Glucocorticoid Receptors and *in Vitro* Responses to Glucocorticoid in Acute Nonlymphocytic Leukemia

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Abstract

Early clinical studies in which glucocorticoids were used alone in the treatment of acute nonlymphocytic leukemia (ANLL) reported a wide range of responses from remission in some patients to dramatic aggravation of the disease in others. In the hopes of identifying those patients likely to derive therapeutic benefit from glucocorticoids, we have studied glucocorticoid receptors and *in vitro* responses to glucocorticoids in 36 previously untreated adults with ANLL. The leukemic blasts of all patients contained glucocorticoid receptors (range, 4,300 to 28,400 total receptor sites per cell; median, 8,800). These receptors were similar in all respects studied to those from a variety of other normal and malignant tissues. There was little difference between receptor levels among the various French-American-British categories. *In vitro* responses to glucocorticoid were observed in leukemic blasts of 26 of 28 cases studied. These responses varied from near complete cell killing to stimulation of proliferation. Since the cells of patients with ANLL have about the same number of receptors as do cells from patients with acute lymphoblastic leukemia and these receptors are capable of mediating physiological responses in *vivo*, it is unlikely that qualitative or quantitative receptor defects underlie the relative resistance to glucocorticoid therapy of ANLL compared to acute lymphoblastic leukemia. However, the broad range of *in vitro* responses and receptor levels suggests that these studies might be useful in identifying those patients with ANLL likely to derive benefit from steroid therapy.

Introduction

Shortly after steroids began to be used in the treatment of acute leukemia, it was noted that patients whose disease was classified morphologically as ALL responded to glucocorticoid therapy considerably better than did those patients classified as acute myelocytic leukemia or ANLL (9, 15, 17, 21). However, among the patients with ANLL were a small number, possibly 10 to 15%, who achieved remission on steroids alone (9, 15, 17). Furthermore, several reports indicated that some patients with ANLL showed rapid and dramatic progression of their disease when given steroids alone (14, 21). The overall poor response of patients with ANLL to glucocorticoids and the occasional patients who appear to be harmed by steroid administration have led many physicians to be reluctant to use steroids in the treatment of this disease.

A large body of data indicates that the cytotoxic effects of glucocorticoids are mediated through the glucocorticoid receptor (for review, see Refs. 1 and 19). *In vitro*, the cytotoxic effects of glucocorticoids occur at concentrations of hormone which partly saturate the receptor. Furthermore, the steroid specificity of the cytotoxic response is similar to the steroid specificity of the receptor. Lastly, cell lines which have been selected for their resistance to glucocorticoid by growth in sublethal concentrations of hormone have either absent or defective glucocorticoid receptors in the vast majority of cases (2, 6, 13, 22). These data suggest that glucocorticoid receptor assays might be useful in identifying patients likely to have a beneficial response to glucocorticoids. Preliminary studies in ALL (5) and non-Hodgkin’s lymphoma (4) indicate that a correlation exists between favorable clinical responses and high levels of glucocorticoid receptors.

Since several chemotherapy protocols presently used in the treatment of ANLL include glucocorticoids, we have studied glucocorticoid receptors and *in vitro* responses to better understand how steroid hormones might be producing their therapeutic effects in ANLL.

Patients and Methods

**Patients.** Neoplastic tissue from 36 patients with previously untreated ANLL were studied. Cells were obtained from the peripheral blood in 16 patients, the bone marrow in 12 patients, and both the peripheral blood and bone marrow in 8 patients. In 5 cases, tissue was studied on more than one occasion. The diagnoses were established by using cytological and cytochemical methods (8, 16) in the absence of any knowledge of the results of either *in vitro* tests or treatment. Each case of ANLL was classified using the FAB classification (3).

**Preparation and Handling of Cell Suspensions.** Samples were drawn from blood or bone marrow and diluted at least 1:5 in Roswell Park Memorial Institute Medium 1640 with penicillin (50 μg/ml), gentamicin (50 μg/ml), glutamine (300 μg/ml), 25 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, and 100 units heparin per ml. Within 24 hr, the leukemic blasts were separated by density gradient centrifugation over Ficoll-Hypaque (7), washed twice in 50 ml of medium at room temperature by centrifugation at 200 × g for 10 min, and resuspended in 50 ml of medium at room temperature. To permit dissociation of endogenous steroid, the cells were incubated at 37°C for 30 to 60 min before being studied. In all...
cases, Wright-stained cytocentrifuge (Shandon Southern Instruments, Sewickley, Pa.) preparations were studied and cases were excluded if there were less than 75% blasts in the final cell suspension.

