Protein expression of the glucocorticoid receptor in childhood acute lymphoblastic leukemia

MELCHIOR LAUTEN, GUNNAR CARIO, GIRMAY ASGEDOM, KARL WELTE, MARTIN SCHRAPPE

Background and Objectives. Early treatment response is a strong predictor for treatment outcome in childhood acute lymphoblastic leukemia (ALL), treated within the protocols of the Berlin-Frankfurt-Münster (BFM) study group. In the ALL-BFM trials, early treatment response is assessed by *in vivo* response to glucocorticoids (prednisone response), the molecular background of which is unknown. Initial *in vivo* resistance to glucocorticoid (GC) treatment in childhood ALL (prednisone-poor response) is associated with a dramatically shorter event-free survival than that found in GC-sensitive patients (prednisone-good responders). The intracellular effects of glucocorticoids are mediated by the glucocorticoid receptor (GR). The protein expression of the GR has been linked to *in vivo* and *in vitro* GC resistance in various diseases treated with GC. However, existing data are conflicting.

Design and Methods. We performed a case-control study for prednisone response to investigate the association of *in vivo* GC resistance and GR protein expression in childhood ALL. GR expression was assessed using Western blot technology.

Results. The median relative GR protein expression of all patients was 0.87. Overall, we did not find different GR protein expression in PPR and PGR patients. GR protein expression was 0.91 in PGR patients versus 0.85 in PPR ones of *in vivo* GC resistance and GR expression.

Interpretation and Conclusions. We conclude that the expression of GR is of minor importance for *in vivo* GC resistance in childhood ALL.

Key words: childhood leukemia, ALL, prednisone response, glucocorticoid resistance, glucocorticoid receptor.

Haematologica 2003; 88:1253-1258
http://www.haematologica.org/2003_11/1253.htm

From the Hannover Medical School, Pediatric Hematology and Oncology, 30625 Hannover, Germany.

Correspondence: Melchior Lauten, MD, Hannover Medical School, Pediatric Hematology and Oncology, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. E-mail: lauten.melchior@mh-hannover.de

Treatment outcome in childhood acute lymphoblastic leukemia (ALL) differs profoundly between low, intermediate and high risk patients. This has led to risk adapted treatment regimens. In ALL trials of the Berlin-Frankfurt-Münster study group (ALL-BFM), risk stratification is based on various parameters, including an *in vivo* glucocorticoid sensitivity test, the *in vivo* prednisone response (PR).[1-4] PR is defined by the number of peripheral leukemic blasts present on day eight of initial prednisone treatment. The threshold value for the distinction between good and poor prednisone response is 1000 blasts/μL. In trial ALL-BFM 90, prednisone-good responders (PGR) had an estimated 82% probability of a 6-year event-free survival (6y-pEFS) in contrast to prednisone-poor responders (PPR), who showed a 6y-pEFS of only 34%.[4] In the 1990s many international studies confirmed the prognostic significance of the early reduction of malignant cell load in hematologic malignancies.[5-8]

The biological background of glucocorticoid (GC) resistance is not known completely. However, the glucocorticoid receptor (GR) has been subject of many *in vitro* and *in vivo* studies, addressing the problem of GC resistance in hematologic malignancies, asthma, and other diseases treated with glucocorticoids.[9-14] Recently, Tissing *et al*. showed that mRNA expression of GR splice variants was not associated with either *in vivo* or *in vitro* GC resistance in childhood ALL.[15] Nevertheless, due to the methodology of his study, a translational defect of GR mRNA could not be excluded. The current study was, therefore, aimed at investigating the association of GR protein expression and prednisone response in a closely matched case-control study of 40 childhood ALL patients.

