Acquired Radioresistance of Hematopoietic Progenitors (Granulocyte/Monocyte Colony-forming Units) during Chronic Radiation Leukemogenesis

Thomas M. Seed and Lillian V. Kaspar

ABSTRACT

Protracted exposure of dogs to low daily doses of whole-body γ-radiation (7.5 cGy/day for duration of life) elicits a high incidence of myeloid leukemia or related myeloproliferative disorders. Under such exposure, vital hematopoietic progenitors [granulocyte/monocyte colony-forming units in agar (CFU-GM)] acquire increased radioresistance along with renewed proliferative capacity at an early phase of evolving myeloid leukemia. To further characterize the expression of acquired radioresistance by CFU-GM, we evaluated the effects of various exposure rates, cumulative radiation doses, and times of exposure and postexposure in several groups of long-lived dogs under two conditions of irradiation: (a) continuous, duration-of-life exposures at dose rates of 0.3–7.5 cGy/day; and (b) discontinuous, fraction-of-life exposures at dose rates of 3.8–26.3 cGy/day, with cumulative doses of 450–3458 cGy and postexposure times of 14–4702 days. Results indicated that (a) under protracted continuous irradiation, the degree of radioresistance expressed by CFU-GM in vitro increased markedly in a biphasic pattern with rising daily rates of exposure; (b) under discontinuous, fraction-of-life exposure regimens, elevated levels of radioresistance were expressed and stably maintained by CFU-GM only following large radiation doses accumulated at high dose rates; and (c) with extended postexposure times, the magnitude of expressed radioresistance appeared to wane. These results continue to support the hypothesis that the acquisition of radioresistance and associated repair functions by vital lineage-committed progenitors, under the strong selective and mutagenic pressure of chronic irradiation, is tied temporally and causally to leukemogenic transformation elicited by radiation exposure.

INTRODUCTION

Under appropriate exposure conditions, ionizing radiation can act as a potent leukemogen, both in animals and in humans (1–3). In the classic radiation leukemogenesis studies by Upton et al. (4–6), the leukemogenic potential of a standard dose of ionizing radiation was shown to be markedly different when the dose was delivered either acutely (in large single doses) or chronically (in small daily doses over an extended period). At a total exposure dose of 300 cGy, for example, acute exposures were demonstrated to be 3-fold more effective than chronic exposures in inducing myeloid leukemia in RF male mice (3). The “response-sparing” (in this case, leukemia-sparing) effect of protracting the course of exposure is commonly attributed to time-dependent repair of radiation-induced damage, regardless of whether the damage is prelethal, premutational, or pretransformational in nature (7).

From these and related studies, as well as from work in our own laboratory with canines and chronic radiation exposure, it is clear that numerous radiological and biological variables modulate the leukemic response of the individual and exposed population at large (3, 8–16). Radiologically, the more important determinants include dose rate, cumulative dose, and duration of exposure; biologically, age, sex, immunohematopoietic competence, and genetic/oncogenetic makeup are clearly important but are not outweighed by factor(s) that selectively control and alter hematopoietic function either during or following radiation exposure. One such factor is the “repair competence” of the hematopoietic organ against radiation damage. In our work with a canine model of chronic radiation-induced myeloid leukemia, we have identified a myeloid leukemia-prone phenotype, of which the characteristic hallmark is a hematopoietic system (specifically, hematopoietic progenitors) that has acquired increased but aberrant repair capacities along with renewed proliferative capacity (11, 12, 17–19). It would seem reasonable that the induction of these preleukemic hematopoietic changes within susceptible animals under chronic irradiation would be dictated by the same factors that control the expression of overt leukemic disease (e.g., dose rate, total dose, etc.).

The intent of this study, therefore, is to characterize the expression of acquired, repair-mediated radioresistance by vital hematopoietic progenitors (CFU-GM) within chronically irradiated dogs, as modulated by (a) rate of exposure, (b) cumulative dose, and (c) times of exposure and postexposure.

MATERIALS AND METHODS

Animals. Outbred beagles were derived from the closed Argonne National Laboratory colony; their status, origin, and general management have been described (20). A total of 105 beagles were used in this study: 34 (28 males, 6 females) served as nonirradiated controls, while the remaining 71 (60 males, 11 females) served as irradiated test animals. All dogs were anatomically and physiologically normal and in good health prior to entry into the experiment(s). These animals were part of larger groups under general toxicological evaluation of the long-term effects of chronic, low-dose irradiation. Various hematopathological aspects of the latter work, including interim survival and leukemia patterns, have been reported (8–13, 15–19, 21–27). These extended animal groups comprised 1079 animals (292 nonirradiated control animals and 787 chronically irradiated animals, of which 369 received continuous radiation exposures and the remaining 418 received fractionated exposures). These extended animal groups were used in this study for the sole purpose of developing and illustrating leukemic incidence rates under various regimes of chronic radiation exposure.

