Elevated Serum Bioactive Prolactin Concentrations in Patients with Systemic Lupus Erythematosus Are Associated with Disease Activity as Disclosed by Homologous Receptor Bioassays

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ABSTRACT. ***Objective.*** To assess the bioactivity of circulating prolactin (PRL) in serum samples from patients with systemic lupus erythematosus (SLE) using 2 novel homologous *in vitro* bioassays, and to correlate PRL bioactivity with lupus activity.

Methods. Serum samples from 98 SLE patients with and without disease activity were tested for immunoreactive and bioactive concentrations of PRL.

Results. Patients with active disease exhibited higher bioactive serum PRL levels in homologous bioassays ($p \leq 0.013$). In contrast, bioactivity in Nb2 cells was similar between patients with and without activity. The bioactive/immunoreactive PRL ratio (BA/IA) in homologous bioassays was significantly higher in patients with both clinical manifestations and serological indicators of lupus disease activity. SLE patients with idiopathic hyperprolactinemia (HPRL) and macroprolactinemia (MPRL) had low SLEDAI scores, and the BA/IA ratio in homologous bioassays was significantly lower compared to those with idiopathic HPRL and no MPRL. There was a negative but significant correlation between MPRL and BA/IA in homologous bioassays ($p < 0.001$), but not when the heterologous bioassay was employed.

Conclusion. Elevated serum bioactive PRL levels revealed by homologous bioassays were associated with disease activity, as well as with specific organ involvement. Big big PRL or macroprolactin is a PRL variant with reduced bioactivity towards its homologous receptor, and this altered bioactivity may contribute to the lower disease activity and absence of symptoms related to HPRL in SLE patients. These novel data must be considered in future studies to establish a relationship between PRL and disease activity in SLE. (First Release May 15 2007; J Rheumatol 2007;34:1514–21)

Key Indexing Terms:
PROLACTIN MACROPROLACTIN PROLACTIN BIOASSAYS
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Prolactin (PRL) is a polypeptide hormone primarily secreted by the anterior pituitary gland. Although the best known biological functions of PRL are linked to lactation and reproduction, the hormone is also involved in other physiological processes including immunoregulation[1,2]. The effects of PRL are mediated by its interaction with a specific cell-surface membrane receptor, the PRL receptor, which belongs to class 1 of the cytokine receptors. This superfamily includes receptors for several interleukins (IL-2, IL-3, IL-4, IL-6, and IL-7), growth hormone (GH), granulocyte-colony stimulating factor, leukemia inhibitory factor, erythropoietin, and thrombopoietin, among others[1–3]. In addition, PRL receptors are widely distributed throughout the immune system[1].

The first report of the association between PRL and systemic lupus erythematosus (SLE) was noted in men[4], and high serum PRL levels have been associated with lupus activity in humans and in experimental models of SLE[5–11].

Serum PRL from healthy subjects and most patients with hyperprolactinemia (HPRL) circulates in several isoforms, of which 3 major isoforms are identifiable by gel filtration chromatography. The major circulating PRL isoform is a 23 kDa single-chain polypeptide, little PRL (monomeric or free PRL), which comprises up to 80% of total PRL; the 2 other isoforms, big PRL (45–50 kDa) and big big PRL (> 100 kDa), circulate in lesser amounts[12]. These isoforms result from posttranslational modifications of the PRL molecule (aggregates of monomeric PRL and PRL bound to binding proteins), which may differently alter the biological and immunological properties of the hormone[12]. It is well recognized that the molecular heterogeneity of PRL is present in sera from patients with SLE[13–17]. Predominant presence of big big PRL, a phenomenon termed macroprolactinemia (MPRL), has been reported in ~40% of SLE patients with idiopathic HPRL[13]. Although the nature of macroprolactinemia is unclear, recent evidence indicates that big big PRL is mostly an IgG-23 kDa PRL complex (i.e., anti-PRL autoantibody-monomeric PRL)[18–20]. Independently of the nature of big big PRL (i.e., due or not due to anti-PRL autoantibodies), clinical symptoms of HPRL, such as amenorrhea and galactorrhea in women and impotence in men, are usually absent in patients with MPRL[13–15,20–22]. Interestingly, SLE patients with MPRL exhibit less clinical and serological evidence of disease activity than those without MPRL[13,14,16,17,23].

