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HEMATOPOIETIC TISSUE REPAIR UNDER CHRONIC LOW DAILY DOSE IRRADIATION

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ABSTRACT

The capacity of the hematopoietic system to repair constantly accruing cellular damage under chronic, low daily dose gamma irradiation is essential for the maintenance of a functional hematopoietic system, and, in turn, long term survival. In certain individuals, however, such continuous cycles of damage and repair provide an essential inductive environment for selected types of hematopathologies, e.g., myeloid leukemia (ML). In our laboratory we have been studying temporal and causal relationships between hematopoietic capacity, associated repair functions, and propensities for hematologic disease in canines under variable levels of chronic radiation stress ($0.3\text{--}26.3\text{ cGy d}^{-1}$). Results indicate that the maximum exposure rate tolerated by the hematopoietic system is highly individual-specific (three major responding subgroups identified) and is based largely on the degree to which repair capacity, and, in turn, hematopoietic restoration, is augmented under chronic exposure. In low-tolerance individuals (prone to aplastic anemia, subgroup 1), the failure to augment basic repair functions seemingly results in a progressive accumulation of genetic and cellular damage within vital progenitor marrow compartments (particularly marked within erythroid compartments) that results in loss of reproductive capacity and ultimately in collapse of the hematopoietic system. The high-tolerance individuals (radioaccommodated and either prone- or not prone to ML, subgroup 2 & 3) appear to minimize the accumulating damage effect of daily exposures by extending repair functions, which preserves reproductive integrity and fosters regenerative hematopoietic responses. As the strength of the regenerative response manifests the extent of repair augmentation, the relatively strong response of high-tolerance individuals progressing to patent ML suggests an insufficiency of repair quality rather than repair quantity. The kinetics of these repair-mediated, regenerative hematopoietic responses within the major subgroups are under study and should provide useful insights into the nature of hematopoietic accommodation (or its failure) under greatly extended periods of chronic, low-daily-dose ionizing radiation exposure.

INTRODUCTION

There are numerous health risks associated with extended space travel, not the least of which include radiation exposure-induced pathologies. Organ systems with strong self-renewing functions (e.g., reproductive and blood-forming tissues) would be principal targets for such radiation injury. Induction patterns for a given pathology depend on both the level of radiation exposure and the genetic constitution of the individual being exposed. In terms of radiation exposure levels, it has been estimated that astronauts living and working on space platforms in earth orbits are chronically exposed to approximately 0.81 mGy per day [1]. Greatly extended resident times (about 100 days or greater) would result in sizable cumulative radiation doses.

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The pathologic consequences of such chronic, low-daily dose exposures are uncertain and ill-defined. We have developed a canine model for the analyses of selected types of blood disorders (e.g., myeloid leukemia and aplastic anemia) induced under chronic, low-level ionizing irradiation /2/. With this model, we have identified distinct preclinical stages and cellular-events associated with evolving blood disease /2/.

In this paper, we attempt to demonstrate relationships between an individual's predisposition to a given hematopathology and the relative radiation responsiveness of principal cellular targets within hematopoietic tissues (bone marrow). Specifically, we ask, "Do response rates (rates of suppression and recovery) reflect pathologic tendencies under chronic radiation stress?". The apparent answer to this question is a qualified "Yes."

METHODS

Animal type and number Analyses of hematopoietic responses under chronic, low-daily-dose irradiation is based on an experimental animal model described in detail elsewhere /3/. In brief, intentionally outbred, young adult (about 400 days of age) beagle dogs from the closed Argonne canine colony were selected for testing; all were deemed in good health and were anatomically normal. For this study, a total of 67 dogs (49 males and 18 females) were selected for analyses of specific hematopoietic functions of interest (sequential quantitation of marrow stem cells). These animals were selected from an extended group assigned to generic chronic radiation (122 test animals) and nonirradiation (22 controls) treatment protocols, but sufficiently assayed for both type and number of specific hematological parameter functions of interest (e.g., quantitation of marrow stem cell levels).

