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HEMATOPOIETIC CELL CRISIS: AN EARLY STAGE OF EVOLVING MYELOID LEUKEMIA FOLLOWING RADIATION EXPOSURE

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Under select radiological conditions, chronic radiation exposure elicits a high incidence of myeloproliferative disease, principally myeloid leukemia (ML), in beagles. Previously we demonstrated that for full ML expression, a four-stage preclinical sequence is required, namely (I) suppression, (II) recovery, (III) accommodation, and (IV) preleukemic transition. Within this pathological sequence, a critical early event has been identified as the acquisition of radioresistance by hematopoietic progenitors that serves to mediate a newfound regenerative hematopoietic capacity. As such, this event "sets the stage" for

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preleukemic progression by initiating progression from preclinical phase I to II. Due to the nature of target cell suppression, the induction of crisis, and the outgrowth of progenitors with altered phenotypes, this preleukemic event resembles the "immortalization" step of the in vitro transformation sequence following induction with either physical and chemical carcinogens. The radiological, temporal, and biological dictates governing this event have been extensively evaluated and will be discussed in light of their role in the induction and progression of chronic radiation leukemia.

INTRODUCTION

Myeloid leukemia (ML) is a prominent, late arising pathologic consequence of ionizing radiation exposure (BEIR, (1980)[1], Upton (1977)[2]). Although the induction of ML by moderate to high doses of ionizing radiation delivered acutely to the whole body is clear and unambiguous in a variety of species, including man, the inductive potency of low doses, especially those delivered over protracted periods, remains questionable (BEIR, (1980)[1]). The latter presents a problem in attempting to model by extrapolation the carcinogenic risks associated with low dose/dose rate radiation exposures. A practical solution to this informational deficit is to develop a better understanding of the basic mechanisms of low dose/dose rate leukemogenesis in order to build scientifically sound, realistic models of carcinogenic action at low dose/dose rate radiation exposures. In this regard, we have developed a canine model for chronic radiation leukemogenesis, in which the preclinical stages of evolving ML can be evaluated in cellular and molecular terms relative to the inductive and promoting effects of protracted low dose of exposures (Seed, *et al.* (1977)[3], Tolle, *et al.* (1979)[4], Fritz, *et al.* (1985)[5]). Using this model, we have identified and partially characterized a four preclinical phase sequence: I) suppression; II) recovery; III) accommodation; and IV) preleukemic transition (Seed, *et al.* (1978)[6]). The

leukemogenic potency of a given chronic radiation regimen has been shown to be largely dictated by the initial level of suppression and the subsequent strength and quality of functional hematopoiesis during recovery (Seed, *et al.* (1982)[7], (1989)[8]). In this study, we examined the quantitative and qualitative features of prerecovery- (precrisis) and postrecovery- (postcrisis) phase progenitors relative to progenitors obtained from nonirradiated (control) dogs. Due to similarities between the induced changes in hematopoietic progenitor responses during the initial two phases (suppression and recovery) and the well described processes of mammalian cell transformation in vitro, we argue here that such similarities might not be merely superficial, but might be tied to common sequences characteristic of transforming mammalian cells in general.

MATERIALS AND METHODS

Animals

Outbred beagles in this study were derived from the closed Argonne National Laboratory colony (for status, origin, and general management, see Norris, *et al.* (1968)[9]. For survival data and determination of leukemia incidence, a total of 203 dogs were used in groups of 122 experimental and 81 control animals. (Sixteen of the experimental animals and 54 of the controls were historical groups.) All dogs were in good health and were approximately 400 days of age at the time of initiating the study. The animals in this study were part of a larger group that has been and is currently under general toxicological evaluation for the long-term effects of chronic, low-dose irradiation (Seed, *et al.* (1985)[10]). Various hematopathological aspects of the latter work, including interim survival and leukemia patterns, have been reported previously (Seed, *et al.* (1977, 1978, 1980, 1981, 1982a, 1982b, 1984, 1985, 1989)[3,6,7,11-16]; Tolle, *et al.* (1979)[4]; Fritz, *et al.* (1985)[5].

