NUMBER AND PROLIFERATIVE ACTIVITY OF PRECURSOR CELLS OF GRANULOCYTES AND MACROPHAGES IN MICE

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It was shown by a modified agar-culture method that the number of precursor cells of granulocytes and macrophages in the bone marrow, spleen, and embryonic liver of mice is 60-80, 20-50, and 0.4-2 per 10^5 cells, respectively. The proliferative activity of these precursors, as reflected in the figure for "thymidine suicide," is twice as high in the bone marrow and embryonic liver as in the spleen.

KEY WORDS: hematopoietic tissue cultures; precursors of granulocytes and macrophages; DNA-synthesizing cells.

When hematopoietic cells are grown in culture, they form colonies in nutrient agar medium consisting of clones, arising from one single cell or colony-forming unit (CFU) in the culture. The suggestion has been made that CFUs belong to the class of hematopoietic semistem cells differentiating into granulocytes, i.e., that they are an intermediate stage between polypotent stem cells and myeloblast – monoblasts [4]. Determination of these early precursors in hematopoietic tissues is of great importance to the study of the mechanisms regulating hematopoiesis.

Data on the relative number of precursor cells of granulocytes and macrophages in the bone marrow, spleen, and embryonic liver of mice and of the proliferative activity of these cells are given in this paper.

EXPERIMENTAL METHOD

CBA mice of both sexes, aged 2-4 months, were used. Embryonic liver was obtained from 17-19-day embryos of CBA and C57BL mice. The number of CFUs was determined by a modified method of cloning hematopoietic cells in semisolid 0.3% nutrient agar medium, fully described earlier [1]. Medium in which kidney cells from 7-14-day mice had been cultivated was used as the colony-stimulating factor (CSF). The cultures were grown in a closed system (Leighton's tubes) in an atmosphere of air containing 10% CO_2 at 37° C. After 7-10 days the colonies were counted under an inverted microscope (magnification 25). Cell aggregates containing not less than 50 cells were regarded as colonies. Each sample was reproduced twice. Differences between the results of two tests did not exceed ±10%. Proliferative activity of the precursor cells was studied by the "thymidine suicide" method in vitro, the basis of which is that, given high specific activity of the isotope, it kills only those cells in whose DNA it has been incorporated. In the present investigation thymidine-H^3 with specific activity of 11 Ci/mmole (USSR) and 22.55 Ci/mmole (Czechoslovakia) was used in a concentration of 100 μCi/ml. Incubation was carried out at 37° C for 20 min. Full details of the method were described previously [2]. To prevent further incorporation of thymidine-H^3 into DNA the cell suspension was washed with 50 volumes of cold medium No. 199 containing nonradioactive thymidine (100 μg/ml).

EXPERIMENTAL RESULTS

Hematopoietic cells from all three sources formed colonies of the usual type: granulocytic, macro-

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TABLE 1. Number of Developing Colonies Compared with Number of Explanted Mouse Hematopoietic Cells

Expt.	Source of cells								
	bone marrow			embryonic liver			spleen		
	number of explanted cells	number of colonies	CFUs per 10^5 cells	number of explanted cells	number of colonies	CFUs per 10^5 cells	number of explanted cells	number of colonies	CFUs per 10^6 cells
1	11 550	9	82	24 000	11	46	248 000	1	4
	23 100	20	87	48 000	24	50	496 000	2	5
	49 000	39	77	96 000	48	50	744 000	4	5
	99 000	62	62	192 000	96	60	992 000	6	6
	198 000	157	78				1 980 000	12	6
2	50 000	36	68	23 000	5	22	520 000	3	6
	100 000	64	63	46 000	10	22	1 040 000	5	5
	150 000	97	63	92 000	19	21	2 080 000	15	7
	200 000	136	68	184 000	37	20	2 600 000	21	8
3							1 770 000	25	14
							3 540 000	45	13
							5 310 000	70	13
4							480 000	12	24
							860 000	24	24
							1 920 000	44	23
							3 840 000	84	22

TABLE 2. Proliferative Activity of Precursor Cells of Mouse Granulocytes and Macrophages [number of colonies (M ± m) per 10^5 cells]

