Acute Lymphoblastic Leukemia

Expression of the glucocorticoid receptor and its isoforms in relation to glucocorticoid resistance in childhood acute lymphocytic leukemia

***In vitro* prednisolone resistance is a poor prognostic factor in the treatment of childhood acute lymphoblastic leukemia (ALL). In a cohort of 54 children with ALL, a lower expression of the glucocorticoid receptor (GR), but not the relative expression levels of the GR-α, GR-β and GR-P isoforms, was associated with *in vitro* prednisolone resistance.**

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In vitro and *in vivo* glucocorticoid (GC) resistance are poor prognostic factors in the treatment of childhood acute lymphoblastic leukemia (ALL). *In vitro* resistance is an adverse risk factor, even for patients with a good *in vivo* response.[1,2] Glucocorticoid-induced apoptosis is initiated after GC binds to the glucocorticoid receptor (GR), and hence a low level of expression of the GR has been hypothesized to be associated with GC resistance. Dexamethasone-binding studies did indeed show that a low receptor number correlated with a high rate of induction failure and relapse in childhood ALL.[3] In contrast, two recent studies suggested that the level of GR expression is not linked to *in vivo* and *in vitro* GC resistance in childhood ALL.[4,5]

Besides the functional GR-α isoform, the GR gene also encodes for isoforms that are unable to bind GC, i.e. GR-β and GR-P (Figure 1). Some studies reported a dominant negative effect (GR-β) and positive effect (GR-P) on GR-α function, whereas other studies could not confirm these findings.[3] In the present study we investigated the correlation between the absolute expression level of GR as well as its isoforms and cellular resistance to GC. To this aim, GR-α, GR-β and GR-P mRNA levels were measured in 54 primary pediatric ALL samples using a quantitative real-time reverse transcription polymerase chain reaction strategy as previously described.[6] Forty-two of these patients were eligible for a paired analysis of prednisolone-sensitive versus prednisolone-resistant cases matched for age (1-9 years and >10 years), immunophenotype (T- or precursor-B ALL) and white blood cell count at diagnosis ($<50\times10^9$/L and $>50\times10^9$/L). Patients with t(9,22) and t(4,11) positive ALL were excluded. Patients were defined as being *in vitro* prednisolone-sensitive or resistant by the MTT assay, using the same criteria for sensitivity (LC_{50} <0.1 μg/mL) and resistance (LC_{50} >150 μg/mL) as previously observed to be of prognostic value.[2]

Our data revealed that GR-α is the predominant isoform, representing 71% of total GR expression. The mRNA level of GR-α strongly correlated with the protein level determined by Western blotting using a polyclonal anti-human GR antibody and anti-β-actin to control for equal protein loading (Spearman's correlation 0.950, $p<0.01$, n=9). The expression of GR-β and GR-P isoforms accounts for 0.1% and 29% of total GR expression, respectively, which is comparable to levels reported in the literature.[5,7] Since the expression of GR-β is very low,

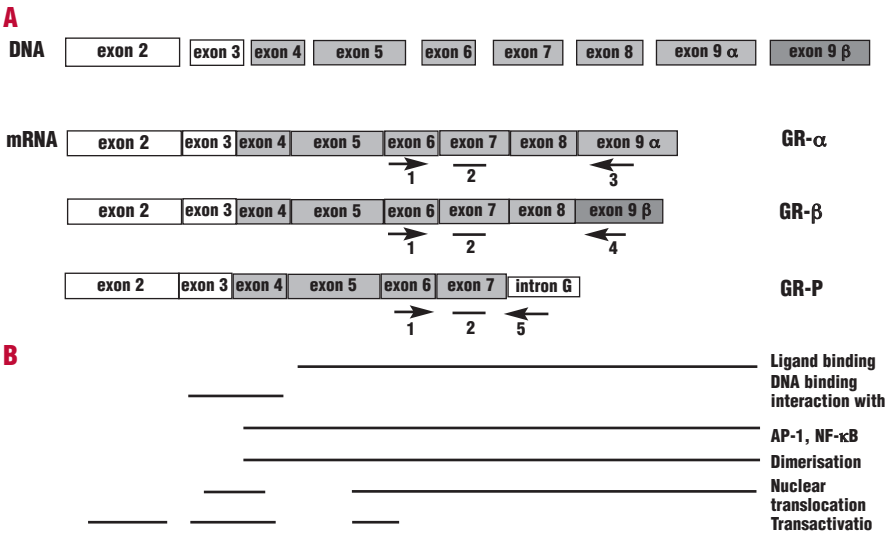

Figure 1. Schematic overview of the glucocorticoid receptor (GR) gene and GR-α, GR-β and GR-P isoforms. A. The location of the primers and probes used in this study are indicated. 1: forward primer for GR-α, GR-β and GR-P, 2: probe for GR-α, GR-β and GR-P, 3: GR-α reverse primer, 4: GR-β reverse primer, 5: GR-P reverse primer. B. Relative position of the functional domains of the GR.

