Abnormal Glucocorticoid Receptors in Acute Leukemia Cells

By Ronald McCaffrey, Anne Lillquist, and Richard Bell

In normal tissues, $^3$H-triamcinolone acetonide ($^3$H-TA) labeled glucocorticoid receptors can be resolved into 2 components by DEAE chromatography: peak I elutes at 0.04 M salt and peak II at 0.22 M salt. By glycerol gradient centrifugation, peak I is 3.5S and peak II is 8.5S. Peak I binds to DNA, while peak II does not. Blast cell $^3$H-TA-binding macromolecules in 27 of 62 cases of acute leukemia had DEAE binding characteristics identical to those of normal tissues; the remaining 35 cases were abnormal. In these cases there was either a single DEAE component by DEAE chromatography: peak I elutes at 2-2.5S, whereas in 5 cases with normal peak I-peak II ratios, the S values were 3.5S and 8.5S, respectively. We hypothesize that those leukemias with abnormal binder characteristics cannot respond to glucocorticoid therapy.

CLINICAL TRIALS with single agent glucocorticoids in acute leukemia established several years ago that the majority of cases of acute lymphoblastic leukemia were responsive with 65% achieving initial remission, whereas patients with acute myeloblastic leukemia were usually resistant, with only 15%-20% of cases responding.1 3 No direct comparisons between glucocorticoid receptor status and clinical outcome were made in these early clinical trials. Based on data from cell culture systems, where mutations to a glucocorticoid-receptor binding site may be resolved into 2 components, one might have predicted that the presence or absence of such receptors in leukemic cells would be a major determinant of clinical glucocorticoid responsiveness. In recent studies, where receptor measurements have been made, such a straightforward relationship has not been demonstrated: virtually all cases of untreated acute leukemia have been shown to have blast cell glucocorticoid receptor material, with overlapping ranges for myeloblastic and lymphoblastic disease.5 11 This lack of concordance between receptor status and clinical outcome gives rise to questions about the physiologic significance of the cellular components being measured as “receptors” in standard quantitative assays. We have hypothesized that these quantitative assays measure a heterogeneous group of binding macromolecules, of which only a subset functions as physiologic mediators of glucocorticoid action. We have therefore initiated a series of studies comparing certain biochemical and biophysical characteristics of glucocorticoid binders in normal cells with those in leukemia cells to determine if abnormal, dysfunctional binding macromolecules exist in leukemic cytosols.

MATERIALS AND METHODS

Reagents

$^3$H-triamcinolone acetonide ($^3$H-TA), 22 Ci/m mole, was purchased from Amersham, Arlington Heights, Ill.; Sephadex G-25 low-amplitude peaks eluting across the entire gradient (5 cases). The abnormal single peak material failed to bind to DNA in 5 cases (of 5 studied), whereas peak I material from 5 cases (of 5 studied), showing normal peak I-peak II ratios, bound normally to DNA. In 3 cases (of 3 studied), the abnormal single peak material had an S value of 2-2.5S, whereas in 5 cases with normal peak I-peak II ratios, the S values were 3.5S and 8.5S, respectively. We hypothesize that those leukemias with abnormal binder characteristics cannot respond to glucocorticoid therapy.

Cells and Tissues

Normal tissues. Thymus, spleen, lymph node, and lung from 3-5-day-old calves were obtained at a local slaughter house, placed on dry ice immediately after death, and trimmed of fat and connective tissue. If not used immediately, tissues were stored as aliquots (approximately 10 g of whole tissue) at −90°C. For certain experiments, single cell suspensions were made from fresh tissue as previously described,12 and stored as pellets at −90°C if not used immediately. Bone marrow mononuclear cells were harvested on LSM from BALB/C mice and used immediately. Fresh human thymus was obtained from a 7 yr old child undergoing cardiac surgery and stored as whole tissue at −90°C. Human peripheral blood mononuclear cells were harvested on LSM from CPD anticoagulated whole blood from normal adults and stored as pellets at −90°C.

