



PROTRACTED RADIATION-INDUCED ALTERATIONS IN HEMATOPOIETIC REPAIR AND RECOVERY

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Abstract

Pathologic predisposition of beagle dogs under chronic, low daily dose (7.5 cGy day⁻¹) whole-body gamma irradiation has been studied relative to molecular repair and hematopoietic competency. Molecular repair, assessed by a microscopy-based unscheduled DNA synthesis (UDS) response, was measured within proliferative and nonproliferative marrow myeloid elements of dogs with markedly different hematopoietic capacities (low capacity, aplasia-prone [AA⁺] versus high capacity, myeloproliferative disease-prone [MPD⁺]) under protracted radiation stress. Results indicated that protracted exposure elicited a net increase in UDS-repair capacity that was largely independent of exposure duration. This enhanced capacity resulted from the increased strength of the UDS signal together with an expanded number of positively responding cells. The combined response was strong in primitive blasts and weak in more differentiated myelocytic cells. The UDS repair response of the MPD⁺ dogs was significantly greater than that of the AA⁺ animals and was clearly modified relative to the controls. These results suggest that both resiliency and pathologic potential of the hematopoietic system under protracted radiation stress is, in part, associated with an augmentable DNA repair within the more primitive myeloid marrow elements.

1. INTRODUCTION

There is increased awareness concerning the relationship(s) between repair sufficiency and the potency of a given toxicant to cause pathology [1]. In a number of well documented genetic diseases of man (e.g., xeroderma pigmentosum, ataxia telangiectasia) insufficient repair capacity has been related clinically to a hypersensitivity to a variety of physical and chemical toxicants, as well as with predisposition to several significant pathologies, including cancer [2].

In the above mentioned pathologies, preexisting genetic/epigenetic lesions are clearly tied, and perhaps drive the noted changes in toxicant-directed repair capacity. This statement, however, begs the question as to the nature and meaning of toxicant-mediated repair responses within 'normal' individuals outwardly lacking signs of genetic/epigenetic-based disease. It appears that the normal individual's repair capacity, in terms of both magnitude and fidelity, can be differentially altered under varying parameters of toxicant exposure [3,4]. However, the late pathological consequences of this toxicant-elicited repair modifications are unclear and need to be clarified.

We have previously identified subgroups of experimental dogs having distinct predispositions to several types of radiation-induced pathologies [5], and related differences in the magnitude and plasticity of hematopoietic repair under chronic toxic stress [6].

In this study we have utilized UDS as a repair correlate in an attempt to monitor and quantitate repair capacity within essential bone marrow progenitors of individual animals subjected to protracted irradiation and prone to several major pathologies of interest.

2. METHODS

Young adult beagle dogs (17) were segregated into control (nonirradiated) groups (6) and test (chronically irradiated) groups (11). Test animals were given whole-body, ^{60}Co gamma irradiation, delivered in a near continuous mode at a daily dose rate of 7.5 cGy day^{-1} (22 hrs each day) for duration of life. Average times of testing (day zero = radiation initiated) for the various irradiated subgroups, along with age-matched controls were as follows: AA⁺, 116 ± 4 days; preleukemic syndrome variant of AA (AA/PLS), 116 days; control #1, 112 ± 6 days; MPD⁺, 1117 ± 184 days; nonMPD, 965 days; and control #2, 1098 ± 208 days. Control animals were comparably caged and handled, but maintained in a shielded anteroom during the course of the experiment. Exposures (and sham exposures) were started when the animals were approximately 400 days of age. Descriptions of animal husbandry, the radiation exposure facility, and dosimetric methods have been given in detail in previous publications [5,7].

Blood samples were taken on a periodic basis, with blood cell counts and differential counts determined by standard procedures. Marrow samples were collected and processed as previously indicated [5,8,9]. The measured endpoints included: blood cell counts and selected marrow functions; i.e., repair, assayed by a microscopy/autoradiographic UDS-based technique [8]. Nuclear grain counts ranging between 1-100 grains per nuclei were scored as UDS positive; nuclear grain counts above 100 were considered to S-phase; zero grains per nuclei were recorded as UDS negative [8].

3. RESULTS

3.1 Blood response patterns under protracted radiation exposure

Figures 1A-E illustrate differences in blood response patterns exhibited by the various irradiated subgroups relative to the nonirradiated control group. These differences were substantial. The aplastic anemia-prone (AA⁺) subgroup, along with the AA/PLS variant, exhibited low hematopoietic capacity under protracted irradiation, as indicated by the progressive, ultimately fatal, pancytopenic condition following relatively short exposure (<300 days). The myeloproliferative disease-prone (MPD⁺) subgroup, and the nonMPD variant, showed high hematopoietic capacity, as indicated by the resistance to acute anemia and by partial restoration of vital leukocytes and platelet blood levels.

3.2 Change in net UDS capacity of myeloid elements

Figures 2 A-D illustrate the change from normal in net UDS capacity of the marrow elements of dogs under protracted irradiation. All irradiated subgroups exhibited increased net UDS capacity at the level of the primitive marrow progenitors (marrow blasts), but not at the level of mature myeloid cells. Only in one subgroup, the AA/PLS variant, was the net UDS capacity of immature myeloid cells elevated relative to unirradiated controls (Fig 2B).

