Chronic Radiation-induced Alteration in Hematopoietic Repair during Preclinical Phases of Aplastic Anemia and Myeloproliferative Disease: Assessing Unscheduled DNA Synthesis Responses

Thomas M. Seed and Susan M. Meyers

ABSTRACT

Protracted, low-daily-dose γ-ray exposure (3.8–7.5 cGy/day) segregates canines into separate survival- and pathology-based subgroups by the early elicitation of distinct, repair-mediated hematopoietic repair pathways. In this study, we verified the blood and marrow responses of two major subgroups prone to either aplastic anemia or myeloproliferative disease, along with two variants, and extended our analyses of hematopoietic repair to include studies of DNA repair in bone marrow blasts using an autoradiographically based unscheduled DNA synthesis (UDS) assay. The myeloproliferative disease-prone subgroup exhibited extended survival (>200 days), related to partial, gradual restoration of blood leukocyte, platelet, and marrow progenitor levels following an initial phase of acute suppression. Marrow blasts taken during the restoration phase showed expanded and qualitatively modified UDS relative to marrow blasts of age-matched control animals. The amount of UDS per blast showed expanded and qualitatively modified UDS relative to marrow blasts of age-matched control animals. The amount of UDS per blast (signal strength) increased significantly, as did the number of UDS-positive cells and their sensitivities to high-dose UV induction and 1-β-D-arabinofuranosylcytosine chemical inhibition. A non-evolving myeloproliferative disease-prone variant having prolonged survival (>200 days) and restored blood cells and marrow progenitor levels also had marrow blasts with expanded UDS responses, but these were uniquely evoked by low (but not high) doses of UV inducer. The aplastic anemia-prone subgroup was characterized by short survival (<200 days), progressive decline (without restoration) in all measured blood and marrow compartments, and largely nonsignificant changes in UDS responses of marrow blasts. A variant of this aplastic anemia-prone subgroup (with comparable short survival due to markedly ineffective hematopoiesis, but expressing select preleukemic features) exhibited reduced numbers (relative to age-matched controls) of highly responsive, UDS-positive marrow blasts (in terms of UDS signal strength and increased sensitivity to 1-β-D-arabinofuranosylcytosine-induced UDS inhibition). From these observations we conclude that: (a) the UDS response of marrow blasts, a correlate of hematopoietic progenitorial repair, is altered differentially within selected subgroups of animals under chronic radiation exposure; and (b) the nature of altered UDS repair response patterns appears to be largely related to the preclinical status/predisposition of the individual animal and thus may provide prognostically useful information in the clinical monitoring of chronically irradiated individuals with minimal but evolving hematologic disease.

INTRODUCTION

An increased awareness is being developed concerning the relationship(s) between DNA repair sufficiency and the potency of a given toxicant to cause pathological conditions (1). In a number of well-documented genetic diseases of man (e.g., xeroderma pigmentosum, ataxia-telangiectasia, Bloom’s syndrome, Fanconi’s anemia, Cockayne’s syndrome, etc.), insufficient DNA repair capacity has been related clinically to a hypersensitivity to a variety of physical and chemical toxicants, as well as causally associated with a marked predisposition to several significant pathological conditions, including cancer (2). Multiple gene locus-based defects in various steps along the major repair pathways (e.g., excision, recombination, post-replication repair pathways) appear responsible for promoting selected types/frequencies of DNA lesions and chromosomal rearrangements and, in turn, manifesting increased cell death, mutation, and transformation (3).

The potential for multiple sites of repair dysfunction lends itself to a range of repair capacities in patients suffering from such genetic diseases. Cells of patients with xeroderma pigmentosum, for example, exhibit a wide range of, but generally suppressed, UDS activity and, in turn, an overall reduced repair capacity stemming from altered DNA polymerase function (4). Xeroderma pigmentosum complementation groups B and G exhibit UDS responses less than 10% of the normal control levels, whereas groups C, E, and F have UDS levels between 10 and 50% of control levels, and a variant subgroup, group D, has nearly normal UDS responses. By contrast, assays of xeroderma pigmentosum repair function based on measures of “end step” ligase activity often show “normal” levels of activity (1). Such differential, repair pathway-specific changes have been associated with other disease states as well (e.g., in therapy-induced leukemias, the capacity to repair toxicant-elicited DNA lesions is compromised due to differential alterations along the repair pathway; UDS-related DNA polymerase activity is amplified, whereas strand ligase activity is suppressed) (5).