The glucocorticoid receptor is a ligand-dependent transcription factor that codes for a diversity of proteins.[16] To date, six isoforms are known: GR-α, GR-β, GR-γ, GR-P, GR-A and GR-B.[17] GR-α and GR-β were first described in 1985[18] and GR-α has since been well characterized as the functional receptor, which comprises an amino-terminal transactivation domain, a central DNA-binding domain and a carboxy-teminal end containing the hormone-binding domain.[19-21]

Even though the structure of GR-β appears highly homologous to that of GR-α (identical through amino acid 727), GR-β does not bind glucocorticoids and is transcriptionally inactive.[17] Nevertheless, reports on the function and the biological importance of GR-β are still conflicting.[22-25] *In vitro* studies using transfected cell

lines[21,26] and *in vivo* studies in asthma, rheumatoid arthritis, inflammatory bowl disease and ALL[12,13,25,27,28] could not clarify the importance of GR-β so far.

GR-γ RNA expression in childhood ALL has been investigated by Beger *et al*. Using an *in vitro* stimulation assay she showed longer GR-γ RNA up-regulation in prednisone-poor responders than in prednisone-good responders after *in vitro* stimulation with dexamethasone.[29]

In a different study investigating GR-P expression levels in hematologic malignancies it was suggested that GR-P increases the activity of GR-α. The authors concluded that, by increasing GR-α activity, GR-P could be involved in GC resistance during GC treatment.[21] The function of GR-A has not yet been subject of investigations.

Comparative *in vivo* studies investigating the protein expression of GR isoforms and their association with GC resistance in childhood ALL have not yet been published. Unfortunately, studies on GR isoform protein expression are generally hampered by technical problems (*see discussion*). Therefore, the current study investigated the protein expression of the sum of all GR isoforms using an isoform unspecific antibody.

Design and Methods

Patients and study design

Our study was designed as a case-control study for prednisone response to assess the association of glucocorticoid resistance and glucocorticoid receptor protein expression in childhood ALL. Cases were patients with poor *in vivo* prednisone response (n=20), controls were patients with good *in vivo* prednisone response (n=20). The study utilized spare peripheral blood and bone marrow samples as well as clinical data from patients enrolled in the ALL-BFM 90 and ALL-BFM 95 trials. Criteria used to match the patients in the two groups were initial white blood cell count (WBC; <10,000, 10,000-<50,000 and ≥50,000 leukocytes/μL), sex, immunophenotype (T-ALL, non T-ALL) and age at diagnosis (<1 year, 1-9 years and ≥10 years) (Table 1). All patients who did not die from progressive disease or relapsed ALL had a follow-up of at least five years.

Fifteen matched patient pairs of the current study were also investigated for heat shock protein 90 (Hsp90) expression in a different study, which was recently published.[30] The Hsp90 data of these patients will be referred to in the discussion.

Cell samples

The spare bone marrow and peripheral blood samples analyzed in this study were taken from ALL patients at diagnosis before administration of glucocorticoids. Peripheral blood samples had to have a minimum of 70% leukemic blasts prior to gradient centrifugation. Mononuclear cells were isolated using gradient centrifugation (FicoII-Paque, Amersham Biosciences, Freiburg, Germany). After isolation, cells were frozen in Roswell Park Memorial Institute medium (RPMI-1640) containing 10% dimethyl sulfoxide (DMSO).

Table 1. Distribution of the clinicopathological features in patients with childhood ALL, divided into two groups according to *in vivo* response to prednisone.

	Prednisone Poor response n=20 (100%)	*Prednisone Good response n=20 (100%)*
Sex		
male	14 (70.0)	14 (70.0)
female	6 (30.0)	6 (30.0)
Age at diagnosis		
<1 year	–	–
1-9 years	15 (75.0)	15 (75.0)
≥ 10 years	5 (25.0)	5 (25.0)
Initial WBC count (/μL)		
<10,000	–	–
10,000-<50,000	4 (20.0)	4 (20.0)
≥ 50,000	16 (80.0)	16 (80.0)
Immunophenotype		
non T-ALL	10 (50.0)	10 (50.0)
T-ALL	10 (50.0)	10 (50.0)

Protein isolation

Protein from BM and peripheral blood mononuclear cells was isolated using a standard protocol for extraction of cytosolic and nuclear proteins.[31] Slight modifications of the protocol included the NP40 concentration (0.1% instead of 0.2%), omission of (4-(2-aminoethyl)-benzolsulfonylfluoride (AEBSF) and an incubation period of 10-30 minutes after resuspension into Dignam buffer A. The concentration of protein was measured photometrically directly before Western blotting.

