STIMULATION OF RECOVERY OF HEMATOPOIESIS IN TOTALLY IRRADIATED ANIMALS BY REPOPULATION WITH HEMATOPOIETIC CELLS IN THE EARLY PERIOD AFTER IRRADIATION

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UDC 576.8.077.35

Experiments on rats and mice receiving whole-body irradiation showed that repopulation with hematopoietic cells from the femoral bone marrow in the early period after irradiation promotes the restoration of hematopoiesis. The number of myelokaryocytes and blood cells was restored more rapidly in rats, while in the mice the number of endogenous colonies in the spleen was greater than in the control.

Previous investigations have shown that puncture of the bone marrow or intraosseous injection of physiological saline in totally irradiated rats or mice in the early period after irradiation is followed by the more rapid recovery of hematopoiesis and, in some cases, by a higher survival rate [1, 3]. It has been postulated that the observed beneficial effect is due to repopulation with intact stem cells, thereby creating better conditions for the restoration of hematopoiesis.

The object of the present investigation was to continue the study of the use of postradiation repopulation to stimulate regeneration of hematopoiesis.

EXPERIMENTAL METHOD

August and Wistar rats aged 3 months were irradiated with γ rays from a Co^{60} source in doses of 450 and 600 R, respectively, at a dose rate of 70 R/sec. During the first 1-2 h after irradiation, the right femur was punctured in some of the animals under ether anesthesia by the method described previously [2]. The puncture needle was inserted through the distal end of the femur into the medullary canal, and the structure of the bone marrow was destroyed by a few vigorous movements, as a result of which about 50% of the myelokaryocytes were flushed out into the blood stream. Blood analyses and investigations of the femoral and tibial bone marrow were carried out at various times during the 30 days after irradiation.

Male CBA mice aged 12 weeks were irradiated under the same conditions in doses of 600 or 850 R. In some animals, during the next 2 h repopulation with bone marrow cells from both femora was carried out, and the animals were sacrificed 11 days later in order to investigate hematopoiesis and to count the number of endogenous colonies of hematopoietic cells in the spleen [11].

In all the experiments animals of the control groups were anesthetized with ether and the synovial pouch of the knee joint was punctured at the same times as in the experimental animals.

RESULTS

Recovery of hematopoiesis in the femoral and tibial marrow of the experimental (repopulated) Wistar rats took place faster than in irradiated, untreated animals (Table 1). It is interesting to note that even in

Laboratory of Experimental Hematology, Division of Radiation Pathophysiology, Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 10, pp. 98-101, October, 1970. Original article submitted January 12, 1970.

TABLE 1. Hematological Indices of Wistar Rats after Irradiation in Dose of 600 R

Nature of procedure and time of investigation		Number of rats	Myelokaryocytes (in millions)			Megakaryocytes (in thousands)	Platelets (in thousands/mm³)	Leukocytes (in thousands/mm³)
			left femur	right femur	tibia			
Control		10	240	246	142	10,9	900	15,2
10-th day	Irradiation	6	35	—	23	—	32	1,7
	Irradiation + repopulation	6	51	37	23	—	52	1,9
20th day	Irradiation	5	122	—	61	1,5	540	4,7
	Irradiation + repopulation	6	137	127	71	3,7[1]	678[1]	5,9
30-th day	Irradiation	6	164	—	98	5,5	680	9,4
	Irradiation + repopulation	6	227[1]	195	113	9,7[1]	850[1]	13,8[1]

Note. Here and in Tables 2 and 3, the symbol[1] denotes results differing significantly from the corresponding control values ($P < 0.05$). The number of megakaryocytes was counted in the tibial marrow.