Irradiation

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 (Norris, *et al.* (1968)[9], Williamson, *et al.* (1968)[17]). Animals were irradiated for duration-of-life at a daily dose rate of 7.5 cGy per 22-h day. Dosimetric methods and calculations have been outlined in detail elsewhere (Williamson, *et al.* (1968)[17]). The animals were caged, fed, watered, and clinically examined and manipulated as described previously (Tolle, *et al.* (1979)[4], Norris, *et al.* (1968)[9]).

Hematology

Hemograms were performed periodically by standard methods on each animal under test (Tolle, *et al.* (1982)[18]). For morphological and functional analyses of bone marrow with time and cumulative radiation dose, marrow biopsies and aspirates were performed at about 100-day intervals.

Bone Marrow Assays

Bone marrow collection: Marrow samples were obtained from either the ilia or humeri of irradiated and nonirradiated dogs by "snap" aspirations. Granulocyte/monocyte progenitors (CFU-GM) were enriched from the marrow samples by density gradient sedimentation procedures (Seed, *et al.* (1982a)[7]).

Quantitation of GM-progenitors: 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 (Seed, *et al.* (1982a)[7]). This method uses a "feeder" layer (containing 10^6 buffy coat leukocytes from control donor dogs and 10% pooled plasma from lethally irradiated [400 cGy] 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. Colonies were counted with an inverted light microscope.

Radiosensitivity testing of GM-progenitors: The radiosensitivity of progenitors was assessed in vitro using inhibition of colony formation as the measured end point (Seed, *et al.* (1982a)[7]). In brief, aliquots of CFU-GM-enriched marrow cell suspensions (about 10^6/ml) were irradiated in vitro with graded doses (0-300 cGy) of ^{60}Co gamma rays delivered at 25 cGy/min. In selected studies, duplicate progenitor-enriched samples were irradiated with JANUS fission neutrons (0.85 MeV mean energy) at 15 cGy/min to total doses of 0-75 cGy (Seed and Kaspar (1990)[19]). Descriptions of the general dosimetric procedures for these mammalian cell irradiations have been described elsewhere (Grahn, *et al.* (1972)[20]). The surviving fraction of CFU-GM within each cell sample was determined by the soft agar cloning procedure. The degree of radiation-induced CFU-GM death 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. From linear regression analyses, the radiobiological response parameters of lethality rate (*Do*), sublethal damage capacity (*Dq*), and subcellular target number (*n*) have been calculated.

Assaying repair capacity: The cellular repair capacity of GM-progenitors was assessed in vitro in terms of cell survival and recovery following either a standard "split-dose" protocol (40 cGy + 40 cGy split dose, interfractional intervals of 0-24 h), or by "variable dose-rate" protocol (25 cGy min^{-1} or 0.25 cGy min^{-1} irradiations to total doses of 0-150 cGy). DNA damage and repair properties were assessed within progenitor-enriched marrow samples using "single strand breaks" (SSBs) as the measured end point via an alkaline elution microfluorometric technique originally described by Meyn and

Jenkins (1983)[21]). Unscheduled DNA synthesis assays were performed via an autoradiographic method described by Lewensohn, *et al.* (1982)[22]).

Cytogenetic assays: Chromosome spreads were prepared and analyzed by standard protocols (LeBeau (1984)[23]).

RESULTS

Survival times and hematopathological responses in selected animal subgroups.

Two distinct survival patterns are observed in dogs subjected to continuous, low daily dose gamma irradiation at 7.5 cGy/day. The first pattern, common to 63% of the irradiated animals, is characterized by relatively short survival (average survival time of 213 days) due to the induction of predominantly aplastic anemia (AA), complicated at times with septicemia. The second pattern, expressed by 37% of the animals, is characterized by long-term survival (average survival time of 911 days) and the high frequency induction of a complex of hematoproliferative diseases whose major component is myeloid leukemia (Table 1). Approximately 43% of these long-surviving animals develop ML.