Leukemia cells. Sixty-two patients with acute leukemia were studied, 57 at the time of first diagnosis, prior to the institution of any therapy, and 5 at relapse prior to reinduction therapy. No patient had received steroid medication within the 2 wk prior to study. Diagnoses were based on classical clinical and laboratory criteria.12 Twenty-six patients (22 new cases, 4 relapses) had acute lymphoblastic leukemia (ALL); 20 (19 new, 1 relapse) had acute lymphoblastic leukemia (ALL); 20 (19 new, 1 relapse) had acute lymphoblastic leukemia (ALL).
myeloblastic leukemia (AML); and 16 had blastic chronic myelogenous leukemia (CML).

Among the 26 ALL patients there were 14 children aged 3-16 yr; the remaining 12 were adults aged 18-70 yr. In 20 cases (10 children, 10 adults) peripheral blood blast cells were studied. White blood cell counts in these patients ranged from 7800 to 360,000 per cu mm, with blast cell percentages of 75%-90%. In 4 of these patients, simultaneous bone marrow aspirates, containing more than 90% blast cells, were also studied. The remaining 6 ALL patients (4 children and 2 adults) had circulating white cell counts of less than 10,000/cu mm, with less than 50% blast cells. In these, bone marrow aspirates, containing more than 90% blast cells, were studied. Terminal deoxynucleotidyl transferase (TdT) activity was determined2 on 24 of the 26 ALL samples (2 childhood ALL samples were not tested for TdT); all were TdT-positive. Multiple markers3 were determined on 9 of the 14 childhood ALL samples. One of these had T-cell disease (HTA+, cALLa-, Ia-), the eighth had the phenotype HTA-, cALLa+, Ia+, TdT-; and the ninth had the phenotype HTA+, cALLa+, Ia+, TdT+. Marker data, beyond TdT determinations were not obtained on the 12 adult cases.

Among the 20 AML cases there was one child age 12 yr; for the remaining 19 cases the age range was 22-73 yr. Circulating white cell counts in these 19 cases ranged from 6000 to 121,000/cu mm; all samples contained at least 80% blast cells. In one of these 19 cases a simultaneous marrow aspirate, containing 85% blast cells, was also studied. The 20th AML patient had a peripheral blood white cell count of 6000/cu mm, with 30% blast cells; a marrow aspirate, containing 95% blast forms, was studied in this patient. All 20 AML samples were TdT-negative; additional market studies were not performed.

The 16 blastic CML patients ranged in age from 30 to 73 yr. All were Philadelphia chromosome-positive. White cell counts ranged from 37,000 to 200,000 cu mm; all contained at least 45% blast cells. Nine were TdT-positive; 7 were TdT-negative. Additional marker studies were not performed.

LSM sedimentation15 was used for harvesting and enriching blast cells from all samples. After extensive washing in PBS (see below) the blast cells were stored as pellets (containing between 2 x 10^6 and 2 x 10^7 cells), and stored at -90°C until studied. In 2 AML cases, peripheral blood blast cells were studied immediately after collection.

Cytosol Preparation and Labeling

The procedures used were modified from the broken cell labeling system of Sakaue and Thompson,14 as previously described. Briefly, aliquots of cytosol, prepared in KD buffer (5 mM K2HPO4, pH 7.6, with 0.5 mM dithiothreitol), containing 15-20 mg protein/ml, were incubated with 3H-TA at 2 x 10^-4 M for 2 hr. Unbound steroid was removed by absorption with charcoal and Sephadex G-25 filtration: quantitation of total cytoplasmic 3H-TA binding was based on radioactivity in the G-25 void volume and expressed as binding sites per 10^6 cells in the starting material. Specific binding was defined as the difference between the amount of 3H-TA binding in the presence and absence of a 300-fold excess of radioinert TA. At 2 x 10^-4 M, 3H-TA specific binding was 93%-97% of total binding, and near saturation was achieved after 2 hr at 4°C. Activated receptor complexes were generated by shifting aliquots of labeled cytosols after G-25 filtration from 4°C to 20°C for varying time periods up to 60 min.