In the above pathological conditions, preexisting genetic/epigenetic lesions clearly underlie and perhaps promote the toxicant-directed changes in repair capacity. An important question that arises from the latter is, “What promotes the toxicant-mediated repair responses within ‘normal’ individuals that outwardly lack signs of genetic/epigenetic-based diseases?” It would appear that the normal individual’s repair capacity, in terms of both magnitude and fidelity, can be differentially altered under varying parameters of toxicant exposure (dose, dose rate, exposure time, etc.) (6–8). The work by Tuschl et al. (9) indicates that selected repair functions (UDS/polymerase responses) can be significantly amplified after prolonged courses of very low dose toxicant exposures (i.e., two qualities of ionizing radiation). The late pathological consequences of this amplified toxicant-elicited repair is, however, unclear and needs to be examined.

We have previously identified distinct subgroups of experimental, outbred dogs within a closed colony with selected predispositions to various types of hematopathological conditions (e.g., AA, myeloid leukemia, and related MPD) under chronic ionizing radiation exposure (10). Further work demonstrated that such pathological predispositions were largely based on differences in the magnitude and plasticity of hematopoietic repair under chronic toxic stress (11–13). This relationship serves as a basis of a working hypothesis suggesting that early aberration of repair of chronic hematopoietic injury mediates and promotes early stages of evolving myeloproliferative disease.

The intent of this study, therefore, was to use UDS as a repair correlate in an attempt to monitor and qualitatively assess repair capacity in essential bone marrow elements of chronically irradiated...
individual animals prone to the major pathological conditions of interest, namely, AA, myeloid leukemia, and related MPD.

MATERIALS AND METHODS

Animals

Outbred dogs (beagles) used in this study were derived from the closed Argonne National Laboratory colony. Details concerning the origin, status, and management can be found elsewhere (14). A total of 17 dogs were used in this study: 6 animals (5 males, 1 female) served as nonirradiated controls; while the remaining 11 (9 males, 2 females) served as irradiated test animals (Table 1). All animals were anatomically and physiologically normal and 536 ± 27 (SE) days old at the time they entered the study. These animals were part of a larger group of animals that had been under general toxicological evaluation for the long-term effects of chronic, low-dose irradiation. Various hemato pathological aspects of the latter work, including interim survival and leukemic patterns, have been reported previously (10–13, 15–24).

Irradiation

All test animals were chronically exposed to low daily doses (7.5 cGy/day) of whole-body 60Co γ-rays. Exposures were carried out (starting on the designated experimental day zero) in a near-continuous mode (22 h/day) and were generally extended for the duration of life or until a preset total exposure dose had accumulated (19). The exposure dose accumulations for each of the groups under study are given in Table 1. Descriptions of the chronic radiation exposure facility, as well as the dosimetric methods and calculations, have been given in detail in previous publications (25).

Hematology

Standard blood hemograms were developed periodically on every animal under test by using standard methods (18).

Bone Marrow Aspirations, Progenitor Enrichments, and Cell Cloning

Bone marrow samples were obtained from either the ilia or humeri of irradiated and nonirradiated dogs under general anesthesia by "snap" aspiration with pediatric spinal tap and a 25-ml syringe containing anticoagulant (4 ml 1.1% EDTA-0.7% NaCl). A total of 19 bone marrow specimens were collected and processed specifically for UDS/repair function analyses (Table 1).

Stock leukocyte/progenitor-enriched cell samples were prepared from the collected marrow samples by an initial Ficoll-sodium diatrizoate density gradient centrifugation, the slides were stained with Giemsa.

Microscopic Analyses of Autoradiographs.

Microscopic analyses of autoradiographs were analyzed with light microscopy by using a standardized photographic grain-counting procedure (30). In brief, UDS response of either single cells or populations of cells was semiquantitatively estimated by a direct, visual count and fixation, the slides were stained with Giemsa.

