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PROBING ALTERED HEMATOPOIETIC PROGENITORS OF PRELEUKEMIC DOGS WITH JANUS FISSION NEUTRONS

T. M. Seed and L. V. Kaspar

Radiation Hematology Group
Biological and Medical Research Division
Argonne National Laboratory
Argonne, Illinois 60439 USA

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INTRODUCTION

Ionizing radiation is a well documented leukemogen for a variety of species, including man (1,2). However, the leukemogenic potency of low doses of ionizing radiation, especially for doses protracted over time, is not well documented. As a consequence, the health risks associated with such low-dose exposures are unclear (i.e., it's uncertain as to the extent to which protracted courses of low-level exposure contribute to the "spontaneous" leukemic rate). Prior to the advent of the "residential radon" problem, most public health workers considered radiation-induced leukemia a rare event and, mainly, the provenance of acute accidental or medically based exposures (1). Recently, a study has provided some epidemiological evidence that as high as 13-25% of all the spontaneous myeloid leukemias in humans might be attributed to chronic exposure within the home to radon (3). Despite these rather startling estimates, the high degree of uncertainty in these assessments impairs the meaningful assignment of leukemogenic potency for low levels of ionizing radiation. One practical way to reduce the uncertainty is by developing a better understanding of the basic leukemogenic mechanisms mediated by ionizing radiation exposure. The latter would be based, in part, on the subsequent development of dose-dependent response models for fundamental hematopoietic target cell responses.

Toward this goal of developing basic insights to mechanisms of radiation leukemogenesis, we have developed a canine model that responds to protracted courses of low-daily-dose gamma irradiation with high incidences of myeloproliferative disease (MPD), principally myeloid leukemia (4,5). Using this model system, we have identified and partially characterized a four-phase preclinical sequence in the induction of MPD, including 1) suppression, 2) recovery, 3) accommodation, and 4) preleukemic transition (6,7). Further, we have identified within this sequence, a critical "early" hematopoietic target cell event that appears to promote progression of the initial preclinical phase to the second preclinical phase (8,9). This key target cell event is characterized by the acquisition of increased radioresistance to low-LET gamma rays by granulocyte/monocyte-committed progenitors (CFU-GM). In order to gain further insight into the

basis of this critical event, the acquired survival responses of preleukemic progenitors have been probed *in vitro* with high-LET fission neutrons.

Considering the possibility that densely ionizing neutrons induce a different quality of genomic lesion than low-LET photon radiation, and also noting that the preleukemic progenitor's new acquisition of increased radioresistance is, in part, the product of the strong selective pressure of chronic, low-LET irradiation *in vivo*, we asked whether or not the preleukemic progenitors process neutron-induced lesions differently than gamma-ray lesions.

MATERIALS AND METHODS

Animals. Outbred beagles in this study were derived from the closed Argonne National Laboratory colony, the status, origin, and general management of which have been described in detail elsewhere (10). A total of 22 dogs were used in this study: 14 were nonirradiated controls, while 8 were chronically irradiated and served as test animals. All dogs were in good health and approximately 400 days of age at the time of initiating the study. Selected aspects of the study have been previously reported (11). In addition, the animals comprising this study were part of a larger group that has been, and is currently, under general toxicological evaluation of the long-term effects of chronic, low-dose irradiation (12). Various hematopathological aspects of the latter work, including interim survival and leukemia patterns, have been reported previously (4-9, 13-17).

Irradiations. Chronic, whole-body ^{60}Co gamma-ray exposures were carried out using "live-in" animal radiation facilities, whose design and operation have been described in detail elsewhere (10,18). Animals were irradiated for the duration of life at a daily dose rate of 7.5 cGy over 22 h/d. Dosimetric methods and calculations have been outlined in detail elsewhere (18). The animals were caged, fed, watered, and clinically examined and manipulated as described previously (5,10).

Hematology. Hemograms were performed periodically by standard methods on each animal under test (19).

