Clinical Implications of Glucocorticoid Receptor Studies in Childhood Acute Lymphoblastic Leukemia

By R. Mastrangelo, R. Malandrino, R. Riccardi, P. Longo, F. O. Ranelletti, and S. Iacobelli

We have performed in parallel, in 19 children with acute lymphoblastic leukemia, a quantitative determination of glucocorticoid levels, in vitro steroid induced inhibition of nucleic acid precursors, and a short-term clinical trial of corticosteroids alone, before the treatment was given, which included corticosteroids and other drugs. From our results it appears that high glucocorticoid receptor levels in acute lymphoblastic leukemia of children do not guarantee a clinical response to corticosteroids. On the other hand, glucocorticoid receptors may turn out to be of value in predicting a poor response to corticosteroids only if their levels are considerably low.

In the last decade the prognosis in acute lymphoblastic leukemia of childhood, has markedly improved and “cure” has been reported in an increasing number of cases. This goal implies considerable risk related to the aggressive treatment used. It has, however, become clear that Acute Lymphoblastic Leukemia (ALL) is a heterogeneous disease and there is heterogeneity in the response and the duration of the response to chemotherapy among different patients.

Corticosteroids are widely employed in the management of ALL in children and their use may be associated with serious side effects.1 Obviously, it would be of value to discriminate in advance glucocorticoid responsive and nonresponsive cases of ALL, and restrict the use of corticosteroids to beneficial situations. In this context, it is of interest that binding to cytoplasmic receptors has recently been found to be the preliminary step in glucocorticoid effect on the leukemic lymphoblasts.2 In an attempt to identify, in ALL of children, those patients likely to respond to corticosteroids, we have performed in parallel a quantitative determination of glucocorticoid receptor (GR) levels, in vitro steroid induced inhibition of nucleic acid precursors, and a short-term clinical trial of corticosteroids before treatment was given, which included corticosteroids and other drugs.

From our results it appears that GR may turn out to be of value in predicting a poor response to glucocorticoids only if their levels are considerably low.

MATERIALS AND METHODS

Case Materials

The patients included in this study were classified as ALL on the basis of the morphological appearance of the leukemic cells and the negative results of the myeloperoxidase reaction. At the time of the study, 13 consecutive patients with ALL had never received any treatment, and 6 patients were studied in relapse and have received chemotherapy that was discontinued at least 5 days before any investigation was performed. These patients had been off glucocorticoids for at least 1 mo. The age of the patients ranged from 2 to 15 yr.

All patients except 2 at diagnosis and 2 out of 6 patients in relapse were examined for B and T cell characteristics.3,4

Isolation of Cells

The number of blasts in each bone-marrow and pleural effusion sample exceeded 90% in all cases and varied from 60% to 99% in samples of peripheral blood. Leukocytes were isolated from defibrinated venous blood through sedimentation in Dextran mixtures essentially as described by Terenius.6

In 3 patients the experiments were performed on bone-marrow samples. The marrow was thoroughly aspirated with a Pasteur pipette to obtain a single cell suspension that was diluted in isotonic saline and then sedimented through Dextran mixtures. After sedimenting at 160 g for 6 min, the leukocytes were resuspended in 5 ml of 0.2 M NaCl and then an equal volume of 1.6 M NaCl was rapidly added. This treatment lysed almost all contaminating red blood cells. Cell pellets were resuspended in Dulbecco mod. Eagle’s medium supplemented with 25 mM Hepes, 100 U/ml penicillin and 100 μg/ml streptomycin (DME). The cell viability, estimated by Nigrosine exclusion procedure, was always above 90%.

Glucocorticoid Receptor Analysis

Leukocytes obtained as described above were suspended at a density varying between 10 and 30 x 10⁶ cells/ml in DME. Aliquots of 0.1 ml of the cell suspension were delivered into 10 x 75-mm plastic tubes, containing six different concentrations of [3H]-triamcinolone acetonide (25 Ci/mM, the Radiochemical Center, Amsterdam) alone or in combination with 100-fold molar excess of nonradioactive triamcinolone acetonide. After incubation for 60 min, at 37°C, 1 ml of cold phosphate buffered saline (PBS) was rapidly added to each tube. Cell pellets were collected by centrifugation at 160 g for 6 min, then washed twice with PBS and extracted with 1 ml of 70% ethanol; extracts were transferred to liquid scintillation vials and counted with 8 ml of Lumagel (Lumac System, Switzerland). Bindings sites per cell and equilibrium dissociation constant were obtained from Scatchard analysis with the aid of a top desk computer Olivetti P6060.