Animal Irradiation. All irradiated dogs were housed in standard-size fiberglass cages just prior to (approximately 2 weeks) and throughout the course of exposure. All experimental procedures, including the initiation of chronic radiation exposure (designated as day 0), were begun when the animals reached young adulthood (i.e., about 400–500 days of age). Dogs receiving a preset total dose of irradiation (i.e., discontinuous, fraction-of-life exposures) were maintained during the postirradiation period in wire pens. Control animals were caged similarly. Irradiations were carried out in specially designed live-in exposure units in agar; GM, granulocyte/monocyte colony-forming unit in agar; GM, granulocyte/monocyte colony-forming unit in agar; ML, myeloid leukemia; MPD, myeloproliferative disorders; SLD, sublethal damage; D0, dose estimate to reduce cell survival by e−2; Dn, dose estimate to reduce cell survival below 100%.

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2 To whom all requests for reprints should be addressed, at Radiation Hematology Group, Biological and Medical Research Division (B1M/202), Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439-4833.
A table of data and text from a scientific paper discussing the acquisition of radioresistance of CFU-GM.

Table 1: Test groups, number and sex of animals, number of marrows sampled, and radiobiological and temporal variables

<table>
<thead>
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<th>Test Group I. Continuous duration-of-life exposures</th>
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<tr>
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Test Group II. Discontinuous fraction-of-life exposures

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<th>No. dogs</th>
<th>Sex (M/F)</th>
<th>No. marrows sampled</th>
<th>Age at start (days)</th>
<th>Age at testing (days)</th>
<th>Age range at testing (days)</th>
<th>Dose rate (cGy)</th>
<th>Total dose (cGy)</th>
<th>Exposure time (days)</th>
<th>Postexposure time (days)</th>
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<td>3.75</td>
<td>3318</td>
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* Average values ± SD.
* One sample (1/11) excluded from final analyses due to unacceptable low progenitor plating efficiency.
* Three animals (3/24) excluded from final group due to poor marrow/progenitor yields.
* Twelve samples (1/11) excluded from final analyses due to unacceptable low progenitor plating efficiencies.

The cloning method uses a "feeder" layer (containing 10^6 buffy coat leukocytes from control donor dogs and 10% pooled plasma from acutely irradiated (400 cGy) dogs) to support CFU-GM colony formation in the upper agar layer (containing 0.5 × 10^6 to 1 × 10^6 target marrow cells/ml). Plates were incubated in 5% CO_2 at 37°C for 8 days. Colonies were counted using an inverted light microscope. Colony type was checked by double-esterase cytochemical staining of whole-mounted agar slabs of randomly selected cultures. Colonies were predominantly and consistently (>90%) of the granulocyte/monocyte mixed variety. Colony counts per 10^6 marrow cells plated were used to estimate concentrations (or surviving fractions) of CFU-GM within given marrow cell preparations.

To assess the radiosensitivity of CFU-GM in vitro, aliquots of stock cell suspensions were irradiated with ^60^Co γ-rays at 25 cGy/min to total doses of 0, 10, 25, 50, 75, 150, or 300 cGy. The surviving fraction of CFU-GM within each cell sample was determined by the cloning procedure described above. The radiation-induced CFU-GM killing at each dose level was assessed in terms of inhibition of clonogenic activity relative to the zero-dose control. Minimum cloning efficiencies of more than 10 CFU-GM/10^5 marrow cells plated (at zero dose) were required for the analysis and inclusion of dose-response data analyses. Minimum cloning efficiencies were not reached in approximately 5% of the total marrow samplings; these samples were restricted to marrows obtained from animals under irradiation rates of 3.75 and 7.5 cGy/day delivered in the continuous duration-of-life exposure mode. From the calculated fraction of surviving CFU-GM at each of the radiation doses, dose-response curves were constructed via linear regression analyses. The response parameters of D_0, D_∞, and subcellular target number (n) were derived from these analyses.