Western blotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with two gels at a time. Forty micrograms of total cell lysate protein were separated on 10% SDS gels for 90 minutes at 180V (Owl Scientific Inc., Wobum, USA). Every gel was run with 40 μg protein from HeLa S3 cells to normalize protein quantity. The gels were transferred to nitrocellulose membranes (Trans-Blot Transfer Medium, BioRad Laboratories, Rich-

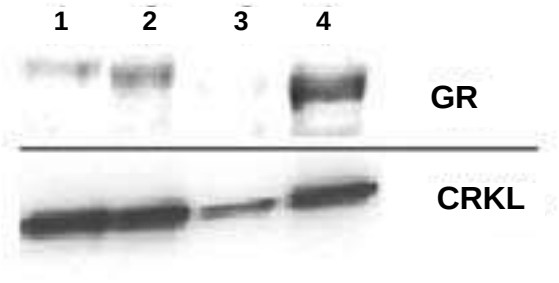

Figure 1. Western blot for GR and CRKL in childhood ALL patients with good and poor *in vivo* response to glucocorticoids (GC). Lanes 1 and 2 show the GR and CRKL expression for patients with good *in vivo* GC response. Lane 3 represents a patient with poor *in vivo* GC response. Lane 4 demonstrates GR and CRKL expression in HeLa S3 cells.

Table 2. Median GR expression shown for the total group of patients and for the groups divided according to *in vivo* response to prednisone.

	Median GR expression[a] (P_{25}-P_{75})	*p*
Total group		
N=40	0.87 (0.58-1.33)	
Prednisone response		
Good (n=20)	0.91 (0.55-1.33)	
Poor (n=20)	0.85 (0.61-1.32)	n.s.

[a]*Relative to the normalized expression of the target protein in HeLa S_3 cells.*

mond, USA) and blotted in parallel at 20V for 2 hours. After blotting, gels (Commassie) and membranes (Ponceau S) were stained to document blotting failure. The membranes were incubated with a polyclonal isoform unspecific GR antibody (sc-1003, SantaCruz Biotechnology, Santa Cruz, USA) at 4°C overnight. After washing (three times with PBS-T), membranes were incubated with a horseradish-peroxidase labeled goat-anti-rabbit secondary antibody (sc-2030, SantaCruz Biotechnology) at 4°C overnight. Subsequently, the membranes were incubated with a polyclonal CRKL antibody (sc-9005, SantaCruz Biotechnology) at 4°C over night. Washing and incubation with the secondary antibody was performed as described above. Every gel was run with HeLa S3 cells as positive controls and with pre-stained markers (Wide range color marker, Sigma, Deisenhofen, Germany). Finally, the bands were visualized on a X-ray film (Cronex 5, AGFA, Mortsel, Belgium) using the electro-chemo-luminescence method according to the manufacturer's instructions. Bands of the GR protein appeared at approximately 90kD, bands of the CRKL protein at approximately 40kD. An example of the Western blots is shown in Figure 1. After scanning of the X-ray film, image analysis was performed on a Macintosh iMAC computer using the public domain National Institutes of Health (NIH) Image program (available on the Internet at *http://rsb.info.nih.gov/nih-image*).

Statistical analysis

Frequencies were calculated for descriptive purposes. GR protein expression was calculated normalized to CRKL and relative to the GR expression in HeLa S3 cells. The correlation between median GR protein expression and prednisone response was calculated by logistic regression analysis and a χ^2 test. Computations were performed using SAS software (SAS-PC Version 6.04, SAS Institute Inc., Cary, NC, USA).

Results

We performed a case-control study for *in vivo* prednisone response on 20 paired patients' samples to assess the association of GC resistance and GR protein expression in childhood ALL. The clinicopathologic features of all patients investigated in our study are shown in Table 1. The median relative GR protein expression of all patients was 0.87. Overall, we did not find different GR protein expression in PPR and PGR patients (Table 2). GR protein expression was 0.91 in PGR patients versus 0.85 in PPR ones. In addition, subgroup analysis for risk factors known to have an impact on event-free survival in childhood ALL (sex, age at diagnosis, initial WBC count, immunophenotype) revealed that even within these subgroups GR protein expression did not differ significantly between the PPR and PGR groups (Table 3).