Chronic low dose rate exposures Animals assigned to the test group were housed in a specially designed chronic low-level radiation exposure facility and exposed in a near-continuous mode to ^{60}Co gamma rays at daily dose rate of 7.5 cGy per 22 h day. Exposures were carried out for duration of life, starting at approximately 400 d of age. Nonirradiated (control) animals were caged and handled in an identical fashion, except for their housing in an adjacent, shielded anteroom.

Clinical and pathologic assessments All dogs were periodically monitored for general clinical status via clinical examinations, weight checks, blood analyses. All primary pathologies, either contributing or directly responsible for the death of the animal, were determined by a combination of standard hemato- and/or histopathologic assessments /2,3/.

Hematopoietic progenitor assays Hematopoietic progenitor (GM-CFU) assays were performed periodically by in vitro soft agar cloning methods previously described /3/. Bone marrow samples were isolated by aspiration from either the iliac crest or the epiphyseal region of humeri from individual animals. Light buoyant density fractions of marrow cells were prepared and assayed for GM-CFU content /3/. Numbers of granulocyte/monocyte-committed progenitors (GM-CFU) per number of bone marrow isolated and plated were estimated from the number of granulocyte/monocyte (GM) colonies generated from 10^5 marrow cells plated per dish. A high-efficiency double-layer procedure, with a "feeder" underlayer and "target cell" overlayer, was employed for the majority of tests. Cloning efficiencies determined by the older, less efficient single layer procedure were adjusted by the use of constant, $k = 2.18$.

Analytic procedures Marrow progenitor responses of both chronically irradiated dogs and nonirradiated controls were analyzed within overlapping time frames that corresponded to an "early suppressive" period (about 0-200 d) and a 'late recovery' period (about 200-800 d) (Figure 1). Sequentially obtained progenitor marrow levels for individual animals during these two time frames were determined and subjected to linear regression analyses. Standard, time/phase-dependent responses were determined for each animal and subsequently pooled with the responses of animals of common groups. Such groups consisted of: nonirradiated controls and chronically irradiated test animals displaying either short survival (<200 d; 200-300 d; or 300-500 d) subgroups prone to aplastic anemia [AA], or long survival (>500 d) and prone to either myeloid leukemia [ML]; or diseases other than ML [non-ML]. Average rates, along with confidence intervals for rates of progenitorial suppression or recovery (Figure 1), were determined

by standard methods (Instat statistical program). Comparisons between average group rates were made by the Student's *t* test.

Fig. 1. Generic response modeling approach for the initial suppressive hematopoietic phase and subsequent secondary recovery phase.

RESULTS

Rates of Suppression Under Chronic Radiation Exposure

Rates of suppression of hematopoietic progenitors (GM-CFU) within bone marrow aspirates from the three major subgroups under study (i) short surviving, AA-prone animals; (ii) long-surviving, ML-progressing animals; and (iii) long-surviving, non-ML-progressing animals — were comparable and not significantly different (Figure 2). However, when the AA-prone subgroup was subdivided based on length of survival (i.e., <200 d; 200-300 d; or 300-400 d), the most sensitive of the AA-prone animals (<200 d survival) exhibited significantly higher suppression rates either of the responses of two slightly longer-lived AA-subgroups (Figure 3) or the very long-lived non-ML prone subgroup (Figure 2). By contrast, rates of progenitor suppression within the ML-progressing subgroup was not significantly different from any of these subgroups, owing to the large response variance noted (Figure 2).

Rates of Recovery Under Chronic Radiation Exposure

Marked differences in recovery rates of GM-committed progenitor marrow levels were noted in the various subgroups under test. The long-lived, chronically irradiated dogs that developed ML exhibited significantly stronger recovery than the comparably long-lived subgroup of animals that developed and died of other pathologies (non-ML pathologies) (Figure 4). Within the latter non-ML subgroup, differences in recovery rates were noted as well: animals that developed and died with lymphoproliferative-like syndromes exhibited the lowest recovery rates, whereas those bearing solid tumors (carcinomas and sarcomas) showed higher rates (Figure 5).

