Eur J Cancer Clin Oncol, Vol. 25, No. 10, pp. 1463–1466, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

Quantitative Transplantation Assays of the Rat Rhabdomyosarcoma BA1112 Isografts into the WAG/Rij Y Rat and Xenotransplantation into the Athymic NCr(nu/nu) Nude Mouse

HERMAN D. SUIT,*‡ SARA ROCKWELL,† ANTHONY ZEITMAN,* VLATKO SILOBRCIC,*§
ELIZABETH A. PORTER,† JONATHAN RAMSAY* and ROBERT SEDLACEK*

**Edwin L. Steele Laboratory for Radiation Biology, Department of Radiation Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, U.S.A. and †Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.*

Abstract—*Quantitative tumor cell transplantation assays have been performed to compare the transplantability of rat rhabdomyosarcoma BA1112 into isologous WAG/Rij Y rats and athymic NCr(nu/nu) nude mice. The end-point was the* TD_{50} *or the number of viable tumor cells which would transplant the tumors into half of the recipients. At Yale, two sets of 2-fold dilutions were prepared, one was sent to the MGH by Air Express. That afternoon, concurrent assays were performed at Yale using the WAG/Rij Y rat and at MGH using the NCr(nu/nu) mouse. The* TD_{50} *values were the same for iso- and xenotransplantation. Furthermore, the* TD_{50}*s in rats and mice were unaffected by standard immunization procedures prior to challenge of the* TD_{50} *assay. The BA1112 (*10^7 *trypan blue excluding cells) grew to* 10–12 mm *and then completely regressed if transplanted into NCr(nu/+) mice which had received* 6 Gy *whole body irradiation but did not grow in control NCr(nu/+) mice. The times for the BA1112 to grow to* 10 mm *were the same in normal or preimmunized WAG/Rij Y rats or NCr(nu/nu) mice and in* 6 Gy *WBI NCr(nu/nu) mice. All of the experimental data show that the xenogenic NCr(nu/nu) mice accept the BA1112 as readily as do the isologous WAG/Rij Y rats.*

INTRODUCTION

QUANTITATIVE INTERPRETATION of the responses of human tumor xenografts growing in the athymic nude mouse to treatment by chemotherapeutic agents or radiation must consider the confounding effect of the residual and low level immune rejection reaction(s) against xenografts [1, 2]. A major interest for us has been the effectiveness of the immune rejection reaction by the nude mouse against foreign tissue grafts, and the extent to which such reaction could be abrogated by immune suppressive procedures. As the initial component of our research using the athymic nude mouse to assess quantitatively the growth and the radiation response of foreign tumors, the transplantability of three spontaneous tumors of the C3H/Sed mouse was compared for transplantation into normal or immune modified syngeneic C3Hf/Sed or allogeneic athymic NCr(nu/nu) nude mice [3]. The experimental finding was that the allogeneic NCr(nu/nu) mice accepted the tumor grafts as readily as the C3Hf/Sed mice, i.e. the allogeneic tumor transplant system could not be used for the intended purpose. We report here the sequel which was designed to assess the transplantability of the BA1112 rat rhabdomyosarcoma into syngeneic WAG/Rij Y rats or into xenogeneic athymic NCr(nu/nu) nude mice.

Accepted 12 May 1989.
†Andres Soriano Professor of Radiation Oncology, Harvard Medical School, U.S.A.
§Present address: Institute of Immunology, Rockefellerova St. 2-10, 4100 Zagreb, Yugoslavia.
This work supported in part by Grants from DHHS CA13311 and CA35215.

MATERIALS AND METHODS

The general plan of these assays was to perform concurrent TD_{50} assays using serial dilutions prepared from one suspension of tumor cells and to score the results according to a common protocol. The TD_{50} represents the number of cells which on the average would be expected to transplant the tumor to half of the recipients. Suspensions of the tumor cells were prepared at Yale by a mechanical

and enzyme method which has been described previously [4, 5]. This suspension was then used to prepare serial 2-fold dilutions. Lethally irradiated (120 Gy) BA1112 cells were added to each dilution in order that the total number of cells in each inoculum be 10^5. The resulting cell suspensions were then divided into two parts, and one set was sent by Air Express to Boston. At approximately the same time in the afternoon of that day, the transplantations were performed at Yale into WAG/Rij Y rats and at MGH into NCr(nu/nu) mice. For these transplantation assays, an inoculum volume of 0.1 ml was injected into the axillary space. For the isotransplants the right and left axillae were employed; for the xenotransplants the right axilla was used.

