JCI The Journal of Clinical Investigation

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J Clin Invest. 1973;52(7):1715-1725. https://doi.org/10.1172/JCI107353.

Research Article

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ABSTRACT The first known step in steroid hormone action is the association of the steroid with specific cytoplasmic steroid-binding proteins (SBP). Using a competitive binding assay, we detected, quantified, and partially characterized such a SBP in cytosol from glucocorticoid-sensitive human lymphoblastic leukemic blasts. The affinity of steroids for the SBP was directly related to their known killing potency. For example, steroids without glucocorticoid effect such as androstenedione, etiocholanolone, and tetrahydrocortisol were unable to displace radiolabeled dexamethasone from the SBP in the binding reaction. The dose-response curve for in vitro inhibition of [^{3}H]thymidine uptake in leukemic blasts correlated closely with the binding affinity of glucocorticoids to the SBP, providing additional support for an essential physiologic role for SBP in steroid action. SBP activity was either greatly diminished or absent in glucocorticoid-resistant cells. Six patients who intially had SBP in their blasts and were responsive to combinations of drugs including glucocorticoids no longer had SBP activity detectable at a time when they no longer responded to combinations of drugs including glucocorticoids. In vitro [^{3}H]thymidine uptake was not inhibited by steroids in leukemic blast cells lacking SBP activity. Other patients who had received some antileukemic therapy including glucocorticoids and who still had SBP in their leukemic blasts, were still responsive to drug combinations that included glucocorticoids. This appears to be the first study demonstrating glucocorticoid receptors in a human tissue.

This work was presented in part at the Annual Meeting of the American Society of Hematology, December 1972, Hollywood, Fla.

Received for publication 7 November 1972 and in revised form 20 February 1973.

INTRODUCTION

The binding of steroid hormones to highly specific, high affinity cytoplasmic receptor proteins has been suggested as the first step in steroid hormone action in many systems (1–12). These steroid-binding proteins (SBP)[1] appear to be the determinant of target tissue specificity (4, 6, 7, 12). In such cells, the kinetics of association and dissociation between steroid and SBP are compatible with observations of the time course of hormone action (10, 13, 14). Furthermore, the affinity of various steroids for a given target cell SBP correlates well with known biologic potency of the steroid in that system (12–16, 17–22). A SBP has been demonstrated in cytosol from rat thymocytes (8). When those cells are exposed to glucocorticoid in sufficient concentration to bind significantly to this SBP eventual cell lysis results (12). Various mouse lymphoma lines, which have been propagated in culture and which are lysed by glucocorticoids, have also been shown to have cytoplasmic SBP (18, 23). In steroid-resistant cell lines derived from these lymphomas, marked decreases in SBP were found (18, 19). Others have reported decreases in SBP activity accompanying loss of steroid responsiveness in cultured hepatoma cells (24) and mouse fibroblasts (5, 25). Recently, a promising attempt has been made to correlate estrogen responsiveness of human breast carcinoma in vivo to the presence of specific cytoplasmic estrogen-binding receptor proteins present in tumor homogenates (26). It therefore seemed appropriate to investigate whether human leukemic lymphoblasts might contain an identifiable SBP.

[1] *Abbreviations used in this paper:* SBP, steroid-binding protein; PBS, phosphate-buffered saline pH 7.4; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia.

Glucocorticoids have been used in the therapy of acute leukemia for years (27–29). Many investigators have studied the mechanism of glucocorticoid effect in normal and leukemic lymphoid tissue (30–38). Unfortunately in some cases the doses used have been so large that the responses may well have been due to nonspecific effects (13, 34). Normal human lymphocytes are glucocorticoid resistant compared with lymphocytes from mouse and rat (30, 34). Studies of glucocorticoid effect in human normal and neoplastic lymphoid tissue have been concerned with alterations in uptake of energy substrate or energy-substrate utilization (31), changes in uptake of amino acids (39), and decreased incorporation of amino acids and nucleosides into macromolecules (32–34, 37, 38). Overall, tests of this kind have proven to be both time consuming and somewhat disappointing in assessing glucocorticoid response.

We therefore undertook an investigation of human acute lymphoblastic leukemic (ALL) blasts, a tumor with known glucocorticoid responsiveness, in an attempt to identify, quantify, and characterize a SBP. The significant morbidity and mortality associated with glucocorticoid therapy, particularly in the treatment of human malignancy is well known (40). With this in mind, the development of a rapid and reliable in vitro test that might predict steroid responsiveness in human leukemic cells would be of value.

METHODS

Leukemic and normal blood cells were obtained either by conventional leukophoresis or by use of the IBM-NCI Continuous Cell Separator (41). Cells were sedimented in 5% dextran (maximum mol wt 140,000) and washed in cold 10 mM phosphate-buffered isotonic saline pH 7.4 (PBS). The cells were either used immediately or stored frozen in liquid nitrogen, in Eagle's minimal essential medium supplemented with 15% fetal calf serum, 4 mM glutamine, and 10% dimethyl sulfoxide (42). Upon thawing, virtually all of the cells remain viable. Just before use, the cells were thawed and washed twice in PBS. Similar results were obtained with fresh or frozen cells. Identical results were also obtained in fresh lymphoblasts from peripheral blood which were examined after hypotonic lysis of the red blood cells and washing of the remaining cells in PBS twice.

