EFFECT OF THE THYMUS ON THE ABILITY OF HEMATOPOIETIC STEM CELLS TO RECOVER AFTER SUBLETHAL RADIATION INJURY

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UDC 612.419.014.482-06:612.438

After fractional irradiation of the bone marrow of mice (300 rad *in vitro* + 300 rad *in vivo*) damage to the hematopoietic stem cells is substantially less if the interval between irradiations is 5 h than if the interval is 30 min, as a result of recovery from sublethal radiation injury (the Elkind repair effect). In thymectomized adult mice ability of hematopoietic stem cells to undergo Elkind repair is severely disturbed 2.5-5 months after the operation. Transplantation of the thymus abolishes the effect of thymectomy.

KEY WORDS: *hematopoietic stem cells; irradiation; Elkind repair, thymectomy.*

The role of the thymus in differentiation of T lymphocytes has recently been established [7, 12]. The role of the thymus in differentiation of hematopoietic stem cells (colony-forming units — CFU) in the myeloid rather than the lymphoid direction is less clear. Data in the literature are few in number and contradictory in nature. The bone marrow of neonatally thymectomized mice contains fewer exogenous CFU than normal bone marrow, but more endogenous CFU [1, 13, 15]. According to some data thymectomy in adult mice increases the number of stem cells in the bone marrow (by the exocolonization test) [11], but according to others it reduces or increases their number (by the endocolonization test) [2, 4]. The proliferative activity of bone-marrow CFU of adult thymectomized mice is unchanged [9, 10]. Konoplyannikov [3] demonstrated a disturbance of the ability of endogenous CFU to recover from radiation injury in thymectomized mice.

In the present investigation the method of exogenous CFU was used to study the ability of hematopoietic stem cells to recover from sublethal radiation injuries — the Elkind repair effect [8] — in thymectomized adult mice. The possibility of abolishing the effect of thymectomy by transplantation of the thymus also was studied.

EXPERIMENTAL METHOD

Female CBA and (CBA × C57BL) mice from the Stolbovaya nursery were used. The number of exogenous CFU was determined by the method of Till and McCulloch [15]. The recipients were irradiated with a dose of 1300 rad from a ^{137}Cs source. The test suspensions were injected intravenously and the number of colonies in the spleen was counted after 8 days.

Donor mice were thymectomized at the age of 8-10 weeks by the method of Boyse et al. [5]. Completeness of removal of thymus was verified when the bone marrow was taken. Mice with remnants of thymus were not included in the experiments. During mock thymectomy on mice aged 8-10 weeks the thorax was opened and closed without removal of the thymus.

The thymus was transplanted 4-4.5 months after thymectomy: one lobe of the thymus from newborn (1 day old) mice was transplanted [15] beneath the capsule of the left kidney. The animals were used as donors of bone marrow 1 month after transplantation.

Preliminary experiments showed that the ability of the CFU to undergo intracellular repair could be observed after two irradiations, each of 300 rad, separated by an interval of 5 h. Accordingly the main experiments were carried out as follows: The recipients were irradiated in a dose of 1000 rad and the test cell suspensions in a dose of 300 rad *in vitro* (dose rate 150 rad/min). After injection of the cells the recipients were further irradiated in a dose of 300 rad, either immediately or 5 h after the injection. The recipients thus

Laboratory of Bone Marrow Culture and Transplantation, Central Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 584-586, November, 1978. Original article submitted December 16, 1978.

