SELF-SUPPORT OF MIGRATING HEMATOPOIETIC STEM CELLS

I. L. Chertkov, O. A. Gurevich, and G. A. Udalov

UDC 612.419

A study of the number of stem cells in splenic colonies and of marked chromosomes in the progeny of the stem cells that recirculating colony-forming units from peripheral blood or from a focus of ectopic hematopoiesis have reduced self-support potential compared with settling (from bone marrow) colony-forming units. The powers of differentiation of the two subpopulations of stem cells were identical.

KEY WORDS: hematopoietic stem cells; migration; self-support.

Hematopoietic stem cells are found in the blood of normal mice [4, 5]. It has been suggested that they are part of a single population of hematopoietic stem cells which have accidentally left the hematopoietic organs [1]. On the other hand, there is evidence that circulating stem cells are a special subpopulation, differing from the settled hematopoietic stem cells in, for example, proliferative activity [3]. The reduced ability of hematopoietic stem cells from the blood to support themselves has been reported [6]. This could imply that the "older" stem cells migrate into the blood and that a mechanism exists in the bone marrow to maintain the increased probability that stem cells which have passed through a cycle of intensive proliferation migrate into the blood stream.

In the investigation described below these findings were confirmed and the ability of stem cells from blood, which have repopulated a newly formed focus of ectopic hematopoiesis , also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA (subsequently described as O), CBAT6T6 (subsequently T6T6), and F_1 (CBA × CBAT6T6) (subsequently T6) mice. To obtain foci of ectopic hematopoiesis, femoral bone marrow, irradiated in vitro in a dose of 1000 rad to destroy the donor's hematopoietic cells, was implanted beneath the capsule of the kidney of a recipient anesthetized with hexobarbital. Cells from the foci were obtained after 1.5-2 months. The hematopoietic stem cells were counted by the splenic colonies method [7] in mice irradiated in a dose of 1300 rad. Self-support of stem cells from different sources was determined by counting colony-forming units (CFUs) in splenic colonies and with the aid of chromosomal markers. In the first case, cells from bone marrow or an ectopic focus were injected into irradiated intermediate recipients in a dose of $4 \cdot 10^4$; blood cells were injected either in a dose of 10^6 washed leukocytes (Table 1), experiment No. 1, or as 0.3 ml blood (about $2.5 \cdot 10^6$ leukocytes; Table 1, experiment No. 1). Some of the intermediate recipients were killed 8-10 days later and the number of colonies counted in their spleens. The remaining recipients were killed on the 12th day and their spleen cells were injected, in doses equivalent to 0.05-1.0 colony, into the final irradiated recipient. The number of colonies in the latter gave the number of CFUs in a 12-day colony in the intermediate recipient. Some donors were irradiated sublethally in a dose of 400 rad or in a dose of 1300 rad followed by injection of syngeneic bone marrow in a dose of 10^7 cells 1.5 months before the hematopoietic cells were taken from them. To study the proliferative potential of the hematopoietic stem cells, cells from bone marrow, ectopic foci of hematopoiesis, or peripheral blood from animals carrying various chromosomal markers were injected into karyologically irradiated recipients; the recipients themselves were always unmarked. To abolish the effect of possible differences in the rate of proliferation cells of individual sublines, different marker combinations were always used in the experiments (Table 2). Since proliferation of cells of all three sublines was identical, the results are described together.

Laboratory of Bone Marrow Culture and Transplantation, Central Institute of Hematology and Blood Transfusion, 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. 87, No. 6, pp. 579-582, June, 1979. Original article submitted July 28, 1978.