Table 1. Characteristics of experimental groups of beagles: numbers, sex, age, radiation exposure conditions, and sampling parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental status</th>
<th>No. of animals</th>
<th>Sex (M/F)</th>
<th>No. of tests</th>
<th>Age SE</th>
<th>Test time a</th>
<th>Test time range</th>
<th>Radiation exposure rate c</th>
<th>Exposure dose d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls 1</td>
<td>Nonirradiated, &lt;200 days</td>
<td>2</td>
<td>2/0</td>
<td>3</td>
<td>706 ± 6</td>
<td>112 ± 6</td>
<td>106–125</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Controls 2</td>
<td>Nonirradiated, &gt;200 days</td>
<td>4</td>
<td>3/1</td>
<td>4</td>
<td>1639 ± 157</td>
<td>1098 ± 208</td>
<td>568–1437</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Test 1</td>
<td>Chronic irradiated, &lt;200 days</td>
<td>4</td>
<td>4/0</td>
<td>4</td>
<td>600 ± 55</td>
<td>116 ± 4</td>
<td>106–125</td>
<td>0.075</td>
<td>8.68 ± 0.305</td>
</tr>
<tr>
<td></td>
<td>AA-prone</td>
<td>1</td>
<td>1/0</td>
<td>1</td>
<td>683</td>
<td>119</td>
<td>119</td>
<td>0.075</td>
<td>8.93</td>
</tr>
<tr>
<td>Test 2</td>
<td>Chronic irradiated, &gt;200 days</td>
<td>5</td>
<td>3/2</td>
<td>6 a</td>
<td>1668 ± 149</td>
<td>1117 ± 184</td>
<td>568–1676</td>
<td>0.075</td>
<td>83.71 ± 13.76</td>
</tr>
<tr>
<td>Non-MPD-prone</td>
<td>1/0</td>
<td>1394</td>
<td>965 b</td>
<td>965</td>
<td>0.075</td>
<td>34.58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Average chronological age (in days) ± SE.  
b Average experimental time (in days) of testing ± SE (day zero = start of exposure or sham exposures).  
c Daily rate of whole-body γ-ray exposures (in Gy, 22 h/day, 7 days/week).  
d Average cumulative exposure doses ± SE at time of testing.  
e Six marrow samples tested with one test censored due to low blast counts.  
f Animal exposed to 461 days and removed from exposure field for 504 days prior to testing at 965 days.
erythroid marrow cells of varying degrees of maturity (blasts, mitotic, postmitotic elements). Raw nuclear grain counts were adjusted for background noise by subtracting the average grain count per equivalent but nonnuclear area. Lightly or moderately labeled cells with nuclear grain counts ranging from 1 to 100 grains/nuclei were scored as UDS positive; heavily labeled cells with nuclear grain counts greater than 100 were considered to be S phase. Cells with no grains per nuclei were recorded as UDS negative.

**Data Analyses**

All response data, regardless of parameter, were analyzed in terms of the time-constrained individual animals which were selectively grouped in terms of both their radiation exposure and hematopathological status (Table 1). Multiple blood and marrow responses for individual animals recorded over specific time intervals were pooled, averaged, and assessed against appropriate control groups. For the blood responses, pooling of response data was done at 50-day intervals for the initial 200 days of exposure and then at 100-day intervals thereafter. These averaged blood responses, along with calculated SEs for the two major subgroups (AA-prone and MPD-prone subgroups) and the two variants were plotted against time of radiation exposure and assessed against comparably treated, nonirradiated controls. For the marrow responses, data were pooled within two time frames (i.e., <200 days or >200 days) for each of the irradiated and control subgroups under test. In the case of the UDS assays, pooled and averaged responses (i.e., UDS signal per cell and number of UDS-positive cells present) of the subgroups were plotted against inductive doses of UV irradiation (i.e., 0, 20, and 200 J/m²). Statistical analyses of the comparative responses of irradiated and nonirradiated subgroups were performed by using Student’s t test, with levels of significance between group responses recorded as values of P.

**RESULTS**

**Hematopoietic Responses of Pathology-prone Subgroups under Chronic Irradiation**

**Blood Responses.** Blood response patterns of the MPD-prone subgroup under chronic irradiation were characterized by marked suppression in circulating levels of platelets and leukocytes during the initial period of chronic radiation exposure (<200 days at 7.5 cGy/day) (Fig. 1A). Erythrocyte levels were maintained at approximately normal control levels (Fig. 1, A and B). With extended exposure (>200 days), recovery occurred within circulating leukocyte and platelets pools (Fig. 1A).

