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Quantitative Transplantation Assays of Spontaneous Tumors of the C3H Mouse as Allografts in Athymic NCr/Sed-*nu/nu* Nude Mice and Isografts in C3Hf/Sed Mice[1]

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ABSTRACT

Three spontaneous tumors of the C3H mouse have been used in a comparison of their transplantability and radiation response (local control) in syngeneic C3Hf/Sed mice and in allogeneic athymic NCr/Sed-*nu/nu* nude mice. The tumors were: MCaIV, a moderately well-differentiated mammary carcinoma; FSaII, a poorly differentiated fibrosarcoma; and SCCVII, a moderately well differentiated squamous cell carcinoma. The tumors were studied as fourth to seventh generation transplants. Assays to determine the number of tumor cells that, on the average, transplant the tumor to half of the recipients or transplant sites (TD_{50}) demonstrated that these 3 tumors transplanted into the s.c. tissue of the NCr/Sed-*nu/nu* as readily as of C3Hf/Sed mice. The TD_{50} for MCaIV was slightly but significantly lower in 4-week-old NCr/Sed-*nu/nu* mice which had received 6 Gy whole body irradiation (WBI) 24 h before transplantation, namely, 5.8×10^4 (95% confidence limits, 4.5–7.6) *versus* 7.8×10^4 (6.0–10.0). The 6-Gy WBI did not affect the TD_{50} for 8- to 10-week-old mice. Similarly, the TD_{50} for SCCVII was lower in 6-Gy WBI NCr/Sed-*nu/nu* recipients (1.5×10^4 *versus* 3.9×10^4). The TD_{50} for FSaII was not affected by 6-Gy WBI. Further, the TD_{50} for FSaII following i.v. injection of tumor cells (transplant to lung) was the same for C3Hf/Sed and NCr/Sed-*nu/nu* mice (this obtained for normal or 6-Gy WBI-treated subjects). The radiation doses which on the average achieve control of half of the MCaIV, FSaII, and SCCVII tumors were lower, higher, and the same in NCr/Sed-*nu/nu* than in C3Hf/Sed mice, respectively. The radiation doses which achieve control of half of the MCaIV and SCCVII tumors were not affected by 6-Gy WBI before transplantation.

INTRODUCTION

Xenografts of human tumors growing in athymic nude mice are used extensively in studies of the pathophysiology of human tumors and their response to various therapeutic strategies (1, 2). In investigations of the response of xenografts to localized radiation, concern has been raised as to the confounding effect of residual immune reactivity by the nude mouse against the xenograft (3). This constitutes a potential difficulty in analysis of results where the radiation dose is sufficient to effect local control of tumor. In that circumstance only a few cells survive the radiation treatment in some of the subjects, and a low level immune reaction against the tumor could affect the result to a significant degree. Experimental studies have shown that the radiation dose required to achieve complete and permanent regression of strongly immunogenic tumors is much lower for tumors growing in normal than in immunosuppressed hosts (4, 5). Further, the required dose increases with the severity of the immune suppression.

One of our research goals is to assess the relative radiation response of the reputedly highly radiation resistant human glioblastoma (6) *versus* the known radiation responsive human squamous cell carcinoma by using xenografts in nude mice. The experimental aim is to characterize radiobiologically these human tumor xenografts when treated at a constant size (and presumably clonogen number) and anatomic site by a standard radiation technique in one laboratory. Concurrently, cell lines from these tumors are to be evaluated radiobiologically *in vitro* with the use of single cells and spheroids. The end points for the *in vivo* studies are to be growth delay and local control. Before initiating the more complex and protracted studies on human tumor xenografts, we planned to evaluate the extent to which immune suppressive procedures could render the nude mouse immunologically blank. Namely, could the perturbing effect of the residual immune capacity of the nude mouse be abrogated and the response of the xenografted tumor be analyzed with minimal allowance for an antitumor immune reaction. For this part of the project, a series of assays have been performed of tumor transplantability and radiation response (local control), using 3 spontaneous tumors as syngeneic transplants in C3Hf/Sed mice or allogeneic transplants into normal or immune modified athymic nude mice.

