Glucocorticoid Receptors and Glutamine Synthetase in Leukemic Sézary Cells

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ABSTRACT

Using a competitive binding assay, we have detected cytoplasmic glucocorticoid receptors in the leukemic cells of several patients with Sézary syndrome, while cells from other patients appear to contain very low or nondetectable receptor levels. The receptors are saturated at approximately $4 \times 10^{-8}$ M $[^{3}H]$dexamethasone, and Scatchard analyses of the binding data indicate a high affinity ($K_d = 6.9 \times 10^{-9}$ M). The cytoplasmic receptors are inactivated at $3^\circ$, but at $4^\circ$ they are relatively stable, even in the absence of glucocorticoids. Competition studies have established the specificity of these receptors. Active glucocorticoids such as dexamethasone, prednisolone, and cortisol (50-fold molar excess) completely block $[^{3}H]$dexamethasone binding, while nonglucocorticoids such as $17\beta$-estradiol and $5\alpha$-dihydrotestosterone have no effect at equivalent concentrations. The antiinducer progesterone is also an effective competitor of $[^{3}H]$dexamethasone binding. The $[^{3}H]$dexamethasone-receptor complex migrates in linear 5 to 20% sucrose gradients (0.4 M KCl) with a sedimentation coefficient of 4S$_{20}$,w. In those cells which contained receptors, the specific activity of glutamine synthetase was increased 2-fold after an 18-hr exposure to dexamethasone ($10^{-8}$ M), whereas no such increase occurred in cells lacking receptor. The presumptive induction of glutamine synthetase activity may thus serve as a marker for functional receptors in leukemic Sézary cells. Retrospective studies (4 patients) suggest a possible correlation between receptor levels and clinical responsiveness to glucocorticoid therapy.

INTRODUCTION

Sézary syndrome is a rare and frequently lethal chronic leukemia characterized by atypical circulating malignant lymphocytes (10), cutaneous infiltration, and an intensely pruritic erythrodermia with the formation of plaques (36). The distinguishing abnormal circulating lymphocyte seen in this leukemia is typically a large cell with a voluminous convoluted nucleus surrounded by a thin rim of cytoplasm (11, 19). Several investigators (6, 7, 19) have shown that the membrane characteristics of Sézary cells are those of T-lymphocytes. Broder et al. (4) have also presented data which suggest that the neoplastic lymphocytes from the majority of patients with Sézary syndrome originate from a subset of T-cells programmed exclusively for helper-like interactions with B-cells in their production of immunoglobulins.

Much of the edema and erythrodermia associated with the Sézary syndrome can be improved by local glucocorticoid therapy, and often the systemic use of glucocorticoids further reduces these symptoms as well as pruritus. Combined therapy including chlorambucil and prednisolone has also been shown to result in freedom from pruritus, followed by partial resolution of the erythroderma and histological disappearance of Sézary cells in many patients (35). Considering that most Sézary patients live an average of 5 years from the onset of symptoms (4), it would be beneficial to determine at which specific stage(s) the malignant lymphocytes are most sensitive to systemic glucocorticoids. Omission of glucocorticoids from the therapy of resistant patients would reduce immunosuppression and thus render these patients less susceptible to infections. Although glucocorticoid hormones elicit a series of profound intracellular responses which ultimately result in the cytosis of lymphoid cells (31), the exact mechanism of this induced lysis is unknown. However, most, if not all, of these effects are mediated through specific cytoplasmic receptors which, when complexed with active glucocorticoids, are translocated to the nucleus where they bind to chromatin (23). Glucocorticoid receptors have been characterized and quantified in acute lymphoblastic leukemia (15, 16), which is also thought to be often of T-cell origin. These studies suggested that in acute lymphoblastic leukemia the presence of functional glucocorticoid receptors may reflect the responsiveness of patients to glucocorticoid therapy. However, in other leukemias, particularly chronic lymphocytic leukemia, in which the level of lymphocyte receptors is frequently comparable to that found in normal lymphocytes, receptor measurements alone appear insufficient to determine glucocorticoid sensitivity (14).

