietal lobe of pentobarbital-anesthetized mice using a guarded 27-gauge needle. Inoculations were made with a Hamilton microliter syringe at a depth of 3 mm from the skin surface. The injection point lay approximately 2 mm anterior to the coronal and 2 mm lateral to the sagittal suture lines. Pilot studies performed using India ink showed no significant extracranial leakage. The immediate mortality of this procedure was virtually zero. Less than 5% of animals displayed neurological signs 48 h after injection. These were sacrificed to avoid later confusion with signs arising from the growth of intracerebral tumor.

In an experiment, mice were randomly assigned to one of the six- to eight-cell dose levels in each TD50 assay. There were 4–10 mice per cell dose in each assay.

**Analysis of Tumor Transplantation Results**

After s.c. tumor cell injection, the animals were examined three times weekly for evidence of tumor at the site of transplantation. A transplant take was scored when the transplanted tumor reached 6 mm in diameter. Transplant take in the cerebrum was detected by the development of progressive neurological signs. These changes were evident 1 to 2 days before death. Moribund animals were sacrificed and the presence of tumor confirmed by necropsy. Histology was performed on a number of brains in each TD50 assay to confirm that the lesions observed macroscopically were indeed tumor. In no instance was an animal, judged clinically as tumor-positive, not found to have intracranial tumor at necropsy. The tumors were usually 3–6 mm in diameter and could be reliably distinguished from normal cerebral tissue with the naked eye. None of the tumor types tested grew as infiltrating lesions, all formed discrete and distinct nodules. Experiments were terminated at 60–80 days for FaDu and 90–120 days for MCalV and HFSal. The tumor take results at each cell dose were tabulated and a logit regression line plotted through the data. From this the TD50 values and their 95% confidence limits were computed (23). TD50 values were compared using these confidence limits and, where they overlapped, a standard Z test.

**Whole-Body Irradiation**

WBI was performed in a Gammacell 137 cesium unit providing a dose rate of ~0.9 Gy/min over the duration of this experiment. A lucite holder accommodating five mice was used for irradiation. The dose for most experiments was 6 Gy and was administered 24 h prior to transplantation. In one set of assays, WBI was given as 19.4 Gy in 10 equal fractions, with three fractions per day at a 4-h spacing. After the final dose on the fourth day each mouse was salvaged by i.v. injection of 10^6 NCr/Sed (nu/nu) bone marrow cells. The marrow was harvested by gently flushing donor femora with Hank's medium. The cell suspension was adjusted to a concentration of 2.5 × 10^6 nucleated cells/ml. The i.v. injection volume was 0.4 ml.

**Immunization Procedure**

Mice were immunized against a tumor by implanting 5 × 10^5 MCalV or FSal, or 4 × 10^5 FaDu viable tumor cells as appropriate into the right hind limb. The growing tumors were measured three times weekly and when they had reached a mean diameter of 8 mm the tumor-bearing leg was amputated. The TD50 assays were performed 1 week later. For DTH testing, mice were immunized against either MCalV, FaDu, or HFSal by giving three injections of 2 × 10^5 lethally irradiated tumor cells (120 Gy) at Days −21, −14, and −7 before challenge with tumor antigen (24). For the first injection, the irradiated tumor cell suspension was admixed 1:1 with complete Freund's adjuvant; 0.1 ml of this mixture (0.05 ml Freund's adjuvant plus 0.05 ml irradiated tumor suspension containing 5 × 10^6 cells) was injected s.c. over each inguinal and axillary region (total = 2 × 10^6 cells). On Days −14 and −7, 0.1 ml of a simple, irradiated, tumor cell suspension (2 × 10^6 cells) was administered i.p. The TD50 assays were performed 1 week after the final injection.

**Preparation of Tumor Antigens**

20 g of tumor tissue were homogenized in 30 ml of Hanks’ balanced salt solution at 2,000 rpm for 45 min. The homogenate was aspirated and centrifuged at 450 × g for 40 min. The supernatant was collected and ultracentrifuged at 100,000 × g for 1 h, and the clear supernatant frozen in 1-ml aliquots.

**Delayed-type Hypersensitivity Testing**

Mice were treated as described by Svboda et al. (25) in groups of eight to 10. On Day 0, the dorso-plantar thicknesses of the right hind feet were measured with a Vernier gauge. The dorsum of the foot was then injected s.c. with 0.05 ml of either tumor antigen solution or Hanks' solution using a 22-gauge needle. Nonspecific reactions subsided over the first 24 h. DTH peaked at 48 h. Repeat measurements of foot thickness were made at 48 h after injection. Any increase in thickness of the feet of test animals was compared with that for control animals using the Mann-Whitney U Test. DTH was scored when the increase in thickness of tumor antigen-injected feet differed significantly (P < 0.05) between control and immunized groups.