Distinct response patterns were evident for irradiated animals with different preclinical disease. The AA⁺ subgroup (Fig 2A) showed the smallest overall gain in net UDS capacity; whereas the AA/PLS variant (Fig 2B) showed the greatest gain. MPD⁺ and the nonMPD variant patterns (Fig 2 C&D) were characterized by substantial gains in net UDS capacity elicited under low UV-dose-induction; but smaller gains in capacity were noted at high dose. The nonMPD variant, in contrast to all other subgroups, exhibited the largest increase in net UDS capacity following low dose UV induction, and the lowest following high dose UV-induction (Fig 2D).

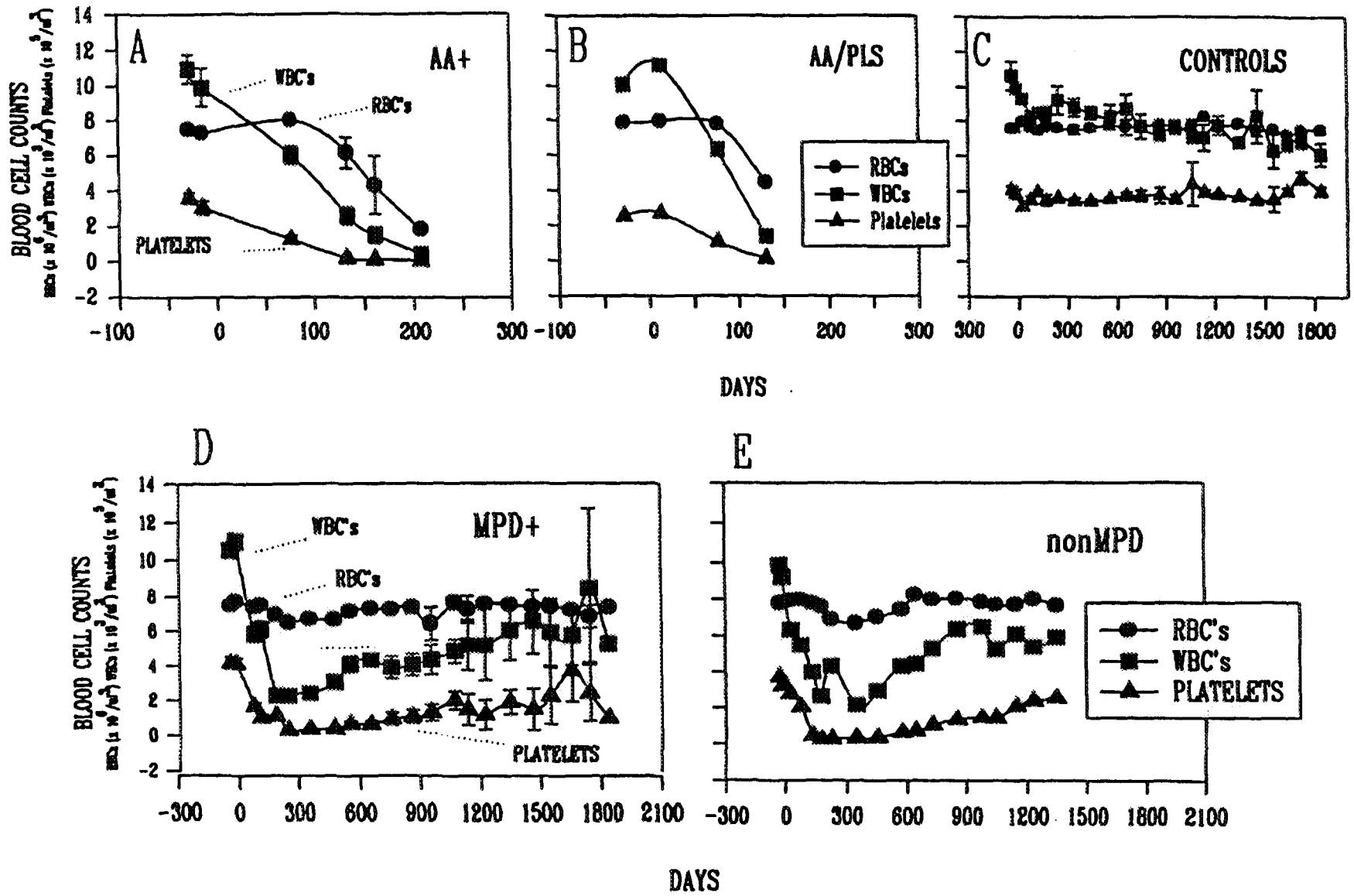


Fig 1. Blood responses of chronically irradiated dogs versus nonirradiated dogs: A) AA⁺; B) AA/PLS; C) controls; D) MPD⁺; and E) nonMPD.

