High Glucocorticoid Receptor Content of Leukemic Blasts Is a Favorable Prognostic Factor in Childhood Acute Lymphoblastic Leukemia


We have previously shown that the number of glucocorticoid receptors (GR) per cell in malignant lymphoblasts from children with newly diagnosed pre-B- and early pre-B-cell acute lymphoblastic leukemia (ALL) has a positive correlation with the probability of successful remission induction (Quddus et al, Cancer Res. 45:6482, 1985). We report now on the long-term outcome for these patients treated on a single protocol with 3 different treatment arms. All of which included glucocorticoid pulses during maintenance therapy. GR were quantitated in leukemic cells from 546 children with ALL at the time of diagnosis. Immunophenotyping studies were performed on all specimens. Prior studies showed that in pre-B- and early pre-B-cell ALL, successful remission induction was associated with a median GR number of 9,900 sites/cell, whereas induction failure was associated with a median receptor number of 4,800 sites/cell. Long-term follow-up of these patients shows an association between higher GR number and improved prognosis. The 5-year event-free survival of 61.0% (SE 2.8%) for patients whose leukemic cells had greater than 8,000 receptors/cell and 47.3% (SE 3.3%) for those with less than 8,000 receptors/cell is significantly different (P < .001). This difference remains significant when adjusted multivariately for blast immunophenotype and clinical risk factors (P < .001) or for treatment type (P < .001). We conclude that GR number greater than 8,000 sites/leukemic cell is a favorable prognostic marker for children with acute lymphocytic leukemia. This finding offers deeper insights into molecular mechanisms of anti-leukemia therapy and suggests that manipulation of steroid receptor number might augment the antitumor response, thus opening new avenues for basic and clinical research.

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GLUCOCORTICOIDS have historically been effective therapeutic agents of therapy for children with ALL. The effect of glucocorticoids has long been understood to be mediated through binding to the glucocorticoid receptor (GR) protein. In recent years, the cloning of the GR gene has led to a detailed description of its biology at the molecular level and to its classification into a superfamily whose members include the steroid, thyroid, and retinoic acid receptors. It is now clear that these receptors comprise a class of ligand-responsive regulators of gene transcription. The intracellular level of GR has been shown to influence steroid responsiveness or resistance, and it has been attractive to hypothesize that the intracellular level of GR in leukemic blasts might correspond to the sensitivity of those cells to steroid-induced lysis.1,8

In childhood acute lymphoblastic leukemia (ALL), in vitro laboratory data, as well as data from single-institution clinical trials with relatively small numbers of patients, have suggested an association of GR level to in vivo efficacy of glucocorticoid-containing combination chemotherapy regimens. GR is found in normal lymphocytes at approximately 2,500 to 5,400 sites/cell,3,4 and in leukemic lymphoblasts, GR levels range from 1,000 to greater than 20,000 sites/cell. In one study of 21 patients with newly diagnosed ALL, GR level less than 6,000/cell was associated with a median survival of 19 months, and a GR level greater than 6,000 with a median survival greater than 31 months.8 In another single-institution study, follow-up of 43 children with newly diagnosed ALL was assessed in light of GR level at diagnosis. These data showed that a GR level less than 16,000 sites/cell was associated with more frequent induction failure and relapse, even when adjusted for clinical variables known to affect prognosis, such as leukocyte count above 100,000 cells/μL, positive erythrocyte rosette test, central nervous system involvement, and presence of a mediastinal mass.9

In view of these encouraging preliminary data, in May 1981 the Pediatric Oncology Group (POG) began a multicenter, prospective study of the prognostic significance of GR levels in leukemic blasts at initial diagnosis of ALL in children. GR levels were quantitated by a whole cell method, and immunophenotyping studies were performed on leukemic cells from 546 children with newly diagnosed pre-B- and early pre-B-cell ALL. Early follow-up data were reported in 1985.3 These data show that a statistically significant correlation exists between GR content and probability of successful remission induction. Of the patients with early pre-B-cell ALL, 291 patients achieved remission status, and 13 did not. The median GR content in the leukemic blasts of those patients who achieved remission was 9,900 recep-
tors/cell, in contrast to a median GR content of 4,800 in those who did not achieve remission (P = .034). The following report concerns the long-term event-free survival (EFS) in this population of patients with ALL.

