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Original Article

Role of abnormal anterior pituitary hormones-growth hormone and prolactin in active systemic lupus erythematosus

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Abstract: Background: The role of anterior pituitary hormones in systemic lupus erythematosus (SLE) remains controversial. Aims and Objectives: We determined the expression levels of human growth hormone (GH), prolactin (PRL), and their receptors in subjects presenting with SLE, and modulation of disease severity. Materials and methods: Forty-seven subjects and ten healthy controls were assessed for possible association between SLE disease activity and levels of serum PRL, GH and thyrotropin-releasing hormone (TRH). In peripheral blood mononuclear cells (PBMC), specific binding and mRNA expression of receptors for GH (GHR), and PRL (PRLR) were determined by receptor-ligand binding assay (RLBA) and RT-PCR. PBMC of recruited subjects were treated with hPRL and rhGH to assess IgG production and antibodies against dsDNA. Results: In active SLE subjects we found elevated PRL and GH levels. Study subject PBMCs displayed augmented GHR and PRLR protein and mRNA expression. Study subjects also showed a positive correlation in serum PRL levels and specific antibodies against dsDNA, SLE disease activity index (SLEDAI), and proteinuria. However, a negative correlation was found between serum PRL levels and complement component C3. We found a positive correlation between specific binding rates of PRLR and GHR and both SLE activity and dsDNA antibody titers. Enhanced IgG and anti-dsDNA secretion was observed in cultured PBMC stimulated by PRL or GH with/without PHA, PWM, IL-2 or IL-10. In active SLE, a close association was found between augmented PRL and GH levels, expression and specific binding activities of PRLR and GHR, and changes in the specific titer of anti-dsDNA. Conclusion: Anterior pituitary hormones play an important role in the pathogenesis of SLE. High levels of growth hormone (GH) and prolactin (PRL) play a role in pathogenesis of SLE, which is correlated with SLE disease activity and antibodies against dsDNA. The mechanism of GH and PRL in SLE was complicated and should be studied further.

Keywords: Growth hormone, prolactin, systemic lupus erythematosus, SLE

Introduction

More than 90% of systemic lupus erythematosus (SLE) patients are female, suggesting an important role for sex hormones [1]. Epidemiological data suggests both pregnant and postmenopausal women are more susceptible to developing SLE [2]. Pregnancy increases SLE incidence, and co-presentation with rheumatoid arthritis (RA) reduces SLE morbidity. Women who have given birth are more likely to develop RA as compared unpregnant women. Additionally, pregnant women are most vulnerable to RA one to three months postpartum, and increased prolactin (PRL) is a risk factor for autoimmune disease [3].

Serum estradiol/testosterone ratio is increased in SLE. Disease activity in female SLE is associated with low testosterone, androstenedione, and dehydroepiandrosterone (DHEA) [4]. Moreover, immune cells express estrogen receptors, and lymphocyte activation by estrogen is associated with SLE disease activity [5]. Anterior pituitary hormones like prolactin (PRL) and growth hormone (GH) are important. PRL affects mammary glands, interferes with gonadotropin, and is broadly immunoregulatory [6]. Serum PRL is higher in pregnant patients with active SLE. Changes in sex hormone levels are controlled by pituitary inhibitors like bromocriptine, which lowers PRL levels and improves the SLE condition [3, 7]. Thus, studies are needed that clarify regulation of SLE disease activity by PRL.

GH and PRL share similar structures and immunomodulatory functions [3]. GH is present in

the pituitary at 50-100 times that of PRL. Secretion of GH affects the synthesis of many factors. Investigations of the relationship between GH and SLE could advance our understanding of SLE severity, and development of novel therapies.

A variety of cells express GH and PRL receptors including T and B lymphocytes [8], endothelial cells, luteal and testicular tissue. GH and PRL receptors share 30% amino acid homology, and GH activates PRLR. Thus, GH and PRL share complex physiological functions, due to their structural or functional receptor diversity.

Both GH and PRL synergistically regulate immunity. Activated lymphocytes produce GH and PRL, which affect IL-2 secretion and its receptor IL-2R. Additionally, GH influences the Th1 and Th2 mediated cytokine switch and regulates immunity [3]. However, contributions made by GH and PRL in SLE remain unresolved. In clinical studies, intra-articular or intravenous administration of somatostatin (act as a factor to inhibit the release of GH, thyrotropin releasing hormone (TRH), and PRL) was found to suppress the inflammatory process in rheumatoid arthritis (RA) [25, 26]. Jacobi AM et al. [27] demonstrated that increased serum level of prolactin (PRL) is present in a subset of SLE patients and correlates with clinical and serological disease activities. Denko et al. [28, 29] investigated the serum level of growth factor (GH) in a group of patients with treated SLE. Treated SLE patients did not have GH levels that differed from age-matched normal female subjects. These researches only studied one kind of anterior pituitary hormones at the same time.

