Elevated Bioactive Prolactin Levels in Systemic Lupus Erythematosus — Association with Disease Activity

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ABSTRACT. ***Objective.*** To assess the possibility that prolactin (PRL) is involved in the pathogenesis of systemic lupus erythematosus (SLE).

Methods. We determined serum PRL levels in 122 serum samples from 78 unselected patients with SLE (73 women, 5 men, age range 16–71 yrs). Disease activity was defined according to Lupus Activity Criteria Count (LACC) and scored by Systemic Lupus Disease Activity Index (SLEDAI). Serum PRL concentrations were determined by immunoradiometric assay (IRMA) and by biological assay (BA) that evaluates Nb2 lymphoma cell proliferation.

Results. Hyperprolactinemia (> 20 ng/ml) was found in 21 patients (26.9%) by IRMA and in 31 (39.7%) by BA. A significant correlation between IRMA and BA PRL levels was found (r_s 0.46, $p < 0.001$). According to LACC, SLE was active in 29 patients and inactive in 49. In those with active disease median PRL levels were higher both by IRMA (18.5 ng/ml, range 2.2–51.2 vs 10.6 ng/ml, range 3.9–29.6; $p < 0.001$) and BA (21.0 ng/ml, range 12.4–84 vs 14.9 ng/ml, range 4.2–46.1; $p < 0.001$). Hyperprolactinemia was associated with active disease in 13/21 patients (61.9%) by IRMA and in 18/31 (58.1%) by BA ($p < 0.01$). SLEDAI scores correlated with PRL levels both by IRMA (r_s 0.5, $p < 0.001$) and BA (r_s 0.41, $p < 0.02$). A followup analysis on serum samples from 44 patients seen again after 6–8 mo confirmed the above results. There was no difference in the rate of different clinical manifestations in hyperprolactinemic and normoprolactinemic subjects, apart from the increased prevalence of malar rash and central nervous system manifestations in the patients with hyperprolactinemia ($p < 0.03$ and $p < 0.01$, respectively).

Conclusion. Hyperprolactinemia was frequently detected in patients with SLE by IRMA and by BA and was associated with disease activity. Our findings suggest that PRL may play a role in the pathogenesis of SLE. (J Rheumatol 2001;28:2216–21)

Key Indexing Terms:
BIOACTIVE PROLACTIN
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It is evident that the endocrine system plays a significant role in the regulation of the immune response. In particular, the relationship between the neuroendocrine peptide prolactin (PRL) and the immune system has been recognized and reviewed[1-3]. Several clinical studies have focused on PRL and its possible involvement in immune dysfunction. PRL levels are higher in women than in men, and elevated prolactinemia has been reported in patients with systemic lupus erythematosus (SLE)[4,5], multiple sclerosis[6], rheumatoid arthritis[7], psoriatic arthritis[8], and acquired immune deficiency syndrome[9], and in patients prior to transplant rejection[10].

There has been growing interest in investigating a possible role of PRL in the pathogenesis of SLE. Surveys of patients with lupus for hyperprolactinemia have produced varying results. Some have reported that in patients with SLE, serum PRL levels are higher than in controls without the autoimmune disease, and the occurrence of hyperprolactinemia (> 20 ng/ml) is a factor that has been commonly found with variable frequency. Some authors report that high PRL levels correlate with active disease in SLE. Others, on the contrary, found that hyperprolactinemia was either not increased in patients with SLE or reported that hyperprolactinemic patients were not prone to active disease (Table 1).

These discrepancies could be linked to the different methods used for PRL determination. It is well accepted that multiple molecular forms of immunoreactive human PRL exist with differences in molecular weight and biological activity. This heterogeneity with differing biological activity may explain in part why radioimmunoassay (RIA) measurements do not always correlate with clinical findings[27,28]. On the other hand, it has been suggested that anti-PRL antibodies give vari-

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able results on serum PRL measurements depending on the immunoassay used[29].

An indirect method to assess serum PRL levels that avoids these inconveniences is the determination of PRL bioactivity with a biological assay (BA), the Nb2 lymphoma cell proliferation. This cell line is dependent on lactogenic hormones for growth, particularly prolactin and human growth hormone (hGH)[30].

Although RIA and BA match closely in euprolactinemic women, discrepancies between the 2 assays have been reported in hyperprolactinemic patients[31,32]. The reliability of RIA in some clinical situations may therefore be questionable.