Data Analyses. All marrow response data, regardless of the parameter under assessment, were analyzed in terms of the time-constrained response of individual animals. Multiple marrow responses for individual animals recorded over specific time intervals were pooled, averaged, and assessed against appropriate control groups. These averaged response values, along with calculated SEs, were plotted against either time of radiation exposure (or postexposure time) or daily rate of exposure. Statistical analyses of the comparative responses of irradiated and nonirradiated animals were performed by using Student's t test, with levels of significance between group responses recorded as values of P.
ACQUIRED RADIORESISTANCE OF CFU-GM

RESULTS

Responses under Continuous, Duration-of-Life Exposure Regimens

Incidence of Myeloproliferative Disease. The overall incidence of MPD within groups of dogs continuously exposed to low daily doses of whole-body γ-irradiation increased from a low value of 1.4% at the lowest dose rate tested (0.30 cGy/day) to a high value of 41.7% at 3.75 cGy/day (Fig. 1). At higher rates (7.5–26.25 cGy/day), the incidence of MPD declined concomitantly with a marked reduction in average survival time resulting from hematopoietically ablative hypoplastic/aplastic marrow diseases. In contrast, eliminating the short-lived dogs (i.e., dogs surviving less than 300 days, which represent 60.3% of the group at 7.5 cGy/day and 73.3% of the group at 12.75 cGy/day) from the analyses, the incidence of MPD continued to rise at the 7.5 and 12.75 cGy/day dose rates, reaching incidences of 45.5% and 50.0%, respectively (Fig. 1). At the highest dose rate tested (26.25 cGy/day), all of the dogs (100%) exhibited ablative marrow responses to chronic radiation exposure and associated short-term survival patterns (<300 days of survival) without any evidence of evolving MPD.

Change in Progenitor Number as a Function of Exposure Time. In long-lived, MPD-prone dogs continuously irradiated under the potent leukemogenic exposure regimen of 7.5 cGy/day, a common time-dependent response pattern of marrow CFU-GM was noted (Fig. 2): a precipitous decline in the number of marrow CFU-GM during the initial period of exposure (~50–125 days) was followed by a gradual recovery to nearly control levels following extended periods of radiation exposure (>700 days).

Change in Progenitor Radioresistance as a Function of Exposure Time. Under a continuous exposure rate of 7.5 cGy/day, the radiosensitivity of marrow CFU-GM from long-lived MPD-prone dogs markedly changed with both time of exposure and preclinical phase progression (Fig. 3). During the initial phase of exposure (<100 days), marrow CFU-GM from these dogs expressed a high degree of radiosensitivity ($D_0 \approx 50$ cGy), whereas following extended exposure periods (>300 days), progenitors expressed markedly increased levels of radioresistance ($D_0 \approx 150$ cGy; $P \approx 0.02$ relative to either age-matched controls or experimental controls less than 100 days). The time frame for the transition between radiosensitive and radioresistant clonotypes was 100–200 days and occurred in concert with the initial preclinical phase transition, i.e., preclinical phase 1 (suppression) to preclinical phase 2 (partial recovery). (See Refs. 13, 17, 19, 23, and 24 for additional information on preclinical phase characteristics.)

The SLD capacity of marrow CFU-GM from long-lived dogs showed similar changes dependent on time of exposure, i.e., marginal SLD capacities ($D_v \approx 10$ cGy) expressed during early phases of exposure (<100 days) and extended capacities ($D_v \approx 180$ cGy) expressed during late phases of exposure (>300 days).
change in radioresistance of GM progenitors of long-lived dogs (>600 days) relative to the daily rate of radiation exposure delivered continuously for duration of life. Average values ± SEs plotted.

Fig. 6. Change in sublethal damage capacity of GM progenitors of long-lived dogs (>600 days) relative to the daily rate of radiation exposure delivered continuously for duration of life. Average values ± SEs plotted.

25-50 cGy) following prolonged exposures (>400 days; \( P \leq 0.02 \)) (Fig. 4).

In contrast to marked changes in radiosensitivity and sublethal damage capacity exhibited by marrow CFU-GM from the long-lived, MPD-prone dogs, marrow CFU-GM from chronically irradiated, short-lived (<300 days) dogs with evolving hypoplastic/aplastic marrow disease(s) failed to exhibit such changes (data not shown).