The nonevolving non-MPD-prone variant exhibited blood response patterns comparable to the evolving MPD-prone subgroup, except that recovery of blood leukocyte levels was more pronounced after 600–1000 days of exposure (Fig. 1C).

The AA-prone subgroup’s blood response patterns were characterized by a progressive, marked decline in circulating levels of not only leukocytes and platelets but also erythrocytes (Fig. 1D). Recovery responses, characteristic of long-surviving MPD-prone animals, were not observed (Fig. 1D).

The AA-PLS variant showed comparable blood response patterns to the AA-prone subgroup (Fig. 1E).

The blood responses of the nonirradiated controls, by contrast with those of the irradiated subgroups, were relatively stable over the extended period of testing (Fig. 1F).

**Marrow Progenitor Levels and Extent of Progenitor Cell Cycling.** The numbers of marrow progenitors committed to granulocyte/monocyte production were markedly reduced in all chronically irradiated subgroups except for the nonevolving non-MPD-prone variant in which chronic radiation exposure was discontinued at an earlier time (after 461 days of exposure) (Fig. 2A). Relative to the age-matched controls, marrow progenitor levels in the AA-prone subgroup and the AA-PLS variant were suppressed by 91 and 95%, respectively (Fig. 2A). The residual surviving AA-prone progenitors exhibited significantly increased cell cycling, suggested by the approximately 30% increase in the S-phase fraction (i.e., the ara-C “suicide-sensitive fraction”), while the extent of cell cycling in the AA-PLS variant progenitors remained slightly below (by ~15%) control levels (Fig. 2B). Marrow progenitor levels of the MPD-prone subgroup during the postradiotherapy phase(s) were higher than those of the nonevolving AA or AA-PLS marrows but still were significantly suppressed (by ~80%) relative to levels seen in nonirradiated control marrows (Fig. 2A). This contrasted the fully recovered, near control levels (~115%) of the nonevolving variant (Fig. 2A). The percentage of MPD-prone progenitors in active cell cycle (in S phase) increased by approximately 11% relative to the controls but remained relatively low compared with the elevated levels observed in AA-prone progenitors.

**Assay of Scheduled and Unscheduled DNA Synthesis Responses of Marrow Elements from Chronically Irradiated and Nonirradiated Subgroups**

**Initial Analyses of Cell Cycle and UDS Response Status of Marrow Blasts and Differentiated Progeny.** Via autoradiographic assay, marrow blasts from the MPD-prone subgroup exhibited a slightly expanded fraction of heavily labeled S-phase cells (~41% blasts in S phase), relative to both the age-matched controls (>200 days) and the nonevolving MPD-prone animal (~31% and ~30% S phase, respectively) (Fig. 3). Marrow blasts from the AA-prone subgroup, by contrast, had a comparable average number of heavily labeled S-phase marrow blasts, relative to the age-matched controls (<200 days). The AA-PLS variant, by contrast, showed a markedly elevated percentage of heavily labeled S-phase cells (~80%) (Fig. 3).

All S-phase blasts, regardless of origin, exhibited marked sensitivity to ara-C treatment and the degree to which semiconservative DNA replication was inhibited (Fig. 3). With the exception of marrow blasts from the non-MPD-prone variant, all blast specimens showed greater than a 97% reduction in S-phase labeling after ara-C treatment (Fig. 3).

In non-S-phase marrow blasts from all control and experimental subgroups, the intensity of nuclear labeling (i.e., the nuclear UDS signal) under either permissive (-ara-C) or nonpermissive (+ara-C) assay conditions consistently increased by severalfold after low-dose UV induction and, to a lesser, more variable extent, after high-dose UV induction (as described below and shown in Figs. 4, 6, 7, and 9). By contrast, less intense UDS signals and fewer UDS-positive cells were observed in the more differentiated marrow elements (data not shown).