In Vitro Sensitivity to Glucocorticoids

One milliliter aliquots of cell suspension in DME were delivered into 14 x 120mm sterile plastic tubes (Falcon) at a density of 2-5 x
10^6 cells/ml. Dexamethasone was added from a stock ethanol solution to achieve the final concentration of 10^{-7}M. The same concentration of ethanol (0.5%) was added to control tubes. After 22 hr at 37°C in a humidified 5% CO2 incubator, 1H-thymidine (2 Ci/mM, the Radiochemical Center, Amersham) was added to a final concentration of 0.1 x 10^{-7}M. One hour later, the cultures were terminated by centrifugation at 200 g for 6 min at 4°C. Cell pellets were then washed twice with 2 ml of ice-cold PBS and extracted with successive washes of 10% trichloroacetic acid (twice), 80% (v/v) of ethanol and ethanol: ethyl ether (1:1, v/v). The dried residue was dissolved in 0.3 ml of formic acid and assayed for radioactivity with the use of 10 ml of Lumagel. The cells were considered to be corticosensitive when the counts per minute in the dried residue was significantly lower than in control cultures (no steroid added).

**In Vivo Glucocorticoid Sensitivity**

In 13 patients at diagnosis a short-term trial of 2–10 days of prednisone, 2 mg/kg/day p.o., was given before combination chemotherapy according to M-IMFRA protocol, which included, for remission induction, prednisone at the same dosage, daily 6-mercaptopurine and weekly Vincristine for a 4-wk period.

For the patients studied in relapse, the administration of prednisone alone, for 4–16 days, was followed by various combinations of drugs to which the patients were thought not to be resistant. Each therapeutic trial included glucocorticoids. The response to glucocorticoid therapy was evaluated, at the end of the third day of treatment, on the basis of the change in the total peripheral blast count, size of liver, spleen, lymphnodes and mediastinal mass, if present. Fifty percent or greater decrease in the number of the circulating leukemic cells and/or in the size of all measurable lesions was considered a positive response. We also monitored all the patients investigated, treated with the M-IMFRA protocol, clinically and hematologically, both in terms of remission induction and remission duration.

Remission induction was evaluated after 4 wk of combination chemotherapy according to the usual criteria.

**RESULTS**

The concentration of glucocorticoid receptors on the leukemic cells of the children investigated ranged from no site per cell, in a single patient, to more than 20,000 sites per cell. Some trends were noted regarding the correlation between the number of GR per cell and the response in vitro and in vivo to corticosteroids for patients with low levels of GR. In order to focus on this tendency, we have divided our patients into two groups according to the GR content per cell. In Table 1 and 2 are shown the patients with GR numbers respectively higher and lower than 4000/cell, in addition to relevant clinical, hematologic, and immunologic features of the two groups. The in vitro inhibitory effect of dexamethasone (1 x 10^{-7}M) on 1H-thymidine incorporation into cellular DNA did not appear to correlate with the number of GR sites per cell (Fig. 1) nor with clinical sensitivity to glucocorticoids.

On the other hand, we visualized three patterns of relationship between the number of receptor sites and the clinical response to corticosteroids. Some patients (N.E., C.M., M.G., M.M., M.V., C.A., M.G., and S.D.) showed a high number of GR and a good clinical response to corticosteroids (Table 1); other patients (A.B., P.C., F.M., and D.A.I.), despite a substantial number of GR, had a minimal or no clinical sensitivity to corticosteroids (Table 1); a third group of patients, with a low number of GR had a poor clinical response to corticosteroids (Table 2). Figure 2 is a graphic representation of a critical patient of the second pattern. Despite a considerable number of GR there

