EARLY POSTRADIATION RECOVERY OF HEMATOPOIETIC STROMAL PRECURSOR CELLS

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Recovery of tissues after irradiation takes place through regeneration of surviving viable stem cells: hematopoietic stem cells (HSC) if the lesion is one causing a "bone marrow" syndrome; stem cells of intestinal epithelium, which enable survival of an animal exposed to an "intestinal" dose range, and so on. The reserves for proliferation of critical organs and tissues can evidently be not only undamaged cells, but also cells which have undergone reversible damage, i.e., which are able to repair the injuries they have sustained.

Irradiation causing radiation damage and, in particular, single-stranded DNA breaks, activates template processes taking place in the cell, i.e., reparative DNA synthesis.

Quantitative parameters of recovery from sublethal radiation injuries were first studied in cultures of Chinese hamster cells [9]. The basic method of investigation of this type of repair is fractionation of the dose of irradiation into two separate fractions with an interval of several hours, not exceeding the duration of the cell cycle. This is necessary to prevent any possible influence of cell proliferation between the two exposures on the total effect of irradiation. By using the method of fractional irradiation it has been shown that self-renewing cell populations and, in particular, HSC [2, 4, 6, 8, 10] and intestinal epithelial stem cells [3, 11, 12], possess high ability for intracellular repair.

The object of this investigation was to study the ability of stromal precursor cells to undergo early postradiation recovery.

EXPERIMENTAL METHOD

Experiments were carried out on male (C57BL × CBA)F_1 mice. Ability of the stromal precursor cells to undergo intracellular repair was studied on a model of recovery as suggested by Elkind, i.e., using a method of fractional irradiation of bone marrow.

The mice serving as bone marrow donors were irradiated *in vivo* in a single full dose (nonfractional irradiation) and also fractionally with an interval of 6 h between the two doses of irradiation. Total doses of irradiation of 10, 12, 14, and 16 Gy were tested. Irradiation was from ^{137}Cs on an IPK gamma-ray apparatus with a dose rate of 0.25 Gy/min [4].

After irradiation the femoral marrow was quickly implanted beneath the renal capsule of an unirradiated syngeneic recipient. The size of the developing ectopic focus was determined from the number of hematopoietic cells in it 4-6 weeks after implantation. In each case cells from 6 to 12 foci were pooled.

The ability of stromal precursor cells to undergo intracellular repair (repair index), was estimated from the ratio of the number of hematopoietic cells formed in heterotopic graphs in groups with fractional irradiation to the number in groups with nonfractional irradiation.

To inhibit repair, mice were given a single intraperitoneal injection of 5 μg/g of actinomycin D (AD) [2].

EXPERIMENTAL RESULTS

After implantation of bone marrow beneath the renal capsule of a syngeneic recipient a heterotopic focus of hematopoiesis was formed. The size of the microenvironment was proportion-

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TABLE 1. Cell Composition of Focus of Heterotopic Transplant Formed by Implantation of Nonfractionally and Fractionally Irradiated Bone Marrow (M ± m)

Total dose of irradiation, Gy	Magnitudes of 1st and 2nd doses, Gy	Number of cells in focus of heterotopic hematopoiesis ($\times 10^6$)			Repair index ($\frac{FI}{NF}$)
		unirradiated bone marrow (control)	nonfractional irradiation (NF)	fractional irradiation (FI)	
10 (n=6)	4+6	10,15±3,20	1,35±10 (13%)	3,27±1,80 (32%)	2,42
10 (n=4)	5+5	12,48±2,10	1,33±0,40 (11%)	4,19±1,00 (34%)	3,33
10 (n=6)	6+4	11,09±3,30	1,42±1,20 (11%)	3,37±1,80 (30%)	2,37
12 (n=4)	6+6	6,19±0,50	0,28±0,05 (4,5%)	1,44±0,20 (23%)	5,29
14 (n=6)	6+8	5,50±2,30	0,27±0,50 (4,9%)	1,27±1,10 (23%)	4,70
16 (n=6)	6+10	5,50±2,30	0,25±0,50 (4,5%)	0,43±0,40 (8%)	1,72

Legend. Here and in Table 2, fraction of stromal precursor cells surviving after irradiation given in parentheses (in % of control); n) number of experiments.

