Endocrine, *vol. 19, no. 2, 139–146, November 2002* 0969–711X/02/19:139–146/$20.00

Antiprolactin Autoantibodies Are Associated with Hyperprolactinemic Status in Men Infected with Human Immunodeficiency Virus

Alfredo Leaños-Miranda[1] and Iris Contreras-Hernández[2]

[1]Hospital General de Zona 1-A Los Venados and [2]Hospital General de Zona 30 Iztacalco, Instituto Mexicano del Seguro Social, México, D.F., México

High serum prolactin (PRL) levels and even hyperprolactinemia are a common finding in human immunodeficiency virus (HIV) infection. However, little is known regarding the mechanisms that may contribute to the rise of PRL. We measured serum PRL levels in 54 HIV-infected and 85 healthy age-matched men. The association between PRL levels among anti-PRL autoantibodies and other clinical variables in HIV-infected men was studied. We also evaluated the changes in serum PRL levels by chromatographic separation (affinity with protein G and gel filtration) after a 10-mg iv bolus of metoclopramide. HIV-infected men had higher serum PRL levels compared with healthy men. Sera from 9 of the 54 (16.7%)HIV-infected men were found to have hyperprolactinemia. Moreover, the anti-PRL autoantibody was present in four of nine (44.4%)HIV-infected men with hyperprolactinemia; it was also associated with hyperprolactinemic status. Serum total PRL levels were higher in HIV-infected men with anti-PRL autoantibodies than hyperprolactinemic HIV-infected men without anti-PRL autoantibodies; by contrast, free PRL levels were lower. In HIV-infected men with anti-PRL autoantibodies, gel filtration showed that big big PRL isoform was present as the predominant circulating form of PRL throughout each measurement after iv metoclopramide. By contrast, the predominant isoform of PRL in serum from healthy men and HIV-infected men who were anti-PRL autoantibody negative was little PRL. On the other hand, high serum total PRL levels were observed at each measurement throughout the metoclopramide test in HIV-infected men with anti-PRL autoantibodies; however, the serum free PRL levels were similar to those found in subjects without anti-PRL autoantibodies. These data demonstrated that anti-PRL autoantibodies are associated with hyperprolactinemic status in HIV-infected subjects, particularly in those with high serum PRL levels.

Received July 30, 2002; Revised August 19, 2002; Accepted August 19, 2002.

Author to whom all correspondence and reprint requests should be addressed: Alfredo Leaños-Miranda, Don Luis #111, Col. Nativitas, CP 03500, México, D.F., México. E-mail: alfredo@intranet.com.mx

Key Words: Human immunodeficiency virus; prolactin; hyperprolactinemia; macroprolactinemia; antiprolactin autoantibody.

Introduction

Many endocrine changes in nearly every hormone have been reported during the course of human immunodeficiency virus (HIV) infection. These changes may be a direct effect of HIV or may be indirectly mediated by opportunistic infections or tumors, or related to drugs used in the treatment of HIV and/or its complications.

Serum prolactin (PRL) levels in HIV-infected patients have been reported to be normal *(1,2)* or higher than in healthy men *(3–5)*. However, the frequency of hyperprolactinemia reported in HIV-infected patients ranges from 7.1% in asymptomatic subjects to 55.5% in acquired immunodeficiency syndrome (AIDS) *(4,5)*. Although the etiology for the increment in PRL concentration and even hyperprolactinemia in HIV infection is poorly understood, there are many contributing factors, including adverse reaction to antiretroviral drugs, opportunistic infections, tumors infiltrating the pituitary gland, and other coexisting medical conditions *(6–8)*.

There are no data on whether autoimmunity may contribute to HIV-related hyperprolactinemia. However, abnormal B-cell function is a feature of HIV infection *(9)* and non-HIV-specific antibodies, including autoantibodies, also appear in HIV-infected patients, but their clinical significance is unclear *(10–12)*. On the other hand, HIV infection and systemic lupus erythematosus (SLE) have a large number of clinical and laboratory features in common *(13,14)*. Additionally, elevated serum PRL levels have been reported consistently in SLE patients, and hyperprolactinemia has occurred in approx 15–31% *(15)*. We recently reported anti-PRL autoantibodies in sera from 40.7% of SLE patients with idiopathic hyperprolactinemia *(16,17)*, and similar cases have been reported in patients without autoimmune disorders *(18–21)*. It has been shown that a significant positive correlation exists between the titers of the anti-PRL autoantibodies and serum PRL levels; the presence of these anti-PRL autoantibodies is associated with hyperprolactinemic status *(16,17,22)*.

The fact that SLE and HIV-infected patients share some clinical and autoimmune features led us to suspect the presence of an anti-PRL autoantibody in HIV-infected men. Autoantibodies against PRL have not been studied previously in HIV-infected men.

The aim of the present work was to determine the frequency of hyperprolactinemia in HIV-infected men, to investigate whether the presence of anti-PRL autoantibodies is involved in its etiology, as well as to characterize HIV-infected men with anti-PRL autoantibodies.

Results

A total of 54 HIV-infected subjects in different stages of disease were studied (23 HIV-infected men without AIDS and 31 men with AIDS). The median serum direct or total PRL of the HIV-infected patients without AIDS was 11.9 μg/L (range: 4.1–148.3; $p = 0.001$ when compared to median healthy men [7.4 μg/L]). The median of AIDS patients was 12.1 μg/L (range: 4.8–169.1; $p = 0.001$ when compared to healthy men). The median in serum total PRL levels between HIV-infected men without AIDS and with AIDS was not statistically significant. Hyperprolactinemia was present in 9 of the 54 studied patients (16.7%; 95% confidence interval [CI]: 6.8–26.6%). The frequency of hyperprolactinemia in HIV-infected patients without AIDS was 21.7% (95% CI: 4.9–38.5%) and in men with AIDS, 12.9% (95% CI: 1.1–24.7%). No significant difference was found in frequency of hyperprolactinemia between HIV-infected patients without and with AIDS ($p = 0.62$). None of the hyperprolactinemic patients had evidence of pituitary adenoma on computed tomography (CT). In a similar fashion, median serum free PRL was significantly higher in HIV-infected patients (both without AIDS [9.0 mg/L, range: 2.4–43.2] and with AIDS [9.5 μg/L, range: 3.0–24.1]) than in healthy men (7.0 μg/L, range: 0.9–16.1; $p \leq 0.001$). When comparing total and free PRL concentrations between patients treated and not treated with highly active antiretroviral therapy, we did not find significant differences ($p \geq 0.29$).