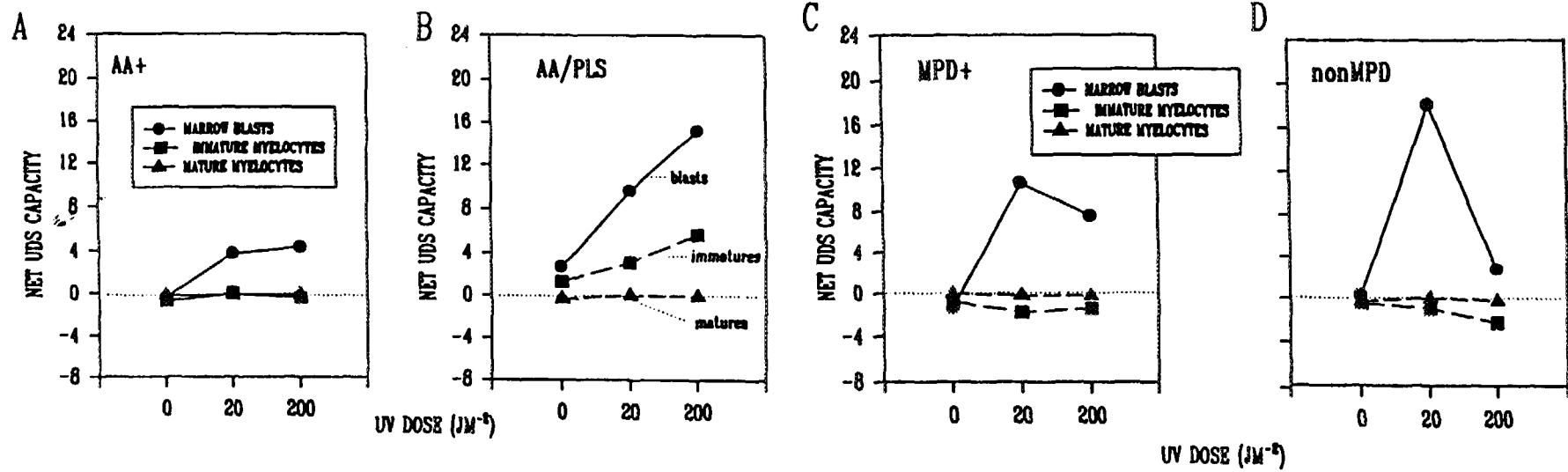


Fig 2. Net difference in UDS repair capacity between irradiated and nonirradiated subgroups: A) AA⁺; B) AA/PLS; C) MPD⁺; and D) nonMPD.

4. DISCUSSION

This study examined the change in hematopoietic repair as a function of protracted gamma irradiation and the preclinical state of evolving hematologic disease. Using net UDS response as a measure of repair, we surveyed several different subgroups of animals with marked differences in survival and hemopathologic tendencies under protracted chronic radiation exposure. Our results showed that the net UDS repair response changed both qualitatively and quantitatively as a consequence of both exposure and the type of developing pathology. The radiation- and pathology-associated changes in the UDS patterns noted in this study are consistent with previous work demonstrating marked differences between these irradiated subgroups when assessed for other hematopoietic repair correlates. The MPD⁺ subgroup is in sharp contrast to the AA⁺ subgroup in terms of repair proficiency of vital marrow progenitors, as manifested by the marked differences in proliferative capacity, radioresistance, split-dose recovery responses, and DNA repair [5,6,8,9]. Repair capacity, acquired under protracted irradiation, is linked temporally to the spontaneous hematopoietic recovery response exhibited by MPD⁺ animals during the initial preclinical phase transition [13]. Both cellular repair and organ system recovery seem to be coordinately constrained by several radiological parameters, most notably by the time of exposure, cumulative radiation dose, and exposure rate [9]. Causal linkages between acquired cellular/molecular repair functions and overall hematopoietic recovery remain uncertain and need to be further developed. Our observations made here with the short lived preleukemic variant (AA/PLS) tend to argue against simple causal relationships between elevated UDS repair signals and hematopoietic recovery under protracted irradiation. The markedly elevated UDS repair signals expressed by this AA/PLS variant in the absence of an effective, sustained hematopoietic recovery response adds to the uncertainty of the causal role played by the UDS response in hematopoietic repair and its misrepair under protracted irradiation. Clearly additional work is needed to resolve these relationships.

With the assumption that the measured UDS response is a reasonable correlate of repair capacity within hematopoietic elements, it then follows that the major differences in UDS patterns exhibited by the various subgroups reflected major differences in the magnitude and inducibility of repair, and also in the resiliency related to both continued toxic insult and saturation.

5. CONCLUSIONS

Protracted gamma exposure elicited an enhanced UDS-repair response within the marrow of beagle dogs. The enhanced UDS repair is confined largely to marrow blasts. With the exception of the AA/PLS variant, the enhanced UDS response is virtually absent in both mature and immature myelocytic elements. The magnitude of the change noted in UDS repair appeared more related to the pathologic status of the subgroup, rather than to the duration of exposure. The resiliency of the hematopoietic system under protracted irradiation does, nevertheless, seem to be associated with distinct patterns of augmentable DNA repair within more primitive myeloid marrow elements.

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