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# Effect of Glucocorticoid Therapy on Glucocorticoid Receptors in Children with Autoimmune Diseases

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## ABSTRACT

Low-dose glucocorticoids (GC) achieve their action completely by classical genomic effects, mediated by the glucocorticoid receptor (GCR). In high doses of GC, nongenomic effects have also been found, but it is still unclear to what extent they contribute to a beneficial outcome. In this study, we present a determination of the number of lymphocyte GCR sites and the binding affinity in healthy children and children with autoimmune diseases. We further assess the effect of GC administration, especially of high-dose pulse therapy on the number of binding sites. The number of GCR sites per cell was analyzed with [$^3$H]-dexamethasone radioligand binding assay and binding affinity (Kd given in nM) in peripheral blood mononuclear cells isolated from 48 healthy children and 35 patients. The patients were divided into three groups based on GC treatment: 0 mg/kg (group 1), 0.01–0.3 mg/kg orally (group 2), and 10–15 mg/kg i.v. pulse therapy (group 3) of prednisolone equivalent per day. Gender- and age-independent normal values of 4338 ± 1687 sites/lymphocytes and Kd 6.7 ± 2.2 nM were found. At 3463 ± 1574, the number of receptor sites in patients without GC (group 1) was significantly lower than that of healthy volunteers ($p < 0.05$). In patients receiving GC treatment, this value was reduced to 2952 ± 512 (group 2). Significant down-regulation to a minimum of 479 ± 168 (group 3) was found after pulse therapy compared with untreated patients ($p < 0.01$). In pulse therapy, GC lead to a fast and dramatic receptor down-regulation. We suppose that the increase in therapeutic success of pulse-therapy may partly be mediated through additional nongenomic effects. **(*Pediatr Res* 49: 130–135, 2001)**

**Abbreviations:**
**GCR,** glucocorticoid receptors
**GC,** glucocorticoids
**Kd,** dissociation constant of [$^3$H]dexamethasone
**PBMC,** peripheral blood mononuclear cells
**MP,** methylprednisolone

GC are potent immunosuppressive and anti-inflammatory drugs. Since 1949, they have been used therapeutically in immunologically mediated diseases. Buttgereit *et al.* have recently published a new modular concept to describe pharmacotherapy with GC (1). It is based on the hypothesis that these agents have both genomic and nongenomic effects. Very low doses of GC produce exclusively genomic effects, mediated by the nuclear GCR. With higher doses, additional nongenomic effects occur that are considered to be mediated by membrane-bound receptors (specific nongenomic effects) and/or direct physicochemical interaction with cell membranes (nonspecific nongenomic effects). It is still unclear whether these effects add to a beneficial clinical outcome.

Cortisone is known to have the strongest influence on the number of GCR in lymphocytes. This GCR is an intracellular 94-kD protein, which belongs to the superfamily of nuclear hormone receptors (2, 3). The receptor protein has two isoforms: GCR$\alpha$, which mediates the hormonal effects, and GCR$\beta$, which is incapable of binding to the hormone and acts as the antagonist of GCR$\alpha$ (4).

Normal ranges of GCR in a large collective of healthy children and children with autoimmune diseases are unknown. A few studies with small numbers of patients using different methods have been published (5–7). The aim of this study is to determine comparative data of GCR sites on lymphocytes and binding affinity in healthy children and children with autoimmune diseases under different treatment regimens. Results show for the first time in children the dose-dependent down-

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regulation of GCR content as well as the binding affinity of PBMC after GC administration. We have studied especially the influence of i.v high-dose MP pulse therapy on the GCR density and the fast and dramatic down-regulation of receptors directly after therapy. Therefore, we assume the possible effect of nongenomic action of GC with these very high concentrations.

## METHODS

***Ethics.*** The study was approved by the local ethics committee. Before collecting blood samples, informed consent of parents and patients or volunteers was obtained.