Change in Progenitor Radioresistance as a Function of Exposure Rate. Decreasing the rate of exposure from 7.5 cGy/day to lower dose rates (e.g., about 1.9-0.3 cGy/day) resulted in a substantial reduction (\( P \leq 0.001 \)) in the level of radioresistance of marrow CFU-GM from long-lived, chronically irradiated dogs (656-3773 days of chronic exposure) (Fig. 5). The net reduction in radioresistance was ∼68 cGy (representing about a 40% decline as estimated by the change in \( D_0 \); Fig. 5). The dose rate-dependent decline in radioresistance occurred in a biphasic pattern; between the dose rates of 7.5 and 1.0 cGy/day, the decrement in radioresistance was estimated at ∼6 cGy/cGy of daily dose rate; between 1.0 and 0 cGy/day, the rate of decline was estimated at ∼61 cGy/cGy of daily dose rate (Fig. 5).

The progenitor’s SLD capacity declined from the high levels observed at 7.5 cGy/day (\( P < 0.0001 \)) to control-like levels at 1.88 cGy/day (Fig. 6). A relatively low decrementing rate of ∼2.5 cGy/cGy of the daily dose rate was estimated over this range of higher exposure rates (7.50 to 1.88 cGy/day). At the lower range of exposure rates tested (0.75 to 0 cGy/day), the SLD capacity again was substantially elevated at 0.75 cGy/day relative to the controls (\( P < 0.0001 \)) and decreased at an estimated rate of ∼24 cGy with further unit reductions in the daily dose rate (Fig. 6).

Responses under Discontinuous, Fraction-of-Life Exposure Regimens

Incidence of Myeloproliferative Disease. Low incidences of MPD occurred within the discontinuous, fraction-of-life exposure groups, whether the groups were analyzed in their entirety or in segregated “high-risk” subpopulations, e.g., long-term survivors (Fig. 7). Relatively low peaks of MPD incidence (about 11%) occurred in groups irradiated at 7.5 cGy/day to total doses of 1500 and 3000 cGy. Incidences were further reduced (to about 5%) at lower cumulative doses (1050 and 450 cGy), as well as at both lower (3.8 cGy/day) and higher (12.8 and 26.3 cGy/day) dose rates (Fig. 7).

Change in Progenitor Number as a Function of Postexposure Time. Regardless of initial daily rate of exposure or cumulative radiation dose, at extended postexposure periods, the number of marrow GM progenitors either approached or slightly exceeded levels found in marrow from comparably long-lived, nonirradiated control animals (Fig. 8). These minimal differences between irradiated and nonirradiated animals were not
ACQUIRED RADIORESISTANCE OF CFU-GM

Fig. 9. Sequential change in marrow GM progenitor number during the time surrounding the termination of chronic radiation exposure (7.5 cGy/day for 461 days). Average values ± SEs plotted.

Fig. 10. Comparison of GM progenitor radiosensitivity in long-lived nonirradiated dogs with chronically irradiated (3.75 or 7.5 cGy/day) dogs exposed under either the continuous (con) or discontinuous (dis) exposure modes. Both control responses and dose rate-specific responses represent pooled data sets. Average values ± SEs plotted.

significant, however (P > 0.2). The recovery in progenitor number following termination of chronic irradiation occurred early and rapidly (<100 days), as suggested by the sequential marrow responses of dogs specifically analyzed during this period (Fig. 9).

Change in Progenitor Radiosensitivity as a Function of Post-exposure Time. By comparison with the levels of radiosensitivity of marrow progenitors from long-lived dogs continuously irradiated at 3.75 or 7.5 cGy/day, the radiosensitivity of marrow progenitors from comparably long-lived, chronically irradiated dogs at extended postexposure periods (>3000 days) was markedly reduced (P < 0.01) and not significantly different from that of an age-matched, nonirradiated control group (P < 0.05) (Fig. 10).

Comparable relationships were observed in the low levels of SLD capacity in progenitors from dogs treated under discontinuous exposure regimens (Fig. 11).

The time course of the reduction in progenitor radiosensitivity following termination of chronic irradiation is illustrated in Fig. 12. The high levels of radiosensitivity noted prior to terminating radiation exposure not only continued during the early postexposure period but dramatically increased in one of the two dogs under study. Following extended postexposure times, the latter long-lived animal (bearing the markedly radioresistant CFU-GM) exhibited a prolonged, graded reduction in radiosensitivity, with "control" levels being reestablished at about 880 days postexposure (Fig. 12). Associated with this gradual shift from the acquired radioresistant to the radiosensitive phenotype was the apparent reestablishment of normal hematological parameters. This response pattern was contrasted with the second dog, which retained markedly elevated levels of radiosensitivity during its 56-day postexposure survival course and died of acute leukemia.