MATERIALS AND METHODS

Experimental Assays. These experiments used assays which yield quantitative descriptions of the tumor transplantability, the TD_{50},[2] and the response (local control) to radiation, the TCD_{50}. The TD_{50} is the number of tumor cells which would be expected on the average to transplant the tumor to half of the recipients or transplant sites (in assays where 4 sites were used per recipient). Similarly, the TCD_{50} is the radiation dose which on the average would be expected to achieve control of half of the tumors irradiated.

Experimental Animals. The mice used in this study were bred and maintained in our defined flora, pathogen-free mouse colony. The C3Hf/Sed mouse has been maintained in this colony for 16 years under continuous brother-sister mating. The history and designation of this line of mice has been described (7). The athymic NCr/Sed-*nu/nu* nude mice are a line of outbred Swiss mice which had the BALB/c nude gene inserted by backcross breeding technique. Our breeding stock of the NCr-*nu/nu* and NCr-nu/+ mice were generously provided by Dr. Carl Hansen of the National Cancer Institute and have been maintained in our colony for more than 25 generations. The athymic NCr/Sed-*nu/nu* nude mouse has proven to be very healthy in this colony. We achieve a production index of $\simeq$1.00 with nearly all the nude pups surviving. At age 4–10 weeks they are placed on experiment. The gross mortality incidences among the NCr/Sed-*nu/nu* mice over 120 days in experiment, for causes other than tumors, were 1.5% and 11.6% in control and 6-Gy WBI mice (pooled data from 8 sets of experiments). For the C3H mice the losses were $\simeq$1%.

Tumors and Transplantation Technique. The 3 spontaneous tumors of the C3H mouse used in these studies have been described previously (8). These are: MCaIV, a moderately differentiated mammary adenocarcinoma; FSaII, a poorly differentiated fibrosarcoma; and SCCVII, a moderately differentiated squamous cell carcinoma. They have been studied as fourth to seventh generation transplants. Quantitative cell transplantation assays were performed by using serial dilutions of

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[2] The abbreviations used are: TD_{50}, number of tumor cells on the average expected to transplant the tumor to half of the recipients or transplant sites; TCD_{50}, radiation dose on the average expected to achieve control of half of the tumors irradiated; WBI, whole body irradiation.

suspensions of single cells. Suspensions of cells were prepared by a mechanical method. Cell viability was determined by trypan blue dye exclusion. For the TD_{50} assays, from one cell suspension, 2- to 4-fold dilutions were made and 4–10 animals were given injections of 0.1-ml suspension at each of 5–8 cell dose levels. This was performed at one session on both the C3Hf/Sed and NCr/Sed-*nu/nu* mice. Unless otherwise noted, equal numbers of male and female mice were used in an assay. For the TD_{50} assays, tumors were transplanted into the s.c. tissues of the right flank and more recently the right axilla, one site per animal. In the first assays, transplantation was made into the right and left axilla and flank (4 sites per animal, the same cell dose at each site in an individual mouse). The results from these assays were indicated in Tables 1–3. In those few assays, for any one animal, tumors were excised as they reached 8 mm until transplant take had been scored for 3 sites at the end of the experiment. In the TCD_{50} assays, transplantation was made in the right leg.

Radiation. Local radiation was administered on the day the mean diameter of the tumor was 6 mm by using a specially designed ^{137}Cs irradiator which features parallel opposed 3-cm diameter fields (9). The mice were anesthetized by an i.p. injection of sodium pentobarbital, 0.05 mg/g body weight. To avoid differences with respect to the comparative blood flow and tissue oxygen status between isografts and allografts, all irradiations of tumors were performed under conditions of clamp hypoxia (10). This assured that all of the cells in all of the tumors were hypoxic during the irradiation. The dose rate during the period of these assays was ≃7.5 Gy/min. Whole body irradiation was performed by using a standard AECL Gamma Cell Irradiator with parallel opposed ^{137}Cs sources. Animals were irradiated in groups of 5. The dose rate was ≃0.9 Gy/min.

Immunization. Specific immunization against tumor was attempted by one of two procedures. First, 2×10^7 lethally irradiated cells (120 Gy) were injected at days 21, 14, and 7 before challenge with viable cells (11). For the first injection, the cells were admixed with complete Freund's adjuvant and injected into each groin and axilla. On days −14 and −7, simple suspensions of 2×10^7 cells were injected i.p. This procedure was used unless otherwise indicated. Secondly, a transplanted tumor was allowed to grow to 8 mm in the leg; the leg was amputated and the TD_{50} assay performed 7–10 days later.