**UDS Responses of Marrow Blasts from the MPD-prone Subgroup versus Age-matched Control Subgroup.** Under nonpermissive test conditions, the average UDS signal within marrow blasts of chronically irradiated, post-recovery-phase MPD-prone dogs increased approximately 5-fold after relatively low UV inductive doses of 20 J/m² and increased approximately 4-fold after the high UV inductive dose of 200 J/m² (Fig. 4A). Marrow blasts from age-matched (>200 days) nonirradiated control animals, by contrast, exhibited significantly (P < 0.05) lower UV-induced UDS signals after both low and high UV inductive doses (Fig. 4A). Concomitant with the increased UDS signal of the MPD-prone blast, the number of UDS-positive blasts within MPD-prone marrows increased approximately 10% after low-dose UV induction (20 J/m²), representing about a 20% increase in the number of UDS-positive cells over control levels (Fig. 4B).

Under permissive test conditions, UDS signal strength of the MPD-prone blasts increased from approximately 3.5-fold to approximately 5-fold after low (20 J/m²) to high (200 J/m²) UV inductive doses (Fig. 4C). The latter increase in UDS signal (per blast) was accompanied by sizable increases (10–20%) in the number of UDS-positive cells (Fig. 4C).
Fig. 1. Blood response patterns of the hematopathology-prone subgroups of dogs under chronic radiation exposure and of a nonirradiated control group. The change in circulating blood levels of leukocytes, platelets, and erythrocytes with time is shown. (A) MPD-prone subgroup with evolving disease under chronic radiation exposure; (B) nonirradiated control group over a similar, extended time course; (C) nonevolving non-MPD-prone variant after 461 days of chronic exposure and subsequently left unexposed; (D) AA-prone subgroup with evolving disease under chronic irradiation; (E) AA/PLS variant under chronic exposure, with evolving aplastic marrow disease, complicated with PLS.

4D). By comparison to UDS patterns seen under nonpermissive conditions, marrow blasts of age-matched controls exhibited relatively strong UDS signals (~5-fold increase) after low-dose UV induction but again relatively weak UDS signals (~3.4-fold increase) at high-dose UV induction (Fig. 4D). The number of UDS-positive control blasts remained relatively constant under the different UV exposure conditions (Fig. 4D).

Relative differences in the strength of UDS signals expressed by marrow blasts under nonpermissive (+ ara-C) and permissive (~ara-C) testing conditions changed differentially as a function of UV dose in the MPD-prone and control subgroups (Fig. 5, A and B). In marrow blasts of both subgroups, steady-state UDS signals were marginally enhanced under nonpermissive testing (+ara-C). After UV induction, relative UDS signal strength of control blasts was significantly suppressed at both UV dose levels, whereas the UDS signals of MPD-prone blasts were markedly enhanced at the low-dose UV level (P < 0.036) and only marginally suppressed at the high-dose UV level (Fig. 4, A and B).
specimens derived from chronically irradiated and nonirradiated subgroups of animals under test. (A) Number of marrow hematopoietic progenitors; (B) fraction of hematopoietic progenitors in S phase.

UDS Responses of the Nonevolving Non-MPD-prone Variant.

Under nonpermissive test conditions, the UDS signal of marrow blasts from the non-MPD-prone variant markedly increased (~5-fold) after low-dose UV induction but was not significantly increased after high-dose UV induction, in terms of either the steady-state UDS level or the UDS response of control blasts from nonirradiated animals at the 200-J/m² dose level (Fig. 6A). The number of UDS-positive blasts from the non-MPD-prone marrow increased by approximately 25 and 15% from steady-state levels after both low and high UV inductive doses, respectively (Fig. 6B). At both UV dose levels, the numbers of UDS-positive cells were approximately 25–30% higher than the levels observed in the control marrow blast populations (Fig. 6B).

Under permissive test conditions, UDS signals of non-MPD-prone blasts markedly increased from steady-state UDS levels after low-dose UV induction but were not significantly increased at the high UV inductive dose (Fig. 6C). Relative to the UDS response of the control blasts from nonirradiated dogs at the high UV inductive dose, the non-MPD-prone blasts were less responsive, in terms of both UDS signal strength and numbers of UDS responsive cells (Fig. 6, C and D).

