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COMPARATIVE ANALYSIS OF THE ACTION OF UNPURIFIED MOUSE ERYTHROPOIETIN AND HUMAN RECOMBINANT ERYTHROPOIETIN ON ERYTHROID AND GRANULOCYTIC-MACROPHAGAL PRECURSOR CELLS IN SEMISOLID MOUSE BONE MARROW CULTURES

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Erythropoietin is a circulating polypeptide hormone which regulates proliferation and differentiation of the erythroid branch of hematopoiesis. In recent years the gene of human erythropoietin has been isolated and a recombinant erythropoietin, comparable with natural erythropoietin in its biological and immune properties, has been obtained [3, 8]. This has opened up new prospects not only for the study of erythropoiesis, but also for the treatment of erythropoietin-deficiency anemias.

A comparative study of the action of recombinant and natural erythropoietin on human hematopoietic precursor cells has shown that recombinant erythropoietin, in equal doses, has a stronger stimulating action on erythroid and polypotent precursors than natural erythropoietin [4].

Another source of erythropoietic activity is mouse serum (plasma), enriched with erythropoietin [9]. This readily available preparation of erythropoietin stimulates colony formation from late erythroid precursor cells in mouse and human bone marrow culture [7]. However, the presence of inhibitors of hematopoiesis of different nature in plasma [2] and also the presence of growth factors make the use of such preparations for the study of erythropoiesis in culture more difficult.

The aim of this investigation was to study the action of unpurified preparations of murine erythropoietin and human recombinant erythropoietin on committed precursor cells of mouse bone marrow.

EXPERIMENTAL METHOD

Experiments were carried out on bone marrow from female (CBA × C57BL)F_1 mice weighing 18-20 g. A cell suspension was prepared in α-MEM medium with 20% fetal calf serum (FCS). Bone marrow cells in a concentration of $2 \cdot 10^5$/ml were cultured by Iscove's method [5] in a modification. The culture medium contained methylcellulose ("Fluka") in α-MEM medium in a final concentration of 0.9%, with 30% FCS ("Flow"), 1% L-glutamine, 1% bovine serum albumin (7.5% of fraction V, from "Gibco"). 10^{-4} M 2-mercaptoethanol (2-ME), 2% HEPES buffer, and antibiotics. To stimulate colony formation a conditioning medium was used in a final concentration of 10%; it was obtained during culture of mouse splenocytes ($2 \cdot 10^6$/ml) in medium RPMI-1640, enriched with glutamine, 2-ME, FCS, and inactivated human plasma, each in a proportion of 2.5%; pokeweed mitogen ("Flow") was added in a concentration of 0.75-1%. The conditioning medium obtained after culture for 7 days was centrifuged and filtered (pore diameter 0.45 μ).

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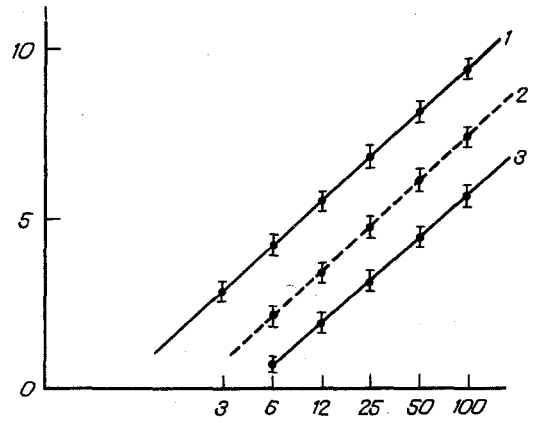

Fig. 1. Dependence of incorporation of ^{3}H-thymidine on concentration of erythropoietic activity in culture medium. 1) Culture stimulated by human recombinant erythropoietin (y = 45x — 38.5); 2) culture stimulated by murine erythropoietin y = 44.6x — 58; 3) culture stimulated by standard erythropoietin (y = 43.8x — 64.5). Abscissa, concentration of serum, supernatant (in μl/ml), and erythropoietin (in mU/ml culture); ordinate, radioactivity, in cpm $\cdot 10^4$).

TABLE 1. Content of PFU-E and CFU-GM in Culture of Mouse Bone Marrow under Stimulation by Different Concentrations of Erythropoietin (M ± m)

Erythropoietin	Erythropoietin concentration in	Number of precursor cells per 10^5 cells	
		PFU-E	CFU-GM
Murine	60	11,7±1,9 / 0	14,3±5,3 / 12,0±2,1
	120	15,2±4,0 / 0	20,9±7,3 / 19,0±4,0
	250	15,4±3,9 / 0	24,9±8,8 / 25,7±6,6
	500	26,3±3,4 / 0	26,1±16,3 / 17,8±5,4
	1000	20,8±4,0 / 0	24,5±8,2 / 13,7±4,8
	2000	10,0±4,2 / 0	18,2±7,7 / 15,9±8,3
Recombinant	60	7,0±3,2 / 0	24,3±6,1 / 6,5±2,8
	120	10,5±4,7 / 0	27,0±9,0 / 12,2±3,6
	250	8,3±3,8 / 0,4±0,2	26,3±9,4 / 17,6±5,6
	500	10,3±2,9 / 1,0±0,3	24,2±2,3 / 16,8±2,4
	1000	15,9±4,0 / 2,5±1,3	26,0±3,0 / 19,7±6,4
	2000	15,5±3,4 / 4,0±2,6	29,0±5,8 / 20,5±5,5

Legend. Numerator — value in the presence of erythropoietin, denominator — value in its absence.