TABLE 2. Effect of AD on Ability of Stromal Precursor Cells to Undergo Intracellular Repair of Radiation Injuries (M ± m)

Treatment of bone marrow donors	Number of cells in focus of heterotopic hematopoiesis ($\times 10^6$)
Control	10,33±3,20
Unirradiated + AD + 6h (n = 6)	14,22±3,80
Nonfractional irradiation:	
10 Gy (n = 6)	0,40±0,60 (3,9%)
10 Gy + 6h (n = 6)	0,75±0,85 (7,3%)
10 Gy + AD + 6h (n = 6)	0,86±0,90 (6,0%)
Fractional irradiation:	
5 Gy + 6h + 5 Gy (n = 6)	1,49±1,20 (14,4%)
5 Gy + AD + 6h + 5 Gy (n = 2)	2,09±0,20 (14,8%)

al to the number of stromal precursor cells and could be estimated from the number of hematopoietic cells formed [1]. Table 1 gives data showing the cell composition of the heterotopic foci formed by fractionally and nonfractionally irradiated bone marrow, and also the fraction of stromal precursor cells surviving after irradiation (as a percentage of the control — unirradiated bone marrow).

Irradiation of bone marrow with a single dose of 10 Gy appreciably reduced the number of stromal precursor cells. According to the results of the six experiments, the surviving fraction was about 12% of the control. In the case of fractional irradiation, whatever the version used, the surviving fraction amounted on average to 32%. In all experiments fractional irradiation revealed high ability of the stromal precursor cells to undergo intracellular repair. The mean value of the repair index was 3.02 ± 0.24.

Irradiation of bone marrow in doses of 12 and 14 Gy gave the same effect. In this case also the stromal precursor cells repaired radiation injuries. The repair indices were virtually equal. The greatest injury was caused by a dose of 16 Gy, but in this case also the repair index was quite high (1.72).

AD increased the mortality of cells due to irradiation and prevented repair processes in nontoxic concentrations [5]. The dose of AD used had a weak stimulating action on stromal precursors (Table 2). The number of cells in the focus of heterotopic hematopoiesis was increased by 1.4 times after treatment both of unirradiated mice and of mice receiving fractional irradiation with AD, when the antibiotic was injected immediately after the first dose of irradiation. That is evidently why it was impossible to demonstrate its clear and lethal effect on intracellular repair. This effect was weakened a little only in the case of recovery after a single dose of irradiation.

The results are evidence that a slowly renewing, highly radioresistant cell population, namely precursor cells of the hematopoietic stroma [7], is capable of early postradiation recovery. Although such recovery was observed after all doses of irradiation, values obtained with doses of 12, 14, and 16 Gy must be treated with reserve, because with nonfractional ir-

radiation in these doses the viable fraction, equal on average to 5% of the control, formed an ectopic focus containing fewer than $0.5 \cdot 10^6$ cells, which is close to the sensitivity of the method. This objection applies to some extent to results obtained with AD also. Meanwhile it was found unexpectedly that AD itself, if injected into intact bone marrow donors, increases the size of the foci of ectopic hematopoiesis formed by their bone marrow, possibly due to the lower sensitivity of precursors treated with AD to the transfer procedure.

For this reason direct proof that mechanisms of DNA repair participate in postradiation recovery of precursor cells of the hematopoietic stroma, observed in this investigation, necessitates approaches of a different kind.

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PROLIFERATION OF HEMATOPOIETIC MICROENVIRONMENT PRECURSORS IN LONG-TERM MOUSE BONE MARROW CULTURES

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The hematopoietic microenvironment is stromal in nature [6] and is created by precursor cells capable of transferring it during implantation of hematopoietic tissues. These precursors have been called "hematopoietic microenvironment transferring units" (HMTU) [1]. During equilibrium hematopoiesis the HMTU are in a resting state and their proliferation cannot be revealed by "suicide" methods. However, the transfer procedure mobilizes HMTU into the cycle, and after 48 h (but not after 24 h) their high sensitivity to cytostatics, which lasts about 2 weeks, can be easily demonstrated [5]. This short-term proliferation is sufficient for the formation of the necessary number of new HMTU to enable construction of a focus of ectopic hematopoiesis, corresponding in size to the initial hematopoietic territory.

A method of long-term bone marrow culture has recently been devised [9]. Maintenance of hematopoiesis in culture depends critically on the construction of a layer of adherent cells, mainly stromal in origin [3], which plays the role of hematopoietic microenvironment. HMTU are contained in the sublayer of long-term bone marrow cultures [2]. Their proliferative state is unknown. Meanwhile the process of sublayer construction is analogous in many respects to the formation of a focus of ectopic hematopoiesis.

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