PROLIFERATIVE POTENTIAL OF CFUs FROM BONE MARROW OF THYMECTOMIZED MICE

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UDC 616.438-089.87-092.9-089.168-07:616.419-076

KEY WORDS: proliferative potential of HSC; thymectomized mice; age.

The basic problem in the understanding of function of hematopoietic stem cells (HSC) can be stated as follows: is the HSC capable of indefinite proliferation [4, 12], or is it restricted to a finite number of divisions [14]. In recent years the evidence has continued to support the theory of clonal succession [1, 2, 5, 10, 13], according to which HSC possess high but limited proliferative potential, and they function by forming successive clones of differentiated cells. On a model of splenic colonies it is basically possible to demonstrate lowering of the prolierative potential of cells responsible for restoration and maintenance of hematopoiesis in irradiated mice [14]. The difficulty of determination of the proliferative potential of HSC and the contradictory nature of most of the data can be attributed to the experimental conditions (one of the possible causes), for virtually all experiments have been conducted on hematopoietic tissue under nonphysiological conditions (repeated passages of HSC to irradiated animals or repeated cycles of regeneration of hematopoiesis without transfer of cells). The second cause is connected with the fact that the splenic colonies method does not determine true HSC, but colony-forming units (CFUs), which, however, if mice are used as the model, are generally considered to be the experimental analog of HSC.

The HSC series is heterogenous. Hematopoletic precursor cells which occupy a higher position in the hierarchic series of HSC (CFUs 11-14 days) possess high proliferative potential, those which are more differentiated (CFUs 7-9 days) have lower potential [7-9].

Whatever view is held on proliferative potential, it is clear that HSC have an adequate reserve to produce an adequate number of hematopoietic cells throughout the life of animal or man.

The following problem according arises: what is the cause of the age-related changes in function of the hematopoietic tissue, for example, of anemia in old individuals. Are they caused by a disturbance of the immanent properties of HSC and, in particular, of proliferative potential and differentiation, or are the changes observed based on age-related differences in function of the hematopoietic microenvironment, regulating HSC proliferation. Nonclinical changes in hematopoiesis in old individuals are perhaps the result of a normal physiological process, caused by an age-dependent change in the balance of growth factors.

In the investigation described below dependence of the proliferative potential of CFUs on age was investigated in animals, and the possible involvement of the thymus in regulation of the properties of CFUs was studied on a model of adult thymectomized (TE) mice. The proliferative potential was defined as the ability of CFUs from 11-day colonies to produce daughter CFUs.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57BL/6)F_1 mice aged from 1.5-2 months (at the beginning of the experiment) to 25 months. Thymectomy was performed on donor mice at the age of 2-2.5 months by the method in [3]. Complete removal of the thymus was verified before bone marrow was taken. The content of CFUs

All-Union Hematologic Scientific Center, Ministry of Health of Russia, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences A. I. Vorob'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 208-209, August, 1992. Original article submitted January 17, 1992.

 0007-4888/92/0008-1196$12.50

TABLE 1. Proliferative Potential of CFUs (number of daughter CFUs in 11-day splenic colonies) from Bone Marrow of Intact and Thymectomized Mice (M ± m)

Age of bone marrow donors, months	CFUs-11 per spleen in primary recipients	Dose of colonies from spleen of primary recipients injected	CFUs-8 from spleen in secondary recipients	Number of daughter CFUs per colony
		Intact mice		
1,5	7,40±0,98	0,1	6,86±0,72	69
3	10,00±1,35	0,2	19,10±2,14	96
6,5	8,80±1,74	0,1	6,00±0,81	60
8,5	9,60±0,93	0,1	6,67±0,39	67
20	9,00±0,71	0,1	9,20±0,64	92
24	7,55±0,60	0,2	23,90±1,43	120
25	8,25±1,31	0,1	8,25±0,89	83
		Thymectomized mice		
6,5	4,60±1,43	0,1	7,00±1,15	70
8,5	10,50±1,80	0,1	8,20±0,80	89
10,5	8,25±1,38	0,1	10,20±1,53	51
20	8,80±1,16	0,1	13,47±0,84	135
24	7,73±0,65	0,2	19,22±0,97	96
25	8,40±1,17	0,1	7,83±0,85	78