Immunometric methods that are commonly used to determine serum PRL are largely blind to changes in the patterns and proportions of PRL isoforms, which may potentially influence both the net *in vivo* biological activity of the hormone and the clinical features of PRL-related disease states. Another method to assess serum PRL is the measurement of circulating bioactive PRL. Although the Nb2 cell proliferation assay is sufficiently sensitive to measure bioactive PRL in human serum, it is not always completely satisfactory because the origin of the Nb2 cells (rat) raises the question of species-specificity when using ligands from other species[24]. In addition, it has been shown that the heterologous Nb2 cell proliferation bioassay is apparently unable to detect differences in PRL bioactivity among the various forms of human circulating prolactin[25,26]. In contrast, 2 recently developed homologous *in vitro* bioassays for human PRL (hPRL) measurement of bioactive PRL in serum have been shown to be valuable tools for the study of human lactogens that may be applied to clarify the discrepancies in estimates of PRL bioactivity resulting from the use of the Nb2 cell proliferation bioassay[25,26].

We applied these homologous *in vitro* bioassay systems to measure circulating bioactive PRL concentrations in samples from patients with SLE, and attempted to correlate bioactive PRL levels with disease activity.

MATERIALS AND METHODS

The study protocol was approved by the Human Ethical Committee and Medical Research Council of the Instituto Mexicano del Seguro Social. Written informed consent was obtained from all subjects, who voluntarily consented to participate in the study.

A group of 98 consecutive Mexican mestizo patients who fulfilled 4 or more of the American College of Rheumatology revised criteria for SLE[27] were included. To avoid possible interference in the *in vitro* bioassays, only patients taking low-dose prednisone (≤ 10 mg/day) and without treatment with immunosuppressors were included. A venous blood sample was drawn between 9:00 and 11:00 AM under basal conditions and without hormonal or drug stimulus. All serum samples from patients with lupus activity were collected before modifications in treatment were undertaken. Sera were stored at –35°C until used. None of these patients had obvious causes of HPRL. Disease activity was classified according to the SLE Disease Activity Index (SLEDAI)[28]. For the purpose of this study, any value > 4 was considered active disease.

Determination of direct or total serum PRL levels. PRL concentration in serum was measured by an immunoradiometric assay (RIA-gnost Prolactin; CIS Bio International, Gif-sur-Yvette, France) as described[8]. Normal levels of PRL are 5–20 ng/ml. Intraassay and interassay coefficients of variation were 5.7% and 6.8%, respectively.

Determination of serum free or monomeric PRL levels. Free PRL was extracted from the serum using polyethylene glycol (PEG), as described[8,13,29]. This procedure precipitates and removes high molecular weight PRL isoforms (big PRL and big big PRL)[29].

Gel filtration chromatography and affinity chromatography. Gel filtration was performed on Sephadex G-100 superfine columns (60 × 1 cm; Pharmacia Biotech, Uppsala, Sweden) as described[29]. Affinity chromatography for IgG was performed using 1 ml protein-G Sepharose columns (HiTrap G, Pharmacia LKB, Uppsala, Sweden) as described[13,29]. Immunoreactive PRL present in eluent fractions was determined by an ultrasensitive enzyme immunoassay[30]. Serum samples were considered to contain anti-PRL autoantibodies when the percentage of PRL retained by the protein-G Sepharose column was > 3.8% (this value represents the mean + 3 SD obtained from sera of 30 healthy pregnant women without MPRL as confirmed by size exclusion chromatography).

Homologous in vitro bioassays of PRL. The first bioassay, referred to as HPL-9 bioassay[25], uses human embryonic kidney-derived 293 (HEK-293) stably transfected with plasmids carrying the cDNA encoding the long isoform of human PRL receptor (hPRLR) and the PRL-responsive LHRE-luciferase reporter gene[25] (LHRE, lactogenic hormone response element, is the DNA binding element of the signal transducer and activator of transcription Stat5[31], one of the major signaling proteins activated by the activated PRLR). The second assay, the Ba/F3-LP bioassay[24–26], is a proliferative assay that has been used to characterize the relative bioactivity of wild-type PRL and several PRL analogs in structure-function studies[24]. These homologous hPRL *in vitro* bioassay systems for measurement of circulating bioactive PRL in serum samples were performed using a procedure as described[25].