Blood response patterns.

Representative blood response patterns for the short-surviving, AA-prone dogs and the long-surviving, ML-prone dogs are shown in Figs. 1 and 2, respectively. The response of the AA-prone animal is characterized by a singular, suppressive phase in which the major circulating blood elements (i.e., erythrocytes, platelets, and total blood leukocytes) progressively decline to fatal levels following 100-150 days of chronic radiation. In contrast, the long-lived, ML-prone dog exhibits a multiphasic, differential cell response

pattern, in which circulating blood leukocytes and platelets, but not erythrocytes, are initially suppressed and later partially restored.

Marrow progenitor responses.

The origin of the differential blood response patterns lies in the bone marrow and, to a large extent, at the level of the hematopoietic stem cell and the early lineage-committed progenitors. Marrow progenitors committed to granulocyte/monocyte differentiation (CFU-GM) appear particularly susceptible to pathological insult arising from chronic, low-dose irradiation in vivo and seemingly can be used to monitor and to "track" damage within critical myeloid compartments.

Key characterizing features of progenitors obtained from the ML-prone dog, both prior to and following hematopoietic crisis, are listed in Table 2.

Quantitative changes: The AA-prone animal under chronic irradiation exhibits a progressive depletion of the GM-progenitor (CFU-GM) compartment (0.5% day^{-1}). The rate of depletion accelerates with time of exposure, which shortens the time required to deplete this vital compartment below the survival threshold (10%). In contrast, the ML-prone animal exhibits a lower, decelerating rate of depletion as it enters hematopoietic crisis. The vital threshold level is approached but not substantially breached (Fig. 3). With a slower rate of GM-progenitor depletion, additional time is afforded to the hematopoietic system for the subsequent repair and recovery (Fig. 3). Within a relatively short time period (i.e., 100-200 days from the nadir of the initial suppressive response), the GM-progenitor compartment in the marrow of the ML-prone dog is partially restored to about 33% of its original (preirradiation) level. Further restoration of the GM-progenitor compartment occurs with subsequent preclinical phase progression, despite continued daily irradiation.

Clonal properties: GM-progenitors from AA-prone animals, as well as from pre- and postcrisis phase ML-prone dogs, consistently give rise to a stable clonotype, namely the G/M mix colony. Further, GM-progenitors retain GM-CSA-dependence during these early pre- and postcrisis periods. However, during a late preclinical phase (phase IV, preleukemic transition), GM-progenitors from a select number of preleukemic dogs express GM-CSA independence. In terms of colony size and the ratio of colony-to-cluster formation, these parameters are substantially reduced with progression into hematopoietic crisis and are subsequently increased following crisis (Table 2).

Radiosensitivity: Precrisis GM-progenitors of either AA- or ML-prone dogs generally retain a high degree of radiosensitivity to low-LET photon radiation (*Do* values averaged over the entire precrisis period are 72 cGy for both AA- and ML-prone marrow progenitors). However, with pathological progression into the acute phase of hematopoietic crisis, progenitors of ML-prone dogs begin to exhibit increased radioresistance. With progression into postcrisis preclinical phases (preclinical phases II and IV), the radioresistance is further increased (average *Do* value of 170 cGy for preclinical phase II-IV progenitors) (Table 2). Qualitatively similar differences in fission neutron radiosensitivity are noted between precrisis and postcrisis phase progenitors of ML-prone dogs, although much higher levels of sensitivity are expressed and the magnitude of change between pre- and postcrisis phase samples is small compared to the change noted for gamma-irradiated samples (i.e., neutron *Do* values increased from 31 to 38 cGy for pre- and postcrisis phase samples, respectively). By comparison, in a single test of precrisis phase progenitors from an AA-prone dog, resistance to fission neutrons was slightly increased. Due to differences in the level resistance expressed by pre- and postcrisis phase progenitors subjected to high- and low-LET radiations, the calculated "relative biological effectiveness" values (RBEs) substantially increased in ML-prone dogs,

with pathological progression from the control hematopoietic state to crisis to postcrisis phases of the evolving disease (i.e., RBE values increased from 2.8 to 4.4 to 5.5, respectively).