MATERIALS AND METHODS

Patient selection and immunophenotyping. Materials, methods, and characteristics of the patient population in this study have been previously reported in detail.9 Informal consent was obtained, and patients were registered on the POG Acute Leukemia in Childhood (ALinC) 13 protocol (POG 8036).10,11 Patients enrolled on ALinC 13 between May 1981 and July 1984 were eligible for inclusion in the GR study, although enrollment in the GR study was not required for entry into ALinC 13. All patients were less than 22 years of age. Patients with central nervous system involvement, age less than 1 year, or with the presence of the Philadelphia chromosome were excluded. Only leukemia samples that contained an adequate number of viable blasts (>5 x 10^6) were studied. Immunophenotyping studies were performed by previously reported methods; major immunophenotype assignments included T-cell, B-cell, pre-B-cell, or early pre-B-cell ALL. Only patients with pre-B- and early pre-B-cell ALL were eligible for this protocol, and only these patients are included in this report. Successful remission induction was defined as fewer than 5% lymphoblasts in a normocellular marrow with no evidence of extramedullary disease 6 weeks after commencement of vincristine and prednisone therapy in patients with pre-B- or early pre-B-ALL.

GR assay. Before initiation of chemotherapy, 3 to 5 mL of bone marrow was aspirated into a heparinized syringe and added to 40 mL RPMI 1640 medium supplemented with 20% fetal bovine serum, penicillin (50 U/mL), and streptomycin (500 μg/mL). Samples were shipped by express mail to the reference laboratory at the Johns Hopkins Hospital at ambient temperature from participating POG-member institutions. Lymphoblasts were purified from marrow by Ficol-Hypaque centrifugation, and glucocorticoid binding was assessed in duplicate or triplicate by whole cell binding of [3H]dexamethasone at 5 different concentrations of ligand. The cell purification and assay techniques have been presented in detail in our previous report.4 Data reported therein confirmed sample-to-sample reproducibility of the assay. These data also showed the stability of the GR in control samples stored in RPMI 1640 medium at room temperature for 1 to 7 days, replicating or exceeding shipping conditions of patient specimens to the reference laboratory by express mail.

Treatment. All patients described in this study were treated on ALinC 13, which has been previously described,10,11 and were entered on study between May 1981 and January 1986. After induction with vincristine and prednisone, good-risk patients were randomized between 2 treatment arms, a standard continuation therapy (S) with 6-mercaptopurine and methotrexate plus prednisone pulses every 4 months or a standard continuation therapy plus intermediate dose methotrexate pulses (SAM) every 2 months. Poor-risk patients were randomized among 3 treatment arms: S, SAM, or a third regimen composed of standard therapy and rotating agents, each combination of which included prednisone. Therapy lasted 3 years. The cutoff for this analysis is November 1990, yielding a follow-up range of 6.3 years to more than 9 years.

Statistical methods. The major analysis is based on EFS, the time from attainment of complete remission to the earliest time of relapse, death from any cause, or last clinical contact. Induction failures are scored as failures at time zero. Comparisons of subgroups with respect to EFS were made using the two-sided log rank test.13 EFS was estimated by the method of Kaplan-Meier14 using standard errors of Peto et al.15 Qualitative and quantitative clinical characteristics were compared using the Pearson χ² test16 and Kruskal-Wallis test,16 respectively. Secondarily, we studied remission duration with exclusion of the 29 induction failures. Because the remission-duration analysis agreed qualitatively with the EFS analysis, we limit this report to EFS. Lastly, follow-up data to October 1992 was analyzed, and results agreed qualitatively with those for the November 1990 cutoff. Because the later follow-up data is not complete, we limit the present analysis to the November 1990 cutoff.

RESULTS

A total of 899 children with newly diagnosed pre-B- or early pre-B-cell ALL on the POG ALinC 13 study were eligible for enrollment on this GR study. Of these patients, a sufficient number of viable leukemic blasts was provided for GR analysis from 546 patients (61%); 282 specimens were unsatisfactory (31%), and specimens were not provided by the member institutions for 71 patients (8%). Of these 546 patients, 292 remain in continuous, complete remission as of November 1990. We evaluated characteristics of the patients for whom GR data could be obtained, compared with those for whom no GR data was obtained. These patient characteristics are analyzed and shown in Table 1. Those patients with GR data are more likely to have a high leukocyte count and poor prognostic clinical features.