The relative strength of UDS signal elicited by blasts of the non-MPD-prone variant is moderately enhanced under the nonpermissive test conditions, not only at steady state but also at the two UV dose levels (Fig. 5C). The latter contrasts the relatively marked suppression of UDS signal strength of control blasts noted under nonpermissive test conditions after UV exposures (Fig. 5B).

UDS Responses of the AA/PLS Variant. Under nonpermissive test conditions, UDS signal strength of the AA/PLS marrow blasts from the prerecovery phase increased approximately 4- and 5-fold at the low and high UV inductive doses, respectively (Fig. 7A). By comparison to the UDS response of marrow blasts of age-matched, nonirradiated control dogs, the latter represents approximately a 2.5-fold increase at both UV dose levels (Fig. 7A). The number of UDS-positive AA/PLS blasts at steady state was elevated (~20%) relative to control levels but declined (~15%) after low-dose UV exposure and remained near control levels after high-dose UV exposure (Fig. 7B).
UDS SIGNAL: MPD-prone

(A) UDS SIGNAL: CONTROL (≥200d)

(B) UDS SIGNAL: non-MPD-prone

Fig. 5. Relative change in strength of UDS signal displayed by marrow blasts under nonpermissive versus permissive test conditions. (A) MPD-prone blasts; (B) control blasts from age-matched (≥200 days) control animals; (C) nonevolving non-MPD-prone variant blasts.

Under permissive test conditions, the steady-state UDS signal of AA/PLS blasts was substantially higher (~4-fold) than the UDS signal of blasts from age-matched controls (Fig. 7C). The strength of the UDS signal within AA/PLS blasts was further extended approximately 4- and 2.5-fold after low and high UV dose induction, respectively (Fig. 7C). The percentage of UDS-positive AA/PLS blasts, however, remained low, below control levels (~20–25% below control levels), both at steady state and after UV dose induction (Fig. 7D).

The relative strength of UDS signal elicited by blasts of the AA/PLS variant is markedly suppressed under the nonpermissive test conditions, not only at steady state but also at the two UV dose levels (Fig. 8A). The latter contrasts UDS signal patterns of control blasts expressed under the two testing conditions (Fig. 8B).

UDS Responses of the AA-prone Subgroup versus Age-matched Controls. The UDS signal of marrow blasts of AA-prone dogs during the prerecovery phase increased under nonpermissive conditions about 2.5-fold after both low- and high-dose UV induction, representing modest (~1.5-fold) but nonsignificant (P < 0.25) increases over the control UDS signals generated by marrow blasts from age-matched nonirradiated animals (Fig. 9A). The number of UDS-positive cells marginally increased (~5–10%) from steady-state levels after UV exposure but remained at levels below (~10%) those observed for the controls (Fig. 9B).

Under permissive test conditions, similar differences were noted between the response patterns exhibited by AA-prone and control blasts, with the exception of the reduced UDS signal strength of the AA-prone blast after high-dose UV induction (Fig. 9C).

UDS signal expressed by AA-prone blasts at steady state and after high-dose UV exposure was modestly enhanced under nonpermissive test conditions, whereas at low-dose UV exposure, UDS signal remained unaffected (Fig. 8C).

DISCUSSION

The intent of this study was to survey the change in hematopoietic repair under chronic radiation exposure. Using UDS as a correlate of repair, our results suggested that hematopoietic repair capacity was indeed altered quantitatively and qualitatively by chronic, low-daily-dose radiation exposure. UDS repair responses changed not only with time and exposure conditions but also with the type of evolving hematopathology. For example, the nonextended, control-like UDS of the AA-prone subgroup, with its ineffective hematopoietic recovery responses, was in marked contrast to the extended but aberrant UDS of the MPD-prone subgroup and its strong hematopoietic recovery and survival patterns.

Comparable exposure-related changes in UDS have been observed in humans subjected to protracted, very low daily doses of ionizing radiation of several different qualities (low linear energy transfer photon irradiation, high linear energy transfer α particles) (6, 7, 9). In one study of individuals exposed to protracted doses of γ-rays, a radiation-dependent response threshold was indicated: the UDS was significantly extended in a subgroup with relatively high dose accumulations (~0.014 cGy/month); but in another subgroup having lower cumulative doses, no such enhanced UDS was observed (7). The extension of UDS response and a corresponding decline in the rate of induced sister chromatid exchange suggested enhanced repair within the subpopulation exposed at the higher dose rate (7).

