PROPERTIES OF ERYTHROPOIETIN-INDEPENDENT PRECURSOR CELLS OF THE ERYTHROID SERIES OF MOUSE BONE MARROW

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The development of methods of cloning hematopoietic cells in vitro has led to the discovery of morphologically unidentifiable erythroid precursor cells, capable of forming erythroid colonies in the presence of erythropoietin preparations in the culture medium [5, 6, 12]. By the use of syngeneic serum as stimulator during culture of mouse bone marrow [1, 2], two types of erythroid precursors have been discovered: the colony-forming unit (CFU_{en}) and the burst-forming unit (BFU_{en}), yielding colonies and bursts without the addition of erythropoietin. According to some properties (times of discovery, absence of erythropoietin requirement, dimensions of the colonies) these precursors differ from those described in the literature.

The object of this investigation was to study the response of erythroid precursors during stimulation of erythropoiesis in vivo and to investigate proliferative activity of new erythroid precursors.

EXPERIMENTAL METHOD

Bone marrow of (CBA × C57BL)F_1 mice was cultured in a plasma clot in the presence of 10% mouse serum without the addition of erythropoietin [2]. Post-transfusion polycythemia was induced in mice by two intraperitoneal injections of an 80% suspension of washed mouse erythrocytes in a dose of 1 ml per mouse.

TABLE 1. Detection of Erythroid Precursors during Bone Marrow Culture in the Presence of Polycythemic Mouse Serum

Mouse serum	Number of precursors/10^5 cells			
	CFU_{en}		BFU_{en}	
	$M \pm m$	n	$M \pm m$	n
Normal	57±9,3	6	18±1,7	6
Polycythemia	56±7,0	6	22±4,2	6

TABLE 2. Changes in Number of Erythroid Precursors during Stimulation of Erythropoiesis In Vivo

Treatment of bone marrow donors	Number of precursors/10^5 cells			
	CFU_{en}		BFU_{en}	
	$M \pm m$	n	$M \pm m$	n
Control	11,5±2,79	9	8,0±1,78	9
Blood loss	15,0±4,2	4	7,2±2,5	4
Polycythemia	25,0±2,60	9	8,7±1,5	9
Polycythemia + 2 i.u. erythropoietin	21,0±5,7	9	9,0±1,91	9
Polycythemia + blood loss	15,0±5,0	5	12,5±2,4	5

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Erythropoiesis was stimulated in vivo either by blood loss (2% of body weight) or by injection of erythropoietin (2 units per mouse). Bone marrow was obtained 24 h after stimulation.

In order to study the radiosensitivity of the erythroid precursors, a suspension of bone-marrow cells was irradiated with ^{137}Cs γ-rays on the IPK apparatus with a dose rate of 100 rads/min.

The proliferative activity of the erythroid precursors was determined by the "thymidime suicide" method [7]. After incubation of 4×10^6 cells/ml with ^3H-thymidine (100 μCi/ml) for 25 min at 37°C the cells were washed twice with medium containing 100 μg/ml of nonradioactive thymidine. Proliferative activity was assessed from the number of surviving colony-forming cells.

EXPERIMENTAL RESULTS

Serum of polycythemic mice with suppressed production of endogenous erythropoietin, in a concentration of 10%, did not lower the cloning efficiency of the erythroid precursors (Table 1).

During culture of bone marrow from mice with posttransfusion polycythemia (i.e., with inhibited erythropoiesis) a significant increase was observed in the number of CFU_{en}, whereas the number of BFU_{en} was indistinguishable from the control (Table 2). Injection of erythropoietin (2 units per mouse) did not change the number of CFU_{en} and BFU_{en} in the bone marrow of the polycythemic mice. In mice subjected to acute blood loss against the background of polycythemia, the number of CFU_{en} in the bone marrow decreased a little (but not significantly) compared with that in polycythemic mice, but the number of BFU_{en} increased (also not significantly). Acute blood loss in normal mice had virtually no effect on the number of CFU_{en} and BFU_{en} (Table 2).

Determination of proliferative activity showed that the proportion of precursors in the S-period of the cell cycle (killed by ^3H-thymidine) was under 50% for CFU_{en} and BFU_{en} (Table 3). The system used revealed two precursors: CFU_{en}, forming colonies on the 2nd-3rd days of culture, and BFU_{en}, giving bursts on the 5th day of culture. It was suggested previously [1, 2] that CFU_{en} and BFU_{en} detectable on bone marrow culture in the presence of syngeneic serum without the addition of an erythropoietin preparation are erythropoietin-independent in nature. The facts described in this paper confirm this hypothesis. In particular, polycythemic serum, with no endogenous erythropoietin, stimulated both precursors just as effectively as the erythropoietin-enriched serum of animals subjected to blood loss.

