Antiprolactin Autoantibodies in Systemic Lupus Erythematosus: Frequency and Correlation with Prolactinemia and Disease Activity

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ABSTRACT. ***Objective***. To determine in patients with systemic lupus erythematosus (SLE) (1) the frequency of antiprolactin (anti-PRL) autoantibodies, and (2) the relationships among anti-PRL autoantibodies, serum prolactin (PRL) levels, and lupus activity.

Methods. In a cross sectional study 259 consecutive patients with SLE were tested for serum PRL levels and anti-PRL autoantibodies based on disease activity.

Results. The frequency of anti-PRL was 5% (13/259), and all SLE patients with anti-PRL had hyperprolactinemia. There was lupus activity in 110 patients (42.5%) and there was no significant difference in frequency of anti-PRL autoantibodies between patients with or without lupus activity (5.5 vs 4.7%; p = 0.99). Only a high level of serum PRL was associated with lupus activity independent from other studied variables (p = 0.024). There was a negative but nonsignificant correlation between the titers of anti-PRL autoantibody and SLEDAI (r_s = –0.16, p = 0.59). Anti-PRL positive patients had higher levels of serum PRL than anti-PRL negative patients (33.2 ± 13.8 vs 11.6 ± 13.2 ng/ml; p = 0.0001) and a significantly different frequency of hyperprolactinemia (100 vs 11.4%; p = 0.00001).

Conclusion. The presence of anti-PRL autoantibodies was associated with hyperprolactinemic status and high serum PRL levels; these data suggest that anti-PRL autoantibodies could be the cause of hyperprolactinemia in a subset of patients with SLE. An increase in serum PRL levels proved to be an important independent factor related to lupus activity, but there was no relationship between anti-PRL autoantibodies and lupus activity. (J Rheumatol 2001;28:1546–53)

Key Indexing Terms:
PROLACTIN ANTIPROLACTIN AUTOANTIBODY HYPERPROLACTINEMIA
SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY

Systemic lupus erythematosus (SLE) is a complex disease that is characterized by both B cell and T cell abnormalities, especially during the active phase of disease, causing an excessive production of autoantibodies. Immune response abnormality in SLE may result from the interplay of genetic, environmental, and hormonal factors.

Lactogenic hormone of anterior pituitary prolactin (PRL) has effects on immune response[1–9]. Elevated serum PRL levels have been reported consistently in patients with SLE, and hyperprolactinemia (HPRL) (> 20 ng/ml) occurred in about 15–31%[10–14]. In contrast, in healthy women from 14 to 43 years of age reported frequency has been 3%[15].

In experimental studies in animal models, there is sufficient evidence that PRL has a deleterious effect on autoimmune diseases and aggravates disease activity in female NZBxW mice[5]. In contrast, clinical reports have provided contradictory data on the relationship between PRL and lupus activity. Some studies support the idea of an association between serum PRL levels and clinical and serological activity[10,14,16], but other studies were unable to confirm this relationship[12,13,17].

We recently found anti-PRL autoantibodies in sera from 40.7% of SLE patients with idiopathic HPRL. Interestingly, these patients have shown less clinical and serologic lupus activity than SLE patients with idiopathic HPRL who were anti-PRL autoantibody negative[18]. Low statistical power of the studies[19], lack of control of variables that influence lupus activity or serum PRL levels[19,20], the accuracy of techniques to measure real concentrations of PRL[21], and the presence of anti-PRL autoantibodies[18] may account for the inconsistencies reported in studies of the association between serum PRL levels and lupus activity.

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The previous data led us to reevaluate the relationship between serum PRL levels and disease activity in a larger number of patients with SLE. We investigated (1) the frequency of anti-PRL autoantibodies, and (2) the relationship between serum PRL levels and lupus activity, taking into account the influence of the presence of the anti-PRL autoantibody, as well as characterizing the SLE patients with anti-PRL autoantibodies. Additionally, we examined the effect of anti-PRL autoantibodies on the measurements of PRL using radioimmunoassay (RIA), the technique most widely used compared to immunoradiometric assay (IRMA).

MATERIALS AND METHODS

The Human Ethical Committee and Medical Research of the Institute approved the study protocol and informed written consent was obtained from all subjects who participated voluntarily in this study.

