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LONG-TERM HEMATOPOIETIC STEM CELL DAMAGE AFTER EXTERNAL IRRADIATION WITH X RAYS

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Abstract

We have investigated the functionality of the lympho-hematopoietic stem cells long-term (9 months) after the irradiation (X rays) of mice at different stages of development, by means of a competitive bone marrow repopulation assay. Our data revealed that a dose of 1 Gy was only capable of inducing significant long-term failures in the functionality of the primitive repopulating cells in mice irradiated at the young-adult stage (12 week-old), but not in mice irradiated at the late stages of foetus development (17 day-old foetuses) nor at the early development of the embryo (4 day-old embryos). The differential generation of long-term stem cell defects as a function of the age was confirmed in mice irradiated with 3 Gy. While no significant effects in the long-term repopulating cells were observed in 4 day-old embryos, significant repopulation deficiencies were observed in this population when mice were irradiated at the 17 day of foetus development, and more markedly at the adult stage of growth. These data offer new evidence about the influence of the developmental stage of the animal on the generation of residual hematopoietic dysfunctions by external irradiation, with particular relevance to the very primitive lympho-hematopoietic stem cells.

Introduction

All the hematopoietic blood cells are generated by a number of committed progenitors, which are characterized by a limited life-span, and which therefore depend on a very small population of self-renewing hematopoietic stem cells (HSCs). The HSC compartment is, however, a non-homogeneous population and although early data suggested that the CFU-S population represented the HSCs of the mouse, recent observations have evidenced that other more primitive populations with long-term lympho-hematopoietic repopulating capacity can be separated from the CFU-S population [1].

Marked differences in the hematopoietic damage produced by the contamination with α-emitting radionuclides or by exposures to low LET radiation have been observed during the embryonic, foetal, neonatal and adult stages of the life of experimental animals [2-6]. These studies showed that, in comparison to γ-radiation, α-particles are extremely damaging to developing hematopoiesis, indicating that a higher RBE than that considered for stochastic effects, should be given to α-particles when the number of hematopoietic progenitors is considered as a biological end-point [3]. In particular, the analysis of the CFU-S population in mice contaminated with α-emitting radionuclides suggested that the hematopoiesis of the embryo is up to 1,000 fold more sensitive than the postnatal hematopoiesis [2].

Given that the bone marrow (BM) competition assay is currently the experimental procedure which more closely defines the functional properties of the self-renewing HSCs [7], we have investigated by this procedure the functionality of the HSC compartment in mice which had been irradiated with X rays at different stages of growth.

Material and Methods

Mice and X-irradiation: B6CF1 (♀C57Bl/6 x ♂Balb/c) mice were used throughout these experiments. Pregnant females (corresponding to the 4th and 17th day of embryonic growth), and young adult mice (12 week-old) were total body irradiated (TBI) using a Philips MG 324 X-ray machine, at 300 kV, 10 mA, HVL: 3.2 mm Cu and a dose rate of 1.03 Gy/min.

Competitive Bone Marrow Repopulation Assay: To determine the functionality of the lympho-hematopoietic SCs, experiments of BM competition were conducted [7,8]. In these assays, BM cells from irradiated male mice were mixed together with equivalent amounts of BM from non-irradiated females and transplanted into myeloablated female mice [8]. Recipients of the chymeric BM were killed at 9 months post-transplantation, and their BM and thymus were dissected and processed for DNA extraction. The repopulating capacity of the irradiated BM was then determined by dot-blot hybridization using a probe for the Y chromosome (See figure 1).

Results and Discussion

Four day-old embryos, as well as 17 day-old foetuses and 12 week-old mice were irradiated with doses of 1 Gy and 3 Gy of X rays. The times at which mice were irradiated correspond to significant periods in the development of the mouse hematopoietic system [9], and were used previously to investigate the hematopoietic effects induced by the contamination with α-emitting radionuclides and irradiation with low LET radiation [2,5]. Although the previous studies have investigated the hematopoietic long-term effects induced by ionizing radiation during different stages of development, our research is focused on a long-term repopulating precursor, closely related to the self-renewing lympho-hematopoietic stem cell.

To investigate deficiencies in the functionality of this precursor as a result of the irradiation, the repopulating capacity of a test BM sample was evaluated with respect to a reference BM which was co-transplanted with the test BM. As shown in the protocol represented in figure 1, the lympho-hematopoietic cells generated by the test BM sample were distinguished from the reference and the residual surviving cells of the recipient by genetic analyses, using a probe for the Y chromosome. With the aim of evaluating long-term hematopoietic defects produced as a residual consequence of the irradiation, the functionality of the HSCs was assayed at 9 months post-irradiation. On the other hand, with the aim of investigating the functionality of a very primitive lympho-hematopoietic repopulating cell, the competitive repopulating capacity of the irradiated BM was tested, both in the thymus and the BM of recipients, also in the long-term (9 months) post-transplantation.

