Potentiation of Erythropoiesis In Vitro by Dexamethasone

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A B S T R A C T The effect of dexamethasone on erythropoiesis was examined in vitro. Hematopoietic cells from 13-day mouse fetal livers were cultured for 48 h in the presence or absence of erythropoietin and erythroid colonies enumerated. Colony formation occurring in cultures containing no added erythropoietin was inhibited by the incorporation of antierythropoietin antibody, suggesting that these colonies formed in response to endogenous hepatic erythropoietin. Maximal colony formation was observed with 0.5 U/ml of sheep erythropoietin. Dexamethasone increased erythroid colony formation with peak stimulation at 10^{-9} M. Dexamethasone potentiation was most marked in cultures containing less than maximally stimulating concentrations of erythropoietin. The cells required only a brief exposure to glucocorticosteroid to exhibit the augmented cloning capacity, and dexamethasone stimulation was inhibited by progesterone (10^{-6} M). A comparable response to dexamethasone was observed in cultures of adult murine and human bone marrow erythroid progenitors, implying that the phenomenon is not peculiar to fetal cells and is not dependent on the presence of fetal hepatocytes. These data suggest that erythroid progenitor cells possess a glucocorticoid receptor mechanism that can modulate the response to erythropoietin in vitro.

INTRODUCTION

Although erythropoietin is the primary humoral regulator of red blood cell production in mammals, relatively little is known about the factors modulating the interaction of erythropoietin with target cells (1). Erythropoietin presumably interacts with a receptor on the surface of certain red cell progenitors (2). Stimulation of these erythropoietin-sensitive cells results in a series of replicative and maturational steps culminating in the production of mature erythrocytes. Various hormones are known to influence hematopoiesis (3). Androgenic

steroids and other steroids with a 5β-H configuration increase red cell production both in vivo and in vitro by direct and indirect mechanisms (4). Androgenic steroids appear to stimulate granulopoiesis as well (5, 6).

The role of glucocorticosteroids in erythropoiesis is uncertain. Glucocorticoids have been used in the treatment of aplastic anemia and may improve red cell production in a congenital abnormality of erythropoiesis (Diamond-Blackfan syndrome). We report here that dexamethasone potentiates the erythropoietin-stimulated proliferation of red cell progenitors in vitro. The dexamethasone effect may involve an augmented sensitivity of progenitor cells to erythropoietin in vitro.

METHODS

Fetuses of white Swiss-Webster mice were obtained at $12\frac{1}{2}$–$13\frac{1}{2}$ days' gestation. The fetal livers were dissected out and single cell suspensions prepared by repeated aspiration through a no. 25 needle. The method of Iscove et al. (7) was used for cloning erythroid progenitors in vitro. Cells were plated in enriched alpha medium with 30% fetal calf serum and antibiotics, with a supporting matrix of 0.8% methyl cellulose. Dithiothreitol (10^{-4} M) or α-thioglycerol (10^{-4} M) was included in all plates (8, 9). For most experiments, 10^4 viable nucleated cells were dispersed in 1-ml cultures in 35-mm plastic dishes (Falcon Plastics, Division of Bioquest, Oxnard, Calif.). Additions to the cultures included Step 3 sheep plasma erythropoietin (Connaught Laboratories, Toronto, Canada) and human urinary erythropoietin (provided by the Blood Resources Division, Heart and Lung Institute, National Institutes of Health). Dexamethasone, prednisone, and progesterone (Sigma Chemical Co., St. Louis, Mo.) were dissolved in 100% ethanol and diluted to appropriate concentrations with complete tissue culture medium. Control cultures were prepared containing equivalent concentrations of ethanol. Rabbit antierythropoietin antibody was a gift of Dr. A. Ichiki, Knoxville, Tenn. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 48 h. Erythroid colonies of eight cells or more were enumerated by using an inverted microscope. Colonies were stained with benzidine after transfer to slides to confirm their erythroid nature (7). Similar cultures were prepared with young adult Swiss-Webster mouse bone marrow. Normal human bone marrow was obtained from informed volunteers by aspiration and the nucleated cells were isolated by centrifugation in Wintrobe tubes. The same culture technique was used for