TABLE 2. Hematological Indices of August Rats after Irradiation in a Dose of 450 R

Nature of procedure and time of investigation		Number of rats	Myelokaryocytes (in millions)			Megakaryocytes (in thousands)	Platelets (in thousands/mm³)	Leukocytes (in thousands/mm³)
			left femur	right femur	tibia			
Control		10	128	130	84	36,2	1 020	5,9
15-th day	Irradiation	11	27	—	17	3,1	38	1,4
	Irradiation + repopulation	8	36[1]	30	25	3,9	23	1,9
30-th day	Irradiation	6	131	—	106	22,0	710	3,6
	Irradiation + repopulation	6	148	144	107	34,0[1]	880[1]	5,1[1]

the right femur, from which about half of the myelokaryocytes were flushed during the repopulation procedure, their number was restored more rapidly than in the irradiated, untreated animals. The number of megakaryocytes and also the numbers of platelets and leukocytes were restored more rapidly.

The results of the study of hematopoiesis in August rats irradiated in a dose of 450 R are given in Table 2. On the 15th day after irradiation the number of myelokaryocytes in the femoral marrow and the number of leukocytes in the blood of the treated animals were higher than in the untreated. On the 30th day, recovery of the number of myelokaryocytes was observed in the animals of both groups; the number of megakaryocytes, platelets, and leukocytes in the treated animals was significantly higher.

Some hematological indices of the CBA mice after irradiation in doses of 600 and 850 R are given in Table 3. The number of endogenous colonies in the treated mice following both doses of irradiation, like the numbers of myelokaryocytes, leukocytes, and platelets, was higher than in the untreated animals.

Repopulation with hematopoietic cells in the early period after irradiation of totally irradiated animals thus had a beneficial effect on the restoration of hematopoiesis as a whole.

At present nothing more than suggestions can be made regarding the mechanism of the therapeutic effect of repopulation with hematopoietic cells after irradiation. It is known, for example, that hematopoietic

TABLE 3. Hematological Indices and Growth of Endogenous Colonies in Spleen of CBA Mice on 11th Day after Irradiation in Doses of 600 and 850 R

Nature of procedure and dose of irradiation		Number of mice	Weight of mice (in g)	Weight of spleen (in mg)	Number of colonies		Myelokaryocytes in tibia (in millions)	Leukocytes (thousands/mm^3)	Platelets (in thousands/mm^3)
					total	mean			
Control		20	21.5	74	–	–	11.5	8.4	1025
600 R	Irradiation	22	20.2	24.1	54	2.4	5.2	0.7	122
	Irradiation + repopulation	25	17.3	24.6	144	5.8[1]	8.8[1]	1.5	195
850 R	Irradiation	23	20.3	22.0	8	0.35	–	–	–
	Irradiation + repopulation	26	20.6	23.1	45	1.73[1]	–	–	–

stem cells, whose presence is proved both by growth of colonies of hematopoietic cells in the spleen of irradiated recipient mice after injection of circulating blood leukocytes [9], and by the restoration of hematopoiesis in irradiated dogs and guinea pigs treated with transfusions of packed leukocytes [6, 10], are constantly found in the blood of experimental animals of different species. It can accordingly be postulated that circulation of the stem cells in the blood stream is an essential part of the life cycle of these cells. After irradiation, the ability of the stem cells to escape into the circulating blood is disturbed, perhaps due to injury of the stem cells themselves, and to disturbances of the hemodynamics and cytoarchitectonics of the bone marrow. It has been shown, for example, that during the first few hours after irradiation, even in comparatively small doses (300 R), virtually no stem cells can be found in the circulating blood [5]. It is also well known that screening part of the bone marrow has a much less marked therapeutic effect than screening followed by repopulation with the protected cells [4], and the formation of endogenous colonies in the spleen of irradiated mice takes place on a much smaller scale after partial protection of the bone marrow than if the same number of unirradiated marrow cells is injected intravenously (repopulation) into totally irradiated recipient animals [8, 12]. Isolated investigations showing the effectiveness of therapeutic administration of autologous bone marrow obtained after uniformly distributed, whole-body irradiation have also been published [7].

Hence, both under normal conditions and in radiation sickness, the escape of hematopoietic stem cells into the circulating blood is evidently an important condition for the maintenance of hematopoiesis. The beneficial effect of repopulation with marrow cells after irradiation is probably connected with the artificial flushing out of intact hematopoietic cells (in particular, stem cells) into the circulating blood stream, with the subsequent formation of foci of hematopoiesis by these cells.

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