Prolactin enhances the in vitro production of IgG in peripheral blood mononuclear cells from patients with systemic lupus erythematosus but not from healthy controls

A M Jacobi, W Rohde, H-D Volk, T Dörner, G-R Burmester, F Hiepe

Abstract

Objectives—Recent evidence suggests that prolactin (PRL) plays a part in the pathogenesis of systemic lupus erythematosus (SLE). Because B cell hyperreactivity and autoantibodies are characteristic hallmarks of SLE, this study aimed at assessing the impact of this pituitary hormone on IgG production by stimulating peripheral blood mononuclear cells (PBMC) with PRL.

Methods—PBMC from 11 patients with SLE assessed by the ECLAM score and eight healthy controls were incubated with PRL and cultured for seven days. IgG production was measured by enzyme linked immunosorbent assay (ELISA).

Results—Spontaneous IgG production of SLE PBMC was significantly enhanced compared with that found in healthy controls. After PRL stimulation, the IgG concentrations of supernatants from SLE PBMC were significantly higher than those of unstimulated PBMC (median 394 ng/ml). Of note, the physiological concentration of PRL (20 ng/ml) induced IgG production more effectively (median 1139 ng/ml) than PRL at 100 ng/ml (median 1029 ng/ml). In contrast, preincubation with PRL did not stimulate IgG production in normal PBMC. A significant correlation between PRL induced IgG production and the disease activity (ECLAM) of the patients with SLE was seen. Moreover, the maximum amount of PRL induced IgG depended on the serum PRL concentrations of the patients with SLE.

Conclusions—The results suggest that PBMC from patients with SLE have an extraordinarily high susceptibility to PRL, showing the most striking effect at a concentration usually found in vivo. This indicates a potential role for mild hyperprolactinaemia in the pathogenesis of SLE, influencing both IgG production and disease activity.


Methods

Patients and healthy controls

The study included 11 patients (eight female, three male) who fulfilled the American College of Rheumatology criteria for SLE and eight healthy subjects (six female, two male, blood
Donors. The patients with SLE were aged 17–57 years (median 30) and controls were aged 19–54 (median 30.5). The disease activity of patients with SLE was assessed according to the European Consensus Lupus Activity Measurement score (ECLAM score),

<table>
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<th>Patient</th>
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<th>Sex</th>
<th>PRL* (ng/ml)</th>
<th>ECLAM score</th>
<th>ANA†</th>
<th>ENA*</th>
<th>aCL</th>
<th>IgG*</th>
<th>IgG* CL-IF*†</th>
<th>Raised</th>
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*PRL = prolactin; ANA = antinuclear antibodies; ENA = extractable nuclear antigens; aCL = anticardiolipin antibodies, positive (+) >50 U/ml, ++ >100 U/ml; aDNA = anti-dsDNA ELISA, positive >6 AU; CL-IF = anti-dsDNA antibodies determined by Crithidia luciliae immunofluorescence technique; ESR = erythrocyte sedimentation rate; GNS = central nervous system.
†Reciprocal titre, + means positive or present; − means negative or not present.

Donors. The patients with SLE were aged 17–57 years (median 30) and controls were aged 19–54 (median 30.5). The disease activity of patients with SLE was assessed according to the European Consensus Lupus Activity Measurement score (ECLAM score),

**Table 1** Characteristics of the patients with systemic lupus erythematosus analysed, clinical manifestations of the disease and other signs of disease activity underlying the ECLAM score.