Changes noted in progenitor's radiosensitivity during pre- and postcrisis phases are modified to varying degrees by pretreatment in vitro with select radiosensitizing agents prior to irradiation in vitro; e.g., cytosinearbinoside (Ara-C) pretreatment with acute, single doses that effectively inhibits DNA synthesis and increases the radiosensitivity (*Do* values) of postcrisis phase progenitors of ML-prone dogs by about 28% but has little effect on precrisis phase progenitors. In contrast, similar Ara-C pretreatment of precrisis phase progenitors from AA-prone dogs marginally reduces radiosensitivity by ~10%.

All progenitors, regardless of origin or pathophysiological status, fail to exhibit enhanced radiosensitivity following pretreatment with metronidazole (i.e., an established hypoxic cell sensitizer).

Repair/recovery capacity: Repair/recovery capacities of progenitors from ML-prone dogs are markedly altered under chronic irradiation, with progression into and out of hematopoietic crisis. This has been shown through a series of both cellular and molecular repair assays. Cellular repair functions, as assayed in vitro by "split-dose" protocols, appear significantly expanded, as evidenced by the expression of both new and elevated survival maxima. During precrisis, progenitor survival is elevated relative to control responses at all interfractional periods beyond 3 h (5-25 h) and with markedly extended survival at 7 h; during postcrisis, survival is significantly elevated at all tested intervals (1-24 h) and with peak survival occurring at 3 and 10 h. Precrisis progenitors from AA-prone dogs fail to show such marked survival enhancements with dose

fractionation: survival is marginally but not significantly enhanced at interfractional periods of 3-24 h.

A second type of cellular repair assay, namely survival enhancement at very low dose rates (0.25 cGy/min versus the normal dose rate of 25 cGy/min), provided additional data indicating that both pre- and postcrisis progenitors from ML-prone dogs have enhanced cellular repair capacity relative to progenitors obtained from either nonirradiated control animals or from AA-prone dogs.

Results of these cellular repair assays support the concept that marked decline in the progenitor's radiosensitivity (increase in radioresistance) following hematopoietic crisis is, in part, a reflection of an enhanced "sublethal damage capacity" (SLD) that is largely repair-mediated. In this regard, selected physicochemical agents, such as Ara-C and high-LET fission neutrons, have been shown to effectively quench the extended SLD capacity observed within postcrisis phase progenitors of ML-prone animals.

The extended SLD capacity of postcrisis phase progenitors is associated with, and most likely causally related to, enhanced DNA damage repair capacity, as evidenced by the results of two molecular assays (i.e., alkaline elution single-strand break [SSB] repair assay and autoradiography unscheduled DNA synthesis [UDS] assay). With the former assay, the "slow" SSB repair component of the overall DNA repair process has been shown to be enhanced quantitatively by about 15% within postcrisis-phase, progenitor-enriched marrow cells, whereas within precrisis-phase marrow cells (both AA- and ML-prone), slow SSB repair capacity is reduced below controls due to extremely slow repair rates. With the UDS assay, the inducible (with UV, gamma rays, or melphalan) component of a repair process appears to be markedly enhanced within postcrisis-phase progenitors, but only marginally enhanced within precrisis-phase progenitors.

Cell cycle properties: The extent of progenitor cell cycling during the pre- and postcrisis phases has been estimated using Ara-C "suiciding" protocols. During precrisis, the fraction of cycling cells (i.e., the fraction of progenitors in S-phase of the cell cycle) increases with time of irradiation and with pathological progression into the more acute phase of hematopoietic crisis. The extent of cycling during precrisis is somewhat higher in AA-prone animals than in ML-prone animals. During the postcrisis phase, the degree of progenitor cycling declines concomitantly with the extent of hematopoietic recovery and accommodation.