A group of 259 consecutive Mexican mestizo patients who fulfilled 4 or more of the American College of Rheumatology revised criteria for the classification of SLE[22] was studied. They were seen at the Lupus Clinic of the Hospital de Especialidades, Centro Médico Nacional Siglo XXI, in México City from September 1996 to April 1997. A venous blood sample was drawn between 9:00 AM and 1:00 PM. Sera were separated and stored at –35ºC until used. Recent medications as well as those taken on the day of the evaluation were recorded. Other conditions associated with elevated PRL were noted. Disease activity was classified according to a published index (SLE Disease Activity Index, SLEDAI)[23]. For this study, any value above 0 was considered active disease. The treatment prescribed for each patient was classified in one of 4 categories: (1) no steroids or immunosuppressives; (2) 15 mg of prednisone daily or less; (3) more than 15 mg of prednisone daily, and (4) immunosuppressives with or without prednisone. The PRL was measured by double antibody RIA (Diagnostic Products Corp., Los Angeles, CA, USA) under basal conditions without hormonal or drug stimulus. The limit of sensitivity of the assay was 1.4 ng/ml, normal levels were between 5 and 20 ng/ml, and HPRL was defined as > 20 ng/ml. Intraassay coefficient of variation was 5.7% and interassay coefficient of variation was 6.8%.

Anti-PRL autoantibody screening. Protein-A Sepharose CL-4B was used to immunoprecipitate the PRL-IgG complex as described[18] and expressed as ratio of serum PRL bound to IgG (RPRL-IgG). Serum samples were judged to contain anti-PRL autoantibodies when the result displayed a ratio that exceeded 1.55%. This result represented the mean + 3 SD from 24 healthy women (14 nonpregnant women and 10 pregnant women).

Extraction of anti-PRL autoantibodies free of endogenous PRL, and free and total PRL. As the presence of anti-PRL autoantibodies has been reported as interfering with double antibody RIA and causing falsely low PRL measurements, we evaluated this possibility using another PRL assay [IRMA (RIA-gnost Prolactin, CIS Bio International, Gif-sur-Yvette, France)] in sera from patients according to the presence or absence of anti-PRL autoantibodies. For this, we carried out the following procedures. First we obtained serum with anti-PRL autoantibody free of endogenous PRL. Briefly, the serum (1.5 ml) was incubated with 6 ml of 0.1 mol/l glycine-HCl buffer (pH 2.2) containing 0.25% of dextran T70 (Pharmacia Fine Chemicals, Uppsala, Sweden) and 2.5% of charcoal activated powder for 1 h at 4ºC with continuous shaking. With this procedure, PRL is dissociated from the autoantibody and the free PRL is absorbed by the dextran coated charcoal[24]. The mixture was then centrifuged at 3000 rpm for 30 min at 4ºC and the supernatant was neutralized with 2 mol/l Tris-HCl buffer (pH 8.5). Finally, IgG was precipitated with ammonium sulfate at 33% and the precipitate was resuspended in phosphate buffered saline and dialyzed. The concentration of IgG was measured by turbidimetry and adjusted to have a final concentration of 5 mg/ml of IgG. Second, free and total PRL were extracted from sera according to the method used in insulin extraction in sera with anti-insulin antibodies[25]. PRL concentrations in supernatants after extraction procedure and serum were measured using RIA and IRMA. Interassay and intraassay coefficients of variation for all tests were less than 7%.

Statistical analysis. The significance of differences between continuous variables was determined by non-paired Student test (or Mann-Whitney U test for non-normal distribution variables). The significance of differences between categorical variables was determined by chi-square test with Yates' continuity correction (or Mantel-Haenszel chi-square with linear tendency for variables with more than 2 categories). Finally, multiple logistic regression models were used to adjust the relationship observed between each studied variable and lupus activity. The log-likelihood method was used to determine the best multiple logistic regression model.

The linear relationship between the anti-PRL autoantibody and SLEDAI was assessed by Spearman correlation coefficient and linear relationship between serum PRL levels by RIA and IRMA was assessed by Pearson correlation coefficient. A 2-tailed $p < 0.05$ was considered statistically significant.

RESULTS

General description. The study sample consisted of 259 patients (242 women and 17 men). Mean age was 35.6 ± 11.0 years (range 16–66) and mean disease duration was 108.8 ± 103.0 mo (range 1–447, median 76). Clinical and demographic characteristics of 259 SLE patients are shown in Table 1. Mean serum PRL by RIA was 12.7 ± 14.0 ng/ml (range 0.4–111.9, median 8.5). HPRL was found [15.8%, 95% confidence interval (CI) 11.4–20.2%] in 41 patients. HPRL was present in 3/17 men (17.6%, 95% CI 0–35.7%), similar to the women 38/242 (15.7%, 95% CI 11.1–20.3%).