Source of cells	Number of experiments	Treatment				Percent of CFUs which died
		control	thymidine-H^3	thymidine	thymidine H^3 + thymidine	
Bone marrow	1	36	17	36	37	52
	5	71±8	38±4	—	—	47±2,5
Embryonic liver	1	97	58	89	87	40
	4	65±6	39±11	—	—	40±0,5
Spleen	1	3,1	1,9	3,0	2,9	29
	4	2,2±0,4	1,6±0,4	—	—	27±2,6

phagal, and mixed [1]. Since the number of colonies formed was a linear function of the number of cells explanted, it was possible to determine the number of precursor cells of granulocytes and macrophages in the various hematopoietic tissues. Data on the relative number of CFUs in the bone marrow, spleen, and embryonic mouse liver are given in Table 1. Clearly, the linear relationship held good for explanation of between $1 \cdot 10^4$ and $2 \cdot 10^5$ embryonic liver and bone-marrow cells and from $2 \cdot 10^4$ to $5 \cdot 10^6$ spleen cells. On the whole the highest numbers of CFUs were found in the bone marrow: 60-80 per 10^5 cells. They were half as numerous in the embryonic liver. Of all the hematopoietic organs studied, the spleen contained fewest CFUs, only 5-20 per 10^6 cells, two orders of magnitude less than were present in the bone marrow.

In all three tissues the CFUs formed actively proliferating cell populations. As Table 2 shows, thymidine-H^3 caused death of 40-50% of the CFUs in the bone marrow and embryonic liver, and 20-30% of CFUs in the spleen. The effect of radioactive thymidine was highly specific. Nonradioactive thymine itself is nontoxic for the CFUs (Table 2), and it virtually completely abolished the cytotoxic action of the thymidine-H^3. It thus follows that at any moment about half of the CFUs in the bone marrow and embryonic liver and about a quarter of the CFUs in the spleen were in the S-period of the cell cycle.

The results confirm that precursor cells of granulocytopoiesis are present in large numbers in hematopoietic tissues. The relatively smaller number of CFUs in the spleen can evidently be attributed to its higher content of lymphocytopoietic cells than the other two hematopoietic tissues. The discovery of a high content of CFUs in the embryonic liver is particularly interesting. It is well known that granulocytopoiesis is virtually absent in this organ and that most of the hematopoietic cells belong to the erythroid series. We

do not yet know whether the direction of differentiation of hematopoietic stem cells is a purely stochastic process [5] or whether it is determined by induction of hematopoiesis by the microenvironment [6]. Since there is no "demand" for granulocytopoiesis in the embryonic liver, there is no microenvironment to induce myeloid hematopoiesis, and in that case the high content of CFUs found in the embryonic liver is a powerful argument in support of the first hypothesis.

The data showing the lower proliferative activity of CFUs in the spleen than in the bone marrow are important. The rate of proliferation of CFUs is known to depend on the demand for granulocytopoiesis. It has been shown, in particular, that their proliferation is accelerated in the regenerating bone marrow in irradiated animals [3], and it has accordingly been postulated that it is controlled by remotely acting factors, possibly by a colony-stimulating factor [4]. The discovery of different degrees of proliferation of CFUs in the hematopoietic tissues of the same mouse shows that proliferation of CFUs is regulated not only by remotely acting but also by local factors and, in particular, the microenvironment of the spleen has less effect on proliferation of CFUs than that of the bone marrow and embryonic liver. An alternative explanation could be that the population of CFUs is heterogeneous, and that their fraction present in the spleen is distinguished by reduced proliferative activity.

LITERATURE CITED

1. N. F. Kondratenko and S. I. Shereshkov, Probl. Gematol., No. 11, 18 (1974).
2. I. L. Chertkov, L. N. Lemeneva, and O. A. Mendelevich, Probl. Gematol., No. 1, 3 (1972).
3. N. N. Iscove, J. E. Till, and E. A. McCulloch, Proc. Soc. Exp. Biol. (New York), 134, 33 (1970).
4. D. Metcalf and M. A. S. Moore, Haematopoietic Cells, Amsterdam (1971).
5. J. E. Till and E. A. McCulloch, Ann. New York Acad. Sci., 14, 115 (1964).
6. J. J. Trentin, N. Wolf, V. Cheng, et al., J. Immunol., 98, 1326 (1967).