Animals

The xenogeneic recipients were athymic NCr(nu/nu) nude mice. They were derived from NIH Swiss mice into which a BALB/c nude gene had been introduced by backcross breeding techniques. These animals were subsequently maintained in an outbred fashion in the defined flora- and pathogen-free colony at the MGH [6]. The isologous recipients were the WAG/Rij Y rats. These animals were brought to Yale in 1976 from the laboratory of Dr. H.S. Reinhold of Rijswijk, The Netherlands. The rats were bred and maintained in the Therapeutic Radiology Animal Colony at Yale University. For TD_{50} assays on NCr(nu/nu) mice and the first assay on WAG/Rij Y rats, approximately equal numbers of male and female animals were used; for the second assay on the WAG/Rij Y rats females were employed. Animal ages at the start of an assay were 8–10 weeks.

Tumor

The BA1112 tumor arose in the mandible of a WAG/Rij rat in 1962 at 8 months after 8.64 Gy whole body irradiation and bone marrow allografting from BROFO rats [7] in the laboratory at Rijswijk. Histologically, the tumor is a poorly differentiated rhabdomyosarcoma. The tumor was non-immunogenic when tested originally [8] and subsequently at Yale [3].

Immunization

The animals were exposed to growth of the BA1112 tumors to a size of 5 mm diameter, at which time the tumors were removed surgically. The TD_{50} assays were started 6–8 days later. In the NCr(nu/nu) mice an additional procedure was used in one assay. The animals received three injections of 2×10^7 lethally irradiated BA1112 cells (120 Gy) at 7 day intervals: for the first, BA1112 cells admixed with complete Freunds adjuvant [9] were injected into right and left axillary and groin regions; the second and third injections were intraperitoneal without adjuvant. One week later, the injections of viable BA1112 cells were made.

Whole body irradiation (WBIO)

Six Gy was administered to the whole body of the NCr(nu/nu) mice using an AECL Gamma Cell ^{137}Cs irradiator, featuring parallel opposed sources. The mice were placed into a pie-shaped holder, accommodating five mice per session.

End-points and data analysis

The principal end-point for this study was the development of tumor at the transplantation site. Tumor take was scored when the tumor reached 10 mm in diameter. Animals were sacrificed when the tumors reached 10–15 mm; no tumor take was scored after day 50. The transplant take results were tabulated as a function of the number of tumor cells injected. A logit regression was fitted through the transplant take results and the TD_{50} with its 95% confidence limits computed [10].

The other end-point was the time required for tumor to grow to 10 mm in diameter. In one experiment, growth curves were constructed for each tumor. The tumor diameters were measured on a three times per week basis, and the tumor volumes computed on the basis of $(D1 \times D2 \times D3) \div 2$. These tumor volumes were then plotted on a log volume vs. linear time grid. The time for the tumor to reach a volume of 1000 mm^3 was then read from the graph for each tumor.

RESULTS

TD_{50} values for isotransplantation of BA1112 rhabdomyosarcoma into WAG/Rij Y rats and xenotransplantation into athymic NCr(nu/nu) nude mice are given in Table 1. The TD_{50} values were in the range of 1–15 for all experimental protocols. Isotransplantation was equivalent for normal and pre-immunized recipients. Similarly, the TD_{50} for xenotransplantation was the same in normal, 6 Gy WBI, and preimmunized recipients; these TD_{50} values were not different from the TD_{50}s for isotransplantation. Suppression of the NCr(nu/nu) mice by 6 Gy whole body irradiation (WBI) at 24 h before challenge with BA1112 cells caused a small but not significant reduction in the TD_{50}.

