Glucocorticoid Sensitivity and Receptors in Cells of Human Myelogenous Leukemia Lines

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ABSTRACT

The effect of dexamethasone on the clonal proliferation of human acute myelogenous leukemia cells was studied using the two available human acute myelogenous leukemia lines KG-1 and HL-60. The cells were cloned in agar in the presence of colony-stimulating activity with and without dexamethasone. Colony formation by KG-1 cells was inhibited by dexamethasone at a concentration of $10^{-7}$ M, and a 50% reduction occurred at $10^{-8}$ M dexamethasone. Dexamethasone had no effect on HL-60 colony formation. Direct assessment of dexamethasone binding showed that both KG-1 and HL-60 cells have high-affinity glucocorticoid receptors with a mean 10,300 and 16,600 binding sites/cell with a $K_d$ of $3.3 \times 10^{-9}$ M and $4.2 \times 10^{-9}$ M ($r \geq -0.95$), respectively. Binding competition studies confirmed the specificity of these glucocorticoid receptors. No correlation was noted between the number of glucocorticoid receptors and the $in vitro$ inhibition of colony formation. A variety of other steroids was tested and found to have no effect on clonal proliferation of the leukemic cells.

INTRODUCTION

Glucocorticoid hormones have profound effects on mammalian hematopoietic cells (13, 14, 16, 27, 30). These steroids usually enhance $in vitro$ proliferation of red cell precursors and decrease proliferation of the granulocyte-monocyte precursor cells (16, 27, 30) and normal and malignant lymphocytes (6, 8). The effect of glucocorticoids on human acute myelogenous leukemia cell proliferation is unclear (11, 18, 24). We used the 2 available human acute myelogenous leukemia cell lines (KG-1 and HL-60) to study the effects of various steroid hormones on myeloid leukemia proliferation and to define their glucocorticoid receptor-binding characteristics.

MATERIALS AND METHODS

Recently, 2 human acute myelogenous leukemia cell lines KG-1 and HL-60 have been developed (9, 20). Both lines grow in suspension and have been maintained in liquid culture for over 2 years. The KG-1 cell line was established from a patient with erythroleukemia evolving into acute myelogenous leukemia (20). Morphologically, these cultured cells are predominantly myeloblasts and promyelocytes. Over 85% of the KG-1 cells are AS-D-chloroacetate esterase positive, and the line retains the same karyotypic abnormality that was present in the fresh leukemia cells of the patient. The HL-60 cell line was derived from a patient with promyelocytic leukemia. These cells are predominantly at the promyelocyte stage of development and stain positive for peroxidase and AS-D-chloroacetate esterase. The cells retain the karyotype of the fresh leukemic cells and are able to produce tumors in nude mice. The KG-1 and HL-60 cells form myeloid colonies in soft-gel culture and are not infected with Epstein-Barr virus.

The cell lines were maintained in T-flasks (Lux Scientific Corp.) with a medium (Flow Laboratories), 20% fetal calf serum (Grand Island Biological Co.), and penicillin and streptomycin (Grand Island Biological Co.). The cloning studies were performed with cells in the logarithmic phase of growth (2 to 4 days after reseeding in fresh media). Five thousand KG-1 cells and 2000 HL-60 cells were plated in 0.3% agar-containing CSA as previously described (20). The CSA source was conditioned media from a line of T-lymphocytes established from a patient with hairy-cell leukemia (15). The same lot of fetal calf serum and conditioned medium was used for all experiments. The KG-1 cells have a marked response to CSA. In the presence of optimal CSA concentrations, the cloning efficiency was 3%, representing a 32-fold increase in colony number over plates not containing CSA. The HL-60 cells had a cloning efficiency of 7.2% in cultures without CSA, and there was a 2.2-fold increase in colony number when optimal concentrations of CSA were added. The CSA dose-response curves for both KG-1 and HL-60 cells consistently showed maximal colony formation with 5% conditioned medium in the plate. A suboptimal CSA concentration (1%) was used for the steroid studies.

Dexamethasone and other steroids were added directly to the culture dishes. Four to 5 replicate culture plates were incubated in a humidified atmosphere of 5% CO$_2$ in air for 14 days. Colonies with a minimum of 50 cells were enumerated with an inverted microscope. The steroids, dexamethasone, progesterone, 11-deoxy cortisolone, 17$\beta$-estradiol, testosterone propionate, fluoxymesterone (fluoxy 11$\beta$, 17$\beta$-dihydroxy-9a-fluoro-17a-methyl-4-androstene-3-one), 5$\alpha$-dihydrotestosterone, 5$\beta$-dihydrotestosterone, androsterone, and etiocholanolone (Sigma Chemical Co., St. Louis, Mo.) were dissolved in ethanol and diluted to appropriate concentrations with tissue culture medium. Control cultures contained equivalent concentrations of ethanol. Each drug was tested in 3 or more experiments.