We have studied GH and PRL expression and their corresponding receptors on immunocompetent cells obtained from SLE subjects. Further, we have studied hormonal and immunological interactions within the hypothalamic-pituitary (GH, PRL) axis, and the hypothalamic-pituitary-adrenal cortical axis, to gain insights into neuro-endocrine regulation of immune cells. By treating lymphocytes with hPRL and rhGH, we observed secretion of antibodies and anti-dsDNA in experiments designed to determine the role of PRL and GH in SLE disease severity.

Materials and methods

Reagents and equipment

RPMI-1640 media was obtained from Gibco BRL, MD, USA; recombinant human growth hormone (rhGH) was obtained from the Chinese Academy of Sciences cells; lymphocyte separation medium was obtained from Sino-American Biotechnology (China); polyclonal rabbit anti-human IgG was obtained from DAKO; standard human IgG was obtained from Bonding; monoclonal HRP-labeled goat anti-human IgG was obtained from Southern Biotech Associates (USA); monoclonal mouse anti-human IL-10 and mouse anti-human IL-2 were obtained from Megagene Company; rhIL-10 was obtained from R&D Systems Inc.; rhIL-2 was obtained from Bonding; the ANA and ENA assays were obtained from EUROIMMUN, (Germany); TRH was obtained from the Shanghai Institute of Biology (China); recombinant human GH (rhGH) was from the Shanghai Institute of Cell Biology; the chemiluminescent cortisol immunoassay kit was obtained from Acess, (USA); 125^{I}-PRL 42 μCi/μg was obtained from Diagnostics Products Corporation; human PRL (hPRL) was from Sigma; 125^{I}-GH 46 μCi/μg was from the Shanghai Institute of Biological Products.

Study subject recruitment

In a prospective SLE study, 47 subjects were selected (n=5 males, and n=42 females). At Huashan Hospital, Shanghai, China, and ten control subjects were recruited (n=1 male and n=9 females). In the study of hPRL and rhGH-induced IgG and anti-dsDNA antibody secretion by cultured PBMC, 19 SLE subjects (4 males and 15 females) and 6 normal subjects (1 male and 5 females) participated. All participants gave informed consent. The project was approved by the ethics committee (2009LS151).

Inclusion criteria [9]: 1) A positive diagnosis of SLE in accordance with the American Rheumatology Association (ARA) diagnostic criteria of 1982 [3, 10]. 2) Disease activity by the SLE Disease Activity Index (SLEDAI) as described [1, 11]. A score ≥5 was defined as active SLE, a score <5 was quiescent SLE.

Exclusion criteria [9]: 1) Active SLE subjects treated with corticosteroids or immunosuppressants, or quiescent SLE treated with pred-

nisone over 5-15 mg/d. 2) Subjects with abnormal thyroid function or other autoimmune diseases.

Determination of serum hormones

Next day, 4 ml of blood was collected at 8:00 am. Serological analysis of TT3, TT4, FT3 and FT4 (China Nuclear Society) excluded hypothyroidism, and hyperthyroidism. Serum PRL and GH levels were determined by time-resolved fluorescence immunoassay (TrFIA, Wallac inc. Finland). Cortisol was measured by chemiluminescent immunoassay.

TRH test

Following fasting, subjects were injected with 250 μg TRH. Blood was collected prior to injection and 15, 30, 60, and 120 min after injection to quantify GH or PRL levels.

Radioactive receptor-ligand binding assay (RLBA)

RLBA and RIA analysis for GHR of primary lymphocytes were determined as described [4]. Assay of PRLR has been previously described [3, 7]. A Beckman LS 8000 liquid scintillation counter (USA) generated the data.

RT-PCR

Total RNA was extracted from PBMC by Trizol (Gibco-BRL, USA) as described [12]. Primer sequences for RT-PCR were:

GHR: Forward 5'-cccggaaatggtctcactgcca-3', Reverse 5'-tctttgtcaggcaagggcaaggcaaggcag-3'; PRLR: Forward 5'-gtggcatctgcaaccgtttt-cactctgcta-3', Reverse 5'-ccacatggaggtgtactgc ttgccaaagtg-3'; GAPDH: Forward 5'-caccatc-ttccaggagcgag-3', Reverse 5'-tcacgccacagttt-cccgga-3'.

PBMNC isolation

PBMC were isolated as described [13], and resuspended in complete culture medium, in 96-well plates at 105 cells/well/100 μl. Cells were challenged dose-dependently with rhGH, pokeweed mitogen (PWM), IL-2, IL-10, and either anti-IL-2 or anti-IL-10. Seven days later, supernatants were assayed.

Cell proliferation

PBMC were seeded at 2×10^5 cells/well/200 μl in 96-well plates, stimulated with 10 μg/mL PWM and dose-dependently with rhGH over 72 h. At 16 to 20 h before terminating the assay, 3H-TdR (0.5 μCi/well) was added to each well and proliferation measured by liquid scintillation counting.