***Control group of healthy children.*** The control group consisted of 48 healthy children and adolescents aged 5–17 y, 28 girls (median 12 y) and 20 boys (median 10 y), who were admitted to hospital for an elective operation. All of them had negative signs of infection and no medical history of GC therapy. The blood samples were obtained between 0700 and 0800 h. Laboratory studies included peripheral leukocyte and differential counts, C-reactive protein, plasma cortisol, and GCR analysis.

***Patients.*** In total, 35 children and adolescents with autoimmune diseases (19 girls aged 6–16 y, median 12 y, and 16 boys aged 6–17 y, median 13 y) were investigated. All children were consecutive patients seen in our pediatric outpatient clinic or were admitted to the pediatric rheumatologic and respiratory diseases unit with the following autoimmune diseases:

- Juvenile chronic arthritis (JCA). JCA was diagnosed in 23 children using the criteria of the European League Against Rheumatism (8) (3 systemic JCA, 5 RF-negative polyarthritis, 4 oligoarthritis I, 5 oligoarthritis II, 3 psoriatic arthritis, 3 spondylarthropathy).
- Systemic lupus erythematodes (SLE). Three patients met the revised criteria for SLE (9).
- Other autoimmune diseases (OAD): 2 polymyositis (10); 2 Behçet's syndrome (11); 1 microscopic panarteritis (12); 1 recurrent uveitis (13); 1 neonatal onset of multisystemic inflammatory disease (NOMID) (14), 1 familial Mediterranean fever (15); 1 alveolitis (16).

Blood samples were obtained between 0700 and 0800 h in all patients and analyzed for erythrocyte sedimentation rate, C-reactive protein, white blood count and differential counts, plasma cortisol, and GCR analysis.

We reported on a total of 48 measurements of GCR and Kd within the three groups divided on the basis of GC dose. These measurements have been obtained on various occasions from the 35 patients classified according to the above-mentioned diagnostic categories (Table 1).

Criteria for starting, restarting, or increasing GC was active disease.

Sixteen patients were receiving GC; of these, five received a high-dose 3-d course of i.v. MP for the first time during these evaluations. The five patients on the high-dose MP course had GCR analysis both before and the day after therapy, and two patients also had GCR analysis 3 wk after the course of MP pulse therapy.

***Group formation based on GC treatment.*** The patients were divided into the following three groups on the basis of GC treatment and dosage:

- Group 1 consisted of 32 patients (15 boys and 17 girls) with rheumatoid diseases with controlled disease activity currently not requiring GC therapy or patients requiring but not yet treated with GC.
- Group 2 consisted of 11 patients (4 boys and 7 girls) who had a low-dose daily GC therapy of 0.01–0.3 mg/kg body weight per day. Some patients received GC therapy because of high disease activity, others as long-term therapy to control disease activity.
- Group 3 consisted of 5 patients (2 boys and 3 girls) with acute and severe disease activity, who received for the first time a 3-d course of MP at a dose of 10–15 mg/kg/d i.v. over 2–3 h.

***Preparation of PBMC.*** After a complete physical examination, blood samples were taken for routine and laboratory research analysis. Peripheral blood was collected in heparinized syringes and PBMC were isolated by density centrifugation using the Ficoll-Hypaque technique. Specifically, a sample of 15–20 mL of blood was withdrawn into heparinized tubes. In LeukoSep® tubes with a porous filter disc (Esquire, Zurich, Switzerland), the blood was diluted 2-fold with modified Hanks' balanced salt solution (HBSS pH 7.4; Sigma Chemical Co., St. Louis, MO, U.S.A.) and layered over Ficoll-Hypaque. Density centrifugation was performed at 400 g for 20 min. The PBMC-enriched interphase was isolated and diluted with 30 mL HBSS buffer. The cells were incubated for 40 min at 37°C in a shaking bath. This procedure is usually used to remove residual exogenous and endogenous cortisol completely (7, 17–20). Centrifugation was then performed at 400 g for 8 min. The cell pellet was washed with 35 mL of HBSS buffer and the final pellet was resuspended in 3 mL HBSS buffer. The PBMC