Diagnosis was established in all cases using standard morphologic diagnostic criteria. All patients in this study with ALL were under 30 yr of age and had received no therapy of any kind at the time of study except as noted. No treated patients had received any glucocorticoid therapy within 10 days of their inclusion in this study.

Binding assays for SBP were performed using the method of Baxter, Rousseau, and Tomkins (9, 10) with certain modifications. The binding buffer was supplemented with 20% glycerol, as this was found to increase the sensitivity of the assay at low protein concentrations, probably by stabilizing the binding protein itself. Centrifugation of the cytosol was 100,000 *g* for 60 min. All incubations were for 2 h at 4°C except for experiments examining the time course of binding. In this assay the extract to be assayed is incubated with various concentrations of [^{3}H]dexamethasone, (22 Ci/mM; Amersham/Searle Corp., Arlington Heights, Ill.) or [^{3}H]cortisol (88 Ci/mM; Amersham/Searle Corp.) in the presence or absence of a 1,000-fold excess of nonlabeled dexamethasone or cortisol respectively, except as noted. Total counts minus noncompetible counts are taken to represent specifically bound steroid (9). Using the assay with these modifications, it was shown that the amount of charcoal added was sufficiently in excess to adsorb all unbound steroid. For example, if [^{3}H]dexamethasone was added to heat-inactivated SBP, followed by charcoal adsorption, greater than 99.99% of the counts were removed. Secondly, the extent of binding was not altered by further additions of charcoal. Thirdly, similar results were obtained when nonprotein-associated steroid was removed by washing the SBP on cellulose filters. Fourthly, the specifically bound dexamethasone or cortisol was linearly related to the protein concentration for a given extract under the conditions of this assay as shown in Fig. 1. It is not considered likely that endogenous steroids would interfere in this assay to any extent. Any small amounts of endogenous plasma glucocorticoids should be removed in the washing process. Nonspecifically cell-bound glucocorticoids should dissociate from the cells during the preparation of the extract (13), and most specifically bound steroid should dissociate during the 60 min centrifugation as described in the results. Any remaining SBP-associated glucocorticoid will be displaced from the SBP by the large excess of competing [^{3}H]dexamethasone as shown in the results.

[^{3}H]Thymidine incorporation studies were performed by suspending the cells at a concentration of $1–2 \times 10^6$ per 35 mm Petri dish in Eagle's minimal essential medium supplemented with 10% autologous complement-free human serum and 4 mM glutamine plus varying concentrations of dexamethasone. The cells were incubated 18 h at 37°C in a humidified 5% CO_2 incubator. They were then exposed to [^{3}H]thymidine (0.3 μM, sp. act 14 Ci/mM; New England Nuclear, Boston, Mass.), for 2 h. The cells were harvested, washed twice in cold PBS, precipitated for 30 min in the cold with 10% trichloroacetic acid containing 2 mM nonradioactive thymidine, the precipitates collected on Millipore filter discs and counted in toluene-phosphor in a Beckman LS 250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) (efficiency 25–27%).

RESULTS

SBP in human leukemic lymphoblasts. Using the competitive assay described, highly specific cytoplasmic steroid-binding activity was detected in human leukemic lymphoblasts. This binding molecule is a protein (see below). Fig. 2 shows a typical binding curve for increasing concentrations of radiolabeled cortisol at a protein concentration of 9.6 mg/ml for the cytoplasmic extract. At 4°C the SBP becomes saturated with [^{3}H]-cortisol at a steroid concentration of about 2.5×10^{-6}M. This greatly exceeds the concentration of plasma-free cortisol with which the SBP is in equilibrium since transcortin has a higher affinity for cortisol than does the SBP (see below). At might be anticipated, binding of glucocorticoids to lymphoblast SBP is insignificant at normal physiologic levels of glucocorticoid (43, 44) at 4°C. In studies of SBP in rat thymocytes (13, 45),

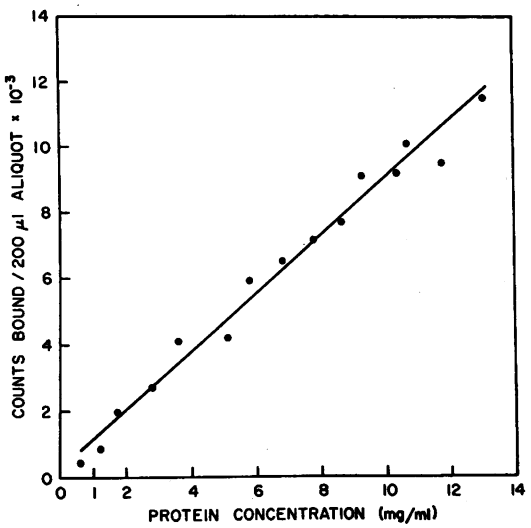

FIGURE 1 Relation of specific binding at 4°C to cytoplasmic extract concentration. Specifically bound [^{3}H]dexamethasone was determined using a steroid concentration of 3.4×10^{-7} M and dilutions of an ALL blast cell cytoplasmic extract.