PRL-dependent Nb2 lymphoma cell proliferation bioassay. The Nb2 cell proliferation bioassay was performed as described[14].

In all bioassays, measurement of bioactive PRL in serum samples was determined at 3 or more dose levels, in triplicate incubations. Anti-hGH antiserum (at final dilution 1:4000; National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA) was routinely added to the incubation medium in order to ascertain the specificity of the hPRL response. The sensitivity of the HPL-9 bioassay is 1–2 ng/ml, and of the Ba/F3-LP and Nb2 cell proliferation bioassays 400 pg/ml and 3.5 pg/ml, respectively[25]. Mean within- and between-assay coefficients of variation were ≤ 6.5% and ≤ 9.8%, respectively.

Statistical analysis. The significance of differences between continuous variables was determined by nonpaired Student t test (or Mann-Whitney U test for non-normally distributed variables). Differences between categorical variables were determined by chi-square test with Yates's continuity correction (or Fisher's exact test for small samples). The relationship

between relative amounts of big big PRL and the ratio of bioactive to immunoreactive PRL was assessed by Spearman's correlation coefficient. A 2-tailed $p < 0.05$ was considered statistically significant.

RESULTS

The study population consisted of 98 patients with SLE (93 women, 5 men). Mean age was 33.0 ± 10.5 years and median disease duration 54.5 months (range 1–414).

Relationship between serum immunoreactive and bioactive PRL levels and lupus disease activity. On the basis of SLEDAI score (> 4 points), 43/98 patients (43.9%) were identified as having lupus activity; the median score was 4 (range 0–37). Comparisons in demographic data, serum concentrations of immunoreactive and bioactive PRL, and ratios of bioactive to immunoreactive direct PRL (BA/IA; as a measure of PRL biopotency) between patients with active and inactive disease are shown in Table 1. There were no significant differences in mean age between active and inactive patients. The mean disease duration was significantly shorter in active patients. There was no significant difference in serum direct PRL levels between active and inactive patients (22.9 ± 17.4 vs 19.9 ± 28.8 ng/ml; p = 0.49). In contrast, the mean serum free PRL level was significantly higher in active than in inactive patients (17.9 ± 18.1 vs 10.6 ± 6.0 ng/ml; p = 0.008). Mean serum concentrations of bioactive PRL measured by homologous bioassays were significantly higher in active patients ($p < 0.001$). Similarly, the median of serum bioactive PRL levels as assessed in Nb2 cells was higher in active patients than in inactive patients (20.0 vs 14.1 ng/ml), but the difference was not statistically significant (p = 0.14). On the other hand, the mean BA/IA ratio in all bioassays was significantly higher in active

Table 1. Demographic data, serum concentrations of total and free immunoreactive PRL, and serum bioactive PRL concentrations disclosed by 3 *in vitro* bioassays (transactivation assay in HPL-9 cells and Ba/F3-LP and Nb2 cell proliferation assays), as well as ratios of bioactive to immunoreactive direct PRL (BA/IA) in patients with inactive and active SLE as evaluated by different cutoff points of the SEDAI score.