0007-4888/79/8706-0611 $07.50

TABLE 1. Self-Support of CFUs in Splenic Colonies of Irradiated Mice Receiving Hematopoietic Cells from Bone Marrow, Peripheral Blood, or a Focus of Ectopic Hematopoiesis (M ± m)

No. of experiment	Cell donor	Source of CFUs	Primary recipient (number of colonies per spleen)	Secondary recipient: number of cells injected (in colony equivalence)	Secondary recipient: number of colonies per spleen	Secondary recipient: number of CFUs per colony	P (compared with bone marrow of inact donors)
	Intact	Bone marrow	11,7±0,9	1,0	Confluent	39	
				0,2	7,7±0,9		
		Focus of ectopic hematopoiesis	12,4±0,7	1,0	48,0±2,2	36	Not significant
				0,2	6,8±1,4		
		Blood	1,4±0,3	0,225	15,1±2,1	64	Not significant
				0,045	2,7±0,6		
	Irradiated 400 rad	Bone marrow	15,4±1,8	1,0	36,4±2,3	42	Not significant
				0,2	9,7±1,6		
		Blood	1,6±0,4	0,5	21,7±2,0	50	Not significant
				0,1	5,6±1,0		
	Chimera	Bone marrow	11,7±2,4	1,0	18,8±3,3	29	Not significant
				0,2	7,6±2,0		
		Blood	3,0±0,5	1,0	41,0±1,6	42	
				0,2	8,7±1,3		
2	Intact	Bone marrow	9,8±0,8	0,2	36,9±1,4	185	
		Focus of ectopic hematopoiesis	5,3±0,7	0,2	14,0±1,1	70	<0,001
		Blood	7,2±1,0	0,2	12,1±1,6	60	<0,001

TABLE 2. Karyologic Characteristics of Hematopoietic Cells Used

No. of experiment	Source of CFUs	Genotype of donor's cells (number of CFUs injected given in parentheses) in different groups: I	II	III
1	Bone marrow	T6T6 (6,3)	T6 (8,6)	0 (5,7)
	Blood	T6 (1,7)	T6T6 (2,5)	T6 (1,7)
	Heterotopic hematopoietic focus	0 (3,5)	0 (3,5)	T6T6 (6,3)
2	Bone marrow	T6 (440)	T6T6 (195)	
	Heterotopic hematopoietic focus	T6T6 (270)	T6 (180)	

Each recipient received approximately the same number of CFUs from different sources (Table 2). The number of CFUs was determined in the recipients' bone marrow, spleen, and thymus (3 or 4 at each time) and the number of metaphases with the different markers was determined 7, 14, and 21 days after injection of small doses of CFUs and 10 days and 1, 2, and 4 months after injection of large doses of CFUs. Colcemid, in a dose of 10 μg/g, was injected intraperitoneally into the mice 1-1.5 h before sacrifice; chromosome preparations were obtained as in [2].

EXPERIMENTAL RESULTS

As the data in Table 1 show, the method of recording the number of CFUs in primary colonies formed by hematopoietic stem cells from different sources is not very reliable for the determination of self support of the CFU. In experiment No. 1 statistically significant differences were not found in the self support of recirculating and settled CFUs; no difference likewise was found by means of this test between intact stem cells and sublethally irradiated (400 rad) cells or cells which had gone through a cycle of intensive proliferation (radiation chimeras). However, despite the absence of differences in the number of CFUs in the colonies, the size of the colonies formed by bone marrow was larger than that of colonies produced by recirculating CFUs. Meanwhile, in experiment 2 self support of stem cells from bone marrow was significantly greater than of migrating cells.

More accurate results were obtained by direct comparison of repopulation of the hematopoietic tissue with the progenies of settled and recirculating CFUs by means of chromosomal markers. In the experiments of series I each recipient received a mixture of CFU from all three sources (bone marrow, ectopic focus, blood). Altogether 6-8 CFUs from bone marrow, 3-6 CFUs from an ectopic focus, and 2-3 CFUs from peripheral blood was injected. Each recipient received altogether 13-15 CFUs, the equivalent (as regards the number of CFUs) to $5 \cdot 10^4$ bone marrow cells. This dose is significantly less than sufficient to give complete protection to mice, and investigations could be carried out only during the first three days after injection of the cells (Fig. 1). The results for all hematopoietic organs studied (bone marrow, spleen, thymus) showed