We determined serum PRL levels both by immunoradiometric assay (IRMA) and the Nb2 method in a group of patients with SLE, and correlated these concentrations with disease activity.

MATERIALS AND METHODS

Patients and controls. Seventy-eight randomly chosen patients (73 women, 5 men, median age 31 yrs, range 16–71) fulfilled at least 4 American College of Rheumatology (ACR) revised criteria for SLE[33]. PRL concentrations were determined on 122 consecutive serum samples by IRMA and BA. These patients were seen at least once at the Rheumatology Department of Second University of Naples from January 1997 to December 1999. Forty-four of these patients were seen again after 6–8 months. At each examination disease activity was defined according to the Lupus Activity Criteria Count (LACC)[34] and scored by the SLE Disease Activity Index (SLEDAI)[35]. All serum samples were taken at 8:00 AM and kept frozen until the hormone assays were performed. At the time of serum collection, all patients were receiving variable doses of corticosteroids (3 patients methylprednisolone pulse therapy), 32 antimalarials, and 20 azathioprine.

The control group consisted of 20 healthy non-obese subjects (18 women, 2 men), median age 28 years (range 19–60).

No patient or control was receiving drugs known to be associated with increased prolactin secretion (chlorpromazine, metoclopramide, etc). Patients with disorders known to be associated with hyperprolactinemia were excluded.

Determination of serum PRL levels. IRMA. Basal serum PRL concentrations were determined by IRMA (RIA–gnost Prolactin, CIS-Bio International, Gif-Sur-Yvette, France). The limit of assay sensitivity was 0.06 ng/ml. The intraassay and interassay coefficients of variation were 1.2–2% and 2.2–4.0%, respectively. Normal PRL levels are 2–20 ng/ml. Hyperprolactinemia was defined as serum PRL levels > 20 ng/ml[36].

Determination of serum PRL levels. Biological assay. Rat Nb2 lymphoma cells were a generous gift from Prof. P.A. Kelly (Faculté de Médecine-Paris V). The Nb2 cells, from an estrogen treated male rat, were cultured in RPMI 1640 medium containing 10% horse serum, 10% inactive fetal calf serum (FCS) (56°C for 30 min), 50 mM ß-mercaptoethanol, 20 mM Hepes, 500 U/ml penicillin, and 500 μg/ml streptomycin.

Twenty-four hours before the PRL bioassay, the cells were washed and transferred (1×10^6 cells/ml) to culture medium with 10% horse serum and 1% FCS to slow the rate of cell replication. After 24 h of starvation, cells were washed and resuspended (2×10^5 cells/ml) in phenol red free RPMI 1640 with only 10% horse serum; 200 μl of this suspension was added to 3 wells for each serum sample or prolactin standard to be tested. Frozen sera aliquots from different patients were diluted from 1:5 to 1:100, depending on the expected prolactin concentration, with medium without FCS. Fifty microliters of varying sera dilutions or standard or medium (for control wells) were added to wells of a 96 well microtest plate and incubated 3 days at 37°C with 5% CO_2 and 95% humidity. Cells in the microtest plates were resuspended by mixing several times with a multichannel micropipette and allowed to settle for 30 min. After settling, optical densities of each well were measured in a Titerteck Multiskan MCC/340 at 340 nm.

Human prolactin (hPRL-RP-2), a gift from the National Institute of Diabetes and Kidney Diseases (NIDDK), was diluted in preservative assay buffer (0.01 M NaH_2PO_4 and Na_2HPO_4; 0.14 M NaCl; 0.1% bovine serum albumin, BSA).

A standard curve for PRL concentration (from 30 pg to 10^4 pg/ml) was plotted against optical density, the hormone concentration expressed in $\log_{10}$ pg/ml prolactin. Unknown concentrations of PRL in the serum samples were calculated by measuring the hormone concentrations from the standard curve. To exclude the possible interference of serum growth hormone in the assay, a rabbit polyclonal antibody against hGH (anti-hGH-IC3; NIDDK) was added to each sample. The intraassay and interassay coefficients of variation were 1.3–2.9% and 3.7–6.2%, respectively.

To exclude possible interference of other serum factors in Nb2 proliferation, some samples from hyperprolactinemic patients were tested on Nb2 proliferation assay in the presence of a specific anti-PRL antibody (anti-hPRL-3; NIDDK). A significant reduction of cell proliferation was observed (data not shown).

