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
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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>PRL* (ng/ml)</th>
<th>ECLAM score</th>
<th>ANA*†</th>
<th>ENA*</th>
<th>aCL*</th>
<th>aDNA*</th>
<th>CL-IF*†</th>
<th>Raised ESR*</th>
<th>Decrease of C3/C4</th>
<th>Fever/ fatigue</th>
<th>Skin</th>
<th>Joint</th>
<th>Haematological</th>
<th>Kidney</th>
<th>GNS*</th>
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<td>21</td>
<td>M</td>
<td>6.4</td>
<td>3</td>
<td>2560</td>
<td>Ro/La/Sm</td>
<td>++</td>
<td>129.7</td>
<td>16</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>–</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>M</td>
<td>10.1</td>
<td>7</td>
<td>160</td>
<td>Ro/La/Sm</td>
<td>++</td>
<td>80</td>
<td>32</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>3</td>
<td>57</td>
<td>F</td>
<td>10.8</td>
<td>4</td>
<td>1280</td>
<td>Ro/La</td>
<td>+</td>
<td>11.6</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>La</td>
<td>++</td>
<td>225</td>
<td>128</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>1280</td>
<td>Ro/La/Sm</td>
<td>++</td>
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<td>32</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>39</td>
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<td>21.2</td>
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<td>320</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Ro/La</td>
<td>+</td>
<td>–</td>
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<td>+</td>
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<td>8</td>
<td>48</td>
<td>F</td>
<td>19.8</td>
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<td>10240</td>
<td>Ro/La</td>
<td>++</td>
<td>140</td>
<td>128</td>
<td>+</td>
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<td>39.5</td>
<td>3</td>
<td>640</td>
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<td>–</td>
<td>16</td>
<td>2</td>
<td>+</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>11</td>
<td>25</td>
<td>F</td>
<td>15.4</td>
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<td>1280</td>
<td>Ro/La</td>
<td>+</td>
<td>26.3</td>
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<td>–</td>
<td>+</td>
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</tr>
</tbody>
</table>

*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 lucilis 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 analysed according to the European Consensus Lupus Activity Measurement score (ECLAM score),³³ assessing clinical as well as serological findings (table 1).

Venous blood was taken for the detection of serum concentrations of PRL. At the same time, citrate blood (40 ml) was obtained for preparation of the PBMC cultures. Patients who had obvious causes for hyperprolactinaemia—for example, pregnancy, prolactinoma, hypothyroidism, advanced renal insufficiency (creatinine > 180 µmol/l), or patients receiving drugs with a side effect of increase in PRL, were excluded from the study. Six patients were receiving treatment with intravenous cyclophosphamide and prednisone, three were receiving methylnisolone bolus treatment, and one patient was receiving azathioprine alone. Another patient did not receive any immunosuppressive treatment at the time of the study. None of these immunosuppressive drugs influence the serum PRL concentration.

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 measurement score (ECLAM score), assessing clinical as well as serological findings (table 1).

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**CELL CULTURES**

PBMC were isolated from citrate blood samples by Ficoll-Hypaque separation according to standard protocols³⁹ (Seromed, Berlin, Germany), including three washing steps with phosphate buffered saline (PBS, pH 7.4; Seromed). The PBMC (1 × 10⁶ 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, 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 µg/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 µg/ml.

**DETERMINATION OF THE IgG CONCENTRATION IN THE CULTURE SUPERNATANTS**

The IgG concentration of the supernatants was determined by a one step enzyme immunoassay, “Human IgG SURALISA” (Serum Diagnostika GmbH, Dolgenbrot, 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 those 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.
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...
Enhancement of IgG production in PBMC
culture supernatants of PBMC from patients with SLE and the PRL serum level.

When the PRL serum levels of the patients with SLE were considered, a difference in IgG production between the groups with normal and raised serum PRL was found which was not statistically significant. The IgG concentrations were higher in cell culture supernatants of hyperprolactinaemic patients with SLE regardless of the concentration of incubated PRL, though the differences were not significant (p=0.0536 (c_m=20 ng/ml) and p=0.2468 (c_m=100 ng/ml); Mann-Whitney U test) because of the small number of patients analysed.

Discussion
This study analysed the influence of PRL on 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.

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 PBMC from patients with SLE and normal controls to PRL in vivo might be that SLE PBMC express a high number of PRL receptors by prior activation.

In contrast, the influence of PRL on the apoptosis of activated peripheral blood mononuclear cells from systemic lupus erythematosus (SLE) patients has been shown to be a cause of raised PRL levels in patients with SLE owing to the reduced clearance of the macromolecule or its impaired penetration to the hypothalamus and a subsequent disturbance of the hypothalamic pituitary feedback mechanism. Another possible explanation is the influence of PRL on the apoptosis of (auto)antibody-producing B cells. It has been shown that PRL increases the expression of anti-apoptotic proteins, such as Bcl-2 or Bax, in vitro.

Furthermore, it has been postulated that the influence of PRL on the apoptosis of B cells might be that SLE PBMC express a high number of PRL receptors by prior activation.

However, it must be noted that the PRL receptor is highly expressed in lymphoid tissues, and the number of PRL receptors may be increased in SLE PBMC, which could contribute to the observed increased susceptibility of PBMC to PRL in SLE.

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 PBMC 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 PBMC 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 PBMC than on the question whether there are increased levels of serum PRL in SLE.

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centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl 1968;97:77–89.


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