Bone Marrow Aspiration and Progenitor Cell Enrichment. Bone marrow samples were obtained from either the ilia or humeri of irradiated and nonirradiated dogs by "snap" aspirations. Marrow samples were collected at time intervals corresponding to the initial phase of exposure (125 days), as well as during early and late postrecovery phase periods (564 ± 151 days, and 1,036 ± 185 days), respectively, and subsequently subjected to cytomorphologic and progenitor cell analyses (9). Granulocyte/monocyte progenitors (CFU-GM) were enriched from the marrow samples by Ficoll-sodium diatrizoate density gradient (1.077 g/l) sedimentation procedures (9). CFU-GM-enriched mononuclear cell fractions were collected, washed, and adjusted to final working cell concentrations of 10^6 cells/ml.

Marrow cell irradiations and progenitor survival estimates. Duplicate series of aliquoted stock CFU-GM-enriched marrow cell suspensions (10^6/ml) were prepared and maintained at less than 10°C prior to, during, and following *in vitro* irradiation. The first series was irradiated with ^{60}Co gamma rays at 25 cGy/min, while the second series was irradiated with JANUS fission neutrons (0.85 MeV mean energy) at 15 cGy/min. The total doses for both series ranged from 10-300 cGy. Unirradiated aliquots served as zero-dose controls in all experiments. Descriptions of the general dosimetric procedures have been given elsewhere (20). The surviving fraction of CFU-GM within each cell sample was determined by a soft agar cloning procedure described below (9). The degree of radiation-induced CFU-GM lethality was assessed in terms of inhibition of clonogenic activity. Dose/survival response curves were constructed from the calculated fraction of surviving CFU-GM at each radiation dose, using either simple linear regression or a modified program that allows for the analysis of biphasic responses. For the latter, survival responses at low doses (less than 100 cGy) were assessed independently from the survival responses seen at high doses (greater than 100 cGy). The intersection of the two responses defined the dose/survival level parameters for the expression of the secondary response. The Y-axis intercept

for the back-extrapolated secondary response served to define the size of the minor subpopulation. From the linear regression analyses, the radiobiological response parameters of lethality rate (*Do*), sublethal damage capacity (*Dq*), and subcellular target number (n) have been made.

The number of viable CFU-GM within either original marrow cell preparations or those irradiated *in vitro* were assayed by a modified Pike/Robinson double agar layer cloning method (9). The cloning method uses a "feeder" layer (containing 10^6 buffy coat leukocytes from control donor dogs and 10% pooled plasma from 400 cGy lethally irradiated dogs) to support CFU-GM colony formation in the upper agar layer (containing 0.5-1 x 10^5 "target" marrow cells/ml). Plates were incubated in 5% CO_2 at 37°C for eight days. Colony growth was enumerated with an inverted light microscope.

RESULTS

Blood Responses. Changes in circulating blood levels of granulocytes, monocytes, and erythrocytes of chronically irradiated dogs (7.5 cGy/day) and in nonirradiated controls are shown in Figs. 1a-1c. These blood responses display two characteristic response traits of dogs prone to develop myeloproliferative disease (MPD) under chronic irradiation: first, the change in granulocyte and monocyte blood levels with time of exposure is more pronounced than that in erythrocyte levels; and second, for the granulocyte and monocyte responses, distinct response phases appear to be expressed in a temporally consistent manner. These phases included 1) suppression, 1-250 days of exposure; 2) partial recovery, 250-350 days; 3) postrecovery accommodation, 350-700 days; and 4) postrecovery, preleukemic transitions, 700-1000 days (Figs. 1a,1b).

Quantitation of bone marrow progenitors during distinct preclinical phases. Bone marrow levels of hematopoietic progenitors committed to granulocyte/monocyte differentiation (CFU-GM) were assayed both in irradiated and nonirradiated dogs during the four preclinical periods (Table 1). Marrow progenitor concentrations were markedly suppressed, relative to nonirradiated

controls, during the initial exposure period and were later partially restored to 66% and 53% of control values during early and late postrecovery periods, respectively.

Radiosensitivity of hematopoietic progenitors. Quantitative changes in marrow progenitors during the prerecovery and postrecovery periods were associated with marked shifts in radiosensitivity to low-LET gamma rays (Fig. 2). Progenitors taken from select animals during the suppressive period exhibited appreciably reduced radiosensitivity (i.e., increased radioresistance) relative to progenitors from nonirradiated controls (Fig. 2; Table 1). The average radiation dose required to exponentially reduce progenitor survival to a 0.37 fractional survival level increased from 77 cGy to 142 cGy, a net gain in radioresistance of 65 cGy. Progenitors taken from irradiated dogs, either shortly following hematopoietic recovery or following much longer periods, exhibited further reductions in radiosensitivity (Table 1). Net gains in radioresistance during these early and late postrecovery periods were 93 cGy and 118 cGy, respectively.

