

The Murine Ependymoblastoma: Growth Pattern and Survival in C57B1/6J Mice

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SUMMARY: The murine ependymoblastoma is a transplantable tumor of cerebral origin. The growth pattern and survival times of the murine ependymoblastoma implanted peripherally and intracranially in non nude C57B1/6J mice have been found to be predictable and consistent when examined by means of Tumor Cell Dose Assessment (end point solution), Tumor Growth and Survival Assessment. The results suggest that a greater tumor cell dose is required to generate peripheral tumor take than brain tumor take. This difference may result from a greater immunologic response to tumor implanted peripherally than into the immunologically privileged brain.

RÉSUMÉ: L'épendymoblastome murin est une tumeur transplantable d'origine cérébrale. Le tableau de croissance et les temps de survie de cet épendymoblastome (lorsqu'implanté en périphérie ou de façon intracrânienne chez la souris C57B1/6J non-nue) furent trouvés constants et prévisibles si examinés par les méthodes d'évaluation de dose cellulaire tumorale, de croissance cellulaire et de survie. Les résultats indiquent qu'il est nécessaire d'employer un plus grand dosage de cellules tumorales pour produire une tumeur périphérique qu'une tumeur cérébrale. Cette différence dépend peut-être de la présence d'une réponse immunologique plus importante à la périphérie.

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Murine brain tumors were originally induced by Seligman and Shear (1939) by the intracerebral implantation of methylcholanthrene. The murine ependymoblastoma was induced and serially transplanted intracerebrally by Zimmerman and Arnold (1941). This experimental tumor has been used extensively to test the tumoricidal activity of chemotherapeutic agents which might have application in the treatment of human malignant brain tumors (Ausman et al., 1970; Geran et al., 1974; Muller and Tator, 1978; Muller et al., 1980; Shapiro, 1971; Shapiro et al., 1970). The model has also been used in the assessment of radiotherapeutic fractionation (Muller and Shin, 1982), and drug distribution studies (Levin et al., 1970; Schwartz et al., 1972; Tator, 1972; Wassenaar and Tator, 1976).

The mouse ependymoblastoma is relatively easy and inexpensive to maintain by serial transplantation. It grows readily in subcutaneous (s.c.), intramuscular (i.m.) and intracerebral (i.c.) locations; and, it has a remarkably constant growth pattern when implanted i.c. or peripherally. Autochthonous models, in which a tumor is induced by oncoviruses or chemical carcinogens, have long latency periods, a low level of predictability and may require containment. Nitrosourea induced tumors were noted also to have the disadvantage of multiplicity and the occurrence of tumors outside of the central nervous system. The advantages of the autochthonous models include the presence of a natural tumor blood supply and the absence of traumatic tumor implantation (Crafts and Wilson, 1977). Xenographic transplantation into athymic "nude" mice has the disadvantages of long latency, unpredictable tumor take and relative expense. However, the ability to assess human tumor tissue may outweigh these disadvantages (Bullard and Bigner, 1980).

We have examined the growth pattern and the survival times of the murine ependymoblastoma implanted peripherally and i.c. in non nude C57B1/6J mice and have found the growth and survival patterns to be predictable and consistent.

MATERIALS AND METHODS

Animals

C57B1/6J mice were purchased from the Jackson Laboratories, Bar Harbour, Maine. Generally, 6 - 12 week old female mice weighing 17 - 21 grams were used in all experiments. The mice were housed 10 per cage and allowed food and water ad libitum.

Tumor Cell Preparation

A murine ependymoblastoma was obtained from the laboratory of Dr. C.H. Tator and maintained by serial s.c. transplantation in our laboratory since 1978. Subcutaneous tumors weighing approximately 2 grams were harvested, mechanically disaggregated in phosphate-buffered-saline (PBS), filtered and concentrated by centrifugation (1200 rpm x 10 minutes). The cell suspensions were then diluted in PBS to yield the desired concentration and cell counts were made with a hemocytometer.

Tumor Inoculation

Intracerebral tumors were produced by the i.c. injection of 2-6 μ l of tumor cells suspension with a micrometer syringe via a small stereotactic frame as previously described (Wassenaar et al., 1972). Intramuscular tumors were produced by the direct injection of 100 μ l of tumor cell suspension into the left lower limb thigh musculature.