GR-site number is shown to correlate with the number of treatment failures (observed v expected) by log rank analysis, as shown in Table 2. The data appear unadjusted (univariate), adjusted multivariately for treatment type, and adjusted multivariately for POG risk group and blast immunophenotype. Based on stepwise analysis, the most significant division appears between patients whose blast GR number was less than 8,000 sites/cell versus those whose number was greater than 8,000 sites/cell (P < .001). In all 3 cases, after adjusting for this division, no significant division emerges at P < .05.

The EFS curves are shown in Fig 1, stratified according to GR content greater or lesser than 8,000/cell. Patients whose

| Table 1. Comparison of Patients for Whom Lymphoblast GR Number Was or Was Not Determined |
|-----------------------------------|--------------|----------------|---------------|----------------|
| Patient characteristics          | Not Sent     | Unsatisfactory | Satisfactory  | P Value |  |
| Total number                      |              |                |               |         |  |
| Standard prognosis                | 44 (82)      | 176 (62)       | 289 (63)      | .021†   |  |
| Female                            | 34 (48)      | 131 (46)       | 256 (47)      | .96*    |  |
| Median leukocyte count (×10^9/L)  | 9.4          | 5.6            | 13.1          | .001†   |  |
| (P < .01)                         | 4.7          | 4.8            | 4.4           | .53†    |  |
| EFS comparison                    |              |                |               |         |  |
| Treatment failures                | 28           | 104            | 254           | .011†   |  |
| Expected failures                 | 32.4         | 128.6          | 225.0         | .050    |  |

Designation of prognosis is assigned by previously determined criteria.10 Prognosis was assigned as either standard or poor. Analysis was performed on groups of eligible patients for whom no specimen was sent, for whom specimens were provided but were unsatisfactory, and for whom the specimens were satisfactory for accurate GR determination. Percentages are given in parentheses.

* Pearson’s χ².
† Kruskal-Wallis test.
‡ Logrank test.
Table 2. Correlation of GR Sites and Number of Treatment Failures (Logrank Analysis)

<table>
<thead>
<tr>
<th>GR No. (sites/cell)</th>
<th>No. of Patients</th>
<th>Failed</th>
<th>Expected†</th>
<th>Expected†</th>
<th>Expected†</th>
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<td>0-2,000</td>
<td>25</td>
<td>14</td>
<td>11.0</td>
<td>12.3</td>
<td>11.0</td>
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<tr>
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<td>67</td>
<td>37</td>
<td>26.9</td>
<td>29.2</td>
<td>27.6</td>
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<td>40</td>
<td>30.5</td>
<td>27.2</td>
<td>30.0</td>
</tr>
<tr>
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<td>70</td>
<td>35</td>
<td>32.2</td>
<td>30.4</td>
<td>34.1</td>
</tr>
<tr>
<td>8,001-10,000</td>
<td>72</td>
<td>31</td>
<td>33.9</td>
<td>34.9</td>
<td>32.5</td>
</tr>
<tr>
<td>10,001-12,001</td>
<td>62</td>
<td>25</td>
<td>30.7</td>
<td>31.4</td>
<td>28.8</td>
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<tr>
<td>12,001-14,001</td>
<td>49</td>
<td>17</td>
<td>26.0</td>
<td>24.8</td>
<td>24.3</td>
</tr>
<tr>
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<td>131</td>
<td>55</td>
<td>62.8</td>
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</table>

† Unadjusted.  †† Adjusted for treatment.  ‡‡ Adjusted for risk and phenotype.

DISCUSSION

In addition to its prognostic value, the determination of tumor cell content of other members of the steroid-receptor superfamily has established importance in several malignancies. For example, levels of estrogen receptor in breast cancer cells provides an important predictor of clinical response to hormonal therapy. In addition, the status of the retinoic acid receptor appears to indicate responsiveness to retinoic acid therapy in acute promyelocytic leukemia (APL). Cultured APL cells differentiate into nonmalignant cells in response to retinoic acid. Clinical complete remissions have been observed in this disease with all-trans retinoic acid, and it is now known that a specific chromosomal translocation common in APL affects the gene for the retinoic acid receptor in a way that influences the response to this agent.