Cytogenetic alterations: During precrisis, marked alterations in chromosome number are mainly limited to the progenitor-enriched marrow elements of the AA-prone animal and are not a prominent feature of marrow elements of the ML-prone animal. However, with increasing time following hematopoietic crisis, the extent of hypodiploidy associated with evolving ML increases and is marked during late preclinical/clinical phases.

In terms of total scorable lesions (breaks, gaps, deletions, and Robersonian-like fusions), the highest frequencies are observed within marrow of the AA-prone animal progressing toward hematopoietic crisis. In contrast, marrow of the ML-prone animal shows lower frequencies during precrisis and, following crisis, elevated frequencies for selected types of lesions.

In terms of nonrandom structural lesions, a first chromosome lesion (1q+) appears largely pathology-specific and clonal in nature. This lesion is characterized by an extended, homogenous staining region near the distal portion of the long arm of the first chromosome. The 1q+ lesion is initially noted at low frequency as ML-prone dogs progress into the acute hematopoietic crisis phase and increases in frequency with passage out of crisis into late preclinical phases.

DISCUSSION

The intent of this study has been to examine the possibility that the initial two hematopoietic phases of evolving myeloid leukemia induced by chronic radiation -- namely, acute suppression and subsequent partial recovery (preclinical phases I and II) -- are akin to the precrisis and postcrisis phases that occur as an integral part of mammalian (fibrocytic/epithelial) cell transformation in vitro.

In the absence of exposure to a given transforming agent (e.g., SV40 virus, DEN, etc.), in vitro-cultured normal human diploid cells exhibit finite mitotic capacities and thus temporally defined life spans (Hayflick and Moorehead (1961)[24]). Upon exposure to a transforming agent, such cultures undergo a series of cytopathologic and proliferative changes collectively called transformation (Girardi, *et al.* (1965)[25]). Major cellular changes within the transforming sequence include: 1) the initial cytotoxic/cytopathic cell response; 2) enhanced compensatory proliferation of surviving cells; 3) subsequent massive cell degeneration and cell death resulting in a generalized loss of culture viability (referred to as "crisis"); and 4) the outgrowth and proliferation of small numbers of rare surviving cells with markedly altered characteristics in selected cultures. The most prominent of the latter alterations include unlimited growth potential (immortalization) and an enhanced propensity for neoplastic cell progression (Little (1985)[26]). The observed time constraints placed on the "naturally occurring" transformation sequence tends to foster the concept that the timing and control of successive stages of cell transformation and neoplastic cell progression is regulated internally by the targeted cell.

In an analogous fashion, the hematopoietic system of dogs responds to chronic exposure to the transforming agent (gamma rays) by an initial phase of cytopathic suppression coupled with a largely ineffective compensatory proliferation of progenitorial elements, resulting in a time-dependent progression into a "crisis" phase. The dominant

crisis response in vivo is overwhelming marrow cell death, followed by hematopoietic failure and death due to aplasia. Like precrisis mammalian cells in vitro, precrisis progenitors from AA-prone dogs in vivo exhibit high frequencies of cytogenetic lesions along with either enhanced or unadjusted physiochemical sensitivities that appear to augment progenitor cell death.

The minor hematopoietic crisis response pattern expressed in vivo by the smaller number of chronically irradiated ML-prone dogs displays characteristics seen in vitro during the normal course of mammalian cell transformation. The most prominent of these common features is the rare surviving clonotype with altered proliferative and mitotic potential that emerges during the acute phase of crisis and rejuvenates the dying culture or failing hematopoietic tissue (Seed, *et al.* (1982a, 1989, 1990, 1987, 1986)[7,12,19,27,28]). In both systems, postcrisis phase cells clearly acquire a set of new, survival-promoting traits. For example, in the case of SV40 virus transformation of normal human diploid cells, precrisis phase cells serve as hosts for the production of infectious and lytic viruses, whereas in postcrisis phase cells where the virus is genomically integrated, infectious virus production is shut off, blocking cytopathic responses due to viral infection and allowing unrestricted proliferation of the immortalized cells (Girardi, *et al.* (1965)[25]).