Variable	SLEDAI > 4		
	No (n = 55)	Yes (n = 43)	p
Age, yrs, mean ± SD	34.6 ± 10.9	31.0 ± 9.7	0.09*
Disease duration, mo, mean ± SD	103.0 ± 89.4	60.8 ± 63.12	0.01*
Women (%)	54 (98.2)	39 (90.7)	0.17†
Direct PRL, ng/ml, mean ± SD	19.9 ± 28.8	22.9 ± 17.4	0.49*
Free PRL, ng/ml, mean ± SD	10.6 ± 6.0	17.9 ± 18.1	0.008*
PRL by HPL-9, ng/ml, mean ± SD	10.1 ± 4.9	22.6 ± 15.8	< 0.001*
PRL by Ba/F3-LP, ng/ml, mean ± SD	14.1 ± 8.0	23.9 ± 14.9	< 0.001*
PRL by Nb2, ng/ml, median (range)	14.2 (1.8–248.9)	20.0 (5.8–86.3)	0.14††
BA/IA in HPL-9, mean ± SD	0.51 ± 0.28	1.01 ± 0.25	< 0.001*
BA/IA in Ba/F3-LP, mean ± SD	0.68 ± 0.39	1.09 ± 0.28	< 0.001*
BA/IA in Nb2, mean ± SD	0.96 ± 0.38	1.18 ± 0.36	0.004*
	SLEDAI > 10		
	No (n = 76)	Yes (n = 22)	
Direct PRL, ng/ml, median (range)	17.4 (5.3–281.7)	18.4 (9.6–79.5)	0.36††
Free PRL, ng/ml, mean ± SD	13.1 ± 7.4	20.8 ± 16.9	0.03*
PRL by HPL-9, ng/ml, mean ± SD	11.8 ± 6.8	28.6 ± 18.5	< 0.001*
PRL by Ba/F3-LP, ng/ml, mean ± SD	15.4 ± 8.8	28.7 ± 17.4	< 0.001*
PRL by Nb2, ng/ml, median (range)	14.5 (1.8–248.9)	26.4 (9.8–86.3)	0.91††
BA/IA in HPL-9, mean ± SD	0.62 ± 0.34	1.12 ± 0.15	< 0.001*
BA/IA in Ba/F3-LP, mean ± SD	0.78 ± 0.38	1.15 ± 0.31	< 0.001*
BA/IA in Nb2, mean ± SD	0.99 ± 0.37	1.28 ± 0.37	0.002*
	SLEDAI > 15		
	No (n = 90)	Yes (n = 8)	
Direct PRL, ng/ml, median (range)	17.0 (5.3–281.7)	27.8 (11.6–79.5)	0.41††
Free PRL, ng/ml, mean ± SD	13.3 ± 7.6	32.5 ± 21.4	0.04*
PRL by HPL-9, ng/ml, mean ± SD	13.4 ± 8.6	40.1 ± 23.0	0.013*
PRL by Ba/F3-LP, ng/ml, mean ± SD	16.4 ± 9.5	41.0 ± 19.5	0.009*
PRL by Nb2, ng/ml, median (range)	16.0 (1.8–248.9)	37.4 (24.0–86.3)	0.30††
BA/IA in HPL-9, mean ± SD	0.70 ± 0.37	1.11 ± 0.06	0.002*
BA/IA in Ba/F3-LP, mean ± SD	0.83 ± 0.18	1.24 ± 0.42	0.005*
BA/IA in Nb2, mean ± SD	1.03 ± 0.38	1.32 ± 0.35	0.06*

* Non-paired Student t test, † Fisher's exact t test, †† Mann-Whitney U test.

patients than in inactive patients ($p < 0.004$). Similar findings were found when higher SLEDAI cutoff scores (at > 10 and at > 15) were used to compare serum concentrations of immunoreactive and bioactive PRL (Table 1).

A significant positive correlation was present between SLEDAI and the BA/IA by HPL-9 ($r_s = 0.66$, $p < 0.001$), BA/IA by Ba/F3-LP ($r_s = 0.52$, $p < 0.001$) and serum free PRL levels ($r_s = 0.44$, $p < 0.001$), and BA/IA by Nb2 ($r_s = 0.27$, $p = 0.008$). In contrast, there was no correlation between SLEDAI and serum direct PRL levels ($r_s = 0.074$, $p = 0.47$).

Association between BA/IA and clinical and serological findings in SLE patients. To determine the clinical relevance of the new homologous *in vitro* PRL bioassays and the classical Nb2 cell proliferation assay, a comparison was made among the BA/IA of the 3 bioassays and the different clinical manifestations and serologic findings of the SLE patients. As shown in Table 2, the BA/IA, determined by either the HPL-9 or Ba/F3-LP cell bioassays, was significantly higher in patients exhibiting 8 clinical and serological measures of disease activity (neurological manifestations, arthritis, renal involvement, mucocutaneous manifestations, serositis, hematological manifestations, hypocomplementemia, and anti-dsDNA; $p \leq 0.04$). In contrast, the BA/IA as assessed by the Nb2 cell bioassay was significantly higher in patients with arthritis, renal involvement, and mucocutaneous manifestation ($p \leq 0.003$).

