DIFFERENTIAL RESPONSE OF EARLY ERYTHROPOIETIC AND GRANULOPOIETIC PROGENITORS TO DEXAMETHASONE AND CORTISONE*

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It is well known that glucocorticoids can affect the concentration of leukocytes in peripheral blood as well as the integrity of lymphoid tissues (1, 2). Less well known, however, is their influence on the bone marrow and, particularly, the degree to which these hormones contribute to the modulation of hematopoiesis in normal and disease states. Cortisone in high, nonphysiologic dosage was found by Cardinali et al. (3) to depress mitotic activity of erythroid and myeloid components of hamster bone marrow. Metcalf (4) observed that high doses of cortisone also decreased the number of presumptive granulopoietic stem cells (CFU-C)[1] in mouse bone marrow in vitro and in vivo and decreased the concentration of colony-stimulating activity (CSA) in mouse serum. A depression of CFU-C was also reported in mouse marrow cultures treated with dexamethasone (5). The effects of glucocorticoids on committed erythroid stem cells are less well characterized, however. Singer et al. (6) noted a marked inhibitory effect of cortisol on a class of erythroid progenitors (CFU-E) in rat bone marrow, whereas Golde et al. (7) found a definite stimulatory effect of dexamethasone on CFU-E in mouse fetal liver cultures and a slight stimulatory effect in adult mouse marrow cultures. Results of in vivo studies also differ on the effect of glucocorticoids on erythropoiesis. According to Malgor et al. (8), dexamethasone increased the erythroid component of bone marrow. Because nephrectomy and anti-erythropoietin antibody abolished the response, increased production of erythropoietin was implicated as the mediator. On the other hand, Gordon et al. (9) have shown that prednisolone did not affect erythropoiesis in normal adult mice and that high doses of prednisolone were, in fact, inhibitory.

To gain further insight into the possible role of glucocorticoids in the regulation of hematopoiesis, we studied dose-response relationships with dexamethasone and cortisone, focusing on both the very early erythroid progenitors (BFU-E) and CFU-C. Our data reveal that BFU-E are much more sensitive than CFU-C to inhibition by low doses of dexamethasone or cortisone.

Materials and Methods

Mice. Adult female CF_1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used in all experiments.

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[1] *Abbreviations used in this paper:* BFU-E, early committed erythroid progenitor; CFU-C, committed granulopoietic progenitor; CFU-E, late committed erythroid progenitor; CSA, colony-stimulating activity.

TABLE I

Influence of Dexamethasone on BFU-E and CFU-C in Individual and Replicate Experiments *

Dexametha-sone	Colonies per 4×10^5 bone marrow cells (mean $\pm$ SE)		
	BFU-E	BFU-E	CFU-C
nM	*8 days*	*14 days*	*8 days*
0	35.2 ± 6.1(4)	61.8 ± 13.9 (4)	127 ± 6.6 (4)
0.2	33.0 ± 5.8	58.4 ± 7.2	
0.6	27.6 ± 2.6	40.4 ± 2.2*	
2.0	23.6 ± 2.6*	27.7 ± 2.8*	116 ± 6.5
2.0	24.3 ± 1.8*	44.5 ± 6.7*	110 ± 6.7
2.0	20.1 ± 1.9*	41.4 ± 4.3*	126 ± 7.4
5.0	10.5 ± 1.0*	31.3 ± 2.4*	
20	4.6 ± 1.5*	10.5 ± 1.2*	95.2 ± 6.6*
20	2.6 ± 1.1*	4.9 ± 2.4*	80.3 ± 5.7*
20			86.5 ± 11.3*
200	4.2 ± 1.5*	6.3 ± 1.7*	
200	3.5 ± 1.0*	6.2 ± 0.7*	53.8 ± 5.1*
200	0.9 ± 1.0*	1.2 ± 3.6*	19.1 ± 3.8*
800			30.0 ± 3.3*

* Significantly different from control values ($P < 0.05$).

Cell Culture. Bone marrow cells were flushed from femurs with 1.0 ml of Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.). A single cell suspension was made by passage through a 25-gauge needle; cells were counted on a hemocytometer. The bone marrow was cultured with alpha medium supplemented with 10% fetal calf serum (Grand Island Biological Co.), 10% horse serum (Flow Laboratories, Inc., Rockville, Md.) and 10% trypticase soy broth (Grand Island Biological Co.), and 10^{-4} M 2-mercaptoethanol in 1% methyl cellulose (10–12). Quadruple cultures containing 4×10^5 cells each were incubated at 37°C in an atmosphere of 100% relative humidity and 5.5% CO_2. Assays for CFU-C and BFU-E were carried out simultaneously on the same bone marrow sample. Cultures for BFU-E contained 5 IU of erythropoietin (Step III Connaught). Cultures for CFU-C contained 1/16 maximal concentration of CSA prepared from L-cell conditioned medium (13). After 8 days of incubation colonies containing 50 or more cells were scored with a dissecting microscope at a magnification of $\times$ 40. They were returned to the incubator and scored again at 14 days. Verification of erythroid bursts was determined with 0.2% tetramethyl benzidine (Sigma Chemical Co., St. Louis, Mo.) in 10% acetic acid and 0.12% H_2O_2 applied directly to cultures (14). Hemoglobin-containing colonies stained fully after 3 min, whereas peroxidase-positive granulocytic colonies stained after 10 min.