In 4 of 54 sera, a significant amount of immunoreactive PRL was retained on the protein G-Sepharose column (65.4 ± 6.9%). In the 50 remaining sera, nearly all immunoreactive PRL passed through the protein G–Sepharose column (0.4 ± 0.8%, range: 0.0–3.1%). Subsequently, anti-PRL autoantibodies were detected in 4 of the 54 HIV-infected men (7.4%; 95% CI: 0.4–14.4%). By contrast, among 9 patients with hyperprolactinemia, 4 had anti-PRL autoantibodies (44.4%; 95% CI: 12.0–76.8%) compared with 0 of 45 patients without hyperprolactinemia (odds ratio: 38.3; 95% CI: 3.2–1849; $p = 0.0005$). Moreover, HIV-infected men with anti-PRL autoantibodies had significant higher serum total PRL levels than hyperprolactinemic HIV-infected men who were anti-PRL autoantibody negative (123.4 ± 60.6 vs 32.9 ± 14.6 μg/L; $p = 0.014$), whereas free PRL levels were lower (median of 8.6 and range of 7.6–9.5 vs median of 24.1 and range of 13.4–43.2 μg/L; $p = 0.016$) (Fig. 1).

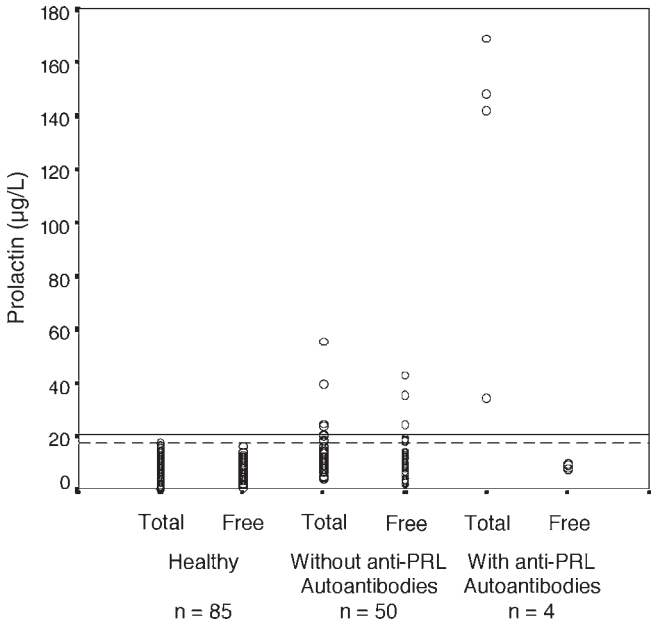

Fig. 1. Serum total and free PRL levels in healthy men and HIV-infected men with and without anti-PRL autoantibodies. The solid horizontal line indicates the mean ± 3 SD of total PRL levels from total healthy men; sera with a value over this cutoff were considered to have hyperprolactinemia. The dashed horizontal line denotes the mean ± 3 SD of free PRL levels from total healthy men.

Gel filtration profiles of immunoreactive PRL in the sera of four HIV-infected men (three homosexual, two without AIDS and one with AIDS; and one heterosexual without AIDS) showed the presence of big big PRL (≥100 kDa) as predominant circulating isoform of PRL in all cases (72.3 ± 7.7%). By contrast, nearly all PRL immunoreactivity was eluted at the position of little PRL (23 kDa) in 6 healthy men (74.9 ± 4.7%) and in 19 HIV-infected men without anti-PRL autoantibodies (78.9 ± 8.6%).

Serum PRL responses to iv metoclopramide in 6 healthy men, 11 HIV-infected men who were anti-PRL autoantibody negative, and 4 HIV-infected men who were anti-PRL autoantibody positive are shown in Fig. 2. All three groups had an increase in serum total PRL and free PRL levels in response to metoclopramide. Increments in serum total PRL levels were statistically significant in both healthy men and HIV-infected men without anti-PRL autoantibodies ($p \leq 0.03$ vs basal), but not in HIV-infected men with anti-PRL autoantibodies ($p = 0.07$ vs basal). Additionally, HIV-infected men with anti-PRL autoantibodies had significantly higher serum total PRL levels than healthy men and HIV-infected men without anti-PRL autoantibodies at any given sampling time throughout the test ($p \leq 0.001$). By contrast, increments in serum total PRL levels were similar between healthy men and HIV-infected men with-

