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GENERATION AGE AS A FACTOR DETERMINING THE USE OF HEMATOPOIETIC STEM CELLS

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KEY WORDS: hematopoietic stem cell, self-renewal, hydroxyurea.

The population of hematopoietic stem cells (HSC) is heterogeneous as regards its degree of self-renewal [2]. It is considered that this heterogeneity is due to differences in the generation age of the stem cells, i.e., differences in the number of divisions through which they have passed. It has been postulated that the older stem cells, i.e., those which have passed through mitosis more often, undergo differentiation first [3, 4]. This hypothesis, which has now become almost canonical, regarding the organization of release of stem cells into differentiation depending on their generation age, is in fact based only on the results of the study of self-renewal of HSC after exposure to cytostatics. In particular, repeated exposure to hydroxyurea, which kills cells in the synthetic period of the cell cycle, leads to selection of the youngest stem cells, with a high degree of self-renewal; the older stem cells are mobilized more easily into the cycle of depopulation of the hematopoietic system caused by the first injections of hydroxyurea, and die when subjected to its action during subsequent injections [3, 4]. These data are of fundamental importance for the understanding of the mechanisms of regulation of HSC, more especially because the conclusion that the proliferative potential of stem cells is directly connected with their generation age is not immune from criticism.

For the foregoing reasons it was decided to undertake an experimental verification of the generation-age hypothesis of the use of HSC.

EXPERIMENTAL METHOD

Female C57BL/6 and hybrid (CBA × C57BL/6)F_1 (abbreviated hereafter to F_1) mice aged 8-12 weeks were used. HSC were determined by cloning in the spleen of mice [5] irradiated in doses of 10 Gy (C57BL/6) or 13 Gy (F_1). Under these conditions the number of endogenous colonies did not exceed 0.2 per spleen. Self-renewal of HSC was characterized by the number of CFUs in the pool of splenic colonies and their number in individual 11-day colonies were determined as described previously [1]. Hydroxyurea in a dose of 1 mg/g body weight was injected intraperitoneally into the mice either strictly in accordance with the scheme [3], i.e., five times altogether 32, 26, 10, 7, and 2 h before removal of bone marrow, or six times every 12 h or every 15 h, followed by removal of bone marrow 2 h after the last injection.

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TABLE 1. Self-Renewal of HSC Surviving after Repeated Injections of Hydroxyurea (M ± m)

Mice	Treatment received by donors of bone marrow	Number of bone marrow cells injected into intermediate recipient	Number of colonies per spleen in intermediate recipient	Number of CFUs per femur of donors	Number of CFUs per 11-day splenic colony	P
C57BL/6	—	$4 \cdot 10^4$	9,5±2,0	2138±450	155±18	
	Hydroxyurea, five times in the course of 32 h	$2 \cdot 10^4$	3,0±0,7	435±101	41±11	<0,001
F_1	—	$6 \cdot 10^4$	25,4±2,4	7874±744	79±7	
	Hydroxyurea six times every 12 h	$6 \cdot 10^4$	12,9±2,0	634±98	80±8	
	Hydroxyurea six times every 15 h	$6 \cdot 10^4$	12,3±1,0	615±50	33±5	<0,001

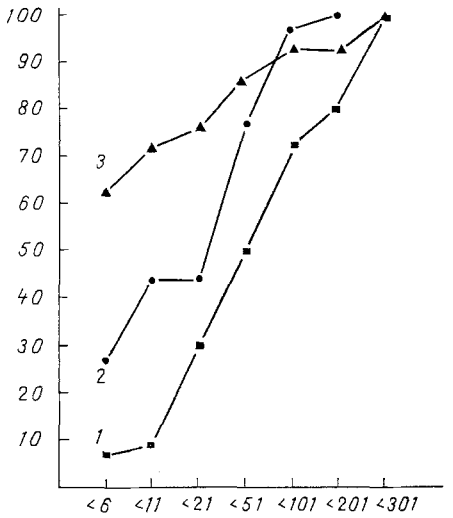

Fig. 1. Cumulative curves of distribution of CFUs according to degree of self-renewal. Abscissa, number of daughter CFUs in individual 11-day splenic colonies; ordinate, fraction of individual colonies with a given content of CFUs. 1) CFUs of bone marrow of normal mice (61 colonies), 2) CFUs of bone marrow of mice receiving five injections of hydroxyurea in the course of 32 h (30 colonies), 3) CFUs of peripheral blood of normal mice (30 colonies).