**Measurement of Serum Levels of PRL**

All blood samples were obtained with minimal trauma by venepuncture of the cubital vein between 9.00 am and 12.00 am, with a previous period of fasting and resting. Serum was separated and stored at −20°C until the hormonal assay was performed. Serum PRL was determined by enzyme linked immunosorbent assay (ELISA, Elias-Medizintechnik GmbH, Freiburg, Germany). The cut off serum PRL concentration for the assay was 15 ng/ml for samples by Ficoll-Hypaque separation according to standard protocols (Seromed, Berlin, Germany), including three washing steps with phosphate buffered saline (PBS, pH 7.4; Seromed). The PBMC (1 × 10^6 cells/ml) were resuspended in medium (RPMI 1640, Seromed) containing 5% fetal calf serum (Seromed), streptomycin/penicillin (10 U/ml, Seromed), and r-PRL (20 and 100 ng/ml) (Genzyme Corporation, Cambridge, USA). Subsequently, the cells were cultured in a CO₂ incubator (5% CO₂) at 37°C for seven days. Thereafter, cell culture supernatants were collected and stored at −20°C until measurement of the IgG concentration.

**In Further Control Experiments**

In further control experiments, lipopolysaccharide (LPS, Sigma, Berlin, Germany) was added to the PBMC cultures of 3/11 patients and of two healthy controls to assess an endotoxin related effect of the PRL on the IgG production of PBMC. The endotoxin concentration of PRL used was 0.02 ng/µg, according to the product information given by the supplier (Genzyme Corporation). To estimate the potential effect of the LPS contained in the r-PRL in more detail, PBMC were also incubated with the endotoxin LPS (2 pg/ml) at a concentration comparable with that expected in a solution of medium containing PRL at 100 ng/ml. In addition, LPS was also added at a concentration of 10 pg/ml.

**Determination of the IgG Concentration in the Culture Supernatants**

The IgG concentration of the supernatants was determined by one step enzyme immunoassay, “Human IgG SURALISA” (Seramun Diagnostika GmbH, Döllgenbrot, Germany) using a sheep high affinity polyclonal IgG antibody.

Statistical analysis was performed with GraphPad Prism 2.01 for Windows 95. The median IgG concentration of the cell culture supernatants and the median PRL level and disease activity of the patients with SLE were analysed. The Mann-Whitney U test was used to compare the IgG concentrations of the cell culture supernatants of patients with SLE and of healthy controls. Subsequently, the effect of PRL on the IgG production of SLE PBMC was assessed by comparing the IgG concentrations before and after incubation with PRL using the non-parametric Wilcoxon test with Bonferroni correction. In addition, the correlation of IgG production with disease activity in SLE was assessed by Spearman’s rank correlation test.

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Results

Serum PRL concentrations were raised in six patients with SLE, while five had normal levels. Table 1 shows characteristics and serum PRL concentrations of the patients with SLE studied.

SPONTANEOUS IgG PRODUCTION IN PBMC CULTURES

The IgG concentration was significantly higher in cell culture supernatants of PBMC from patients with SLE (median 394 ng/ml, range 132–1734) than in supernatants of PBMC from healthy controls where the IgG concentrations were below the detectable concentration of the assay used (<150 ng/ml) (p<0.0001, Mann-Whitney U test).

INFLUENCE OF PRL ON IgG PRODUCTION

After incubation with PRL at an overall physiological concentration (20 ng/ml), a remarkable increase in the IgG production of SLE PBMC was detected (fig 1). The IgG concentration was significantly higher (median 1139 ng/ml, range 223–2316) than that determined for PBMC without stimulation (p=0.001, Wilcoxon test).

The IgG concentrations of the supernatants of PBMC from patients with SLE after incubation with PRL at a concentration of 100 ng/ml were also significantly higher (median value 1029 ng/ml, range 215–2390) than the basal concentration of IgG (p=0.002, Wilcoxon test). Most notably, the increase in IgG production was significantly lower than that seen after incubation with PRL at the physiological concentration of 20 ng/ml (Wilcoxon test and Bonferroni correction p=0.043).

In contrast, such an increase in IgG production was not detected in the PBMC obtained from healthy controls either after incubation with PRL at the physiological (20 ng/ml) or at the high (100 ng/ml) concentration with values below 150 ng IgG/ml. However, in one of the eight blood donors a mild increase in the IgG concentration after incubation with PRL was detected (spontaneous <150 ng/ml v PRL at 20 ng/ml: 272 ng/ml and v PRL at 100 ng/ml: 264 ng/ml).