**Table 1.** *Distribution of patients according to categories and GC groups*

| | JCA ($n$ = 23) | | SLE ($n$ = 3) | | OAD ($n$ = 9) | | Total ($n$ = 35) (patients) | |
|---|---|---|---|---|---|---|---|---|
| Diagnoses | $n_p$ | $m$ | $n_p$ | $m$ | $n_p$ | $m$ | $n_p$ | $m$ |
| Group 1 | 20 | 21 | 2 | 2 | 6 | 9 | 28 | 32 |
| Group 2 | 4 | 5 | 2 | 2 | 4 | 4 | 10 | 11 |
| Group 3 | 0 | 0 | 0 | 0 | 5 | 5 | 5 | 5 |
| Total ($n$ = 48) (GCR analysis) | 24 | 26 | 4 | 4 | 15 | 18 | 43 | 48 |

$n$, number of patients; $m$, number of GCR measurements and Kd.

suspension consisted of lymphocytes and 3%–15% monocytes, as determined by fluorescence-activated cell sorter analysis. Trypan blue staining revealed more than 95% viable cells.

***Determination of receptor density and affinity.*** Established [$^3$H]dexamethasone radioligand binding assay and Scatchard analysis were used as described (7, 17–21) to determine the number of GCR sites per cell and receptor binding affinity (Kd). In brief, following PBMC preparation as described above, we added to 300 $\mu$L of cell suspension (containing about $2 \times 10^6$ PBMC; a Coulter counter was used in each experiment to determine exact cell numbers), 200 $\mu$L of [$^3$H]-labeled dexamethasone (6,7[$^3$H]; specific activity 40–60 Ci/mmol; DuPont De Nemours, Brussels, Belgium) diluted in the same HBSS medium. Four concentrations of tritium-dexamethasone (2.5, 5, 10, 20 nM) were used to achieve a complete binding curve. Identical aliquots were incubated in the presence of a 1000-fold excess of unlabeled dexamethasone to determine nonspecific binding.

Incubation of all aliquots was performed at 37°C for 40 min with continuous shaking and then stopped by adding 2 mL ice-cold $MgCl_2$ solution. Samples were centrifuged at 800 g for 2 min and washed three times with 800 $\mu$L of PBS at 4°C. The final pellet was resuspended in 1.6 mL of isotonic NaCl solution and transferred into 6 mL of liquid scintillation cocktail (Serva, Heidelberg, Germany). All incubations were performed in duplicate. Radioactivity was measured for 3 min in a scintillation counter (model Wallac 1410, Wallac Oy, Turku, Finland). The number of GCR sites per cell was calculated by the Scatchard method (21), using computer-assisted linear regression.

***Plasma cortisol.*** Plasma cortisol levels were estimated with a commercial RIA (Immunotech-Coulter, Marseille, France).

The assay cross-reacted with 6 $\alpha$-MP at 0.27%, with prednisone at <0.1%, and with prednisolone at 6%. The intra-and interassay coefficients of variation were between 3.1% and 5.8% and 5.3% and 9.2%, respectively, for values between 36 and 740 nmol/L.

***Statistical methods.*** All results for number of GCR are expressed as geometrical mean/SD. Dissociation constant (Kd) and plasma cortisol levels are expressed as mean/SD. Results between the groups were compared using the Mann-Whitney test. Probability values of <0.05 were considered to be statistically significant.

## RESULTS

***GCR content and dissociation constant in healthy controls.*** In 48 healthy children (20 boys and 28 girls, aged 5 to 17 y, median 11 y), the number of GCR was 4338 ± 1687 sites/lymphocyte and the mean binding affinity showed a Kd of 6.7 ± 2.2 nM (Fig. 1). There was no statistical difference between males and females and no correlation with age was found.

***GCR content and Kd in patients with autoimmunologic-mediated diseases.*** In group 1, the number of GCR and Kd in 32 patients (15 boys and 17 girls, aged 6 to 16 y, median 12 y) not receiving steroid therapy was significantly lower than in healthy children ($p < 0.02$). The geometrical mean was 3463 ± 1574 sites/lymphocyte for GCR. Dissociation constant Kd was lower, with 5.2 ± 2.1 resulting in higher binding affinity than in healthy children (Fig. 1).