Table 1. Demographic data and clinical characteristics of 259 patients with SLE.

Variables	
Age, yrs mean ± SD	35.6 ± 11.0
Women (%)	242 (93.4)
Disease duration, mo, median	76
Treatment with chloroquine (%)	67 (25.9)
Category of treatment (mean ± SD)	2.4 ± 1.1
1 (%)	56 (21.6)
2 (%)	114 (44.9)
3 (%)	31 (12.0)
4 (%)	58 (22.4)
Prednisone, mg/day, median	5
Active disease (%)	110 (42.5)
SLEDAI, median	0
Prolactin, ng/ml, median	8.5
HPRL (%)	41 (15.8)
Associated conditions that increase prolactin	
All (%)	38 (4.7)
Without HPRL (%)	24 (11.0)*
With HPRL (%)	14 (34.1)‡

HPRL: hyperprolactinemia.

*Seven amitriptyline, 6 estrogens, 5 chronic renal failure, 3 verapamil, 2 acute renal failure, and 1 primary biliary cirrhosis.

‡Three chronic renal failure, 2 pregnancy, 2 haloperidol, 1 acute renal failure, 1 primary hypothyroidism, 1 amitripyline, 1 chlorpromazine, 1 domperidone, 1 verapamil, and 1 chronic active hepatitis C.

Among the 41 patients with HPRL, in 14 (34.1%) there was an identifiable condition that could account for the increased PRL level (secondary HPRL): 6 attributable to the use of medications, 4 to renal failure, 2 to pregnancy, one to chronic active hepatitis C, and one to hypothyroidism (thyroid stimulating hormone > 50 IU/ml). In 27 patients (65.9%), no cause could be identified that would explain the presence of HPRL (idiopathic HPRL).

Frequency of anti-PRL autoantibodies. According to RPRL-IgG, 13 patients were identified with anti-PRL autoantibodies. The frequency in the entire study group was 5.0% (95% CI 2.3–7.7%) and 100% of patients with anti-PRL autoantibodies had HPRL.

Relationship between anti-PRL autoantibodies and serum PRL levels in lupus activity. On the basis of the SLEDAI index (≥ 1 point), 110/259 patients (42.5%) were identified with lupus activity. The mean score was 6.9 ± 6.4, median 4. The comparison in demographic and clinical characteristics between active and non-active patients is shown in Table 2. In univariate analysis, the presence of anti-PRL autoantibody or RPRL-IgG did not display a significant difference between active and non-active patients. However, the statistical power of study was 4.6% in the difference found between the presence of anti-PRL autoantibodies and lupus activity. Active patients were younger and their disease duration was shorter; in addition, this group had a higher frequency of HPRL and serum PRL levels than non-active patients ($p \leq 0.036$). Within logistic regression models, the first included all variables with statistical significance ($p < 0.05$) in univariate analysis. The category of treatment and doses of prednisone were included based on a chosen level of significance; these are not theoretically associated with the activity, but rather are indirect indexes of appropriate classification of lupus activity. In addition, the titers of anti-PRL autoantibodies were included because patients with anti-PRL autoantibodies had a higher frequency of HPRL and serum PRL levels. Therefore, the category of treatment, doses of prednisone, and RPRL-IgG were included to determine whether the other variables would remain independent with respect to variables with statistical significance in univariate analysis. The complete and reduced models are shown in Table 3; the complete and reduced models had a similar likelihood ratio (chi-square = 2.13, p = 0.35). The test of goodness of fit showed that the data were adjusted to the models (p = 0.17). The final model used (reduced) showed that only the PRL was an independent factor associated with lupus activity (coefficient = 0.0252, 95% CI 0.0033–0.0470, p = 0.024) and the remaining variables were not associated with lupus activity. Similarly, the dose of prednisone remained significant, interpreted as a variable of appropriate classification of lupus activity.

Correlation between the titers of anti-PRL autoantibody and lupus activity. In patients with anti-PRL autoantibodies there was a negative correlation, albeit not significant, between the titers of anti-PRL autoantibodies expressed as RPRL-IgG and the SLEDAI score ($r_s = -0.16$, p = 0.59) (Figure 1).

Characterization of patients with anti-PRL autoantibodies. To characterize SLE patients with and without anti-PRL autoantibodies, a comparison was made among their demographic, laboratory, and clinical variables (Table 4). In patients with anti-PRL autoantibodies we found the following characteristics: serum PRL levels measured with RIA were significantly higher (33.2 ± 13.8 vs 11.6 ± 13.2

Table 2. Demographic and clinical variables in patients with inactive and active SLE.