Analytical Procedures

All procedures were performed at 4°C as previously described. Briefly, 3H-TA-labeled materials were applied to DEAE cellulose columns (2 x 6 cm) and DNA cellulose columns (2 x 3 cm) equilibrated with KD buffer. After sample application the columns were washed with KD buffer until flow-through radioactivity was cleared, and developed with 30 ml linear gradients of 0.005-0.4 M K2HPO4 in KD buffer. Fraction size was 1 ml and 200 A aliquots were used for radioactive counting. Binding to DNA cellulose was calculated from the difference between the radioactivity applied to the column and that eluted by the salt gradient, and expressed as a percentage of the total 3H-TA-labeled material applied.

Linear gradients of 10%-30% glycerol in KD buffer were used for isokinetic sedimentation analyses of 3H-TA-labeled complexes eluting from DEAE columns, as previously described. All samples (500 A) were layered on 4.6 ml gradients in polyallomer tubes and spun at 48,000 rpm for 17 hr in a SW 50.1 rotor. Five-drop fractions were collected from the bottom of the gradients and used for radioactivity measures. Scientists established in relation to standard 14C markers which were run on companion gradients and processed identical.

RESULTS

DEAE Chromatography

Normal Tissues

Receptors from all normal cytosols (peripheral blood cells, bone marrow, spleen thymus, lymph nodes, and lung) eluted as 2 species from DEAE cellulose columns. Shown in Fig. 1 are the patterns obtained with normal human thymus, human peripheral blood cells, and mouse bone marrow cells. We call this two-peak profile the normal tissue pattern, and refer to the 0.04 M salt component as peak I and the 0.22 M salt component as peak II. Radioactivity in both peaks was macromolecularly bound, as defined by Sephadex G-25 filtration, and was lost equivalently from both peaks (93%-97% reduction in both peaks) when the incubations were done in the presence of a 300-fold excess of radioinert TA. By OD at A280, a major protein peak (about 35% of the applied total protein) was eluted in the peak I area with less than 4% of the applied A280 material eluting across the peak II area. Similar data have been reported by Sakaue and Thompson for normal rat tissues, and HTC and LA-9 cell lines.14

Leukemia Cells

Of the 26 ALL samples studied, 16 (62%) had DEAE chromatography characteristics identical to those found in normal tissues (Fig. 2A). In the remaining 10 cases, the DEAE patterns were abnormal. In 6 of these 10 cases, the abnormality was that the macromolecular bound 3H-TA material eluted from the DEAE as a single peak at 0.04 M salt (Fig. 2B). The remaining 4 cases (who had quantitatively the lowest binding among the 26 ALL cases studied) were abnormal in that the 3H-TA-labeled material eluted from the DEAE as multiple peaks across the entire gradient (Fig. 2C). All 14 childhood ALL samples had the
normal tissue DEAE pattern. Of the 12 adult ALL samples, only 2 had the normal tissue profile.

Among the 20 AML samples studied, 8 (40%) showed the normal tissue DEAE characteristics, while the remaining 12 (60%) showed the abnormal single 0.04 M peak species, which was also seen in 6 of the ALL cases (Fig. 2B). No examples of multiple peaks were seen with the AML samples. The single childhood AML sample showed the abnormal single peak binder.

Among the 16 blastic CML cases, only 3 (19%) had the normal tissue DEAE pattern. Of the remainder, 12 cases (75%) had the abnormal single peak binder and 1 case has the multiple low amplitude peak pattern.

TdT status was not related to DEAE pattern; of the 9 TdT-positive cases, 3 had the normal peak I–peak II pattern and 6 had the abnormal single peak pattern. These data are summarized on Table 1.