Similar changes in UDS have been noted within subgroups of patients with certain types of hematopathologies. Elevated UDS and suppressed capacity to repair DNA strand breaks were noted in the lymphocytes of leukemic patients during secondary relapse of acute disease following primary therapy-induced remissions (5). By contrast, patients remaining in primary remission failed to exhibit such changes.

The pathology-associated UDS patterns observed in this study are consistent with the differential responses of other hematopoietic repair correlates (12, 31, 32). Vital marrow progenitors of the MPD-prone subgroup manifest an aberrant repair proficiency under chronic radiation exposure, as evidenced by increased proliferative capacity, increased radioresistance, amplified and pattern-modified split-dose re-
Fig. 6. UDS responses of marrow blasts of the non-MPD-prone variant relative to the UDS responses of marrow blasts from age-matched (>200 days) controls. A-D, measured endpoints and test conditions as stated previously for Fig. 4.

...UDS responses, and extended capacity to repair single-stranded breaks in radiation-damaged DNA. By contrast, progenitors of the AA-prone subgroup manifest a marked repair deficiency, evidenced by progressive decline in the progenitor's proliferative capacity, retention of high radiosensitivity, control-like split-dose recovery patterns, and reduced single-stranded DNA break repair capacity. These previously reported characteristics, combined with our new findings, continue to support the concept that the selected subgrouping of dogs is based, in part, on inherent differences in both constitutive and inducible hematopoietic repair capacities.

In this study, the varying UDS response patterns expressed by the various subgroups suggest underlying differences in the levels and/or activities of essential DNA repair enzymes (e.g., DNA polymerases). Undoubtedly, these differences influence overall hematopoietic repair/recovery potential under chronic irradiation. A repair-deficient phenotype (e.g., AA prone) would have a basal insufficiency to extend UDS/polymerase activity under genotoxic stress. Coupled with low activity levels of DNA ligase or other DNA repair enzymes, such enzymatic insufficiency might be rate limiting for the effective repair of essential genes within radiation-damaged and rapidly proliferating hematopoietic progenitors. By contrast, a repair-proficient phenotype (e.g., MPD-prone) would have overextended UDS/polymerase activity under chronic genotoxic stress, sufficient for continual repair of damaged vital genes and promotion of cell survival, but not without the adverse complications of increasing the frequencies of gene mutations (due to the error-prone nature of DNA polymerase β) and subsequent leukemic cell transformations.

It is clear from this and previous studies (12, 31) that hematopoietic repair potentials can be measured and sequentially monitored over a time course of chronic radiation exposure, with differences used to identify individuals at risk to several types of hematopathologies (e.g., aplastic anemia or myeloproliferative disease). What is not clear, however, is whether or not these same repair functions can be used to identify individuals at risk prior to radiation exposure. Resolving this question, however, will require additional studies.

Repair capacity acquired under chronic radiation exposure is linked temporally to the spontaneous hematopoietic recovery response exhibited by MPD-prone animals during the initial preclinical phase transition (30, 31). Both cellular repair and organ system recovery seem to be coordinately constrained by several radiological variables, most notably the time of exposure, cumulative radiation dose, and exposure rate (32). Causal linkages between acquired cellular/molecular repair and overall hematopoietic recovery remain uncertain. In fact, our observations here with the AA/PLS variant appear to add to this uncertainty, arguing against simple causal relationships. Nevertheless, the process of hematopoietic recovery under toxic stress is
measured end points and conditions of testing as stated previously. Responses of marrow blasts from age-matched (<200 days) nonirradiated controls. An alternative explanation might be selective defect(s) in one or more of either the polymerase or non-polymerase components comprising the repair/recovery system (e.g., suppressed DNA ligase function). An alternative explanation might be that, due to the unique nature and/or extent of genic lesions accrued by the variant under chronic irradiation, there is simply an insufficiency of repair of essential, critically damaged hematopoietic genes, despite the exaggerated DNA polymerase-mediated UDS response noted.