Dexamethasone (Sigma Chemical Co.) was dissolved in 100% ethanol and diluted $1/10^4$– $1/10^7$ with distilled water. The exact concentration was determined spectrophotometrically by measuring absorption at 250 nm and assuming $\epsilon = 1.4 \times 10^4$ $M^{-1}cm^{-1}$ (15). 50 μl of solution was added to each culture. As a control for the effects of ethanol, the same volume of $1/10^4$ dilution of ethanol was added to the plates not containing dexamethasone. Cortisone (Sigma Chemical Co.) was dissolved in 100% ethanol and diluted $1/10^3$–$1/10^5$ before addition of 50 μl to each culture. The final vol of all cultures was 1.15 ml.

In a separate experiment bone marrow was incubated in Hanks' balanced salt solution containing various concentrations of dexamethasone for 1 h before plating.

Statistical Analysis. Individual colony counts were normalized to the mean of control data from four replicate experiments. Differences between means were analyzed with Student's two-tailed t test.

Results

The results of individual and replicate studies of BFU-E and CFU-C sensitivity to dexamethasone revealed that the erythropoietic progenitors were considerably more sensitive than the granulopoietic (Table I). The overall pattern of the log dose-
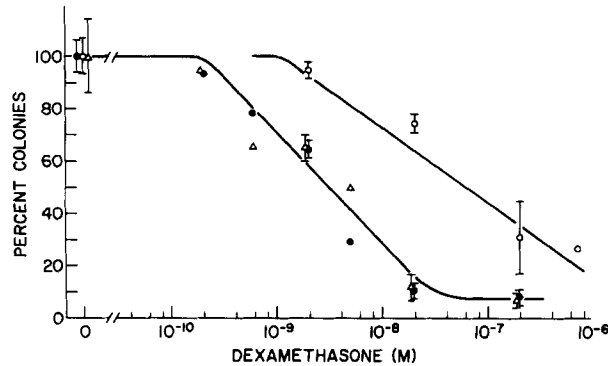
FIG. 1.   BFU-E and CFU-C as a function of dexamethasone concentration in the culture medium. (Percent colonies, mean of two to three experiments ± SE.) Differences between percent BFU-E and percent CFU-C were significant at all concentrations of dexamethasone ($P < 0.01$) except 0.2 nM. ●, BFU-E, 8 days; △, BFU-E, 14 days; ○, CFU-C, 8 days.
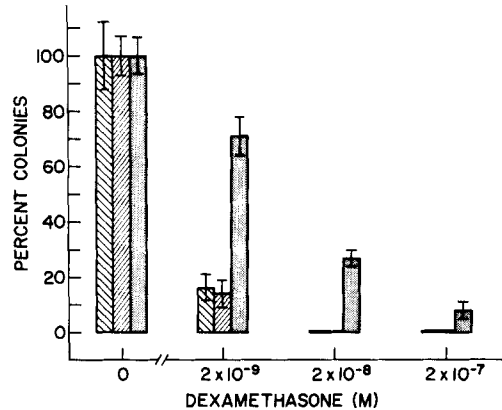

FIG. 2.   Effect of dexamethasone treatment before and during bone marrow culturing. (Percent colonies ± SE.) Differences between percent BFU-E and percent CFU-C were significant at 2 nM and 20 nM ($P < 0.0025$) and at 200 nM ($P < 0.01$). ▨, BFU-E, 8 days; ▧, BFU-E, 14 days; ▥, CFU-C, 8 days.

response relationship was sigmoidal, with an apparent threshold for BFU-E at $\approx 2 \times 10^{-10}$ M dexamethasone and a linear component (slope = -40, correlation coefficient = 0.95, $P < 0.01$) that became asymptotic at about 90% BFU-E inhibition with concentrations of dexamethasone above 2×10^{-8} M. As shown in Fig. 1, the dose-response relationship for CFU-C was similar. Although the linear component (slope = -30, correlation coefficient = 0.95, $P < 0.01$) was not significantly different from that found for BFU-E, the curve was shifted to the right by at least an order of magnitude along the dose axis, the dexamethasone concentration for 50% inhibition being 60×10^{-9} M for CFU-C compared to 3×10^{-9} M for BFU-E. The BFU-E response to dexamethasone was similar for the 8- and 14-day colony counts.

When bone marrow was treated with dexamethasone for 1 h before plating, with continued exposure to the glucocorticoid after plating, the inhibitory response was increased for both BFU-E and CFU-C, but their differential sensitivity was not affected (Fig. 2).