**Mixing Experiments**

In order to investigate the generation of the abnormal single peak binder, an aliquot of leukemic cells (from a case of common ALL) was mixed in a one-to-one ratio with an aliquot of AML cells showing the abnormal single peak pattern. This cell mixture was
Fig. 3. DEAE chromatography of $^3$H-TA-labeled glucocorticoid receptors from human leukemic blast cells. (A) The normal tissue pattern derived from a case of childhood common ALL ($7.2 \times 10^8$ cells); (B) the abnormal single peak pattern derived from a case of AML ($7 \times 10^8$ cells); (C) the chromatogram obtained from mixing $7.2 \times 10^8$ cells from case A with $7.0 \times 10^8$ cells from case B. Data shown represent actual cpm/0.2-ml aliquots of 1-ml fractions.

Cytosols from 5 leukemias (3 childhood common ALL, 1 AML, and 1 CML) were lysed to generate a mixed ALL–AML homogenate and processed through the $^3$H-TA labeling and DEAE chromatography procedures. The binders maintained their individual identities (Fig. 3): the “mixed” DEAE chromatogram shows the individual peaks existing in additive ratios. In a second such mixing experiment (data not shown), an aliquot of AML cells with the normal tissue pattern mixed with an aliquot of single-peak AML cells also showed a DEAE chromatogram with additive peak ratios. Thus it does not appear that the factor(s) responsible for the generation of the abnormal single peak material affect the normal tissue-type binders. This suggests that the abnormal single-peak pattern is “stable” and not generated during the in vitro manipulations.

Heat Activation

DEAE Chromatography

Cytosols from 5 leukemias (3 childhood common ALL, 1 AML, and 1 CML) that showed the normal tissue peak I–peak II DEAE profile continued to behave like normal tissues when DEAE chromatography was performed after heat activation. Under these conditions, with normal tissues, the DEAE chromatographic patterns progressively change from peak II to peak I predominance with increasing duration of exposure of the labeled cytosol to 20°C before chromatography. This alteration has been shown to be due to the conversion of peak II complexes to material having peak I characteristics. The DEAE profiles of 1 of these 5 normal pattern leukemic samples before and after 30 min at 20°C is illustrated in Fig. 4 A and B. Cytosols from 5 other leukemias (1 adult ALL, 1 AML, and 3 CML) showing the abnormal single peak pattern were similarly studied. As shown for 1 representative case (Fig. 4 C and D), cytosols showing the abnormal single peak pattern, maintain this pattern after heating to 20°C for periods of up to 60 min.

DNA Cellulose Binding

Sufficient cells were available from 10 leukemic cases to study DNA cellulose binding. Five of these (3 childhood common ALL, 1 AML, 1 CML) had the normal tissue DEAE characteristics and 5 (1 adult...
Fig. 5. DNA cellulose chromatography of peak I material from heated cytosols. (A) The elution from DNA cellulose of heat-activated peak I complexes from a case of childhood ALL with the normal DEAE pattern. In the example shown, 34% of the applied 3H-TA material eluted at 0.1 M salt; (B) the elution from DNA cellulose of heated peak I material from a case of childhood AML with the abnormal single peak DEAE pattern. In the example shown, 3% of the applied 3H-TA material eluted at 0.04 M salt. For reference, the elution from DNA cellulose of heat-activated peak I material from calf thymus is shown in C. In the example shown, 37% of the applied material eluted at 0.1 M salt. Data shown represent actual cpm/0.5-ml aliquots of 1-ml fractions.

ALL, 1 AML 3 CML) had the abnormal single peak profile.

Activated peak I complexes from the 5 leukemic cytosols that showed normal initial DEAE chromatograms also bound normally to DNA cellulose columns (Fig. 5A). In contrast, the labeled complexes from the 5 leukemias with the single peak pattern showed insignificant binding to DNA cellulose either before or after heat activation (Fig. 5B). For reference, DNA binding of activated peak I complexes from a typical normal tissue (calf thymus) is shown in C. In the example shown, 37% of the applied material eluted at 0.1 M salt. Data shown represent actual cpm/0.5-ml aliquots of 1-ml fractions.