Significant shifts in progenitor's radiosensitivity to high-LET fission neutrons were observed as well, but the magnitude of those shifts were small by comparison (Fig. 3). The major fraction of progenitors, regardless of marrow source, displayed a high degree of radiosensitivity over a rather narrow, low-dose range (10-100 cGy). Relative to controls, progenitors from prerecovery and postrecovery preclinical phases expressed a small but significant stepwise reduction in radiosensitivity. *Do* values increased from 28 cGy for control progenitors to 31 cGy for the prerecovery phase progenitors, and to 38 cGy and 37 cGy for progenitors of early and late postrecovery phases, respectively (Table 1). The latter represented net increases in radioresistance of 3 cGy, 10 cGy, and 9 cGy for prerecovery and early and late postrecovery phase progenitors, respectively.

The biological effectiveness of fission neutrons (10-75 cGy dose range) and gamma rays (10-300 cGy dose range) differed for the three preclinical states analyzed (Table 1). The postrecovery phase progenitors yielded the largest effectiveness ratios (4.6 and 5.5), whereas naive progenitors from nonirradiated dogs exhibited the lowest ratios (2.8).

Size and radiosensitivity of a minor marrow progenitor subpopulation. An examination of the control progenitor responses to fission neutrons over the entire dose range (10-300 cGy) revealed a biphasic survival pattern (Fig. 4). The initial steeply sloped survival response seen at low neutron doses (10-75 cGy) broke to a more shallow response at a dose 115 cGy and at a survival level of 1.9% (Fig. 4). The break in the survival response is assumed to be due to the expression of a second progenitorial subpopulation, characterized by its small size (2.2%) and its marked radioresistance. Under chronic irradiation and in preclinical progression, this minor subpopulation grows in size from 2.2% in nonirradiated controls, to 3.8% in the prerecovery phase, to 4.4% in early postrecovery phases, to 14.9% during the late postrecovery phases, and it increases its radiosensitivity as well (Fig. 4).

DISCUSSION

This study was initiated with the intent of examining alterations in radiosensitivity of hematopoietic (GM-committed) progenitors from chronically irradiated dogs during preclinical phases of evolving myeloproliferative disease (principally, myeloid leukemia). Major shifts in radiosensitivity were observed, especially in terms of the progenitor's *in vitro* sensitivity to gamma rays (i.e., the homologous quality of radiation to which the animal was exposed) where the magnitude of radioresistance (assessed in terms of lethality responses, i.e., D_0 values) rose by roughly 100 cGy during the posthematopoietic recovery or crisis phase (preclinical phase III). Previously, we have shown that such acquired progenitor responses are both time-of-exposure-dependent and pathology-specific (8,9,21). Radioresistance is selectively acquired in a well-defined time frame of exposure (150-250 days), but only by dogs destined to survive the initial, acute hematopoietic crisis phase and to progress into subsequent preleukemic phases (preclinical phases III and IV). By contrast, radiosensitive dogs with shortened survival courses under chronic irradiation and a proneness to develop aplastic anemia fail to exhibit both this specific progenitorial acquisition and a battery of other new traits (e.g., enhanced cellular repair) that

affect cell survival (8,22). These latter observations have led us to suggest that the underlying molecular and cellular processes involved in the phenotypic expression of new progenitor characteristics are keys to the promotion of early evolving preleukemic syndromes.

Can we gain any insight into the nature and mechanisms of these MPD-specific progenitorial acquisitions by probing with high-LET fission neutrons? We initially reasoned that if the progenitor's acquired radioresistance to low-LET gamma rays is largely repair-mediated, then comparable responses to densely ionizing fission neutrons, with known repair-suppressing action, might be effectively quenched. Our observations of neutron-induced lethality responses seem to support this contention, at least in part. Progenitors consistently exhibited low resistance (high sensitivity) to fission neutrons over the narrow low-dose range of 0-75 cGy. In contrast to the sizable increases in gamma-ray resistance in progenitors of the MPD-prone dogs, only small incremental increases were noted in terms of neutron resistance. Undoubtedly, such differences in survival responses elicited by the two different radiation qualities are directly related, not only to the magnitude and quality of the initial cellular damage but also to differences in processing and repair of that damage by progenitors with distinct pathophysiological potentials. It is of interest that the sublethal damage (SLD) capacity, as a correlate of cellular repair potential (assessed by the *Dq* dose parameter), is either substantially reduced or ablated by fission neutrons in progenitors from both nonirradiated and chronically irradiated, MPD-prone animals (i.e., comparing *Dq* values from the gamma-ray data set to the neutron data set [Table 1]). The greatest level of SLD suppression (27 cGy) is seen in progenitors from the late postrecovery phase.