EXPERIMENTAL METHOD

After injection of hydroxyurea by the scheme in [3] the number of CFUs in the bone marrow was reduced about by 80% (Table 1). Self-renewal of the preserved CFUs not only was not increased but was significantly reduced. In this connection a more intensive scheme of administration of hydroxyurea was used (six times, every 12-15 h), when the total duration of cytostatic treatment was increased from 32 to 62-77 h. The number of CFUs after these conditions was reduced to 7% of initially, i.e., three times greater than during treatment by the scheme in [3]. In this case also, self-renewal of the preserved CFUs, as will be clear from Table 1, was neither increased nor significantly reduced.

It can be tentatively suggested that the CFUs preserved after the action of hydroxyrea possess a high degree of self-renewal, but their presence was masked by the more numerous CFUs with low proliferative potential, temporarily protected against terminal differentiation by the toxic effect of the hydroxyurea. This hypothesis was tested by the study of the self-renewal spectrum of individual CFUs (Fig. 1). Under the influence of hydroxyurea the decrease in self-renewal of the CFUs was due not to expansion of the subpopulation of CFUs with a low degree of self renewal, but by an equivalent decrease in proliferative potential of all subpopulations of stem cells; moreover, not only an increase in the fraction of CFUs with a low degree of self renewal was observed, but disappearance of the CFUs most capable of self-renewal. In the case of selection of any subpopulation of CFUs the angle of slope of the

curve would be bound to change, but this did not happen. For comparison, the characteristics of a subpopulation of CFUs in which the class of stem cells with low self-renewal is shown and the different angle of slope of the curve of distribution of CFUs by self-renewal will be clearly apparent.

It is difficult to explain why these results differ from data in the literature [3, 4]. The line of mice may perhaps be relevant. In the investigations cited above mice of line BALB/c were used and daughter CFUs were recorded in 12-day colonies (in 11-day colonies in the present investigation). Whatever the case, the data given above do not settle the question of whether the mechanism of use of stem cells depends on their generation age.

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ULTRADIAN BIORHYTHMS IN MOUSE BONE MARROW

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KEY WORDS: ultradian fluctuations; bone marrow; cytologic composition.

One of the most probable methods of spatiotemporal organization of differentiation processes and repopulation of the blood cells is through a single system of feedback manifested as biorhythms. Circadian fluctuations in the number of blood cells have been known for a long time [12]. Circadian fluctuations in the number of bone marrow karyocytes have been found in man and rats [4, 15]. A circadian biorhythm has been described for the blood erythropoietin level [17]. Meanwhile, biorhythms with a period of several hours (ultradian) are evidently more commensurate with the more rapid processes of new formation and migration of cells. However, the system of ultradian biorhythms has not yet been adequately studied. Ultradian fluctuations in blood hormone concentrations are known [13]. Ultradian fluctuations in the number of lymphocytes in the thymus, spleen, and lymph nodes were discovered recently [7]. Ultradian fluctuations in activity of cell mediators regulating migration and proliferation of lymphoid cells and of interferon in the culture fluid of normal and immune lymphocytes also have been found [8, 10].

The existence of ultradian fluctuations in the number of karyocytes in mouse bone marrow was established by the investigation described below and ultradian changes were found in the relative percentage and absolute number of cells belonging to the various branches of hematopoiesis.

EXPERIMENTAL METHOD

Mice of line AK (female) obtained from the "Stolbovaya" Nursery or bred by the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and selected for weight (±1 g), were kept four or five animals to a cage. Every hour during the experiment, mice from one cage were killed simultaneously by cervical dislocation and the femora were removed from them: the left — to count the number of karyocytes, the right — to prepare films. The distal epiphysis of each left femur was removed along the epiphyseal disk. The needle of a tuberculin

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