Determination of serum GH levels. IRMA. In 49 patients, hGH serum concentrations were determined by a solid phase 2 site IRMA (ELSA-hGH, CIS-Bio International). The limit of assay sensitivity was 0.04 ng/ml. The intraassay and interassay coefficients of variation were 2.3–2.8 and 3.2–4.4, respectively. Normal levels of hGH are 0–10 ng/ml.

Statistical analysis. Comparisons between groups were made by Mann-Whitney U test. The significance of the correlations was determined using Spearman's rank correlation coefficient. Fisher's exact test was used to determine the association between lupus disease activity and hyperprolactinemia. All p values < 0.05 were considered statistically significant. Data are given as median (range) or means ± SD.

RESULTS

A statistically significant correlation between IRMA and BA PRL levels was found (r_s 0.46, $p < 0.001$) in all samples (Figure 1).

In the 78 SLE patients serum PRL levels measured by IRMA were between 2.2 and 51.2 ng/ml (mean 15.2 ± 9.1), PRL levels by BA were between 4.2 and 84 ng/ml (mean 22.2 ± 14.6).

In women PRL levels measured by IRMA and BA ranged from 3.9 to 51.2 ng/ml (mean 15.7 ± 9.0) and from 5.6 to 84 ng/ml (mean 22.4 ± 14.9), respectively. In men the values

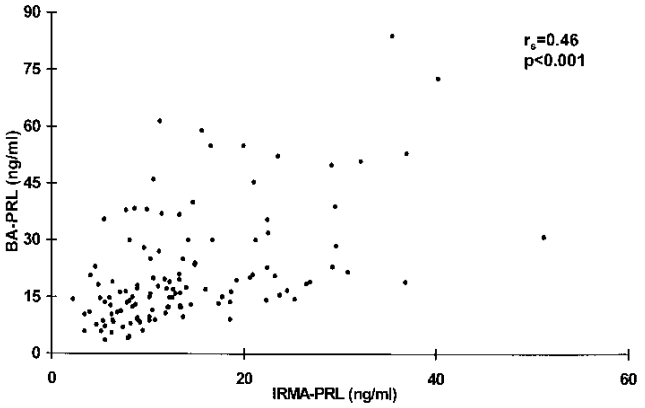

Figure 1. Correlation between serum PRL levels measured by Nb2 bioassay (BA) and by immunoradiometric assay (IRMA) in 122 samples from patients with SLE. Value of r_s (Spearman's rank correlation) is given.

Table 1. Studies on serum prolactin levels and SLE.

Authors	SLE Patients with Serum PRL Levels > 20 ng/ml, %	Patients, M/F	Correlation with Disease Activity
Lavalle[4]	85.7	8/0	ND
Folomeev[11]	100	29/0	ND
Jara[5]	22.2	0/45	Yes
Pauzner[12]	19.5	12/70	No
Buskila[13]	15.9	4/59	No
El-Garf[14]	9	33 prepubertal	No
Neidhart[15]	30	0/29	ND
Ostendorf[16]	2.2	14/168	No
Mok[17]	35	3/69	No
Pacilio[18]	30.6	3/46	Yes
Rovensky[19]	31	4/31	No
Ferreira[20]	37.5	1/23	No
Jimena[21]	27.7	0/36	No
Leanos[22]	15.8	259	ND
Miranda[23]	42	1/25	Yes
Mok[24]	13	31/0	No
Scali[25]	18	168	Yes
Zoli[26]	20	0/20	Yes

ND: not determined.

ranged from 2.2 to 20.9 ng/ml (mean 8.2 ± 7.4) and from 4.2 to 35.5 ng/ml (mean 19.6 ± 11.5).

In controls, PRL levels measured by IRMA were between 3.4 and 16.2 ng/ml (mean 8.9 ± 3.2), PRL levels by BA were between 3.2 and 18.4 ng/ml (mean 12.8 ± 2.7). Thus all controls presented normal serum levels (< 20 ng/ml) of PRL.

According to the LACC, SLE was active in 29 patients and inactive in 49. Median PRL levels by IRMA were 18.5 ng/ml (range 2.2–51.2) in active disease and 10.6 ng/ml (range 3.9–29.6) in non-active disease ($p < 0.001$). Median PRL levels by BA were 21.0 ng/ml (range 12.4–84) in active disease and 14.9 ng/ml (range 4.2–46.1) in non-active disease ($p < 0.001$) (Figure 2).