Change in Progenitor Radiosensitivity as a Function of Exposure Rate and Cumulative Radiation Dose. An extended survey of the radiosensitivity progenitor phenotype within the marrow of long-lived dogs showed that discontinuous, fraction-of-life exposure regimens were largely ineffective in promoting (or maintaining) the radioresistant trait (Fig. 13). Only at the relatively high dose rate of 12.75 cGy/day and doses of 1050 and 1500 cGy were moderately (but significantly) radiosensitive CFU-GM observed (P ≤ 0.001) (Fig. 13). Dose rates (<12.75 cGy/day), as well as the lower cumulative doses, consistently failed to elicit (or maintain) levels of radiosensitivity comparable to those observed under continuous irradiation.

The marrow progenitor’s SLD capacity was not extended significantly from control levels (as seen under continuous, duration-of-life exposures) at the time of testing and as a consequence of prior chronic irradiation, despite the relatively
high exposure rates (3.8–26.3 cGy/day) and sizable total cumulative doses of 450–1500 cGy (P < 0.2) (Fig. 14).

DISCUSSION

Previously we had demonstrated that chronic exposure of dogs to low daily doses of whole-body γ-radiation induced a high incidence of myelodysplasia that often progressed to overt ML (10, 13). Under optimal radiological conditions (e.g., 7.5 cGy/day for duration of life), the frequency of these late-arising myelodysplastic syndromes rose to well over 50% in selected subgroups of dogs (11). We further demonstrated through sequential analyses of hematopoietic function that a critical preclinical event in the evolution of the myelodysplastic syndrome involved an early phase of aberrant hematopoietic repair that mediated a broadly based hematological recovery following an initial phase of acute hematopoietic suppression (17). Furthermore, the induction of the hematopoietic repair/recovery process(es) under chronic irradiation seemingly served to segregate the entire exposed population into distinct subpopulations with characteristic hematopathological tendencies (e.g., one subpopulation was prone to aplastic anemia, whereas another was prone to myeloid leukemia). Subsequent analyses demonstrated that the hematopoietic progenitors of the ML-prone dogs taken either during or following recovery exhibited marked change not only in clonal activity but also in radiation sensitivity.

The original intent of this study was to evaluate the effect of altering selected radiological and temporal variables (i.e., dose rate, total dose, etc.) of chronic irradiation on the expression of radioresistance by hematopoietic progenitors in ML-prone dogs. The results presented here clearly indicate that select changes in exposure variables affected not only the acquisition but also the stability of the rare progenitor phenotype (i.e., radioresistant progenitors), along with associated changes in induction frequencies of myelodysplasia and preleukemic and leukemic syndromes. First, the extent of exposure strongly dictated the induction frequency; i.e., the more prolonged the exposure, the higher (and apparently the more stable) the acquired radioresistance by GM progenitors. At the extremes, for example, the induction frequencies for the aberrant progenitor clone type and myelodysplastic syndrome ranged from 0% to 100% as the duration of exposure (e.g., at 7.5 cGy/day) was extended from 60 to 400 days. Minimum (threshold) exposure times of about 125 days were required for the progenitor event to be expressed (at the 7.5 cGy/day dose rate).

Like the exposure time variable, the daily dose rate strongly influenced the expression of the acquired progenitor response as well; i.e., the higher the daily rate (within the range of 0.3–7.5 cGy/day), the higher the rate of expression of the aberrant progenitor phenotype. However, at the higher dose rates (12.8 cGy/day or higher), long-term survival was often compromised to the extent that minimum exposure times were not reached, thus curtailing progenitorial expression.

The total cumulative dose is inexorably linked to both the time of exposure and the daily dose rate. As such, the cumulative dose-dependent threshold for the expression of the progenitorial event appeared to be about 1000 cGy, as indicated by analyses of progenitor responses from dogs under both continuous and discontinuous radiation regimens. It is important to note that these time and radiation dose thresholds closely correspond to the noted thresholds for leukemia induction. In this regard, it is remarkable that despite the range of dose rates and exposure times, cumulative doses as large as 450 cGy consistently failed to elicit either the aberrant progenitor response or evidence of evolving leukemic disease in these dogs.