Discussion

In 1983 Pui *et al*. investigated GR-binding sites in blasts of patients with relapsed ALL and found no association with clinical characteristics, initial response to treatment, or duration of remission.[32] Since then, expression of GR and its isoforms has been subject of various investigations in patients with a broad spectrum of diseases that are treated with glucocorticoids.[21,26-28,35] In some of these studies, GR-β and GR-P were suggested to inhibit GR-dependent activation of transcription and, therefore, contribute to GC resistance. In one study examining leukemic blasts from 13 ALL patients and peripheral blood mononuclear cells from 9 healthy volunteers, the ALL samples showed

Table 3. Median GR expression in subgroups of patients with childhood ALL, further divided according to *in vivo* response to prednisone.

		Median GR expression[a] (P_{25}-P_{75})	
	No. of patients	*Poor PDN*[b] *response*	*Good PDN*[b] *response*
Sex			
Male	28	0.68 (0.55-1.21)	0.98 (0.67-1.28)
Female	12	1.20 (0.88-1.48)	0.70 (0.50-1.60)
Age			
1-9 years	30	0.86 (0.59-1.35)	0.88 (0.53-1.36)
≥ 10 years	10	0.83 (0.64-1.52)	1.13 (0.70-1.46)
Initial WBC count (/μL)			
10,000 – 49,999	8	0.86 (0.73-1.38)	1.06 (0.55-1.45)
≥ 50,000	32	0.78 (0.58-1.32)	0.91 (0.55-1.31)
Immunophenotype			
Non T-ALL	20	1.03 (0.80-1.48)	1.19 (0.78-1.56)
T-ALL	20	0.67 (0.43-1.22)	0.74 (0.53-1.05)

PDN: prednisone. [b]PDN: relative to the normalized expression of the target protein in HeLa S_3 cells. All differences of GR expression between the two responder groups were statistically not significant.

reduced GR-α protein expression with no concomitant decrease in GR-β compared to the findings in healthy controls. In addition, GR-α expression was lower in mononuclear leukocytes of T-cell lineage than in leukocytes from the B-cell precursor immunophenotype. The authors considered that the reason for the reduced sensitivity to glucocorticoids in T-ALL might be different expression of GR isoforms in certain immunophenotypes.[25]

The mechanism responsible for the putative influence of GR splice variant expression on GC resistance has been suggested to be an activation defect of the GC-GR complex. After entering the cell, glucocorticoids bind to the GR within the cytoplasm. Subsequently, the GC-GR complex moves to the nucleus and binds to glucocorticoid responsive elements (GRE) from GC responsive genes within the nucleus. The binding of the GC-GR complex with GRE is dependent on the formation of GR homodimers, which must be formed by two liganded GR.[33] The formation of a GR heterodimer (e.g. GR-α GR-β) seems to prevent induction of transactivation or transrepression of GC responsive genes. Furthermore, competitive binding of GR-α and GR-β or GR-P to co-activators or co-repressors has been suggested to play a role in GC resistance.[22]

The investigation of protein expression of GR isoforms is hampered by biological as well as technical problems. For example, Haarman and colleagues found that GR-β protein is not detectable in primary ALL cells, whereas it is detectable in CEM-C7 cells.[34] In addition, Beger *et al*. described that sequences from GR-α, GR-β and GR-P can form the carboxy-terminal end of GR-γ and that GR-γ RNA expression may be up-regulated for longer in PPR samples than in PGR ones after *in vitro* stimulation with dexamethasone.[29] Beger´s study reveals a fundamental problem concerning the interpretation of studies examining differential protein regulation of GR isoforms. GR isoform specific antibodies are commercially available only for GR-α and GR-β. However, since we are now aware that GR-γ exists with the carboxy-terminal ends of various other GR isoforms, and since the structure of GR-γ may differ from each of these isoforms by only 1 amino acid within the DNA binding domain (arginine), we must conclude that the GR-α and GR-β specific antibodies currently used are highly likely to recognize not only the respective specific GR isoform, but also GR-γ. Thus, potential effects of GR-α or GR-β on GC resistance could have been mimicked by a GR-γ effect. We think that, currently, it is not possible to study GR isoform protein expression using an isoform specific antibody approach.

