Acta Paediatrica Hungarica, 28 (2), pp 127—135 (1987)

Clinical significance of glucocorticoid receptors in acute leukaemia. Preliminary observations in Hungary and review of the literature

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Glucocorticoid receptor (GR) levels were quantitated in leukaemic blast cells separated from peripheral blood of 15 children with acute lymhocytic leukaemia (ALL) and of 6 children with acute non-lymphocytic leukaemia (ANLL). Using a whole-cell assay, it was found that specific (^{3}H)-dexamethasone binding exhibited a wide range in both types of acute leukaemia: GR levels scattered between 0–22,346/cell and 0–8772/cell in ALL and ANLL patients, respectively. In this paper we discuss our observations together with current knowledge on GR levels in leukaemic blast cells and their relationship to glucocorticoid sensitivity and disease outcome.

Based on the inhibitory effects on human lymphoid tissues the glucocorticoid hormones are widely used together with other cytotoxic drugs in various combined chemotherapy regimens in acute lymphoblastic leukaemias (ALL) in childhood. As it is known from early clinical trials, glucocorticoids alone are capable of inducing complete remission (CR) in this disease with response rates ranging from 19 to 76% according to various studies [10, 18, 31, 35, 37] while in adult type ALL glucocorticoids are less effective [10]. Some patients, however, are unresponsive at the onset or become resistant to further steroid (and combined) treatment during the course of the disease. In the case of acute non-lymphoid leukaemias (ANLL) the response rate is about 15% of the initial cases both in children and adults [10, 37].

Similarly to other steroid hormones the glucocorticoids exert their effects through binding with specific intracytoplasmic proteins, the glucocorticoid receptors (GR) [7]. Ample evidence has accumulated during the past two decades indicating that the receptors are rate-limiting in glucocorticoid hormone action [for review, see 32].

Since the first promising results of Lippman and coworkers, who showed that low GR levels are associated with more frequent and early relapses in childhood ALL [26], several groups have reported on similar experiments; these data were extensively reviewed [1, 13] but the results remained somewhat controversial, particularly

when attempts were focussed on correlating the in vitro glucocorticoid sensitivity of blast cells with their GR content. Therefore, we have decided to study the children in our department either with freshly diagnosed leukaemia or relapse.

Patients and Methods

Patients

Fifteen patients suffered from ALL, 6 from ANLL. The diagnosis was based on clinical findings, cytologic examination of Giemsa-stained marrow and blood smears as well as on cytochemical and immunologic reactions. Chromosome studies supported the diagnosis in some cases. Blast cells were classified according to FAB criteria [9]. ALL patients were divided into two prognostic groups at the time of diagnosis. Using the numerical BFM risk factor score [24] patients having a value equal to or less than 1.20 were considered to be at "standard risk", while those having a score above 1.20 were considered to be at "high risk". The clinicohaematologic characterization of our patients is reported in Tables I and II. Patient No 15 in Table I was a 5 years old foreign boy, who was treated for ALL for 8 months abroad but further classification of the disease was not available. All the patients were treated according to the protocols of the Hungarian Leukaemia Study Group for Children. The therapy was individualized to suit some relapsed patients who failed to respond to the protocol. Patient No 1 in Table I received a short-term single drug steroid pretreatment (20 mg prednisolone/m^2 body area/day) because of extreme leukocytosis and organomegaly.

GR assay

Mononuclear cells (MN cells) were separated from heparinized whole blood on Ficoll gradient according to Boyum [4], washed and suspended in ice-cold Hank's balanced salt solution and used immediately for receptor assay. Viability of cells was over 95% in every case. H^3-dexamethasone (38 Ci/mmol, The Radiochemical Centre, Amersham, England) binding on whole cells was measured in the absence and in the presence of 1000-fold excess of unlabelled hormone (Sigma Chemical Co.) as described previously [22]. The determinations were performed in triplicate. Radioactivity was measured with Nuclear Chicago ISOCAP 300 radiospectrofluorometer. The number of receptor per cell was calculated using a single saturating concentration (20 nM) of the hormone [23].

Results

GR levels of 9 freshly diagnosed, 10 relapsed ALL cases, and 7 ANLL cases (four times at the onset and three times in relapse) were determined. Results are demonstrated in Figure 1. For 20 normal children the range of GR sites per MN cell was 1099–4266 with a mean of 2308, and with a median of 2115.