**Measurement of Glucocorticoid Receptor Sites.** The method used to measure nuclear glucocorticoid receptors has been previously described in detail (12). Briefly, a cell suspension containing approximately $1 \times 10^8$ cells/ml in Roswell Park Memorial Institute Medium 1640 was incubated with 10 different concentrations of $[6,7-^3H]$dexamethasone, hereafter called $[^3H]$dexamethasone, specific activity about 35 Ci/mmol; New England Nuclear, Boston, Mass.) from 0.5 to 100 nM. After a 30-min incubation at 37° with gentle shaking, the cells were broken by a 1:60 dilution in 1.5 mM MgCl$_2$ at 3°, and the radioactivity bound to the resulting nuclear pellet was determined. Nonsaturable binding was assayed by a parallel incubation of cell suspension with 1 μM $[^3H]$dexamethasone. The number and affinity of binding sites were estimated by Scatchard analysis (20).

This method was used to measure nuclear receptors in cells of 43 normal and leukemic individuals. Since the results were found to agree closely with the numbers of molecules saturably bound per cell after incubation with a single near-saturating concentration of $[^3H]$dexamethasone (40 nM), with and without 2 μM unlabeled dexamethasone, this latter method was used in the cells of 26 patients.

The method used to measure cytoplasmic glucocorticoid receptor sites has also been described in detail (12). Briefly, cells were incubated with 40 nM $[^3H]$dexamethasone, with and without 2 μM unlabeled dexamethasone for either 30 min at 37°, or 120 min at 3°, depending on the purpose of the assay. Cells were then broken by a 1:6 dilution in dextran-coated charcoal suspended in 1.5 mM MgCl$_2$ at 3°, and the radioactivity present in the supernatant was determined. The dextran-coated charcoal adsorbs free steroid, while leaving the bound steroid in the supernatant. To correct for nonsaturable binding, the cpm bound in the presence of 2 μM dexamethasone were subtracted from those bound in the absence of competing hormone. The cpm bound per 20 μl of cell suspensions were converted to molecules bound per cell. Receptor sites per cell were then calculated from these values by extrapolating to infinite steroid concentrations (11, 18). Total receptor sites per cell were calculated as the sum of the cytoplasmic and nuclear receptor sites per cell.

**Translocation of the Hormone-Receptor Complex from Cytoplasm to Nucleus.** Cytoplasmic and nuclear receptor sites were first assayed in a cell suspension after a 2-hr incubation of cells with $[^3H]$dexamethasone (40 nM), with and without unlabeled dexamethasone (2 μM). After a 2-hr incubation at 3°, duplicate 20-μl aliquots were removed from each tube and were used to measure cytoplasmic and nuclear binding, as described above. The cell suspension was then transferred to a 37° water bath, and the incubation was continued for 30 min, after which cytoplasmic and nuclear binding were again assayed. Translocation was scored as positive if nuclear binding increased while cytoplasmic binding decreased on warming the cells to 37°.

**In Vitro Responses to Glucocorticoid.** In vitro sensitivity to glucocorticoid was measured by using the uptake of glucose, incorporation of leucine, uridine, and thymidine, and cell viability by using methods previously described (12). Briefly, for assays of isotope incorporation, cells ($1 \times 10^6$/ml) were incubated in quadruplicate, with and without 100 nM dexamethasone for 20 hr at 37°. Radiolabeled leucine, uridine, and thymidine were then added, and the incubation was continued for 4 hr. The cells were then harvested on glass fiber filter paper, and the isotope incorporation was determined by liquid scintillation counting. For assay of cell survival, cells ($5 \times 10^5$/ml) were incubated in quadruplicate in the presence and absence of dexamethasone (400 nM) at 37°. At 96 hr, samples were removed for cell counts and determination of viability by trypan blue dye exclusion. a-Glucose transport was measured as previously described (12). Results for in vitro sensitivity studies have been expressed as percentage change from the values obtained when cells were incubated without dexamethasone.

**Statistical Methods.** Differences in continuous variables between groups of patients were evaluated using a Mann-Whitney test. To determine the statistical significance of in vitro glucocorticoid responses, a t test was used to compare the results of quadruplicate incubations in the presence and absence of dexamethasone.