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Tumor Cell Dose Assessment (End Point Dilution) in I.M. Implanted Tumors

In each of 10 experiments, 5 serial dilutions of tumor cell suspension were injected into 20 i.m. sites in normal mice (1000 assessable sites in total). The tumor cell concentration varied from 1.6×10^3 to 9.3×10^6 per $100\mu\text{l}$. The mice were observed for the development of tumor for 100 days. The percentage of positive injection sites in each group was plotted against the log of the cell count injected; and the TD50 (the number of tumor cells which produce tumor growth in 50% of the injection sites) was calculated by least squares regression.

Tumor Growth and Survival Assessment in I.M. Injected Mice

Twenty-four groups of 10-20 mice were injected with 4.0 to 8.0×10^6 tumor cells into the left thigh musculature. Forty-eight groups of 10-64 mice (993 mice total) were implanted with 1.3×10^5 to 1.5×10^6 tumor cells intracerebrally. The tumor bearing mice were observed daily for survival and were weighed a minimum of three times weekly. The percent cumulative survival was plotted against time in days after tumor implantation and the median day of death calculated. Mice surviving longer than 100 days after tumor implantation were considered long term survivors and for the sake of further calculations their survival time was considered to be 100 days.

The growth of the i.m. tumors were assessed 3-5 times weekly by measuring the maximum diameter of the tumor bearing leg by ascertaining the best fit through a series of premeasured rings; the diameter was read directly from the ring of best fit. The mean diameter for each experimental group was plotted against time in days after tumor inoculation. The time in days required for the tumor to increase the mean diameter of the leg by 25%, 50% and 100% was calculated.

In all experiments the day of tumor cell implantation was considered day 1. A different tumor cell suspension was used in each experiment.

RESULTS

Tumor Growth and Survival Times in I.M. Tumor Bearing Mice

Table 1 summarizes the growth pattern and survival times of 260 i.m. tumor bearing mice in 24 groups. The mean times (\pm S.D.) for a 25%, 50%, or 100% increase in mean leg diameter were 13.1 ± 3.0 , 15.3 ± 3.4 , and 19.9 ± 4.5 days respectively. Figure 1 shows the growth pattern in 13 consecutive groups.

The mean (\pm S.D.) median day of death MDD was 36.4 ± 9.7 days. The MDD derived from pooling all the individual survival times of 260 mice was 36 days; the mean

survival was 38.1 ± 15.9 days. Ninety-two percent of the mice died between the 10th and 60th day after tumor inoculation (Fig. 2.). A single mouse in each of two groups (0.8%) died before the mean diameter of the tumor bearing legs of that group exceeded an increase of 25%, in four groups a total of 7 deaths (2.7%) occurred before the mean diameter increase exceeded 50%, and, in seven groups a total of 10 deaths (3.8%) occurred before the mean diameter exceeded 100%.

The linear relationship between MDD and time to increase 25% was described by $Y = 0.1449X + 7.8480$ ($r = 0.459$, $p < 0.05$). The linear relationship between MDD and time to increase 50% was best described by $Y = 0.2274X + 7.0306$ ($r = 0.641$, $p < 0.02$); and, the linear relationship between MDD and time to increase 100% was best described by $Y = 0.3211X + 8.1680$ ($r = 0.687$, $p < 0.001$). The slope of the regression line increased as the end-point diameter enlarged.

Survival Time in I.C. Implanted Mice

Fig. 3 shows the percent cumulative survival against time in days after the i.c. implantation of $1.3 - 15.0 \times 10^5$ tumor cells into 993 mice in 48 groups of 10 to 64. Less than 3% of the mice died prior to the 15th day after implantation and less than 3% died after the 60th day. There were 2.5% long term survivors. The MDD was 32 days for the whole group and the mean survival was 33.8 ± 15.0 days.

Tumor Dose Assessment in I.M. Tumor Bearing Mice

Table 2 summarizes the tumor cell doses and the percent tumor take in the end point dilution assessment of 10 groups of mice. The mean TD50 (\pm S.D.) was $1.40 (\pm 0.01) \times 10^5$.