Relationship among molecular heterogeneity of PRL, bioactive PRL levels, and lupus disease activity. To determine the relationship between molecular heterogeneity of PRL and bioactive PRL on the basis of lupus disease activity, gel filtration chromatography and affinity chromatography on a protein-G column were applied to all sera from SLE patients with idiopathic HPRL (defined as serum total PRL value > 20 ng/ml and no known cause for HPRL). Idiopathic HPRL was found in 35/98 patients (35.7%). Gel filtration chromatography profiles in 12/35 (34.3%) sera showed a predominant pattern of MPRL or big big PRL (≥ 50% of immunoreactive total PRL) with MW ~150 kDa. In all samples, the presence of MPRL was due to anti-PRL autoantibodies, as confirmed by affinity chromatography studies (Table 3). In the sera from SLE patients without MPRL, most PRL (89.2 ± 11.7%) eluted as monomeric 23 kDa PRL (little PRL) and no or minimal amounts of PRL (1.9 ± 0.8%) coeluted with the IgG by protein-G column. The comparison in demographic and clinical variables and measurements of PRL between SLE patients with and those without MPRL are shown in Table 3. The median SLEDAI score was significantly lower in patients with MPRL than in patients without MPRL: 0 (range 0–8) versus 10 (range 0–37), respectively ($p < 0.001$). SLE patients with MPRL had significant elevations in serum direct PRL and bioactive PRL levels measured by Nb2 cells; in addition, these patients showed a significant decrease in serum free PRL concentrations, bioactive PRL levels by homologous bioassays, and BA/IA determined by homologous bioassays. The mean BA/IA measured in Nb2 cells was higher in patients without MPRL than in patients with MPRL (1.16 ± 0.40 vs 1.06 ± 0.15, respectively), but the difference did not reach statistical significance.

Correlation between percentage of big big PRL and BA/IA. The results derived from the 3 bioassays suggested that the presence and relative amounts of big big PRL may influence the BA/IA as assessed by the homologous bioassays (HPL-9 and Ba/F3-LP cell bioassays), but not by the heterologous bioassay (Nb2 cell bioassay). To examine this issue further and to confirm that MPRL had low biological activity in the homologous bioassays, a series of correlations between the percentages of big big PRL present in samples from SLE patients with idiopathic HPRL and the BA/IA derived from each bioassay were performed (Figure 1). A highly significant negative correlation was present when bioactive PRL was assessed by the homologous bioassays ($r_s = -0.86$, $p < 0.001$ and $r_s = -0.76$, $p < 0.001$ for the HPL-9 and Ba/F3-LP cells, respectively) but not by the heterologous bioassay ($r_s = -0.19$, $p = 0.38$).

Table 2. Ratios of bioactive to immunoreactive direct PRL (BA/IA) in SLE patients disclosed by 3 *in vitro* bioassays (transactivation assay in HPL-9 cells and Ba/F3-LP and Nb2 cell proliferation assays) and according to the presence of various clinical and serological measures.

Variable	Bioactivity:Total PRL Immunoreactivity Ratio								
	HPL-9:Immunoreactive Direct PRL			Ba/F3-LP:Immunoreactive Direct PRL			Nb2:Immunoreactive Direct PRL		
	No	Yes	p	No	Yes	p	No	Yes	p
Neurological manifestations	0.72 ± 0.37	1.00 ± 0.12	0.002	0.85 ± 0.41	1.03 ± 0.08	0.004	1.05 ± 0.39	1.11 ± 0.22	0.76
Arthritis	0.65 ± 0.35	1.07 ± 0.24	< 0.001	0.80 ± 0.41	1.13 ± 0.17	0.001	1.00 ± 0.37	1.29 ± 0.37	0.004
Renal involvement	0.64 ± 0.35	1.08 ± 0.18	< 0.001	0.79 ± 0.38	1.16 ± 0.33	< 0.001	1.00 ± 0.36	1.29 ± 0.39	0.003
Mucocutaneous manifestations	0.66 ± 0.36	0.97 ± 0.28	0.001	0.81 ± 0.39	1.05 ± 0.38	0.013	1.00 ± 0.37	1.25 ± 0.37	0.008
Serositis	0.71 ± 0.37	1.06 ± 0.22	0.01	0.85 ± 0.41	1.04 ± 0.15	0.03	1.05 ± 0.39	1.12 ± 0.21	0.68
Hematological manifestations	0.69 ± 0.36	1.09 ± 0.18	< 0.001	0.84 ± 0.40	1.12 ± 0.20	0.003	1.04 ± 0.39	1.16 ± 0.29	0.37
Hypocomplementemia	0.57 ± 0.35	0.92 ± 0.30	< 0.001	0.72 ± 0.41	1.04 ± 0.30	< 0.001	0.98 ± 0.38	1.14 ± 0.37	0.05
Anti-dsDNA	0.62 ± 0.32	0.81 ± 0.35	0.03	0.81 ± 0.33	0.98 ± 0.23	0.04	1.07 ± 0.42	1.04 ± 0.34	0.73