For most of our previous studies, however, positive correlations between enhanced hematopoietic repair and subsequent hematopoietic recovery have been consistently observed. In these studies, the recovery-proficient hematopoietic phenotype of the MPD-prone animal has been characterized by extended but aberrant repair and localized at the level of the lineage-committed hematopoietic progenitor (32). Localizing the extended repair function to the more primitive hematopoietic progenitors clearly increases the likelihood that repair enhancement and hematopoietic recovery are causally associated (via a repair-mediated promotion of progenitor viability). It is also possible that the repair-mediated sparing of progenitor cell death might ultimately be causally tied to early stages of evolving MPD by virtue of a progressive shift in balance of the progenitor's self-renewal and differentiation functions; i.e., self-renewal would be progressively promoted by a repair-mediated retardation of differentiative flow, normally enhanced by ionizing radiation exposure (32, 33). Another possibility is that the acquired progenitorial repair might be "error-prone," thus rendering bearing stem cells hypermutable, with increased tendency to transform. The latter suggestion is supported in part by work that demonstrates carcinogen-induced repair as being defective and error prone and serving to enhance both mutational and transformational frequencies in vitro (2, 34, 35). Further, it has been shown that at least one of the UDS-related repair enzymes, namely DNA polymerase β, is inducible with DNA-damaging agents (36), with relatively low fidelity in its proof reading activity, and appears at high constitutive levels in leukemic cells expressing multiple-drug resistances (38). Although the newly acquired repair functions under chronic irradiation probably provide the driving force for both early hematopoietic recovery and the subsequent aberrant progenitor responses essential for full MPD expression, it is certainly feasible that the noted
“acquired” repair is simply a manifestation of a radiation-induced change in age structure of selected progenitorial marrow compartments (38). Under this scenario, the older progenitors (having lower repair and proliferative potentials) would be lost first through the reproductive demands and differential pressures exerted by chronic irradiation, leaving a younger subset of progenitors with greater repair, self-renewal, and proliferative potentials.

With the assumption that the measured UDS response is a reasonable correlate of repair capacity within hematopoietic elements, it follows that the major differences in UDS patterns exhibited by the various subgroups reflects not only sizable differences in the magnitude and inducibility of repair but also differences in the resiliency of the repair system to both toxic insult and to its saturation. For example, UDS response of the MPD-prone subgroup tested during postrecovery phase periods was markedly and uniquely augmented by ara-C treatment under nonpermissive test conditions. Such acquired changes in repair responses might well suggest major differences in the nature and overall extent of genic damage between subgroups. Massive lesions (large “patch,” >100 base pair lesions), not repairable at fidelity rates essential for cell survival, might elicit the ara-C-sensitive repair dominated by polymerase α in chronically irradiated, repair-deficient blasts (AA-prone and AA/PLS variant).

Further, the possible differential involvement of the two polymerases in repair responses might well suggest major differences in the nature and overall extent of genic damage between subgroups. Massive lesions (large “patch,” >100 base pair lesions), not repairable at fidelity rates essential for cell survival, might elicit the ara-C-sensitive repair dominated by polymerase α in chronically irradiated, repair-deficient blasts (AA-prone and AA/PLS variant). Smaller, more repairable lesions produced under chronic irradiation might elicit a greater contribution from ara-C-insensitive polymerase β in error-prone, but survival-sparing, hematopoietic repair.

In conclusion, two major observations have been made: (a) UV-inducible UDS response of marrow blasts of chronically irradiated, MPD-prone dogs during the postrecovery period is significantly extended in terms of signal strength per cell as well as numbers of UDS-responding cells; (b) differences in UV induction patterns of UDS in marrow blasts from MPD-prone dogs, relative to other pathological and control subgroups, suggest that selectively modified UDS-associated repair capacity has been acquired by progenitors under chronic irradiation. These observations are consistent with the hypothesis that an acquired aberrant progenitorial repair is tied to early phases of evolving MPD under chronic γ-ray exposure.

REFERENCES

Chronic Radiation-induced Alteration in Hematopoietic Repair during Preclinical Phases of Aplastic Anemia and Myeloproliferative Disease: Assessing Unscheduled DNA Synthesis Responses

Thomas M. Seed and Susan M. Meyers


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/53/19/4518

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/53/19/4518. Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.