GR concentration of our ALL cases scattered between 0–22,346 specific binding sites per cell (mean 4182, median 1134). The new patients had values ranging between 748–22,346 (mean 7114, median 4714), while patients in relapse had values ranging between 0–11,621 (mean 1715, median 529). We have chosen two arbitary limits, 1000 and 10,000 receptor sites per cell, as indicated by horizontal lines in the Figure. According to the blast cell receptor concentration, patients were divided into three groups, the "high", "intermediate", and "low GR level group", having more than

TABLE I

Clinical and Haematologic Characterization of ALL Cases

Case No.	FAB	BFM	Age (yr)	Sex	Leukocytes (G/l)	Disease outcome	
		(at onset of leukaemia)				CR	R
		"High GR level group"					
1	L_1	1.70	4	M	70.0		+
2	L_1	1.10	3	M	4.2		+
3[r]	L_1	0.32	15/12	F	6.2		+
		"Intermediate GR level group"					
4	L_1	1.10	10	F	59.4	+	
5	L_1	0.53	5	F	2.8	+	
6	L_1	1.26	9	M	5.6	+	
7	L_1	1.22	8/12	M	25.0		+
8	L_1	0.90	4	M	30.0	+	
9[r]	L_1	0.83	6	F	3.0	+	
10	L_1	1.22	6	M	3.8	+	
		"Low GR level group"					
11[r]—12[r]	L_3	2.20	3	F	100.0		+
13—14[r]	L_1	1.48	7	M	84.0		+
15[r]	n.a.	n.a.	5	M	n.a.		+
16[r]	L_2	1.30	3	F	29.		+
17[r]	L_1	0.32	15/12	F	6.2		+
18[r]	L_1	1.10	3	M	4.2		+
19[r]	L_1	1.18	4	F	12.0		+

ALL = acute lymphocytic leukaemia, L_{1-3} according to FAB criteria [9].
BFM = risk factor according to the BFM group [24].
M = male, F = female, CR = complete remission, R = relapse or refractaer to therapy.
yr = year(s),
[r] indicates that GR assay was performed at time of relapse in these cases.
Cases No 2 and 18, 3 and 17, 11 and 12, and 13 and 14 represent the same patients in different phases of their disease.

TABLE II

Clinical and Haematologic Characterization of ANLL Cases

Case No.	FAB	Age (yr)	Sex	Leucocytes (G/l)	Disease outcome	
	(at onset of leukaemia)				PR	R
1[r]	M_2	12	M	3.0		+
2	M_4	7	M	18.6		+
3	M_{5B}	7	M	40.0		+
4—5[r]	M_1	3	M	22.0		+
6[r]	M_2	11	M	67.0	+	
7	M_1	5	M	4.8		+

ANLL = acute non-lymphocytic leukaemia, M_{1-6} according to FAB criteria [9], yr = year(s)
M = male, PR = partial remission, R = relapsed or refractaer to therapy.
[r] indicates that GR assay was performed at time of relapse in these cases.
Cases Nos 4 and 5 represent the same patient in different phases of the disease.

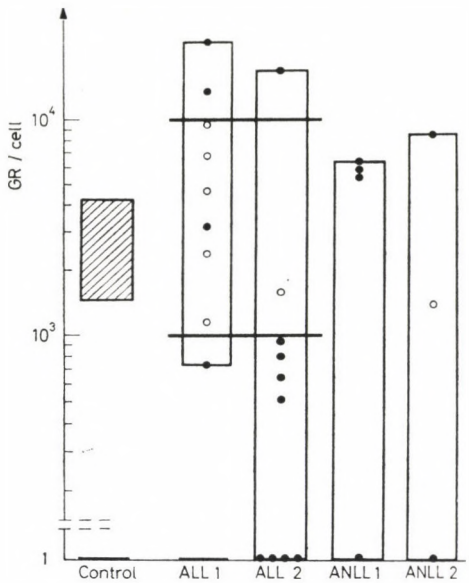

FIG. 1. Control: GR number of mononuclear cells of 20 healthy children. ALL 1: GR number of 9 ALL patients at diagnosis. ALL 2: GR number of 10 relapsed ALL patients. ANLL 1: GR number of 4 ANLL patients at diagnosis. ANLL 2: GR number of 3 relapsed ANLL patients. ○: open symbols represent patients in remission since GR determination; ●: shaded symbols represent patients who were resistant to therapy or suffered relapse(s) since GR determination; Two horizontal lines divide the patients demonstrated on the 2nd and 3rd slope into "high", "intermediate" and "low" GR level groups.