IgG Determination [1]

PBMC were challenged with PWM (10 μg/mL), and dose-dependently with rhGH at 10-12, 10-11, 10-10, 10-9, 10-8, 10-7, and at 10-6 mol/L, rhIL-2 at 100 μg/200 μl, rhIL-10 at 2 ng/200 μl, anti-IL-10 mAb at 100 ng/200 μl, and anti-IL-2 mAb at 100 ng/200 μl. Regression equation was calculated based on logarithmic values of IgG (mg) and the OD values. Thus IgG levels were determined, which corresponded to supernatant OD values. An EXL-800 automatic microplate reader (USA) generated the data.

Determination of IgG and anti-dsDNA in PBMC cultures

Supernatants were incubated with DNase I (pH 7.4 in PBS) at 37°C, 90 min, and $MgSO_4$ as an adjuvant at final concentrations of 0.1 mg/ml, and Mg^{2+} at 2 mM. To terminate enzymatic reactions, 20 mM EDTA was added. Digested DNA was quantified by spectrophotometry. Anti-dsDNA antibodies were quantified by specific radioimmunoassay (China Institute of Atomic Energy).

ELISA procedure by Avidin-Biotin-Complex (ABC)

Wells were pretreated with 0.5% protamine sulfate, and then coated with nDNA (50 μl/ml). Wells were blocked with 100 μl of ELISA blocking solution (Jingmei, China) at 37°C, 2 h, and then washed once. Next, each well was incubated with 50 μl DNase I treated supernatant for 2 h, 37°C, followed by biotinylated anti-human IgG antibody at 37°C, 1 h. The ABC complex was developed at 37°C, 30 min, following which substrate was added at 100 μl/well at 37°C, 20 min for color development. The assay was terminated with 2 M H_2SO_4 and the OD was measured at a wavelength of 490 nm. The IL-2 ELISA kit was obtained from Jingmei (China).

Statistical analyses

Data were expressed as mean ± S.D., and analyzed by one-way ANOVA, Student's T-test, Chi-

Table 1. GH, PRL and their receptors in SLE and healthy persons (Mean ± SD)

Group	GH	PRL	GH-R	PRL-R
Active SLE	6.35±7.18	14.45±7.25*	3.44±2.05	5.03±2.51*
Quiescent SLE	2.03±3.47	11.79±8.02	1.28±1.19	3.18±2.26
Healthy controls	2.50±2.27	7.08±6.26	0.99±0.96	2.62±1.05
ANOVA	P<0.01	P<0.01	P<0.01	P<0.01

Note: *There was no statistically significant difference between active SLE and quiescent SLE group.

Table 2. Immune response and TRH changes in 34 patients with active SLE

Group	n	ANA titer ↑	dsDNA↑	C3↓	RNP+	SM+
TRH increase	29	15	24	18	5	5
TRH normal	5	0	1	0	3	1
χ^2		3.06	5.42	4.09	2.60	0.14
P value		>0.05	<0.05	<0.05	>0.05	>0.05

Note: The rate of response to TRH was positively correlated with SLEDAI, dsDNA antibody titer increase, C3 decrease (P<0.05, Chi-square correlation test).

Table 3. ACTH, cortisol and ratio of (GH+PRL)/C

Group	n	ACTH (pg/mL)	Cortisol (μg/mL)	PRL+GH/Cortisol
Active	17	1.00±0.00*	7.17±1.71*	3.08±0.92*
Quiescent	13	1.84±2.09*	6.88±1.01*	1.43±0.43*
Control	11	4.80±4.75	15.06±5.54	0.93±0.40

Note: *P<0.01 versus controls by T test.

square test, linear regression and logistical regression (SPSS version 9.0 (SPSS Inc, Chicago, IL, USA). An alpha value of *P*<0.05 was considered statistically significant.

Results

Study subjects

Forty seven SLE patients were recruited (age 15-53 years, mean 34.1±2.5 years). The average disease duration was 3 years (range 1 month to 12 years). 34 subjects presented with active SLE and 13 with quiescent disease. The control group included 10 normal healthy subjects (age 18-50 years; mean age 30 years). Both control and study subjects were age- and sex-matched for this study.

Correlation of serum PRL and GH levels with SLE

Serum PRL levels in 34 active SLE subjects (13.43±5.65, P<0.01) and 13 quiescent SLE subjects (11.86±5.07, P<0.01) were significantly higher than normal controls (7.38±3.09). There was no difference in the serum levels of PRL seen in SLE active as compared SLE quiescent subjects (**Table 1**). Serum GH levels in the active SLE (n=34) (6.27±5.18) were significantly higher than levels seen in either quiescent SLE (n=13) (2.32±3.07, P<0.01) or control subjects (2.51±3.12, P< 0.01). There were no differences in the levels of GH seen in quiescent SLE subjects or controls (**Table 1**).