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**Table 1. Clinical and Hematologic Features in a Group of Patients With GR/Cell > 4,000**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>WBC</th>
<th>Blasts</th>
<th>Blasts (%)</th>
<th>Sample Site</th>
<th>Blasts (%)</th>
<th>Sample Site</th>
<th>Surface GR Sites/Cell</th>
<th>GR Therapy Steroid Duration (Days)</th>
<th>Response In Vitro to Steroid Therapy Alone</th>
<th>Response In Vivo to Steroid Therapy Alone</th>
<th>CR</th>
<th>Duration of CR (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A.B.</td>
<td>7</td>
<td>F</td>
<td>11,700</td>
<td>73</td>
<td>73</td>
<td>PB</td>
<td>20,791</td>
<td>Null</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>*</td>
<td>No</td>
<td>–</td>
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<tr>
<td>2. N.E.</td>
<td>4</td>
<td>M</td>
<td>3,600</td>
<td>3</td>
<td>99</td>
<td>BM</td>
<td>17,684</td>
<td>Null</td>
<td>3</td>
<td>–</td>
<td>+</td>
<td>§</td>
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<tr>
<td>3. C.M.</td>
<td>4</td>
<td>M</td>
<td>62,000</td>
<td>99</td>
<td>99</td>
<td>PB</td>
<td>15,000</td>
<td>Null</td>
<td>2</td>
<td>–</td>
<td>+</td>
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<tr>
<td>4. M.G.</td>
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<td>M</td>
<td>42,000</td>
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<td>99</td>
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<td>–</td>
<td>+</td>
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<td>5. P.C.</td>
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<td>M</td>
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<td>77</td>
<td>PB</td>
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<td>+</td>
<td>§</td>
<td>Yes</td>
<td>4†</td>
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<td>6. M.M.</td>
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<td>M</td>
<td>15,000</td>
<td>0</td>
<td>99</td>
<td>PE</td>
<td>7,150</td>
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<td>M</td>
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<td>76</td>
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<td>8. C.A.</td>
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<td>M</td>
<td>18,000</td>
<td>72</td>
<td>72</td>
<td>PB</td>
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<td>9. F.M.†</td>
<td>12</td>
<td>M</td>
<td>7,800</td>
<td>60</td>
<td>60</td>
<td>PB</td>
<td>5,433</td>
<td>T</td>
<td>16</td>
<td>–</td>
<td>–</td>
<td>*</td>
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<td>10. D.A.I.§</td>
<td>2</td>
<td>M</td>
<td>30,000</td>
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<td>88</td>
<td>PB</td>
<td>5,289</td>
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<td>–</td>
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<td>11. M.G.</td>
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<td>F</td>
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<td>95</td>
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<td>+</td>
<td>+</td>
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<td>5</td>
<td>M</td>
<td>12,000</td>
<td>80</td>
<td>80</td>
<td>PB</td>
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<td>4</td>
<td>–</td>
<td>+</td>
<td>No</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; PB, peripheral blood; PE, pleural effusion; GR, glucocorticoid receptors; CR, complete remission; ND, not done.

*Patients treated longer than 3 days also showed a negative response at the end of steroid therapy alone.
†Died in relapse.
§A plus sign signifies 50% or greater decrease in the number of circulating leukemic cells and/or in the size of all measurable lesions.
‖With combination chemotherapy following the short-term course of steroid therapy alone.
Table 2. Clinical and Hematologic Features in a Group of Patients With GR/Cell < 4,000

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>WBC</th>
<th>Sample Site</th>
<th>PB (%)</th>
<th>Blast Site</th>
<th>Blast (%)</th>
<th>Sample Site</th>
<th>GR Sites/Cell</th>
<th>Surface Markers</th>
<th>Duration Steroid Therapy Alone (days)</th>
<th>Response In Vitro to Steroid Therapy Alone</th>
<th>Response In Vivo to Steroid Therapy Alone</th>
<th>CR (mo)</th>
<th>Duration of CR</th>
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<tbody>
<tr>
<td>13</td>
<td>M.M.</td>
<td>15 M</td>
<td>300,000</td>
<td>PB</td>
<td>99</td>
<td>3,260</td>
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<td>No</td>
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<td>14</td>
<td>R.D.</td>
<td>4 M</td>
<td>71,000</td>
<td>PB</td>
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<td>2,994</td>
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<tr>
<td>15</td>
<td>D.A.S.</td>
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<td>16,500</td>
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<td>45</td>
<td>2,207</td>
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<td>No</td>
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<tr>
<td>16</td>
<td>M.L.</td>
<td>9 M</td>
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<td>PB</td>
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<td>1,430</td>
<td>ND</td>
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<td>16</td>
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<tr>
<td>17</td>
<td>M.J.</td>
<td>6 F</td>
<td>3,500</td>
<td>PB</td>
<td>90</td>
<td>980</td>
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<td>6</td>
<td>No</td>
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<tr>
<td>18</td>
<td>D.M.V.</td>
<td>7 M</td>
<td>130,000</td>
<td>PB</td>
<td>99</td>
<td>688</td>
<td>Null</td>
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<td>No</td>
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<tr>
<td>19</td>
<td>L.F.</td>
<td>10 M</td>
<td>4,300</td>
<td>PB</td>
<td>98</td>
<td>Not detectable</td>
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<td>No</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: GR, glucocorticoid receptors; PB, peripheral blood; BM, bone marrow; CR, complete remission; ND, not done.

