CELLULAR REPAIR OF SUBLETHAL RADIATION INJURIES TO TWO CFU-S SUBPOPULATIONS FROM FETAL MOUSE LIVER AND ADULT MOUSE BONE MARROW

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It has recently been shown that among splenic colony-forming units (CFU-s) there exist various subpopulations of cells, differing in their proliferative potential and their ability to differentiate. For instance, half of the CFU-s from various hematopoietic sources (bone marrow, fetal liver), forming colonies 7-8 days after injection of hematopoietic cells into lethally irradiated recipients, consists of unipotent (predominantly erythroid) precursors, whereas virtually all 11-12-day CFU-s are polypotent [2, 5]. It has been shown that 7-day and 11-day CFU-s from both adult and fetal tissues differ in proliferative potential [4], self-maintenance, and also radiosensitivity [1, 6]. Meanwhile such an important aspect of the radiobiology of stem cells (CFU-s in particular) as ability to repair sublethal radiation injuries (SRI) has been inadequately studied. We know that 9-10-day exogenous CFU-s from adult mouse bone marrow are highly capable of repairing SRI [7], but the ability of precursor cells earlier in the histogenetic series (11-day CFU-s) from bone marrow and fetal liver to repair SRI has not been studied. The aim of this investigation was to compare ability of 8- and 11-day CFU-s containing in the two principal hematopoietic organs in the pre- and postnatal periods of development of mice, namely fetal liver (FL) and bone marrow (BM), to repair SRI.

EXPERIMENTAL METHOD

Adult (CBA×C57BL/6)F_1 mice aged 6-10 weeks and 14-18 day fetuses of these same hybrids were used. Bone marrow cells were flushed out of the femora with medium 199 and suspended by means of a syringe; FL were minced in medium 199 by means of a glass homogenizer.

Repair of radiation injuries was studied on Elkind's model of repair [3], adapted to hematopoietic tissue [7].

Recipient mice were irradiated in a dose of 6 Gy on an IPK (^{137}Cs) γ-ray source with a dose rate of 0.20 Gy/min, after which the irradiated animals (10 mice per group) were given an injection of bone marrow cells in a dose of 5×10^6 cells per mouse, or FL cells in a dose of 3×10^6 cells per mouse (for counting 8-day colonies) and in a dose of 6×10^6 cells per mouse (for counting 11-day colonies). Half of the experimental mice were irradiated in a single dose of 6 Gy 1 h after transplantation of the cells (the nonfractional irradiation group — NF). The remaining recipient mice were irradiated fractionally with intervals of 5 h between two equal doses of irradiation, each of 3 Gy — the fractional irradiation group (FI). Thus the recipient mice in all experiments received a total dose of irradiation of 12 Gy and the injected cells a dose of 6 Gy (either in a single dose or in two fractions separated by an interval of 5 h). Colonies were counted in the spleens on the 8th and 11th days after transplantation of the hematopoietic cells. The repair index (RI) was calculated as the ratio of the number of colonies formed after FI and the number formed after NF. The average number of endogenous colonies in all experiments was 0.13 ± 0.07 colony per spleen.

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TABLE 1. Repair of Sublethal Radiation Injuries in 8- and 11-Day CFU-s from Mouse Fetal Liver (FL) and Adult Bone Marrow (BM) (number of colonies per spleen, M±m)

Expt. No	Source of hemato-poietic cells	8-day CFU-s			11-day CFU-s		
		NF	FI	RI	NF	FI	RI
1	14-day FL	ND	ND	ND	5,80±0,79	6,78±1,05	1,17
2	15-day FL	4,56+0,69	6,40±1,11	1,40	4,50±1,08	5,0±0,98	1,11
3	17-day FL	5,67±1,15	10,10±1,89	1,78	4,25±1,25	6,25±0,71	1,47
4	18-day FL	8,0±1,27	13,14±2,03	1,64*	7,60±1,50	9,0±1,64	1,18
1	BM	ND	ND	ND	16,80±1,22	19,0±1,20	1,13
3	BM	4,17+0,79	13,71±2,20	3,29*	7,43±0,72	11,57±1,06	1,56*
4	BM	2,0±0,44	6,22±0,10	3,11*	4,83±0,83	10,44±0,75	2,16*

Legend. NF) Nonfractional irradiation, FI) fractional irradiation, RI) repair index, ND) no data. Asterisk indicates that differences are significant. In experiments Nos. 1, 3, and 4, ability to repair sublethal radiation injuries of CFU-s from FL and BM was investigated in parallel tests.

EXPERIMENTAL RESULTS

Data on repair of SRI by the two CFU-s subpopulations from the fetal and adult sources of hematopoietic cells are given in Table 1. The doses of cells from FL and BM injected were chosen on the basis of the radiosensitivity parameters for CFU-s subpopulations from the corresponding tissues. Since 8-day CFU-s (CFU-s-8) from FL are more radioresistant than 11-day CFU-s (CFU-s-11; D_0 1.5-1.8 Gy and 1.0 Gy respectively), the dose of transplanted cells for obtaining the calculated number of colonies differed by a factor of 2 (3×10^6 and 6×10^6 cells/mouse for CFU-s-8 and CFU-s-11 respectively). CFU-s-8 from BM, on the other hand, are more radiosensitive than CFU-s-11, but the values of D_0 for these precursor cells from BM did not differ so significantly (1.0 and 1.2 Gy for CFU-s-8 and CFU-s-11 respectively); to obtain the calculated number of colonies at both times of recording, the dose of cells injected was therefore 5×10^6 cells/mouse.

FU-s-8 from FL also have weak ability to repair SRI compared with CFU-s-8 from adult mouse BM (their average RI was 1.61 and 3.2 respectively). Compared with CFU-s-8, CFU-s-11 from FL had even weaker ability to repair SRI (the average value of RI based on results of four experiments was 1.23). Polypotent precursor cells (CFU-s-11) from BM also exhibit weaker ability to repair SRI (the mean value of RI was 1.62) compared with CFU-s-8 from this same hematopoietic tissue. Comparison of RI for CFU-s-11 from BM and FL showed that polypotent precursor cells from adult hematopoietic tissue repair SRI significantly better.

The results are evidence that, irrespective of the source of hematopoietic cells (fetal or adult), 11-day (polypotent) CFU-s are significantly less able to repair SRI than 8-day CFU-s (some of which, as was stated above, are unpotent cells). Both early and more mature fetal CFU-s possess lower repair parameters than CFU-s from adult animals. It is evident that mechanisms of SRI repair are formed in the antenatal period, and reach full development in the postnatal period. Their activity also differs in cells at different levels of the hierarchy of hematopoietic precursors.

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