The glucocorticoid sensitivity of various subclasses of T-cells has also been studied using the mouse thymus as a model system. Cantor and Boyse (8) have shown that specific Ly alloantigens are expressed on lymphoid cells undergoing thymus-dependent differentiation. Although helper (Ly 1$^{+}$) and killer/suppressor (Ly 1$^{-}$) medullary cells are generally considered relatively resistant to glucocorticoids, their immature immunoincompetent precursors in the cortex are very sensitive (2). Other data suggest that certain mature immunocompetent T-lymphocytes can retain their glucocorticoid sensitivity. Waldmann and Broder (34) have found that in a subset of hypogammaglobulinemia patients, the abnormality of immunoglobulin synthesis is

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3 The trivial names used are: prednisolone, 1,4-pregnadien-11$\beta$, 17$\alpha$, 21-triol-3,20-dione; cortisol, 4-pregn-11$\beta$, 17$\alpha$, 21-triol-3,20-dione; predni- sone, 1,4-pregnadien-17$\alpha$, 21-diol-3,11$\beta$, 20-trione; estradiol, 1,3,5(10)-estra- trien-3,17$\beta$-diol; dihydrotestosterone, 5$\alpha$-androstan-17$\beta$-ol-3-one; cortico- lone, 4-pregn-17$\alpha$, 21-diol-3, 20-dione; progesterone, 4-pregn-3,20-dione.

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associated with a disorder of regulatory suppressor T-cells which may be very sensitive to glucocorticoids.

Since the majority of Sézary cells are helper lymphocytes, these cells afforded us the opportunity to study the glucocorticoid receptors in another subset of immunocompetent T-cells. The present study was thus undertaken to determine whether leukemic Sézary cells contain cytoplasmic glucocorticoid receptors and, if so, to determine the affinity and specificity of these receptors. Since several lymphoid mutants have been described which possess cytoplasmic glucocorticoid receptors but are resistant to glucocorticoids (29, 30), we were also interested in establishing an assay which would reflect the presence of functional receptors in Sézary cells. Since the induction of glutamine synthetase by glucocorticoids is strictly dependent on the presence of functional cytoplasmic receptors (32), we have studied the possible induction of this enzyme in Sézary cells.

MATERIALS AND METHODS

Source of Sézary Cells. All patients used in this study (NIH Clinical Center and Georgetown University Hospital) were diagnosed as Sézary syndrome patients by standard clinical and cytological criteria. All suffered from pruritic erythroderma and had atypical circulating Sézary lymphocytes, and skin biopsies revealed Sézary cells infiltrating the dermis. None of the patients had received chemotherapy of any type which included glucocorticoids within 2 weeks of their inclusion in this study. Leukocytes were obtained from Patients 1, 2, 3, 4, and 6 by leukapheresis using an IBM-National Cancer Institute continuous cell separator (12). Following leukapheresis, the cells were collected by centrifugation, and the serum was aspirated. Peripheral blood was obtained from Patients 5 and 7. Hypotonic lysis of RBC was achieved by pipetting cells repeatedly in cold distilled water for 75 sec and was terminated by the addition of an equal volume of 2× phosphate-buffered saline (KCl, 0.4 g/liter; KH₂PO₄, 0.4 g/liter; NaCl, 16.0 g/liter; and Na₂HPO₄•7 H₂O, 4.32 g/liter). The washed leukocytes were used immediately or were resuspended in RPMI* (prepared by NIH Media Unit) supplemented with 20% heat-inactivated fetal calf serum, 4 mM glutamine, and 10% dimethyl sulfoxide (Fisher Scientific Co., Fair Lawn, N. J.) and were then frozen in an acetone-dry ice bath and aliquoted into borosilicate glass tubes (Corning Glass Co.). All subsequent incubations were performed at 4°C in a Kynorack (Streck Laboratories Inc., Omaha, Nebr.) for 6 hr, except for experiments examining the time course of binding and the effects of elevated temperatures. Experiments designed to study the time course of specific binding indicated that an incubation period of 6 hr was required to reach equilibrium. In the standard binding assay, 200-μl aliquots of the cytosol were incubated with increasing concentrations of the synthetic glucocorticoid [³H]dexamethasone (26 Ci/mmol; American/Searle Corp., Arlington Heights, Ill.) in the presence or absence of a 500-fold excess of nonlabeled dexamethasone. Following the incubation, 25 μl of dextran-coated charcoal suspension were added; each tube was agitated on a Vortex mixer for 10 sec and then centrifuged at 3000 rpm for 10 min in a refrigerated Sorvall RC-3 centrifuge. Three 50-liter aliquots of the resultant clear supernatant were then added to 10 ml of Aquasol Scintillation Cocktail (New England Nuclear, Boston, Mass.) and were counted in a Beckman LS-255 scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.) with an average counting efficiency of 45% for tritium. Total counts minus noncompetable counts were then taken to represent specifically bound dexamethasone (1). Final results are expressed as pmol [³H]dexamethasone specifically bound per mg of cytoplasmic protein. Protein determinations were performed according to the method of Lowry et al. (18) using, as the standard, bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) dissolved in the binding buffer. It is unlikely that endogenous glucocorticoids would interfere to any extent in this standard binding assay, since small amounts of free endogenous glucocorticoids would be removed during the washing process and any specifically bound steroid would be displaced from the receptor by the large excess of competing [³H]dexamethasone. Although it is possible that the cytosols were contaminated by very small amounts of serum proteins, [³H]dexamethasone binds only to intracellular receptors and not to serum corticosterone-binding globulin (transcortin) (17).