Association of SLE disease severity and hypothalamic-pituitary GH, and PRL

Elevated PRL and GH were found in 29 of 34 active SLE subjects after TRH stimulation (P<0.01, Figures S1 and S2) as compared quiescent SLE (2/13) patients. The basal serum levels of both PRL and GH were elevated in 18 of 34 active SLE subjects as compared quiescent SLE (3/13). In addition, 34 active SLE subjects exhibited an increased response rate after TRH stimulation, which was positively correlated with dsDNA antibody titer and down-regulated Complement C3 (C3, P<0.05, **Table 2**). In active SLE, serum TSH levels were significantly higher as compared with quiescent SLE or control groups (P<0.01). Marked differences in serum TSH levels were not seen between SLE quiescent subjects and control groups.

Association of SLE disease activity and the hypothalamic-pituitary-adrenal axis

Without corticosteroid treatment, both active SLE and quiescent SLE had lower levels of serum ACTH and cortisol than the control group (P<0.01). We observed no significant difference between active and quiescent SLE groups. The ratio of (PRL+GH)/cortisol in active SLE was significantly higher than that seen in quiescent SLE and the control groups (P<0.01). Additionally, the ratio was significantly different between quiescent SLE and the control group (P<0.01, **Table 3**). By linear regression analysis, we noted that in 17 subjects presenting with

Table 4. PRLR total binding rate and specific binding rate in SLE patients and controls (Mean ± SD)

Group	N	TB (%)	SB (%)	ANOVA
SLE active	24	15.346±6.988	5.035±2.513	F_{SB}=5.334
				P=0.003
SLE quiescent	22	14.036±6.540	3.188±2.262	F_{TB}=7.906
				P=0.01
Control	15	8.194±1.475	2.620±1.057	

Note: Using Q test for comparison between SB in active and quiescent SLE and healthy persons P<0.0001, the remaining P>0.05.

Table 5. Ratio of PRLR mRNA expression in PBMC from SLE patients and controls PBMC

Group	N	Mean ± SD	ANOVA
SLE active	18	0.854±0.550	F=20.93
SLE quiescent	14	0.575±0.431	P<0.0001
Control	16	0.195±0.134	

Note: Using Q test for comparison: Active vs. Control P=0.0001, Active-Quiescent P=0.002, Quiescent-Control P=0.019.

Table 6. GHR total binding rate and specific binding rate in SLE patients and controls (Mean ± SD)

Group	N	TB (%)	SB (%)	ANOVA
SLE Active	24	13.75±5.67	3.44±2.05	F_{SB}=15.28
				P=0.0001
SLE Quiescent	22	11.40±4.59	1.28±1.19	F_{TB}=4.875
				P=0.005
Control	15	8.82±0.87	0.99±0.96	

Note: Using Q test for comparison: TB: Active vs. Control P=0.002, the remaining P>0.05. SB: Active vs. Control P<0.001, Active vs. Quiescent P<0.001, Quiescent vs. Control P>0.05.

active SLE, and not receiving corticosteroids, the ratio of (GH+PRL)/cortisol was positively correlated with the dsDNA antibody titers (r=0.8921, P<0.01, (Figure S3).

Association of PRLR and GHR binding capacity and expression with SLE disease activity

Specific binding (SB) of PRLR in 34 subjects with active SLE (5.16±2.41, P<0.01) and 13 subjects with quiescent SLE (4.21±2.03, P<0.05) was markedly higher than the control group (2.59±1.06, **Table 4**). The SB of PRLR in either active or quiescent SLE was very similar (**Table 4**). The levels of PRLR mRNA in 34 subjects with active SLE (0.86±0.43) was significantly higher when compared with 13 subjects with quiescent SLE (0.56±0.31, P<0.05) and control group (0.19±0.13, P<0.01, **Table 2**). Additionally, in the SLE quiescent stage, the PRLR mRNA expression levels were significantly higher than the PRLR expression levels seen in the control group (P<0.01, **Table 5**).

In terms of the specific binding of GHR in 34 subjects with active SLE who did not receive corticosteroids, the SB of GHR (3.35±2.01) was significantly higher than that seen for 13 subjects with either quiescent SLE (1.34±1.21) or the control group (0.98±0.94, P<0.01). By contrast, both quiescent SLE subjects and the control group showed no significant difference in the SB of GHR (**Table 6**). Additionally, in 34 subjects with active SLE, expression levels of GHR mRNA (0.76±0.43) were shown to be significantly higher than that seen for 13 subjects with either quiescent disease (0.47±0.26, P<0.05) or the control group (0.36±0.17, P<0.01 and **Table 7**). The mRNA expression levels in quiescent SLE and in the control group were similar.