The protein data of this study support the RNA data published by Tissing *et al*., who investigated GR-α, GR-β and GR-P RNA expression as well as the expression of the sum of all GR isoforms (GR-total) in a case-control study of prednisone response in childhood ALL at diagnosis. In that study there was no differential expression of any GR isoform RNA.[36] Twelve of the patients of the current study (6 matched pairs) were also investigated in the study by Tissing *et al*. This now allows us to hypothesize that a defect of GR mRNA translation as a cause of GC resistance in childhood ALL can be excluded by the current study to a certain extent. However, our study does not definitively exclude that GR expression could influence outcome of childhood ALL, since the initial co-existence of small cell fractions with distinct GR expression patterns cannot be ruled out. The analysis of resistant blasts after 1 week of GC treatment could be a next step to approach this issue.

In a recently published study we showed that differential expression of hsp90, the main protein of the GR activating heterocomplex, does not con-

tribute to GC resistance in childhood ALL.[30] Fifteen matched pairs of the current study were also investigated in the hsp90 study. Therefore, we calculated hsp90/GR ratios for each of the 15 responder pairs to look for an imbalance between hsp90 and GR expression in each of the patients. GC resistance in patients with normal GR expression could occur, for instance, because of low hsp90 expression and thus low GR activating capacity. However, hsp90/GR ratios were not significantly different in sensitive and resistant patients (*data not shown*).

To summarize, using Western blot technology we did not find a correlation between GR protein expression and GC resistance in childhood ALL In addition, there was no difference in the ratio of hsp90 to GR expression in GC sensitive and resistant ALL patients. Further studies might focus on elucidating activation defects of the GC-GR complex in detail. In particular, *in vivo* stimulation studies focusing on the dynamics of GR expression under GC treatment would be helpful to clarify, once and for all, the role of GR expression in GC resistance in childhood ALL.