hepatoma cells (10), and mouse fibroblasts (46) very potent glucocorticoids such as dexamethasone and triamcinolone have a much higher affinity for SBP than does cortisol. Similar binding studies on leukemic blasts performed using [^{3}H]dexamethasone indicate that the SBP is saturated at a steroid concentration of about 2.5×10^{-7} M. A parenteral dose of dexamethasone of 5 mg could easily elevate the plasma steroid concentration to 5×10^{-7} M (47, 48), more than enough to saturate the SBP. Further increases in steroid concentration have no effect on the amount of specifically bound steroid. Providing that the SBP-glucocorticoid complex dissociates freely under the reaction conditions (Fig. 6A) the use of greater than saturating amounts of [^{3}H]dexamethasone in the binding reaction prevents any significant interference by endogenous steroid. The insert in Fig. 2 shows a Scatchard plot (49) of the specific binding data shown in the figure. The straight line that is obtained is consistent with a single class of receptor molecules of uniform steroid affinity being detected in the assay. In the experiment shown, 0.26 pmol of cortisol were specifically bound per mg of cytoplasmic protein. Assuming one steroid molecule bound per receptor the receptor concentration was 2.5×10^{-9} M. If one estimates there to be 1×10^{-8} mg of cytoplasmic protein per cell (by measuring the protein concentration in the 100,000 *g* supernate for a known number of cells) one can calculate 1.5×10^5 receptors per cell. From the intercepts of the Scatchard plot, a dissociation constant of 1.3×10^{-8} M is obtained for the reaction cortisol + SBP $\rightleftharpoons$ cortisol-SBP complex (49). The dissociation constant calculated for dexamethasone using similar techniques is 7×10^{-9} M. The dissociation constant for cortisol with respect to transcortin is 1.6×10^{-9} M (50). All of the constants were determined at 4°C. It was not possible to perform the binding reaction at 37°C because of the instability of the binding protein (see below). Thus, whether the same relationship between transcortin and SBP-binding affinities holds at 37°C is not known.

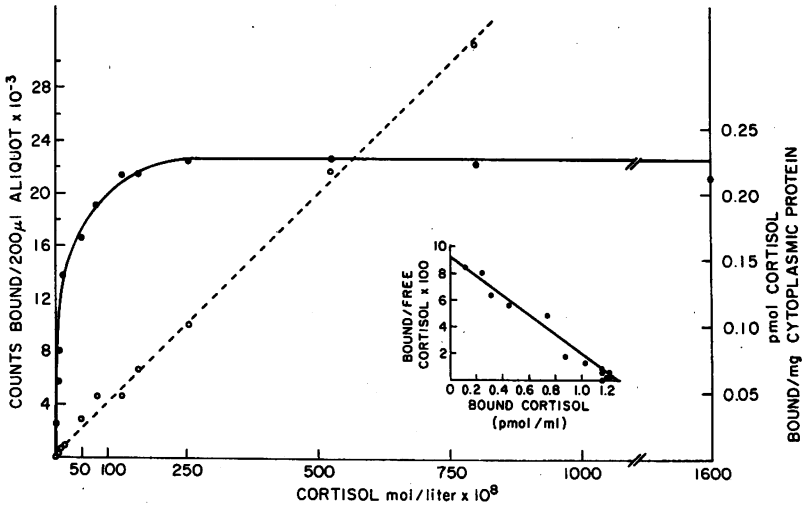

FIGURE 2 Specific binding of [^{3}H]cortisol at 4°C to cytoplasmic extract from lymphoblasts from the same patient shown in Fig. 1. The free steroid concentration is determined by subtracting the bound steroid from the initial amount added to the incubation. The dotted line shows nonspecifically bound noncompetible cortisol which is the background. The insert shows a Scatchard plot of the specific-binding curve.