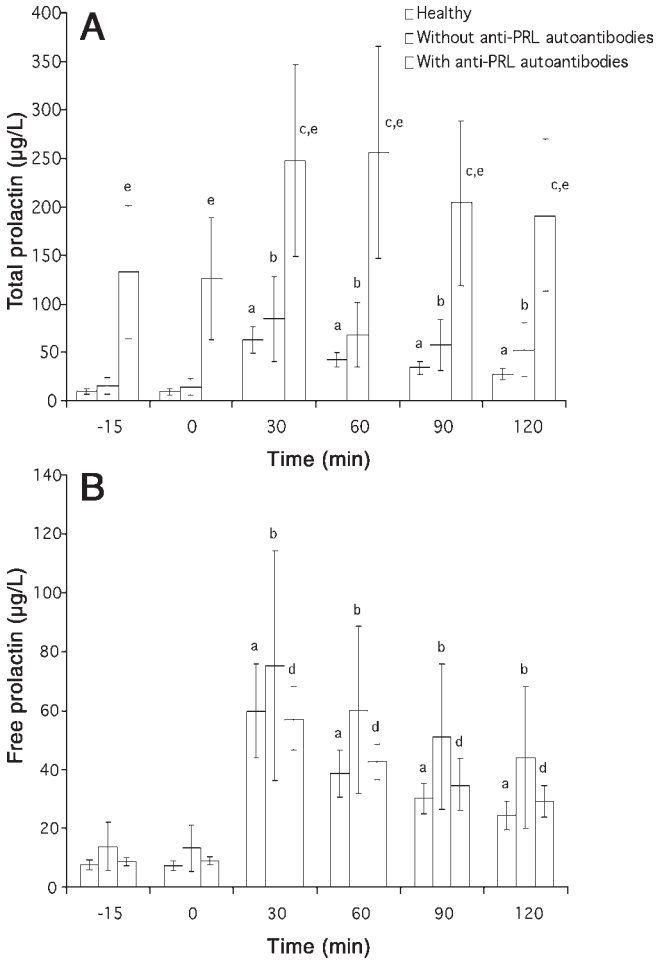

Fig. 2. Response of a 10-mg iv bolus of metoclopramide on serum total PRL levels (**A**) and on serum free PRL levels (**B**) in 6 healthy men, in 11 HIV-infected men without anti-PRL autoantibodies, and in 4 HIV-infected men with anti-PRL autoantibodies. Each bar represents the mean ± SD. $^{a}p \leq 0.001$, $^{b}p = 0.03$, $^{c}p = 0.07$, $^{d}p \leq 0.01$vs basal; $^{e}p \leq 0.001$ vs healthy men and HIV-infected men without anti-PRL autoantibodies.

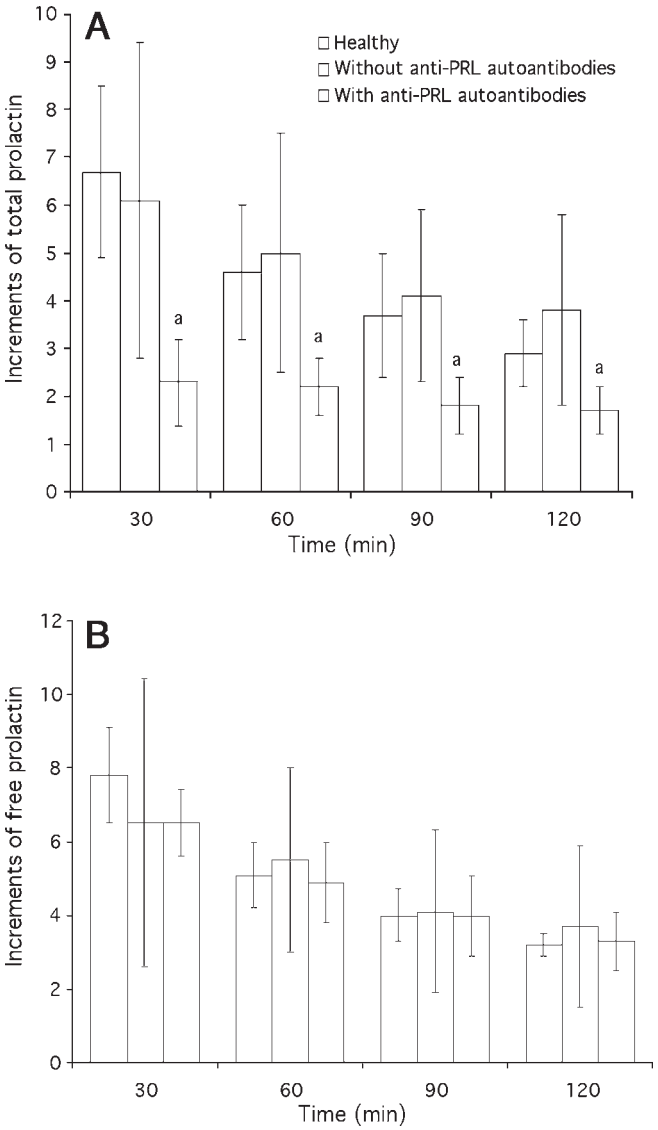

Fig. 3. Fold increases in serum total PRL (**A**) and free PRL (**B**) in comparison to basal value in response to a 10-mg iv bolus of metoclopramide. Each bar represents the mean ± SD. $^{a}p \leq 0.018$ vs healthy men and HIV-infected men without anti-PRL autoantibodies.

out anti-PRL autoantibodies at each sampling time ($p \geq 0.45$). Likewise, increments in serum free PRL levels were statistically significant in all groups ($p \leq 0.03$ vs basal). However, there was no difference in serum free PRL levels among the three groups at each sampling time ($p \geq 0.12$).

We also analyzed the fold increases in total and free PRL in comparison with its basal value in response to metoclopramide (Fig. 3). In HIV-infected men with anti-PRL autoantibodies, fold increases in total PRL during the test were significantly lower than in healthy men and HIV-infected men without anti-PRL autoantibodies ($p \leq 0.018$). By contrast, free PRL fold increases were not significantly different among the three groups ($p \geq 0.15$).

