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EFFECT OF ERYTHROCYTE BREAKDOWN PRODUCTS ON STEM CELLS AND ERYTHROPOIETIN FORMATION

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In experiments on CBA mice and albino rats the effect of erythrocyte breakdown products (EBP) on the number of colony-forming units (CFU), differentiation of stem cells, and erythropoietin production was studied. After three or four injections of EBP to normal or lethally irradiated (1000 rad) mice, no changes in the number of CFU or in differentiation of the stem cells were observed after transplantation of bone marrow. Daily administration of EBP to mice for 3 days before irradiation (1000 rad) and bone marrow transplantation led to an increase in the number of colonies in the recipients' spleen, mainly on account of colonies of erythroid type. Injection of EBP into the animals did not change the erythropoietic activity of the blood serum. The possible role of EBP in the mechanism of autoregulation of erythropoiesis is discussed.

KEY WORDS: erythrocyte breakdown products; stem cells; erythropoietin.

The stimulating action of erythrocyte breakdown products (EBP) on erythropoiesis can now be taken as proven [3, 4, 6, 8, 11-13]. However, the mechanism of this stimulating action of the erythrocyte breakdown products is not clear. Some workers postulate that their effect is mediated through increased formation of erythropoietins [2, 8, 13]. A direct stimulating action of EBP on the proliferation of bone marrow erythroblasts also has been suggested [1, 2, 4, 5, 12].

Some aspects of the mechanism of action of EBP on erythropoiesis, notably their effect on hematopoietic stem cells, and also on erythropoietin production were studied in this investigation.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice weighing 20-25 g and on albino rats weighing 100-150 g. EBP were obtained from erythrocytes of these animals by washing, hemolysis with distilled water (1 volume erythrocytes + 3 volumes water), and freezing and thawing three times. Homologous EBP were injected intraperitoneally in a dose of 1 ml into rats and 0.2 ml into mice.

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TABLE 1. Indices of Erythropoiesis in Mice Receiving EBP

Time of investigation	n	Hematocrit index, %	Erythrocytes, millions/μl	Reticulocytes, %	Erythroblasts, millions
Before experiment	6	38,0±2,6	7,8±0,4	1,8±0,1	1,69±0.2*
On 4th-5th day	10	46,2±4,0	8.9±0,6	4,3±0,6†	2,56±0,3†
		122%	115%	240%	152%

*Index in normal mice (n = 10) in one femur.
†$P \leq 0.05$ relative to same index before experiment.

The number of colony-forming units (CFU) in the bone marrow and spleen was determined by the method of Till and McCulloch [15]. The types of colonies were determined by histological examination of sections of the spleen of the recipient mice.

The experiments to study the effect of EBP on CFU were carried out in the following three variants. In the first the number of CFU was determined in the bone marrow and spleen of mice receiving homologous EBP for 3 days. The animals of this group were killed on the third to fifth day after the first injection during the period of maximal activation of erythropoiesis. In the second variant of the experiments the effect of EBP was studied on colony formation in normal bone marrow after its transplantation into lethally irradiated mice. EBP were injected in the above dose into the recipients of the bone marrow simultaneously with the graft and 2, 4, and 6 days later. By carrying out the experiments in this way it was possible to assess the effect of the EBP on differentiation of stem cells. In the third variant of the experiments the recipients of normal bone marrow were mice which had been given EBP on the 3 days before lethal irradiation. In these experiments the effect of the test factor on the ability of the spleen to adsorb transplanted CFU could be determined.

The effects of EBP on erythropoietin production were studied in experiments on rats. The erythropoietic activity of the plasma was assessed from the reticulocyte response in polycythemic rats and from the incorporation of ^{59}Fe into the erythrocytes of the animals.

EXPERIMENTAL RESULTS

Injection of EBP caused stimulation of erythropoiesis in the mice, as shown by a tendency for the hematocrit index and the erythrocyte count to rise, and a significant increase in the reticulocyte count and also in the number of erythroblasts in the bone marrow (Table 1).

To study the mechanism of the changes observed the effect of EBP on hematopoietic stem cells was investigated. In mice with erythropoiesis stimulated by injection of EBP (first series of experiments) the number of CFU was not increased either in the bone marrow or in the spleen, when calculated per 10^5 transplanted cells or per whole spleen or femur. On microscopic investigation no significant changes likewise were found in the distribution of the colonies by histological type.