Hyperprolactinemia (> 20 ng/ml) was found in 21 patients (26.9%) using IRMA and in 31 (39.7%) by BA. The mean serum PRL levels in these instances were 27.5 ± 7.5 ng/ml (range 20.6–51.2) with IRMA and 35.7 ± 14.5 ng/ml (range 20.2–84) with BA. Hyperprolactinemia was significantly associated with active disease (by LACC) in 13 cases (61.9%) by IRMA and in 18 (58.1%) by BA ($p < 0.01$).

The SLEDAI median was 8.0 (range 0–16) in IRMA hyperprolactinemic cases (n = 21) vs 2.0 (range 0–24) in the remaining cases (n = 57) ($p < 0.001$). Moreover, the median was 8.0 (range 0–16) in the BA hyperprolactinemic cases (n = 31) vs 2.0 (range 0–24) in the remaining cases (n = 47) ($p < 0.001$).

A positive correlation resulted between SLEDAI and serum PRL levels by IRMA (r_s 0.5, $p < 0.001$) and BA (r_s 0.41, $p < 0.02$) (Figure 3).

A further analysis carried out on the serum samples obtained from 44 followup patients confirmed these results.

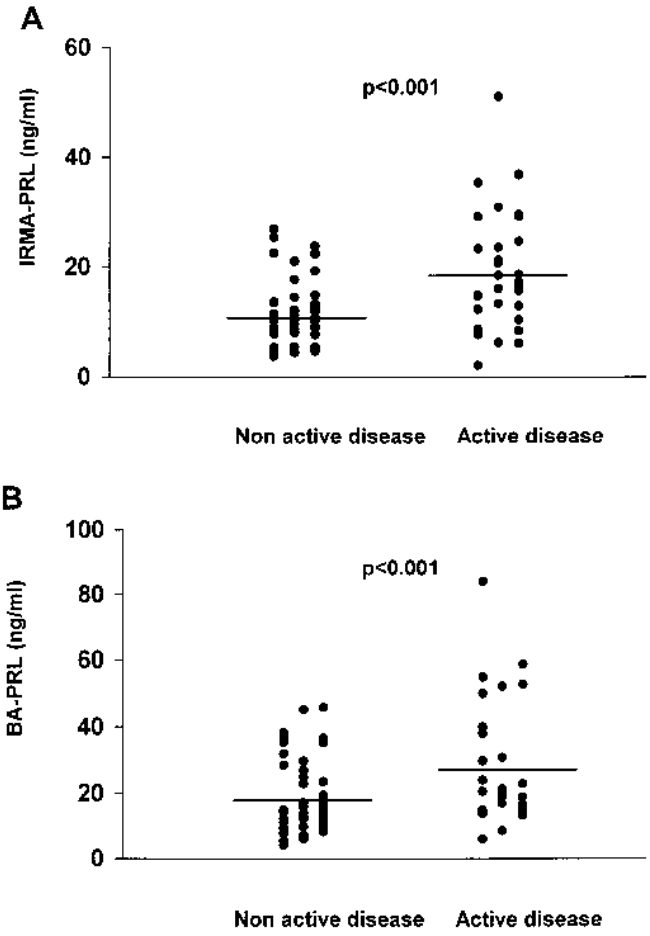

Figure 2. Distribution of serum PRL levels in 78 patients determined by IRMA (A) and by BA (B) according to SLE activity (by LACC).

By the LACC, SLE was active in 16 patients and inactive in 28. Median PRL level by IRMA was 19.9 ng/ml (range 6.3–51.2) in active disease cases and 8.65 ng/ml (range 4–14) in non-active disease ($p < 0.001$). Median PRL level by the BA was 31.0 ng/ml (range 12.4–72.8) in active disease cases and 12.5 ng/ml (range 3.6–20.6) in non-active disease ($p < 0.001$). The SLEDAI score was higher in the 7 IRMA hyperprolactinemic patients (median 13.5, range 8–22) than in the remaining 37 IRMA normoprolactinemic patients (median 2.0, range 0–12) (r_s 0.57, $p < 0.001$). Moreover, the SLEDAI score was higher in the 14 BA hyperprolactinemic patients (median 12, range 0–22) than in the remaining 30 BA normoprolactinemic patients (median 2, range 0–12) (r_s 0.51, $p < 0.001$).

The prevalence of different clinical manifestations in hyperprolactinemic and normoprolactinemic subjects is shown in Table 2. In hyperprolactinemia, determined by either IRMA or BA, a higher prevalence of malar rash and central nervous system (CNS) involvement was found ($p < 0.03$ and $p < 0.01$, respectively).