was determined by the method in [15]. Colonies were counted in spleens of mice irradiated in a dose of 12 Gy (^{137}Cs source, Blood Transfusion Institute). The number of endogenous colonies did not exceed 0.2 per spleen. The number of mice in a group of recipients was usually 8-15. To determine proliferative potential of CFUs, cells of the test bone marrow were injected intravenously into lethally irradiated mice. The number of colonies in the spleen was counted 11 days later, and the pool of spleen cells was homogenized and injected into lethally irradiated secondary recipients in a dose of 0.1-0.2 colony per mouse. The number of colonies of secondary recipients was determined after 8 days. The proliferative potential, i.e., the number of daughter CFUs per colony, was determined by appropriate calculations.

EXPERIMENTAL RESULTS

The number of daughter CFUs in 11-day splenic colonies from bone marrow of TE mice was studied at different postoperative times (from 6.5 to 25 months) after thymectomy. Intact mice of the same age as the TE mice served as the control. In that way it was possible to control the proliferative potential of CFUs from the bone marrow of animals with natural involution of the thymus and the experimental (TE) group of animals. The results are given in Table 1.

Analysis of the number of daughter CFUs in 11-day colonies formed by bone marrow of intact mice showed that the change in the number of daughter CFUs did not correlate with the age of the mice. The values obtained were about equal. On average, based on the results of seven experiments, the proliferative potential from bone marrow of this group of mice was 84 ± 8.

A similar pattern, i.e., no change in proliferative potential with age, also was observed for CFUs from bone marrow of TE mice. The parameter of CFUs chosen for study likewise was not under the influence of the thymus. About equal values were obtained 6.5 and 25 months after thymectomy. On average, based on the results of six independent experiments, the proliferative potential was 87 ± 12, and did not differ significantly from that in the control (intact group of mice).

The results confirm the view that regulation of the properties of HSC is age-independent. The most conclusive data, opposed to the view that the proliferative potential of CFUs decreases during aging of the animal, were obtained in [6]. Identity of the proliferative potential of HSC for animals of all three age groups was established by the method of competitive repopulation, i.e., injection of a mixture of bone marrow from young, adult, and old mice,

Another approach to the problem, namely the use of adult TE mice as experimental model, presupposed, on the one hand, that aging of the animal and involution of the thymus (a phenomenon invariably accompanying aging) are interdependent, and on the other hand, it could be decided whether factors of thymic nature regulate the properties of HSC. The attempt to establish a relationship of cause and effect between aging of the animals and involution

of the thymus with respect to one of the determinant qualities of HSC proved to be unsuccessful. Removal of the thymus during its period of maximal activity does not affect an important parameter of HSC such as its proliferative potential. It is not clear, however, whether the thymus and its derivatives are in fact involved in the regulation of the property of HSC chosen for study, or whether a change in proliferative potential of HSC is not recorded under conditions of stable hematopoiesis.

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EFFECT OF HIGH ELECTROLYTE CONCENTRATIONS ON THE PLASMA MEMBRANE AND ITS GLYCOCALYX

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UDC 612.014:576.314].014.462.08

KEY WORDS: plasma membrane; glycocalyx; leukocytes; tumor cells; hypertonic solution.

Among all the membranes that play an important role in the life of animal cells, several can be picked out and, in particular, the plasma membrane, which performs several functions including transport of nutrients and of inorganic ions into and out of the cell. An active role in these functions is played by the outer juxtamembranous layer, or glycocalyx, which has the function of a cation exchanger [1]. There are many different ways of isolating membranes in order to study their activity physicochemically. Most frequently these methods include destruction of the cell and isolation of fragments of the plasma membrane from homogenates in various ways, such as centrifugation in a density gradient [2]. In our investigations the observation and study of the behavior of these membranes were linked with investigation of the mechanism of halo formation around living cells. In our view, a halo is formed

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 210-212, August, 1992. Original article submitted November 24, 1991.

0007-4888/92/0008-1198$12.50