In relapse.

* Patients treated longer than 3 days also showed a negative response at the end of steroid therapy alone.

† With combination chemotherapy following the short-term course of steroid therapy alone.

was no in vitro response and no significant clinical response at the dosage of 60 mg/m² and 120 mg/m².

Too few cases have been studied for each single pattern to further correlate GR concentration with other parameters. It is noteworthy, however, that patients with low receptor levels and no in vivo response to corticosteroids either tend to have a very high WBC at the time of the initial presentation or were in relapse at the time of the study.

The follow-up on the patients investigated at diagnosis is reported in Table 1 and Table 2. Of the 13 patients who received single agent glucocorticoid therapy followed by the M-IMFRA chemotherapy protocol, 10 obtained a complete remission and 3 did not. The duration of complete remission ranged from 4 mo to 16 mo.

DISCUSSION

In ALL, studies attempting to correlate GR on the leukemic cells with clinical response to corticosteroids present some difficulties, since virtually all patients with acute leukemia are presently treated with at least two drugs, including corticosteroids. Only unresponsive cases may be presumed to be resistant to corticosteroids.

Six such patients studied by Lippman, with a cytoplasmic assay, all showed a very low number of GR. More recently, Homo et al., using a whole cell assay, have attempted to relate GR to the short term in vivo response to glucocorticoid therapy, before starting combined chemotherapy. No significant differences were found between glucocorticoid responsive and nonresponsive patients, in terms of GR. However, only a few patients were investigated in this manner and no comparison was made between the number of receptor sites per cell with the magnitude of the response to corticosteroids for each single patient.

The apparent absence of correlation may merely reflect the misleading determination of the mean value for the two groups of patients. In this connection, our findings support the above interpretation.

In our experience, the presence of even a substantial number of GR does not necessarily indicate a clinical response to corticosteroids. On the other hand, patients with a low number of GR, in agreement with Lippman's results, appeared invariably resistant to a short-term course of glucocorticoids. These observations are not surprising, if one considers the many steps involved in glucocorticoid hormone action distal to the initial binding of glucocorticoids to the receptors. Previous clinical studies in breast cancer have also suggested a similar pattern of relationship between estrogen receptors and response to specific hormonal treatment. Moreover, cell lines from S-49 lymphoma cells and similar cell lines have been described, which contained GR and were resistant to glucocorticoids, whereas cell lines deficient in GR

Fig. 1. Percentage inhibition of ³H-Thymidine incorporation after incubation with Dexamethasone (10⁻¹⁰M) as a function of GR sites per cell.
were all resistant to glucocorticoids either in vivo or in vitro.\textsuperscript{13,15}

The main clinical implications of the above observations concern the utility of GR assays in predicting response to corticosteroid therapy. If our results are confirmed by further studies, GR may turn out of value for the selection of those patients with ALL in whom only untoward reactions of corticosteroids may be expected without beneficial effects.

According to Lippman,\textsuperscript{8} a close correlation also exists in ALL of children between the levels of glucocorticoid activity at diagnosis and the lengths of the complete remission. For the majority of our patients investigated at the time of diagnosis it is premature to attempt a correlation between glucocorticoid levels and prognosis.

All we can say is that one patient (A.B.), with a high number of receptors did not even achieve a complete remission and a second patient (P.C.) with a substantial number of GR relapsed 4 mo after diagnosis. On the other hand, one child (M.L.), with 1400 GR sites/cell continues to be in complete remission 16 mo after diagnosis. These data contrast with Lippman’s results.

\textbf{ACKNOWLEDGMENT}

We are grateful to Dr. F. Aiuti, III Clinica Medica-Università degli Studi di Roma, for the determination of B and T markers.

\textbf{REFERENCES}

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Clinical implications of glucocorticoid receptor studies in childhood acute lymphoblastic leukemia

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