DEAE profiles fell within this range, with the bound material eluting (as shown in Fig. 5A) as single peak at 0.1 M salt. This material was shown to represent macromolecular-bound 3H-TA by filtration over Sephadex G-25. The flow-through and wash fractions from normal tissues and leukemic samples with normal DEAE profiles contained, on average, 40% of the applied 3H-TA radioactivity (range 35%–45%). By Sephadex G-25 filtration, most of this 3H-TA remained bound to macromolecules, less than 20% was free 3H-TA. In contrast, the flow-through and wash fractions from those leukemias showing no affinity for DNA contained 55%–70% of the applied 3H-TA radioactivity, most of which also remained bound to macromolecules. With all cells and tissues, both normal and leukemic, 30%–40% of the applied 3H-TA radioactivity remained firmly bound to the column and could not be eluted even with 1 M salt. With plain cellulose, this tight background binding also occurred: 65%–70% of the applied material appeared in the flow-through, while the remainder stayed firmly bound even with 1 M salt. The determinants of this tight binding are unknown.

Peak II complexes, generated from normal tissues, fail to bind to DNA cellulose. After heat activation of this peak II material (which converts peak II complexes to material with peak I characteristics), its affinity for DNA cellulose increases to an average of 20%.

In the 5 leukemic cases with normal tissue DEAE characteristics studied for DNA cellulose binding, direct activation of peak II complexes resulted in DNA cellulose binding, which ranged from 26% to 32% (average 29%) of the applied material.

Isoelectric Sedimentation

DEAE-derived peak I and peak II complexes from normal tissues have sedimentation coefficients on glycerol gradients of 3.5S and 8.5S, respectively. Similar analyses were performed on 5 leukemic samples with normal DEAE chromatograms (3 common ALL, 2 AML). Peak I and peak II complexes from these cases were also 3.5S and 8.5S, respectively. In contrast, the 3H-TA-labeled material from 2 leukemic samples (1 adult ALL, 1 childhood AML, 1 CML) showing the abnormal single peak DEAE pattern had a sedimentation coefficient of 2–2.5S (Fig. 6).

Quantitative Binding

Total and specific 3H-TA binding for samples with normal tissue DEAE chromatographic profiles were compared with samples showing the abnormal single peak pattern (see Table 1). Under the conditions used here (single point determinations at $2 \times 10^{-8} M)$
In 4 additional cases (1 adult ALL, 2 AML, 1 cases.

cells, a normal tissue DEAE of up to 11 mo at -90°C. With both fresh and frozen
steroid therapy, were studied. In these the DEAE
studies. In 2 AML patients, cell aliquots were studied
blastic CML) repeat peripheral blood
examples encountered no
immediately after harvesting and
after
pattern to another in a total of 42 repeat DEAE
from
peripheral blood cell aliquots were available for study.
8 patients with the normal DEAE
patterns were concordant. In 8 patients with the normal DEAE
pattern and 5 with the single peak pattern, multiple
peripheral blood samples were available for study, the
In these, the DEAE
profiles
were identical to those of normal tissues by DEAE chroma-
tography, the mean 3H-TA binding was 798 femtomoles per 10^8 blast cells (range 152–
13,320). For samples with the abnormal single peak
DEAE pattern the mean was 500 femtomoles per 10^8 blast cells (range 188–16,280). In 5 additional
leukemic samples stored at -90°C in 10% DMSO, we
failed to detect any 3H-TA binding, even after the
removal of the DMSO by standard techniques. No samples stored in DMSO are therefore included in this
study.