Variables	Inactive, n = 149	Active, n = 110	p
Age, yrs, mean ± SD	37.3 ± 11.0	33.4 ± 10.7	0.005*
Women (%)	139 (93.3)	103 (93.6)	0.89‡
Disease duration, mo, median	103	63.5	0.0001§
Last menses prior to evaluation, days, median	26.5	20.0	0.14§
Category of treatment, mean ± SD	2.2 ± 1.1	2.6 ± 1.0	0.0004§
1 (%)	44 (29.5)	12 (10.9)	
2 (%)	67 (45.0)	47 (42.7)	0.003‖
3 (%)	5 (3.4)	26 (23.6)	
4 (%)	33 (22.1)	25 (22.7)	
Treatment with chloroquine (%)	36 (24.2)	31 (28.2)	0.56‡
Prednisone, mg/day, median	5.0	10.0	0.00001§
Creatinine clearance, ml/min/1.73 m^2, mean ± SD	89.3 ± 30.3	85.5 ± 32.5	0.33*
Prolactin, ng/ml, median	6.8	11.1	0.00001§
Hyperprolactinemia (%)	17 (11.4)	24 (21.8)	0.036‡
Associated conditions that increase prolactin (%)	23 (15.4)	15 (13.6)	0.82‡
Ratio of serum PRL bound to IgG, mean ± SD	0.62 ± 1.6	0.58 ± 1.6	0.83*
Anti-PRL autoantibody (%)	7 (4.7)	6 (5.5)	0.99‡

PRL: prolactin. *Non-paired Student's t test; ‡chi-square test; §Mann-Whitney U test; ‖ chi-square Mantel-Haenszel tendency linear test.

Table 3. Variables associated with lupus activity (logistic regression models).

Variable	Total Model Regression Coefficient	Total Model 95% CI	Total Model p	Final Model Regression Coefficient	Final Model 95% CI	Final Model p
Constant	–0.5416	–1.8318 to 0.7487	0.411	–0.6780	–1.7748 to 0.4188	0.226
Prolactin, ng/ml	0.0337	0.0064 to 0.0610	0.016	0.0252	0.0033 to 0.0470	0.024
Age, yrs	–0.0101	–0.0395 to 0.0194	0.504	–0.0087	–0.0380 to 0.0205	0.558
Disease duration, mo	–0.0028	–0.0065 to 0.0009	0.139	–0.0025	–0.0061 to 0.0011	0.172
Prednisone, mg/day	0.0593	0.0288 to 0.0898	0.0001	0.592	0.0321 to 0.0862	0.0001
Category of treatment	–0.0340	–0.3312 to 0.2632	0.823			
RPRL-IgG %	–0.1345	–0.3220 to 0.0529	0.160			

RPL-IgG: ratio of serum prolactin bound to IgG.

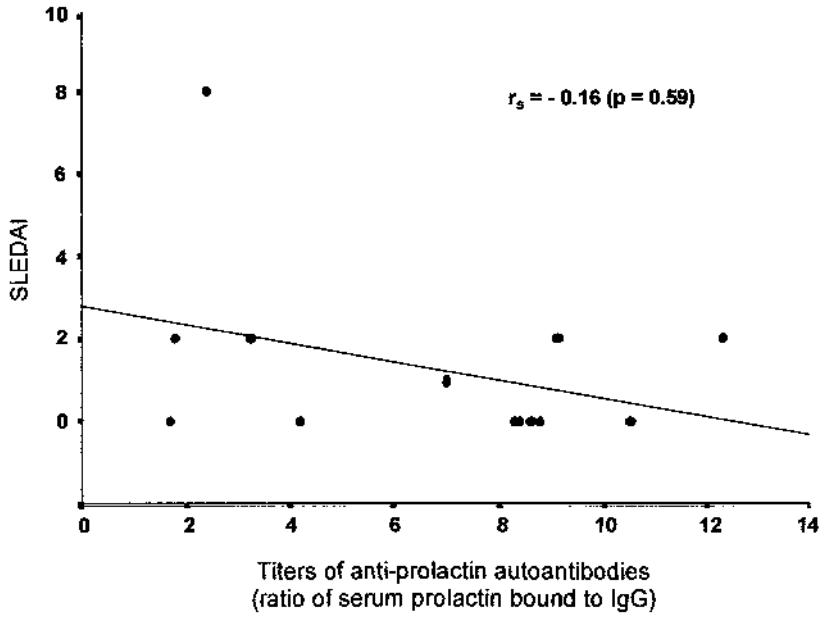

Figure 1. Relationship between titers of anti-PRL autoantibody and SLEDAI scores in 13 patients with SLE and anti-PRL autoantibodies.