The times for BA1112 tumors to grow to 10 mm in average diameter in WAG/Rij Y rats and NCr(nu/nu) mice are presented in Table 2. Using this end-point, the growth of tumor after injection of 4, 8, or 16 cells was comparable for isotransplantation into normal or immunized rats or xenotransplantation into normal, immunized, or 6 Gy WBI treated NCr(nu/nu) mice.

Table 1. TD_{50} *values for the isotransplantation and xenotransplantation of the rat BA*1112 *tumor*

Exp. No.	Tumor transplant cell dose 50				
	WAG/Rij rats		NCr(nu/nu) mice		
	Untreated	Immunized	Untreated	Immunized	Irradiated
1	3 (2...5)* [90]	—	15 (6...40) [65]†	—	4 [64]
2	4 (2...8) [68]	2 (1...4) [66]	2 (1...5) [40]	2 (1...10) [29]	1 (1...3) [34]
3	—	—	2 (1...4) [54]	2 (1...4) [29]	2 (1...2) [39]
Pooled 1–3	4 (2...5)	2 (1...4)	2 (1...5)	2 (1...5)	1 (1...2)

*TD_{50} (95% confidence limits).
†No. of mice (one transplant site per mouse).
No. of transplant sites (two per rat).

Table 2. *Days for BA*1112 *to grow to* 10 *mm diameter*

Host status	WAG/Rij Y rat		Athymic NCr(nu/nu)		
	Normal	Immunized	Normal	6 Gy WBI	Immunized
No. cells injected					
1	34 (2)	31.6 (3)	35.8 (4)	33.3 (3)	
2	31 (1)	33.5 (4)	34.0 (4)	35.0 (6)	37.2 (5)
4	32.6 (5)	31.7 (6)	34.0 (7)	32.0 (10)	31.0 (6)
8	33.2 (5)	30.7 (7)	30.1 (7)	30.3 (10)	34.2 (4)
16	30.0 (7)	31.4 (7)	27.8 (5)	29.0 (5)	28.0 (6)
32	31.1 (7)	30.3 (7)	—	28.8 (5)	24.0 (4)
64	26.3 (7)	29.4 (8)	23.4 (5)	—	23.0 (3)
128	26.3 (8)	22.9 (8)	—	—	—

Number of tumors in parentheses.

Transplantability of BA1112 was assessed in NCr(nu/+) mice (heterozygous for the nude gene complex). Following injection of 10^7 trypan blue excluding BA1112 cells, no tumors appeared in normal NCr(nu/+) mice. However, in 6 Gy WBI NCr(nu/+) mice, tumors appeared and grew to 10–12 mm and then regressed completely in all subjects. A parallel experiment with the same protocol was conducted using C3Hf/Sed mice and with the same outcome: temporary growth only in 6 Gy WBI subjects.

DISCUSSION

Quantitative transplantation assays (TD_{50} assays) are an effective means for assessment of the relative transplantability of tumor into normal syngeneic and nude xenogeneic recipients. There are few practical options and all involve the use of rodent tumor systems (isotransplantation of tumors of mice, rats, guinea pigs, or hamsters and xenotransplantation into nude mice or rats). Use of canine, feline, equine, etc. tumors would be confined to a very limited and single study of autotransplantation (graded cell doses injected into multiple sites) vs. the full TD_{50} assay for xenotransplantation into the nude mouse or rat. Here we compared the TD_{50}s and times to grow to 10 mm for the rat rhabdomyosarcoma BA1112 when isotransplanted into the WAG/Rij Y rat or xenografted into the athymic NCr(nu/nu) mouse. For this animal tumor system, xenografts were accepted as readily as isografts and they grew to 10 mm in equivalent times. Neither prior immunization nor 6 Gy WBI affected the TD_{50} values.

Nude mice bearing human tumor xenografts develop metastatic tumors quite infrequently in the usual observation periods. In contrast, widely dispersed metastatic tumor appeared following xenotransplantation of BA1112 into the NCr(nu/nu) mice by only a few cells. We had planned to

determine the radiation dose–response curve for local control of the xenotransplanted BA1112. The experiment was not feasible because of the short survival times due to the rapid appearance of metastatic tumors.