Our evidence shows that progenitors from the chronically irradiated dogs (especially those in the postrecovery phases) process, repair, and recover from low-LET radiation injury more efficiently than from high-LET injury. This suggests that the changes in injury repair may be specific to radiation quality or lesion quality. Additional studies on the molecular processing of genomic lesions by high- and low-LET radiations are needed to clarify these differences.

The neutron survival studies also yielded an additional interesting observation that might suggest an alternate mechanism of acquired resistance, one which doesn't directly involve acquired repair functions, but rather the simple selection of a preexisting radioresistant clonal subpopulation. We noted that the survival response for progenitors over an expanded neutron dose range (10-300 cGy) was biphasic: the initial response at low doses (10-75 cGy) was steeply sloped, while the secondary response (at doses greater than 100 cGy) had a more shallow slope (Fig. 4). Often, such biphasic responses are attributed to mixed populations (23). If this is true here as well, then the break in the survival response would indicate the presence of a small, markedly radioresistant GM-progenitor subpopulation within marrow of nonirradiated control dogs. By monitoring its survival characteristics, it would appear that this small subpopulation expands under chronic irradiation, minimally during the prerecovery phase and more extensively following hematopoietic crisis and recovery. Such observations favor the possibility that simple selection of preexisting radioresistant progenitors is a mechanism by which progenitorial populations of large, acquired radioresistance.

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REFERENCES

1. *BEIR Report.* Leukemia. In: The effects on populations of exposure to low levels of ionizing radiation, pp. 398-431, National Academy Press, Washington, DC, 1980.
2. Upton, A. C. Experimental radiation-induced leukemia. In: Radiation-induced Leukemogenesis and Related Viruses, Duplan, J. F., (ed.), p. 37, Elsevier/North Holland Biomedical Press, Amsterdam, 1977.
3. Henshaw, D. L., Eatough, J. P., and Richardson, R. B. Radon as a causative factor in induction of myeloid leukemia and other cancers. *Lancet* 335: 1009-1012, 1990.
4. Seed, T. M., Tolle, D. V., Fritz, T. E., Devine, R., Poole, C. M., and Norris, W. P. Irradiation-induced erythroleukemia and myelogenous leukemia in the beagle dog: Hematology and ultrastructure. *Blood* 50: 1061-1079, 1977.
5. Tolle, D. V., Fritz, T. E., Seed, T. M., Cullen, S. M., Lombard, L. S., and Poole, C. M. Irradiation-induced canine leukemia: A proposed new model. Incidence and pathology. In: Experimental Hematology Today. Baum, S. J., and Ledney, G. D. (eds.), pp. 247-256, S. Karger, New York, 1979.
6. Seed, T. M., Tolle, D. V., Fritz, T. E., Cullen, S. M., Kaspar, L. V., and Poole, C. M. Hematopathological consequences of protracted gamma irradiation in the beagle: Preclinical phases of leukemia induction. In: Late Biological Effects of Ionizing Radiation, pp. 531-545, International Atomic Energy Agency, Vienna, 1978.
7. Seed, T. M., Kaspar, L. V., Fritz, T. E., and Tolle, D. V. Cellular responses in chronic radiation leukemogenesis. In: Carcinogenesis, Vol 10, Huberman, E., and Barr, S. H. (eds.), pp. 363-379, Raven Press, New York, 1985.

8. Seed, T. M., Kaspar, L. V., Tolle, D. V., Fritz, T. E., and Frazier, M. E. Analyses of critical target cell responses during preclinical phases of evolving chronic radiation-induced myeloproliferative disease: Exploitation of a unique canine model. In: Multilevel Health Effects Research: From Molecules to Man, Park, J. F., and Pelroy, R. A. (eds.), pp. 245-255, Battelle Press, Richland, 1989.

