Hormonal Effects on Cell Proliferation in a Human Erythroleukemia Cell Line (K562)

By Charlotte Gauwerky and David W. Golde

We have investigated the hormonal responsiveness of K562 cells using a serum-substituted in vitro clonogenic assay. Dexamethasone inhibited colony formation by the K562 cells, and the inhibitory effect could be reversed by progesterone (10^{-9} M). Fluoxymesterone caused a prominent enhancement of K562 colony growth, whereas estriol had no effect. Stimulation by triiodothyronine was maximal at 10^{-7} M, and the thyroid effect could be abrogated by the beta-adrenergic antagonist butoxamine in equimolar concentrations. Using standard tissue culture conditions, the beta-adrenergic agonist isoproterenol, but not the alpha-catecholamine phenylephrine, enhanced the proliferation of K562 cells. When K562 cells were grown under hormone-depleted conditions, they developed responsiveness to phenylephrine and were no longer stimulated by isoproterenol. DBCAMP and prostaglandins of the E series also caused K562 colony enhancement. Prostaglandin F_{2alpha} had no effect on cell proliferation. Insulin was an effective stimulant of colony formation of K562 cells, as were human growth hormone and ovine prolactin. Bovine growth hormone had no effect. Our results are consistent with the identification of K562 as an erythroid line, and they indicate that K562 cells respond to endocrine hormones in a manner analogous to normal erythroid progenitors.

It is now known that various endocrine hormones have prominent effects on erythropoiesis in vitro and in vivo. Some of the in vivo effects of the endocrine hormones are mediated by the modulation of erythropoietin elaboration, but others are due to direct effects on erythroid precursor cells. The hormonal responsiveness of neoplastic human erythroid cells has been incompletely studied, although there is some information available concerning erythropoietin and glucocorticosteroid responsiveness. The Friend virus-infected murine erythroleukemia cell line has provided an excellent tool for examining hormonal interactions with neoplastic mouse erythroid cells. Until recently, however, an equivalent human erythroleukemia line has not been available.

The erythroid nature of most strains of the K562 human leukemic cell line has been convincingly demonstrated. This cell line was originally established by Lozzio and Lozzio from pleural fluid cells of a patient with CML in blast crisis. These cells have the specific erythroid surface protein glycophorin (A), and they can be induced to differentiate to the normoblast level and to synthesize hemoglobin.

We have used a serum-substituted in vitro clonogenic assay to define the effects of certain endocrine hormones on K562 cell proliferation.

MATERIALS AND METHODS

K562 cells were a gift from Dr. Ronald Billing at UCLA. The cells were maintained in continuous suspension cultures with alpha medium, 20% fetal calf serum and antibiotics. The karyotype of the cells was identical to that originally reported by Lozzio. The erythroleukemia cells were grown in T-75 flasks and fed with fresh medium 1-3 days before plating in Petri dishes. We used the methylcellulose culture system for cloning K562 cells, as previously reported, for Friend erythroleukemia cells. The cells were cloned in 0.8% methylcellulose with alpha medium, 30% fetal calf serum, 10^{-4} M thioglycerol, and antibiotics. In most studies we used a serum-substituted technique to avoid problems with endogenous hormone, hormone binding by serum proteins, and effects of other growth factors. In this system, the fetal calf serum was replaced by 0.5% bovine serum albumin (not delipidated). The cells were added without washing, however, so that the culture dish ultimately contained about 1% serum. The concentration of endogenous hormones present in the final culture medium was at least an order of magnitude below effective growth-promoting levels. Each culture plate was calculated to contain approximately 1.3 ng T_{3}, 0.02 ng T_{4}, 0.115 ng rT_{3}, and 0.005 ng insulin. Bovine growth hormone did not stimulate human red cell precursor or K562 cell proliferation. Together with various hormones, 2 x 10^5 cells were plated per dish in the serum-containing and in the serum-substituted system. Duplicate plates were incubated in a humidified atmosphere at 37°C with 5% CO_{2} in air. After 4-5 days of incubation, clusters of 7 or more cells were counted using an inverted microscope. On the fourth day the mean cloning efficiency in the serum-containing system was 34% and in the serum-free system, 25%. Replicate plates varied by less than 4%.

