Negative Relationship Between Expression of Glucocorticoid Receptor α and Disease Activity: Glucocorticoid Treatment of Patients with Systemic Lupus Erythematosus

XIU LI, FENG-SHAN ZHANG, JI-HUI ZHANG, and JING-YUAN WANG

ABSTRACT. Objective. Glucocorticoid receptor α (GRα) is crucial for glucocorticoids (GC) to carry out their physiological and pharmacological roles. Studies have shown the disorder of GR-GC systems in autoimmune diseases. Our study was performed to test the relationship between GRα expression and disease activity of systemic lupus erythematosus (SLE).

Methods. The responses of 55 patients with SLE to GC were screened. We examined GRα mRNA and protein expression in peripheral blood mononuclear cells from SLE patients and healthy volunteers by reverse transcriptase-polymerase chain reaction and Western blotting.

Results. Expression of GRα in patients with SLE was lower than that in controls ($p < 0.05$). Expression of GRα obviously decreased after administration of GC in the steroid-sensitive group with SLE ($p < 0.05$). Expression of GRα was negatively correlated with SLE Disease Activity Index scores in the steroid-sensitive group with SLE.

Conclusion. The expression of GRα in patients with SLE was low and there was a negative correlation between GRα expression and disease activity; these findings might provide insight into the pathogenesis of SLE and help to screen whether the patient is sensitive to GC treatment. (Heilungkiang Provincial Health Department Guiding Projects Funding. Trial registration No. 2006-094.) (First Release Dec 23 2009; J Rheumatol 2010;2:316–21; doi:10.3899/jrheum.090191)

Key Indexing Terms:
GLUCOCORTICOID RECEPTOR α   DISEASE ACTIVITY
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The pathogenesis of systemic lupus erythematosus (SLE) is complex[1]. Management of SLE is a challenge because no interventions result in a cure. Moreover, exacerbation of the disease can occur after a few months of stable maintenance treatment, and unexpected side effects of the therapies can be as undesirable as the disease.

Glucocorticoids (GC) are the most important therapy in the treatment of SLE. However, responses to GC differ widely from patient to patient. The biological action of GC is mediated through the activation of glucocorticoid receptors (GR)[2]. Human GR exists mainly in 2 isoforms, GRα and GRß, where GRα is the predominant isoform[3]. GRα binds steroids and mediates transactivation of target genes[2]. In contrast, GRß does not bind steroids and is unable to activate GC-responsive genes[3,4]. Some studies have shown that GRß is the dominant inhibitor of GRα activity[3,5].

GC is an important hormone produced by the hypothalamic-pituitary-adrenal axis, which possesses strong immune suppression activity[6]. GC is involved not only in identifying antigen, producing antibody, activating lymphocytes, and releasing cytokines, but also serves to maintain autoimmune endurance. The antiinflammatory and immunosuppressive effects of GC rely on 3 main mechanisms[7]: direct effects on gene expression through binding of GR to GC-responsive elements, i.e., in the induction of annexin I and MAPK phosphatase 1; indirect effects on gene expression through the interactions of GR with other transcription factors, namely nuclear factor-κB and activator protein 1; and GR-mediated effects on second-messenger cascades, i.e., the PI3K-Akt-eNOS pathway. Previous studies[8] found that the level of adrenocorticotropic hormone and cortisol in patients with SLE was normal. Therefore, GR is crucial for enabling GC to carry out its physiological and pharmacological functions in patients with SLE.

Recent studies have shown the turbulence of GR-GC systems in autoimmune diseases[8-12], exemplified by the differing findings in these studies. Andreae, *et al*[9] found the num-

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ber of receptor sites in patients without GC was significantly lower than that of healthy persons; Eggert, et al[10] and Neeck, et al[11] reported that GR expression in peripheral blood mononuclear cells (PBMC) was significantly increased in patients with rheumatoid arthritis (RA) who had never received corticosteroids; van Everdingen, et al[12] and Gladman, et al[13] found no correlation between GR number and RA/SLE disease activity. However, Tanaka, et al[14] reported a negative correlation of GRα expression with SLE disease activity. Moreover, a direct correlation between sensitivity to GC and the concentration of GR in cells has been reported[15,16]. We investigated whether the pathogenesis of SLE and disease activity were related to the expression of GRα.