**FaDu Tumor Infiltrate Analysis**

Single cell suspensions were prepared as described from two to four tumors for each analysis. Nonmalignant cells were separated from tumor cells using Lymphocyte Separation Medium (Organon Teknika, Durham, NC) and centrifugation at 1000 × g for 30 min. A portion of the cell suspension was fixed in 70% ethanol for 1 h and stained with propidium iodide (10 µg/ml) for DNA analysis. The tumor-associated mononuclear cells were incubated with the fluorescent monoclonal antibodies anti-Thy 1.2, -GAM IgG (Becton Dickinson, Mountain View, CA), and -ASGM-1 (Wako Pure Chemical Industries, Wako, TX), and analyzed on an Ortho Spectrum III flow cytometer to determine the proportion of labeled cells for each antibody. The propidium iodide sample was used to determine the proportion of normal and tumor cells using the Mann-Whitney U Test. DTH was scored when the increase in thickness of tumor antigen-injected feet differed significantly (P < 0.05) between control and immunized groups.
TRANSPANTABILITY OF TUMORS INTO BRAIN AND S.C. TISSUES

RESULTS

Isotransplantability of FSAI and MCalV in Normal or Immune-modified C3Hf/Sed Mice. Two tumors, a highly immunogenic fibrosarcoma, FSAI, and a very weakly immunogenic mammary carcinoma, MCalV, were examined in a series of comparative TDso assays (Table 1). The subcutaneous TDso values for MCalV have been given previously (26). For both tumors, the intracerebral TDso was lower than the s.c. TDso. The ratio TDso s.c./i.c. was, in three separate experiments, only slightly higher for FSAI (3.2, 4.2, 6.9) than for MCalV (2.5) (Table 2). Neither the s.c. nor the intracerebral TDso values for MCalV were altered by preimmunization or 6 Gy whole-body irradiation. For FSAI, however, TDso values were greatly increased by preimmunization but reduced only slightly by prior WBI. This was true for the intracerebral as well as the s.c. site (the ratios TDso control/TDso WBI being 2.1 and 2.6 for i.e. and s.c., respectively).

Fig. 1A shows discrete MCalV tumors growing at the right cerebrocerebellar junctions in the brains of C3H mice. The lesions are readily distinguishable with the naked eye. Histological sections are shown in Fig. 1B.

Allotransplantability of MCalV into Control, Preimmunized, and 6 Gy Whole-Body Irradiated NCrf/Sed Nude Mice. When >10⁶ MCalV cells were injected into the s.c. tissues of NCrf/Sed (nu/nu) heterozygous mice, no tumor growth resulted. Temporary growth followed by complete regression occurred if the heterozygotes were first given 6 Gy WBI. MCalV is allogeneic to this strain of mouse and thus rejected. To assess the immunological reactivity, the ratios were 0.6 for s.c. and 1.6 for i.e. preimmunization but reduced only slightly by prior WBI. This was true for the intracerebral as well as the s.c. site (the ratios TDso control/TDso WBI being 2.1 and 2.6 for i.e. and s.c., respectively).

Table 1 Transplantability of FSAI and MCalV into the cerebral and s.c. tissues of syngeneic C3Hf/Sed mice

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Experiment</th>
<th>6GY WBI</th>
<th>Control</th>
<th>Preimmunization</th>
<th>6GY WBI</th>
<th>Control</th>
<th>Preimmunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSAI</td>
<td>1</td>
<td>9.0 × 10⁶</td>
<td>&gt;10⁶</td>
<td>(6.3…12.8)*</td>
<td>2.8 × 10⁴</td>
<td>(1.9…4.0)</td>
<td>5.5 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.2 × 10⁴</td>
<td>1.3 × 10⁴</td>
<td>(1.8…9.9)</td>
<td>1.0 × 10⁴</td>
<td>(0.6…1.6)</td>
<td>&gt;10⁴</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.5 × 10⁵</td>
<td>1.7 × 10⁴</td>
<td>(5.1…8.4)</td>
<td>1.2 × 10⁵</td>
<td>(0.9…1.6)</td>
<td>(1.5…4.4)</td>
</tr>
<tr>
<td>MCalV</td>
<td>1</td>
<td>1.2 × 10⁸</td>
<td>1.4 × 10⁸</td>
<td>(0.8…1.7)</td>
<td>6.8 × 10⁴</td>
<td>(4.9…9.3)</td>
<td>5.0 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.0 × 10⁷</td>
<td>1.9 × 10⁷</td>
<td>(0.8…2.4)</td>
<td>3.2 × 10⁴</td>
<td>(2.4…4.4)</td>
<td>(2.9…8.6)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.9 × 10⁹</td>
<td>3.2 × 10⁹</td>
<td>(2.1…4.4)</td>
<td>2.8 × 10⁸</td>
<td>(1.8…4.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.1 × 10⁷</td>
<td>2.8 × 10⁸</td>
<td>(5.6…8.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, 95% confidence limits.