Data expressed as mean ± SD. * Non-paired Student t test.

Table 3. Demographic and clinical variables, distribution of PRL immunoreactivity in 3 fractions obtained after gel filtration, percentage of retained PRL in affinity chromatography, serum total and free immunoreactive PRL levels, serum bioactive PRL concentrations by 3 *in vitro* bioassays, and ratios of bioactive to immunoreactive direct PRL (BA/IA) in SLE patients with idiopathic hyperprolactinemia according to the absence or presence of macroprolactinemia (MPRL)

Variable	Without MPRL (n = 23)	With MPRL (n = 12)	p
Age, yrs, mean ± SD	30.3 ± 11.7	30.2 ± 6.7	0.97*
Disease duration, mo, mean ± SD	52.7 ± 62.8	50.4 ± 35.0	0.91*
Women (%)	20 (87.0)	12 (100)	0.54†
SLEDAI score, median (range)	10 (0–37)	0 (0–8)	< 0.001††
Big big PRL (%), mean ± SD	3.3 ± 3.1	74.5 ± 14.5	< 0.001*
Big PRL (%), mean ± SD	7.6 ± 5.1	8.3 ± 4.6	0.17*
Little PRL (%), mean ± SD	89.2 ± 11.7	16.8 ± 11.4	< 0.001*
IgG-bound PRL (%), mean ± SD[1]	1.9 ± 0.8	48.4 ± 9.9	< 0.001*
Direct PRL, ng/ml, mean ± SD	33.9 ± 13.9	102.3 ± 70.4	0.006*
Free PRL, ng/ml, mean ± SD	26.9 ± 15.3	14.0 ± 6.4	0.009*
PRL by HPL-9, ng/ml, mean ± SD	29.5 ± 18.7	13.2 ± 4.0	< 0.001*
PRL by Ba/F3-LP, ng/ml, mean ± SD	33.8 ± 14.8	18.1 ± 6.1	< 0.001*
PRL by Nb2, ng/ml, mean ± SD	38.7 ± 19.1	105.1 ± 19.1	0.005*
BA/IA in HPL-9, mean ± SD	0.84 ± 0.34	0.19 ± 0.14	< 0.001*
BA/IA in Ba/F3-LP, mean ± SD	1.01 ± 0.19	0.26 ± 0.18	< 0.001*
BA/IA in Nb2, mean ± SD	1.16 ± 0.40	1.06 ± 0.15	0.40*

[1] PRL retained in protein-G Sepharose column (PRL retained/PRL not retained + PRL reatined × 100%). * Non-paired Student t test, † Fisher's exact t test, †† Mann-Whitney U test.

DISCUSSION

High serum PRL concentrations and even HPRL are common findings in patients with SLE. Nevertheless, clinical studies on the participation of PRL in SLE and its relationship with disease activity have yielded controversial results[5–8,13,32], despite the fact that both clinical trials and studies in experimental models have clearly established a link between PRL and SLE[9–11,33,34]. The majority of clinical studies on SLE patients, however, have measured only serum immunoreactive (direct) PRL levels when attempting to establish an association between levels of this hormone and disease activity, without considering the existence of different PRL isoforms. Molecular heterogeneity of PRL in sera from SLE patients was first described in 1998[13] and subsequent studies confirmed its occurrence[8,14–17,23]. In our recent study involving a large number of SLE patients[23], we found that serum direct or total PRL levels measured by an immunoradiometric assay were not related to lupus activity, whereas the increase in serum levels of free or monomeric PRL and free HPRL were both associated with lupus activity, and also with specific organ involvement. Additionally, we found that the SLE patients with higher relative concentrations of big big PRL or lower amounts of little PRL had less lupus activity. Further, normal or elevated serum free PRL levels may be present in SLE patients with MPRL. Based on these data, we suggested that not only the presence of MPRL but also the levels of serum free PRL may be associated with lupus activity[23].