Several studies have been performed to assess the prognostic value of GR level in neoplastic tissue for a variety of malignancies. Data from chronic lymphocytic leukemia and childhood acute nonlymphocytic leukemia have generally failed to show a convincing association of steroid-receptor level to prognosis. Data have appeared promising in non-Hodgkin lymphoma, but the data have not been consistent. However, several studies have consistently shown that GR quantitation might have promise as a prognostic indicator in childhood ALL and, to a lesser extent, in adult ALL. Alternative methods have recently been used for assessing glucocorticoid sensitivity of ALL blasts in children. An in vitro drug sensitivity assay, the MTT assay, showed a positive association of in vitro glucocorticoid sensitivity to long-term outcome in a small group of patients. In other studies of childhood ALL, reduction of circulating leukemic blasts to less than 1,000 blasts/μL after 7 days of prednisone monotherapy and 1 dose of intra-thecal methotrexate was an important, favorable prognostic factor. Such a therapeutic trial might be interpreted as an in vivo assay for leukemic blast sensitivity mediated in part through GR function.

Thus, ALL has been the only disease in which GR content has appeared consistently to be an independent prognostic factor, although previous data have been limited in terms of patient number and duration of follow-up. The strength of this study involves its large patient number and longer follow-up in the setting of a large, multicenter clinical trial. To minimize potential problems with variability of GR assays in different laboratories or by different methods, all specimens in this multicenter trial were assayed for GR content by a uniform technique in a single reference laboratory. The reproducibility of this assay and stability of the specimens during shipping have been previously established.

Initial data from this trial have related GR content to immunologic subtype of ALL. Leukemic blasts from children with T-cell or B-cell ALL, two subtypes with historically poor prognosis with steroid-containing regimens, have median GR contents (4,000 and 3,200 sites/cell, respectively) lower than those in the better prognosis group, pre-B-cell or early pre-B-cell ALL (8,100 and 9,700 sites/cell, respectively). Thus, lower GR content is associated with poor prognosis immunologic phenotypes of childhood ALL.

In addition, this trial has previously related the rate of remission induction to GR content. Patients who achieved remission had a median GR content of 9,900, whereas patients failing induction had a median GR content of 4,800 (P = .034). Thus, higher GR content is associated with a higher probability of successful remission induction.

This report relates higher GR content in leukemic blasts
to better long-term outcome. GR content of greater than 8,000 sites/cell appears to confer a better-than-average prognosis, and GR content of less than 8,000 sites/cell is associated with a poorer-than-average prognosis. Data from this study validate the conclusions derived from an earlier single institution study with a small number of patients and limited follow-up.

It must be noted that GR data could not be obtained for 39% of the eligible patients enrolled on the POG ALL in C13 study due either to failure of the diagnosing institution to provide a specimen for GR analysis or to inadequate numbers of available blasts in the specimen provided; inadequate numbers were more common in patients with a lower leukocyte count and fewer marrow lymphoblasts. This means that investigation of GR content necessarily biases the study population toward patients with high leukocyte count from whom a more cellular specimen could be obtained, and this bias is evident in the comparison of patient characteristics (Table 1). In turn, the higher leukocyte count for patients with GR data biases this group toward a poorer prognosis, because high leukocyte count is independently associated with a poor prognosis. This information must raise the possibility of selection bias, because the group of patients whose blasts yielded GR data had a worse prognosis (5-year EFS = 53.5% vs 61.7%, cutoff date of analysis November 1, 1990). Mitigating against such bias, there was no significant correlation between leukocyte count and GR content in the specimens assayed. In fact, there is considerable variation in the GR content among patients with the same leukocyte count. Furthermore, multivariate analysis of these data supports GR content as an independent prognostic indicator.

It is interesting that, despite multiagent-combination chemotherapy with a relatively limited dependence on systemic glucocorticoid administration, GR content in leukemic blasts remains a statistically significant factor associated with a favorable prognosis in childhood ALL. Because the other antileukemia agents administered in these protocols act by mechanisms not known to involve GR, this piece of data emphasizes the importance of glucocorticoid effect in the elimination of ALL. It should be noted that each treatment area of this protocol did include a 4-week pulse of prednisone every 4 to 16 weeks during continuation therapy, unlike most contemporary ALL treatment regimens. This component of the therapy conceivably may have increased the selective pressure against cells with higher GR content. Alternatively, it is possible that GR level is a marker for cellular characteristics that might affect the response to the other antileukemia agents used in this treatment protocol. However, recently gained knowledge concerning the function of the GR in lymphocyte cell death supports the hypothesis that GR function is of direct importance in treatment of ALL.