When the effect of EBP directly on colony formation was studied in normal bone marrow (second series of experiments) no significant changes likewise were found either in the number of colonies formed (11.1 ± 2.0 per 10^5 transplanted cells compared with 9.1 ± 1.0 in the control, after injection of physiological saline), or in the distribution of the colonies by histological type.

The results of these two series of experiments thus showed that EBP have no significant effect on the number of CFU in the body or on their differentiation; i.e., hematopoietic stem cells are not the target of action of EBP.

The most interesting results were obtained in the third series of experiments in which mice which were recipients of normal bone marrow received injections of EBP during the 3 days before irradiation and transplantation of bone marrow cells. In this group of animals an increase in the number of splenic colonies was observed. Histological examination showed that this increase was due chiefly to an increase in the number of erythroid colonies and to a lesser degree to an increase in the number of granulocytic colonies and it was accompanied by a decrease in the number of megakaryocytic and mixed colonies (Table 2).

The results can be explained as follows. EBP, although without any significant effect on stem cells, change the functional state of the spleen in such a way that it can receive a larger number of CFU and thus provide more favorable conditions for erythroid differentiation of stem cells.

TABLE 2. Colony Formation in Spleens of Lethally Irradiated Mice Previously Receiving EBP

Recipients of bone marrow	n	Number of CFU per 10^5 cells	Types of colonies			
			E	G	Me	Mi
Mice previously receiving physiological saline	20	7,3±1,0	4,6±0,6	1,6±0,2	0,7±0,2	0,4±0,1
Mice previously receiving EBP	16	13,3±1,3*	10,1±1,3*	3,1±0,4*	0,1±0,1*	0,1±0,1*

Legend: E) erythroid, G) granulocytic, Me) megakaryocytic, and Mi) mixed colonies.
*$P \leq 0.05$ relative to same index in mice receiving physiological saline.

TABLE 3. Effect of Blood Serum on Rats Receiving EBP on Erythropoiesis in Polycythemic Rats

Serum injected*	Number of sera	Reticulocytes, %	^{59}Fe, % incorporation
Serum of intact rats	5	0,1±0,02	0,5±0,2
Serum of rats receiving EBP	5	0,2±0,04	0,6±0,1
Standard erythropoietin C	—	3,5±0,5†	13,8±0,5†

*Each serum was tested on two recipient rats.
†$P \leq 0.01$ relative to action of normal serum.

Considering that erythropoietin has no significant effect on CFU but, at the same time, it stimulates growth of erythroid colonies both in vivo [7] and in vitro [10], a special series of experiments was carried out to study the erythropoietic activity of the blood serum of rats in which erythropoiesis had been stimulated by injection of EBP* (Table 3). The results showed that this serum, when injected into polycythemic rats, did not induce a reticulocyte response in them and did not increase the incorporation of ^{59}Fe into the erythrocytes. Hence it follows that the serum of these animals did not contain erythropoietin. A standard preparation of erythropoietin C, which gave a positive result, was used as the control.

The results of these investigations thus showed that EBP exert their influence on hematopoietic stem cells. However, this action of EBP is unconnected with any direct effect on CFU, as shown by the results of the first and second series of experiments. It likewise cannot be explained on the grounds that EBP exert their effect through the production of erythropoietin, stimulating growth of erythroid colonies, for no change in the serum erythropoietic activity was observed in animals after injection of EBP. The most likely explanation of the action of EBP in stimulating erythropoiesis may be that they change the microenvironment of the hematopoietic cells, as a result of which the stroma of the hematopoietic organs can receive a larger number of CFU, and this favors the formation of a larger number of erythropoietin-sensitive cells. Growth of the erythroid colonies, however, in the future is controlled by erythropoietin. EBP thus have a regulatory action on erythropoiesis at the stem cell level through mechanisms of "short-range" regulation and they do not affect mechanisms of "long-range" regulation of hematopoiesis. This conclusion is confirmed by data showing the stimulating action of phenylhydrazine on erythropoiesis [9] and its radioprotective action [14] in irradiated animals. Phenylhydrazine, which induces hemolysis of erythrocytes, was found to have a radioprotective action only if given 5-7 days before irradiation and to be ineffective if given immediately before or after irradiation. The explanation of this phenomenon may be that the EBP formed by the action of phenylhydrazine change the microenvironment of the stem cells, and a certain length of time, similar to that used in the present experiments, is necessary for this to arise.

*The stroma of erythrocytes was used as the EBP, for the writers showed previously that the stimulating action of EBP is entirely due to the stroma of these cells [5].

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