ng/ml; p = 0.0001); in addition, all had HPRL (100% vs 11.4%; p = 0.00001) and serum globulin levels were higher (3.9 ± 0.92 vs 3.4 ± 0.72 g/dl; p = 0.03). Moreover, these patients were younger (median 29 vs 36 yrs; p = 0.031) and their disease was of shorter duration (median 48 vs 82 mo; p = 0.024). There were no significant differences in the remaining variables.

Effects of anti-PRL autoantibodies in PRL assays. In 9 sera from SLE patients with anti-PRL autoantibodies and 28 sera from hyperprolactinemic SLE patients without anti-PRL autoantibodies, we measured direct, total, and free PRL by RIA and IRMA. Comparison of both assays showed that in patients with anti-PRL autoantibodies, the measurements of direct PRL by RIA were substantially smaller than the measurements of total PRL by RIA. In contrast, the measurements of direct PRL by IRMA were similar to the measurements of total PRL by IRMA, in patients with and without anti-PRL autoantibodies. In the serum from patients with anti-PRL autoantibodies, the determination of free PRL was smaller than the determination of total PRL in both radiometric assays (Table 5). However, the correlation between measurements of direct PRL by RIA and IRMA was significant in patients with or without anti-PRL autoantibodies; this was less so for anti-PRL positive patients versus anti-PRL negative patients (r = 0.90, p = 0.0001 and r = 0.97, p = 0.0001, respectively) (Figure 2).

Additionally, to confirm that the suppressor effect in direct PRL measurement by double-antibody RIA was due to the anti-PRL, a pool of serum free of endogenous PRL was made up from 3 patients with anti-PRL autoantibodies and IgG was concentrated with ammonium sulfate at 33%. This IgG (0.5 mg/100 μl) was added to 100 μl of the stan-

Table 4. Demographic data and clinical and laboratory variables in anti-PRL negative and anti-PRL positive patients with SLE.

Variable	Anti-PRL Negative, n = 246	Anti-PRL Positive, n = 13	p
Age, yrs, median	36	29	0.031*
Women (%)	229 (93.1)	13 (100)	1.0**
Disease duration, mo, median	82	48	0.024*
Last menses prior to evaluation, days, median	23	21	0.49*
Category of treatment, median	2	2	0.79*
Prednisone mg/day, mean ± SD	13.1 ± 23.5	7.5 ± 5.5	0.39§
Creatinine clearance, ml/min/1.73 m^2, mean ± SD	87.9 ± 31.6	84.2 ± 24.2	0.67§
Prolactin ng/ml, mean ± SD	11.6 ± 13.2	33.2 ± 13.8	0.0001§
Hyperprolactinemia (%)	28 (11.4)	13 (100)	0.00001‡
Associated conditions that increase PRL (%)	36 (14.6)	2 (15.4)	1.0‡
RPRL-IgG, median	0.20	8.3	0.00001*
SLEDAI, median	0	0	0.62*
C3 mg/dl, mean ± SD	92.0 ± 31.1	99.8 ± 20.3	0.37§
C4 mg/dl, mean ± SD	18.8 ± 8.0	19.2 ± 5.3	0.85§
Anti-dsDNA IU/ml, mean ± SD	21.9 ± 30.9	27.8 ± 35.8	0.51§
Hemoglobin g/dl, mean ± SD	13.5 ± 2.0	12.5 ± 2.0	0.10§
Platelet × $10^{-4}/\mu$l, mean ± SD	23.6 ± 7.9	26.3 ± 10.3	0.24§
Leukocyte/μl, mean ± SD	5992 ± 2559	5784 ± 2423	0.78§
Lymphocyte/μl, mean ± SD	1509 ± 702	1729 ± 688	0.27§
Serum albumin g/dl, mean ± SD	3.8 ± 0.69	3.8 ± 0.45	0.86§
Serum globulin g/dl, mean ± SD	3.4 ± 0.72	3.9 ± 0.92	0.03§

RPRL-IgG: ratio of serum PRL bound to IgG; *Mann-Whitney U test; ‡Fisher's exact t test; §non-paired Student's t test.