Fig. 2 presents a plot of TDso versus time after transplantation; this demonstrates no obvious differences in latency period for tumor development between the three groups of mice at either transplantation site.

Xenotransplantability of FaDu, U87, and HFSAl into the Brain and s.c. Tissues of Control and Immune-modified NCr/Sed Nude Mice. To assess whether any of the three human tumors were capable of evoking a primary immune response from their nude mouse hosts, TDso assays were performed using the s.c. and cerebral sites (Tables 2 and 3). The ratios of s.c. to i.e. TDso were significantly greater than unity for all three: FaDu, 51.4, 6.3, and >14.7; U87, 26.7; and HFSAl, >50. Whole-body irradiation reduced the s.c. TDso values for FaDu and HFSAl (the ratios of TDso control/TDso WBI were 3.2, 2.6, and 3.2 for FaDu, and 2.9 and 3.0 for HFSAl). The s.c. TDso for U87 was reduced in two separate assays (ratios of 1.7 and 1.6), but significantly so in only one experiment. The s.c. TDso for FaDu was decreased further by raising the dose to 19.4 Gy (10 equal doses, three fractions per day) (Table 4). The ratios TDso 6 Gy/TDso 19.4 Gy WBI were 2.0 and 3.1 in separate experiments. Provided bone marrow rescue was given, there was no significant mortality from the 19.4 Gy. The LDso for WBI given in 10 fractions was 22.8 Gy with an LD10 of 21.0 and an LD90 of 24.7 Gy.

Whilst whole-body irradiation enhanced the s.c. transplantability of FaDu, it had no effect at the i.e. site. This implies that in normal NCr/Sed nude mice primary immunity may be expressed s.c. but is virtually absent in the relatively immune privileged brain.

In two separate assays, the s.c. TDso value for FaDu was increased by preimmunization, but in neither case did this achieve significance.

Fig. 1, C and D, shows photomicrographs of FaDu and HFSAl growing in the brains of nude mice; there is a lack of cellular infiltration and discrete brain-tumor boundaries are evident.

Delayed-type Hypersensitivity Reactions of Immunized NCr/Sed Nude Mice Rechallenged with Tumor Antigen. Results in Tables 2 and 3 clearly show that residual immunity in the nude mouse can influence the xenotransplantation of human tumors. The more closely related allogeneic MCalV did not elicit a

that this allogeneic tumor is not detectably immunogenic in these mice.

The ratios s.c. TDso/i.e. TDso were significantly greater than one for control and WBI mice (4.3 and 4.9). The only ratio not significantly greater than one obtained for preimmunized mice (1.9). Thus, there are nonimmunological factors which contribute to the greater transplantability of MCalV into the cerebral than the s.c. tissue.
Table 2: Relative transplantability of two murine and three human tumors into the cerebral and s.c. tissues of either C3Hf/Sed or athymic NCr/Sed nude mice