Both hPRL and rhGH activated cell proliferation

PBMC proliferation was dose-dependently activated by hPRL. PBMC stimulation with 9 M hPRL showed that hPRL did not affect lymphocyte proliferation in subjects with active SLE, quiescent SLE or in normal controls. However, stimulation of PBMC with PHA plus PRL markedly enhanced proliferation in SLE subjects as compared the control group (P<0.001, Figure S4).

By contrast, dose-dependent stimulation of PBMC was seen following treatment with rhGH. *In vitro* isotope incorporation experiments using GH^{-12} M and GH^{-8} M to stimulate PBMC proliferation, showed that GH exerted only a weak effect on cultured PBMC proliferation. At concentrations greater than GH^{-7} M, lymphocyte proliferation was indistinct. When challenged with $rhGH^{-8}$ M, PBMC from subjects with active SLE showed no obvious proliferation as compared with either quiescent SLE or controls. By contrast, in cultures stimulated with PWM plus GH, we observed significant prolifer-

Table 7. Ratio of GHR mRNA expression in PBMC from SLE patients and controls

Group	N	Mean ± S.D	ANOVA
SLE Active	18	0.77±0.66	F=6.735
SLE Quiescent	14	0.45±0.28	P=0.003
Control	16	0.21±0.12	

Using Q test for comparison: Active vs. Control P=0.008, Quiescent vs. Control P=0.215, Quiescent vs. Active P=0.032.

Table 8. The influence of rhPRL and PWM, IL-2, IL-10 and antibodies on cultured PBMC producing IgG. T-test was used to compare the group with and without adding PRL

IgG (ng/ml)				
	n=19	Mean ± SD	t	P
Control		142.32±52.81		
rhPRL	C	291.41±70.13	-7.40	<0.01
IL-2		216.63±63.15		
IL-2	PRL+	332.98±134.78	-3.41	<0.01
Anti-IL2		153.42±58.36		
Anti-IL-2	PRL+	246.89±68.27	-4.54	<0.01
IL-10		250.09±76.42		
IL-10	PRL+	266.87±79.81	-0.66	>0.05
Anti-IL-10		167.31±69.75		
Anti-IL-10	PRL+	179.05±71.94	-0.51	>0.05
PWM		309.89±98.79		
PWM	PRL+	519.02±182.52	-4.39	<0.01

ation as compared controls (P<0.001, Figure S5).

Production of IgD and anti-dsDNA antibody secretion in PRL or GH stimulated PMNC

After PBMC were cultured for 7 d, IgG levels in the supernatant were measured. A higher level of IgG was found in PBMC from subjects with active SLE as compared the quiescent SLE group or the control group, (P<0.01, Figure S6). In addition, levels of anti-dsDNA antibody in the same supernatant were also measured and the antibody was secreted by stimulation of PBMC with hPRL at 10^{-9} M. Without stimulation, PBMC from the SLE group released greater levels of anti-dsDNA antibody than either the quiescent SLE or the control group (P<0.01).

At physiological concentrations, stimulation of PBMC with hPRL plus either PHA or IL-2, stimulated the secretion of IgG and anti-dsDNA antibody in subjects with both active and quiescent SLE. Additionally, stimulation of cultures with

Table 9. The influence of rhGH and PWM, IL-2, IL-10 and antibodies on cultured PBMC producing IgG. T-test was used to compare the group with and without adding GH

IgG (ng/ml)				
	n=19	Mean ± SD	t	P
Control		141.39±53.93		
rhGH	C	280.66±69.27	-8.53	<0.01
IL-2		243.57±70.03		
IL-2	GH+	255.78±147.92	-1.88	>0.05
Anti-IL2		199.25±140.33		
Anti-IL-2	GH+	220.89±148.03	-1.7	>0.05
IL-10		250.45±79.43		
IL-10	GH+	410.58±161.10	-3.93	<0.01
Anti-IL-10		159.28±66.14		
Anti-IL-10	GH+	225.87±65.17	-2.91	<0.01
PWM		330.75±104.21		
PWM	GH+	554.10±216.19	-6.26	<0.01

PHA, IL-2, IL-10 and PRL exhibited synergistic effects in stimulating PBMC proliferation (**Table 8**). By contrast, anti-IL-2 and anti-IL-10 antagonized the ability of PRL to stimulate cellular proliferation (**Table 9**).

In vitro experiments showed that rhGH at 10^{-8} M could stimulate IgG secretion by PBMC of subjects with SLE. There were also significant differences seen between active and quiescent SLE subjects and normal controls (P<0.01, Figure S6). For example, cultures stimulated with both PWM and rhGH10^{-8} M displayed a higher response than cultures stimulated with rhGH10^{-8} M alone (P<0.01). Similarly, cultures stimulated with both IL-10 and rhGH10^{-8} M displayed a higher response than cultures stimulated by rhGH10^{-8} M alone (P<0.01).