Nonradioactive steroids used in the competition and induction experiments were obtained from the following sources: cortisol, prednisone, prednisolone, 17β-estradiol, and 5α-dihydrotestosterone from Sigma Chemical Co.; dexamethasone from Merck, Sharp and Dohme (West Point, Pa.); and corticosterone from Aldrich Chemical Co. (Milwaukee, Wis.).

Sucrose Gradients. Linear 5 to 20% sucrose gradients were prepared using binding buffer (10% glycerol; 0.4 M KCl) in cellulose nitrate tubes (Beckman Instruments, Inc., Palo Alto, Calif.) with a gradient-former (Hoefler Scientific Instruments, San Francisco, Calif.) and a peristaltic pump (LKB Instruments, Inc., Rockville, Md.). Following adsorption of free [³H]dexamethasone with dextran-coated charcoal, 0.3-ml aliquots of the labeled cytosols were layered onto gradients which were then centrifuged for 18 hr at 149,000 × g in the SW 50.1 rotor of a refrigerated L5-65
Clinica! information and glucocorticoid receptor content in Sézary patients

Patient Sex Age Highest leu-

kocyte count (cu mm) % Lympho-

cytes (% Sézary) Receptor content (pmol [^3H]dexamethasone bound/mg cytoplasmic protein) a Responsiveness to glucocorticoid therapy Induction of glutamine synthetase (EsA'@ In-

duced/ESA control)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Highest leukocyte count (cu mm)</th>
<th>% Lymphocytes (% Sézary)</th>
<th>Receptor content (pmol [^3H]dexamethasone bound/mg cytoplasmic protein) a</th>
<th>Responsiveness to glucocorticoid therapy</th>
<th>Induction of glutamine synthetase (EsA'@ Induced/ESA control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M (64)</td>
<td>80,000</td>
<td>85 (100)</td>
<td>0.42 (K_d = 6.9 x 10^-6 M)</td>
<td>Single agent (prednisone) dramatically reduced Sézary count; combined chemotherapy reduced edema and erythroderma</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 M (46)</td>
<td>12,000</td>
<td>50 (20)</td>
<td>0.04</td>
<td>Chlorambucil + prednisone had no effect on Sézary count or skin lesions</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 F (57)</td>
<td>7,500</td>
<td>33 (75)</td>
<td>0.08</td>
<td>No systemic glucocorticoids given; trimacinolone (topical) improved skin slightly</td>
<td>Not assayed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 M (57)</td>
<td>8,500</td>
<td>50 (100)</td>
<td>None detectable</td>
<td>No chemotherapy prior to leukapheresis</td>
<td>Not assayed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 M (47)</td>
<td>35,000</td>
<td>92 (85)</td>
<td>None detectable</td>
<td>Refractory to combined chemotherapy including glucocorticoids</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 M (46)</td>
<td>258,000</td>
<td>99 (100)</td>
<td>0.13 (K_d = 6.9 x 10^-6 M)</td>
<td>No chemotherapy at time of leukapheresis; developed malignant lymphoma</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 F (46)</td>
<td>46,000</td>
<td>60 (100)</td>
<td>0.07</td>
<td>Refractory to combined chemotherapy including glucocorticoids</td>
<td>Not assayed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Determined using a saturating concentration of [^3H]dexamethasone.

b EsA, specific activity of the enzyme (induced and control) expressed as mean of duplicate determinations (range < 15% of mean).
Glucocorticoid Receptors in Sézary Cells

specific binding activity. At 4°, 100% of the specific binding was preserved after 24 hr if [3H]dexamethasone was present throughout the incubation period. If the cytosol was stored at 4° for 24 hr in the absence of dexamethasone followed by addition of [3H]dexamethasone and an incubation of 6 hr, 77% of the specific binding activity was detected.