MATERIALS AND METHODS

Subjects. Fifty-five female patients with SLE and 20 healthy female controls were included in the trial (No. 2006-094). The average age of the 55 patients was 30 ± 8 years, and the average age of the 20 controls was 27 ± 6 years. All SLE patients met the diagnostic criteria for SLE established by the American Rheumatism Association in 1997[17], and their scores on the SLE Disease Activity Index (SLEDAI) were > 9[18] (Table 1). All SLE patients were steroid-naive. Patients who had been treated with prednisone or cytotoxic drugs were excluded from the study.

The expression of GRα mRNA and protein in PBMC of SLE patients was measured before and after GC administration. All the patients received a GC (methylprednisolone) as part of their routine therapy (without cytotoxic drugs). Patients who at the start of the study had fever, arthritis, arthralgia, myalgia, serositis, skin erythematosus, but not those with organ lesions, received methylprednisolone injection 0.5–1 mg/kg/day in 2 successive weeks; patients with organ lesions, such as renal involvement and obvious vasculitis, were given methylprednisolone injection 1–2 mg/kg/day for 2 weeks. We classified the patients into 2 groups according to their response to methylprednisolone: steroid-sensitive and steroid-insensitive. The criteria for classification in the steroid-sensitive group were remission or partial remission of clinical symptoms, and a decrease in the SLEDAI score after administration of methylprednisolone. The criteria for the steroid-insensitive group were that patients showed no response to GC in the clinic and an unchanging or increasing SLEDAI score.

Preparation of PBMC. Heparinized venous peripheral blood was obtained

Table 1. Clinical and laboratory findings of 55 female patients with SLE.

Characteristic	n (%)
New rash	25 (45)
Arthritis	35 (63)
Mucosal membrane ulceration	25 (45)
Myositis	25 (45)
Vasculitis	15 (27)
Renal involvement (proteinuria, casts, hematuria, pyuria)	36 (65)
Seizure/psychosis	2/2 (3/3)
Pleurisy/pericarditis	21/18 (38/32)
Fever	32 (58)
Alopecia	24 (43)
Thrombocytopenia	32 (58)
Leukopenia	31 (56)
Abnormal ds-DNA	39 (71)
Low complement levels	40 (73)

from all SLE patients and controls. The blood was diluted by addition of an equal volume of 0.9% NaCl. Six milliliters of diluted blood were carefully layered over 3 ml of Lymphoprep (Amersham Pharmacia Biotech, Uppsala, Sweden) and centrifuged at 800 xg for 20 min at room temperature in a swing-out rotor. After centrifugation the mononuclear cells formed a distinct band at the sample interface. The harvested fraction was diluted with buffered RPMI-1640 medium (Gibco BRL, Gaithersburg, MD, USA) to reduce the density of the solution, and the cells were pelleted by centrifugation for 10 min at 250 xg. Platelets were removed by layering the cells suspended in buffered RPMI-1640 medium and centrifugation for 15 min at 350 xg. The pellets were used as mononuclear cells.

Reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was extracted from PBMC of SLE patients and controls with TRIzol reagent (Invitrogen, Eugene, OR, USA) according to the instructions of the manufacturer and quantified by measuring absorbance at 260 nm. Reverse transcription and cDNAs were amplified in the same test tube using the Access RT-PCR system (Promega, Madison, WI, USA). These cDNAs were amplified with the following primer sets: GRα, 5'-CCT AAG GAC GGT CTG AAG AGC-3' and 5'-GCC AAG TCT TGG CCC TCT AT-3'; ß-actin, 5'-CAA CTC CAT CAT GAA GTG TAA C-3' and 5'-CCA CAC GGA GTA CTT GCG CTG-3', as described[3]. The reaction mixture (50 μl) contained 2 μl RNA, 1 μl AMV reverse transcriptase (5 U/μl), 10 μl AMV/Tfl 5x reaction buffer, 1 μl dNTP mix (10 mM of each dNTP), 2 μl of primers (50 pmol each of upstream and downstream primers), 2 μl of $MgSO_4$ (25 mM), 1 μl of Tfl DNA polymerase (5 U/μl), and 31 μl of nuclease-free water. First, cDNA synthesis was carried out at 45°C for 45 min and denaturation at 94°C for 2 min. Next, the PCR program used for GRα was incubated in a thermal cycler at 94°C for 30 s, 50°C for 1 min, and 68°C for 1 min, for 35 cycles; and for ß-actin at 94°C for 30 s, 56°C for 30 s, and 68°C for 30 s for 27 cycles. After final extension for 10 min at 68°C, PCR products were separated on 2% agarose gels containing ethidium bromide. A DL2000 DNA size standard (TaKaRa, Shiga, Japan) was electrophoresed on the same gel to determine product size. The gel was photographed and the amount of GRα and ß-actin from each sample was analyzed by scanning densitometry. To normalize the results, the amount of GRα was divided by the amount of ß-actin.

Western blot analysis. After 2 washes with phosphate buffered saline (PBS), the PBMC were lysed in cold lysis buffer (PBS containing 1% nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate supplemented with 100 μg/ml phenylmethylsulfonyl fluoride and 1 μg/ml aprotinin). After centrifugation at 13,000 xg for 20 min, the supernatants were collected and the total protein concentrations were determined by spectrophotometer, using bovine serum albumin as standard. Proteins (20 μg) were resolved by electrophoresis through a 7.5% polyacrylamide gel and electrophoretically transferred to nitrocellulose membrane. After blocking nonspecific binding sites by immersing the membrane in 5% blocking reagent dissolved in Tris-buffered saline-Tween for 1 h at 37°C, the membrane was incubated overnight at 4°C with polyclonal anti-hGRα at 1:400 dilution (Santa Cruz Biochemical, Santa Cruz, CA, USA). The membrane was washed and then incubated with horseradish peroxidase-conjugated whole antibody at 1:1000 dilution (Amersham Biosciences) for 40 min at 37°C. After washing, the membrane was covered with ECL detection reagents (Amersham Biosciences) for 1 min at room temperature. The membrane wrapped with Pewrap was exposed to X-ray film for 2–5 min, developed, and exposed. The film was photographed and the amount of GRα and ß-actin from each sample was analyzed by scanning densitometry. To normalize the results, the amount of GRα was divided by the amount of ß-actin.

Statistical analysis. Statistical analyses were performed using SPSS v. 15.0 (SPSS, Chicago, IL, USA). All data were expressed as mean ± SD. Differences between groups were evaluated by one-way ANOVA and paired-samples t test; p < 0.05 was considered significant. Correlation between 2 variables was analyzed by Spearman rank correlation analysis.