End Points and Analysis of Results. For the TD_{50} assays, transplant take was scored when the tumor reached 10 mm. Tumor transplant take results were tabulated as a function of cell dose and a logit regression line was fitted through the data (10); the TD_{50} and its 95% confidence limits were then computed. The end point in the TCD_{50} assays was local control at 120 days (absence of palpable tumor). Mice with recurrent tumors were sacrificed at tumor size of 12 mm. All mice surviving at 120 days were sacrificed and subjected to necropsy examination for residual or metastatic tumor. Animals dying before day 120 with local control (*e.g.*, distant metastases, lymphoma) were excluded from the analysis. The tumor control results at 120 days were tabulated, a logit regression line was fitted, and the TCD_{50} value with 95% confidence limits was computed (10).

Statistical Methods. Ninety-five % confidence intervals were used to compare separate TD_{50} or TCD_{50} data. When these overlapped, a standard Z test was used to discriminate statistically significant differences that may still exist.

RESULTS

MCaIV transplanted as readily into the allogeneic 4-week-old NCr/Sed-*nu/nu* mice as into the syngeneic C3Hf/Sed mice as shown by the data given in Table 1. For the 8-week-old mice the TD_{50} was marginally higher in the NCr/Sed-*nu/nu* recipients. The TD_{50} values in both the C3Hf/Sed and the NCr/Sed-*nu/nu* mice tended to be higher for transplantation into MCaIV immunized recipients (achieving significance in experiment 1 for the C3Hf/Sed mice) and lower in 6-Gy WBI recipients. We have examined further for an effect of 6-Gy WBI or immunization to modify the TD_{50} in separate experiments (experiments 2 and 3). For transplantation of MCaIV into C3Hf/Sed mice, the TD_{50} was significantly higher in preimmunized than in 6-Gy WBI animals (Z test; $P < 0.05$). However, neither of those TD_{50} values differed from the TD_{50} for control mice. In comparable, but separate, assays in NCr/Sed-*nu/nu* mice, there was no effect of 6-Gy WBI or preimmunization on acceptance of MCaIV transplants. In experiments performed in the mid-1960s, the TD_{50}s for MCaIV transplanted into C3H/He mice were modestly but significantly increased by prior immunization (5.0×10^4 to 13.3×10^4) (11). However, 4.7-Gy[3] WBI did not affect transplantability into F_1 hybrid mice (12).

The TD_{50} values for SCCVII were the same for syngeneic and allogeneic transplantation into normal recipients (Table 1). The TD_{50} was marginally but significantly lower in 6-Gy WBI NCr/Sed-*nu/nu* mice.

The TD_{50}s for transplantation of FSaII into the s.c. tissue are also shown in Table 1; comparable values were obtained for syngeneic and allogeneic transplantation for the various experiments. The TD_{50} in NCr/Sed-*nu/nu* mice was not modified by 6-Gy WBI or immunization.

In addition, for FSaII, TD_{50} assays were performed by using i.v. (essentially transplantation to lung) injection of viable tumor cells. As shown by Table 2, the TD_{50}s were the same for i.v. injection of tumor cells (transplant to lung) into C3Hf/Sed and NCr/Sed-*nu/nu* mice. Whole body irradiation caused a significant (Z test, $P < 0.05$), lowering of TD_{50} for both syngeneic and allogeneic transplantation. The numbers of tumor nodules at 13–14 days after i.v. injection of FSaII cells were the same in NCr/Sed-*nu/nu* and C3Hf/Sed mice at all cell doses used in both normal and 6-Gy WBI recipients in 2 of 3 experiments. In one experiment, the numbers of tumor nodules were lower in the NCr/Sed-*nu/nu* mice. In each of the 3 assays, the numbers of nodules in lungs were higher in the mice which had received 6-Gy WBI. The mean survival times after i.v. injection of FaII cells were the same for the NCr/Sed-*nu/nu* and the C3H mice. The observed lower TD_{50} values and higher number of tumor nodules in the 6-Gy WBI recipients is not proof that an immune rejection reaction had been suppressed. The phenomenon of greater transplantability in irradiated lung is well known and obtains with nonimmunogenic tumor systems (13).