References

1. Riehm H, Reiter A, Schrappe M, Berthold F, Dopfer R, Gerein V, et al. [Corticosteroid-dependent reduction of leukocyte count in blood as a prognostic factor in acute lymphoblastic leukemia in childhood (therapy study ALL-BFM 83)]. Klin Padiatr 1987;199:151-60.
2. Reiter A, Schrappe M, Ludwig WD, Hiddemann W, Sauter S, Henze G, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 1994;84: 3122-33.
3. Dordelmann M, Reiter A, Borkhardt A, Ludwig WD, Gotz N, Viehmann S, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood 1999;94:1209-17.
4. Schrappe M, Reiter A, Ludwig W-D, Harbott J, Zimmermann M, Hiddemann W, et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood 2000;95:3310-22.
5. Gajjar A, Ribeiro R, Hancock ML, Rivera GK, Mahmoud H, Sandlund JT, et al. Persistence of circulating blasts after 1 week of multiagent chemotherapy confers a poor prognosis in childhood acute lymphoblastic leukemia. Blood 1995;86: 1292-5.
6. Steinherz PG, Gaynon PS, Breneman JC, Cherlow JM, Grossman NJ, Kersey JH, et al. Cytoreduction and prognosis in acute lymphoblastic leukemia: the importance of early marrow response: report from the Childrens Cancer Group. J Clin Oncol 1996;14:389-98.
7. Chessells JM, Bailey C, Richards SM. Intensification of treatment and survival in all children with lymphoblastic leukaemia: results of UK Medical Research Council trial UKALL X. Lancet 1995;345:143-8.
8. Thyss A, Suciu S, Bertrand Y, Mazingue F, Robert A, Vilmer E, et al. Systemic effect of intrathecal methotrexate during the initial phase of treatment of childhood acute lymphoblastic leukemia. The European Organization for Research and Treatment of Cancer Children's Leukemia Cooperative Group. J Clin Oncol 1997;15:1824-30.
9. Pui CH, Costlow ME, Kalwinsky DK, Dahl GV. Glucocorticoid receptors in childhood acute non-lymphocytic leukemia. Leuk Res 1983;7:11-6.
10. Shahidi H, Vottero A, Stratakis CA, Taymans SE, Karl M, Longui CA, et al. Imbalanced expression of the glucocorticoid receptor isoforms in cultured lymphocytes from a patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia. Biochem Biophys Res Commun 1999;254:559-65.
11. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor β-isoform. J Allergy Clin Immunol 2000;105:943-50.
12. Orii F, Ashida T, Nomura M, Maemoto A, Fujiki T, Ayabe T, et al. Quantitative analysis for human glucocorticoid receptor α/β mRNA in IBD. Biochem Biophys Res Commun 2002; 296: 1286-94.
13. Chikanza IC. Mechanisms of corticosteroid resistance in rheumatoid arthritis: a putative role for the corticosteroid receptor β isoform. Ann N Y Acad Sci 2002;966:39-48.
14. Hamilos DL, Leung DY, Muro S, Kahn AM, Hamilos SS, Thawley SE, et al. GRβ expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone. J Allergy Clin Immunol 2001;108:59-68.
15. Tissing WJE, Lauten M, Meijerink J, den Boer ML, Verschuuren AC, Gerdes K, et al. Glucocorticoid receptor splice variants α, β and GR-P and in vivo glucocorticoid resistance in childhood acute lymphoblastic leukemia. Blood 2001;98: 313a[abstract].
16. Yudt MR, Cidlowski JA. The glucocorticoid receptor: coding a diversity of proteins and responses through a single gene. Mol Endocrinol 2002;16:1719-26.
17. Kofler R, Schmidt S, Kofler A, Ausserlechner MJ. Resistance to glucocorticoid-induced apoptosis in lymphoblastic leukemia. J Endocrinol 2003;178:19-27.
18. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, et al. Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 1985;318: 635-41.
19. Oakley RH, Sar M, Cidlowski JA. The human glucocorticoid receptor β isoform. Expression, biochemical properties, and putative function. J Biol Chem 1996;271:9550-9.
20. Oakley RH, Webster JC, Sar M, Parker CR, Cidlowski JA. Expression and subcellular distribution of the β-isoform of the human glucocorticoid receptor. Endocrinology 1997; 138:5028-38.
21. de Lange P, Segeren CM, Koper JW, Wiemer E, Sonneveld P, Brinkmann AO, et al. Expression in hematological malignancies of a glucocorticoid receptor splice variant that augments glucocorticoid receptor-mediated effects in transfected cells. Cancer Res 2001;61:3937-41.
22. Vottero A, Chrousos GP. Glucocorticoid receptor β: view I. Trends Endocrinol Metab 1999;10:333-8.
23. Carlstedt-Duke J. Glucocorticoid receptor β: view II. Trends Endocrinol Metab 1999;10:339-42.
24. de Lange P, Koper JW, Brinkmann AO, de Jong FH, Lamberts SW. Natural variants of the β isoform of the human glucocorticoid receptor do not alter sensitivity to glucocorticoids. Mol Cell Endocrinol 1999;153:163-8.
25. Longui CA, Vottero A, Adamson PC, Cole DE, Kino T, Monte O, et al. Low glucocorticoid receptor α/β ratio in T-cell lymphoblastic leukemia. Horm Metab Res 2000;32:401-6.
26. Moalli PA, Pillay S, Krett NL, Rosen ST. Alternatively spliced glucocorticoid receptor messenger RNAs in glucocorticoid-resistant human multiple myeloma cells. Cancer Res 1993; 53:3877-9.
27. Pujols L, Mullol J, Perez M, Roca-Ferrer J, Juan M, Xaubet A, et al. Expression of the human glucocorticoid receptor α and β isoforms in human respiratory epithelial cells and their regulation by dexamethasone. Am J Respir Cell Mol Biol 2001; 24:49-57.
28. Leung DY, Chrousos GP. Is there a role for glucocorticoid receptor β in glucocorticoid-dependent asthmatics? Am J Respir Crit Care Med 2000;162:1-3.
29. Beger C, Gerdes K, Lauten M, Tissing WJ, Fernandez-Munoz I, Schrappe M, et al. Expression and structural analysis of glucocorticoid receptor isoform γ in human leukaemia cells