TABLE I
Fetal Liver Erythroid Colony Formation In Vitro

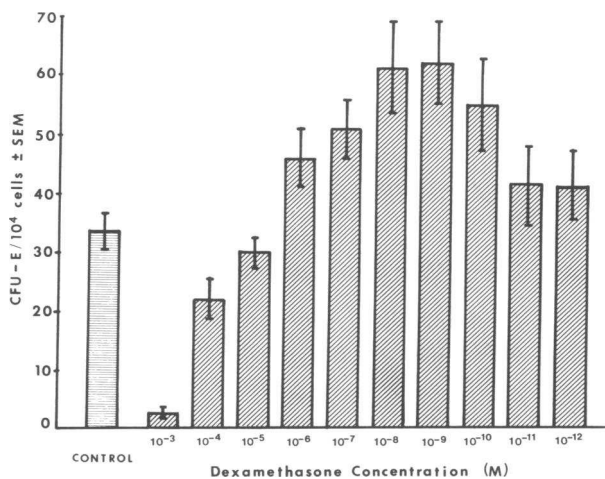| Addition to plate	CFU-E
	per 10^4 cells
—	34
0.5 U erythropoietin	176
100 μl antierythropoietin antibody	4
100 μl antierythropoietin antibody	20
+0.5 U erythropoietin	
Ethanol control (equivalent to	30
10^{-3} M dexamethasone)	
Normal rabbit serum control	32

FIGURE 1 Effect of dexamethasone on erythroid colony formation (CFU-E). Plates contain no added erythropoietin. Bars give the mean values of six experiments and bracketed lines the standard error of the mean.

human cells (7). In the experiments with bone marrow 10^5 cells were plated. The progenitor cell yielding the red cell colonies is referred to as "CFU-E" (colony-forming unit, erythroid) [1] (10, 11).

RESULTS

Some erythroid colonies developed from fetal mouse liver cells plated in methyl cellulose containing no added erythropoietin. The addition of antierythropoietin antibody largely abrogated this spontaneous colony formation (Table I), suggesting that these colonies formed in response to endogenous erythropoietin produced by the fetal liver (12-14) or perhaps also to erythropoietin in the fetal calf serum. Erythropoietin-stimulated erythroid colonies in fetal liver hematopoietic cell cultures were found to be linearly related to the number of cells plated. Dose-response curves consistently showed maximum colony formation with 0.5 U of sheep erythropoietin (Table II). At this dose, colony formation was increased approximately fivefold over control cultures. 100 μl of antierythropoietin antibody serum reduced the erythropoietin-stimulated colony formation to below control levels (Table I). A series of culture experiments were run to test for the effect of ethanol. Ethanol, in concen-

[1] *Abbreviation used in this paper:* CFU-E, colony-forming unit, erythroid.

TABLE II
Erythropoietin Dose Response

Erythropoietin	CFU-E
U	*per 10^4 cells*
0	29 (20–37)
0.01	62 (56–68)
0.05	115 (102–128)
0.10	142 (124–160)
0.50	230 (187–272)
1.00	166 (108–224)

Mean and range are given for two experiments performed in duplicate with sheep erythropoietin.

trations equivalent to dilutions yielding 10^{-3} M dexamethasone, had no detectable effect on erythroid colony formation (Table I).

In plates not containing added erythropoietin, dexamethasone caused an increase in fetal erythroid colony formation over a wide range of concentrations (Fig. 1). Dexamethasone inhibited colony formation at concentra-
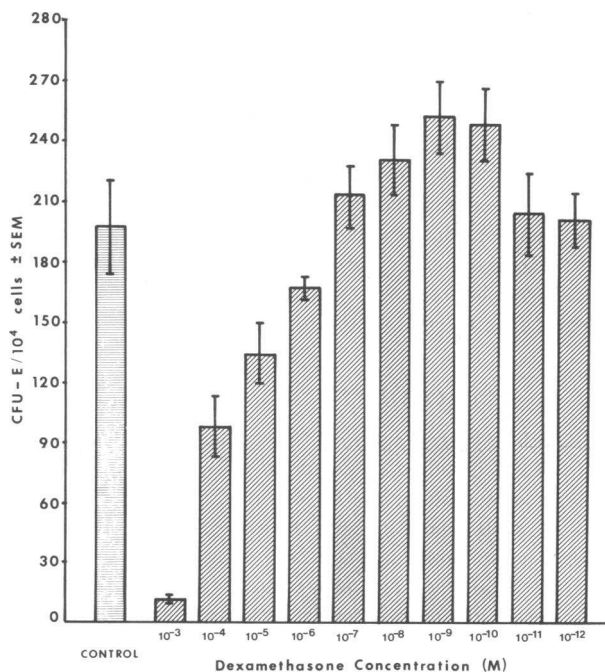

FIGURE 2 Effect of dexamethasone on erythroid colony formation (CFU-E). Plates contain 0.5 U sheep plasma erythropoietin. Bars give the mean values of six experiments and bracketed lines the standard error of the mean.

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Table III

Effect of Prednisone on Erythropoietin-Stimulated Fetal Liver Erythroid Colony Formation

Prednisone	CFU-E
M	$per/10^4$ cells
—	120
10^{-5}	60
10^{-6}	164
10^{-7}	180
10^{-8}	128
10^{-9}	110

Cultures contain 0.5 U sheep erythropoietin.

tions greater than 10^{-5} M. Stimulation was observed between 10^{-6} M and 10^{-10} M, with peak effect (mean of 82% stimulation) noted at 10^{-9} M (Fig. 1).