Studies of $[^{3}H]$dexamethasone binding, competition studies, and Scatchard analysis were performed with whole cells as previously described (29, 34). The binding studies were done...
on the cells during their logarithmic growth phase (2 to 4 days after reseeding in liquid culture).

RESULTS

The effect of dexamethasone on KG-1 and HL-60 colony formation is shown in Chart 1. Dexamethasone inhibited KG-1 colony formation at concentrations equal to $10^{-7}$ M ($p = 0.01$; 2-tailed t test) and reduced clonal growth by 50% at $10^{-5}$ M. The HL-60 cells were resistant to dexamethasone. Dexamethasone ($10^{-5}$ M to $10^{-9}$ M) had no effect on HL-60 clonal proliferation with or without CSA added to the plates. Ethanol in concentrations equivalent to dilutions of $10^{-6}$ M dexamethasone had no detectable effect on colony formation. When the KG-1 cells were exposed to $10^{-6}$ M dexamethasone for 1, 8, or 24 hr, washed twice, and plated, no colony inhibition was observed (102 ± 3, 95 ± 6, and 97 ± 4% of control ± S.E., respectively).

A number of steroids ($10^{-5}$ to $10^{-11}$ M) were tested for their effect upon KG-1 and HL-60 colony formation in soft-gel culture. Progesterone, 11-deoxycorticosterone, testosterone propionate, fluoxymesterone, and testosterone metabolites (5-$\alpha$-dihydrotestosterone, 5-$\beta$-dihydrotestosterone, androsterone, and etiocholanolone) had no discernible effect on colony formation.

Studies of dexamethasone receptor binding were performed on the KG-1 and HL-60 cells. Both leukemia cell lines had high-affinity glucocorticoid receptors. KG-1 and HL-60 showed saturable binding of [3H]dexamethasone with a mean of 10,300 and 15,600 binding sites/cell, respectively. The Scatchard plots of the data gave straight lines ($r = -0.95$; Chart 2), suggesting that a single high-affinity class of cytoplasmic receptors was involved in binding of tritiated dexamethasone.

Specificity of steroid receptor binding was investigated by testing the ability of various unlabeled steroids to compete with [3H]dexamethasone for glucocorticoid binding sites (Chart 3). Biologically active glucocorticoids were able to compete effectively. Unlabeled dexamethasone and hydrocortisone at a 5- and 10-fold molar excess, respectively, competitively blocked [3H]dexamethasone binding. Hydrocortisone competed more effectively than did cortisone for binding sites, which is consistent with the observation that reduction of the 11-keto group is necessary for biological activity. The leukemia cell steroid receptors had little affinity for compounds without glucocorticoid activity such as glucocorticoid metabolites (tetrahydrocortisone), inactive stereoisomers (11-$\alpha$-hydroxycortisone), or other steroids (5-$\alpha$-dihydrotestosterone and estradiol). Progesterone and 11-deoxycortisone, competitive noninducers, were moderately effective competitors for glucocorticoid binding.

DISCUSSION

While prednisone is dramatically effective in the treatment of acute lymphocytic leukemia, glucocorticoids appear to have only marginal activity in human acute myelogenous leukemia although a few patients may improve with large doses (7, 31, 32).

The present studies show that the pure acute myelogenous leukemia cell lines KG-1 and HL-60 have high-affinity glucocorticoid receptors and that the number of receptors/cell was comparable to that found in acute lymphocytic leukemia cells. These receptors were found to have specificity for biologically active glucocorticoids. The receptors competitively bound dexamethasone, hydrocortisone, progesterone and to some extent 11-deoxycortisone, and cortisone, but they did not bind the biologically inactive glucocorticoids tetrahydrocortisone, 11-$\alpha$-hydroxycortisone, or other steroids (estradiol and $\alpha$-dihydrotestosterone). There was a discrepancy between the in vitro biological effect of dexamethasone on KG-1 and HL-60 and the glucocorticoid receptor content of the cells. The HL-60 cells had slightly increased binding affinity and more steroid receptors than did the KG-1 myelogenous leukemia cell population. Nevertheless, the KG-1 cells were more sensitive than were HL-60 cells to the lytic or static effects of the hormone in vitro.