The final group of experiments were assays of local control of 6-mm tumors as a function of radiation dose. The resultant TCD_{50} values are shown in Table 3. TCD_{50} values were significantly lower for MCaIV when growing in normal or 6-Gy WBI NCr/Sed-*nu/nu* mice than in normal C3Hf/Sed mice as shown in two independent experiments. There was, however, no significant effect of the 6-Gy WBI to increase the TCD_{50}. Local control of FSaII required significantly higher doses for allografts in the NCr/Sed-*nu/nu* mice than transplants in the syngeneic C3Hf/Sed mice. For SCCVII, the TCD_{50} values were the same for tumors in C3Hf/Sed and NCr/Sed-*nu/nu* mice. For both MCaIV and SCCVII, the TCD_{50} was unaffected by 6-Gy WBI 24 h before transplantation. This does not support an immune rejection reaction as responsible for TCD_{50} MCaIV being lower in NCr/Sed-*nu/nu* mice. In contrast, the TCD_{50} for FSaII was significantly higher in the NCr/Sed-*nu/nu* than in the C3Hf/Sed mice; namely, the opposite to that expected were the NCr/Sed-*nu/nu* mounting an immune rejection against the FSaII.

[3] Radiations were 250 kVp X-rays. Dose is expressed here in terms of ^{60}Co Gy equivalent units, namely, doses in 250 kVp rads were multiplied by relative biological effectiveness factor of 1/0.85.

Table 1 *TD_{50} values for syngeneic and allogeneic transplantation of MCaIV, FSaII, and SCCVII into the s.c. tissues of control, 6-Gy whole-body irradiated, or "immunized" recipients*

Experiment	Recipient age (wk)	Status	TD_{50} values C3Hf/Sed	TD_{50} values NCr/Sed-*nu/nu*
MCaIV				
1	4	Control	11.0×10^4 (8.6–14.0)[a]	7.8×10^4 (6.0–10.0)
		6-Gy WBI	7.4×10^4 (4.7–12.0)	5.8×10^4 (4.5–7.6)
	8	Control	7.1×10^4 (5.6–8.9)	10.1×10^4 (6.9–15.0)
		Immunized	24.0×10^4 (11.0–51.0)	14.0×10^4 (1.8–10.0)
2 and 3	8–10	Control	14.3×10^4 (8.4–24.4)	9.0×10^4 (4.2–19.3)
		6-Gy WBI	11.8×10^4 (8.3–16.8)	11.2×10^4 (5.8–21.9)
		Immunized	18.7×10^4 (13.3–26.2)	7.0×10^4 (2.9–16.7)
FSaII				
1	4	Control	12.3 (5.3–28.5)	1.0 (0.5–2.0)
2	8–10	Control	2.6 (1.6–4.3) 3.8 (2.1–6.8)	2.0 (1.4–2.8)
		6-Gy WBI		1.2 (0.8–1.6) 1.5 (1.4–2.8)
		Immunized		2.2 (1.3–2.8)
3	10	Control		2.0 (1.4–2.8)
		6-Gy WBI		1.2 (0.8–1.6)
	4	Control		1.3 (1.0–1.8)
		6-Gy WBI		1.5 (1.1–2.1)
SCCVII				
1	4	Control	3.0×10^4 (1.3–11.0)	3.9×10^4 (1.3–11.0)
		6-Gy WBI	$>10^4 < 10^5$	1.5×10^4 (0.6–3.6)

[a] Numbers in parentheses, 95% confidence limits.
[b] Immunized by allowing tumor transplant in leg to grow to 8 mm; the leg was amputated and the TD_{50} assay was performed 7–10 days later.

Table 2 *FSaII tumor nodules in lung at 13–14 days, mean survival times, and TD_{50} values following i.v. injection of FSaII cells into C3Hf/Sed and NCr/Sed-nu/nu mice*

No. of FSaII cells	C3Hf/Sed Normal	C3Hf/Sed 6-Gy WBI	NCr/Sed-*nu/nu* Normal	NCr/Sed-*nu/nu* 6-Gy WBI
	Mean no. of tumor nodules in lungs			
Experiment 1				
4×10^3	50.4 ± 18.8[a]	67.5 ± 37.6	13.2 ± 12.2	28.5 ± 21.0
16×10^3	98.2 ± 65.5	127 ± 31.0	36.8 ± 25.8	51.2 ± 44.4
Experiment 2				
4×10^3	1.8 ± 1.3	3.4 ± 1.4	2.0 ± 1.9	3.3 ± 2
16×10^3	5.0 ± 3.8	12.9 ± 8.0	8.1 ± 5.1	13.6 ± 10.0
Experiment 3				
16×10^3	35.3 ± 12.7	47.5 ± 12.5	33.9 ± 10.9	64.2 ± 23.5
	Mean survival times (days)			
Experiment 2				
4×10^3	27.0	20.0	25.0	24.0
16×10^3	21.0	16.9	16.4	18.0
	TD_{50} values			
Experiment 4	16.5 (7.9–34.5)[b]	5.1 (2.4–10.4)	18.5 (61–56.3)	6.3 (3.5–11.4)