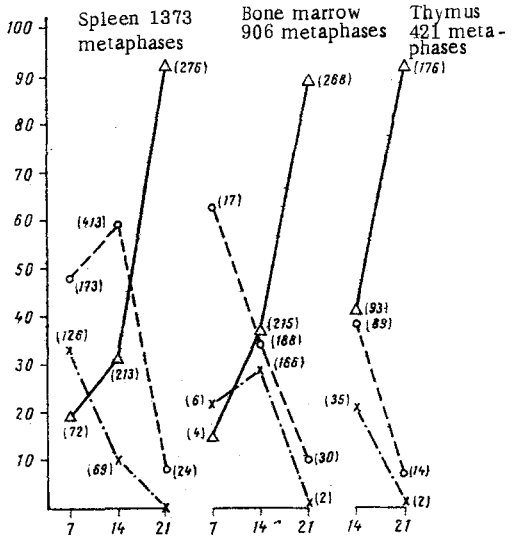

Fig. 1

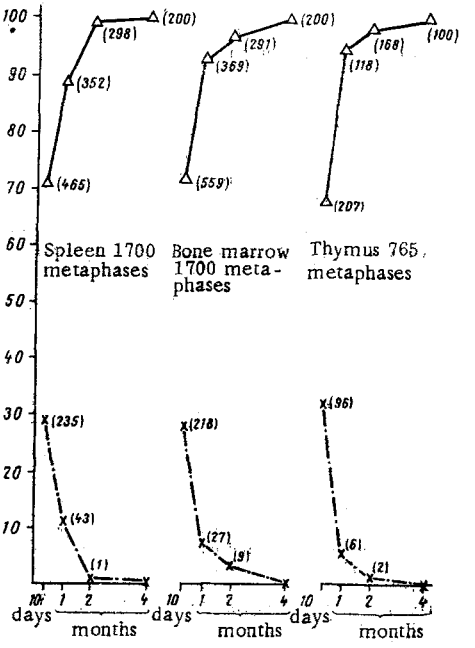

Fig. 2

Fig. 1. Composition of regenerating hematopoietic tissue after injection of small doses of recirculating and settled hematopoietic stem cells. Continuous lines represent progenies of hematopoietic stem cells; broken line – progenies of stem cells from blood; lines of dots and dashes progenies of stem cells from foci of ectopic hematopoiesis numbers in parentheses give absolute number of metaphases analyzed for that same point. Abscissa, time after injection of cells (in days); ordinate, relative number of different markers (in %).

Fig. 2. Composition of regenerating hematopoietic tissue after injection of large doses of recirculating and settled hematopoietic stem cells. Legend as in Fig. 1.

complete agreement. In the first week the existing number of dividing cells belonged to progenies of migrating CFUs. During the second week their contribution fell sharply, and after three weeks about 90% of all metaphases analyzed belonged to the donor of bone marrow. The results thus confirmed the data mentioned above [6]. It was also shown that reduced ability of cell support is characteristic not only of CFUs in the blood, but also of stem cells which have resettled in hematopoietic tissues and in particular, in a focus of ectopic hematopoiesis. Hence it follows that stem cells that take part in repopulation are not selected for increased ability of self support from the total population of migrating CFUs. A stay of 1-1.5 months in a focus of normal hematopoiesis by the migrating stem cells, in contact with the hematopoietic microenvironment, did not restore their ability of self-support.

The results, like those obtained by other workers [6], have two important shortcomings. Since a small dose of cells was injected, first, it was impossible to study the fate of the hematopoietic cells in the later stages; second, with a small dose of cells they had to pass through a very intensive cycle of proliferation in the course of regeneration, and this may be beyond the capacity of recirculating cells. The results may have been different if the demand had been relatively smaller or if the investigation had been carried out at later stages. The possibility of conducting an investigation with high doses of CFUs was limited previously by their small number in the peripheral blood. Now that the identity of migrating CFUs from peripheral blood and from an ectopic focus of hematopoiesis, in which they are just as numerous as in bone marrow, has been established in principle, this difficulty has been overcome. In the experiments of series II each recipient received 200-300 CFUs from an ectopic focus and 200-400 CFUs from bone marrow (Table 2), a total of 400-700 CFUs, equivalent to the number of CFUs in a complete protective dose of bone marrow ($1-2 \cdot 10^6$ cells). The results were in principle the same as in series I (Fig. 2). In all the hematopoietic organs studied the fraction of dividing cells of different origin was the same. Not until 10 days after transplantation did regeneration of hematopoiesis due to progenies of the migrating stem cells result, the contribution of which at this time was only 30% of all dividing cells. By the first month this fraction had fallen to 5-10%, by two months it was below 3%, and by 4 months all the dividing cells were descendants of settled (bone marrow) CFUs.