9. Seed, T. M., Kaspar, L. V., Tolle, D. V., Fritz, T. E. Hemopathologic predisposition and survival time under continuous gamma irradiation: Responses mediated by altered radiosensitivity of hematopoietic progenitors. *Exp. Hematol.* 10: 232-248, 1982.

10. Norris, W. P., Fritz, T. E., Rehfeld, C. E., Poole, C. M. The response of the beagle dog to ^{60}Co gamma radiation: Determination of the LD50/30 and description of associated changes. *Radiat. Res.* 35: 681-708, 1968.

11. Seed, T. M., and Kaspar, L. V. Changing patterns of radiosensitivity of hematopoietic progenitors from chronically irradiated dogs prone either to aplastic anemia or to myeloproliferative disease. *Leukemia Res.* 14: 299-307, 1990.

12. Seed, T. M., and Kaspar, L. V. Hematopoiesis under conditions of chronic toxicity. In: Division of Biological and Medical Research, Research Summary, Argonne National Laboratory Report ANL-85-30, p. 69, 1985.

13. Fritz, T. E., Tolle, D. V., and Seed, T. M. The preleukemic syndrome in radiation-induced myeloid leukemia and related myeloproliferative disorders. In: The Preleukemic Syndrome, Bagby, G. C., Jr. (ed.), pp. 87-100, CRC Press, Boca Raton, Florida, 1985.

14. Seed, T. M., Chubb, G. T., and Tolle, D. V. Sequential changes in bone marrow architecture during continuous low dose gamma irradiation. *Scan. Elect. Microsc.* 1981: 61-72, 1981.

15. Seed, T. M., Chubb, G. T., Tolle, D. V., Fritz, T. E., Poole, C. M., Doyle, D. E., Lombard, L. S., and Kaspar, L. V. The ultrastructure of radiation-induced endosteal myelofibrosis in the dog. *Scan. Elect. Microsc.* 1982: 377-391, 1982.

16. Seed, T. M., Cullen, S. M., Kaspar, L. V., Tolle, D. V., and Fritz, T. E. Hemopathological consequences of protracted gamma irradiation: Alterations in granulocyte reserves and granulocyte mobilization. *Blood* 56: 42-51, 1980.

17. Seed, T. M., Fritz, T. E., Tolle, D. V., Poole, C. M., Lombard, L. S., Doyle, D. E., Kaspar, L. V., Cullen, S. M., and Carnes, B. A., Survival patterns and hemopathological responses of dogs under continuous gamma irradiation. In: Response of Different Species to Total Body Irradiation. Broerse, J. J. and MacVittie, T. J. (eds.), pp. 137-159, Martinus Nijhoff, Boston, 1984.

18. Williamson, F. S., Hubbard, L. B., Jordan, D. L. The response of beagle dogs to protracted exposure to ^{60}Co gamma rays at 5-35R/day-I. Dosimetry. In: Division of Biological and Medical Research Annual Report, pp. 153-156, Argonne National Laboratory Report ANL-7535, 1968.

19. Tolle, D. V., Fritz, T. E., Seed, T. M., Cullen, S. M., Lombard, L. S., Poole, C. M. Leukemia induction in beagles exposed continuously to irradiation: Hematopathology. In: Experimental Hematology Today, Baum, S. J., Ledney, G. D., and Thierfelder, S. (eds.), pp. 241-249, S. Karger, New York, 1982.

20. Grahn, D., Ainsworth, E. J., Williamson, F. S., and Fry, R. J. M. A program to study fission neutron-induced chronic injury in cell tissues and animals utilizing the JANUS reactor of the Argonne National Laboratory. In: Radiobiological Applications of Neutron Irradiations, p. 211, International Atomic Energy Agency, Vienna, 1972.

21. Seed, T. M., Kaspar, L. V., Tolle, D. V., Fritz, T. E. Chronic radiation leukemogenesis: Postnatal hematophathologic effects resulting from *in utero* irradiation. *Leukemia Res.* 11: 171-179, 1987.

22. Seed, T. M., Kaspar, L. V., and Grdina, D. J. Hematopoietic repair modifications during preclinical phases of chronic radiation-induced myeloproliferative disease. *Exp. Hemat.* 14(6): 443, 1986.