By using a concentration of steroid sufficient to saturate the binding protein (usually 4×10^{-7} M dexamethasone) it was possible to determine the picomoles of dexamethasone specifically associated with the SBP and thereby quantify the SBP itself. This measurement was performed in 22 untreated, newly diagnosed patients with acute lymphoblastic leukemia (Fig. 3 "ALL"). Although there is wide variation about the mean value of 0.31 pmol of dexamethasone bound per mg of cytoplasmic protein it is apparent that a significant amount of SBP was detectable in all untreated patients. Duplicate determinations on separately obtained samples from the same patient rarely varied by more than 20%. Peripheral blood lymphocytes obtained from normal volunteers (Fig. 3 "NV") showed very little specific glucocorticoid-binding activity. There was no overlap between leukemic lymphoblasts and normal lymphocytes with respect to binding. Whether these low-binding values in normal lymphocytes represent some steroid-responsive cells with significant binding activity seen against a background of cells without binding, or whether normal lymphocytes have a pattern of uniformly low binding is not known. Some normal lymphocytes show inhibition by glucocorticoids (37, 38). The marked differences in response of "T" and "B" cell functions to glucocorticoids (47, 51), as well as the known heterogenity of circulating lymphocytes (52) would favor the former explanation. A defect in the assay procedure of normal lymphocyte SBP activity is possible but appears unlikely. Clearly normal unstimulated lymphocytes are not the control with which leukemic lymphoblasts should be compared. Rather, equal numbers of blasts from steroid-resistant patients as described below provide a more relevant comparison. Interestingly, we have been able to show an increase in SBP in lymphocytes stimulated to undergo blast transformation with phytohemagglutinin in several preliminary studies.

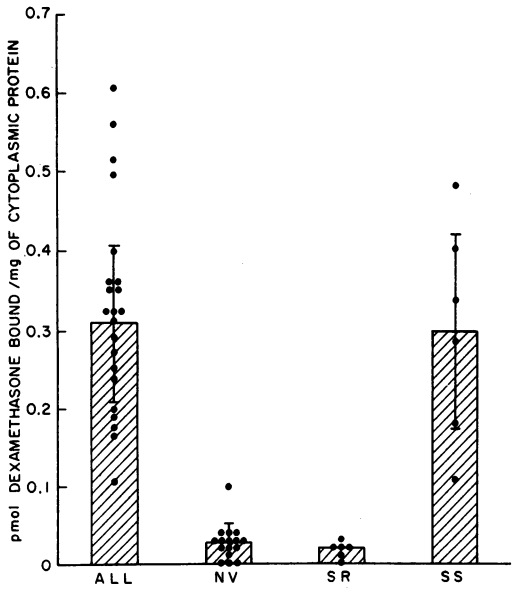

FIGURE 3 The total SBP activity at 4°C is shown for lymphoblasts from 22 untreated patients with acute lymphoblastic leukemia "ALL" ±1 SD; lymphocytes from 16 normal volunteers "NV" ±1 SD; and lymphoblasts from 6 steroid unresponsive patients, "SR"; and 6 patients with ALL who had received some steroid therapy but were still steroid responsive "SS" ±1 SD. Steroid concentrations used were greatly in excess of those needed to saturate the SBP. Some of these patients have been previously presented.

SBP activity was determined in six patients who had been treated with many courses of chemotherapy including glucocorticoids and who no longer responded to this combination of therapy. These patients had received no glucocorticoids within 10 days of study. In these patients SBP was barely detectable (Fig. 3 "SR") in sharp contrast to the untreated group. Stored cells from one of these six patients have been examined at several stages of disease. They had 0.32 pmol of dexamethasone bound per mg of cytoplasmic protein at the time of initial diagnosis. A drug combination including glucocorticoids induced a remission. At the time of his first relapse a repeat determination of his SBP activity revealed 0.40 pmol specifically bound. He once again responded to a drug combination including glucocorticoids. At the time of his second relapse, SBP activity was not detectable in his lymphoblasts. Interestingly, at this time he did not respond to the same drug combination including glucocorticoids.

In contrast to the treated unresponsive patients, six other patients who had received some antileukemic chemotherapy and relapsed, but subsequently responded to a drug combination including glucocorticoids, had SBP determinations performed at the time of their relapse (Fig. 3 "SS"). All of these patients had significant SBP activity in their lymphoblasts in an amount not appreciably different from the untreated patients (0.30 pmol specifically bound compared with 0.31 pmol in untreated patients). These patients have not yet relapsed. This group of patients appears valid for comparison with the steroid-resistant group although these patients were not selected randomly nor was it shown that they were equal to the steroid-resistant group in terms of previous therapy.

The relationship of thymidine uptake to steroid binding. In order to assign a physiologic role to SBP in the mechanism of action of glucocorticoids, there should be a good correlation between binding affinity and the response of the cells to varying concentrations of steroids (9, 23). Inhibition of incorporation of nucleoside precursors into nucleic acids has been shown to be an

early effect of steroids in glucocorticoid-inhibited cells (53, 54). For this reason we measured uptake of [^{3}H]-thymidine by ALL lymphoblasts in short-term tissue culture in the presence of glucocorticoids. Fig. 4A reveals the results of such a study with cells obtained from an untreated patient. It is seen that the concentrations of dexamethasone that are sufficient to saturate the SBP are very close to those that inhibit nucleoside incorporation. Essentially identical results to those shown in Fig. 4A have been obtained in all cells studied from five untreated patients. On the other hand, studies with ALL cells from a steroid-resistant patient shown in Fig. 4B revealed essentially no SBP activity even at concentrations of dexamethasone approaching 10^{-6} M. Similar results were obtained in all four steroid-resistant patients studied in this way. These cells, which have no SBP activity, show no inhibition of incorporation of nucleoside precursor at any steroid concentration studied. These observations provide further support for a physiologic role for SBP in glucocorticoid action in a human tissue.