RESULTS

Expression of GRα mRNA. GRα mRNA was detected in all samples (Figure 1, Table 2). There were 49 patients in the steroid-sensitive SLE group and 6 patients in the steroid-insensitive group. GRα mRNA expression in patients with SLE was significantly lower than in controls before and after steroid treatment ($p = 0.007$, $p = 0.018$, $p = 0.005$ and $p < 0.001$, $p = 0.001$, $p = 0.007$, respectively). The expression of GRα mRNA in the steroid-sensitive group with SLE prior to treatment with GC was higher than that of
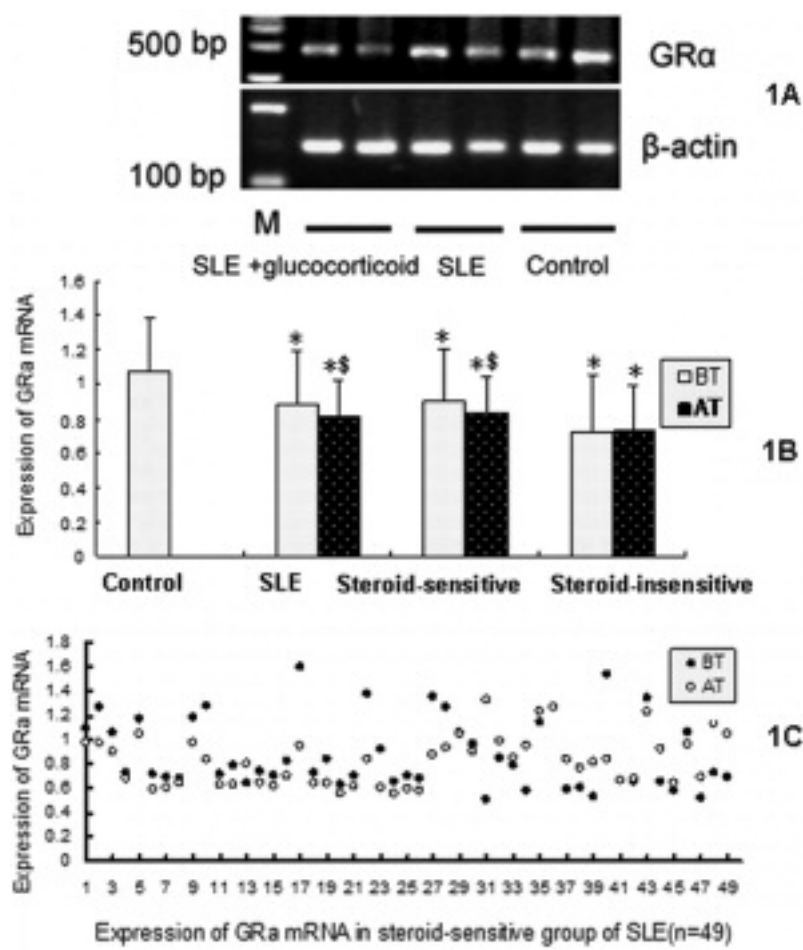

Figure 1. Expression of GRα mRNA. A. RT-PCR results. GRα mRNA expression in patients with SLE was significantly lower than in controls ($p < 0.05$). B and C. Expression of GRα mRNA in the steroid-sensitive SLE group before treatment (BT) with glucocorticoids was higher than that of patients after treatment (AT) ($p < 0.05$). *$p < 0.05$ compared to healthy controls; $^{\$}p < 0.05$ comparing SLE patients before and after steroid treatment.

Table 2. GRα mRNA expression in peripheral blood mononuclear cells from healthy controls and SLE patients.

Group	n	Expression of GRα mRNA Before Treatment	After Treatment
Controls	20	1.07 ± 0.31	—
Patients with SLE	55	0.88 ± 0.31*	0.81 ± 0.21*†
Steroid-sensitive patients	49	0.90 ± 0.30*	0.83 ± 0.21*†
Steroid-insensitive patients	6	0.72 ± 0.33*	0.73 ± 0.26*

Data are expressed as mean ± SD (GRα mRNA/ß-actin). * $p < 0.05$ compared to healthy controls; † $p < 0.05$ comparing SLE patients before and after steroid treatment.

patients after treatment ($t = 2.221$, $p = 0.031$). There were no significant differences in the steroid-insensitive SLE groups before and after steroid treatment ($t = -0.199$, $p = 0.850$). In addition, no significant differences in GRα mRNA expression were found between the steroid-sensitive and steroid-insensitive SLE groups before and after steroid treatment ($p = 0.117$ and $p = 0.408$).