Additional studies suggest that different levels of GR may yield different cellular responses to glucocorticoid. Normal lymphocytes, with a GR content of 2,500 to 5,400 sites/cell, are steroid sensitive yet fully recover after high-dose glucocorticoid therapy. Our previous data have shown that failure of remission induction is associated with a median lymphoblast-GR content of 4,800 sites/cell, a number similar to normal lymphocytes. Similar to normal lymphocytes, these malignant lymphoblasts of relatively low GR content are not completely eradicated during induction therapy. However, successful remission induction is associated with a higher median blast-GR content of 9,900, suggesting that GR content is a predictor of successful tumor kill.

Our current long-term results validate and extend this paradigm, using the more important outcome variable of EFS rather than initial remission induction. The EFS data from this trial suggests that lymphoblasts with GR content less than 8,000 sites/cell are unlikely to be fully eradicated during ALL therapy, whereas higher GR content is associated with probable, complete eradication of the malignant lymphoblast clone. All of these data are consistent with a model in which GR content is associated with differential sensitivity to glucocorticoid, providing a plausible explanation why normal lymphocytes but not malignant lymphoblasts repopulate a patient's lymphoid system after successful leukemia therapy.

In contrast to our study, previous trials have cited 16,000 or 6,000 GR sites/cell as cutoff values for favorable prognosis. In the first case, the cutoff value differs from ours due to a significant difference in study design. The 16,000 figure was derived using successful induction remission as the measured outcome, distinct from this present study, which studies EFS as the outcome. The 6,000 figure was derived from an EFS study, but this was only a single-institution study with smaller patient numbers and relatively short follow-up. Although the differences in such results also might be partially due to differences in patient selection or to different GR assay methodology, these data all show qualitatively that higher GR content is associated with better prognosis. The much larger patient population and longer follow-up in this trial might provide a more accurate assessment of specific cutoff levels of GR content as an independent prognostic indicator in childhood ALL.

Advances in basic research have provided a more precise insight into the molecular mechanism of steroid-induced lymphocytolysis. It is now well established that GR is a ligand-dependent modulator of gene transcription. It is thought that upregulation or downregulation of the transcription of steroid-responsive genes produces the biologic effects of glucocorticoids. Recent data suggest that lymphocytois is mediated at least in part by GR-induced downregulation of specific growth-related genes such as the c-myc proto-oncogene.

Laboratory studies corroborate our finding that the intracellular level of GR is important for steroid sensitivity. An analog of cyclic adenosine monophosphate (cAMP) induces twofold increased expression of GR and restores steroid sensitivity in steroid-resistant cultured rat hepatoma cells, implying a specific GR threshold level for steroid sensitivity. In addition, elevation of cAMP levels enhances glucocorticoid binding and lysis of murine thymocytes. This effect may be mediated in part through cAMP-dependent protein kinase, which appears to cooperate with GR to induce glu-
corticoid sensitivity in steroid-resistant cultured-murine T-cell lymphoma cells. These laboratory data may indicate that a threshold level of GR confers steroid sensitivity, in support of data from our clinical trial. Furthermore, these studies indicate that elevation of intracellular cAMP can increase steroid sensitivity through higher GR activity.

This laboratory data suggest a potential therapeutic approach to increase tumor sensitivity to steroid therapy. Significant elevations of intracellular cAMP levels in lymphocytes have been achieved through the use of theophylline in cultured human cell lines derived from T-cell, B-cell, and plasma-cell malignancies, and in vivo in rats with oral aminophylline, and in humans after a single intravenous infusion of theophylline. However, the potential synergy of glucocorticoids with theophylline on lysis of leukemic blasts in vivo has not been evaluated. Investigation along these lines might present a provocative opportunity for further laboratory and clinical studies. The data appear clear that higher GR levels are associated with better remission induction and better long-term prognosis in childhood ALL. It will be of interest and potential clinical relevance to conduct further laboratory and clinical trials to evaluate the potential therapeutic role of additional agents to modulate GR levels in lymphoblasts.

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APPENDIX

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