10,000, between 1000 and 10,000, and under 1000 specific binding sites per cell. A detailed clinicohaematologic characterization of the patients in the three groups is shown in Table I. As can be seen in Figure 1 and Table I, most new patients (6 of 9 cases) had an intermediate blast cell receptor concentration. Each patient in this group except for one infant was in CR, patient No 9 in her second remission, since the assay was performed at the time of relapse in this case. Most of the relapsed cases displayed low blast cell GR concentration (8 of 10 cases), while only one new case belonged to the "low GR level group". The patients in this group had an unfavourable clinical outcome: they suffered a relapse or repeated relapses since GR assay was performed, or failed to respond to combined cytotoxic therapy. The patients in the "high GR level group" (two new cases and one relapsed case) also had a poor outcome of the disease. The short term prednisolone pretreatment of patient No 1 witnessed for steroid sensitivity, since the 70.0 G/l initial leukocyte count decreased to 0.8 G/l after one week on single drug steroid. Two ALL patients were assayed at diagnosis and relapse. Their blasts were GR positive at the onset with 13,514 and 748

binding sites per cell, cases No 2 and 13, respectively, while no specific steroid binding could be demonstrated at the time of relapse (cases No 18 and 14). One patient had GR positive with 11,621 GR binding sites per cell in her first relapse, and GR negative blasts in her second relapse (cases No 3 and 17.). One patient had similar low receptor concentrations with 938 and 643 GR per cell at the time of her first and second relapse.

The 7 ANLL cases had GR values ranging between 0–8772 specific binding sites per cell (mean 4071, median 5833). The range of GR concentration was 0–6474 (mean 4659, median 5833) per cell for 4 untreated, and 0–8772 (mean 3287, median 1088) per cell for 3 relapsed patients. One patient was assayed at diagnosis and at relapse, he had 5833 and zero GR sites per cell, respectively (Cases No 4 and 5). Both receptor negative cases proved to be resistant to combined chemotherapy. The clinicohaematologic characterization of our ANLL patients is demonstrated in Table II.

Discussion

As the other steroid hormones, glucocorticoids exert their effect in target cells via an interaction with specific receptor molecules. The lipophilic hormone freely enters the cell through the plasma membrane. The receptor is an asymmetric, amphoteric polypeptide similar in most mammalian tissues and species. The widespread regulatory role of glucocorticoids stems from the widespread distribution of the receptor; all tissues, except for red cells and platelets, contain specific GR. Binding of hormone to these receptors results in a temperature and/or ionic strength dependent intramolecular transformation, yielding the activated complex allowing it to bind tightly to acceptor sites within the nucleus. Shortly after reaching the nucleus transcriptional processes are modified and the glucocorticoid effect is mediated via new mRNA synthesis. The altered gene expression results in an altered cellular metabolism. More details of receptor physiology can be found in the review of Rousseau and Baxter [32].

Lymphoid cells show typical changes in vitro: inhibition of glucose and amino acid transport and of macromolecular synthesis accompanied by cell lysis in some cases [29]. The in vivo action of glucocorticoid hormones is more complex. The steroids do not only affect the cells directly but cause changes in cell-to-cell interactions, in lymphocyte traffic and sequestration, in release of soluble mediators and growth factors [8]. "Glucocorticoid sensitivity" can be defined therefore depending on the investigated experimental system. The most often used in vivo and in vitro assays of glucocorticoid sensitivity are listed in Table III. The glucocorticoid effect in various cell types is dependent on many factors and species differences can be observed. If we consider e.g. their lytic effect, human lymphoid cells are "resistant", when compared to rodent lymphoid cells [5]. On the

TABLE III

Different assay methods for the determination of glucocorticoid sensitivity of leukaemia cells

IN VITRO ASSAYS

- cell lysis
- inhibition of transport of hexoses, aminoacides and nucleosids
- inhibition of precursor incorporation into proteins, RNA and DNA
- RNA polymerase activity changes