Specificity of Glucocorticoid Receptors. The specificity of these cytoplasmic receptors was demonstrated by measuring the ability of competing steroid analogs to displace 5 x 10⁻⁸ M [3H]dexamethasone in the binding reaction. The ability of dexamethasone, prednisolone, and cortisol at a 50-fold molar excess to completely displace the specifically bound [3H]dexamethasone (Chart 2A) agrees with the known cytolytic activity of these steroids in lymphoid systems (21). Prednisone (Chart 2B) and cortexolone (Chart 2A), which in rodent thymocytes is an antiglucocorticoid (22), also competed for binding when present at higher molar excesses. Progesterone (Chart 2B), an antiinducer in human lymphoid tissues (15), was also an effective competitor, while 17β-estradiol and 5α-dihydrotestosterone showed weak competition (Chart 2B).

The specificity of this competition for receptor sites is also reflected in sucrose gradient profiles of cytoplasmic extracts. As seen in Chart 3, the [3H]dexamethasone-receptor complex migrated with a sedimentation coefficient of approximately 4S₂₀,₅₀ in linear 5 to 20% sucrose gradients containing 0.4 M KCl. A 500-fold molar excess of unlabeled dexamethasone completely abolished this 4S binding peak,

came saturated at a dexamethasone concentration of approximately 4 x 10⁻⁸ M. Chart 1B shows a Scatchard plot (25) of the specific binding data in Chart 1A. Although there appeared to be some nonlinearity at the lower concentrations of [³H]dexamethasone, this was probably artifactual and may have resulted from failure to achieve equilibrium binding after 6 hr. The straight line, which was drawn by hand for the remaining points, is consistent with a single class of receptors of uniform affinity. The equilibrium dissociation constant (Kₐ) calculated from the slope of the Scatchard plot was 6.9 x 10⁻⁸ M, which agrees with the reported affinity of cytoplasmic glucocorticoid receptors in acute lymphoblastic leukemia (16).

Thermal Lability of Receptors. The cytoplasmic glucocorticoid receptors in Sézary cells were found to be thermostabile. A 30-min preincubation at 37° left only 5.4% of the specific cytoplasmic binding activity relative to a control incubated for 6 hr at 4°, while 30 min at 22° left 67% of the specific binding activity.
the increase in specific activity in Sézary cells is demonstrated in Chart 6. Although dexamethasone (10^{-6} M) induced enzyme activity maximally, other active glucocorticoids such as prednisolone (10^{-6} M) and cortisol (10^{-6} M) also resulted in increased enzyme activity. Nonglucocorticoids such as progesterone and 17β-estradiol failed to increase the specific activity of glutamine synthetase.

**DISCUSSION**

The data presented demonstrate that lymphocytes from some Sézary patients contain significant quantities of cytoplasmic glucocorticoid receptors which are labeled by cytoplasmic glucocorticoid binding. The time course (Chart 4) indicates that maximal enzyme activity was achieved after 18 hr. Chart 5 depicts the dose-response relationship and indicates that maximal enzyme activity was achieved with approximately 10^{-8} M dexamethasone. Half-maximal enzyme activity was achieved at approximately 2.4 \times 10^{-8} M dexamethasone, which is higher than the experimentally calculated concentration required for half-maximal saturation of cytoplasmic receptors (K_d = 6.9 \times 10^{-8} M). The explanation for this apparent discrepancy is unclear. Using several murine T-cell lines which are sensitive to glucocorticoids, we have also found that the equilibrium dissociation constant determined using a whole-cell binding assay is higher than that determined using a cytosol assay (T. J. Schmidt and E. B. Thompson, unpublished data). It is possible that the glycerol added to the homogenization buffer used in the cytoplasmic assay slows the dissociation of [H]dexamethasone from receptors and thus lowers the equilibrium dissociation constant (K_d). The kinetics of this increase in specific activity of glutamine synthetase in Sézary cells are identical to those seen in an established human leukemic cell line (CEM) which contains specific glucocorticoid receptors. It has been shown with the latter cell line, that the basal specific activity of glutamine synthetase is inversely related to the concentration of glutamine in the medium and that dexamethasone always produces a 2.5-fold increase in specific activity regardless of the basal enzyme activity. The specific activity of glutamine synthetase in Sézary lymphocytes by different steroids (10^{-8} M) (single determinations).

