Glucocorticoid Receptors in Normal Leukocytes: Effects of Age, Gender, Season, and Plasma Cortisol Concentrations

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We measured glucocorticoid receptors (GR) in mononuclear leukocytes (MNL) isolated from peripheral blood of 145 apparently healthy volunteers (86 men and 59 women). An age-related decrease in the number of GR was suggested between subjects younger than 20 years and elderly subjects; there was no apparent seasonal variation in GR. Gender difference in the number of GR was not significant, although women showed slightly fewer GR. Eight patients with dermatomyositis/polymyositis were examined to determine whether the number of GR in MNL could be down-regulated by their cognate ligands. The number of GR in MNL from these patients was significantly decreased one month after the initiation of prednisolone therapy. However, in normal subjects, the GR in MNL did not demonstrate circadian variation, in contrast to concentrations of plasma cortisol.

Additional Keyphrases: sex- and age-related effects · dermatomyositis/polymyositis · prednisolone therapy

Glucocorticoids exert numerous biological effects on various cellular and metabolic processes. On entry into target cells, they interact with specific intracellular proteins, e.g., glucocorticoid receptors (GR); the GR then undergo a conformational change that enables a receptor to translocate to the nucleus and bind to specific acceptor sites of DNA.3 The GR thereby modulate the expression of target genes in a tissue-specific manner, resulting in a cascade of biological events (reviewed in I, 2).

Abundant evidence has already been accumulated suggesting the importance of GR determination in assessing therapeutic responsiveness to glucocorticoids in patients with heterologous diseases and in healthy humans (3–9). However, it has become apparent that the relationship between the number of GR and glucocorticoid responsiveness is not always consistent, the reason for which is still unclear. Moreover, as previously reported, the number of GR shows wide variation, attributable to differences in assay procedures or conditions (3–16), which has made it extremely difficult to compare independently performed studies. Taken together, contents of GR have been suggested to be critical in predicting the magnitude of hormone effects in various diseases, but that is not sufficiently proven yet, at least in part because of variation in the GR assay itself. We have already established a simple whole-cell assay of GR in human mononuclear leukocytes (MNL) (9) and have shown that the number of GR in normal human MNL correlates to hormone responsiveness both in vivo (9) and in vitro (9a). To further verify the importance of this assay procedure with regard to clinical application, we have studied a relatively large, apparently healthy population to examine age- or gender-related and seasonal and diurnal variations in the number of GR in normal human MNL.

Subjects and Sampling

Subjects were 145 apparently healthy volunteers: 86 men, ages 18–79 years (mean 24.2, SD 35.1), 59 women, ages 18–77 years (mean 32.3, SD 31.8), and eight women with dermatomyositis/polymyositis, ages 19–36 years (mean 23.5, SD 11.1).

Blood was drawn into heparinized tubes by antecubital venous puncture, generally at 0800 h after an overnight fast, with two consecutive blood samplings at 15-min intervals (the volumes of blood drawn were 60, 5, and 5 mL, respectively). MNL were isolated, without delay, from 55 mL of the initially collected blood sample and analyzed for GR. Plasma was separated from 5 mL of each blood collection and stored at −80 °C until determination of cortisol.

Materials and Methods

Chemicals. [6,7-3H]Dexamethasone (specific activity, 47.5 kCi/mol) and [1,2-3H]cortisol (40 kCi/mol) were purchased from New England Nuclear (NEN) Research Products, Boston, MA. Unlabeled dexamethasone was from Sigma Chemical Co., St. Louis, MO. Ficoll 400 was from Pharmacia, Uppsala, Sweden. Hypaque was from Winthrop Labs., New York, NY. RPMI-1640 medium and fetal calf serum were from Gibco BRL Labs., New York, NY.

Cell preparations. Whole blood was fractionated by one-step gradient centrifugation with Ficoll (80.2 g/L)–Hypaque (1.077 kg/L) at 400 × g for 30 min at 20 °C. The MNL-enriched interphase was washed twice in phosphate-buffered saline, pH 7.4, then resuspended in RPMI-1640 medium and used as MNL in the present study. Viability of cells exceeded 95% during the experiments, as judged by trypan blue dye exclusion.