Experimental polycythemia in mice causes a marked decrease in the number of CFU_{en} in the bone marrow, which is completely restored after injection of 1 i.u. of an erythropoietin preparation into the mice. The number of BFU_{en} in these situations remains unchanged [3, 4, 10, 11, 13]. The behavior of the precursors now discovered was different. Not only did the number of CFU_{en} not decrease in posttransfusion polycythemia but, on the contrary, it increased statistically significantly; injection of erythropoietin into polycythemic mice, and likewise blood loss, did not lead to an increase in the number of CFU_{en}. The number of BFU_{en} in the bone marrow of mice in all forms of stimulation studied remained virtually unchanged.

Furthermore, erythroid precursors discovered during culture under the above conditions differed from erythropoietin-dependent precursors described previously in other properties also. For instance, they are more radio-resistant: for CFU_{en}, D_0 = 206 rads, n (extrapolation number) = 1.3; for BFU_{en}, D_0 = 118 rads, n = 1.4 (the appropriate data will be published in more detail separately). The CFU_{en} also have lower proliferative activity.

Taken as a whole, these results confirm that the two precursors are capable of clonal proliferation irrespective of the presence or absence of the hormone erythropoietin. This agrees with data in previous publications on the existence of erythroid precursors detectable with the aid of factors other than erythropoietin: splenic factor [9], myeloproliferative factor [14], and "burst-promotor activity" [8]. Perhaps in this system

TABLE 3. Effect of ^3H-Thymidine on Erythroid Precursors In Vitro

Treatment of bone marrow donors	Number or precursors killed by ^3H-thymidine, %			
	CFU_{en}		BFU_{en}	
	$M\pm m$	n	$M\pm m$	n
Control	42±12,5	5	46±4,5	3
Polycythemia	44±8,0	3	—	

mouse serum is the source of the non-erythropoietin stimulator of erythroid colony formation. The essential difference of this system is ability to form clones without erythropoietin, whereas in all other cases described above its presence is essential.

LITERATURE CITED

1. T. E. Manakova and S. Yu. Shekhter, Byull. Éksp. Biol. Med., No. 6, 582 (1979).
2. S. Yu. Shekhter and T. E. Manakova, in: Mechanisms of Regulation in the Blood System [in Russian], Krasonyarsk (1978), Part 1, pp. 10-11.
3. C. J. Gregory, E. A. McCulloch, and J. E. Till, J. Cell Physiol., 81, 411 (1973).
4. C. J. Gregory and A. G. Eaves, Blood, 51, 527 (1978).
5. D. S. Heath, A. A. Axelrad, D. L. McLeod, et al., Blood, 47, 777 (1976).
6. N. N. Iscove and F. Sieber, Exp. Hemat., 3, 32 (1975).
7. N. N. Iscove, Cell Tissue Kinet., 10, 323 (1977).
8. G. R. Jonson and D. Metcalf, Proc. Natl. Acad. Sci. USA, 74, 3878 (1977).
9. J. M. Pannaccinlli, G. G. Massa, A. G. Saviane, et al., Acta Haemat. (Basel), 58, 27 (1977).
10. C. Peschle, M. C. Magli, C. Cillo, et al., Br. J. Haemat., 37, 345 (1977).
11. A. D. Tepperman, J. E. Curtis, and E. A. McCulloch, Blood, 44, 659 (1974).
12. K. B. Udupa and K. R. Reissmann, J. Lab. Clin. Med., 89, 278 (1977).

INCORPORATION OF LABELED CORTISOL INTO DIFFERENT TYPES OF CONNECTIVE TISSUE

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Adrenocortical hormones and, in particular, hydrocortisone have a marked effect on the state of the skeletal system and the organ of vision, in which they give rise to metabolic changes [1, 8, 12, 14]. Corticosteroids change the activity of lysosomal enzymes of bone tissue and of the tissues of the eye; activity of glycosidases in different types of connective tissue is variously changed [10]. Investigations [3, 4, 6] have shown that corticosteroids can affect lysosomal enzyme activity in the tissues of the eye, and on that basis it has been postulated that enzyme systems of connective tissue are especially sensitive to the action of hormones. It has also been shown that these tissues are target tissues for the action of cortisol [7]. No investigations into incorporation of steroid hormones into bone and cartilage could be found in the accessible literature. Meanwhile, the possibility of biochemical parallels in the tissue metabolism of the eyes and of bone and cartilage tissues can be deduced from observations showing that lesions of the skeletal system (osteogenesis imperfecta, Paget's disease, osteolathyrism, mucopolysaccharidoses, etc.) are accompanied by disturbances in the organ of vision [2, 5, 13, 15].

The object of this investigation was to study the distribution, dynamics of accumulation, and excretion of cortisol-^{3}H in the cortical and cancellous bones, costal cartilage, and also in the sclera and cornea, in order to examine the specificity of the link between these tissues and the hormone.

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