In the present study, we have confirmed that serum free PRL level is higher in patients with active disease (evaluated by SLEDAI score at > 4, > 10, and > 15) than in patients with inactive disease. By contrast, although the serum direct PRL level was higher in patients with active disease, no clinical significance was observed in lupus activity.

Measurements of serum immunoreactive PRL concentrations in several disease states characterized by HPRL do not always correlate with the clinical findings. Indeed, several investigators have reported that asymptomatic HPRL is frequently associated with the presence of molecular heterogeneity, particularly the predominant presence of big big PRL[15,21,35,36]. Given the lack of symptoms associated with HPRL in the majority of patients with MPRL (independent of its origin)[20] and the finding that the MPRL exhibits normal *in vitro* bioactivity when tested in Nb2 cells[14,22,25,26], it has been proposed that MPRL cannot exert full biological activity *in vivo* since its access to target cells may be restricted due to its greater molecular weight and/or altered net charge[14,16,22]. Nevertheless, the recent development of new homologous *in vitro* bioassays for human PRL[24–26] has highlighted the species-specificity of the biological response observed with various lactogens, suggesting that the use of a heterologous bioassay (i.e., the Nb2 assay) to determine the bioactivity of serum PRL may not always be appropriate, particularly when MPRL is present.

Since molecular heterogeneity of PRL is present in the serum of SLE patients with and without HPRL[13–17,23] and considering that MPRL may in fact represent a PRL variant with reduced bioactivity and thus explain the low disease