 0007-4888/78/8611-1500$07.50

TABLE 1. Effect of Thymus on Ability of Hematopoietic Stem Cells to Recover from Sublethal Radiation Injuries (M ± m)

Expt. No.	Donors	Number of colonies per spleen after injection of 4×10^4 unirradiated bone marrow cells	Number of colonies per spleen after injection of 5×10^6 bone marrow cells irradiated with a dose of 600 rad		Recovery index
			continuous irradiation	fractional irradiation	
1	Intact mice	5,2±1,6	1,9±0,5	5,2±1,6	2,7
2	Intact mice	6,3±0,6	3,4±1,1	7,7±2,0	2,3
	Thymectomized mice (1)	13,0±1,4	3,8±1,0	4,4±1,0	1,2
3	Intact mice	9,3±0,9	9,3±1,5	16,2±1,0	1,7
	Mice undergoing mock thymectomy (2)	13,3±1,8	9,8±1,4	13,4±.	1,3
	Thymectomized mice (3)	12,7±2,6	14,5±6,5	12,0±1,1	0,8
4	Intact mice	8,0±1,0	2,7±0,5	8,3±1,5	3,1
	Mice undergoing mock thymectomy (2)	11,8±1,0	2,6±0,6	9,2±1,9	3,5
	Thymectomized mice (3)	13,5±1,2	5,8±0,9	4,2±1,3	0,8
5	Intact mice	6,2±1,8	12,5±1,5	22,3±2,1	1,8
	Mice undergoing thymectomy (4) + transplantation of thymus (5)	11,7±1,6	7,0±1,2	19,9±1,9	2,8
6	Intact mice	—	11,8±1,4	32,4±2,7	2,7
	Mice undergoing thymectomy (4) + transplantation of thymus (6)	—	6,2±1,2	12,5±2,1	2,0

Legend: 1) time after thymectomy 2.5 months; 2) time after mock thymectomy 36 days; 3) time after thymectomy 5 months; 4) time between thymectomy and transplantation of thymus 4-4.5 months; 5) time after transplantation of thymus 38 days; 6) time after transplantation of thymus 50 days.

always received a total dose of 1300 rad and the test cells a dose of 600 rad either virtually without interruption (continuous irradiation) or with an interval of 5 h (fractional irradiation). The ability of the hematopoietic stem cells to undergo intracellular regeneration was assessed from the ratio between the number of CFU remaining in the groups with fractional irradiation and their number in the groups with continous irradiation.

In all the experiments the group of recipients consisted of 8-12 mice, whose mean survival rate was 70-80%.

EXPERIMENTAL RESULTS

As Table 1 shows, the regime of fractional irradiation chosen clearly revealed the ability of the hematopoietic stem cells of intact mice to undergo intracellular repair of radiation damage. The recovery index of the hematopoietic stem cells (the ratio between the number of colonies formed by bone marrow after fractional irradiation and the number of colonies from bone marrow after continuous irradiation) for the intact donors was 2.4. Mock thymectomy had no significant effect on the ability of the CFU to undergo Elkind repair. In this case the recovery index was 2.4 also.

Thymectomy on adult mice completely abolished the ability of CFU to undergo Elkind repair. The number of colonies after fractional irradiation of bone marrow of the thymectomized mice was not greater than after continuous irradiation. The mean recovery index in this case was 0.9.

Transplantation of the thymus virtually abolished the effect of thymectomy: 1-1.5 months after transplantation the hematopoietic stem cells had almost recovered their ability to carry out intracellular repair of radiation injuries. The recovery index of the CFU of the thymectomized mice with a grafted thymus was 2.4 (Table 1).

The results show that the thymus is essential for the normal functioning of hematopoietic stem cells and, in particular, for maintaining their ability to repair radiation injuries. The ability of CFU to effect intracellular repair is considerably disturbed in old animals [6]. The results now obtained suggest that this effect is due to age involution of the thymus. It is not yet clear how the thymus affects hematopoietic stem cells — whether humorally or by means of cellular factors. It is likewise not clear whether this effect on hematopoietic stem cells is direct or whether the thymus factors and (or) T cells exert their influence through a change in the properties of the hematopoietic microcirculation. Some of these problems may perhaps be solved by investigations currently in progress with transplantation of the thymus in chambers impermeable to cells.

The author is grateful to A. G. Konoplyannikov for valuable advice and consultation.

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