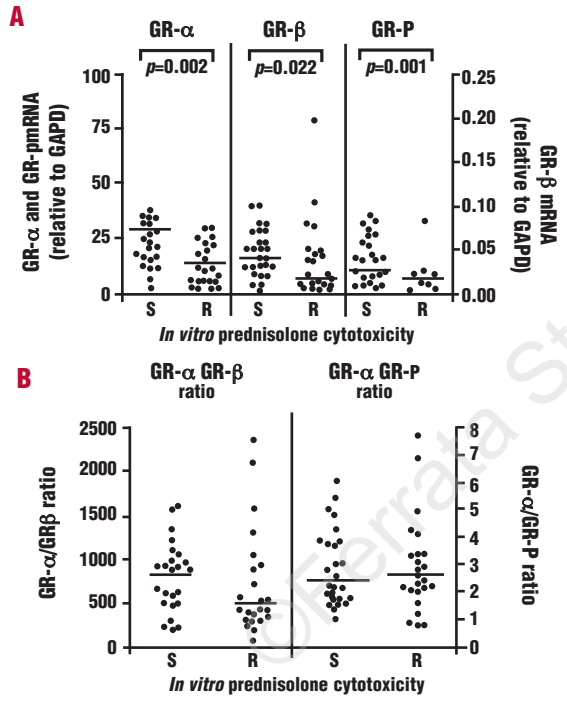

Figure 2 . Expression of the GR-α, GR-β and GR-P isoforms in relation to GC resistance in childhood ALL. A. GR-α, GR-β and GR-P mRNA expression relative to GAPDH (in arbitrary units, AU) in *in vitro* prednisolone sensitive (S; n=29) and resistant (R; n=25) patients. The bar indicates the median value. Significant *p*-values are indicated (Mann-Whitney U test). B. Ratios of GR-α/GR-β and GR-α/GR-P are compared between *in vitro* prednisolone sensitive (S) and resistant (R) cases. GR-α/GR-β ratio (left Y-axis) and GR-alpha/GR-P ratio (right Y-axis). Ratios in *in vitro* prednisolone-sensitive patients did not differ from those observed in resistant patients ($p>0.05$ for each ratio).

it is questionable whether this isoform is of biological relevance in leukemia. The mRNA expression of GR-α was significantly lower in *in vitro* prednisolone-resistant patients than in sensitive patients, both in the total group (n=54, 1.95-fold, Mann-Whitney U test $p=0.002$, Figure 2A) and in the matched group (n=42, 1.6-fold, Wilcoxon's rank test for matched pairs, $p=0.04$). Our results are in contrast to those of Haarman *et al.* who did not observe a correlation between GR-α expression and GC resistance,[5] which might be due to a smaller number of cases as well as the inclusion of patients with intermediate sensitivity to GC.

As for GR-α, the absolute expression levels of both GR-β and GR-P were significantly lower in *in vitro* prednisolone-resistant patients (2.5-fold, $p=0.02$ and 1.6-fold, $p=0.001$ in the total group (Figure 2A) and 1.4-fold, $p=0.05$ and 1.5-fold, $p=0.011$ in the matched group for GR-β and GR-P, respectively). In contrast, the relative (percent) expression of each isoform did not differ between *in vitro* prednisolone sensitive and resistant patients (Figure 2B). These data suggest that the absolute number of receptors is more important for prednisolone toxicity than the relative expression of isoforms in pediatric ALL. GR-β and GR-P are thought to contribute to GC resistance by interfering with GR-α function through formation of non-functional dimers with GR-α or by competing with GR-α for binding to NF-GR-κB and AP-1.[3] Our data imply that, like GR-α, GR-β and GR-P are just naturally occurring splice variants without a selective role in GC resistance.

Two small studies suggested that the GR-γ isoform (with one amino acid insertion in the N-terminal domain of the GR) might be related to GC resistance in childhood ALL.[5,8] However, only 2.8% of total *GR* expression is GR-γ. Furthermore, this amino acid insertion can occur in all C-terminal splice variations (GR-α, GR-β and GR-P), making it very unlikely that this insertion selectively influences GC sensitivity in pediatric ALL.

Taken together, our data suggest that the absolute expression level of GR, but not the relative (percent) expression of the isoforms GR-α, GR-β and GR-P, is

linked to GC resistance in childhood ALL. Since the difference in expression of the receptor is rather small (~2-fold) compared to the >1000-fold difference in resistance to prednisolone between the tested patients, it is likely that other mechanisms may have a more pronounced contribution to GC resistance in pediatric ALL. Other putative causes of resistance may be identified by gene expression profiling.[9,10]

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