Figures 4 and 5 show the distribution of immunoreactive PRL by gel filtration and affinity chromatography after iv metoclopramide. The proportion of big big PRL in HIV-infected men who were anti-PRL autoantibody positive was significantly higher than in healthy men and HIV-infected men without anti-PRL autoantibodies at any given sampling time throughout the test ($p \leq 0.01$) (Fig. 4A); by contrast, the proportion of little PRL was significantly lower ($p \leq 0.01$) (Fig. 4C). On the other hand, in HIV-infected men who were anti-PRL autoantibody positive at 30 and 120 min, the proportions of big big PRL were considerably lower than at the basal value ($p \leq 0.002$); by contrast, the proportions of little PRL increased at 30 and 120 min after administration of metoclopramide ($p \leq 0.03$). There was no difference in the proportion of PRL isoforms between healthy men and HIV-infected men who were anti-PRL autoantibody negative, and these proportions did not change significantly

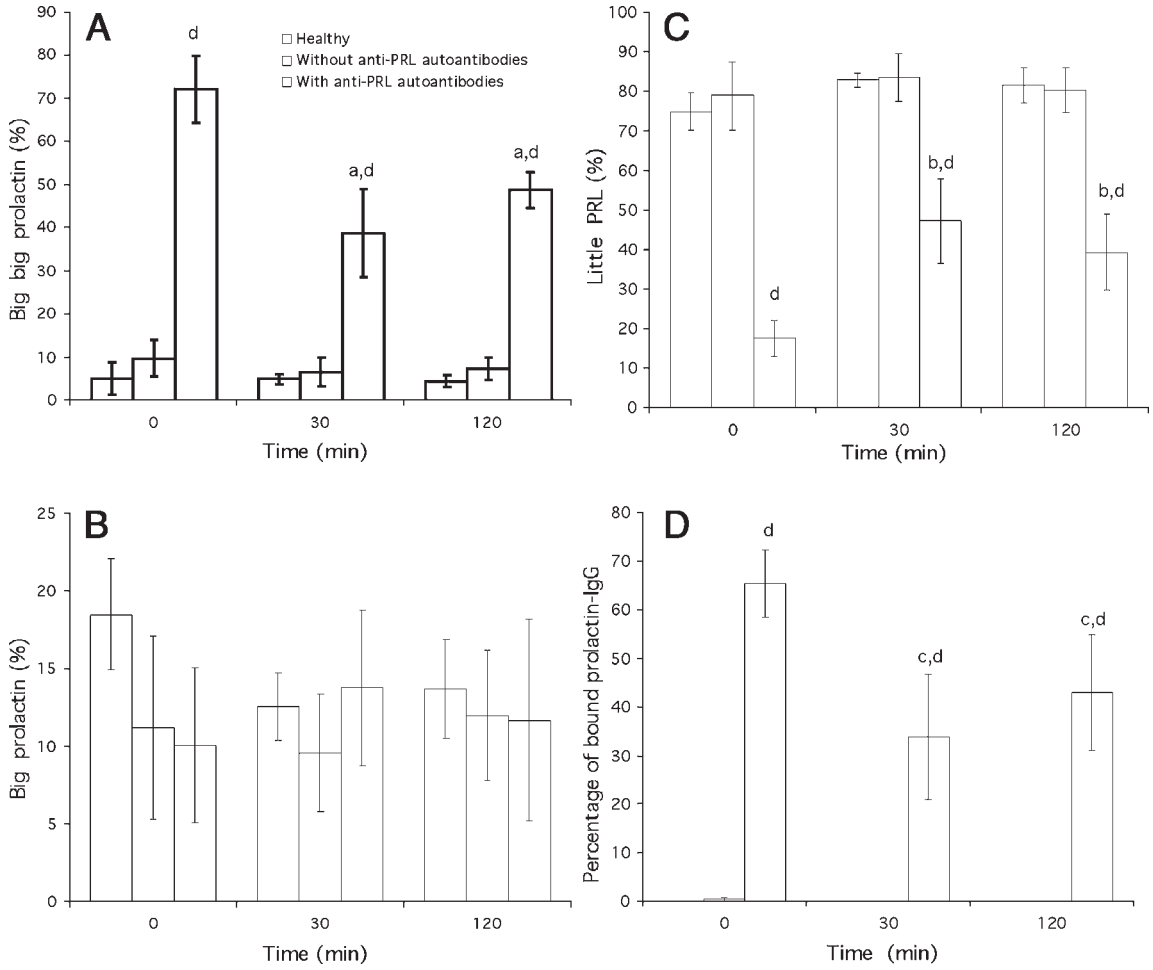

Fig. 4. (**A–C**). Changes in distribution of PRL immunoreactivity in three fractions after gel filtration chromatography in serum from 6 healthy men, 11 HIV-infected men without anti-PRL autoantibodies, and 4 HIV-infected men with anti-PRL autoantibodies after administration of a 10-mg iv bolus of metoclopramide on a Sephadex G-100 column (56 × 1 cm). Samples of 1.5 mL were applied in the column, and 1.3-mL fractions were collected; subsequently, the proportion of each isoform of PRL was determined. (**D**) PRL isoforms include little PRL (20–26 kDa), big PRL (45–50 kDa), and big big PRL (≥100 kDa) (**D**) Changes in percentage of bound PRL-IgG by affinity chromatography on a G-protein Sepharose column (1 mL). Samples (0.2–0.5 mL) were applied in the column and 1-mL fractions were collected. Each bar represents the mean ± SD. $^{a}p \leq 0.002$, $^{b}p \leq 0.03$, $^{c}p \leq 0.007$ vs. basal; $^{d}p \leq 0.01$ vs. healthy men and HIV-infected men without anti-PRL autoantibodies.

after administration of metoclopramide ($p \geq 0.09$) (Fig. 5). Figure 4D shows the percentage of bound PRL-IgG after administration of metoclopramide; there was a decrease in the percentage of bound PRL-IgG at 30 and 120 min ($p \leq 0.01$). By contrast, in healthy men and HIV-infected men the percentage remained the same.

Finally, when comparing the clinical variables between the 4 HIV-infected men with anti-PRL autoantibodies and the 50 HIV-infected men without autoantibodies, there was no difference related to age, CD4, CD8, or viral load ($p > 0.05$). Additionally, we did not find a correlation between serum total or free PRL and CD4, CD8, and viral load ($p \geq 0.17$).