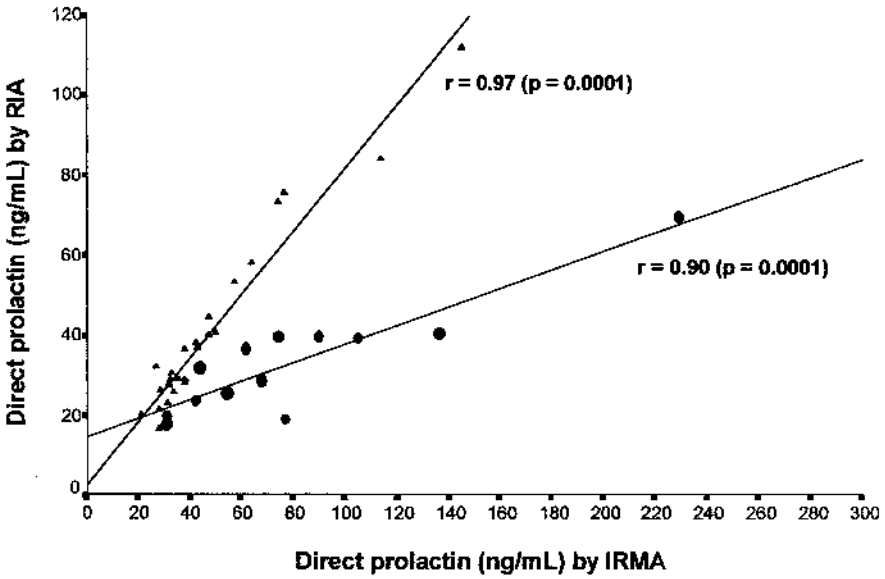

Figure 2. Relationship between direct PRL by RIA and IRMA in patients with anti-PRL autoantibody (●) and anti-PRL autoantibody-negative (▲).

dard PRL (53 ng/ml), and mean recovery was measured with RIA and IRMA (Figure 3). The IgG of the serum pool with anti-PRL was mixed at different concentrations with the standard PRL. The anti-PRL autoantibodies did not affect PRL values measured by IRMA. In contrast, PRL values measured by RIA decreased in a dose dependent manner when IgG from the serum pool was added; at 0.1 mg/100 μl of IgG concentration it had a recovery level of 89%, and at 0.5 mg/100 μl of IgG concentration a recovery level of 37%. Finally, to prove that the anti-PRL autoantibody is an IgG specific to PRL, and not IgG with PRL activity, we prepared serum free of endogenous PRL in 4

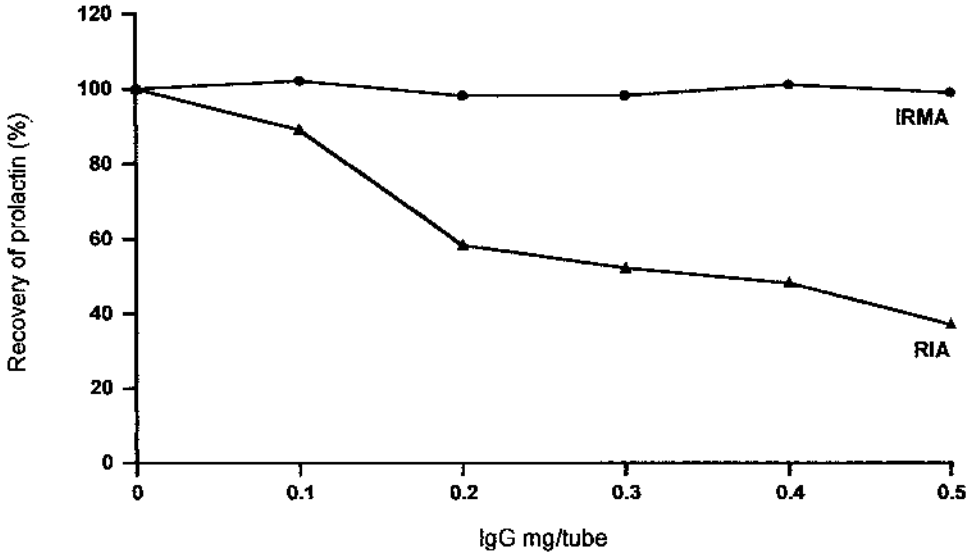

Figure 3. Effects of IgG purified from pool serum with anti-PRL autoantibody on the recovery of prolactin assayed by double antibody RIA and IRMA.