The results show that migrating stem cells do not differ from settled cells in the probability of divergent differentiation, e.g., myeloid and lymphoid, as can be deduced from the identical fraction of the two subpopulations of CFUs in the bone marrow and spleen, where myeloid hematopoiesis predominates during regeneration after irradiation, compared with the thymus, where differentiation is entirely lymphoid. Meanwhile, in their ability to support themselves, migrating CFUs are significantly inferior to settled. Hence it follows that migrating CFUs are not members of a single population of CFUs that have accidentally entered the circulation, but are a special subpopulation of stem cells with reduced proliferative potential. Possibly only stem cells which have already passed through a process of clonal aging, removed from the bone marrow as spent precursors, migrate into the blood stream. The problem of whether a special structural organization is present in the bone marrow to produce displacement of the stem cells during aging into regions from which migration into the circulation is easier requires further study.

LITERATURE CITED

1. N. F. Kondratenko and I. L. Chertkov, Byull. Éksp. Biol. Med., No. 9, 100 (1972).
2. I. L. Chertkov, L. N. Lemeneva, O. A. Mendelevich, et al., Cell Tissue Kinetics, 5, 387 (1972).
3. G. Gidali, I. Feher, and S. Antal, Blood, 43, 573 (1974).
4. J. W. Goodman and G. S. Hodgson, Blood, 19, 702 (1962).
5. J. P. Lewis, M. Passavoy, M. Freeman, et al., J. Cell. Physiol., 71, 121 (1968).
6. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 214 (1961).

INVESTIGATION OF ERYTHROID PRECURSORS BY MOUSE BONE MARROW CULTURE IN A PLASMA CLOT

T. E. Manakova and S. Yu. Shekhter

UDC 612.111.3-08:612.419-085.23

Two types of erythroid precursors were isolated during culture of mouse bone marrow in a plasma clot in the presence of mouse serum and without the addition of exogenous erythropoietin to the medium. The first type of precursor was more similar in its characteristics to the erythroid colony-forming unit described previously. The second type of precursor was an erythroid burst-forming unit, similar in its properties to that described previously. The optimal concentration of mouse serum in the culture medium was 10-15%. The clonal nature of the colonies and bursts described is confirmed by the linear relationship between their number and the cell concentrations in culture.

KEY WORDS: erythroid colony-forming unit; erythroid burst-forming unit; erythropoietin; mouse serum.

Methods of culture of hematopoietic tissue in semisolid media whereby precursors of cells of the erythroid series at different levels of differentiation can be identified have recently been developed [1, 5, 6, 9]. The most mature of them, the erythroid colony-forming unit (CFU-E) gives rise to colonies consisting of 8-32 erythroid cells after culture for 48 h in the presence of low concentrations of erythropoietin. The least mature precursor, the erythroid burst-forming unit (BFU-E), forms either isolated colonies consisting of 64 to 10,000 erythroid cells or colonies consisting of several small cell clusters after culture for 8 h.

This paper describes a system by means of which two precursors can be discovered: One forms erythroid colonies of 8-32 cells on the third day, the other forms erythroid bursts on the fifth day in culture.

Laboratory of Bone Marrow Culture and Transplantation, Central Institute of Hematology and y and Blood Transfusion, Moscow. Laboratory of Experimental and Clinical Hematology, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 582-585, June, 1979. Original article submitted July 28, 1978.

0007-4888/79/8706-0614$07.50