INFLUENCE OF LPS ON IgG PRODUCTION

To exclude the possibility that the observed PRL effect on IgG production is related to an endotoxin contamination of the PRL used, we examined the impact of two LPS concentrations (2 and 10 pg/ml) on IgG production in the PBMC of three patients with SLE and two healthy controls. LPS at a concentration of 2 pg/ml did not alter the IgG production of PBMC from patients with SLE (spontaneous 508 ng/ml v LPS at 2 pg/ml: 515 ng/ml, mean values), whereas LPS at a higher concentration (10 pg/ml) caused a mild increase of the IgG production (mean value 645 ng/ml). However, this difference was not statistically significant (Wilcoxon test) and markedly less than that induced by PRL, especially at a concentration of 20 ng/ml. Moreover, no increase in the IgG production of PBMC from two healthy subjects was seen after incubation with LPS at either of the concentrations (2 and 10 pg/ml).

CORRELATION OF IgG PRODUCTION IN CELL CULTURE SUPERNATANTS OF PBMC FROM PATIENTS WITH SLE AND THE DISEASE ACTIVITY

Further analysis of the spontaneous production of IgG by PBMC and the disease activity of the patients with SLE did not show a significant correlation (r_s=0.5208, p=0.1003, Spearman’s rank correlation). However, the IgG concentrations after incubation with PRL at a

Figure 1 Concentration of total IgG in the supernatants of peripheral blood mononuclear cells (PBMC) obtained from 11 patients with systemic lupus erythematosus (SLE) after incubation with prolactin (PRL) at concentrations of 20 and 100 ng/ml, respectively, and without PRL (0) for seven days. Significant differences were seen between the IgG production by PBMC from patients with SLE after and without stimulation with PRL (20 and 100 ng/ml, respectively) (Wilcoxon test and Bonferroni correction, p=0.043).

Figure 2 Correlation of the IgG concentration of the supernatants of peripheral blood mononuclear cells from patients with systemic lupus erythematosus (SLE) after incubation with prolactin (PRL) at two concentrations (C_{PRL}=20 ng/ml and 100 ng/ml) and the ECLAM score of the patients with SLE analysed.

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Enhancement of IgG production in PBMC
difference from the patients with SLE. The table amounts of IgG, providing a striking detection with PRL, and spontaneously, PBMC increase in IgG production than PBMC from high susceptibility to PRL, with a more marked PBMC of patients with active disease had a is markedly related to the disease activity. activity indicates that the susceptibility to PRL in vitro IgG production and its relation to disease activity in patients with SLE.

Overall, the spontaneous IgG production of PBMC from patients with SLE did not depend on the disease activity and serum PRL levels of the patients examined.

To our knowledge this is the first study analysing the effect of PRL at a concentration of 20 ng/ml, equivalent to the median serum PRL concentration of these patients, on the IgG production of their PBMC which shows a significant enhancement. This effect was also shown for PRL at a higher concentration (100 ng/ml), though the impact on IgG production was less. Remarkably, PRL exerts a greater effect on IgG production at its overall physiological concentration than at 100 ng/ml. This supports the theory of the dimerisation model, which has been suggested not only for the growth hormone receptor but also for the PRL receptor. At high ligand concentrations, receptor dimerisation is blocked because two PRL molecules are bound to one receptor, whereas binding of one molecule/receptor is seen at low ligand concentration.