Discussion

The presence of high serum PRL levels and even of hyperprolactinemia is a common finding in HIV-infected men, yet the cause is uncertain. We found higher serum PRL levels in HIV-infected men than in healthy men, and frequency of hyperprolactinemia in these patients was 16.7%; similar findings were found in previous studies *(3–5)*.

In the present study, we demonstrated that the frequency of anti-PRL autoantibodies in the total HIV-infected men studied was 7.4%. We observed a stronger association between the presence of anti-PRL autoantibodies and hyperprolactinemic status, whereas all HIV-infected men with anti-PRL autoantibodies had hyperprolactinemia. We found that in hyperprolactinemic HIV-infected men, the frequency of anti-PRL autoantibodies was high (44.4%). These results are similar to those found by our group in SLE patients *(16)*. These data support the idea that anti-PRL autoantibodies can also be a cause of hyperprolactinemia, originating a form of secondary hyperprolactinemia.

In serum from healthy subjects and most patients with hyperprolactinemia, the majority of PRL immunoreactiv-

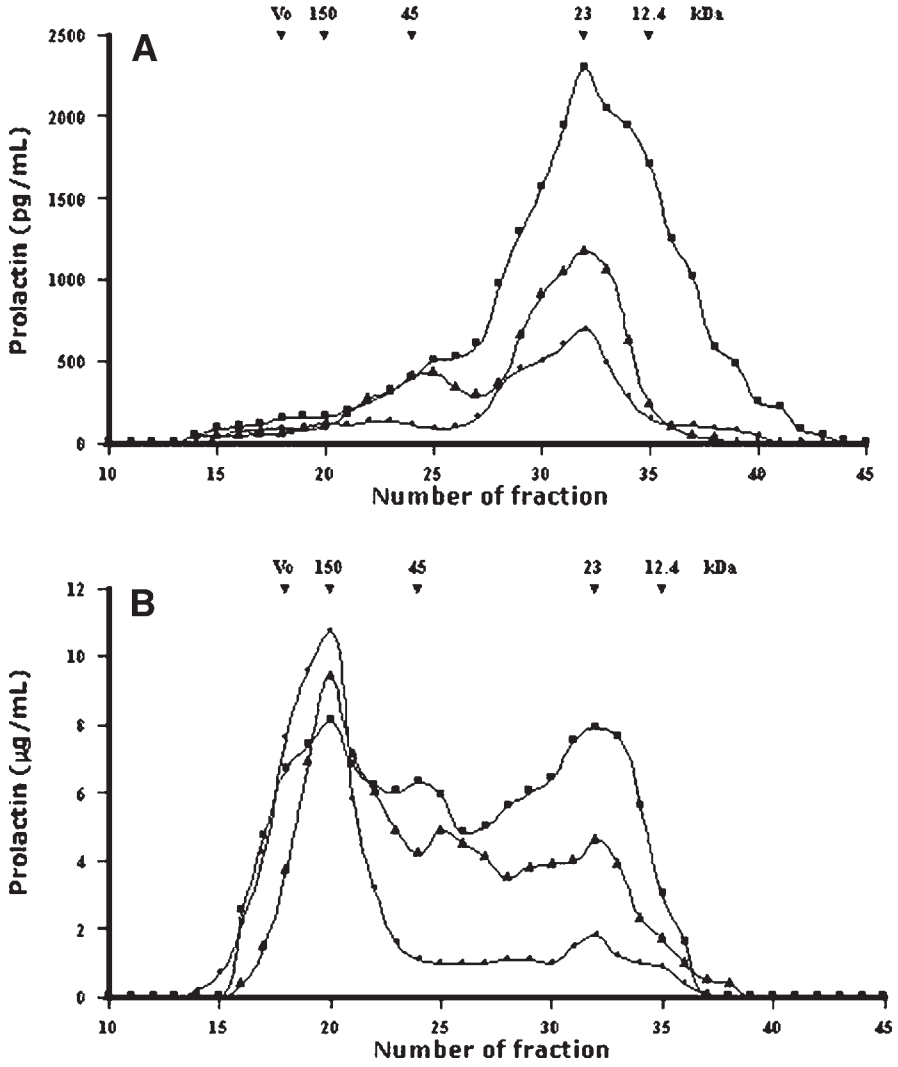

Fig. 5. Representative gel filtration profiles of immunoreactive PRL in serum after administration of a 10-mg iv bolus of metoclopramide on a Sephadex G-100 column (56 × 1 cm). (**A**) Sera from one HIV-infected man with normoprolactinemia whose test was negative for anti-PRL autoantibody; (**B**) sera from one HIV-infected man with anti-PRL autoantibody. Samples (0.5–1 mL) were applied on the column, and 1.3-mL fractions were collected (at basal time, ●; 30 min, ■; and 120 min, ▲).

ity is found by gel filtration chromatography to have an apparent mol wt of 23 kDa (little PRL). Lesser amounts are found in the void volume (big big PRL, mol wt of ≥100 kDa) or in a position corresponding to a mol wt of 45–50 kDa (big PRL). Here we demonstrated that nearly all PRL in sera from men who were anti-PRL autoantibody positive eluted in the fraction with the mol wt of IgG (150 kDa). The predominant presence of big big PRL in the bloodstream is a phenomenon termed *macroprolactinemia (23)*. Interestingly, in HIV-infected men with anti-PRL autoantibodies, gel filtration profiles of PRL in response to metoclopramide showed that the relative proportion of little PRL increased dramatically. This finding could be explained because the release of little PRL (free PRL or monomeric PRL) from the pituitary gland is increased in response to metoclopramide, and part of little PRL binds to anti-PRL autoantibody, leading to an increase in big big PRL. Thus, this PRL isoform may not exert feedback action on pituitary, because it does not gain access to the hypothalamus through the capillary wall owing its high molecular weight. Therefore, an additional release of little PRL by the pituitary gland is required in order to exceed the binding capacity of the autoantibody until little PRL reaches its physiologic level to exert feedback action on the hypothalamic-pituitary axis. This explanation can be supported by the results obtained in the measurements of free PRL after iv metoclopramide, whereas the serum free PRL levels or their increments in comparison to basal value were similar among healthy and HIV-infected men (with or without anti-PRL autoantibodies).