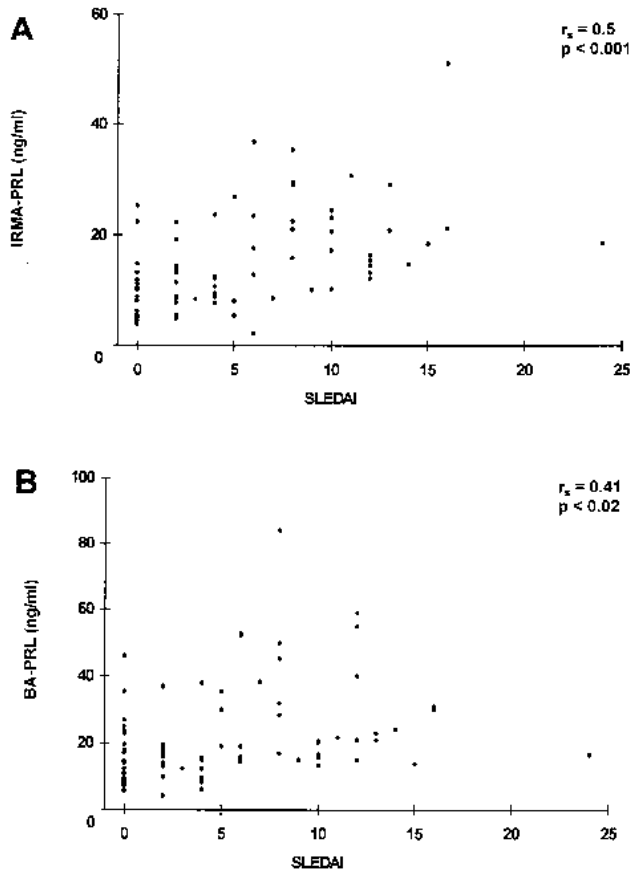

Figure 3. Correlation between SLEDAI scores and serum PRL levels measured by IRMA (A) and by BA (B) in 78 serum samples from patients with SLE.

Moreover, in 49 patients serum levels of growth hormone were between 0.1 and 20.6 ng/ml (mean 3.3 ± 4.5). GH levels did not correlate with PRL levels determined by IRMA and BA. According to LACC, SLE was active in 14 patients and inactive in 35. No significant difference was found in GH levels between patients with active and those with inactive disease (median 13 ng/ml, range 0.1–17.6 vs 1.3 ng/ml, range 0.1–20.6, respectively).

Antimalarial drugs are commonly used in patients with SLE. An *in vitro* study showed that chloroquine inhibited PRL secretion from cultured anterior pituitary cells[37]. We evaluated the effect of antimalarials on PRL levels in our patients. No correlation was found between antimalarial therapy and PRL levels.

DISCUSSION

SLE is an immune complex mediated disease that is more common in women, especially during the reproductive years. The observation that PRL has multiple interactions with the immune system[1-3] has prompted investigators to explore its role in the pathogenesis of immune disorders. In particular, there is great interest in investigating the possibility of a relationship between hyperprolactinemia and SLE[38]. As shown in Table 1, many contrasting reports have been published on this subject. However, in most cases a high number of patients with SLE were found to be hyperprolactinemic, yet only a limited number of authors report positive correlation between hyperprolactinemia and disease activity.

Blanco-Favela, *et al*[39] analyzed 5 studies on this topic and concluded that lupus activity is more frequent in patients with hyperprolactinemia than those without. Studying the hormonal profiles of 16 male patients with lupus, Chang, *et al*[40] found that serum PRL levels were significantly higher than in the normal controls.

In our study, hyperprolactinemia was found by IRMA in 21/78 patients (26.9%) and there was a significant association between high PRL levels and disease activity evaluated by LACC. Moreover, a higher median SLEDAI score in hyperprolactinemia was found, along with a correlation between PRL levels and SLEDAI.

Since hyperprolactinemia is not always associated with SLE and/or with disease activity in the literature, we hypothesized that this discrepancy might be linked to the different methods used for PRL determination. On the basis of the dif-

Table 2. Clinical manifestations in SLE patients with normal prolactinemia and hyperprolactinemia.