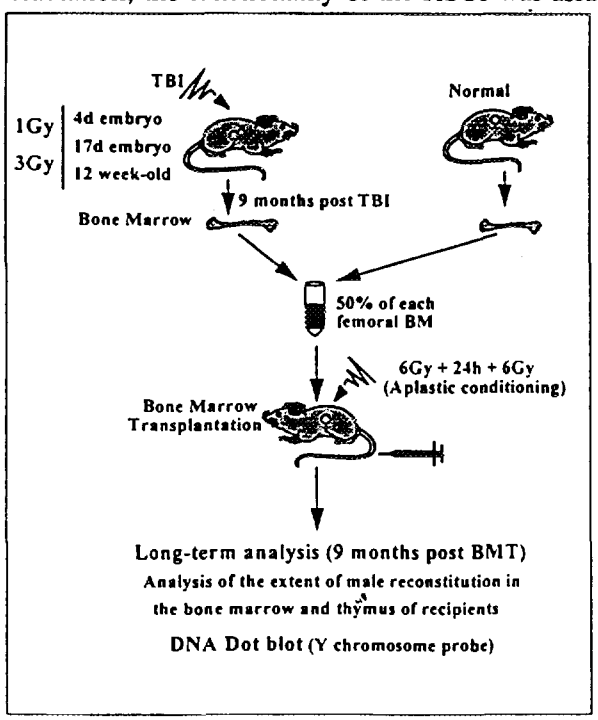

Fig. 1. Experimental procedure for evaluating the competitive repopulation capacity of bone marrow cells after irradiation of mice at different stages of development.

Data in figure 2a shows that only in the case of young-adult irradiated mice, a dose of 1 Gy was capable of inducing a residual defect in the long-term repopulating cells; an effect which was significant when the myeloid BM cells of recipients were analysed. Figure 2b shows that doses as high as 3 Gy were also uncapable of inducing residual effects in the repopulating function of the HSCs in 4 day-old embryos. However, significant residual dysfunctions in the repopulating function of the HSCs were evidenced when 17d old-embryos or adult irradiated mice were analysed. It is of significance, however, that a more marked repopulation defect was observed in the group of adult-irradiated mice than in the foetus-irradiated group, suggesting that the sensitivity of the mouse for generating functional stem cell defects is directly related to the developmental stage of the animal. Either a genetic damage in the self-renewing hematopoietic stem cells [10,11], the generation of a functional damage in the stromal microenvironment [4,12,13,14], or even a proliferative exhaustion of stem cells which survive the irradiation [15], could be involved in the HSC repopulation defects described in this study.

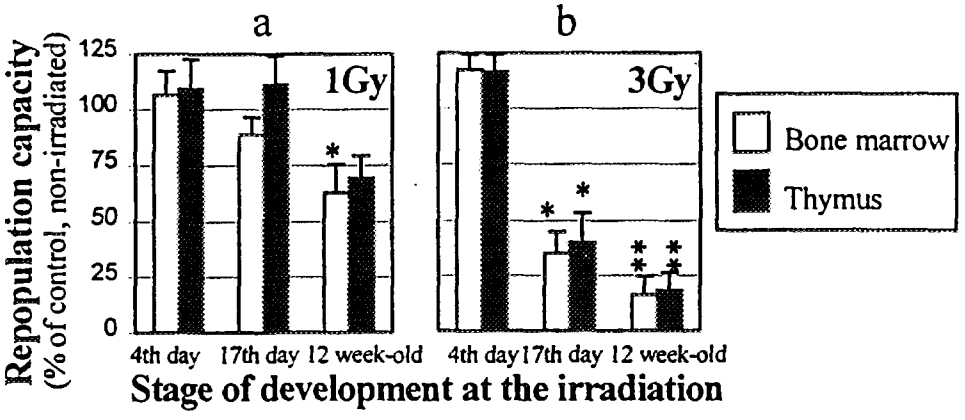

Fig. 2. Analysis of the competitive repopulation capacity of bone marrow samples obtained 9 months after irradiation of mice with a single dose of 1 Gy or 3 Gy-X rays at different stages of development (Data show the mean value ± standard error of 2-6 recipients per point. Significantly different from control, using Student's t-test: * $p<0.05$; ** $p<0.01$).

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