Secretion of IgG was much lower in PBMC cultures of SLE subjects stimulated with anti-IL-10 and rhGH at 10^{-8} M, as compared cultures not stimulated with anti-IL-10 (P<0.01, **Table 9**). Additionally, rhGH stimulated IgG secretion by PBMC cultures of both active and quiescent subjects. Stimulation with PWM, IL-10 and rhGH were synergistic in driving IgG secretion by PBMC, and was almost completely abolished in cultures treated with anti-IL-10 hGH (**Table 9**).

Discussion

SLE predominantly affects women, indicating that sex hormones are involved in disease

pathogenesis [1]. Many studies have revealed that anterior pituitary hormones, including PRL and GH influence disease progression, but their role in SLE severity remains controversial.

In the current study, we prospectively investigated SLE severity in a cohort of active or quiescent SLE subjects and found that in active SLE, levels of PRL and GH were markedly higher than in quiescent SLE or normal controls, an observation which closely matched the SLE disease activity index-SLEDAI. Additionally, active SLE subjects showed increased serum PRL and GH levels in the TRH stimulation test and showed that increased expression and specific binding of PRLR and GHR were also positively correlated with anti-dsDNA antibody titer levels. Additionally, *in vitro* simulation of PBMC with PRL or GH, or together with PHA, PWM, IL-2 or IL-10 augmented IgG and anti-dsDNA secretion, which suggested that not only did PRL and GH modulate host immunity in SLE, but also disease activity.

In a proportion of SLE subjects with normal PRL levels, an increased TRH test response was observed, and they also showed a latently high PRL level [11]. We found active SLE subjects had PRL levels, which were significantly higher than the quiescent SLE and control groups, suggesting that SLE subjects a mildly high PRL was present in SLE subjects. Linear regression analysis showed that high PRL was positively correlated with SLIDAI, dsDNA antibody titers, and proteinuria but negatively correlated with serum C3. In addition, IL-2R expression levels in the high PRL group were significantly augmented as compared the normal PRL group. These observations were consistent with the work of Jara et al. who reported that in patients with SLE, serum PRL levels were positively correlated and ANA titers and the clinical disease activity index [13]. Others have found that application of bromocriptine, which is an inhibitor of pituitary PRL secretion, could control disease severity in SLE in both animal models and human patients [14]. Additionally, SLE patients with high PRL was also found to be associated with the nephritic activity index [2], and others have found that PRL levels in the serum and urine were related to SLE disease activity [8].

Our observations also confirmed a correlation between hyperprolactinemia and SLE. Active pituitary function may give rise to an increase in the secretion of PRL [11, 15]. At the same time, activated immune cells produce a variety of PRL-like peptides [4], which may also augment serum PRL levels. Genetic linkage equilibrium studies have found that female patients presenting with SLE display linkage disequilibrium between the HLA-DR*0301 gene loci and the D6S422 microsatellite linking to PRL [16]. This may partly represent the cause for an abnormal level of PRL seen in SLE. We speculate that in patients presenting with SLE, and under conditions of physiological or environmental stress, there exist currently unknown factors, which are present prior to the onset of active SLE. However, we speculate that prolonged expression of high-levels of serum GH and PRL, accompanied by genetically defective $CD4^+$ Th cells and an impaired immune tolerance of TS cells, collectively "collaborate" to provoke an autoimmune reaction.

Previously, we treated SLE patients with octreotide (OCT), an octapeptide, which not only mimics somatostatin [17], but is 50 times more potent than somatostatin in suppressing GH release. By inhibiting GH and PRL release, SLE disease activity was improved and pro-Th2 type inflammatory cytokine secretion by PBMC in SLE was dampened by OCT [17]. Future studies could help identify whether treatment with OCT could block PRL secretion and expression of its cognate receptor [17]. Future studies could also explore the beneficial effects of OCT administration, Th2 cytokine responses and the reciprocal regulation of both PRLR and GHR expression and circulating GH and PRL levels.

The status of thyroid function will significantly affect the PRL response to TRH stimulation. The TRH test showed that active SLE subjects displayed increased pituitary secretion of both GH and PRL, indicative of a weakened HPA axis activity.

Active SLE subjects also displayed a higher PRL and GH/cortisol ratio than quiescent SLE or normal subjects. This ratio was also positively correlated with the titers of anti-dsDNA antibody. This observation was consistent with that of Neidhart et al. [18] who showed that the PRL/cortisol ratio was associated with the disease activity index in patients with SLE. Moreover, significantly higher serum PRL and GH levels were seen in SLE patients following insulin injection [19], and SLE disease activity

was related to dysfunction of the hypothalamic-pituitary-adrenal axis. SLE patients also had reduced pituitary-adrenal axis activity [20, 21]. Our findings were consistent with these studies. For example, in active SLE, the hypothalamic-pituitary (PRL, GH)-immune cell activities were enhanced, and the hypothalamic-pituitary-adrenal axis activity was weakened. The imbalance seen in these neuroendocrine and immunological networks, may contribute to B cell dysfunction, and autoantibody production.