SLE patients with HPRL (3 with RPRL-IgG > 1.55% and one with normal RPRL-IgG). Figure 4 shows autoradiography after incubation of different concentrations of IgG (25, 50, and 100 μg) on nitrocellulose strip paper with ^{125}I-hPRL. Autoradiography revealed that in IgG from patients with RPRL-IgG higher than 1.55%, the IgG bound to radiolabeled hPRL, while this was not the case with IgG from a patient with normal RPRL-IgG.

DISCUSSION

High serum prolactin levels and even hyperprolactinemia are a common finding in patients with SLE, yet the cause is uncertain. We found HPRL in 15.8% of patients with SLE, similar to the frequency in previous studies[10,12,14]; this frequency was similar between women and men.

We determined that the frequency of anti-PRL autoantibodies in all patients studied was 5%. Interestingly, all patients with anti-PRL autoantibodies had HPRL; these data support previous findings of our group, i.e., that the anti-PRL autoantibodies can also be a cause of HPRL, originating a form of secondary HPRL.

There are 2 possible mechanisms by which autoantibodies could raise serum PRL. First, free PRL is readily filtered by the glomerulus. In contrast, the PRL-autoantibody complex (forming a macromolecule) escapes degradation in the kidney, an important means of PRL catabolism[26,27]. In the same fashion, Hattori showed by clearance studies in rats that the PRL-antibody complex is eliminated more slowly from the bloodstream than free PRL[28]. Second, there is an autoregulatory system between anterior pituitary, hypothalamus, and serum that controls PRL secretion. We propose that the anti-PRL autoantibody could block feedback mechanisms in the system, resulting in

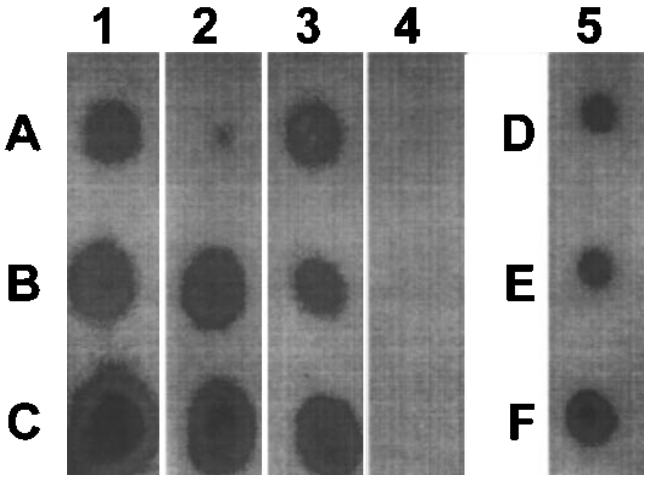

Figure 4. Autoradiography after incubating ^{125}I-hPRL on strip nitrocellulose paper with IgG purified from serum-free PRL with or without anti-PRL autoantibody. Lane 1 to 3: serum from anti-PRL positive patients; Lane 4: serum from one anti-PRL negative patient; Lane 5: polyclonal rabbit antiserum to HPRL (Diagnostic Products Corp., Los Angeles, CA, USA). Concentrations of purified IgG applied on strip nitrocellulose paper (μg): (A) 25, (B) 50, (C) 100. Amount of commercial polyclonal rabbit antiserum to HPRL (μl): (D) 5, (E) 10, (F) 20.

presentation of a false low level of serum PRL to the hypothalamus and pituitary; this point may also be relevant in the presentation of active PRL to receptors in lymphocytes.

The factors that cause production of anti-PRL autoantibody are not understood. However, while genetic and environmental factors could account for the antibody, it must also be considered that changes in the structure of PRL could increase its antigenicity.

Our results, as well as those of Hattori and colleagues[21],

Table 5. Comparison in measurements of direct PRL and its fractions by 2 radiometric assays (RIA and IRMA) in serum from SLE patients with hyperprolactinemia according to the presence of anti-PRL autoantibodies. Data expressed as mean ± SD of the percentage between assay techniques.