Western blot of GRα. GRα protein was detected in all samples (Figure 2, Table 3). GRα protein expression in the steroid-sensitive group with SLE before and after steroid treatment was significantly lower than in controls ($p = 0.033$
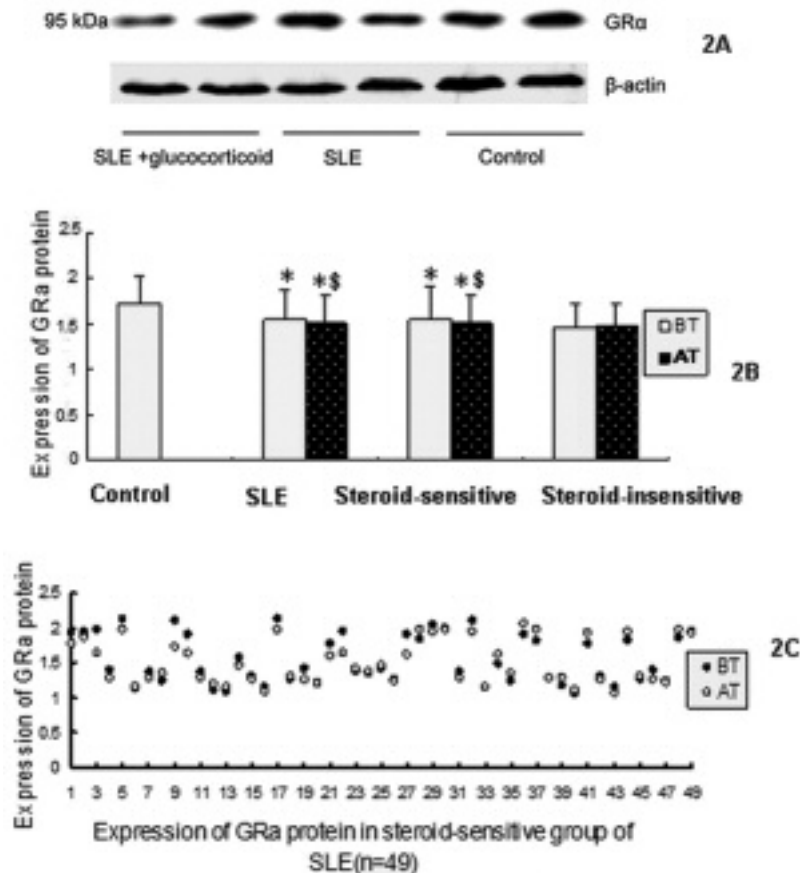
Figure 2. Expression of GRα protein. A. Western blot results. GRα protein expression in patients with SLE was significantly lower than in controls (p < 0.05). B and C. Expression of GRα protein in the steroid-sensitive group with SLE before treatment (BT) with glucocorticoids was higher than that of patients after treatment (AT) (p < 0.05). *p < 0.05 compared to healthy controls; $p < 0.05 comparing SLE patients before and after steroid treatment.

Table 3. GRα protein expression in peripheral blood mononuclear cells from healthy controls and SLE patients.

| Group | n | Expression of GRα Protein | |
		Before Treatment	After Treatment
Controls	20	1.73 ± 0.29	—
Patients with SLE	55	1.54 ± 0.34*	1.51 ± 0.31*[†]
Steroid-sensitive patients	49	1.55 ± 0.35*	1.51 ± 0.32*[†]
Steroid-insensitive patients	6	1.47 ± 0.25	1.48 ± 0.24

Data are expressed as mean ± SD (10 × GRα protein/ß-actin). * p < 0.05 compared to healthy controls; [†] p < 0.05 comparing SLE patients before and after steroid treatment.

and p = 0.010, respectively). There were no significant differences between controls and steroid-insensitive SLE groups before and after steroid treatment (p = 0.085 and p = 0.091). The expression of GRα protein in the steroid-sensitive group with SLE prior to treatment with GC was higher than that of patients after treatment (t = 2.068, p = 0.044). However, there were no significant differences in the

steroid-insensitive SLE groups before and after steroid treatment (t = –0.344, p = 0.745). In addition, there were no significant differences between the steroid-sensitive and steroid-insensitive SLE groups before and after steroid treatment (p = 0.584 and p = 0.822).