Precrisis progenitors from chronically irradiated ML-prone dogs have limited potential that is further restricted by chronic daily irradiation. The marked reduction in the number of progenitors with time of exposure leads toward hematopoietic crisis. The underlying basis of this reduction is the progenitor's relatively high radiosensitivity and marginal capacity to repair the constant cellular damage. Similar to the mammalian cell transformation sequence, rare clonotypes emerge during crisis which have renewed proliferative capacities and novel characteristics that promote the process(es) of

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neoplastic cell progression (Seed, *et al.* (1989)[12]). In contrast, however, to emerging transformed clones, these hematoprogenitor clones is not immortalized per se, but only have extended growth potential. The emergence of altered clones is restricted, both radiologically and temporally; i.e., sufficiently large, cumulative radiation doses (10-15 Gy) delivered in small daily fractions over time (150-200 days) are required in order to cause significant levels of progenitor cell suppression, and, in turn, high emergence frequencies (about 40%) of such clonotypes.

What novel characteristics are acquired by the emerging clone? The most important is extended growth potential, which tends to mimic the "immortalization" step of the transformation sequence. This time-dependent change in growth potential appears causally associated with increased radioresistance, mediated largely by enhanced cellular and molecular repair functions and genetically based on a nonrandom chromosomal rearrangement (1q+ lesion). Presumably, such acquisition(s) fosters a clonal growth advantage and, in turn, an enhanced progenitor cell renewal at the expense of the radiation-induced differentiative flow. Loss (or simply an uncoupling) of these tightly regulated vital cell functions (i.e., self-renewal and differentiation) might be responsible for the immortalization step in cell transformation (von Wangenheim (1987)[29], Sachs (1980)[30]). Although, the precise molecular mechanism responsible for such loss of regulatory control is yet to be defined, genic rearrangements that alter functional protooncogene or cytokine networks are undoubtedly involved (e.g., ras, fms, abl, raf, etc., gene mutations and rearrangements that selectively impart extended growth potential to the targeted progenitor) (Frazier, *et al.* (1989)[31], Pirollo, *et al.* (1989)[32], Rowley (1985)[33], Jacobs (1985)[34], Toksoz, *et al.* (1989)[35]).

How might the crisis-acquired characteristics be linked to the "uncoupling" of the progenitor's vital regulatory processes. If the threshold of radiation-induced

differentiative flow progressively rises due to an increase in radioresistance and associated repair functions following hematopoietic crisis, then the progenitor's self-renewal capacity would necessarily expand to the extent of the "block" in differentiation. In essence, the latter provides a caricature of an evolving preleukemic syndrome. Future studies will be directed toward the identification and characterization of the molecular processes responsible for this extended growth potential.

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TABLES

Table 1. Survival patterns and pathological responses of dogs subjected to protracted, low daily dose irradiation (7.5 cGy day^{-1}).

Table 2. Comparison of key characteristics of hematopoietic progenitors from aplasia-prone dogs and leukemia-prone dogs during both precrisis and postcrisis phase periods.

FIGURE LEGENDS

Fig. 1. Representative blood response pattern of major circulating elements (erythrocytes, leukocytes, platelets) of a chronically irradiated, aplasia-prone dog during progression into the acute, lethal phase of hematopoietic crisis. The upper panel charts the sequential pattern of radioresistance exhibited by marrow progenitors (CFU-GM), both prior to and throughout the course of chronic irradiation. The label "X" represents a normal radiosensitive clonotype.

Fig. 2. Representative blood response pattern of a chronically irradiated dog during the initial precrisis phase and the subsequent postcrisis phase(s) of evolving myeloid leukemia. The upper panel charts the sequential pattern of radioresistance exhibited by marrow progenitors (CFU-GM), both prior to and throughout the course of chronic irradiation. The label "X" signifies the normal radiosensitive clone, whereas "A" signifies the emergence of a radioresistant clone.