Whole-cell assay of GR. Cell counts were adjusted to 1 × 10^9/L in RPMI-1640 medium and the GR were
determined essentially as described before (9). Briefly, 200 μL of suspension was added to all tubes, which had previously received 50 μL of RPMI-1640 medium containing [3H]dexamethasone at final concentrations of 0.5, 1, 2.5, 5, 10, 20, and 40 nmol/L, respectively, with or without a 500-fold molar excess of unlabeled dexamethasone. All tubes were incubated at 37°C with gentle shaking for 2 h. Then, 200 μL of suspension was transferred into a microcentrifuge tube (Microfuge™; Beckman Instruments, Palo Alto, CA) containing 150 μL of silicone oil cushion. After centrifuging the sample at 10,000 × g for 45 s, we cut off the bottom tip of tube, which contained the cell pellet, and placed this in a scintillation counting vial. The radioactivity of the pellet was counted in 10 mL of Aquasol (NEN Research Products) with a Beckman Model LS-9800 scintillation counter, which has an average efficiency of 40% for tritium. All determinations were performed in triplicate. The apparent dissociation constant (Kd) and the number of specific binding sites (Rmax) were determined by Scatchard analysis (17).

Radioimmunoassay of plasma cortisol. We measured cortisol in plasma in duplicate by radioimmunoassay (18) and then calculated the mean value. The detection limit of the assay was 3 nmol/L. The intra- and interassay CVs were <10% and <17%, respectively (18).

Statistical analysis. Linear regression and correlation were calculated by the least-squares method. For comparison of two variables, we used Student's t-test, paired or unpaired where appropriate (19). Results are expressed as mean ± SD unless otherwise specified.

Results

Whole-cell assay for GR in normal human MNL. Rmax and Kd were determined by Scatchard analysis, and individual Rmax results were used as the GR number of the corresponding subject. In five subjects, GR determination was repeated five times in separate experiments, and the intra-assay CV was calculated for Rmax and Kd, respectively. The CV for Rmax ranged from 0.6% to 26.1% (mean 9.8%), and the CV for Kd from 4.4% to 39.2% (mean 15.9%), as shown in Table 1.

Effects of age, gender, and time of year on number of GR in MNL. In 145 healthy subjects, the range of Rmax was 2.5–16.4 fmol/10^6 cells (8.5 ± 2.5, mean ± SD), and Kd was 2.2–15.8 nmol/L (6.8 ± 2.3). When the GR number was determined, we plotted the individual GR number in MNL against age (Figure 1). There was no significant correlation between GR number and age (r = −0.122). However, when subjects were divided into four groups by age (Table 2), group B (age ≥20 and <40 years) showed fewer GR in MNL than did group A (age <20; P < 0.05). Although not statistically significant, groups C (age ≥40 and <60) and D (age ≥60) also showed relatively fewer GR than did group A.

Gender difference was unclear in the GR number in MNL (Figure 2). The range of GR number in MNL in 86 men was 3.3–13.3 fmol/10^6 cells (8.7 ± 2.8), and in 59 women was 2.5–16.4 fmol/10^6 cells (8.1 ± 3.0).

Seasonal variation in the GR number in MNL is shown in Figure 3. There was no apparent relationship between the GR number in MNL and the month of the year when GR were determined.

Down-regulation of GR in MNL in patients undergoing prednisolone therapy. To test whether our method for GR determination detected down-regulation of GR in MNL after exogenous administration of glucocorticoids, we studied eight women with dermatomyositis/polymyositis. Before administration of synthetic glucocorticoid prednisolone, the GR number in MNL ranged from 7.7 to 15.3 fmol/10^6 cells (mean 10.7, SD 2.2). Initial dosages of prednisolone varied from 40 to 60 mg/d (mean 54.9, SD 9.0). One month after initiation of the therapy, GR were determined again in MNL of the corresponding patients. In seven of eight patients, the GR in MNL decreased, and the mean GR number in MNL (7.8 SD

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**Table 1. Precision of Whole-Cell Assay of Glucocorticoid Receptors**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Receptor number (fmol/10^6 cells) ± SD</th>
<th>CV, %</th>
<th>Kd (nmol/L) ± SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.T.</td>
<td>10.41 ± 0.91</td>
<td>9.1</td>
<td>7.93 ± 0.64</td>
<td>10.6</td>
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<tr>
<td>S.A.</td>
<td>9.06 ± 0.06</td>
<td>0.6</td>
<td>7.15 ± 0.31</td>
<td>4.4</td>
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<tr>
<td>D.I.</td>
<td>11.22 ± 0.17</td>
<td>1.5</td>
<td>7.26 ± 1.23</td>
<td>16.9</td>
</tr>
<tr>
<td>H.O.</td>
<td>10.37 ± 2.71</td>
<td>26.1</td>
<td>6.31 ± 2.47</td>
<td>39.2</td>
</tr>
<tr>
<td>D.M.</td>
<td>9.75 ± 1.13</td>
<td>11.6</td>
<td>8.07 ± 0.67</td>
<td>8.3</td>
</tr>
</tbody>
</table>

n = 5 assays for each subject.

