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.


Prolactin (PRL) is a polypeptide hormone with a heterogeneous function and is produced by a variety of tissues in the human body. Besides its production in the pituitary gland, many cells of the immune system can synthesise PRL and respond to PRL transmitted signals with the help of specific PRL receptors using different signal transduction pathways. PRL functions similarly to a cytokine, though it acts not only in an endocrine but also in a paracrine and autocrine manner. PRL is also known to stimulate the humoral and cellular immune response. Thus it has been shown to stimulate the immunoglobulin and autoantibody production of human peripheral blood mononuclear cells (PBMC) from healthy subjects and patients with systemic lupus erythematosus (SLE). On the other hand, PRL is secreted by PBMC from healthy subjects and in even larger amounts by PBMC from patients with SLE. Animal models resembling SLE show a correlation between the PRL levels and disease activity. These studies indicate that PRL may play a part in the pathogenesis of SLE. Furthermore, other studies have reported an increased prevalence of various autoantibodies in patients with hyperprolactinaemia and others found raised PRL levels in patients with autoimmune diseases, most notably, in patients with SLE at a prevalence of 2–31%. However, these studies gave contradictory results for the correlation between PRL levels and the clinical or serological disease activity. Furthermore, it has also been shown that reduction of the PRL level in SLE by bromocriptine, a dopamine agonist, decreases the frequency of lupus flares in patients with SLE and reduces overall disease activity. Because previous studies provided inconclusive evidence about the influence of serum PRL on disease activity and serological abnormalities in SLE, this study determined the influence of PRL at two different concentrations—namely, 20 and 100 ng/ml, on the IgG secretion of PBMC from patients with SLE and healthy controls in vitro to estimate the impact of hyperprolactinaemia on the disease activity. Of note, previous studies showed that up to 31% of patients with SLE have PRL levels between 20 and 100 ng/ml.

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
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</th>
<th>ANA*</th>
<th>ENA*</th>
<th>aCL</th>
<th>IgG*</th>
<th>aDNA</th>
<th>IgG*</th>
<th>CL-IF*</th>
<th>Raised ESR</th>
<th>Decrease of C3/C4</th>
<th>Fever/</th>
<th>Fatigue</th>
<th>Skin</th>
<th>Joint</th>
<th>Haematological</th>
<th>Kidney</th>
<th>GNS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<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>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<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>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<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>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>F</td>
<td>34.3</td>
<td>10</td>
<td>640</td>
<td>La</td>
<td>++</td>
<td>225</td>
<td>128</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>M</td>
<td>20.5</td>
<td>4</td>
<td>1280</td>
<td>Ro/La/Sm</td>
<td>++</td>
<td>34.2</td>
<td>32</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>F</td>
<td>21.2</td>
<td>5</td>
<td>320</td>
<td>Ro/La</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>21</td>
<td>F</td>
<td>24.7</td>
<td>4</td>
<td>1280</td>
<td>Ro/La</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>F</td>
<td>19.8</td>
<td>10</td>
<td>10240</td>
<td>Ro</td>
<td>++</td>
<td>140</td>
<td>128</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>F</td>
<td>39.5</td>
<td>3</td>
<td>640</td>
<td>–</td>
<td>–</td>
<td>16</td>
<td>2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>F</td>
<td>9.5</td>
<td>1</td>
<td>320</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>21.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>F</td>
<td>15.4</td>
<td>6</td>
<td>1280</td>
<td>Ro/La</td>
<td>+</td>
<td>26.3</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*PRL = prolactin; ANA = antinuclear antibodies; ENA = extactable 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; CNS = central nervous system.

†Reciprocal titre, + means positive or present; − means negative or not present.
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<0.001, 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

245

...able amounts of IgG, providing a striking from healthy controls did not produce detect-

tion with PRL, and spontaneously, PBMC

patients with low disease activity. After incuba-

increase in IgG production than PBMC from

PBMC of patients with active disease had a

is markedly related to the disease activity.

cause 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 ana-

lysing 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 sig-

nificant 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 physi-

ological 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 incuba-

tion with PRL, and spontaneously, PBMC

from healthy controls did not produce detect-

able amounts of IgG, providing a striking

difference from the patients with SLE. The

current results provide evidence that the influ-

ence of PRL on IgG production depends on

the disease activity, whereas PRL does not

increase the IgG production of PBMC ob-

ained 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 sig-

ificantly 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 concentra-

tions 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 immuno-

globulin production by PBMC from patients

with SLE. Alternatively, PRL could potentially

permit a more efficient maturation of B cells

into antibody producing plasma cells. Al-

though 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 normoprolactinae-

mic 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)anti-

body 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 vitro might be that SLE PBMC 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 an anti-PRL antibody (IgG) and has been reported to occur frequently in patients with SLE.

Furthermore, it has been postulated 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 Nb2 lymphoma cells. PRL has also been shown to prevent these cells from glucocorticoid-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 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.

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Hi 620/1–1, SFB 421, C4).

References


34 Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one
Enhancement of IgG production in PBMC...
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 and F Hiepe

Ann Rheum Dis 2001 60: 242-247
doi: 10.1136/ard.60.3.242