Properties of the cytoplasmic steroid receptors. The SBP was found to be thermolabile. The lymphoblast SBP when complexed to steroid was completely inactivated by a 30 min incubation at 37°C. A 30 min incubation at 22°C left 80% relative to a control incubated at 4°C. At 4°C about 75% of the binding activity was preserved after 24 h if glucocorticoids were present throughout the incubation period, but none if they were omitted.

A 20 min preincubation at 22°C with dexamethasone and either trypsin (1 mg/ml) a chymotrypsin 100 μg/ml, papain (1 mg/ml), or protease (1 mg/ml) completely destroyed all binding activity. Incubation with muramidase (500 μg/ml), deoxyribonuclease (bovine pancreas 100 μg/ml), ribonuclease (bovine pancreas 100 μg/ml), phospholipase A (100 μg/ml), phospholipase C (500 μg/ml), and phospholipase D (500 μg/ml) had no effect on binding. Therefore, the receptors appear to be proteins and are referred to as such in this report. $HgCl_2$ (10^{-4} M), Triton X-100 (0.4%), NaCl (150 mM) and KCl (1 M) completely destroyed binding activity whereas up to 25% glycerol or 5% ethanol had no effect on the extent of the binding reaction.

Kinetics of the specific cytoplasmic steroid-binding reaction. The association and dissociation reactions for one patient from whom large amounts of material were available were studied. Fig. 5A shows the time course of specific binding of dexamethasone to SBP at 4°C for six different steroid concentrations. It can be seen that for concentrations of steroid sufficient to saturate the SBP, the reaction has approached equilibrium after 2 h. In the binding reaction, dexamethasone

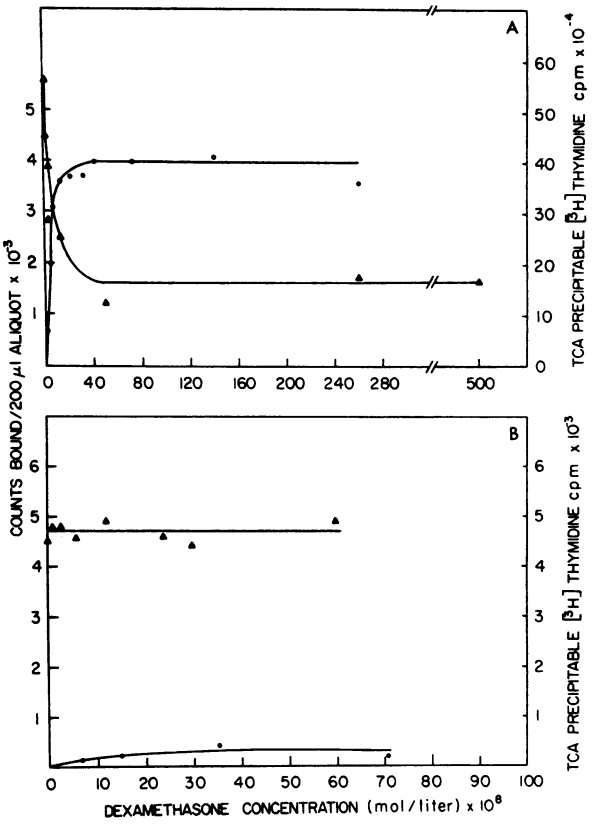

FIGURE 4 (A) Comparison of specific binding of [^{3}H]dexamethasone to the SBP at 4°C (●) with inhibition of [^{3}H]thymidine incorporation at 37°C (▲) as a function of dexamethasone concentration. This data is for lymphoblasts from a patient with untreated ALL. (B) Similar comparison as in Fig. 4A except that in this case the lymphoblasts were from a patient with ALL who clinically also was resistant to glucocorticoids. (●) Specific binding to SBP; (▲) [^{3}H]thymidine incorporation.

+ SBP $\overset{K_1}{\rightleftarrows}_{K_2}$ [dexamethasone-SBP complex] second order kinetics may apply. If this be the case, a plot of time vs. $\log_{10}$ (unbound dexamethasone concentration/unbound SBP concentration) gives a straight line (Fig. 5B), consistent with second order kinetics in the binding reaction (55). Similar straight lines are obtained for the other steroid and receptor concentrations shown in Fig. 5A. From the slope of this line, the association rate constant (K_1) may be calculated to be $3.5 \times 10^7 M^{-1}min^{-1}$.