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**Table 2. Effects of Age on GR Number in MNL**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, years</th>
<th>GR number (fmol/10^6 cells), mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>13</td>
<td>&lt;20</td>
<td>11.4 ± 2.4</td>
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<tr>
<td>B</td>
<td>101</td>
<td>≥20 and &lt;40</td>
<td>9.6 ± 2.6*</td>
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<tr>
<td>C</td>
<td>20</td>
<td>≥40 and &lt;60</td>
<td>8.5 ± 2.8</td>
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<tr>
<td>D</td>
<td>11</td>
<td>≥60</td>
<td>7.9 ± 2.6</td>
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* P < 0.05 vs GR number in group A.
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tions of GR during aging. In the present study, the relationship between age and the GR number in MNL was not significant, partly because of nonuniform distribution of the subjects by age. However, younger subjects tended to have relatively more GR in MNL, as also reported for rats. Plasma cortisol concentrations reportedly do not change considerably during aging (27); thus, developmental change in the GR number probably does not reciprocally complement the age-related changes in plasma cortisol concentrations, but may relate to some other aspects of human development and aging.

Gender differences in the distribution and regulation of GR have also been studied in rats (28), but not in humans. The GR number in the thymus and the liver are lower in female than in male rats (28). Humans show no significant gender difference in plasma cortisol concentrations (29), and the present study also showed no marked difference by gender in the number of GR in MNL. Ovariectomy increases GR concentrations in rats, presumably by abrogating agonistic activity of progesterone (28). Accordingly, to explain these differences between rats and humans, we must consider the possibility that plasma progesterone may also influence the GR number in women, thus masking actual gender difference in GR numbers (28).

GR are down-regulated by their cognate ligands (10, 30, 31). We have shown that administration of prednisolone for one month decreased the GR number in MNL considerably, indicating the reciprocal relationship between the GR number in MNL and plasma concentrations of prednisolone. However, morning concentrations of cortisol in plasma did not show any correlation with the GR number in MNL. Moreover, we failed to demonstrate significant alterations in the GR number in MNL within a day, even though plasma cortisol showed circadian variation. Pardes et al. (5) reported that urinary excretion of 17OHC, not plasma concentrations of morning cortisol, correlated with the GR number in MNL in patients with Cushing disease as determined by whole-cell assay. Doe et al. (11) reported that cytosolic GR reflected the circadian variation of plasma cortisol, but that whole-cell receptors did not change markedly within a day. Given these results, we suggest that physiological secretion of cortisol is hardly sufficient to affect the concentrations of GR within a day, in comparison with therapeutic concentrations of synthetic glucocorticoid prednisolone. From a methodological viewpoint, cytosolic GR assay eliminates GR translocated into the nucleus, which are already exposed to and activated by cortisol, and may reflect the number of GR remaining in the cytosol. By contrast, whole-cell GR assay principally detects all binding sites for glucocorticoids through ideally complete exchange of the ligands, whether the GR are in the cytosol or translocated after activation. Taken together, cytosolic GR concentrations may represent the amount of ligand-free receptor, which promptly fluctuates after activation and translocation of the receptor. On the other hand, concentrations of whole-cell GR may reflect the amount of available receptor protein, which is relatively constant within a day (32). We have recently suggested (manuscript submitted) that the GR number determined by whole-cell assay in MNL reflects individual responsiveness to glucocorticoids in vivo (9) and in vitro (9a) in healthy humans. Moreover, this GR-hormone response relationship is reported in various categories of diseases, in which the GR were determined by whole-cell assay (3–8). Thus, we conclude that individual responsiveness to glucocorticoids is reflected by the concentrations of whole-cell GR in MNL.

In summary, whole-cell assay of GR apparently is not affected by age, gender, season, or physiological secretion of cortisol, and is relevant in assessing the relationship between the GR number and clinical sensitivity to glucocorticoid therapy. Clearly, for practical application, we should examine further the influence of diseases and concomitant administration of other therapeutic drugs on GR determination.

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References

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