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Host</th>
<th>s.c. TD50 control</th>
<th>s.c. TD50 6GY WBI</th>
<th>s.c. TD50 immun.</th>
<th>s.c. TD50 control</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFSal</td>
<td>NCr/Sed (nu/nu)</td>
<td>2.9, 3.0</td>
<td>1.5, 2.4*</td>
<td>51.4, 6.3, &gt;14.7</td>
<td>&gt;50.0</td>
</tr>
<tr>
<td>FaDu</td>
<td>NCr/Sed (nu/nu)</td>
<td>1.7, 1.6*</td>
<td></td>
<td></td>
<td>26.7</td>
</tr>
<tr>
<td>MCalV</td>
<td>C3Hf/Sed</td>
<td>1.2*, 1.1*</td>
<td>1.3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCalV</td>
<td>C3Hf/Sed</td>
<td>2.7, 2.1</td>
<td>30.2, &gt;11.0</td>
<td></td>
<td>3.2, 4.2, 6.9</td>
</tr>
<tr>
<td>FaDu</td>
<td>NCr/Sed (nu/nu)</td>
<td>0.8*</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>FaDu</td>
<td>NCr/Sed (nu/nu)</td>
<td>3.2, 2.6, 3.2</td>
<td></td>
<td></td>
<td>3.5, 4.3</td>
</tr>
<tr>
<td>HFSal</td>
<td>NCr/Sed (nu/nu)</td>
<td></td>
<td></td>
<td></td>
<td>51.4, 6.3, &gt;14.7</td>
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<tr>
<td>U87</td>
<td>NCr/Sed (nu/nu)</td>
<td></td>
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</table>

* Ratio not significantly greater than unity, Z test > 0.05.

Fig. 1. Intracerebrally transplanted tumors. a, MCalV in syngeneic C3Hf/Sed mouse brain showing discrete nodule formation at the right cerebro-cerebellar junction; b, MCalV in syngeneic C3Hf/Sed mouse brain (× 100); c, FaDu in xenogeneic NCr/Sed (nu/nu) mouse brain (× 100); d, HFSal in xenogeneic NCr/Sed (nu/nu) mouse brain (× 100).

demonstrable immune response capable of altering the TD50. To investigate these influences further, the ability of nude mice to respond with delayed-type hypersensitivity on rechallenge with human tumor antigen was tested (Table 5). When control nude mice were challenged in the foot dorsum with any of the three tumor antigen preparations, no significant swelling was visible at 48 h. When nude mice immunized against MCalV were rechallenged no reaction occurred. However, when mice immunized against HFSal were rechallenged with either HFSal or FaDu, antigen-significant DTH was recorded. The FaDu/HFSal cross reactivity shows that the DTH reaction is not directed at tumor-specific antigens.

Mouse Cell Infiltrates in 8–10-mm FaDu Tumors. Of over 1600 FaDu tumors grown in individual nude mice over the course of these experiments, less than 30 (<2%) showed a spontaneous halt in growth or regression. These rare events occurred in control and in whole-body irradiated mice, usually about 2 to 3 months after transplantation. Four such arrested/regressing tumors from control mice were taken at a comparable size (8–10 mm mean diameter) and the proportion of human to mouse cells assessed by flow cytometry. The mouse cell infiltrate was characterized and compared with that of similar sized but progressively growing tumors. A small proportion of cells carry more than one of the test antigens and are therefore detected by more than one antibody; when totalled the percentages total percentage figures, thus, exceed 100. Table 6 indicates that the proportion of mouse cells was greater in the regressing tumors (58 versus 34%) and that of these 16.6% were T-cells as
Table 3  Transplantability of one allogeneic and three human tumors into the cerebral and s.c. tissues of athymic NCr/Sed nude mice

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Experiment</th>
<th>6GY WBI</th>
<th>s.c. TD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Control</th>
<th>Preimmunization</th>
<th>i.c. TD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Control</th>
<th>Preimmunization</th>
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<tr>
<td>MCalv</td>
<td>1</td>
<td>11.2 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>9.0 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>7.0 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2.3 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>(5.8 ... 21.9&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>(4.2 ... 19.3)</td>
<td>(2.9 ... 16.7)</td>
<td>(1.7 ... 3.1)</td>
<td>(1.4 ... 3.2)</td>
<td>(2.4 ... 5.5)</td>
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<tr>
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<td></td>
<td>2</td>
<td>3.7 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>(6.9 ... 13.3)</td>
<td>(14.6 ... 43.6)</td>
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<td>(0.8 ... 6.6)</td>
<td>(4.3 ... 7.7)</td>
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<td></td>
<td>5</td>
<td>2.9 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>4.5 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>(1.7 ... 5.0)</td>
<td>(2.6 ... 7.7)</td>
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<td>(2.4 ... 5.7)</td>
<td>6.0 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>25.1 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
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<sup>a</sup> Numbers in parentheses, 95% confidence limits.
<sup>b</sup> Immunization by amputation of limb bearing 8-mm tumor.
<sup>c</sup> Immunization by injection of 2 x 10<sup>6</sup> lethally irradiated cells.
<sup>d</sup> 49 day data only.