The following hormones were studied: dexamethasone, progesterone, fluoxymesterone, and estriol were obtained from Sigma Chemicals, St. Louis, Mo. Prostaglandin E_{1} (PGE_{1}), PGE_{2}, and PGF_{2alpha} were provided by Dr. J. Pike (Upjohn Co., Kalamazoo, Mich.). Stock solutions were prepared in 100% ethanol and further diluted with alpha medium to the appropriate concentrations. Triiodothyronine (L-T_{3}) (a gift from Dr. I. J. Chopra, Los Angeles), highly purified human growth hormone (HGH), and ovine prolactin (both gifts from Dr. C. H. Li, San Francisco) were solubilized in 0.1 N NaOH and further diluted with phosphate-buffered saline (pH 9.3). The stock solution of porcine insulin (a gift from Dr. C. H. Li) was prepared in 1 N HCl and further diluted in phosphate-buffered saline.

Dibutylryl cAMP, L-isoproterenol, and phenylephrine (all from Sigma Chemicals) were directly dissolved in alpha medium without

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fetal calf serum. Butoxamine (Sigma Chemicals) was solubilized in 100% ethanol and further diluted with alpha medium. For each hormone, control cultures were prepared containing equivalent concentrations of the diluent material.

To investigate the influence of the thyroid state on the response of K562 colony formation to α- and β-adrenergic agonists, K562 cells were grown for 48 hr in alpha medium containing 20% charcoal-absorbed human serum prepared by the method of Larsen. This treatment removes 99% of the thyroid hormone in serum. After preincubation, the response of these hormone-deficient cells to phenylephrine and isoproterenol was tested.

RESULTS

The basic strategy of the studies designed to determine the hormonal responsiveness of K562 cells was to incubate the cells in suspension culture for 48 hr in normal or hormone-depleted serum. The cells were then transferred into methylcellulose cultures containing the hormones to be tested and incubated for 4–5 days, after which K562 colonies were enumerated.

Steroid Hormones

Under the described conditions, dexamethasone was added to the cultures of K562 cells in concentrations from $10^{-11}$ to $10^{-6}$ M using the serum-containing and the serum-substituted system. As shown in Fig. 1, dexamethasone consistently inhibited K562 colony formation at concentrations from $10^{-10}$ to $10^{-8}$ M. The inhibitory effect of dexamethasone was much more prominent in the serum-containing system, and a 50% reduction in colony formation was observed at $10^{-6}$ M. This observation suggests that other serum factors interacted with dexamethasone in inhibiting colony formation. Progesterone was able to reverse the dexamethasone-induced inhibition when added at $10^{-6}$ M concentration to the cultures together with dexamethasone. All further experiments were performed only in the serum-substituted system to avoid interactions from factors in the fetal calf serum.

In order to study the influence of sex hormones, we tested the synthetic testosterone derivative fluoxymesterone at concentrations from $10^{-9}$ to $10^{-3}$ M. In contrast to 17β estriol ($10^{-9}$ to $10^{-4}$ M), which did not influence K562 colony formation, fluoxymesterone was found to be a potent stimulant for K562 colony growth (Fig. 2). With fluoxymesterone, peak stimulation of 156% of control occurred at $10^{-5}$ M.

L-T₃, Adrenergic Agents, and cAMP Inducers

Triiodothyronine was used as the prototype thyroid hormone to investigate the growth-promoting effect on K562 cells. As demonstrated in Fig. 3, L-T₃ was a potent stimulant of colony formation. It was maximally effective at a concentration of $10^{-7}$ M, where peak activity occurred with 145% of control. L-T₃ was found to enhance K562 colony growth only over a very limited range of concentrations, between $10^{-8}$ and $10^{-6}$ M.