23. Alper, T. Cell survival curve shapes: Algebraic expressions. In: Cellular Radiobiology, pp. 44-46, Cambridge University Press, London, 1979.

Table I: Number and radiosensitivity of granulocyte/monocyte-committed hematopoietic progenitors from chronically irradiated and nonirradiated dogs in various preclinical phases of progressing myeloproliferative disease.

MARROW SOURCE	NO. TESTED	NO. CFU-GM ($x10^{-5}$)	RADIATION PARAMETERS						
			Gamma Rays[a]			Fission Neutrons[b]			
			Do	Dq	n	Do	Dq	n	RBE[c]
Nonirradiated Controls	19	120.9[d] ±9.7	77.4 ±5.1	6.1 ±3.1	1.14 ±0.05	27.9 ±1.2	1.6 ±1.1	1.07 ±0.04	2.79 ±0.17
MPD-Prone-I Prerecovery	2	30.6 ±12.1	141.9 ±38.2	(-13.3) (±21.8)	0.88 ±0.17	30.5 ±1.4	(-1.7) (±3.1)	0.96 ±0.095	4.61 ±1.04
MPD-Prone-III Postrecovery, Early	7	79.5 ±16.1	170.8 ±18.3	7.0 ±8.2	1.07 ±0.05	38.3 ±2.8	3.3 ±1.3	1.09 ±0.04	4.62 ±0.62
MPD-Prone-III/IV Postrecovery, Late	7	63.6 ±13.1	195.3 ±51.2	27.1 ±12.9	1.17 ±0.07	37.4 ±3.9	(-2.98) (±7.3)	1.05 ±0.13	5.50 ±1.27

[a]Gamma rays, radiosensitivity parameters estimated via linear regression analysis using full 10-300 cGy dose range.

[b]Fission neutrons, radiosensitivity parameters estimated via linear regression analysis using restricted dose range of 10-75 cGy.

[c]RBE values defined as the ratio of gamma *Do* value/Neutron *Do* value.

[d]CFU-GM values derived from zero-dose point and reported as average value (x 10^{-5} marrow cells plated) ± standard error.

Figure Legends

Figs. 1a-1c. Time-dependent changes in circulating levels of selected blood cell types within chronically irradiated, leukemia-prone dogs and within nonirradiated controls. Fig. 1a shows blood granulocyte responses; Fig. 1b shows monocyte responses; and Fig. 1c shows erythrocyte responses.

Fig. 2. Gamma ray survival responses of GM-progenitors from chronically irradiated dogs during prerecovery and postrecovery phases of progressing myeloproliferative disease. Progenitor responses from nonirradiated dogs are shown for comparison. (Figure is to left.)

Fig. 3. Survival responses of GM-progenitors following fission neutron exposures (10-100 cGy) *in vitro*. Responses of progenitors from chronically irradiated dogs, relative to controls are shown. (Figure is to right.)

Fig. 4. Biphasic survival responses of GM-progenitors following fission neutron exposures at higher doses (>100 cGy). Size and survival characteristics of a minor progenitor subpopulation are shown.

ABSTRACT

Probing Altered Hematopoietic Progenitors of Preleukemic Dogs with JANUS Fission Neutrons. T. M. Seed and L. V. Kaspar. Argonne National Laboratory, Argonne, Illinois.