In addition to examining the induction and expression of the aberrant progenitorial event, the stability of the response was examined. Stability is considered an important variable, since the acquired progenitor response is seemingly tied to an early phase of aberrant hematopoietic repair during evolving myeloid leukemia (17, 18). From the results presented, once the radioresistance phenotype was acquired under continuous irradiation and the daily exposure continued, the phenotype appeared to be extremely stable. This was borne out by the sequential response of selected animals in which multiple marrow samples were collected and tested and the aberrant progenitor phenotype was expressed for prolonged periods (up to hundreds of days).

In contrast, by discontinuing the daily radiation exposure after critical thresholds (time and total cumulative dose) had been reached, the acquired phenotype appeared to become unstable and was eventually lost in the majority of animals during prolonged postirradiation periods. Nevertheless, in a number of animals that had been previously irradiated under the more potent leukemogenic exposure regimens (12.8 cGy/day to cumulative doses of 1050–1500 cGy), the acquired radioresistance phenotype continued to be expressed for extremely long postirradiation periods (e.g., 3300–4300 days; Table 1).

The destabilizing action (in terms of loss of expression of the
radioresistant phenotype) of discontinuing the chronic exposure was only inferred initially by noting the often marked differences in levels of expression within groups exposed to discontinuous versus continuous chronic irradiation. This clonal de-stabilization was subsequently verified within a small number of sequentially assessed animals. This assessment served to highlight the apparent association between the acquisition and maintenance of progenitorial radioresistance and subsequent preleukemic/leukemic transformations and, conversely, the loss of progenitorial radioresistance and the subsequent reversal of any preleukemic changes that might have occurred.

Although the results presented indicate that strong temporal relationships exist between the acquisition of progenitorial radioresistance and the induction and progression of MPD, the question of causal relationships still remains. There are a number of possible causal mechanisms. For example, the acquired radioresistance seen in targeted progenitors under chronic irradiation might be due largely to an enhanced, error-prone repair capacity. As a result of the simultaneous induction and repair/misrepair of genomic lesions, critical genomic errors might accumulate with time and elicit increasingly aberrant but vital progenitorial functions (e.g., self-renewal and differentiation). Although the latter causal mechanism is largely speculative at this point, we have obtained solid evidence that the acquired radioresistance in marrow progenitors is largely mediated by enhanced and aberrant molecular and cellular repair capacities (17, 18, 31). The absolute fidelity of the repair, however, remains to be established. Nevertheless, we as well as a number of other investigators consider misrepair to be a critical pretransformational process associated with radiation carcinogenesis (7, 32, 33).

A related mechanism possibility concerns repair-mediated oncogene activation. Recent work has indicated that selected oncogenes (e.g., fms, abl) as well as the dominant transforming oncogene, n-ras, are not only activated but amplified and overexpressed as well, during patent phases of ML in our chronically irradiated dogs (34). This latter observation raises the obvious possibility that the early preclinical events associated with acquired radioresistance and progenitor repair and recovery are tied to the activation of selected oncogene species. Although this remains to be determined experimentally, the recent studies by other investigators (35) have shown that the transfection of selected oncogenes (i.e., abl, src, erb-b) in both cloned hematopoietic progenitor cells (32D cl 3 cells), as well as clonal stromal fibroblastic cells, impart not only a high degree of radioresistance to relatively low-dose rate γ-irradiation but also the expression of autocrine-like functions (growth-factor independence). It is of interest to note that the progenitorial acquisition (autocrine-like function) elicited by oncogene transfection is also noted in a small number of our chronically irradiated dogs during late preleukemic phases of evolving ML (17).

A third possible mechanism relates to the well-documented cellular radiation effects of "enhanced differentiative flow" and its associated reciprocal suppression of mitotic capacity (36). Considering the possibility that a radiation dose threshold for these effects exists and can be modified through selective environmental and pathophysiological pressures, any change in the radiation threshold would serve to promote a disequilibrium between vital progenitor functions, namely self-renewal and differentiation. Lowering the threshold would enhance "differentiative flow," suppress self-renewal, and promote loss in numbers of viable progenitors (analogous to responses seen in evolving aplasia). In contrast, elevating the threshold would serve to block differentiative flow, enhance self-renewal, and, in turn, promote a gain in number of viable progenitors. At this extreme, this process would provide a caricature of evolving MPD.

In summary, the results presented support the concept that acquisition by marrow progenitors of the aberrant radioresistant phenotype is governed by a select set of temporal and radiological parameters. Furthermore, acquisition of the progenitor trait is associated with evolving myelodysplastic disease and, therefore, may be a useful "high-risk" indicator of impending disease.

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