**Results**

**Glucocorticoid Receptors.** As shown in Table 1, the cells of all 36 patients contained glucocorticoid receptors. The number of total glucocorticoid receptors per cell varied from 4,300 to 28,400, with a median of 8,800. By all of the criteria we were able to study, these receptors were similar to those described in other normal and neoplastic tissue (1, 19). The median dissociation constant (the free concentration of steroid at which the receptors are half-saturated) was 15 nM, with a range of 8 to 21 nM in the 7 patients studied. The steroid specificity of the receptor was identical to that described for normal human

<p>| Table 1 |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Glucocorticoid receptors in leukemic cells of 36 patients with ANLL</th>
<th>Total receptor sites/cell</th>
<th>$K_d$ (nM)</th>
<th>Nuclear translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>Median</td>
<td>Mean</td>
<td>Range</td>
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<tr>
<td>4,300–28,400</td>
<td>8,800</td>
<td>9,000</td>
<td>8–21</td>
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# Calculated for dexamethasone for the cells of 7 patients.

b Scored positive if nuclear binding increased while cytoplasmic binding decreased on warming the cells from 3 to 37°.

**Chart 1.** Correlation between glucocorticoid receptor levels in samples of blood and bone marrow obtained from the same patient at the same time. Results are presented for nuclear, cytoplasmic, and total receptor sites per cell for 8 patients.
Nuclear binding of the hormone-receptor complex was observed in the cells of all patients, with from 34 to 95% of the total receptors present in the nucleus at 37°. In the cells of 13 of 15 patients studied, the ratio of nuclear to cytoplasmic binding increased when cells were warmed from 3° to 37°.

In 8 patients, glucocorticoid receptors were studied on samples of blood and bone marrow drawn on the same day, prior to treatment. As illustrated in Chart 1, similar values were obtained for cytoplasmic, nuclear, and total receptors from different sites of the same patient.

The number of glucocorticoid receptor sites per cell was similar among the various FAB categories of ANLL (Chart 2). In 4 of 5 patients studied, the number of glucocorticoid receptors in samples obtained at relapse was slightly lower than that at diagnosis (median change, —16%) (Chart 3).

**In Vitro Responses to Glucocorticoids.** Table 2 shows the effects of dexamethasone on glucose transport, incorporation of leucine, uridine, and thymidine, and cell viability in 28 patients in whom sufficient cells were available for study. Statistically significant effects for at least one of these assays were observed in the cells of 26 of 28 patients studied. Dose-response relationships were studied with cells of 15 patients. The effects were dose related at concentrations of dexamethasone equivalent to physiological and pharmacological concentrations of cortisol. The half-maximal response occurred at a concentration slightly lower than 10 nm, a concentration very close to the $K_d$ of the receptor for dexamethasone. In separate experiments, these effects were first detected after 2 hr and reached a maximal response by 24 hr. The dose-response relationships and latency of response are characteristic of receptor-mediated responses.

Although in vitro responses to dexamethasone were observed in the cells of nearly all patients, both stimulatory and inhibitory responses were seen. The extremes of this variation are illustrated in Chart 4. Patient A was studied on 3 occasions; in each case, dexamethasone (400 nm) stimulated the leukemic blasts to proliferate. In contrast, the cells of Patient B were rapidly killed by glucocorticoid with 94% cell death in dexamethasone-treated cultures, as compared to only 20% cell death in the control cultures. Dexamethasone stimulated proliferation in 3 of 26 cases and showed cytotoxic effects in the cells of 20 of 26 cases. The viability of cells of 3 patients was not affected by dexamethasone. Stimulatory or inhibitory effects were not clearly related to FAB classification.

**Discussion**

The leukemic cells of all 36 patients with ANLL had glucocorticoid receptors. These receptors were similar in all respects studied to those in other normal and malignant tissues. The numbers of receptors varied widely but were similar to those found by ourselves and others in ALL, a disease which responds to steroid therapy much more frequently than does ANLL. The fact that metabolic effects of glucocorticoid were observed in the cells of 26 of 28 patients studied indicates that these receptors were not defective in their ability to mediate a response. These results suggest that qualitative or quantitative
glucocorticoid receptor defects do not underlie the relative clinical resistance of ANLL compared to ALL to glucocorticoid treatment.

Our studies indicate that ANLL is extremely heterogeneous with respect to in vitro response to glucocorticoid. The cells of 3 of 28 patients were stimulated to proliferate by therapeutic concentrations of glucocorticoid. Similar results have been reported by Cline and Rosenbaum (10). However, in the majority of patients, cytotoxic effects were observed which were similar but less extensive than those we have seen in ALL (12). In one patient, greater than 90% cell killing was observed compared to controls without dexamethasone. The in vitro heterogeneity parallels results seen in early clinical studies in which some patients achieved remission on steroids alone, while others demonstrated rapidly progressive disease. The broad range of responses observed in vitro suggests that these in vitro assays might permit identification of those patients likely to derive therapeutic benefit from glucocorticoids.

References

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