Protracted courses of low daily dose gamma irradiation elicit high incidences of myeloproliferative disease, principally myeloid leukemia (ML), in beagle dogs (Seed et al., Leukemia Res. 11:171, 1987). A Four-phase preclinical sequence in the induction of ML has been described: 1) suppression, 2) recovery, 3) accommodation, and 4) preleukemic transition. Within this sequence, a critical "early" occurring hematopoietic target cell event that promotes progression of preclinical phase I and II has been identified and characterized by an acquisition of increased radioresistance to low-LET gamma rays by granulocyte/monocyte lineage-committed progenitors (CFU-GM). In order to gain further insight into basis of this critical event, the acquired survival response of preleukemic progenitors has been probed, *in vitro*, with high-LET fission neutrons. for these studies, marrow CFU-GM were isolated from chronically irradiated, preleukemic dogs, as well as from nonirradiated controls, subjected to graded doses (0-300 cGy) of either JANUS fission neutrons or 60Co gamma rays, and assayed for survival by a standard cloning assay. Major observations resulting from these assays include the following. First, the previously noted acquired radioresistance of preleukemic CFU-GM to low-LET gamma rays extends to high-LET fission neutrons as well. Relative to control CFU-GM exhibited small but significant increases in radioresistance of about 10 cGy, with an average *Do* value of 38 cGy (+2.3 cGy) for preleukemic CFU-GM and 28 cGy (+1.3 cGy) for the control CFU-GM irradiated within a narrow dose range of 10-75 cGy. Second, at higher neutron doses (150-600 cGy), fractional survival of both control and preleukemic CFU-GM declined nonexponentially, suggesting the existence of a small, radioresistant subpopulation constituting about 2% of the total marrow CFU-GM within normal nonirradiated dogs--but in preleukemic marrow (preclinical phases II-IV), a much larger 15% fraction of the progenitor population. The latter is most likely the result of a normally minor subpopulation gaining a growth advantage due to its inherent radioresistance, and clonally expanding in the strong selective pressure of chronic marrow irradiation, *in vivo*. We speculate that these qualitative/quantitative changes in progenitor function foster the initiation of aberrant regenerative hematopoiesis characteristic of early evolving radiation leukemogenesis.
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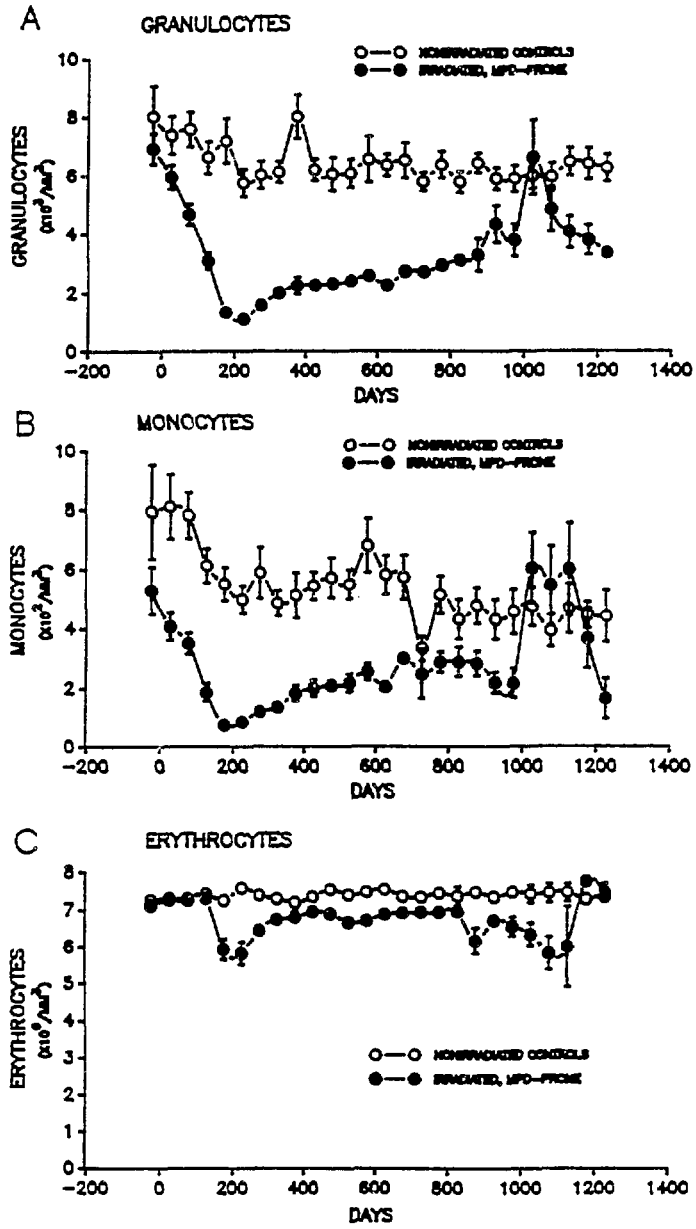