The dissociation reaction of the dexamethasone SBP complex was also studied. After allowing the SBP to become saturated with radiolabeled dexamethasone, a 1,000-fold excess of nonradioactive dexamethasone was added as a chase and specifically bound counts determined. The rapid decrease in specifically bound counts, even at 4°C, shown in Fig. 6A, demonstrates the reversibility of the cytoplasmic-binding reaction. If it

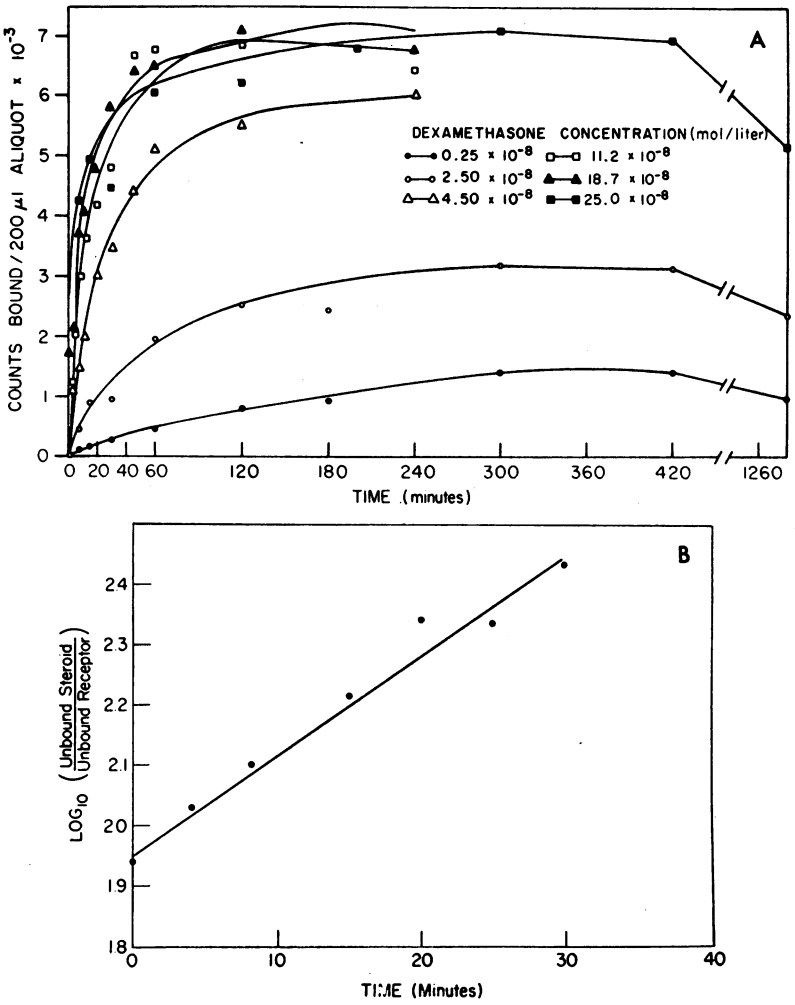

FIGURE 5 (A) Kinetics of association between dexamethasone and lymphoblast SBP. Incubations were at 4°C. (B) Plot of $\log_{10}$ (unbound steroid concentration/unbound receptor concentration) versus time for early time points of the association curve for a steroid concentration of 2.5×10^{-9} M shown in Fig. 5A.

is assumed that the dissociation reaction follows first order kinetics, then a plot of $\log_{10}$ (bound steroid concentration) vs. time should be linear (55) as shown in Fig. 6B. From the slope of this line dissociation rate constant of 1.73×10^{-2} min^{-1} is obtained. From the dissociation and association rate constants, an overall equilibrium (dissociation) constant of 0.5×10^{-9} M can be calculated for the binding reaction as compared with 7×10^{-9} M which was obtained from the equilibrium data for dexamethasone which is not shown.

Affinity of SBP for other steroid analogs. The affinity of various steroids for the SBP was studied in cells from the same patient described in the section above by measuring the ability of competing steroid analogs in 100-fold excess to compete with [^{3}H]dexamethasone in the binding reaction. The concentration of dexamethasone used was 3.8×10^{-7} M. If target tissue specificity is universally determined by SBP, one would expect there to be a good correlation between binding affinity and biologic effect. Table I compares the ability of various steroids to displace dexamethasone in the binding reaction with lymphoblast SBP, with the published results of biologic activity in various other lymphoid systems where known, and with potency of the steroid as an inducer of the enzyme tyrosine aminotransferase in hepatoma tissue-culture cells, another glucocorticoid-response tissue (9). The excellent correlation between binding to this lymphoblast SBP and activity in other lymphoid systems is apparent. The close agreement between steroid analogs in terms of "inhibitory" effects in lymphoid tissue and stimulatory effects in liver is also of interest. Exceptions to the rule

are progesterone and cortexolone which show a marked discrepancy between binding and biologic effect. This is consistent with the observation that association with receptor is only the first of several steps between binding and biologic response (8). There is evidence that the receptor must undergo a temperature-dependent transformation after association with the steroid before uptake by the nucleus can occur (8, 11, 17, 56, 57), a step that does not occur with progesterone and cortexolene (8, 10). Such steroids have therefore been termed "anti-inducers" (21).