DISCUSSION

The murine ependymoblastoma is a stable tumor model derived from cerebral tissue. Our results show that the survival and tumor growth times are predictable and consistent.

The median survival of mice implanted i.c. with $1.3 - 15.0 \times 10^5$ cells was 32 days and the median survival of mice implanted i.m. with $4.0 - 8 \times 10^6$ cells was 36 days; these survival times are very similar in spite of the larger tumor cell doses used in the i.m. groups. Of course, the mechanism of death was quite different in the two groups. The i.c. implanted mice lost weight terminally and displayed neurologic signs such as somnolence, spinning and movement disruption, whereas the i.m. tumor bearing mice gained weight until death; and, their weight gain paralleled the increase in tumor size. On gross inspection the mechanism of death in the i.m. group appeared to be pelvic invasion of the tumor in a high proportion of the mice.

TABLE 1: The Growth Pattern and Survival Times of Tumor Bearing Mice

Experiment Numbers	Number of Mice	Mean Days to Increase Leg Diameter by			Mean Median Day Death
		25%	50%	100%	
24 Groups	260	13.1 ± 3.0	15.3 ± 3.4	19.9 ± 4.5	36.4 ± 9.7

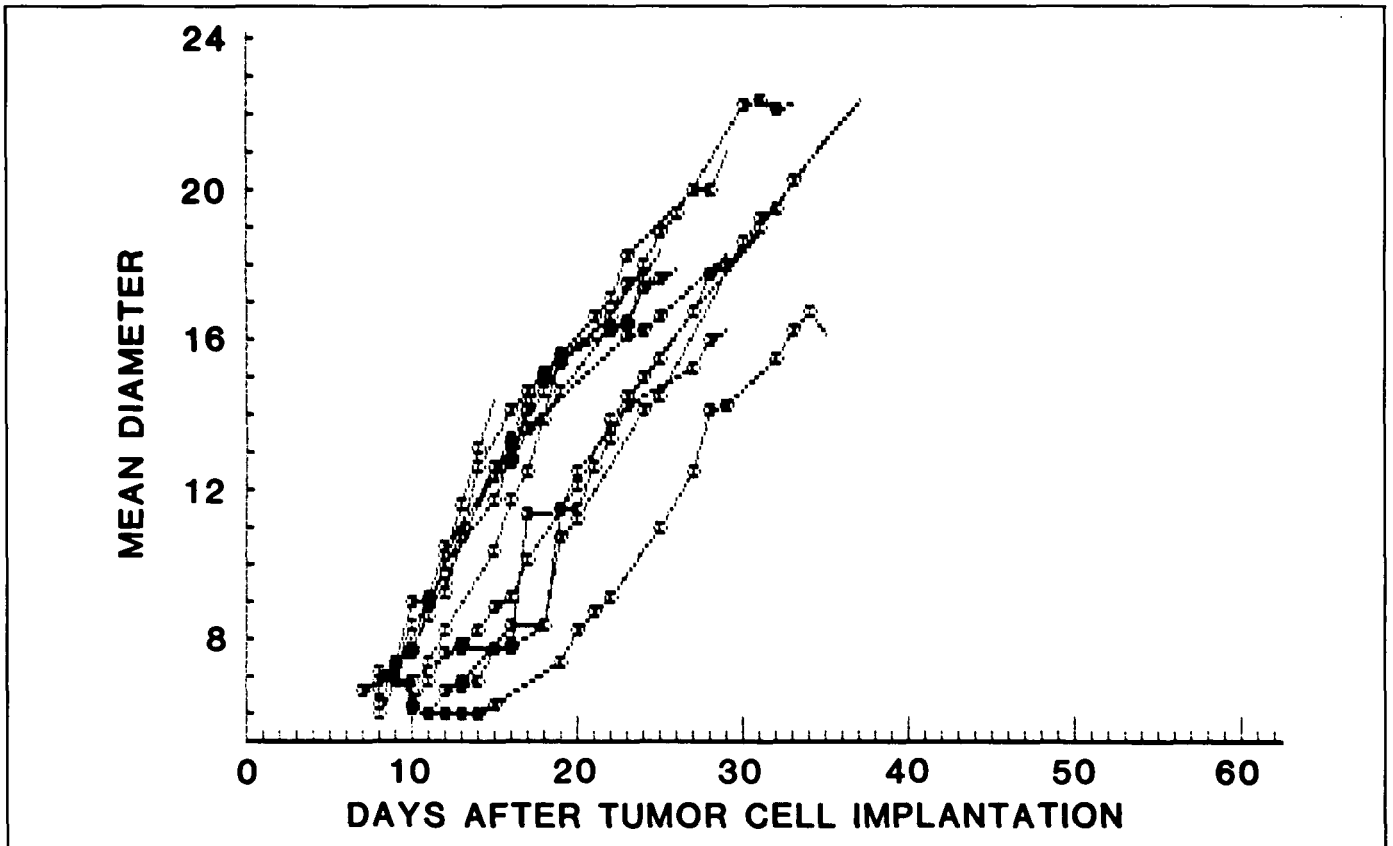


Figure 1 — Mean diameter of tumor bearing limb plotted against days after tumor cell implantation in 13 consecutive experiments.

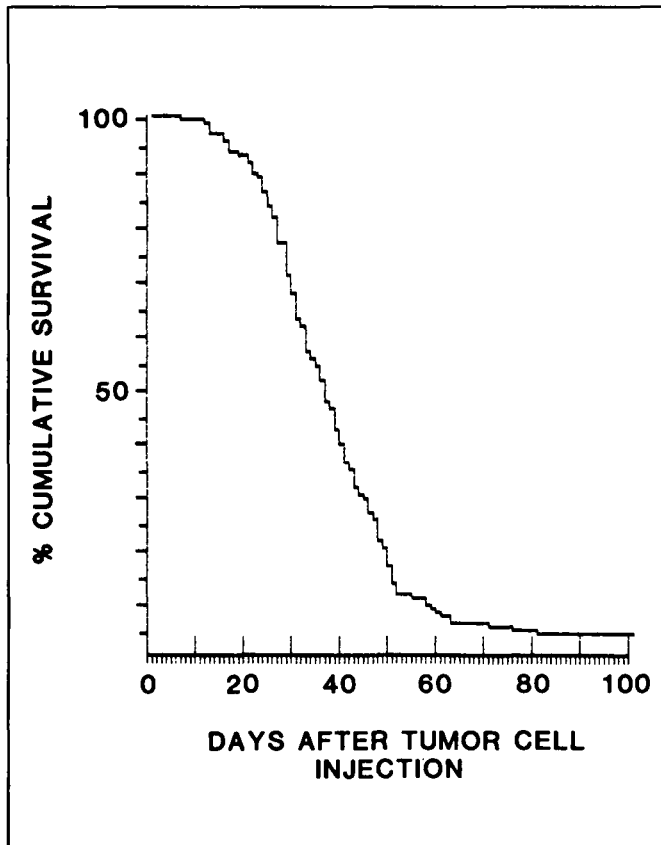


Figure 2 — Percent cumulative survival of intramuscular (leg) bearing mice plotted against time after tumor cell implantation.