	Anti-PRL Negative, n = 28	Anti-PRL Positive, n = 9	p
Direct by RIA/total by RIA	106.4 ± 12.5	64.3 ± 28.1	0.0003*
Direct by IRMA/total by IRMA	120.6 ± 17.7	116.2 ± 23.3	0.55‡
Free by RIA/total by RIA	93.5 ± 11.6	11.7 ± 12.0	0.0001‡
Free by IRMA/total by IRMA	192.2 ± 15.8	24.6 ± 12.5	0.0001‡
Total by RIA/total by IRMA	94.3 ± 9.6	90.0 ± 14.7	0.31‡

*Mann-Whitney U test; ‡non-paired Student's t test.

show that the presence of autoantibodies to PRL could interfere with the real values of PRL when the technique used is double antibody RIA. In contrast, the IRMA technique appears to be more specific and reliable. Our explanation of this phenomenon is similar to that discussed by other researchers. PRL is a polypeptide having several antigenic sites and therefore may be accessible both to the endogenous autoantibody and to the reagent antibody. The autoantibody does not displace the binding of ^{125}I-hPRL to the reagent antibody, but binds to reagent antibody bound and free PRL. Because the second antibody may have a cross reaction with human Ig and/or due to the presence of polyethylene glycol with which the second antibody is mixed, a larger amount of tracer PRL is precipitated, causing artificially low results[21]. This phenomenon has also been described in RIA to determine thyroglobulin, a macromolecule with several antigenic sites, in the presence of antithyroglobulin autoantibodies[29]. In the IRMA technique, in which radiolabeled antibodies associate with the solid phase antibody by forming a complex with PRL as the intermediate, spurious results did not result from the presence of anti-PRL autoantibodies. The reason for non-interference is that PRL may retain full immunoreactivity even when bound to the anti-PRL autoantibody because of the different recognition sites (epitopes).

Clinically, there was no relationship between lupus activity and anti-PRL autoantibodies. However, the frequency of anti-PRL autoantibodies in patients with active disease was 5.5%, only 0.8% higher than in patients with non-active disease (chi-square, p = 0.99). Nonetheless, the statistical power of the study showing whether the difference is significant was very low (power of 4.3%) with the risk of type II or ß-type error (i.e., we could have concluded there were no differences when differences did exist). In the same fashion, although there was a negative correlation between SLEDAI scores and anti-PRL titers, there was no statistical significance. We did not observe clinical significance of anti-PRL autoantibodies in lupus activity. However, in our previous study of the same SLE patients that included only those with idiopathic HPRL (to control the effects of other causes for HPRL) we found that anti-PRL positive patients (whose predominant circulating form is big big PRL, due to PRL-IgG complex) had less serologic and clinical disease activity than anti-PRL negative patients (whose predominant circulating form is little PRL)[18]. In the same fashion, several studies have reported a small number of patients with asymptomatic HPRL due to the presence of anti-PRL autoantibodies[30–32]. Moreover, Hattori found *in vitro* that the PRL-IgG complex had a complete biological action on PRL dependent Nb2 lymphoma cell assay[28]. In contrast, *in vivo* the PRL-IgG complex may not exert sufficient action because it does not easily cross the capillary walls due to its high molecular weight; these data suggest that PRL has attenuated biological activity when it is bound to its autoantibody.

On the other hand, the only factor that maintained significant independence in relation to lupus activity in analysis of logistic regression was a high level of serum PRL, even after including the presence of anti-PRL autoantibodies in the analysis. Although this study showed that PRL is associated with disease activity in patients with SLE, our study has the limitations inherent in a cross sectional design (temporal ambiguity). We therefore did not intend to establish a causal relationship in the sequence of events. However, other evidence supports a temporal causal relationship between serum PRL levels and disease activity: in the NZB/W mouse model[5], series of patients with HPRL later developed SLE[33]; and clinical trials have used bromocriptine to inhibit the secretion of PRL by pituitary gland in patients with SLE, improving their clinical and serologic course[34,35]. All these data suggest that PRL plays an important pathogenic role in disease activity and is not merely an epiphenomenon.

Taken together, the data suggest that both serum PRL level and the detection of individual and/or combinations of PRL isoforms in sera of patients with SLE might prove of value for understanding the clinical or pathologic relevance of PRL in autoimmune diseases. Their biological role is currently under investigation in our laboratory. Also, all these data may explain why some clinical studies failed to find an association between serum PRL levels and disease activity in SLE patients.

We confirmed that the presence of anti-PRL autoanti-

bodies was associated with a hyperprolactinemic state. Elevated serum PRL levels were associated with disease activity independently of other variables. Finally, we found that the presence of anti-PRL autoantibodies interferes with measuring PRL by RIA and showed that the measured levels of this hormone are lower than the real values. In contrast, the IRMA assay led to more reliable results. All these findings should be taken into account in further studies of the relationship between PRL and SLE.

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