Reproducibility of Results

In the five cases where simultaneous marrow and
peripheral blood samples were available for study, the
DEAE profiles (3 normal, 1 single peak, 1 “smear”) were concordant. In 8 patients with the normal DEAE pattern and 5 with the single peak pattern, multiple
peripheral blood cell aliquots were available for study.
In these, the DEAE profiles were “stable.” We
encountered no examples of “switching” from one
pattern to another in a total of 42 repeat DEAE studies. In 2 AML patients, cell aliquots were studied
immediately after harvesting and after storage periods
of up to 11 mo at -90°C. With both fresh and frozen
cells, a normal tissue DEAE pattern was seen in both
cases. In 4 additional cases (1 adult ALL, 2 AML, 1
blastic CML) repeat peripheral blood samples taken
from 1 to 11 days apart, without intervening chemo-
steroid therapy, were studied. In these the DEAE
patterns were concordant (3 normal tissue type patterns, 1 single peak pattern). An interval of up to
22 hr in EDTA anticoagulant tubes at temperatures
between 4°C and 20°C before sample harvesting on
LSM and storage at -90°C appears to have no affect
on the DEAE profile of leukemic cells. Five ALL
samples were held in EDTA at 20°C for 4 hr and 4°C
for 18 hr before harvesting and storage. All 5 exhib-
ited the normal tissue DEAE profile. Likewise, normal
peripheral blood or marrow DEAE profiles are not altered by up to 24 hr at 4°C in EDTA before LSM
harvesting.

Cytoplasmic glucocorticoid receptors can be char-
acterized by a variety of techniques. Among these, ion
exchange chromatography, isokinetic sedimentation
analysis, and affinity for DNA have proven to be
useful in analyzing receptor structure and function. By
DEAE chromatography, labeled cytoplasmic gluco-
corticoid receptors from a variety of normal tissues
can be resolved into 2 components. These
components, referred to as peak I (early eluting) and
peak II (late eluting) are 3.3S and 8.5S, respectively.
Peak I binds to DNA; Peak II does not. Following
activation (by heat, change in ionic strength or pH),
peak II complexes acquire peak I characteristics,
changing from an 8.5S configuration to a 3.5S form,
eluting from DEAE in the peak I area and showing
affinity for DNA. The peak I material that is seen
variably in the initial, “preactivated” DEAE chro-
matograms of normal tissues (Fig. 1) may be
accounted for by slow activation occurring during the
analytical procedures.

The 62 leukemic samples we studied by these tech-
niques segregated into 2 groups: those with normal
tissue binder characteristics (27 cases) and those with
abnormal binders (35 cases). Normal and abnormal
patterns were noted in all three acute leukemia vari-
ants (Table I). Sixteen of 26 cases (62%) of acute
lymphoblastic leukemia, 8 of 20 cases (40%) of acute
myeloblastic leukemia, and 3 of 16 cases (19%) of
blastic CML had 3H-TA-labeled cytoplasmic binders
identical to those of normal tissues by DEAE chroma-
tography. In 30 of the remaining 35 cases, the 3H-
TA-labeled binders eluted as a single species from
DEAE in the peak I (0.04 M salt) region. This
abnormality was present in 10 of the 26 (23%) ALL
cases, 12 of the 20 (60%) AML, and in 12 of the 16
(75%) blastic CML cases. The final 5 abnormal cases
(who quantitatively had the lowest binding in the
series) showed multiple low-amplitude peaks eluting
across the entire DEAE gradient. Four of these multi-
The abnormal single peak material had no affinity for DNA cellulose in 5 of 5 cases studied, whereas affinity for DNA cellulose was normal in 5 other cases (of 5 studied) in which the initial DEAE profiles were normal. Furthermore, in 3 of 3 cases studied, the abnormal single peak DEAE material had a sedimentation coefficient of 2–2.5S. Five samples with normal tissue initial DEAE profiles had peak I–peak II S values identical to those of normal tissues (3.5S and 8.5S, respectively). Thus, while the abnormal single peak DEAE material cannot be distinguished from normal peak I complexes by DEAE chromatography alone, further characterization on DNA cellulose and glycerol gradients clearly differentiate between the two forms. The smaller S value suggests a protease effect in the generation of the abnormal single peak material. Sherman et al., Stevens et al., and Wrange and Gustafsson have independently characterized several proteolytically modified steroid receptor species in which digested receptor fragments continued to have intact ligand binding sites but displayed altered nuclear or DNA affinity. If proteolytic modification of receptor material is responsible for the generation of the abnormal binders we have identified, the proteolysis does not proceed in vitro under the conditions we have used for receptor identification. In the mixing experiments, where blast cells having the abnormal receptor characteristics were homogenized together with blast cells showing normal receptors, the “mixed” normal–abnormal cytosols retained their original DEAE characteristics (Fig. 4). Proteolytic receptor modification is not totally excluded by such mixing experiments, however. We are presently investigating modified extraction and labeling conditions, including the effects of a variety of protease inhibitors, on receptor characteristics.