On the other hand, the decrease in serum free PRL after 30 min was statistically significant and similar among the three groups. Likewise, the decrease in serum total PRL was statistically significant in both healthy men and HIV-infected men without anti-PRL autoantibodies (whose pre-

dominant circulating isoform is little PRL), but not in HIV-infected men (whose predominant circulating isoform is big big PRL). These data suggest that the PRL-IgG complex was cleared from the circulation more slowly than free PRL. We previously demonstrated by clearance studies in rats that the PRL-IgG complex is eliminated more slowly from the bloodstream than serum containing only monomeric PRL *(24,25)*, postulating that this could be an additional mechanism in which PRL is retained in circulation for a long time, contributing to hyperprolactinemic status.

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 or might be a response to increased secretion of PRL by lymphocytes and/or the pituitary gland in an immunologically distorted milieu.

Although our study showed that anti-PRL autoantibodies are associated with hyperprolactinemic status in HIV-infected men, it has the limitation inherent in a cross-sectional design (e.g., temporal ambiguity); we therefore did not intend to establish a causal relationship between the presence of anti-PRL autoantibodies and hyperprolactinemic status. However, big big PRL can be made in vitro by mixing 23-kDa PRL with serum (depleted of endogenous PRL after treatment with bromocriptine) from an SLE woman with anti-PRL autoantibodies *(24)*. However, the establishment of a temporal relationship between anti-PRL autoantibodies and hyperprolactinemia must be proven clearly in follow-up studies.

Although our results suggest that the anti-PRL autoantibody itself is the cause of hyperprolactinemia in the majority of HIV-infected men, mainly in those with very high serum PRL levels without secondary causes of hyperprolactinemia, the mechanisms to explain the rise in serum PRL levels in the remaining HIV infection are still unclear. In this regard, Montero et al. *(5)* found that serum PRL levels and frequency of hyperprolactinemia were higher in patients with concomitant infections than in symptom-free patients. However, these findings cannot be supported by us to explain the high serum PRL levels, because none of our patients had concomitant infections on the day of the evaluation. On the other hand, hyperprolactinemia has been associated in patients under treatment with protease inhibitors *(6)*, but this should be considered with caution because four of four reported patients also took drugs related to the increase in serum PRL level. Our results did not show differences in serum PRL levels and use of protease inhibitor–based anti-retroviral treatment; Montero et al. *(5)* found similar results. One possible explanation for the rise in serum PRL levels in HIV-infected patients is from an extrapituitary source. This possibility arises from the following facts: First, there is the presence of the receptor for PRL and production of PRL by human peripheral blood mononuclear cells (PBMCs) and human T- and B-cell lines *(26–28)*. Second, both non-stimulated and stimulated PBMCs from SLE patients (similar to HIV-infected subjects, they have chronic pathologic immune stimulation) have an increased production of PRL *(29)*. Third, high serum PRL levels have been observed in patients with acute myeloid leukemia, and it was suggested that the elevated serum PRL may be owing to release of PRL from the leukemia blast cells, which were shown to contain PRL *(30)*.

However, to date nothing is known concerning the synthesis and secretion of PRL by lymphoid cells during the course of HIV infection; nonetheless, it is possible that in HIV-infected subjects, for some unknown reason, the expression and secretion of PRL by lymphocytes might be abnormally increased, which in turn causes an increase in the serum PRL. Further studies are needed to investigate the synthesis and secretion of PRL by lymphocytes from HIV-infected subjects; this is currently under investigation by our group.

Interestingly, our studied HIV-infected men with anti-PRL autoantibodies lacked the clinical symptoms of hyperprolactinemia, despite having extremely high serum PRL levels (three had serum PRL levels >100 μg/L: 148.3, 169.1, and 142.0 μg/L), such as impotence and/or galactorrhea, and their pituitary CTs were normal. In this regard, we previously demonstrated that the PRL-IgG complex from SLE patients was fully active in vitro; we concluded that the absence of hyperprolactinemia symptoms can be explained by the large molecular size of the complex, which impedes PRL from easily passing through the capillary walls to reach their target tissues *(30)*. In addition, this conclusion can be supported by the fact that concentrations of serum free PRL (or little PRL, which is the PRL isoform associated with clinical symptoms of hyperprolactinemia) in HIV-infected men who were anti-PRL autoantibody positive were similar to those found in normoprolactinemic subjects (whose predominant circulating isoform is little PRL).

Conversely, a recent report by Parra et al. *(31)* on a small group of HIV-positive men suggested the existence of clearly diminished hypothalamic dopaminergic tone. However, our findings argue against this conclusion: the results of our study support the existence of normal dopaminergic hypothalamic tone in HIV-infected men in the setting of similar response of pituitary free PRL to iv metoclopramide. When we measured both the serum free PRL levels and their fold increases in comparison to basal value in response to metoclopramide, we found no differences among the groups of men studied; this reinforces the idea that concentration of serum free PRL (or little PRL) is the PRL isoform most important to exert feedback action on the hypothalamic-pituitary axis. By contrast, Parra et al. *(31)* did not take into account the presence of anti-PRL autoantibodies, measurements of free PRL levels, and the different isoforms of PRL in their study. Therefore, we propose that free PRL levels are the best parameter to evaluate dopaminergic tone.

Finally, we did not uncover the clinical significance of serum PRL levels in HIV-infected men; however, this could be owing in part to a reduced number of studied patients. Further studies on the clinical significance of increases in

serum PRL and their isoforms during the course of HIV infection are needed.