Correlation between expression of GRα and SLEDAI score. The SLEDAI score in the steroid-sensitive group with SLE after treatment with GC was lower than that of patients before treatment (t = 10.60, p < 0.001; Figure 3). There were no significant differences in SLEDAI scores in the steroid-insensitive SLE group before and after treatment with GC (t = –2.236, p = 0.076). There was a negative correlation between GRα mRNA/protein expression and the SLEDAI score (20.67 ± 4.13) in the steroid-sensitive SLE group before treatment with steroids. After treatment with steroids, expression of GRα mRNA and GRα protein was also negatively associated with the SLEDAI score (13.53 ± 4.16). However, there were no correlations between GRα mRNA/protein expression and the SLEDAI score (22.50 ± 3.94 and 24.0 ± 4.10) in the steroid-insensitive SLE group
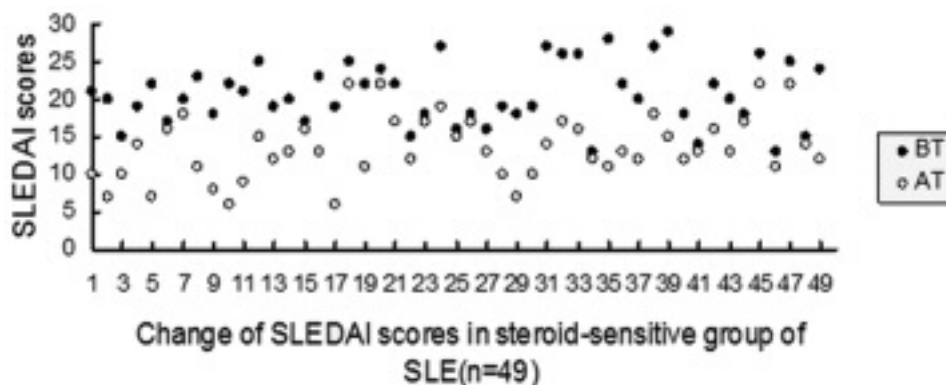
Figure 3. SLEDAI scores in steroid-sensitive SLE patients were lower after glucocorticoid treatment (AT) than before treatment (BT) (p < 0.05).
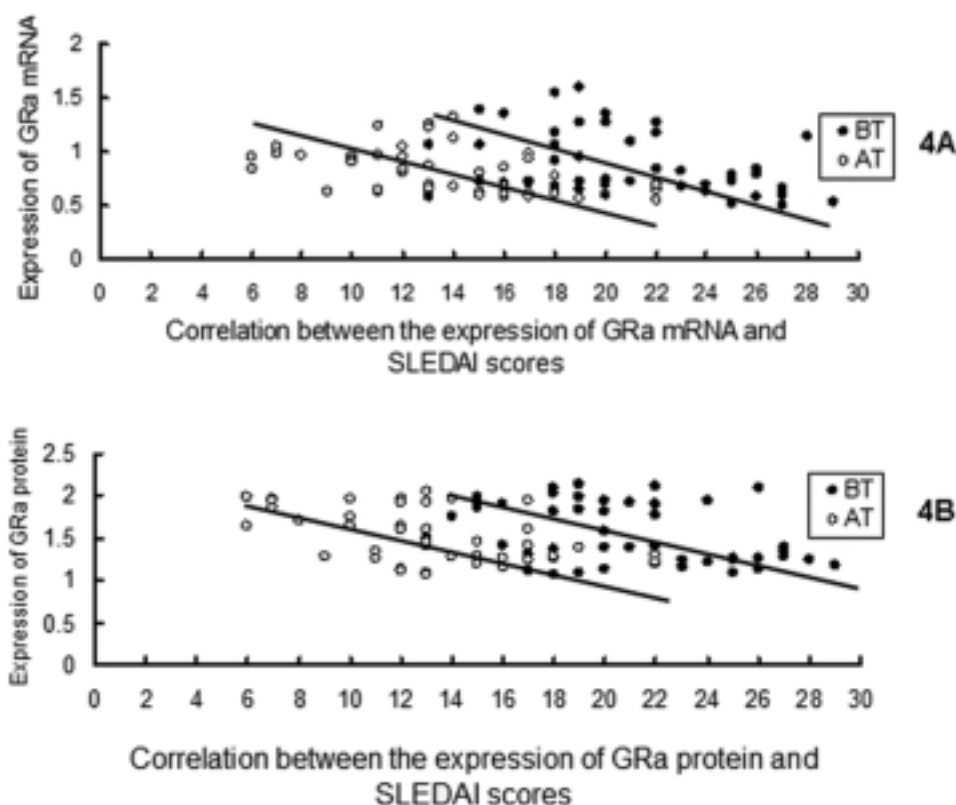