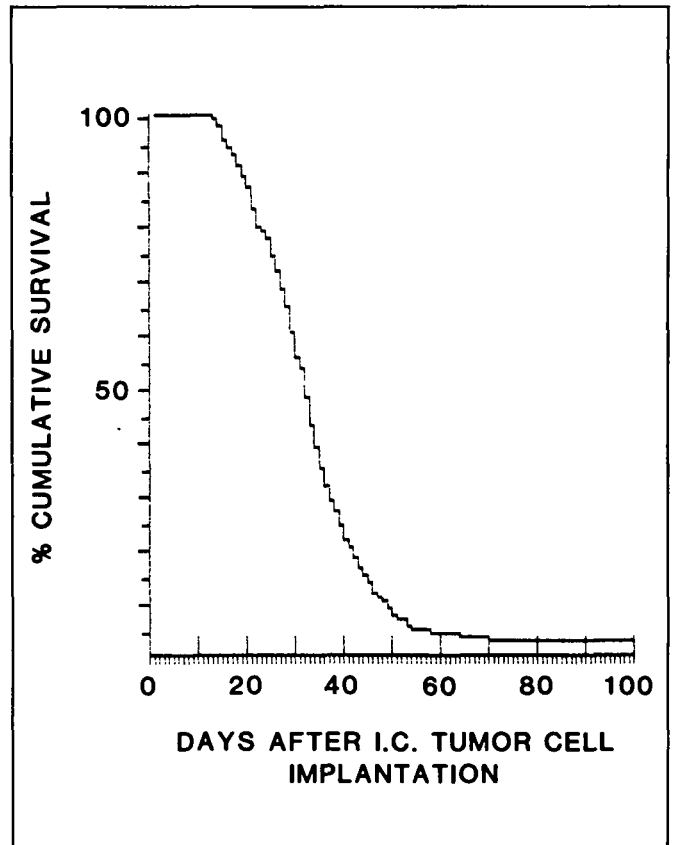


Figure 3 — Percent cumulative survival of intracranial tumor bearing mice plotted against time after tumor cell implantation.

TABLE 2: Assessment of Rate of Tumor Take After I.M. Implantation

Experiment Number	T.D. 50 x 10 ⁵
1	0.63
2	1.00
3	2.47
4	0.794
5	1.58
6	1.41
7	1.58
8	1.00
9	2.24
10	1.26

Five tumor cell doses per experiment
 Twenty tumor assessment sites per dose
 Tumor Cell Dose Range per 100 μ l
 (1.6 x 10³ — 9.3 x 10⁶)

The TD50 value in the i.m. tumor bearing mice was 1.4 x 10⁵ cells; whereas the i.c. tumor bearing mice had virtually a 100% tumor take at doses over 10⁵ tumor cells. Muller and Tator (1977) have shown that 10⁴ tumor cells result in a greater than 90% tumor take when implanted i.c. These results suggest that a greater tumor cell dose is required to generate peripheral tumor take than brain tumor take. A possible explanation of this difference may be a greater immunologic response to tumor implanted peripherally than into the immunologically privileged brain even in syngeneic tumors.

Our data suggests that the i.m. survival times are similar to the survival times for i.c. implanted mice, although an increase in tumor dose may be required. Our results are compatible with those of Suzuki and Tanaka (1980) who used a transplantable Rous virus induced murine glial tumor. They reported a mean survival of 26.2 days after i.c. implantation and 27.8 days after s.c. implantation. The survival times which we observed in the i.c. implanted mice were similar to those reported elsewhere. Ausman et al. (1970) reported a median day of death of 29 days from a sample of 642 mice implanted intracerebrally with a small fragment of tumor tissue; and Wassenaar et al. (1973) reported a MDD of 27.5 in a sample of 200 mice implanted i.c. with tumor cell suspension.

Experimental tumor models have been used extensively to screen potentially useful anti-cancer agents for many years. Experimental tumors of different tissue or species origin may have different responses to the same chemotherapeutic agent. For example, the murine glioma was sensitive to dihydrogalactitol while the rat 9L gliosarcoma was much less sensitive (Levin et al, 1970). And, the same experimental tumor may have different sensitivities to the same agent when the tumor is located in an i.c. as opposed to a peripheral location. For example, cyclophosphamide is effective against the L1210 leukemia when the tumor is implanted s.c. but it is not effective when the tumor is implanted i.c. (Chirigos, 1964). Because of the con-

sistent growth pattern of the ependymoblastoma when implanted peripherally and because of the relationship between MDD and time for tumor increase, chemotherapeutic agents can be tested in both i.c. and peripheral locations in order to ascertain whether the location of the tumor or its sensitivity influence the effectiveness of the agent.

The murine ependymoblastoma has been used extensively in drug screening trials of the National Cancer Institute (Geran et al, 1974). Generally, the results in this model have been consistent with the findings in human clinical brain tumor trials. The nitrosoureas and procarbazine are effective in prolonging survival in ependymoblastoma bearing mice while mithromycin, cyclophosphamide and methotrexate are ineffective (Shapiro, et al, 1970; Tator et al, 1970). These results parallel the human clinical experience (Walker et al, 1974).

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