In group 2, 11 patients (4 boys, 7 girls, median 13 y) who had low-dose oral GC therapy (0.01–0.3 mg prednisolone/kg/d), binding sites of GCR were lower than in patients without GC treatment. The geometrical mean of 2952 ± 512 sites/lymphocyte and mean values for Kd of 4.9 ± 1.6 nM were determined, but significance was not reached compared with untreated patients (see Fig. 1).

In group 3, five patients (2 boys and 3 girls, median 12 y) were treated with MP pulse therapy over a 3-d course, with i.v. doses of 10–15 mg/kg/d. Baseline values of GCR and Kd before treatment were comparable with those of patients in group 2, because three of the patients had prior oral low-dose GC therapy. Measurement on the day after pulse therapy determined significant reduction to values of 479 ± 168 sites/lymphocyte—only one-sixth of the baseline values. In two patients, the binding assay was performed 3 wk after pulse therapy and showed a return to normal values with 3373 and 3584 sites/lymphocyte, respectively, comparable with other patient groups (Fig. 2).

There were no differences between the different diseases. Within the patient groups, Kd did not differ significantly. Other publications also found no difference in quantity of GCR in relation to age or gender (17, 21).

***Plasma cortisol levels.*** Healthy children in the control group had plasma cortisol levels within normal limits (140–665 nmol/L). No correlations with gender and age were found.

Children in group 1 (patients without GC therapy) had plasma cortisol levels toward the lower end of the normal range, with 290 ± 144 nmol/L.

In group 2 (GC treatment of 0.01–0.3 mg/kg/d), some patients had suppressed plasma cortisol levels. Five patients had values below 140 nmol/L, with 99.6 ± 15.7 nmol/L, but the remaining six patients had plasma cortisol levels within normal limits, with 222 ± 78.8 nmol/L.

Children receiving pulse therapy (group 3) had strongly suppressed plasma cortisol levels of 47 ± 35 nmol/L, which normalized 3 wk after therapy (Table 2).

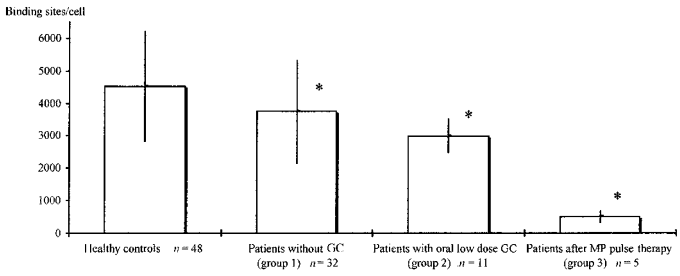


**Figure 1.** GCR content in healthy children and children with autoimmune diseases. In a large collective of 48 healthy boys and girls, GCR number and dissociation constant were determined by radioligand binding assay and Scatchard analysis. They were compared with children with autoimmune diseases. Patients had significantly lower GCR numbers than healthy controls. Patients with oral low-dose GC treatment (0.01–0.3 mg/kg/d) showed fewer receptor numbers than patients without treatment (NS). After i.v. MP pulse therapy (10–15 mg/kg/d for 3 d), a fast and dramatic down-regulation of receptor content was found in five patients. *$p < 0.02$ compared with healthy children.

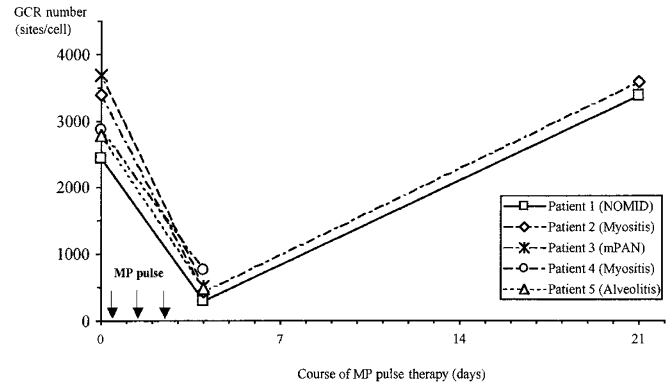


**Figure 2.** Number of GCR in patients before and after MP pulse therapy (10–15 mg/kg/d). Five patients with severe outbreaks of their autoimmune diseases were successfully treated with MP pulse therapy. The number of GCR were down-regulated to only one-sixth of baseline values directly after pulse therapy, but measurements of two patients showed a return to normal values after 3 wk.