DISCUSSION

The nude mouse has value as a system for assessing the in vitro efficacy of a variety of antineoplastic therapies. There are, nonetheless, serious difficulties with the model. An important one is the uncertain impact on the observed tumor response by the low level immune reactivity of nude mice. An optimal model for experimental therapeutics would have either no or a weak, but well defined, residual immune reactivity against the tumor.
The experiments described here examined the magnitude of the residual reactivity and assessed the efficacy of whole-body irradiation in minimizing it. Other immunosuppressive strategies are currently being assessed.

The TD50 assay was chosen for the ease of quantitating small differences in resistance to transplantation between normal and immune-modified recipients (24, 26). The data obtained using two syngeneic tumors in C3Hf/Sed mice demonstrate this. For both tumors the TD50 values obtained for transplantation in s.c. tissue were significantly higher than for cerebral tissue. The s.c./i.c. TD50 difference was larger in three separate experiments for FSaI (s.c./i.c. ratios of 3.2, 4.2, and 6.9) than for MCAIV (2.5). The small difference in the s.c./i.c. ratio between FSaI and MCAIV indicates that nonimmunological factors account for an important component of the enhanced i.c. transplantability. This interpretation is supported by the report of Peters who showed that the s.c. TD50 of two spontaneous murine tumors (a carcinoma and a sarcoma of CBA mice) could be reduced by simply mixing the viable cells with a homogenate of syngeneic brain tissue prior to transplantation (27).

That the intracerebral TD50 for FSaI could be slightly but significantly reduced by WBI and greatly increased by immunization, whereas values for the nonimmunogenic MCAIV remained unchanged, indicates that: (a) primary immunity may be expressed at the cerebral site in an immune-competent animal, and (b) the brain does not confer detectable protection against a vigorous secondary response in an immune-competent animal. This work supports studies by Scheinberg who showed that some rejection of foreign intracerebral tumor can occur after systemic immunization (28). Together they demonstrate that the immunologically privileged status of the brain tissue is far from complete. Evidence that the brain may, nevertheless, still offer protection from a weaker primary response comes from the many studies in which human tumors have been grown intracerebrally in thymic normal rabbits, guinea pigs, and mice (29), and from the work of Epstein (11) and Drewinko (13) who saw enhanced take of human lymphomas and colon carcinomas transplanted i.c. in nude mice.

The data obtained for MCAIV in earlier work (26) demonstrated that this tumor can be transplanted with comparable ease into allogeneic nude and syngeneic intact hosts. Equivalent iso- and allotransplantability was also demonstrated for two other spontaneous murine tumors, SCCVII and FSaI (a squamous cell carcinoma and a fibrosarcoma). That MCAIV did not arouse a detectable immune response from nude mice was shown in the present study by (a) a TD50 preimmunized/TD50 WBI ratio close to unity at both s.c. and i.c. sites, (b) by a TD50 s.c./TD50 i.c. value in control nu/nu mice that was close to the ratio obtained in syngeneic hosts, and (c) absence of DTH on rechallenge. We have also performed a series of TD50 assays using the rat rhadomyosarcoma BA1112 as an isotransplant into WAG/Rij rats and as a xenotransplant into control, whole-body irradiated, or specifically immunized NCR/Sed nude mice (30). BA1112 showed the same s.c. TD50 values for both its syngeneic rat hosts and the xenogeneic nude mice. The TD50 values obtained from WBI and immunized mice indicate that this tumor, despite its origin in a foreign species, is also not detectably immunogenic in the NCR/Sed (nu/nu) animal.

These results contrast with those for the human tumors FaDu, U87, and HFSal (Tables 2–6). This suggests that large histocompatibility differences between tumor and the nude mouse are necessary in order to elicit an immune response detectable even by this quantitative assay technique. For the three human tumors, the intracerebral TD50 was substantially and significantly lower than the s.c. TD50. The difference ranged from 6- to 51-fold in different assays. Such a wide range might reflect limitations in the resolution of the assay; however, the constant values obtained for FaDu on repeated intracerebral testing (Table 3) indicate that this is not the case. Most of the variation seen when comparing i.c. and s.c. TD50 values occurs because of variability in transplantation into s.c. tissue. A convincing explanation for this phenomenon is not known to the authors. The TD50 value for FaDu transplanted s.c. was not reduced by prior admixture of the tumor cell suspension with either a brain tissue homogenate or with lethally irradiated cells.4

WBI reduced the s.c. TD50 for FaDu into nude mice three-fold but had no influence on the TD50 in the immune-protected i.c. site. This argues, firstly, that WBI may decrease the host’s influence on s.c. transplantability, and secondly, that the i.c. value may approach an “ultimately” low TD50 for that tumor in nude mice. If so, in assessing the effect of any immunosuppressive measure on nude mice, the degree to which the s.c. TD50 can be brought towards the i.c. TD50 may be a yardstick of its success. The studies with MCAIV indicate that the ratio may not go below 2–3 because of nonimmunological factors which favor transplantation intracerebrally. The implication of the high s.c./i.c. ratio for FaDu still detectable after 6 Gy WBI, is that a low level of either radiation-resistant or rapidly recovering immune reactivity persists against the s.c. transplanted tumor.