DISCUSSION

The present studies reveal that human acute leukemic lymphoblasts from patients still responsive to steroid therapy contain a cytoplasmic protein capable of binding glucocorticoids with an affinity appropriate to the response of these cells to steroids. Furthermore, the kinetics of association and dissociation described in the present study are adequate to allow for the known time course of steroid effect in these cells (8, 21, 22, 31, 54, 58, 59). The steroid-unresponsive cells examined in this study lack binding activity. These findings suggest a physiologic role for this SBP in glucocorticoid action in humans. As previously mentioned, highly specific steroid-receptor molecules have been found in many other steroid-responsive tissues. For reasons which are not clear however, when steroid unresponsiveness occurs, it is frequently the initial binding step which appears to be affected (5, 15, 18, 24–26). For example, Pratt and Ishii found that mouse fibroblasts which were no longer growth inhibited by glucocorticoids had a loss of 80–90% of steroid-binding activity (25). They and co-workers have succeeded in partially characterizing this SBP as a high molecular weight (~620,000) substance containing both protein and phospholipid based on its sensitivity to phospholipase as well as proteases (46). The lymphoblast SBP described here was not sensitive to phospholipase action under the conditions we employed. Rosen, Fina, Milholland, and Rosen studied a transplantable lymphocarcoma (P1798) which was glucocorticoid responsive (59). Repeated treatments with cortisol led to the emergence of a resistant tumor line which showed a decrease of over 90% in detectable cytoplasmic steroid-binding molecules. Aronow and Gabourel first developed a hydrocortisone-resistant subline of a mouse lymphoma in vitro (60). Using a similar technique, Baxter, Harris, Tomkins, and Cohn studied several mouse lymphoma lines in culture which are lysed by glucocorticoids. Glucocorticoid-resistant cells appeared in their cultures with a frequency of 10^{-5}–10^{-6} (19). In these resistant cells cytoplasmic steroid-binding activity was found to be reduced from 50 to 90% (19). Rosenau, Baxter, Rousseau, and Tomkins were able to separate the cytoplasmic-binding step from nuclear uptake of steroid (18). By recombination experiments they showed that nuclei from steroid-resistant cells took up steroids normally when mixed with cytoplasm from sensitive cells whereas nuclei from steroid-sensitive cells did not show uptake of steroid when mixed with cytoplasmic extract derived from resistant cells.

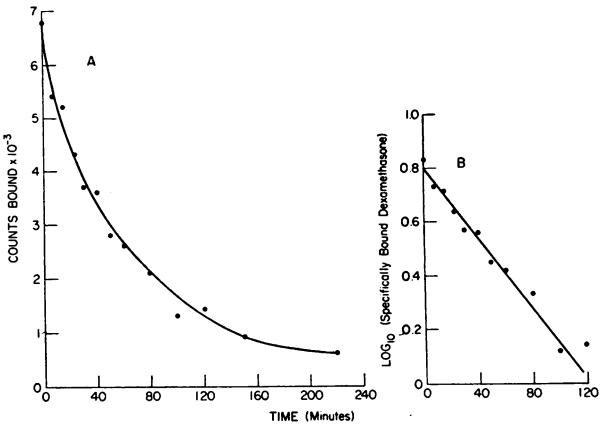

FIGURE 6 (A) Kinetics of dissociation of the lymphoblast SBP-dexamethasone complex at 4°C. After a 1 h incubation with radioactive dexamethasone a 1,000-fold excess of nonradioactive dexamethasone was added to the incubation mix and specific binding determined at the times shown. (B) Plot of $\log_{10}$ (bound steroid) vs. time for the data shown in Fig. 6A.

There has been much recent interest in the responses of lymphoid cells to glucocorticoids (17, 22, 31–39, 54, 57–67). Some early events in the action of steroids on lymphoid tissue have included a decreased uptake of substrate, particularly glucose (17, 31, 62, 63), decreased transport of amino acids (39, 64) and decreased incorporation of precursor small molecules into protein and nucleic acids (22, 32, 33, 37, 38, 53). All of these early effects apparently require protein and RNA synthesis in that they can be inhibited by actinomycin D and cycloheximide (39, 57). This has led to the hypothesis that some key RNA or protein factor is induced by steroid interaction with the nucleus (8, 68).

Several previous attempts have been made to measure in vitro cytotoxicity of steroids using one of the above parameters (30, 32, 33, 36–39). Schrek incubated normal lymphocytes and those from patients with chronic lymphocytic leukemia (CLL) with prednisolone for 7 days, estimating cell viability at the end of the incubation (30). He observed variable cytotoxicity in CLL cells. Normal lymphocytes usually remained viable during the study. This is consistent with our observation of minimal binding activity in normal lymphocytes. Claman, Moorhead, and Benner have also reported a high degree of steroid resistance in human

TABLE I

Comparison of Binding Affinity for Lymphoblast Binding Protein with Biologic Activity of the Steroids in Inhibitory (Lymphoid) and Stimulatory (Hepatoma) Systems