**Table 2.** *GCR content, Kd, and plasma cortisol levels*

| | Number of GCR (sites/lymphocyte: geom. mean/SD) | Dissociation constant (Kd in nM mean/SD) | Plasma cortisol level (nmol/l: mean/SD)* |
|---|---|---|---|
| Healthy controls ($n$ = 48) | 4338 ± 1780 | 6.7 ± 2.2 | 382 ± 184 |
| Girls ($n$ = 28) | 4292 ± 1789 | 6.2 ± 2.2 | 375 ± 206 |
| Boys ($n$ = 20) | 4404 ± 1811 | 7.3 ± 2.0 | 392 ± 153 |
| Group 1: Patients with no GC ($n$ = 32) | 3463 ± 1574 | 5.2 ± 2.1 | 290 ± 144 |
| Girls ($n$ = 17) | 3229 ± 1211 | 5.5 ± 2.0 | 278 ± 149 |
| Boys ($n$ = 15) | 3748 ± 1885 | 4.9 ± 2.2 | 236 ± 91 |
| Group 2: Patients with oral GC ($n$ = 11) (0.01–0.3 mg/kg/d) | 2952 ± 512 | 4.9 ± 1.6 | 166 ± 85 |
| Girls ($n$ = 6) | 2852 ± 563 | 4.9 ± 1.5 | 161 ± 58 |
| Boys ($n$ = 5) | 3086 ± 427 | 4.7 ± 1.9 | 180 ± 155 |
| Group 3: Patients before pulse therapy ($n$ = 5) | 3004 ± 500 | 5.9 ± 3.1 | 403 ± 117 |
| Patients 1 d after pulse therapy ($n$ = 5) (10–15 mg/kg/d) | 479 ± 168 | 4.4 ± 2.1 | 47 ± 35 |
| Patients 3 wk after pulse therapy ($n$ = 2) | 3373<br>3584 | 6.0<br>3.1 | 120<br>360 |

* Normal values: 140–665 nmol/L.

## DISCUSSION

In this study, we present for the first time comparative normal values of GCR and the binding affinity of PBMC in a large collective of healthy children. In this group, we determined 4338 ± 1687 GCR sites per lymphocyte with a Kd of 6.7 ± 2.2 nM. No correlations with gender or age were found. Of special interest is that numbers of GCR in the children below 9 y of age and in the older subjects were similar. This suggests that no significant change in GCR content occurs with the onset of puberty.

Early reports of hematological malignancies provide the first data about GCR content in healthy adults and children. In the various reports, binding sites of GCR in blood lymphocytes were found to be in a range from 3000 to 7000 sites per cell with a Kd of around $10^{-8}$ M (22). Tanaka *et al.* (19) described the effects of age, gender, and season on GCR in normal leukocytes of 145 healthy subjects aged 18–78 y. They did not find a difference of receptor number related to season, circadian rhythm, plasma cortisol, or gender, but reported a decrease of GCR in healthy volunteers over the age of 20. Schlaghecke *et al.* (23) determined GCR in 100 healthy adult men and women, respectively, but did not find a correlation with gender or season. However, they found a significant difference in the circadian rhythm of GCR, with higher numbers at 2300 h. They could not confirm an age-related decrease of GCR and the binding affinity did not differ between the groups. They further compared healthy adults and patients with rheumatoid arthritis concerning the difference in GCR content and binding affinity. An important finding was that the number of GCR was significantly higher in healthy volunteers (5619 ± 1369 binding sites per lymphocyte) than in patients with rheumatoid arthritis (2159 ± 492) who were not being treated with GC. We also measured significantly lower receptors in the group of patients with autoimmune diseases without current GC treatment (group 1), compared with healthy children. We measured GCR in group 1 at 3463 ± 1574 sites per lymphocyte and Kd was 5.2 ± 2.1 nM. It is interesting that two-thirds of these patients had a juvenile chronic arthritis as underlying autoimmune disease, which is similar to Schlaghecke's group of patients with rheumatoid arthritis. In contrast, Sanden *et al.* (17) very recently determined significantly more GCR in adult patients with autoimmune diseases, compared with healthy adult volunteers. However, this group consisted predominantly of patients with SLE, and only 20% had rheumatoid arthritis, which could explain the different findings.