In conclusion, we demonstrated that anti-PRL autoantibodies are associated with hyperprolactinemic status in HIV-infected men. The presence of anti-PRL autoantibodies or serum PRL levels was not associated with the stage of disease, CD4 and CD8 cell counts, or viral load.

Materials and Methods

Subjects

The local human ethical committee and medical research of the Iztacalco General Hospital No. 30 of the Instituto Mexicano del Seguro Social approved the study protocol, and informed written consent was obtained from all subjects who participated voluntarily.

A group of 54 consecutive HIV-infected men was studied, with ages ranging from 23 to 70 yr (mean ± SD: 37.1 ± 9.1 yr) and duration of HIV infection from 1 to 119 mo (mean ± SD: 36.6 ± 27.9 mo). HIV infection was demonstrated by seroreactivity (enzyme-linked immunosorbent assay [ELISA]) and confirmed by Western blot. No patient had evidence of autoimmune or endocrine disorders, nor of taking drugs or having any concomitant disease known to increase serum PRL levels. These patients were seen at the AIDS Clinic at Iztacalco General Hospital No. 30 of the Instituto Mexicano del Seguro Social from January to March 2001. Clinical and demographic data were recorded. HIV infection was classified with or without AIDS according to the 1993 revised Centers for Disease Control classification *(32)*. A venous blood sample was drawn between 7:00 and 8:00 AM, after an overnight fast. The sera were separated, aliquoted, and stored at –35°C until used. As a control, we used 85 healthy men blood donors (within the same age range) to establish the norm range of serum PRL level.

Experimental Protocol

All HIV-infected patients with anti-PRL autoantibodies, as well as 11 HIV-infected patients who were anti-PRL autoantibody negative (6 with normoprolactinemia and 5 with hyperprolactinemia), and 6 healthy men were studied in an identical fashion. After a 10-h overnight fast, an indwelling peripheral catheter was placed in the antecubital vein between 8:00 and 9:00 AM and kept permeable with a slow infusion of 0.9% saline solution. After a 30-min rest, two basal samples of blood were obtained at 15-min intervals (–15 and 0 min) and thereafter at 30, 60, 90, and 120 min after the administration of a 10-mg iv bolus of metoclopramide (an antidopaminergic drug). At each sampling time, the first 0.3 mL of blood was discarded to avoid a dilution error. The sera were separated and stored at –35°C until used.

Assay for PRL

PRL concentrations in serum and fractions by chromatography on gel filtration and affinity were measured by an ultrasensitive enzyme immunoassay (EIA) for human PRL developed in our laboratory to measure small amounts of PRL. Polystyrene 96-well plates (Nunc, Roskilde, Denmark) were coated with 100 µL/well of monoclonal antibodies specific for human PRL (American Qualex, San Clemente, CA) at concentrations of 3 µg/mL in 0.1 *M* carbonate-bicarbonate buffer, pH 9.0. Plates were incubated for 2 h at 37°C and stored at 4°C until used. Optimum blocking conditions for nonspecific binding were achieved using 300 µL/well of 5% skimmed milk in phosphate-buffered saline (PBS), pH 7.4, containing Tween-20 at 0.05% (PBST), for 2 h at 37°C. After washing five times with PBST, the samples were added in duplicate at different dilutions to ascertain parallelism with the standard curve. The plates were left for 1 h at 37°C, then washed five times with PBST, and subsequently incubated with rabbit antiserum against highly purified human pituitary PRL (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK] hPRL B-3, kindly supplied by Dr. A. F. Parlow) generated in our laboratory at a 1:2000 dilution. Following 1 h of incubation at 37°C, the plates were washed five times with PBST and subsequently incubated with a peroxidase-conjugated goat antirabbit serum (Dako, Carpinteria, CA), at a 1:2000 dilution for 1 h at 37°C. Plates then were washed five times with PBST before the reactions were revealed with *o*-phenylene diamine (Sigma, St. Louis, MO). Optical density was read at 492 nm using an ELISA reader (MR 5000: Dynatech, Chantilly, VA). The minimal detectable quantity was 0.018 µg/L, and the intra- and interassay coefficients of variation were 4.5 and 6.8%, respectively. Using the same methodology as previously reported *(17)*, the anti-PRL autoantibody was found not to interfere with present EIA for PRL.

The mean serum PRL level from 85 healthy men was 8.3 ± 4.0 µg/L (range: 0.6–17.4 µg/L). Hyperprolactinemia was defined as a serum PRL level of >20.3 µg/L; this result represents the mean ± 3 SD of healthy men. Free PRL was extracted from the serum using polyethylene glycol, as previously described *(16,33)*.

Detection of Anti-PRL Autoantibodies

Affinity columns for IgG (a protein-G column, HiTrap G; Pharmacia LKB, Uppsala, Sweden) as previously described *(16)* were used for detecting anti-PRL autoantibodies. Serum samples were judged to contain anti-PRL autoantibodies when the proportion of PRL retained on the column exceeded 4.2%. This result represents the mean ± 3 SD of 20 healthy pregnant women at different stages of pregnancy who had little PRL as the predominant circulating species of PRL demonstrated by gel filtration chromatography.

Gel Filtration Chromatography

Gel filtration was performed using a column (56 × 1 cm) of Sephadex G-100 Superfine (Pharmacia) as previously described *(16)*, and PRL immunoreactivity from each fraction was determined by EIA.

Statistical Analysis

Data were expressed as mean ± SD or median (range). Differences between two continuous variables were compared by nonpaired student's *t*-test (or Mann-Whitney *U* test for nonnormally distributed variables). Differences among ≥3 groups for continuous variables were compared by one-way analysis of variance (ANOVA) followed by multiple comparisons procedures (Scheffe's method for nonpaired samples or Tukey's procedure for paired samples) or by the Kruskal-Wallis one-way ANOVA for nonnormally distributed variables (using Mann-Whitney U test for nonpaired samples or Wilcoxon signed rank test for multiple comparisons). Differences between proportions were compared by χ^2 analysis or Fisher's exact test. A two-tailed p value of <0.05 was considered significant.