Competing steroid	% [^{3}H]Dexamethasone¶ bound in presence of 100-fold excess of competing steroid	Biologic activity of competing steroid in lymphoid systems*	Reference	Biologic activity in hepatoma tissue culture cells	Reference
Dexamethasone	0	Optimal inducer	19	Optimal inducer	21
				Suboptimal inducer§	21
Progesterone	0	Anti-inducer§	19	Anti-inducer	21
5β-dihydrocortisol	0	Unknown		Unknown	
Hydrocortisone	0	Optimal inducer	17	Optimal inducer	21
Triamcinolone	6	Optimal inducer	17	Optimal inducer	‖
Aldosterone	12	Unknown		Optimal inducer	10
Prednisolone	22	Optimal inducer	‖	Optimal inducer	‖
Deoxycorticosterone	24	Suboptimal inducer	12	Optimal inducer	21
Corticosterone	24	Optimal inducer	16	Optimal inducer	10
Cortexolone	31	Anti-inducer	8	Unknown	
2α-Hydroxycortisol	40	Inactive	18	Unknown	
17α-Methyltestosterone	40	Unknown		Anti-inducer	21
Spironolactone	57	Unknown		Unknown	
Prednisone	69	Inactive‡	‖	Unknown	
		Inactive§	22		
Testosterone	74	Anti-inducer	18	Anti-inducer	21
Cortisone	84	Inactive‡	23	Anti-inducer	21
19-Nortestosterone	84	Unknown		Unknown	
17β-Estradiol	94	Inactive‡	22	Anti-inducer	21
Etiocholanolone	100	Unknown		Unknown	
Tetrahydrocortisol	100	Inactive	12	Inactive	21
Androstenedione	100	Inactive	22	Inactive	21

* The classification of biologic activity is that of Samuels and Tomkins (21).

‡ Because of the method used in assaying biologic effect, the possibility that these steriods are anti-inducers cannot be excluded.

§ Conflicting results published.

‖ Unpublished results.

¶ Dexamethasone concentration was 3.8×10^{-7}M.

thymocytes based on a [^{51}Cr] release assay (34). Werthamer and Amaral have shown a steroid-induced early decrease in uridine incorporation in CLL cells which was greater than that seen in normal lymphocytes (36). Cline and Rosenbaum studied uridine incorporation in ALL cells incubated for 24 h in the presence or absence of glucocorticoids (32, 33). They concluded that in vitro cytoxicity was partially successful in predicting in vivo steroid effect but not in predicting remission induction. Harris studied the specificity of response of mouse lymphoma cells to various steroids (23). The results obtained closely parallel the binding data for these steroids in human lymphoblasts given in Table I.

Although glucocorticoids alone induced remission in only some patients with ALL almost all will show some clinical response to steroid therapy (27, 28, 40, 69, 70). This is consistent with our observation of the presence of binding protein in the untreated newly diagnosed group of patients. There is ample evidence that several antileukemic drugs used in combination may provide a more effective therapeutic regimen (29). In this very setting however, tumor resistance to a single drug may be undetected if response to others persists. It would presumably be of real benefit if one could eliminate the ineffective drug from the regimen at this point thus avoiding needless toxicity. This is particularly true in the case of glucocorticoids because of their many side effects which are poorly tolerated by leukemic patients (29, 69, 71). We initially sought steroid-unresponsive cells in patients who were no longer responsive to the whole drug combination. Therefore, we cannot estimate as yet how many patients are resistant to steroids while responsive to other drugs in the combination.

We must emphasize that further prospective study will be necessary to prove whether a determination of SBP will provide an accurate prediction of glucocorticoid effect in ALL blasts. It will be noted that initially

all patients with ALL had SBP detectable in their lymphoblasts. This correlates well with the fact that steroids will have an ameliorating effect in most untreated patients with ALL. We would expect that in the future, at least some previously untreated patients may present with cells lacking SBP activity. [^{3}H]Thymidine incorporation was inhibited by steroids in all five of the untreated patients studied in this way. The origin of the steroid-resistant lymphoblasts which inevitably seem to arise in patients receiving repeated courses of therapy remains unknown. Whether they arise from a preexisting subpopulation which eventually overgrows or from variants arising due to therapy must still be discovered. Cultured lymphocytoid cells (18) gave rise to resistant cells at a rate too high to be compatable with simple recessive mutations in a diploid locus. This kind of phenomenon has also been described by others (72). Pursuit of this baffling problem in mammalian genetics is important in seeking more effective means of drug therapy.

In patients with acute myeloblastic leukemia (AML), the response rate to glucocorticoids is much lower and then only at relatively high doses of drug (27, 28, 40, 68). One might therefore hope that if an SBP were detectable in AML, binding studies on untreated patients might be able to identify steroid-responsive patients and even provide some guidelines for doses needed to saturate the SBP. In preliminary studies, we have found that cytoplasmic extracts from myeloblasts of previously untreated patients frequently have no glucocorticoid-binding activity whereas extracts from a few patients have SBP activity. The significance of these findings awaits further observations.

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