	IRMA			BA		
	Normal PRL, n = 96 n (%)	High PRL, n = 26 n (%)	p	Normal PRL, n = 78 n (%)	High PRL, n = 44 n (%)	p
Rash	11 (11.5)	8 (30.7)	0.03	7 (8.9)	12. (27.3)	0.01
Mucosal ulcers	2 (2.01)	0	NS (0.6)	2 (2.5)	0	NS (0.4)
Arthritis	2 (2.1)	1 (3.8)	NS (0.5)	2 (2.5)	1 (2.3)	NS (1.7)
Serositis	0	2 (7.6)	0.04	0	2 (4.5)	NS (0.2)
Kidney involvement	19 (19.8)	8 (30.7)	NS (0.3)	15 (19.2)	12 (27.3)	NS (0.4)
Psychosis or seizures	4 (4.2)	8 (30.7)	< 0.001	3 (3.8)	9 (20.4)	< 0.01
Vasculitis	8 (8.3)	4 (15.4)	NS (0.3)	7 (8.9)	5 (11.3)	NS (0.7)

IRMA: immunoradiometric assay, BA: biological assay. p Values evaluated by Fisher's exact test. NS: not significant.

ferent biological activity of multiple forms of immunoreactive and bioactive human prolactin, we evaluated serum PRL levels by IRMA and BA.

The Nb2 lymphoma cell bioassay, developed in 1980 by Tanaka and coworkers[30], is a method that has the advantages of both the radioimmunological assay (sensitivity and specificity) and bioassay (direct measurement of a cellular response).

In our study with low or moderate levels of plasma PRL there was agreement between IRMA and BA; however, at high levels, plasma PRL bioactivity slightly but significantly exceeded the radioimmunoactivity. This difference was also observed in other laboratories[41,42].

It has been reported that the antiproliferative effect of glucocorticoids could interfere with Nb2 cell growth, but this effect was reversed in the presence of prolactin[43]. Moreover, in our patients we found a positive correlation between steroid or azathioprine doses and elevated BA serum PRL levels that rules out a possible interference of these drugs on Nb2 cell proliferation (data not shown).

Our data obtained by biological assay confirmed the association between hyperprolactinemia and SLE activity.

The source of excessive circulating PRL in patients with SLE is still undefined. Some of these patients have prolactinomas[44] and others have hyperprolactinemia secondary to recognized causes such as drugs, hypothyroidism, or renal failure[45]. Our SLE patients who had elevated serum prolactin did not have identifiable causes of hyperprolactinemia. The elevated prolactin may result from lymphocyte and thymocyte PRL. It is well known that human lymphocytes are capable of producing PRL, and it is believed that this prolactin acts locally to stimulate lymphocyte proliferation in an autocrine or paracrine manner[2,46]. Moreover, it is interesting to note the increased prevalence of CNS manifestations in patients with hyperprolactinemia. Our findings are in agreement with data presented by Jara, *et al*[47] and El-Garf, *et al*[14], who reported a possible relationship between PRL levels and CNS manifestations. An explanation for this phenomenon could be the intrathecal synthesis of interleukin 6 (IL-6) in SLE patients with CNS involvement[47], because it has been reported that IL-6 is a stimulator of PRL secretion[48]. In a disease state, high circulating levels of proinflammatory cytokines (IL-6) might activate the pituitary to produce excessive amounts of PRL.

The data reported by Leanos, *et al*[22] suggest that PRL attenuates biological activity when it is bound to its antibody. It is likely that anti-PRL autoantibodies inhibit PRL action in the target cell by interfering with hormone receptor binding in the outer membrane. All these findings may explain why some clinical studies find no associations between serum PRL levels and disease activity in SLE. Further studies are needed to investigate the correlation between disease activity and the presence of specific isoforms of circulating PRL.

We hypothesized that multiple molecular forms of immunoreactive human PRL could manifest differences in biological activity. Our results using both IRMA and BA show that correlation between PRL levels and disease activity is significant. This correlation is confirmed in patients seen again after 6 to 8 months, giving strength to the association between serum PRL levels and lupus disease activity.

The efficacy of bromocriptine, a hypoprolactinemic drug, in treating patients with active SLE[38,49] lends further support to the involvement of PRL in SLE, although recent studies showed an effect of bromocriptine treatment in normoprolactinemic patients with SLE[50].

In summary, hyperprolactinemia detected by IRMA or BA is likely associated with disease activity in SLE. Although cause and effect remain to be established, hyperprolactinemia may be one means by which SLE disease activity is exacerbated, so this hormone does play a significant role in the immunoregulation of the disease.

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