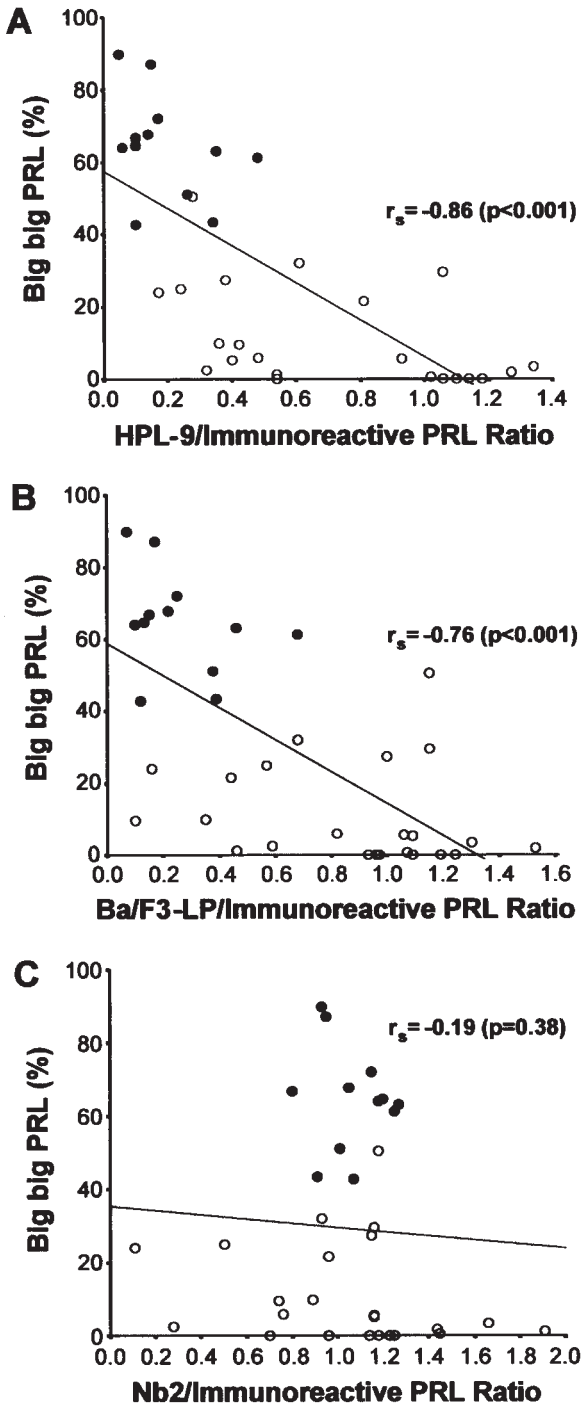

Figure 1. Relationship between the percentages of big big PRL (determined by gel filtration chromatography) present in serum samples from 35 SLE patients with idiopathic hyperprolactinemia — 23 without macroprolactinemia (○) and 12 with macroprolactinemia (●); and the ratio of bioactive to immunoreactive direct PRL in HPL-9 cells (A), in Ba/F3 cells (B), and in Nb2 cells (C).

activity and lack of HPRL symptoms in SLE patients, we applied 2 novel homologous *in vitro* bioassays to measure bioactive PRL concentrations in sera from patients affected by this disorder. Our results showed that serum bioactive PRL concentrations were significantly higher in patients with active disease than in those with inactive disease, whenever bioactive PRL was measured by any of the homologous bioassays. The finding that lupus activity did not correlate with bioactive PRL levels as determined by the Nb2 cell-based bioassay is in agreement with data from Cruz, *et al*[16], who found a similar BA/IA between SLE patients with and those without lupus activity. Further, when the BA/IA was employed to compare sample potency between patients with active and inactive disease, we found that for the homologous bioassays this ratio was higher in patients with multiple organ involvement and serological markers of active disease at the 3 SLEDAI cutoff points, whereas for the heterologous bioassay the ratio was higher at only 2 cutoff points and in patients with arthritis, renal involvement, and mucocutaneous manifestations exclusively. Thus, the applications of homologous and heterologous bioassays allowed identification of distinct subgroups of SLE patients according to the severity and state of disease.

It has been reported that SLE patients with HPRL and MPRL have less lupus activity than patients with HPRL but without MPRL[14,16,23]. We thus analyzed and compared samples from SLE patients with idiopathic HPRL according to absence or presence of MPRL. We found that 34.3% of SLE patients with idiopathic HPRL also had MPRL, and that all patients with MPRL presented anti-PRL autoantibodies, confirming and extending previous findings[13]. We also confirmed that SLE patients with MPRL had less lupus activity than patients without MPRL[13,14,16]. Interestingly, among samples from SLE patients without MPRL, the BA/IA was similar (close to unity), regardless of the bioassay employed, proving that monomeric PRL is bioactive and that its levels in serum concentrations may be reliably measured either by heterologous or homologous PRL receptor-based bioassays. This finding contrasts with the results in samples from SLE patients with MPRL, in whom measurements of the BA/IA showed low level of activity in both homologous receptor bioassays, while in the Nb2 bioassay they showed higher level of activity. Moreover, we also found a negative correlation between the relative amounts of big big PRL and the BA/IA obtained through homologous bioassays, while there was no correlation between big big PRL and the BA/IA using the heterologous bioassay. These data suggest that the Nb2 assay is reliable for measuring serum bioactive PRL concentrations when monomeric PRL is the main circulating PRL isoform, but not when the other PRL isoforms, including macroprolactin, are present in significant amounts. Indeed, we[25] and others[26] have documented that the low bioactivity exhibited by samples from patients with MPRL in both homologous assays may actually originate from the fraction of monomeric PRL (or free PRL) present in the unfractionated or fractionated samples. Although the mechanism(s) subserving the low bioactivity

of macroprolactin in the homologous bioassays remains to be determined, our results emphasize the species-specificity of PRL isoforms on PRL receptor activation. Further, the data indicate that IgG-bound PRL has low intrinsic activity, thus challenging the prevailing hypothesis that macroprolactin cannot exert its full biological activity *in vivo* because of its restricted access to target tissues.

Our study demonstrates that elevated serum bioactive PRL concentrations are associated with SLE activity and specific organ involvement, as revealed by homologous *in vitro* bioassays. Macroprolactin, a PRL variant with reduced bioactivity toward its homologous receptor, may contribute to the lower disease activity and absence of symptoms related to HPRL in patients with SLE. In accord with previous studies[14,16,23], our results provide evidence that the presence of MPRL could be useful as a marker of lupus inactivity and serum free PRL levels for monitoring the response to treatment.

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