Consistent with our studies, high PRL levels stimulate SLE and normal healthy subjects [19], and both PHA and PWM stimulate increased PRL release by PBMC from SLE patients. Spontaneous PRL secretion by B cells was proposed [22]. Additionally, rPRL stimulated SLE PBMC secrete IgG [20]. Consequently, SLE patients not only secreted higher antibody levels than normal controls, and both disease activity and IgG levels were positively correlated.

Autoimmunity is characterized by neuroendocrine dysfunction and prompt therapy could improve or terminate it. Therapeutic strategies should aim at immunoregulation, utilizing neuroendocrine peptide, receptor agonists or antagonist. Thus, PRL and PRLR may play immunoregulatory roles in SLE, by altering the $CD4^+/CD8^+$ ratio [15], or activating PBMC-mediated IgG and IgG-dsDNA secretion [21]. Additionally, BRC treatment of NZB/W mice improved glomerular nephritis, and serological markers including anti-dsDNA, decreased CIC and disease activity [21], and only $CD4^+$ T cells expressed high PRLR levels. Additionally, PRL promotes increased IL-4 and IL-6 secretion by Th2 cells and antibody production by B cells [22-24]. Resent research [4] showed that OCT (octreotide, an analog of somatostatin that suppresses the release of GH) inhibited PBMC proliferation and PBMC secretion of IL-6, IL-10 and IFN-g stimulated by GH. Treatment of patients with OCT resulted in clinical improvement in SLE. This research only studied one kind of anterior pituitary hormones-growth hormone, but not mentioned the other kind of anterior pituitary hormones-prolactin.

In conclusion, we explored the potential involvement of immuno-endocrine circuits in SLE. Future studies will determine immunomodulatory functions of PRL and GH, and the role played by regulatory T cells (Tregs) and innate immunity in modulating $CD4^+$ Th1- and Th2-mediated responses, which should advance design of new therapeutics (e.g. Octreotide) in SLE.

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Disclosure of conflict of interest

None.

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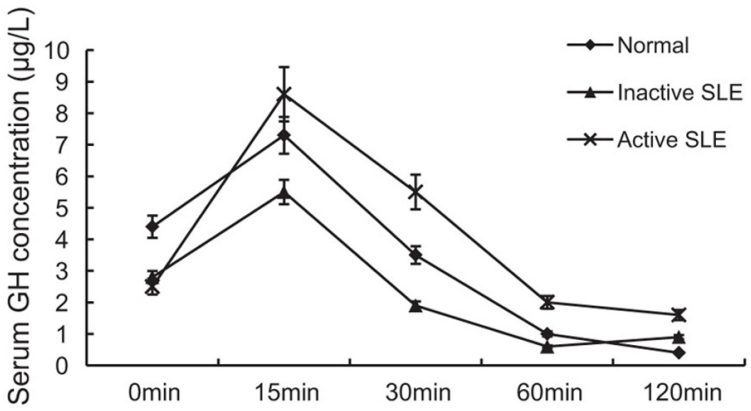

Figure S1. Serum GH levels at indicated time points after TRH injection.

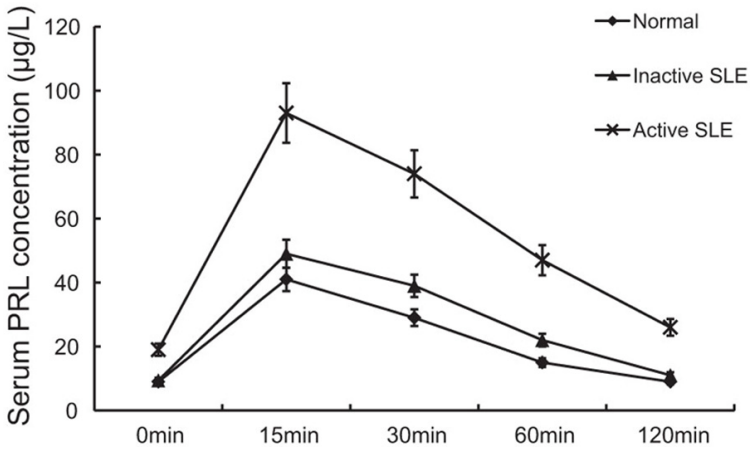

Figure S2. Serum PRL levels at indicated time points after TRH injection.