using an isoform-specific real-time polymerase chain reaction approach. Br J Haematol 2003;122:245-52.
30. Lauten M, Beger C, Gerdes K, Asgedom G, Kardinal C, Welte K, et al. Expression of heat-shock protein 90 in glucocorticoid-sensitive and -resistant childhood acute lymphoblastic leukaemia. Leukemia 2003;17:1551-6.
31. Bahr MJ, Vincent KJ, Arthur MJ, Fowler AV, Smart DE, Wright MC, et al. Control of the tissue inhibitor of metalloproteinases-1 promoter in culture-activated rat hepatic stellate cells: regulation by activator protein-1 DNA binding proteins. Hepatology 1999;29:839-48.
32. Pui CH, Costlow ME, Dahl GV, Rivera G, Murphy SB. Response of recurrent acute lymphoblastic leukemia to glucocorticoids: serial studies of receptor content, in vivo cytokinetic changes and clinical responses. Leuk Res 1983;7:747-53.
33. Tsai SY, Carlstedt-Duke J, Weigel NL, Dahlman K, Gustafsson JA, Tsai MJ, et al. Molecular interactions of steroid hormone receptor with its enhancer element: evidence for receptor dimer formation. Cell 1988;55:361-9.
34. Haarman EG, Kaspers GJ, Pieters R, Rottier MM, Veerman AJ. Glucocorticoid receptor α, β and γ expression versus in vitro glucocorticoid resistance in childhood leukemia. Blood 2002;100:322a[abstract].
35. Christodoulopoulos P, Leung DY, Elliott MW, Hogg JC, Muro S, Toda M, et al. Increased number of glucocorticoid receptor-β-expressing cells in the airways in fatal asthma. J Allerg Clin Immunol 2000;106:479-84.
36. Tissing WJE, Lauten M, Meijerink J, den Boer ML, Gerdes K, Beger C, et al. Glucocorticoid receptor isoforms α, β and GR-P in relation to glucocorticoid resistance in childhood ALL. Medical and Pediatric Oncology 2002;39:321a[abstract].

Pre-publication Report & Outcomes of Peer Review

Contributions

GC was involved in planning the study and writing and reviewing the manuscript. GA performed the protein expression experiments. KW and MS took part in planning the project. ML was responsible for designing the case-control study, for the statistical analysis and for writing the report.

We gratefully acknowledge the excellent co-operation of all participants of trials ALL-BFM 90 and ALL-BFM 95 as well that of all doctors and nurses at the participating medical centers.

Disclosures

Conflict of interest: none

Redundant publications: parts of the results were presented at the 5th International Symposium on Leukaemia and Lymphoma, Amsterdam, Netherlands (oral presentation, abstract #O28). The abstract of the presentation was published in Leukemia (Leukemia 2003;17:665).

Funding

This study was partly supported by the Deutsche Leukämie Forschungshilfe, Bonn, Germany and the Madeleine-Schickedanz-Kinderkrebsstiftung, Fürth, Germany.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received June 17, 2003; accepted October 3, 2003.

In the following paragraphs, Professor Cazzola summarizes the peer-review process and its outcomes.

What is already known on this topic

Early treatment response is a strong predictor for treatment outcome in childhood acute lymphoblastic leukemia (ALL), treated within the protocols of the Berlin-Frankfurt-Münster (BFM) study group. In the ALL-BFM trials, early treatment response is assessed by *in vivo* response to glucocorticoids (prednisone response), the molecular background of which is unknown.

What this study adds

This paper reports negative results, suggesting that the expression of glucocorticoid receptor does not influence prednisone response in childhood acute lymphoblastic leukemia.