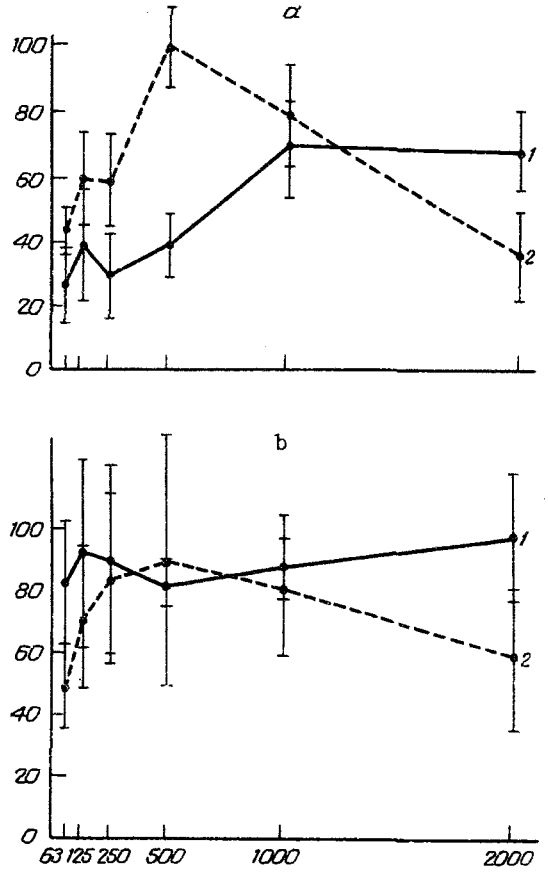

Fig. 2. Action of recombinant human erythropoietin and murine erythropoietin on colony and burst formation in mouse bone marrow culture: a) PFU-E, b) CFU-GM. Abscissa, erythropoietin concentration (in U/ml); ordinate, number of bursts or colonies per 10^5 bone marrow cells (in % of maximal).

Either the supernatant of CHO cells with addition of recombinant human erythropoietin gene* — unpurified recombinant erythropoietin, or the serum of anemic mice (unpurified murine erythropoietin) was used as the source of erythropoietin. To prepare anemic serum enriched with erythropoietin, (DBA × BALB)F_1 mice were used and were irradiated on a γ-apparatus in a dose of 6 Gy; next day they were given an intraperitoneal injection of phenylhydrazine hydrochloride (PH) in a dose of 60 mg/kg [9]. After 7-8 days the serum or plasma obtained from the mice was sterilized by filtration (pore diameter 0.22 μ), poured out in volumes of 1 ml, and kept at —20°C. To stimulate erythroid colonies, samples of erythropoietin were added to the culture in a concentration of 0.06-2.0 U/ml.

Colonies and bursts of erythroid (PGU-E) and granulocytic-macrophagal (CFU-GM) precursor cells were formed in clonogenic cultures of mouse bone marrow under stimulation by erythropoietin and conditioning medium in optimal concentration (Table 1). The number of PFU-E increased with an increase in concentration of the hormone in the culture medium from 0.1 to 1.0 U/ml. The maximal effect was achieved with murine erythropoietin in a concentration of 0.5 U/ml and human recombinant erythropoietin in a concentration of 1 U/ml. With a higher level of erythropoietic activity in the culture medium the number of erythroid bursts did not increase, but a plateau (recombinant preparation) or a decrease in their number (anemic mouse serum) was observed (Fig. 2a). Meanwhile the number of CFU-GM was unchanged fol lowing the action of different concentrations of both erythropoietin preparations (Fig. 2b), i.e., their development was maintained virtually entirely by the conditioned medium and not by erythropoietic activity.

Thus the investigation showed that unpurified preparations of human recombinant erythropoietin and murine erythropoietin are effective stimulators of clonogenic erythroid precursors from mouse bone marrow in culture in vitro. Preparations of murine erythropoietin were found to be more effective than samples of human recombinant erythropoietin.

However, growth of erythroid bursts with erythropoietin in mouse serum in a concentration over 0.5 U/ml was depressed, probably because of the presence of inhibitory factors in it. The absence of side effects even with the unpurified supernatant of recombinant erythropoietin obtained from CHO cells makes it preferable for use in the study of erythropoiesis in man and animals.

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