Most notably, the correlation of in vitro IgG production of PBMC from patients with SLE caused by incubation with PRL and the disease activity indicates that the susceptibility to PRL is markedly related to the disease activity. PBMC of patients with active disease had a high susceptibility to PRL, with a more marked increase in IgG production than PBMC from patients with low disease activity. After incubation with PRL, and spontaneously, PBMC from healthy controls did not produce detectable amounts of IgG, providing a striking difference from the patients with SLE. The current results provide evidence that the influence of PRL on IgG production depends on the disease activity, whereas PRL does not increase the IgG production of PBMC obtained from normal controls. This is consistent with the observation of Lahat et al, who found that PRL at a concentration of 0.2–100 ng/ml affected the immunoglobulin production of anti-IgM stimulated or interleukin 2 (IL2) stimulated B cells from healthy subjects but did not affect unstimulated cells. Preactivation of the cells seems to be required for the PRL dependent enhancement of IgG production. In contrast, Gutiérrez et al showed that PRL enhances the immunoglobulin production of PBMC from healthy subjects more strikingly than from PBMC of patients with SLE. This difference might be accounted for by the higher PRL concentration used, far exceeding the physiological range of serum PRL levels in healthy subjects.

The results of our study indicate that the significantly increased production of IgG by PBMC from patients with SLE cannot be ascribed to PRL alone. Thus the PBMC from the patients with SLE produced high amounts of IgG spontaneously that could be further enhanced by incubation with PRL. In general, there must be a regulatory defect of B cells in SLE, or of the interaction of the PBMC from patients with SLE, causing a more or less unregulated production of immunoglobulin and, probably, autoantibodies. Obviously, PRL can enhance this immunoglobulin production in vitro, though it is not uniquely responsible for the induction of immunoglobulin or autoantibody production at PRL concentrations between 20 and 100 ng/ml. The lack of enhanced immunoglobulin production by PBMC from normal donors after incubation with PRL suggests that either preactivation by the disease or inherited cellular defects account for the observed abnormalities of the immunoglobulin production by PBMC from patients with SLE. Alternatively, PRL could potentially permit a more efficient maturation of B cells into antibody producing plasma cells. Although further proof is needed, the induction of IL2 receptors by PRL, as previously shown in rat splenocytes, can increase the generation of antibody producing cells as IL2 is known to permit the maturation of plasma cells.

If these in vitro results are transferable to an in vivo situation, patients with SLE with mild hyperprolactinaemia, representing up to one third of all patients with SLE (unpublished data), provide conditions for an enhanced immunoglobulin production and autoantibody generation. Although somewhat speculative, this might explain the positive therapeutic effect of bromocriptine in normoprolactinaemic patients, as described previously and the frequently observed exacerbation of SLE during pregnancy.

The impact of PRL on IgG production in vitro seemed to depend on the disease activity. Therefore, the data suggest that the extent of PRL induced enhancement of the (auto)antibody production in SLE is correlated with the disease activity in vivo. To what extent PRL itself is responsible for an increase in disease activity and its origin in patients with SLE remains uncertain. However, the results
suggest that low serum PRL levels indicate a low risk of disease exacerbation.

One explanation for the observed difference in the susceptibility of PMBC from patients with SLE and normal controls to PRL in vitro might be that SLE PMBC express a high number of PRL receptors by prior activation in vivo. Alternatively, they may, like CLL-B cells, have the ability to receive additional signals transmitted by high molecular weight PRL by specific mechanisms of receptor function or of intracellular signal transduction. High molecular weight PRL consists of PRL bound to a macromolecule or its impaired penetration to SLE owing to the reduced clearance of the hypothalamic pituitary feedback mechanism. Another possible explanation is the influence of PRL on the apoptosis of lymphoma cells. PRL has also been shown to prevent these cells from glucocorticoids-induced apoptosis, raising the question whether slightly raised serum PRL levels in patients with SLE decrease the therapeutic effect of steroid treatment.

The results of the current study suggest an impact of mild hyperprolactinaemia on the immunoglobulin production and disease activity in SLE. As far as we know, no data have been published about the effect of PRL at an overall physiological concentration on PMBC from patients with SLE that indicate a potential role for borderline serum PRL or mild hyperprolactinaemia in aggravating or perpetuating the disease. However, this impact of PRL is strongly related to the disease activity of the patients and probably related to an increased susceptibility of PMBC to PRL in patients with disease flares. In further studies our attention needs to be focused more on the detailed mechanisms underlying the increased IgG response to PRL in SLE PMBC than on the question whether there are increased levels of serum PRL in SLE.

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