In the presence of maximally stimulating concentrations of erythropoietin, the dexamethasone effect on numbers of CFU-E was less pronounced. With 10^{-9} M dexamethasone, colony formation increased by a mean of 27% in the presence of 0.5 U of sheep erythropoietin (Fig. 2). A similar pattern was observed with prednisone, though peak stimulation occurred at 10^{-7} M (Table III).

The glucocorticoid stimulation of erythroid colony formation in vitro did not require that the hormone be present in the tissue culture plate. The effect was observed when the cells were exposed to dexamethasone for 1 h, washed, and then plated. Since progesterone is known to block glucocorticoid binding to the receptors of other types of cells (15) and interfere with the transport of the receptor complex to the nucleus (16), experiments were performed to ascertain the effect of simultaneously added progesterone on the glucocorticoid stimulation of erythropoiesis. Progesterone (10^{-6} M) was found to abrogate the glucocorticoid effect on the numbers of fetal erythroid colonies (Table IV). This

Table V

Effect of Dexamethasone (10^{-9} M) on Erythropoietin Dose Response (Fetal Liver Erythroid Cells)

Erythropoietin	CFU-E		% augmentation*
	Dexamethasone	Control	
U	per 10^4 cells		
0	44	20	120
0.01	120	68	77
0.05	236	128	84
0.10	264	160	65
0.50	300	272	10
1.00	268	224	20
2.00	236	192	22

$$* \frac{\text{Dexamethasone} - \text{control}}{\text{control}} \times 100.$$

effect was observed in cultures containing no erythropoietin as well as in those containing maximally stimulating concentrations of erythropoietin. Progesterone itself at 10^{-6} M was found to have no effect on erythropoiesis but was toxic at 10^{-5} M.

In an attempt to define the mechanism of dexamethasone-stimulated erythropoiesis, erythropoietin dose-response experiments were repeated with and without dexamethasone (10^{-9} M). These studies showed that the glucocorticoid augmentation of erythropoiesis was greatest at levels of erythropoietin below those causing maximal stimulation (Table V). Similar phenomena were observed with human and sheep erythropoietin. Dexamethasone did not increase erythroid colony formation in the cultures containing antierythropoietin antibody (Table VI), implying that the steroid had no direct stimulatory effect.

Studies were performed with adult mouse bone marrow in order to be certain that the observations were not peculiar to the fetal liver hematopoietic cell cul-

Table IV

Effect of Dexamethasone, Progesterone, and Erythropoietin on Erythroid Colony Formation (Fetal Liver Erythroid Cells)

Dexamethasone	Progesterone	Erythropoietin	CFU-E
M	M	U	$per 10^4$ cells
—	—	—	40
10^{-9}	—	—	88
—	10^{-6}	—	48
10^{-9}	10^{-6}	—	48
—	—	0.5	144
10^{-9}	—	0.5	232
10^{-9}	10^{-6}	0.5	136
—	10^{-6}	0.5	144

Table VI

Effect of Antierythropoietin Antibody on Dexamethasone Response (Fetal Liver Erythroid Cells)

Erythropoietin	Antierythropoietin antibody	Dexamethasone	CFU-E
U	μl	M	$per 10^4$ cells
—	—	—	48
—	50	—	24
—	—	10^{-9}	88
—	50	10^{-9}	28
0.5	—	—	180
0.5	100	—	44
0.5	50	—	124
0.5	—	10^{-9}	244
0.5	50	10^{-9}	116

TABLE VII

Effect of Dexamethasone on Adult Mouse Marrow CFU-E

	CFU-E
	per 10^5 cells
Control	3±2
Dexamethasone (10^{-9} M)	10±2
Erythropoietin (0.5 U)	140±24
Erythropoietin (0.5 U) + dexamethasone (10^{-9} M)	191±33
Erythropoietin (0.5 U)	95
Erythropoietin (0.5 U) + dexamethasone (10^{-9} M)	140
Erythropoietin (0.5 U) + dexamethasone (10^{-9} M) + progesterone (10^{-6} M)	100

Mean±SE given for seven experiments, plates contain α-thioglycerol (10^{-4} M).

ture system. Murine marrow cells showed an erythropoietin dose-response effect like that of fetal liver cells. Maximal stimulation occurred at 0.5 U of sheep erythropoietin. Dexamethasone (10^{-9} M) caused a substantial number of colonies to appear in cultures not containing added erythropoietin (Table VII). The dexamethasone-induced augmentation of cloning in cultures to which erythropoietin was added was comparable to that seen in the fetal liver experiments and the dexamethasone effect was blocked by progesterone (Table VII).