[a] One standard deviation.
[b] Numbers in parentheses, 95% confidence limits.

Table 3 *TCD_{50} values for 6-mm diameter isotransplants and allotransplants of MCaIV, FSaII, and SCCVII treated by single doses under clamp hypoxia*

Tumor	TCD_{50} at 120 days C3Hf/Sed	NCr/Sed-*nu/nu*	NCr/Sed-*nu/nu* (6-Gy WBI)
MCaIV	70.1 (66.9–73.4)[a]	58.6 (52.2–65.7)	60.7 (58.3–63.2)
	65.9 (64.5–67.3)	62.5 (59.6–65.4)	63.4 (61.1–65.9)
Pooled	67.6 (66.0–69.2)	61.5 (58.6–64.5)	61.8 (60.0–63.5)
FSaII	70.3 (65.3–75.6)	83.6 (79.2–88.2)	
	72.2 (69.4–75.1)		
	77.6 (74.9–80.4)	82.5 (80.1–84.9)	
SCCVII	66.6 (63.8–69.5)		67.6 (64.5–70.9)
		64.7 (51.1–81.9)	69.9 (64.3–75.9)
	74.1 (71.6–76.6)	71.3 (66.6–76.3)	70.8 (66.7–75.1)

[a] Numbers in parentheses, 95% confidence limits.

DISCUSSION

MCaIV, FSaII, and SCCVII transplanted virtually as readily into the s.c. tissue of normal allogeneic athymic NCr/Sed-*nu/nu* nude mice as into syngeneic C3H mice. This was also true for transplantation to the lung for FSaII, using i.v. injection of tumor cells, using TD_{50}, mean survival time, or number of tumor nodules at 14 days as the end points. Immune modification of the recipients before challenge with viable tumor cells effected only slight changes in TD_{50} values in either syngeneic or allogeneic mice.

The TCD_{50} value would be expected to decrease with effectiveness of the host immune reaction to the tumor. In these experiments, the TCD_{50} values for MCaIV, FSaII, and SCCVII growing in NCr/Sed-*nu/nu* mice were lower, higher, and the same, respectively, than for these three tumors growing in the syngeneic C3Hf/Sed hosts. There is no basis for expecting a higher TCD_{50} for a spontaneous tumor growing in allogeneic nude mice than in syngeneic mice as was found for FSaII in repeat experiments. The TCD_{50} values for MCaIV were 10% lower ($P < 0.05$) when growing in NCr/Sed-*nu/nu* mice. If that lesser TCD_{50} reflected an active immune rejection reaction participating in tumor eradication, a higher TCD_{50} would have been expected for MCaIV transplanted into 6-Gy WBI mice. This was not found. Similarly, 6-Gy WBI had no effect on the TCD_{50} for SCCVII in NCr/Sed-*nu/nu* mice. As noted earlier, 6-Gy WBI was associated with a lower TD_{50} in NCr/Sed-*nu/nu* mice for SCCVII but not for MCaIV. These observed differences in TCD_{50} values for syngeneic and allogeneic transplantation may reflect limitations in the experimental assays and/or be due in part to unknown differences in number of clonogens in tumors measured to be 6 mm growing in the normal and the nude mice.

In conclusion, these results from quantitative tumor cell transplantation and radiation response (local control) assays using MCaIV, FSaII, and SCCVII indicate only very small

differences between the syngeneic C3Hf/Sed and allogeneic NCr/Sed-*nu/nu* mice. Reports from other laboratories have clearly shown that xenografting of human tumor into nude mice is facilitated by whole body irradiation, antisera to natural killer cells, etc. (14, 15). We interpret our present findings as showing that the histocompatibility differences between tumors of the C3H mouse and the NCr/Sed-*nu/nu* mice are not sufficient for the planned research; namely, to assay for changes in immune reactivity following various immune suppressive procedures designed to render the nude mouse essentially immunologically blank to challenge by human tumor xenografts. This assumes that our nude mice would react immunologically against a human tumor xenograft. To test this, experiments need to be performed that use stronger histocompatibility differences between tumor and nude mouse recipient than obtains for these allografted tumors. Studies are planned using xenografts of rat and human tumors transplanted into normal and immune modified athymic nude mice.

