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SELF-MAINTENANCE CAPACITY OF HEMATOPOIETIC CFUs IN THE LATE STAGES AFTER LONG-TERM IRRADIATION

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KEY WORDS: colony-forming units; spleen; irradiation.

Long-term exposure to harmful factors such as ionizing radiation or cytostatic agents leads to an irreversible decrease in the stem-cell pool of the hematopoietic system [2, 9] and also, perhaps, of other cell renewal systems. The mechanism of this residual damage is not yet clear. The size of the stem-cell population is determined primarily by the self-maintenance capacity of these cells [4, 6]. It has been shown that this unique property of polypotent CFUs (units forming colonies in the spleen) may be damaged as a result of chronic irradiation [5, 10].

The aim of this investigation was to study the ability of mouse bone marrow CFUs to maintain their own population in the late stages after long-term external irradiation.

EXPERIMENTAL METHOD

CBA mice aged 9-11 weeks were irradiated daily on an experimental ^{137}Cs γ-ray source in a dose of 0.5 Gy (exposure dose rate $0.34 \cdot 10^{4}$-$0.44 \cdot 10^{-4}$ A/kg) up to a total dose of 10 Gy. The number of CFUs in the bone marrow of the irradiated mice was determined by the exocolonization method [11]; the recipients were irradiated on an ÉGO-2 apparatus in a dose of 9.2 Gy. The self-maintenance capacity of the CFUs was determined by the number of CFUs in individual colonies formed by stem cells of the animals studied [8, 12]. For this purpose, a cell suspension was prepared from the bone marrow of mice of the experimental and control groups and injected into lethally irradiated recipients in a dose of 8-9 CFUs per mouse. After 10 days 12 to 15 colonies were isolated from the spleens of these primary recipients, and each was carefully freed from surrounding tissue. A cell suspension was prepared from the contents of each colony in 0.6-0.8 ml of medium, and this was injected in equal amounts into three or four lethally irradiated secondary recipients. Nine days later the number of colonies growing on the surface of their spleens was counted, and the number of CFUs in each separate colony was determined. Self-maintenance capacity of the CFUs was studied 3, 6, and 12 months after irradiation in a total dose of 10 Gy. Three repetitions of the experiments were done at each time of the investigation, and three donors (experimental mice and intact mice of the same age) and 10 to 12 primary recipients were used in each of them. Ability of CFUs of the bone marrow to maintain their own population was compared in the experimental and control animals, and also in mice of the different experimental groups, by calculating the mean number of CFUs per colony and their distribution among the colonies studied.

In a separate series the proliferative activity of bone marrow CFUs was studied during and after long-term irradiation. The number of CFUs in the period of DNA synthesis was found with the aid of [^{3}H]thymidine with high specific activity, as described previously [3]. To determine the percentage of CFUs taking part in mitosis, three or four experiments were carried out at each time of the investigation.

The results were subjected to statistical analysis by Student's t test ($P \leq 0.05$).

EXPERIMENTAL RESULTS

Daily irradiation of CBA mice in a total dose of 10 Gy led to a statistically significant decrease in their mean life span (624 ± 24 days compared with 749 ± 34 days in the control; $P < 0.05$). Pools of morphologically

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TABLE 1. Number of CFUs in Individual Splenic Colonies and Frequency of Colonies (in %) with Different Numbers of CFUs

Time after irradiation, months	Group of animals	Number of colonies studied	Number of CFUs per colony	Distribution of number of CFUs per colony							
				0	1—5	6—10	11—15	16—20	21—30	31—40	40
Control		96	39,1±5,5	0	5,2	32,3	13,4	15,7	9,5	9,5	14,4
3	Experiment	40	8,4±8,2*	20	45	5	5	15	—	5	5
6	Experiment	50	15,0±3,0*	6	5	28	20	10	18	11	2
12—15	Experiment	50	16,1±2,4*	4	4	24	20	12	20	12	4

Legend. Here and in Table 2: *) $P < 0.05$ compared with control.

TABLE 2. Proliferative Activity of Bone Marrow CFUs from Irradiated and Intact CBA Mice

During irradiation		After end of irradiation (10 Gy)		
total dose, Gy	per cent of CFUs in S-period	time of investigation, months	group of animals	per cent of CFUs in S-period
0 (control)	3,6±3,4	1	control	3,2±3,4
2,5	60,0±15,0*		Experiment	30,5±6,7*
10	46,0±9,7*	3	Experiment	1,3±1,7
		6		4,9±2,3

identifiable hematopoietic cells and peripheral blood cells of these animals were completely restored 1-3 months after the end of irradiation. Residual radiation damage was found in the stem-cell pool, just as in mice of a different line irradiated under similar conditions [2]. The number of CFUs in the experimental CBA mice did not exceed 60% of the age norm by the end of their life.