Figure 4. A. Correlation between GRα mRNA and SLEDAI scores in patients before treatment (BT) and after treatment (AT) with glucocorticoid. There was a negative correlation between GRα mRNA and protein expression (B) and the SLEDAI score in the steroid-sensitive SLE group before treatment and after treatment.

before and after treatment with glucocorticoid (Figure 4, Table 4).

DISCUSSION
In order to survey the relative pathogenesis of SLE, we examined GRα mRNA and protein expression in PBMC from SLE patients and healthy controls. GRα mRNA and protein expression in PBMC was significantly lower in patients with SLE than in controls. This finding suggests that the expression of GRα is decreased in patients with SLE.

We found that GRα mRNA and protein expression in the steroid-sensitive group with SLE after treatment with GC was significantly lower than that in patients before treatment with GC. This is supported by reports that GC can down-regulate GRα[19-21]. We also found that expression of GRα in the steroid-sensitive group with SLE before treatment with GC was negatively associated with the SLEDAI score, which was similar to results described by Tanaka, *et al*[14]. Our findings suggest that the lower the expression of GRα, the more severe the disease expression will be and the larg-

Table 4. Correlation between GRα mRNA/protein expression and SLEDAI score.

| | Steroid-sensitive Group, n = 49 | | | | Steroid-insensitive Group, n = 6 | | | |
| | Before Treatment | | After Treatment | | Before Treatment | | After Treatment | |
	r	p	r	p	r	p	r	p
GRα mRNA and SLEDAI	–0.295	0.040	-0.537	< 0.001	0.600	0.208	0.200	0.704
GRα protein and SLEDAI	–0.319	0.026	–0.413	0.003	–0.257	0.623	–0.371	0.468

er the therapeutically effective dose required for treatment. This may also explain why patients with higher GRα expression are sensitive to GC[8,14]. Among the steroid-sensitive SLE patients after treatment, the expression of GRα was also negatively associated with the SLEDAI score. We did not simultaneously analyze the relationship between GRα and all other measures in SLE patients before and after GC treatment because the exposure factors were different in SLE patients treated with GC. This observation may explain why patients' SLEDAI scores did not increase and why the illness did not worsen, even though the expression of GRα was lower after GC treatment. In our study, there were no correlations between the expression of GRα and those described above in the steroid-insensitive SLE group. The reason for this might be that the number of steroid-insensitive patients in the study was relatively low (6 patients).

In summary, our results suggest that decreased GRα plays a significant role in the pathogenesis of SLE. The results indicate that it was important to determine the level of GRα expression in PBMC of SLE patients in order to predict the state of illness, the prognosis, the patient's sensitivity to GC, and the therapeutically effective dose. In this way, detection of GRα may provide a valuable guideline for glucocorticoid treatment in SLE.

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