To study the interrelationship between L-T₃-induced colony enhancement of K562 and β₂-adrenergic receptors, the β₂-adrenergic antagonist butoxamine ($10^{-9}$–$10^{-5}$ M) was added, together with $10^{-7}$ M L-T₃, to the culture plates. Figure 4 shows that butoxamine abrogated the L-T₃-stimulated colony enhancement completely at equimolar concentrations.
Fig. 3. Effect of L-T$_3$ (Δ-Δ) and l-isoproterenol (○-○) on colony formation by K562 cells. Data derived from 4 experiments performed in duplicate (mean ± SEM).

Thus, a $\beta_2$-mediated mechanism can be inferred for L-T$_3$-induced colony enhancement of K562.

To compare the effects of $\alpha$ and $\beta$ catecholamines on K562 colony formation, phenylephrine was used for $\alpha$-adrenergic and isoproterenol for $\beta_1$$\beta_2$-adrenergic specificity. Both substances were tested at concentrations from $10^{-9}$ to $10^{-5}$ M. Isoproterenol was found to potentiate K562 colony formation with maximal activity of 140% of control at $10^{-7}$ M; however, phenylephrine was ineffective at all concentrations tested (Figs. 3 and 5).

To determine whether alterations of the endocrine state could influence the response to the specificity of the catecholamine, K562 cells were made hormone deficient by growth in alpha medium containing 20% charcoal-absorbed human serum. After 48-hr preincubation, the reaction of K562 cells with isoproterenol and phenylephrine was again determined using serum-substituted tissue culture conditions. As shown in Fig. 5, the $\beta$ agonist isoproterenol was no longer capable of enhancing K562 colony formation. However, K562 responded now to the $\alpha$ agonist phenylephrine, which was tested at concentrations from $10^{-9}$ to $10^{-5}$ M. Peak activity occurred at $10^{-7}$ M with 140% stimulation above control. This response was identical to the stimulation induced by isoproterenol under normal culture conditions. K562 cells grown in charcoal-treated serum were also tested for response to L-T$_3$. Colony formation was maximally enhanced by L-T$_3$, at $10^{-7}$ M as observed under usual tissue culture conditions (data not shown).

Since adrenergic receptor activity is linked to the adenylate-cyclase cAMP system, we looked at the effect of dbcAMP and various prostaglandins known to be associated with cellular cyclic nucleotide activity. All compounds were added to the culture plates at $10^{-9}$ to $10^{-5}$ M. The results are illustrated in Fig. 6. Dibutyryl cAMP was found to enhance K562 colony formation with optimal effect of 136% of control at $10^{-7}$ M. Prostaglandins of the E series (E$_1$ and E$_2$) also potentiated the clonal growth of K562 (120%–130% of control at $10^{-7}$ M). Prostaglandin F$_{2\alpha}$ had no effect on colony formation over a range of concentrations from $10^{-9}$ to $10^{-5}$ M.

**Insulin and Other Polypeptide Hormones**

Polypeptide hormones known to influence normal erythroid colony growth were tested for their effect on K562 colony formation under serum-substituted culture conditions. Highly purified insulin was tested at concentrations between 0.1 and 10 ng/ml. As shown
in Table 1, a clear stimulatory effect of insulin on K562 colony growth was seen at 0.1 ng/ml. Maximal potentiation was detected at a physiologic level of insulin (1 ng/ml), with peak activity of 150% of control. Insulin concentrations above 1 ng/ml caused a rapid decrease in potentiation of K562 colony formation. Table 1 also shows that human growth hormone and ovine prolactin were able to stimulate the clonal growth of K562 cells maximally at 100 ng/ml. Bovine growth hormone was inactive (data not shown).

Table 2 summarizes the identified effects of various hormones on colony formation of normal granulocytic and erythroid precursor cells, Friend erythroleukemia cells, and human erythroleukemia cells (K562). The table is derived from our own data and those of other investigators.

Table 1. Effects of Polypeptide Hormones on K562 Colony Formation

<table>
<thead>
<tr>
<th>Polypeptide Hormone</th>
<th>Concentration (ng/ml)</th>
<th>K562 Colonies (as Control ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>0.1</td>
<td>114 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>147 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>104 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>102 ± 1.1</td>
</tr>
<tr>
<td>Human growth hormone</td>
<td>25</td>
<td>122 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>130 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>159 ± 2</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>134 ± 1</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>25</td>
<td>108 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>116 ± 2</td>
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<td>130 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
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*Data derived from 3 experiments performed in duplicate (mean ± SEM).