Human bone marrow erythroid colony formation was also increased in the presence of dexamethasone (10^{-9} M) (Table VIII). The degree of augmentation at maximal stimulating levels of erythropoietin was comparable to the results obtained with murine tissues.

DISCUSSION

Although the 5β-H steroids are known stimulators of red cell production (4, 17), the relationship of glucocorticosteroids to erythropoiesis is uncertain. Addison's disease in man is associated with normocytic, normochromic anemia (18), and a congenital erythroid hy-

TABLE VIII

Effect of Dexamethasone on Human Bone Marrow CFU-E

	CFU-E
	per 10^5 cells
Control	5 (0–9)
Dexamethasone (10^{-9} M)	19 (16–22)
Erythropoietin	
0.01 U	25 (12–37)
0.05 U	32 (14–49)
0.1 U	42 (22–62)
0.5 U	48 (26–70)
1.0 U	60 (34–84)
1.0 U + dexamethasone (10^{-9} M)	74 (54–93)

Mean and range of two experiments performed in duplicate with human urinary erythropoietin.

poplasia of children (Diamond-Blackfan syndrome) is frequently responsive to low doses of glucocorticoids (19). In animals, glucocorticosteroids and ACTH have been reported to stimulate erythropoiesis (20–22) but inhibition has also been observed (23, 24) On the basis. of observations in nephrectomized rats, Malgor and co-workers (22) concluded that dexamethasone stimulated erythropoiesis by an effect on erythropoietin production. Glucocorticoids are also reported to stimulate erythropoiesis in amphibians (25). Cortisone has been found inhibitory to granulopoiesis both in vivo and in vitro (26).

Mouse fetal liver is a rich source of erythropoietin-responsive cells (27) and has been used in assays for erythropoietin (28). We used a cloning assay employing fetal liver cells in order to assess the effect of dexamethasone on mammalian erythropoiesis in vitro. Erythroid colonies formed in vitro without the addition of exogenous erythropoietin. Since these colonies did not form when an antibody to erythropoietin was included in the culture plate, we assumed that these colonies were generated in response to endogenous erythropoietin produced by the fetal liver cells (12–14) or perhaps in part to erythropoietin in the fetal calf serum. Exogenous erythropoietin markedly stimulated colony formation in vitro, and peak activity was observed at a concentration of 0.5 U/ml sheep erythropoietin. Higher doses of sheep erythropoietin yielded fewer colonies and this may have been due to toxic impurities in the erythropoietin preparation (7).

Dexamethasone produced a marked augmentation of colony formation in vitro. The dexamethasone-induced increased cloning was most prominent at suboptimal levels of erythropoietin. It seems likely that the dexamethasone effect was mediated via a glucocorticosteroid receptor mechanism in the erythropoietin-responsive cells. Only a brief exposure to steroid was required to exhibit the stimulatory effect and the dexamethasone stimulation was ablated by appropriate concentrations

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of progesterone. Progesterone is known to block glucocorticoid activity by interfering with glucocorticoid receptor binding and function (15, 16). Also, dexamethasone did not increase colony formation in the presence of antierythropoietin antibody.

Erythropoietin is thought to act via a specific receptor mechanism on certain red cell progenitors (2, 27). The present studies suggest that these cells may also have a glucocorticoid receptor mechanism. The addition of dexamethasone appears to increase the sensitivity and response to erythropoietin in vitro and does so maximally in concentrations equivalent to physiologic levels of hydrocortisone. The comparable response of adult mouse and human marrow erythroid progenitors to dexamethasone implies that the phenomenon is not peculiar to fetal cells and is not dependent on the presence of fetal hepatocytes in the culture plates. One possible explanation for these observations is that there is an interaction between the postulated receptor mechanism for glucocorticosteroid and erythropoietin on red cell progenitors. Glucocorticosteroid modulation of the interaction between hormones and their receptor mechanisms has been described in several systems. Recently, glucocorticoids were shown to modulate the sensitivity of the rat testicular Leydig cell to chorionic gonadotropin (hCG) (29). Gospodarowicz demonstrated a permissive effect of glucocorticoid on the stimulation of 3T3 cells by fibroblast growth factor (30). Progesterone was inhibitory in the 3T3 system as well.

Since concentrations of dexamethasone higher than those equivalent to physiologic concentrations of hydrocortisone did not augment erythropoiesis, there is little in these in vitro experiments to suggest that glucocorticoids would be of use as a "general" stimulus to erythropoiesis. On the other hand, diseases involving alterations in the function or interaction of these postulated receptor mechanisms might be affected by exogenous glucocorticosteroid.

ACKNOWLEDGMENTS

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