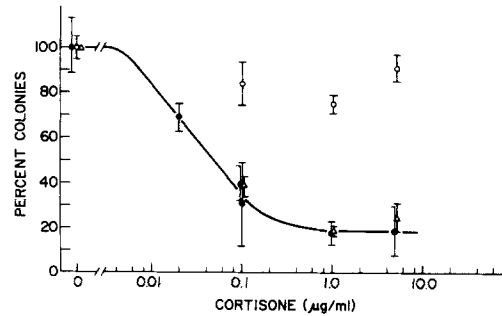The effect of cortisone was generally similar to that of dexamethasone (Fig. 3);

Fig. 3. BFU-E and CFU-C as a function of cortisone concentration in the culture medium. (Percent colonies ± SE.) Differences between percent BFU-E and percent CFU-C were significant at all concentrations of cortisone ($P < 0.0025$). ●, BFU-E, 8 days; △, BFU-E, 14 days; ○, CFU-C, 8 days.

BFU-E was inhibited 60% by 0.1 µg/ml. The contrast between the BFU-E and CFU-C responses to cortisone was even more striking than that seen with dexamethasone. There was little CFU-C inhibition with low doses of cortisone, the maximum being about 18% at 1 µg/ml.

Discussion

In many tissues, the catabolic action of glucocorticoids results in decreased synthesis and increased degradation of protein, RNA, and DNA. Glucocorticoids are also known to inhibit amino acid and glucose uptake. Although the inhibition of BFU-E and CFU-C activity described here may merely reflect the general catabolic effect of this class of hormones, the large difference in sensitivity between BFU-E and CFU-C indicates that the inhibition by glucocorticoids was specific rather than general. Although we observed less CFU-C inhibition at lower doses of glucocorticoids than reported previously (5), our systems were sufficiently different particularly in source and concentration of CSA to preclude direct comparison; it is known that CFU-C consist of subpopulations that respond selectively to CSA of diverse origin (16, 17). By using a fairly low CSA concentration, we may have selected for a sensitive population of CFU-C. This is perhaps a more realistic physiologic condition than that obtained with maximal stimulation at high CSA concentration. CFU-C that are more difficult to trigger and require a higher concentration of CSA may also be more prone to being turned off. The CFU-C response we observed with cortisone agrees closely with that seen by Metcalf (4) at a similar concentration.

The basis of the large differential glucocorticoid sensitivity of BFU-E and CFU-C is not known. It may reflect differences in the number of receptors available for interaction with glucocorticoids or differences in the consequences of their binding by glucocorticoids. Despite the relative insensitivity of CFU-C, their macrophage progeny are quite sensitive, the specific binding of glucocorticoids being correlated with decreased secretion of elastases and other enzymes (18). It is possible that differences in heterogeneity of the cell population may also be a factor. In contrast to CFU-C, which are presumed to have a broad age distribution, BFU-E are considered to constitute only very early progenitors and may be representative of a more homogeneous committed stem cell population.

BFU-E are thought to generate a pool of more differentiated erythroid progenitors known as CFU-E; dexamethasone has been reported to increase the number of CFU-

E in adult mouse bone marrow by 36 ± 24% (7). Because of the considerable standard error, this increase is of questionable significance. In several experiments, we observed a similar but statistically insignificant increase in CFU-E with dexamethasone treatment. If an increase in CFU-E indeed occurs, the effect of glucocorticoids on the late erythroid precursor pool would tend to counteract the effect on the early erythroid pool. Differential control of precursor pools in series would provide an efficient mechanism for dampening the effect of glucocorticoids and perhaps for fine tuning of the size of the erythron.

Our findings suggest that the size of the granulocyte-macrophage progenitor pool is relatively unaffected by physiologic concentrations and fluctuations of glucocorticoids. Because of the rapid turnover of the vital granulocytic elements in peripheral blood, this is of more than passing interest. On the other hand, whatever the reason behind the effect, transient changes in proliferation of committed erythroid stem cells due to physiologic fluctuations of glucocorticoid concentration would have a negligible impact because of the much slower turnover of circulating erythrocytes.

Summary

The sensitivity of erythropoietic (BFU-E) and granulopoietic (CFU-C) progenitor cells to dexamethasone and cortisone was studied in cultures of mouse bone marrow. Although the log dose-response relationships had a similar form, the BFU-E were much more sensitive than the CFU-C to either glucocorticoid. The dexamethasone concentration for 50% inhibition was 3×10^{-9} M for BFU-E and 60×10^{-9} M for CFU-C. The differential sensitivity to cortisone was even greater, with 60% inhibition of BFU-E and 18% inhibition of CFU-C at 0.1 μg/ml. These findings suggest a specific rather than a general response to glucocorticoids and indicate that granulocyte-macrophage progenitors are less affected than early erythroid progenitors by physiologic concentrations of these hormones.

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