DISCUSSION

Endocrine hormones and hormonal mediators are known to have important influences on erythropoiesis. Compounds prominently affecting erythroid progenitors include steroids of various classes,23 thyroid hormones,24 cyclic nucleotides,25 prostaglandins of the E family,26 catecholamines,2728 and certain polypeptide hormones.29 We studied the hormonal modulation of human erythroleukemia cells using the only available human erythroleukemic cell line, K562. This cell line is composed of erythroleukemic cells that have been characterized in detail.1521 Using a short-term clonogenic assay we observed colonies consisting of 7 or more cells that could evolve from cells at a maturation level analogous to proerythroblasts. The addition of a stimulating factor for colony formation was not necessary in our culture system, although it is reported that K562 cells respond to high concentrations of erythropoietin.21

We used dexamethasone as a prototype glucocorticosteroid and found that dexamethasone inhibited K562 colony growth in a manner similar to our previous observations with Friend leukemia cells.14 The inhibitory effect could be abrogated by appropriate concentrations of progesterone, suggesting that dexamethasone acted through the usual glucocorticoid receptor mechanism. These results differ from our earlier studies on fresh neoplastic erythroid cells in man, where dexamethasone potentiated human erythroid colony formation from bone marrow of patients with polycythemia vera, preleukemia, and erythroleukemia.910 Likewise, the reported effects of dexamethasone on normal erythroid colony growth in man and in animals are contradictory. We and others observed that dexamethasone stimulated erythropoiesis,63031 while several investigators have reported that
dexamethasone inhibited normal murine erythroid colony formation. The reason for this discrepancy is not immediately obvious, although steroid effects on an interacting cell population may be important. Dexamethasone blocks differentiation in Friend cells and has a modest inhibitory effect on Friend cell proliferation. In regard to other steroid hormones, we observed that K562 colony growth was prominently stimulated by fluoxymesterone. The concentration of fluoxymesterone showing maximal activity was $10^{-6} \ M$, similar to that described for normal erythroid precursors.

The effect of various thyroid hormones on normal erythroid colony formation has been intensively investigated. The K562 cells responded to L-T$_3$ in a manner analogous to normal erythroid progenitors, and the thyroid effect was associated with a functioning $\beta$ receptor mechanism. Our results also confirm that the response of cells to adrenergic agents is regulated by the thyroid state. Corresponding to the findings of Popovic and coworkers that erythroid colonies (CFU-E) grown from hypothyroid dog bone marrow were no longer potentiated by isoproterenol, we were able to simulate the hypothyroid state in vitro by growing K562 cells in hormone-depleted serum. Under these conditions, the K562 cells became responsive to the $\alpha$-adrenergic agent phenylephrine and lost responsiveness to $\beta$-adrenergic catecholamines, suggesting that $\beta$-adrenergic receptor activity is dependent on normal hormonal conditions. Although charcoal treatment of serum causes depletion of various hormones, it is most likely that for the experiments reported thyroid hormones are relevant variables.

The K562 cells are stimulated by prostaglandins of the E series and dbcAMP, as are normal erythroid precursors. In contrast, dbcAMP and PGE$_2$ are inhibitory for normal and granulocyte-monocyte colony-forming cells. K562 cells responded to insulin in a manner similar to normal erythroid precursors and Friend erythroleukemia cells, which showed peak stimulation at physiological concentration. Ovine prolactin also had a stimulatory effect on K562 cells and on Friend cell colony formation. We previously showed that growth hormone stimulated erythropoiesis in vitro in a species-specific manner, but had no effect on granulopoiesis. K562 colony growth was also promoted by human growth hormone, but bovine growth hormone was inactive. The Friend cells were stimulated by both human and bovine growth hormone.

The K562 human erythroleukemia cell line is an excellent model system for studying hormonal interactions with neoplastic human red cell precursors. The studies reported here show that the K562 cells respond to endocrine hormones and their mediators in a manner analogous to other erythroid progenitors.

REFERENCES

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