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* J. M. Harmon and E. B. Thompson, manuscript in preparation.
plasmic glucocorticoid receptors, whereas cells from other Sézary patients appear to contain low or undetectable levels of such receptors. This continuum of glucocorticoid receptor levels is analogous to the situation seen with estrogen receptors in human breast cancer. Obviously, it is not possible to identify precisely the glucocorticoid-receptor concentration which demarcates a receptor-positive from a receptor-negative cytosol. Those glucocorticoid receptors detected in these helper T-lymphocytes appeared very similar in their saturability, affinity, thermolability, and specificity to glucocorticoid receptors described in other human leukemic and lymphoid cells. Considering that only a small number of patients were studied, it is not possible to draw conclusions concerning the value of receptor determinations in predicting clinical responsiveness to glucocorticoid therapy. Patient 1, whose cells contained levels of receptor several times greater than those found in any other patient, did in fact respond to prednisolone alone and in combination with other drugs; and Patient 5, in whose cells no receptor was detected, was refractory to combined therapy. However, Patients 2 and 7 were found to have measurable receptor levels and yet were refractory to glucocorticoid therapy. Obviously, further prospective studies with additional untreated patients would be required to ascertain whether receptor levels can provide an accurate prediction of glucocorticoid-mediated cytolysis of Sézary cells.

Inasmuch as the levels of cytoplasmic receptor in the cells from some Sézary patients were very low or undetectable, it is possible that the majority of the unbound receptors in these cells were inactivated during homogenization, perhaps due to the release of proteolytic enzymes or other inactivators. However, mixing experiments in which receptor-negative and receptor-positive cytosols were utilized indicated that the receptor-negative cytosols lacked factors which could inactivate receptors in the receptive-positive cytosols. In interpreting the receptor data for each patient (Table 1), one must also consider the percentage of leukocytes which were Sézary cells. In Patients 2, 3, and 4 where the leukocyte fraction consisted of other cell types in addition to lymphocytes, the relatively low receptor levels may reflect this cellular heterogeneity. With Patients 1 and 6, whose leukocyte fraction consisted primarily of Sézary lymphocytes, it is not possible to differentiate between a homogeneous population of Sézary cells, all of which contain the same concentration of receptors, and a heterogeneous population, in which some subpopulations contain extremely high receptor levels.

Although loss of glucocorticoid sensitivity in the S49 cultured mouse lymphoma cell line is frequently associated with the loss of functional cytoplasmic receptors, resistant mutants which contain normal cytoplasmic receptors but are defective in nuclear transfer of the glucocorticoid-receptor complex have been described (29, 30). Thus, it was clear that a functional test which reflected the nuclear uptake of the cytoplasmic glucocorticoid-receptor complexes detected in Sézary cells would strengthen the physiological role of these receptors as mediators of cytolysis. Considering that the ability of glucocorticoids to induce specific enzymes in a variety of cells is dependent on the presence of functional receptors (25, 32), we felt that the ability of dexamethasone to increase the specific activity of glutamine synthetase through a presumed inductive mechanism might serve as such a functional test in Sézary cells. This enzyme has been studied extensively in chick embryo retinal tissue where it can be induced prematurely by dexamethasone (21, 24). Induction in this tissue requires new protein and RNA synthesis (20) and increases levels of specific translatable mRNA (28). Induction of glutamine synthetase by glucocorticoids has also been reported in L-cells (32) and rat hepatoma tissue culture cells (9). Since we have found a number of mouse and human B- and T-cell lines which are lysed by glucocorticoids but show no induction of glutamine synthetase, it is clear that induction of this enzyme is not required for glucocorticoid killing of lymphoid cells (unpublished data). As previously mentioned, glutamine synthetase is inducible in a human leukemic cell line (CEM). Approximately 40 glucocorticoid-resistant mutants of this cell line have been analyzed and found to be noninducible for glutamine synthetase. These data suggest that the induction of glutamine synthetase in this cell line is a marker for functional receptors and glucocorticoid-mediated killing. The data presented here suggest that induction of glutamine synthetase may also serve as a marker for functional glucocorticoid receptors in Sézary cells.

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REFERENCES


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