Many of the biological effects of steroid hormones are triggered by the interaction of the steroid with high-affinity receptors (4, 5, 12, 17, 19). The hormone-receptor complex enters the nucleus, binds to sites on chromatin, and induces specific gene transcription. Steroids cause growth inhibition and death of certain cells. However, the precise mechanism of glucocorticoid inhibition of cell growth is not known.

Studies with mutant S49 mouse lymphoma cells show that the high-affinity steroid hormone receptors clearly play a role in glucocorticoid hormone-induced cell death (1, 33). Also, estrogen receptor analysis is critical in predicting response to endocrine therapy in human breast cancer. Those tumors lacking estrogen receptors do not respond to endocrine treatment. Patients whose tumors contain estrogen receptors have a 50 to 70% response to endocrine therapy (26). Several investigators have shown that the biological sensitivity of acute lymphocytic leukemia cells to glucocorticoids in vitro and in vivo is related to their steroid receptor content (22, 25). However, human lymphoblast cell lines that possess high-affinity glucocorticoid receptors have been isolated, but they are resistant to the cytotoxic effects of glucocorticoids (23).

Previous investigations have shown that glucocorticoids inhibit the normal murine marrow myeloid colony-forming unit in vitro (27, 28, 30). Golde et al. (14) found that $10^{-6}$ M dexamethasone caused a 55% inhibition of the mouse bone marrow

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myeloid colony-forming unit. Progesterone, a competitive steroid noninducer, abrogated the dexamethasone effect, suggesting that the glucocorticoid inhibition of colony formation was mediated by a glucocorticoid receptor mechanism.

The relationship between glucocorticoid response and receptor content in human myelogenous leukemia cells is not clear. One study found that glucocorticoid-binding receptors were present in 3 of 16 acute myelogenous leukemia patients (24). Leukemia cells from the 3 patients whose cells were receptor positive showed decreased DNA, RNA, and protein synthesis in the presence of dexamethasone. The cells from the receptor-negative leukemia patients had no in vitro response to the glucocorticoid. Another study found that cells from 17 of 18 patients with acute myelogenous leukemia had the same number of steroid-binding sites as did acute lymphocytic leukemic cells (11). The lymphocytic leukemia cells, however, were more sensitive to the biological effects of dexamethasone. Krystosek and Sachs (21) studied steroid receptors in clones of murine myelogenous leukemia cells that differed in ability to mature in the presence of dexamethasone. Those clones that did not mature after steroid exposure had the same number of cytoplasmic and nuclear glucocorticoid receptor sites as did those dexamethasone-induced differentiated clones.

Glucocorticoid resistance can occur in cells that have high-affinity cytoplasmic receptors. In vitro studies have shown that there can be defects in transfer of the receptor-steroid complex to the nucleus, defects in correct nuclear localization, or abnormalities in reactions subsequent to nuclear localization of the complex (33). Some effects of steroids on cells may be independent of the interaction of the steroid with its cytoplasmic receptors (2, 3). For example, Breslow et al. (3) have shown that human fibroblasts derived from normal individuals and from patients with testicular feminization syndrome are killed to the same degree by the steroid dihydrotestosterone even though the fibroblasts from the testicular feminization patients did not contain high-affinity androgen receptors.

In the present study using the 2 available acute myelogenous leukemia lines, the ability of a potent glucocorticoid to inhibit myeloid leukemia cell proliferation in vitro was not related to the number of high-affinity glucocorticoid receptors present in the cells. It is possible that the HL-60 cell resistance to steroid cytotoxicity is due to an abnormality of receptor-steroid complex activity subsequent to receptor binding. In a recent study, however, it has been shown that the expression of Fc receptors by HL-60 cells can be half-maximally inhibited by $10^{-8}$ M dexamethasone (10). This effect observed at a low hormone concentration suggests a process mediated by glucocorticoid receptors. Another possible explanation of HL-60 steroid resistance with respect to clonal growth is that the HL-60 population is heterogeneous and that the clonal cells which proliferate in soft-gel culture have fewer specific glucocorticoid-binding sites. Finally, it is possible that the KG-1 glucocorticoid sensitivity to cytotoxicity is mediated by a mechanism unrelated to the glucocorticoid receptors in human acute myelogenous leukemia cells.

ACKNOWLEDGMENTS

The authors wish to thank Leslie Lowe for her excellent technical assistance and Dr. Steven Collins and Dr. Robert Gallo for generously providing the HL-60 cells.

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