The TD50 values obtained for FaDu in 19.4 Gy-fractionated WBI nude mice were significantly lower in repeated experiments than those obtained after the 6-Gy single dose. NK cells have been reported, on the basis of studies performed up to 20 hours after in vitro irradiation, to be relatively radioresistant compared with other lymphoid subgroups (31, 32). They are, however, not totally resistant. Hochman observed that NK activity was undetectable in the spleens of mice 2 weeks after 7 Gy WBI (33). Similar results have been reported from this laboratory following 6 Gy WBI (15). The increase in dose from 6 Gy to 19.4 Gy will reduce further the numbers of intact lymphoid cells in each category and hence, result in further reduction in immune reactivity.

Whilst the transplant rejection reaction of conventional immune-competent animals is T-cell mediated, the mechanisms inhibiting tumor growth in nude mice (T, B, NK, macrophage, etc.) have not been fully defined. Because of the athymic state, the enhancing effect of pretreatment with either cyclophosphamide (2, 3, 7, 34), antilymphocyte serum (4, 5), WBI (6), or corticosteroids (9), on human tumor take rates has been ascribed to suppression of non-T-cell immunity. Data from this laboratory have demonstrated that, despite the absence of a thymus, NCR/Sed nude mice retain a readily detectable population of cells in the spleen staining with a monoclonal antibody directed against the Thy. 1.2 T-cell marker (15). At 8 weeks of age, 23% of splenocytes from heterozygous nu/+ females stain for this antigen. The figure is only 2.7% for nu/nu females, but as much as 8.3% for nude males. Other workers have reported similar T-cell numbers in nude mice (9). A subset of NK cells weakly expresses thymic antigens, but they alone do not account for the “T” cells found in nude mice which are capable of performing a range of normal, and exclusively, T-cell functions. The most characteristic of these is the capacity to generate allo- and xeno-, and H-2-restricted cytotoxicity (16, 17).

Table 5 illustrates that nude mice have an immunological

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4 A. L. Zietman, unpublished data.
memory and are capable of mounting a DTH reaction on rechallenge with human tumor antigen. This phenomenon, in conventional mice, is principally T-cell mediated. DTH was expressed against both human tumors investigated but not against the allogeneic MCAv. WBI depressed the s.c. TD50 for the two human tumors, but not for MCAv. Further, T-cell numbers in the spleens of these nude mice are severely and chronically suppressed by a single dose of 6 Gy (15). These findings are consistent with the notion that a principal effect of 6 Gy WBI may be against an active T-cell residuum.

The importance of the residual T-cell population was also indicated by the ~2% of FaDu tumors which had either ceased to grow or had begun to regress. The host cell infiltrate was more abundant in the static than the progressively growing tumors and contained a higher proportion of cells staining for the Thy. 1 marker. T-cell immunity is classically described as “adaptive,” meaning that it can be enhanced by previous exposure to a specific antigen. Table 3 shows that in two separate experiments using two different methods of immunization, the s.c. TD50 was higher than controls, but in neither case to a significant degree. After WBI all lymphoid subgroups concerned with resistance to a major histocompatibility complex-mismatched tumor (T, B, and NK) are depleted and, together with the nonimmunological enhancement associated with this procedure, a perceptible alteration in TD50 is seen. By contrast, immunization does not augment NK activity, nor does it stimulate any nonimmunological inhibition. It is, therefore, conceivable that, though present, any changes in immune activity brought about by this procedure are below the level of detection by the TD50 assay.

Our conclusion is that detectable, but low magnitude, immune reactivity remains in the nude mouse and that this influences the xenotransplantability (especially in the s.c. tissue) of some human tumors. Whole-body irradiation and intracranial transplantation are useful strategies to enhance the tumor take rate.

REFERENCES

Quantitative Studies on the Transplantability of Murine and Human Tumors into the Brain and Subcutaneous Tissues of NCr/Sed Nude Mice


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