Fig. 3. Quantitative changes in marrow GM-progenitors from chronically irradiated dogs, prone to either aplastic anemia or to myeloid leukemia.

TABLE 1. SURVIVAL TIMES AND CAUSES OF DEATH OF CONTINUOUSLY GAMMA IRRADIATED (7.5 CGY/DAY) BEAGLES[a]

Parameter	Pathology/Survival Patterns	
	Short AA-Prone (<300 days)	Long ML-Prone (>300 days)
1. Number Displaying/Total	59/94	35/94
2. Percent	62.8	37.2
3. Mean Survival Time	213 (±44)	911 (±488)
4. Range of Survival Times	122-298	305-1966
5. Cause of Death (%)		
(a) Septicemia/Aplasia	11.9	5.7
(b) Aplasia	83.1	25.7
(c) Hemoproliferative Disease	1.7	57.1
- myeloproliferative dycrasias	-0-	5.7
- myelofibrosis	1.7	2.9
- myeloid leukemia	-0-	42.9
- lymphocytic leukemia	-0-	2.9
- lymphoma	-0-	2.9
(d) Solid tumors (nonhematopoietic)	-0-	2.9
(e) Other	3.3	8.6

[a]Of 122 irradiated (7.5 cGy/day) dogs included in this study, 94 are dead and are included in this table. The 28 remaining dogs are alive and are in various phases of evolving aplasia or hemoproliferative disease.

TABLE 2. CHARACTERISTIC OF PRECRISIS AND POSTCRISIS PHASE HEMATOPOIETIC PROGENITORS FROM LEUKEMIA-PRONE DOGS UNDER CHRONIC GAMMA IRRADIATION

CHARACTERISTIC	Progenitor Phase/Source[a]	
	Precrisis	Postcrisis
Clonal Properties		
Concentration	Progressively Reduced	Variable, Phase Specific (II Low/Increasing; III Subnormal/Oscillating; IV Subnormal/Rising)
Pre-radiation	Slightly Elevated	Slightly Elevated
Clonal Type	G/M Mixed	G/M Mixed
Size	Progressively Reduced	Variable, Phase Specific (II Small, III Large, IV Very Small)
Colony/Cluster Ratio	Progressively Reduced	Variable, Phase Specific (II Low, III Normal, IV Very Low)
CSA Dependence	Normal	Variable, Phase Specific (II-III Normal, IV Selectively Reduced)
Physical Properties		
Buoyant Density	≤1.077 g/l	≤1.077 g/l
Radiosensitivity		
Gamma (*Do*)	Normal (~72 rads)	Markedly Decreased (~170 rads)
Post-Ara-C (DMF)	No Change (~1.0)	Moderately Increased (~1.4)
Post-Metronidazole (DMF)	N.D.	No Effect (~1.0)
Neutrons (*Do*)	Slightly Decreased (~31 cGy)	Decreased (~38 cGy)
RBE (γ/n)	Increased (Ic ~4.6)	Increased (II-IV 4.6-5.5)
Chemical Sensitivity		
ARA-C (Chronic Exposure)(DMF)	N.D.	Increased (~42%)
Cell Cycle Properties		
S-Phase Fraction	Increased (10-30%)	Decreased (5-15%)
Repair/Recovery Capacity		
Split-Dose Response	Enhanced 7 hr max	Enhanced 3 and 10 hr maxs
SLD Capacity	Normal	Variable, Expanded
PDL Capacity	Normal	Expanded (?)
DNA-SSB - Slow Repair	Reduced	Expanded (~15%)
UDS - Inducible Capacity	N.D.	Expanded (~55%)
Cytogenetic Properties		
Ploidy	Diploid	Increased Hypoploidy (~12%)
Aberrancy Frequency	Slightly Increased (~4%)	Increased (~14%)
Nonrandom Lesions (1q+)	Increased (~15%)	Markedly Increased (25-50%)

[a]Progenitor characteristics and responses listed are relative to comparable responses of control progenitors from nonirradiated dogs.

END

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