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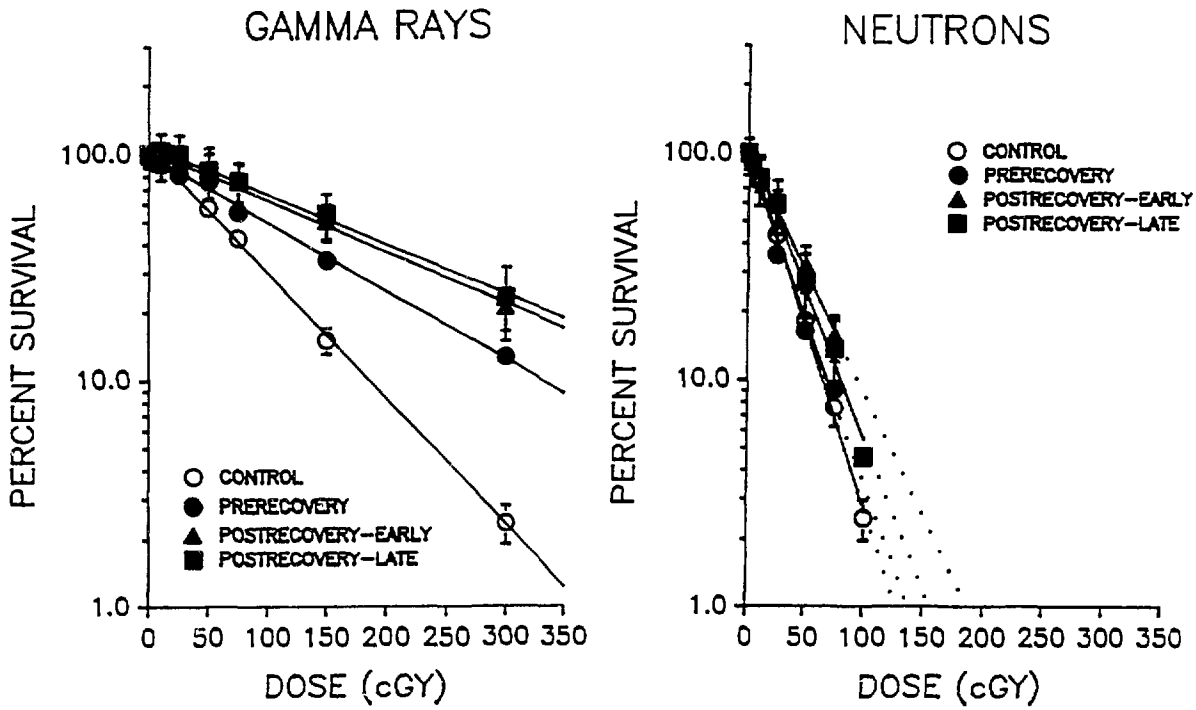

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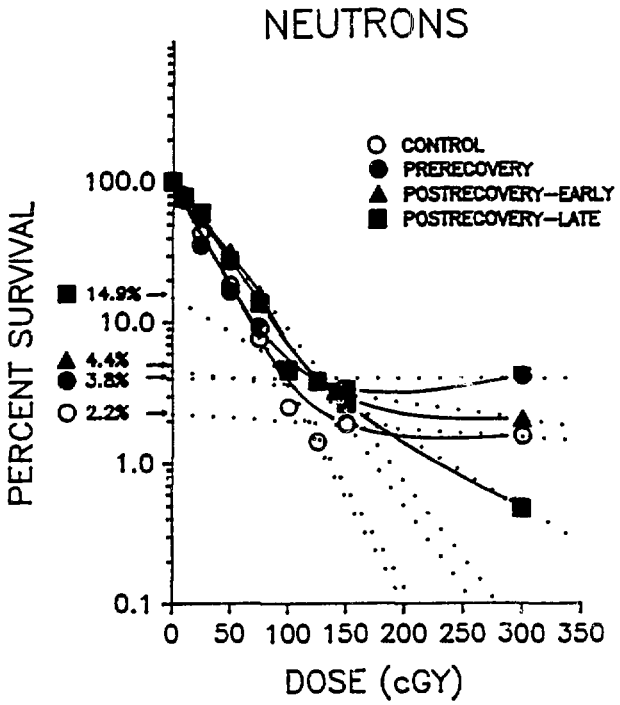

Fig. 4. Biphasic survival responses of GM-progenitors following fission neutron exposures at higher doses (>100 cGy). Size and survival characteristics of a minor progenitor subpopulation are shown.