Many authors have observed down-regulation of GCR after GC administration. Schlechte *et al.* (1982) and Shipman *et al.* (1983) showed a decrease of receptor number in healthy volunteers after GC administration. The down-regulation was rapid and return of receptor number to baseline required up to 2.5 wk (24, 25).

Several mechanisms of GCR down-regulation have been reported. It is well known that the most important factor in down-regulation of GCR is the modulation of GC themselves. One explanation for this negative feedback is the protection of tissue from possible damage due to excessive and extended GC action (26). Further, tight regulation of activity of the GCR is suggested because of the wide spectrum of positive and negative gene expression by the GCR. There are transcriptional, posttranscriptional, and posttranslational effects described as mechanisms of down-regulation. On the transcriptional level, GC decrease GCR mRNA significantly after 2–3 h. This has been observed in all cells and tissues that undergo down-regulation of GCR protein. Reduction of the GCR protein level itself requires at least 12–24 h after the start of therapy (4, 26–33). Activated GCR have also been shown to bind to sites of coding DNA and/or mRNA, rather than within the GCR promoter, which lacks consensus glucocorticoid responsive element sites (34–36). The result is down-regulation by inhibition of transcription and/or by reduction of mRNA stability and translatability (37). An additional mechanism of down-regulation is the reduction of GCR protein half-life in the presence of GC (38).

In the present study, we found GCR down-regulation in patients who received oral low-dose GC treatment up to 0.3 mg/kg/d (group 2), but this did not reach significance. Griese did not find a significant difference between only a small number of healthy and asthmatic children; but when a short course of prednisolone therapy was administered in the asthmatics, significant reduction in GCR number was seen (5). Hampl *et al.* could not determine any significant difference in GCR content between healthy children and children with systemic diseases under long-term GC therapy with doses of 0.04–1.4 mg/kg prednisolone per day (6), but they used a different method. In our laboratory, Sanden *et al.* reported for the first time dose dependency in GCR down-regulation. It was shown that GC administration from doses of 0.25–1 mg/kg/d results in significant reduction of GCR in lymphocytes *in vivo*. Doses >1 mg/kg/d, usually achieved in pulse therapy in adults, resulted in even greater down-regulation of the GCR. Nevertheless, very low doses of oral prednisolone (up to 0.25 mg/kg/d) did not lead to a significant reduction of binding sites (17). This is similar to our findings that the patients of group 2, taking very low oral prednisolone doses (up to 0.3 mg/kg/d), showed a (nonsignificant) down-regulation compared with patients with no steroid treatment. It seems that down-regulation of GCR requires doses above 0.25 mg/kg/d. The exact dose-equivalent of prednisolone, where receptor reduction starts, still has to be established.