IN VIVO ASSAYS

- blast cell reduction
  a. response to glucocorticoid monotherapy
  b. response to glucocorticoid containing combined chemotherapy
- clinical outcome
  a. achievement of complete remission (CR)
  b. CR duration
  c. frequency of relapses
  d. survival

other hand, lymphoid cells in a given species are heterogeneous regarding their glucocorticoid sensitivity. For example, cortical thymocytes are more sensitive than peripheral, lymph node, and bone marrow derived lymphocytes, or isolated mature T-cells to the inhibitory action of steroids using the nucleoside incorporation assay [2, 6, 14, 16, 21, 37]. These differences might be related to the stage of cell maturation [17]. Similar observations have been reported for leukaemic lymphoblasts [15] and myeloblasts [11]. Since glucocorticoids play a central role in the treatment of ALL, several authors attempted to explore the mechanisms involved in the leukaemia cell–steroid interactions. It soon appeared that the presence or absence of blast cell GR is a critical factor in mediating the hormone responses involved in the remission-induction process [7]. Interestingly enough, receptor deficiency is far the most common cause of glucocorticoid resistance in established animal and human cell lines, when compared to the other possible abnormalities in the different steps of the hormone-receptor interaction [34]. According to the results of several authors, isolated peripheral blood leukocytes from most of the newly diagnosed ALL patients contained GRs. When assayed by the whole cell method, the number of specific binding sites scattered between 1000 and about 20,000 per cell, showing a somewhat brighter range than what was found in normal blood lymphocytes possessing 3000–7000 GR sites per cell. Dissociation constants for glucocorticoids were similar in the patients' cells to those determined in the cells of healthy persons [13]. The use of cytosolic assay resulted in a marked underestimation of the GR count [27]. Receptor negative cases

were more common in previously treated patients [25]. A straightforward relationship between blast cell GR content and the parameters of in vitro sensitivity cannot, however, be established. Most of the in vivo responsiveness studies were not easily interpreted because of the simultaneous use of other cytotoxic drugs, so it was difficult to ascertain whether the observed decrease of the blast cell count was due to the glucocorticoid, to some of the other agents, or to the combination schedule. To overcome this difficulty, several groups used short-term single agent glucocorticoid therapy. In such studies GR levels were reported to be slightly higher in the responsive patients, that were defined on the basis of at least a 50% decrease in the peripheral blast cell number [3, 12, 15, 19, 27]. Lippman and coworkers [26] were the first to compare the GR level of ALL children before any treatment with the outcome of long term combined chemotherapy. They demonstrated that patients with low receptor number have a short remission. Mereover they could correlate high receptor levels with "null" cell feature and low receptor levels with T-cell phenotype. They found that the GR level was an independent prognostic variable in childhood ALL. More recent studies also confirmed the poor prognostic significance of low GR levels in ALL children [for review, see 1, 13]. In contrast, such a relationship could not be established in adult type ALL [3].

The well-known restricted response rate to glucocorticoids in the cases of ANLL was attempted to explain on the basis of the blast cell receptor content. It has now well been established that myeloid and lymphoid leukaemia cells possess essentially equivalent specific binding sites. The biologic significance of the GR level in ANLL is much more uncertain than it is in childhood ALL. For instance, marked stimulation of precursor incorporation by glucocorticoids has been shown in myeloid leukaemia cells *in vitro* [30], while some authors indicated that the GR level is a useful predictor of prognosis in ANLL [35]. We could demonstrate the presence of GR in 5 out of 7 ANLL cases. Myeloblasts had a similar GR concentration than did lymphoblasts. Both our receptor negative patients failed to respond to the combined cytotoxic treatment.

The present observations of ALL patients are consistent with the view that a reduction in GR concentration is an important mechanism by which leukaemia cells lose their sensitivity to glucocorticoids. This hypothesis is supported by the following data. First, all the patients in the "low GR level group" had an unfavourable course of the disease. Second, 8 out of 10 investigated relapsed cases belonged to this group. Further evidence for this view is provided by Cases Nos 2 and 3. These patients had a high GR concentration, while at the time of their fatal relapse no specific binding was detectable. More intriguing is the poor clinical outcome of the patients in the "high GR level group". Some data [7, 28] clearly demonstrate that

"high" receptor numbers do not guarantee a responsive disease. The GR level might represent a biological marker of cell maturation and rate of growth in ALL, and thus may be related to the response to combined chemotherapy. Case No 1 supports this hypothesis, since this patient was sensitive to glucocorticoid as evidenced by the successful short-term prednisolone pretreatment. In the other two cases in the "high GR level group" glucocorticoid resistance could not be ruled out. Recent studies demonstrated GRs with abnormal physicochemical properties in lymphoid blast cells [1].

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Received 20 March, 1986

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