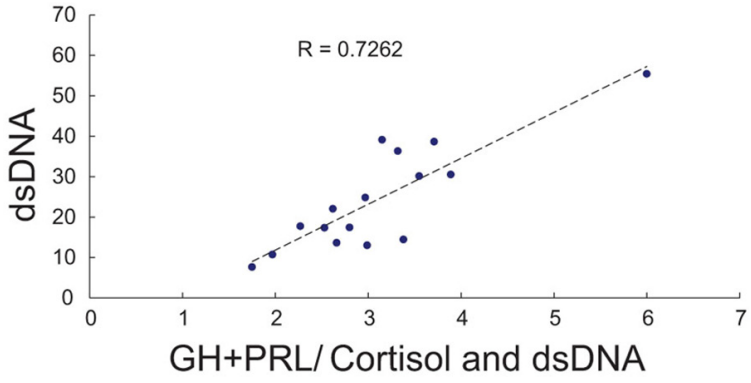

Figure S3. Linear correlation between (GH+PRL)/Cortisol and dsDNA titer.

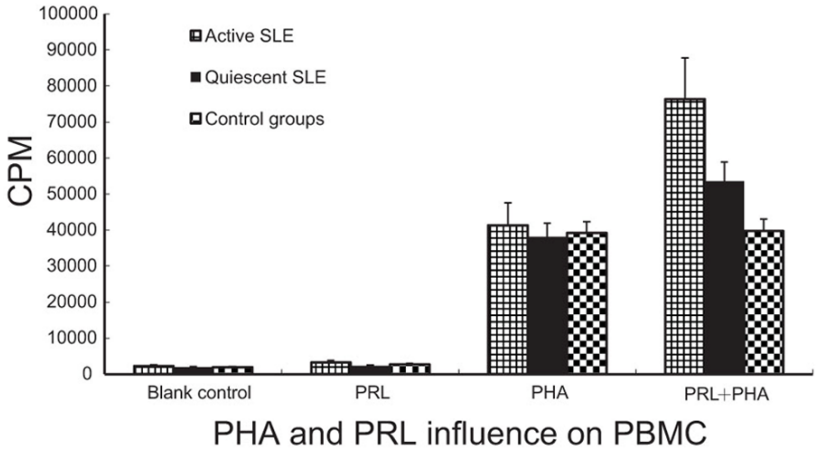

Figure S4. PHA and PRL influence on PBMC (3H-TdR incorporation)* ANOVA analysis was used to compared the active SLE, quiescent SLE and control groups. (P<0.01).

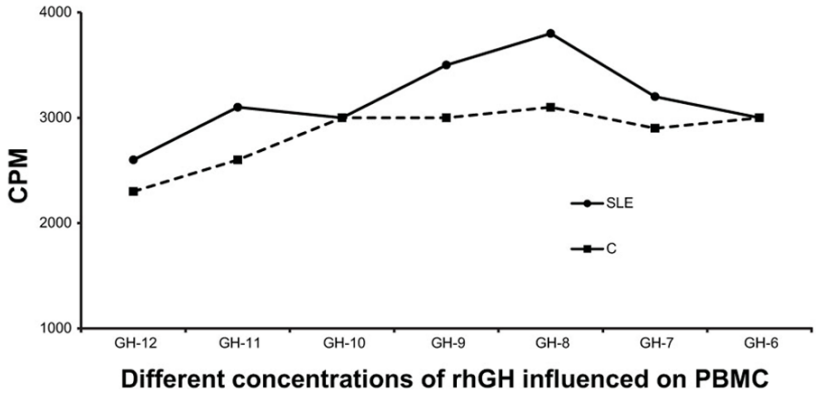

Figure S5. Different concentrations of rhGH influenced on PBMC proliferation measured by CPM.

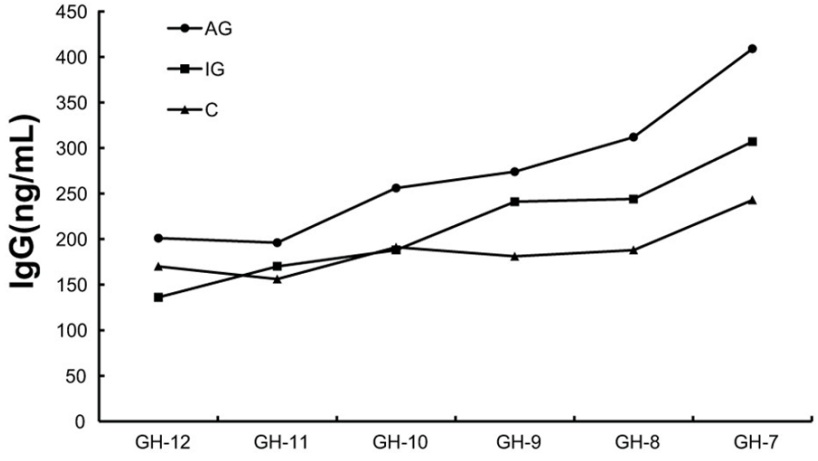

Figure S6. The influence of different concentrations of rhGH on IgG production. AG: Active SLE, IG: quiescent SLE, C: controls. GH-7 represented 10-7 mol/L.