The mean number of CFUs per colony was identical in intact mice of the three control groups (aged 6-9 and 15-18 months). The distribution profiles of the number of CFUs in the colonies also were similar for these animals. Accordingly, the values obtained for mice of the control groups in these tests were pooled. As the data in Table 1 shows, in all splenic colonies formed by hematopoietic CFUs of intact mice which were analyzed, CFUs were found.

About 50% of the colonies contained from six to 15 CFUs, and more than 40 CFUs were found in 14% of the colonies.

The mean number of CFUs and their distribution among the individual splenic colonies growing after injection of bone marrow CFUs of irradiated mice differed appreciably from the corresponding values in intact mice. When bone marrow was injected 3 months after irradiation the mean number of CFUs per colony was 4.5 times less than the control value ($P < 0.05$). In 20% of colonies no CFUs were found, and most colonies (45%) contained from 1 to 5 CFUs (Table 1). A tendency was noted for the number of CFUs to increase in colonies formed by stem cells of irradiated mice 6 and 12 months after irradiation, compared with the previous time of testing ($P > 0.05$), but the mean number of CFUs per colony under these circumstances remained 2.5 times less than the control values ($P < 0.05$). However, the difference in the distribution of the number of CFUs among individual colonies compared with the control was less marked than in the earlier stages of the experiments. Nearly half of all colonies tested contained 6 to 15 CFUs, and only in 4-5% of colonies were no CFUs found.

The decrease in the self-maintenance capacity of hematopoietic CFUs of the irradiated animals can be explained, first, by the preceding stimulation of their proliferative activity, leading to exhaustion of the stem-cell pool [6, 10]. In the process of daily irradiation all the bone marrow CFUs which survived were in a state of proliferation, the rates of which remained increased for at least 1 month after the end of irradiation also (Table 2).

Increased proliferative activity of the hematopoietic CFUs led to restriction of their ability to maintain their own population after acute irradiation or exposure to other harmful factors [1, 7, 9, 10]. Radiation-induced injuries to CFUs followed by their elimination may also have contributed to the disturbance of the self-maintenance capacity of the CFUs in the irradiated animals [13].

Prolonged and repeated irradiation thus induces a lasting decrease in the ability of hematopoietic CFUs to maintain their own population. The disturbance of this unique property of CFUs is evidently linked with an irreversible decrease in the stem-cell pool of the hematopoietic system after irradiation.

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CIRCADIAN RHYTHMS OF SEX STEROIDS IN FEMALE BABOONS DURING PROLONGED HYPOKINESIA

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The widespread character of hypodynamia in modern society necessitates a comprehensive study of the effect of this extremal factor on women and on the reproduction system in particular. The most adequate model for research of this kind is provided by female baboons (Papio hemadryas), in which the basic parameters of activity of the hypothalamic–hypophyseal–gonads system are very close to those in man [1, 3]. Since the circadian rhythm of secretory activity of the gonads is one of the most important characteristics of functional integrity of the hypothalamic–hypophyseal–ovarian system [8], experiments were carried out on female baboons in order to study circadian rhythms of hormonal activity of their ovaries and adrenals during prolonged clinostatic hypokinesia. This paper describes the results of a comparative study of circadian rhythms of the estradiol, progesterone, testosterone, and cortisol levels in the peripheral blood plasma of unrestrained female baboons and of baboons whose movements were restricted, in different phases of the menstrual cycle.

EXPERIMENTAL METHOD

Experiments were carried out on 10 mature fertile female baboons weighing 12-16 kg, aged 5-10 years, with a stable biphasic menstrual cycle lasting 28-35 days. The cycles were monitored by noting swelling of the "genital skin," which is the target tissue for estrogens and reflects proliferative processes developing in the reproductive system in the follicular phase of the cycle. To study circadian rhythms of the steroid levels (control) blood samples were taken from monkeys unrestrained in their cages, with natural alternation of daylight and darkness, every 6 h for the 24-h period starting from 12 noon in the follicular (6th-8th day) and luteal (6th-

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