It is also possible, although unlikely, that the abnormal single peak material is somehow generated during the interval between storage at –90°C and assay. We have not yet had an opportunity to study a fresh (unfrozen) sample containing the abnormal single peak material. Two fresh samples were studied in this series; both had a normal peak I–peak II DEAE profile. Future studies will define whether the abnormal single peak can be identified in fresh unfrozen cells.

We did not encounter any examples of “activation-labile” receptor complexes, as reported by Schmidt, Harmon, and Thompson, for the steroid-resistant human leukemia cell line CEM-4R4. We heat-activated a total of 5 leukemic samples with normal initial DEAE profiles; none showed receptor lability following activation. We anticipate, however, on the basis of the CEM-4R4 data that examples of this receptor abnormality will be uncovered in future patients.

Our speculation is that leukemias with abnormal binder characteristics may be incapable of responding to glucocorticoid therapy. The trend in our data on the distribution of normal–abnormal receptor phenotypes among patients with the major variants of acute leukemia is beginning to show statistical concordance with historical data from the single-agent era on glucocorticoid responsiveness among these variants. However, our speculation will require more cogent validation than the simple establishment of statistical concordance with historical data. The 62 patients in our series all received multiple agent chemotherapy, and we have therefore made no attempt to correlate clinical outcome with receptor characteristics. We are presently studying the relationship between receptor phenotype and responsiveness to single-agent prednisone in an animal model using the spontaneous lymphoblastic leukemia-lymphoma of domestic cats and dogs. This disease, which has many similarities to lymphoblastic disease in humans, shows a 40% response rate to single agent glucocorticoid therapy. In preliminary studies we have identified both the normal tissue and the abnormal single peak DEAE receptor patterns from the blast cells of these animals. In this animal model we will directly correlate receptor characteristics with therapeutic outcome.

The establishment of a clearly defined responder phenotype for glucocorticoid receptors in leukemia cells would permit restriction of the use of glucocorticoids to those patients in whom such receptors could be identified, thus eliminating unnecessary exposure to what may sometimes be serious systemic glucocorticoid toxicity. The ability to unequivocally identify physiologically functional receptors would also allow a rational pursuit of detailed molecular investigation of
the mechanisms involved in steroid-induced remissions in leukemia.

ACKNOWLEDGEMENT

The assistance of Dr. Stanley Marks during the early phase of this study is acknowledged. We are indebted to Drs. P. Beardsley, F. Bunn, R. Burgess, P. Chervenick, F. Coco, J. Crowley, J. Cuttner, H. Finkel, J. Glass, R. Hellwig, R. Mayer, W. Moloney, P. Quesenberry, T. Pisciotta, K. Rai, J. Rappeport, D. Rosenthal, S. Sallan, A. Sawitsky, R. Shudduck, R. Sullivan, H. Weinstein, and L. Weintraub for providing us with the clinical samples analyzed in this study, and to Dr. T. A. Lister for providing the samples stored in DMSO.

REFERENCES

Abnormal glucocorticoid receptors in acute leukemia cells

R McCaffrey, A Lilquist and R Bell