Fig. 2. (upper left) Rates of suppression of marrow progenitors (GM-CFU's) for the three major groups under test; i.e., AA, ML, and nonML subgroups.

Fig. 3. (upper right) Rates of suppression of marrow progenitors for AA subgroups.

Fig. 4. (lower left) Rates of recovery of marrow progenitors for ML and nonML groups.

Fig. 5. (lower right) Rates of recovery of marrow for nonML subgroups.

DISCUSSION AND CONCLUSIONS

Analyses of the rates of bone marrow progenitor suppression and recovery responses of dogs under chronic, low-daily-dose (7.5 cGy d^{-1}) gamma irradiation indicate a high degree of individual variability, due largely to differences in inherent hematopoietic radiosensitivity and pathologic predispositions. It is clear, however, that there are correlations between rates of progenitor suppression and recovery and subsequent hematopathologic progression and survival of the individual. It is also clear that subgroups of individuals with common progenitor sensitivities and hemopathologic predispositions exist and can be identified. For example, the most hematopoietically sensitive animals (i.e., AA-prone animals with survival courses less than 200 d) exhibited significantly higher initial rates of suppression than did either of the two AA-prone subgroups with slightly extended survival times, or the long-lived subgroup that eventually developed and died of a battery of non-myeloid leukemic syndromes. By contrast, the long-lived ML subgroup exhibited a high rate of suppression, not significantly different from any of the AA-subgroups. Recovery rates exhibited by the ML-subgroup were significantly higher than those of the non-ML subgroup and in turn infinitely higher than the recovery deficient responses of the AA subgroups.

Do these terrestrially based, chronic gamma-ray-exposure experiments in dogs have any relevance to the radiological problems associated with extended space travel by man? Indeed they may. First, the pathologic uncertainties associated with chronic, low-level radiation exposure, particularly at the level of the individual, are sufficiently great to warrant a better understanding of the health risks, and in particular, the cancer risks. Chronic, low-level radiation exposure represents one of the more serious hazards facing astronauts during extended (or multiple) space missions, with multiple assignments accentuating overall health risks and in particular cancer risks /4/. Nevertheless, there are valid questions concerning differences in radiation qualities (e.g., high Z particles and protons versus low-LET gamma rays), as well as differences in species. In terms of the long-term mortality and cancer risk, findings in proton-irradiated nonhuman primates /5/ indicate that overall life-shortening is influenced primarily by dose rather than the proton energies, and that a major contributor of this life-shortening is cancer (but mainly of the nonleukemic type). This is in contrast to the incidence of high myeloid leukemia in the chronically gamma-irradiated animals reported here, but not so in terms of other comparable studies in which dogs were irradiated at extremely low daily rates ($0.3\text{-}1.9 \text{ cGy d}^{-1}$) /5/. Common patterns of ML induction have been shown at these lower dose rates as well /7/; i.e., at exposure rates that might reflect and model "worst-case" exposure scenarios that astronauts might experience following intense solar particle shower events /8/.

Considering the above, the limitation of using chronically delivered low-LET gamma rays instead of high-LET protons or heavy ions (HZE particles) might indeed be minimal due to the relatively low consensus estimates for the weighting factor (1-1.1) for protons /4/, as suggested by the findings of the USAF/NASA proton bioeffects project /6/.

As to the question of species differences, the hematopoietic organ systems of the two species (canine/human) are extremely comparable, both in terms of overall structure and function, as well as their radiation-induction sensitivities to the prominent hematopathologies /7/.

There is an urgent need to develop a new generation of risk analyses parameters based on the "individual", and not solely the population at large. In this regard, the canine model presented might well serve as a useful vehicle in an attempt to identify early hematopoietic response indicators for the various radiation-induced blood disorders, regardless of whether the chronic exposure stems from terrestrial or galactic sources.

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