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REFERENCES

1. Fogh, J., and Giovanella, B. C. The Nude Mouse in Experimental and Clinical Research, Vol. 2. New York: Academic Press, 1982.
2. Sordat, B. Immune-deficient Animals. 4th International Workshop on Immune-Deficient Animals in Experimental Research, Chexbres, 1982. Basel: S. Karger, 1984.
3. Rofstad, E. K., Wahl, A., and Brustad, T. Radiation response of human melanoma multicellular spheroids measured as single cell survival, growth delay, and spheroid cure: comparisons with the parent tumor xenograft. Int. J. Radiat. Oncol. Biol. Phys., *12:* 975–982, 1986.
4. Suit, H. D., and Order, S. Considerations in the interactions of radiation and immunotherapy. *In:* Interaction of Radiation and Host Immune Defense Mechanisms in Malignancy. Conference, March 1974, Greenbrier, WV. BNL 50418. Brookhaven, NY: Brookhaven National Laboratory, 1974.
5. Stone, H. B., Peters, L. J., and Milas, L. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. J. Natl. Cancer Inst., *63:* 1229–1235, 1979.
6. Walker, M. D., Strike, T. A., and Sheline, G. E. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. Int. J. Radiat. Oncol. Biol. Phys., *5:* 1725–1731, 1979.
7. Sedlacek, R. S., Orcutt, R. P., Suit, H. D., and Rose, E. F. A flexible barrier at cage level for existing colonies: production and maintenance of a limited stable anaerobic flora in a closed inbred mouse colony. *In:* S. Sasaki, A. Ozawa, and K. Hasimoto (eds.), Recent Advances in Germ Free Research, pp. 65–69. Tokyo: Tokai University Press, 1981.
8. Suit, H. D., Sedlacek, R. S., Silver, G., and Dosoretz, D. Pentobarbital anesthesia and the response of tumor and normal tissue in the C3Hf/Sed mouse to radiation. Radiat. Res., *104:* 47–65, 1985.
9. Hranitzky, E. B., Almond, P. R., Suit, H. D., and Moore, E. B. A cesium-137 irradiator for small laboratory animals. Radiology, *107:* 641–644, 1973.
10. Suit, H. D., Shalek, R. J., and Wette, R. Radiation response of C3H mouse mammary carcinoma evaluated in terms of cellular radiation sensitivity. *In:* Cellular Radiation Biology, M. D. Anderson Hospital and Tumor Institute, Houston, TX. pp. 514–530. Baltimore, MD: Williams & Wilkins Co., 1965.
11. Suit, H. D., and Silobrcic, V. Tumor-specific antigen(s) in a spontaneous mammary carcinoma of C3H mice. II. Active immunization of mammary tumor agent free mice. J. Natl. Cancer Inst., *39:* 1121–1128, 1967.
12. Silobrcic, V., and Suit, H. D. Tumor-specific antigen(s) in a spontaneous mammary carcinoma of C3H mice. I. Quantitative cell transplants into mammary tumor agent positive and free mice. J. Natl. Cancer Inst., *39:* 1113–1119, 1967.
13. Peters, L. J., Mason, K. A., and McBride, W. H. Pitfalls in the use of the lung colony assay to assess T-cell function in irradiated mice. Br. J. Cancer, *36:* 386–390, 1977.
14. Ohsugi, Y., Gershwin, M. E., Owens, R. B., and Nelson-Rees, WA. Tumorigenicity of human malignant lymphoblasts: comparative study with unmanipulated nude mice, antilymphocyte serum-treated nude mice, and X-irradiated nude mice. J. Natl. Cancer Inst., *65:* 715–718, 1980.
15. Habu, S., Fukui, H., Shimamura, K., Kasai, M., Nagai, Y., Okumura, K., and Tamaoki, N. *In vivo* effects of anti-asialo GM1. I. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. J. Immunol., *127:* 34–38, 1981.