GC are important anti-inflammatory and immunosuppressive drugs with three distinct effects: genomic, specific nongenomic, and unspecific nongenomic. It is widely appreciated that GC act mostly *via* receptor-mediated effects (39) (classical genomic effects). For these genomic effects, GC bind to the ubiquitously expressed cytosolic GCR. The activated steroid-receptor complex is then translocated to the nucleus, where the synthesis of important regulating proteins, *e.g.* lipocortin-1, an inhibitor of phospholipase $A_2$, is initiated. The steroid-receptor complex also interacts with transcription factors (activating-protein-1 and nuclear factor-$\kappa$B), resulting in decreased synthesis of certain proteins, such as proinflammatory cytokines. These genomic actions of GC are observed at any therapeutic concentration, but occur not earlier than 30 min after receptor binding (1), whereas nongenomic effects have been shown to occur only at high doses (40). From *in vitro* experiments it is suggested that at doses below 250 mg prednisolone equivalent per day, nongenomic effects are of minor concern (41, 42). Increasing the dose of GC leads to additional nongenomic-mediated effects (1). Nongenomic effects occur rapidly (within seconds to a few minutes) and result mainly from interaction with cell membranes. It is believed that specific nongenomic actions are mediated by steroid-selective membrane-bound receptors, which leads to a second messenger cascade. Nonspecific nongenomic actions of GC are direct physicochemical effects on cellular membranes (43). In therapeutically relevant concentrations, MP instantaneously inhibits $Ca^{2+}$ and $Na^{+}$ ions cycling across the membranes and decreases intracellular free calcium concentrations, but has little effect on protein synthesis (44). Further effects are a decreased phospholipid turnover in the cell membranes and a decreased production of free radicals (43). These direct effects are suggested to be mediated by direct actions on biologic membranes and are supposed to interfere with activation and maintenance of immune cells. Therefore, we believe that with the administration of high doses of GC we take additional advantage of the nongenomic effects, which then may mediate to a better result in quickly combating the acute and intense immune responses.

Severe autoimmune disorders in childhood often require prolonged treatment with GC. The therapy shows the same side effects as in adults with one major additional problem: it also inhibits linear growth. MP pulse therapy is a very effective treatment in autoimmune-mediated diseases (45–48), with relatively mild and transient side effects in children (49) and is also believed not to interfere with linear growth (50, 51). This therapy is especially important if rapid and strong control of disease activity is needed.

We studied five patients with different underlying autoimmune diseases, who had an acute and severe outbreak of the disease and consequently underwent MP pulse therapy. Before starting pulse therapy, the baseline number of GCR corresponded to that of patients with low-dose oral GC (group 2). Measurement on the day after the 3-d course of pulse therapy showed that the high concentration of GC produced receptor saturation and led to a rapid and dramatic down-regulation of receptors. These high concentrations of GC consequently meet only one-sixth of the baseline values of GCR. We have observed that by adhering to the usual interval of 3-4 wk between pulses, the number of GCR do recover from down-regulation, as shown by analysis of two patients. Compared with group 2 (patients receiving low-dose oral GC), the doses of the administered GC in pulse therapy are evidently much higher and result in a dramatic down-regulation of the GCR. The reason for this decrease in binding sites could be that the much higher doses of hormone recruit more GCR and quantitatively more genomic effects are produced, which then lead to a more profound down-regulation. However, it cannot be excluded that the appearance of additional nongenomic effects of GC at high doses contribute to this down-regulation by as yet unknown mechanisms.

The cause for our findings that children with autoimmune diseases without GC therapy have lower GCR numbers than healthy controls remains unclear. The current understanding of a regulatory degradation of the number of GCR requires GC themselves, which act by the described mechanisms of down-regulation. However, in the patients of group 2 we found plasma cortisol levels that were at the lower end of the normal range, with few patients having slightly decreased levels (Table 2). Several authors have described similar findings in adult rheumatoid arthritis patients who have low plasma cortisol levels that are still within the normal limits (52). A potential pathogenetic mechanism in chronic inflammatory disorders could be a hypofunction of the hypothalamic-pituitary-adrenal axis, as several investigators have suggested in patients with rheumatoid arthritis (53).

Therefore, it can only be speculated that either inherent genetic factors or active inflammatory mechanisms lead to a decrease in GCR numbers in children with autoimmune disorders. A possible mechanism could be the increased levels of

proinflammatory cytokines and/or other inflammatory mediators.

In conclusion, the present study shows that children with autoimmune diseases have lower GCR numbers on PBMC than healthy children. There is no clinical doubt of the therapeutic effectiveness of the treatment with MP pulse therapy, despite the significant subsequent decrease in binding sites for the hormone. We support the hypothesis that the success of pulse therapy may partly be mediated by additional nongenomic effects.

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