This differed markedly from the very low frequency of distant metastasis reported previously in the WAG/Rij Y rat after local irradiation of 7.5 mm tumors by Moulder et al. [11], who generated complete dose–response curves for local control using observation periods of 180–250 days. This appears to reflect a spontaneous change in the characteristics of the serially transplanted tumor line. A dose–response assay for tumor control in WAG/Rij Y rats, begun during the course of the second TD_{50} experiment reported here, had to be terminated because of widespread metastases. A return to frozen BA1112 stock yielded a BA1112 line having the non-metastatic behavior originally described for this tumor.

Subsequent experiments in this laboratory have demonstrated that the NCr(nu/nu) mouse are capable of immunological reactivity against human tumor xenografts [12]. The TD_{50} for subcutaneous transplantation was higher by factors of 2–3 than for transplantation into the 6 Gy treated mice. For the three human tumors tested, the subcutaneous TD_{50} was significantly lower after 6 Gy whole body irradiation. In addition, delayed-type hypersensitivity was displayed against the human tumors but not against an allogeneic murine tumor. Therefore, the histocompatability differences between human and mouse are sufficient to evoke an immune response in the NCr(nu/nu) animals, the differences between rat and mouse are not necessarily so.

Acknowledgements—The authors are pleased to acknowledge the excellent work by the research technologists Richard Waite and Scott Malcolm. We are very appreciative of the effort by Claire Hunt in the preparation of this manuscript.

REFERENCES

1. Lees RK, MacDonald HR. Ability of nude mice to generate alloreactive, xenoreactive, and H2 restricted cytotoxic T-lymphocyte responses. In: Sordat B, ed. *Immune-deficient Animals*. Karger, Basel, 1984, 7–11.
2. RB Herberman. Natural cell-mediated cytotoxicity in nude mice. In: Fogh J, Giovanella B eds. *The Nude Mouse in Experimental and Clinical Research*. New York, Academic Press, 1978.
3. Suit HD, Sedlacek RS, Zietman A. Quantitative transplantation assays of spontaneous tumors of the C3H mouse as allografts into athymic NCr(nu/nu) nude mice. *Cancer Res* 1988, **48**, 4525–4528.
4. Martin DF, Rockwell S, Fischer JJ. Development of an *in vitro* assay for the survival of cells suspended from BA1112 rat sarcomas. *Eur J Cancer Clin Oncol* 1983, **19**, 791–797.
5. Reinhold HS. A cell dispersion technique for use in quantitative transplantation studies with solid tumours. *Eur J Cancer* 1965, **1**, 67–71.
6. Sedlacek RS, Suit HD, Mason KA, Rose EA. Development and operation of a stable limited defined flora mouse colony. *7th ICLAS Symposium, Utrecht, 1979*. Stuttgart, G. Fischer, 1980, 197–201.
7. Kal HB. *Responses of a Rat Rhabdomyosarcoma and Rat Skin to Irradiation with Gamma Rays and* 15 MeV *Neutrons at Low Dose Rates*. Publication of the Radiobiological Inst. of the Organization for Health Research TNO, Rijswijk, The Netherlands, 1974, 29 pp.
8. Reinhold RS. Quantitative evaluation of the radiosensitivity of cells of a transplantable rhabdomyosarcoma in the rat. *Eur J Cancer* 1966, **2**, 33–42.
9. Suit HD, 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* 1967, **39**, 1121–1128.
10. Suit HD, Shalek RJ, Wette R. Radiation response of C3H mouse mammary carcinoma evaluated in terms of cellular radiation sensitivity. In: *Cellular Radiation Biology*. Baltimore, Williams & Wilkins,1965, 514–530.
11. Moulder JE, Fischer JJ, Milardo R. Time–dose relationships for the cure of an experimental rat tumor with fractionated radiation. *Int J Radiat Oncol Biol Phys* 1976, **1**, 431–438.
12. Zietman A, Suit H, Ramsay J, Silobrcic V, Sedlacek R. Quantitative studies on the transplantability of murine and human tumors into the brain and subcutaneous tissues of NCr/Sed nude mice. *Cancer Res* 1988, **48**, 6510–6516.