Acknowledgment

We are indebted to the NIDDK-National Hormone and Pituitary Program (Dr. A. F. Parlow) for the human PRL reagent.

References

1. Dobs, A. S., Dempsy, M. A., Ladenson, P. W., and Polk, B. F. (1988). *Am. J. Med.* **84,** 611–616.
2. Merenich, J. A., McDermott, M. T., Asp, A. A., Harrison, S. M., and Kidd, G. S. (1990). *J. Clin. Endocrinol. Metab.* **70,** 566–571.
3. Croxson, T. S., Chapman, W. E., Miller, L. K., Levit, C. D., Senie, R., and Zumoff, B. (1989). *J. Clin. Endocrinol. Metab*. **68,** 317–321.
4. Graef, A. S., Gonzalez, S. S., Baca, V. R., et al. (1994). *Clin. Immunol. Immunopathol.* **72,** 390–393.
5. Montero, A., Giovannoni, A. G., and Sen, L. (2000). *Medicina (Buenos Aires)* **60,** 427–430.
6. Hutchinson, J., Murphy, M., Harries, R., and Skinner, C. J. (2000). *Lancet* **356,** 1003–1004.
7. Shaw, J. A., Sawers, H. A., Strachan, F. M., and Bevan, J. S. (1997). *J. R. Soc. Med.* **90,** 274–275.
8. Kleinberg, D. L., Noel, G. L., and Frantz, A. G. (1977). *N. Engl. J. Med.* **296,** 589–600.
9. Lane, H. C., Masur, H., Edgar, L. C., Whalen, G., Rook, A. H., and Fauci, A. S. (1983). *N. Engl. J. Med.* **309,** 453–458.
10. Muller, S., Richalet, P., Laurent-Crawford, A., et al. (1992). *AIDS* **6,** 933–942.
11. Kordossis, T., Piha, L., Guialis, A., Kosmopoulou, O., Tzioufas, A. G., and Moutsopoulos, H. M. (1997). *Clin. Exp. Rheumatol.* **5,** 185–188.
12. Massabki, P. S., Accetturi, C., Nishie, I. A., da Silva, N. P., Sato, E. I., and Andrade, L. E. (1997). *AIDS* **11,** 1845–1850.
13. Kopelman, R. G. and Zolla-Pazner, S. (1988). *Am. J. Med.* **84,** 82–88.
14. Kaye, B. R. (1989). *Ann. Intern. Med.* **111,** 158–167.
15. Blanco-Favela, F., Quintal-Alvarez, G., and Leaños-Miranda, A. (1999). *J. Rheumatol.* **26,** 55–59.
16. Leaños, A., Pascoe, D., Fraga, A., and Blanco-Favela, F. (1988). *Lupus* **7,** 398–403.
17. Leaños-Miranda, A., Pascoe-Lira, D., Chávez-Rueda, K. A., and Blanco-Favela, F. (2001). *J. Rheumatol.* **28,** 1546–1553.
18. Hattori, N., Ishihara, T., Ikekubo, K., Moridera, K., Hino, M., and Kurahachi, H. (1992). *J. Clin. Endocrinol. Metab.* **75,** 1226–1229.
19. Leite, V., Cosby, H., Sobrinho, L. G., Fresnoza, A., Santos, M. A., and Friesen, H. G. (1992). *Clin. Endocrinol.* **37,** 365–372.
20. Cavaco, B., Leite, V., Santos, M. A., Arranhado, E., and Sobrinho, L. G. (1995). *J. Clin. Endocrinol. Metab.* **80,** 2342–2346.
21. Pascoe-Lira, D., Duran-Reyes, G., Contreras-Hernández, I., Manuel-Apolinar, L., Blanco-Favela, F., and Leaños-Miranda, A. (2001). *J. Clin. Endocrinol. Metab.* **86,** 924–929.
22. Hattori, N., Ikekubo, K., Ishihara, T., Moriderea, K., Hino, M., and Kurahachi, H. (1994). *Eur. J. Endocrinol.* **130,** 438–445.
23. Garnier, P. E., Aubert, M. L., Kaplan, S. I., and Grumbach, M. M. (1978). *J. Clin. Endocrinol. Metab.* **47,** 1273–1281.
24. Leaños-Miranda, A., Pascoe-Lira, D., Chávez-Rueda, K. A., and Blanco-Favela, F. (2001). *J. Clin. Endocrinol. Metab.* **86,** 2619–2624.
25. Leaños-Miranda, A., Chávez-Rueda, K. A., and Blanco-Favela, F. (2001). *Arthritis Rheum.* **44,** 866–875.
26. Pellegrini, I., Lebrun, J. J., Ali, S., and Kelly, A. P. (1992). *Mol. Endocrinol.* **6,** 1023–1031.
27. Montgomery, D. W., Shen, G. K., Ulrich, E. D., Steiner, L. L., Parrish, P. R., and Zukoski, C. F. (1992). *Endocrinology* **131,** 3019–3026.
28. Matera, L. (1997). *Neuroimmunomodulation* **4,** 171–180.
29. Larrea, F., Martínez-Castillo, A., Cabrera, V., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 3664–3669.
30. Hatfill, S. J., Kirby, R., Hanley, M., Ribicki, E., and Bohm, L. (1990). *Leuk. Res.* **14,** 57–62.
31. Parra, A., Ramírez-Peredo, J., Coutiño, B., et al. (1999). *J. Lab. Clin. Med.* **133,** 70–74.
32. Centers for Disease Control and Prevention. (1992). *MMWR Morb. Mortal. Wkly. Rep.* **41(RR-17),** 1–19.
33. Leaños-Miranda, A., Pascoe-Lira, D., Chávez-Rueda, K. A., and Blanco-Favela, F. (2001). *Lupus* **10,** 340–345.