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Effect of elevated serum prolactin concentrations on the immunophenotype of human lymphocytes, mitogen-induced proliferation and phagocytic activity of polymorphonuclear cells

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Abstract. It has been suggested that the immune system is an important target tissue of prolactin (PRL). We therefore investigated several immune parameters in nine patients with chronically elevated serum prolactin concentrations. The immunophenotype of lymphocytes, mitogen-induced lymphocyte proliferation and phagocytic activity of polymorphonuclear cells were determined under high serum prolactin levels and 2 weeks after treatment with dopamine agonists. An increased CD4/CD8 ratio in the hyperprolactinaemic patients could be detected compared with healthy control subjects, which remained high after treatment and did not seem to correlate with serum prolactin concentrations. Peripheral blood B lymphocytes showed an increased expression of CD69 in the treated group but not in untreated patients compared with healthy control subjects. Interleukin 2 receptor, CD45RO, transferrin receptor or HLA-DR expression of CD4 or CD8 cells, as well as oxidative burst and phagocytic activity of granulocytes, were not affected in the patients with prolactinomas. Lymphocyte transformation response to phytohaemagglutinin *in vitro* was found not to be influenced by elevated prolactin levels except at the highest mitogen concentration tested. These data together with previous reports suggest that, although PRL is required for lymphocyte maturation to achieve normal immune function, elevated PRL levels do not lead to an 'overstimulation' of the immune system in men.

Keywords. Flow cytometry, granulocytes, lymphocytes, oxidative burst, phagocytosis, prolactin.

Introduction

Evidence has accumulated in support of the hypothesis that the immune system is an important target of pituitary prolactin and that it may play a physiological role in the regulation of humoral and cell-mediated immune response [1,2]. Receptors for PRL on human lymphocytes have been identified on T cells and B cells, and the addition of PRL *in vitro* enhances interferon (IFN)-γ production of mononuclear cells [3–6]. In addition, data in rodents show an impaired immune regulation after hypophysectomy, which can be restored by the administration of PRL [7]. However, most reports describe the lack of PRL, and only a few studies deal with *in vivo* examinations in humans. As previously reported, we have been unable to demonstrate an effect of elevated serum PRL concentrations on cytokine production, serum immunoglobulin levels and natural killer cell activity [8]. The aim of the present study was to examine the influence of elevated PRL concentrations on lymphocyte phenotype and proliferation as well as on the phagocytic activity of granulocytes. We therefore analysed those parameters in nine patients with prolactinomas before and 2 weeks after treatment with dopamine agonists (DAs) known to suppress pituitary PRL secretion.

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Patients and methods

Patients and healthy controls

Nine sex- and age-matched patients with hyperprolactinaemia (four women and five men, mean age 46 years, range 26–67; Table 1) and 10 healthy control subjects (five women and five men, mean age 50 years, range 29–75) were studied after informed consent was obtained.

Lymphocyte phenotyping, mitogen-induced lymphocyte proliferation, phagocytic and oxidative burst activity of granulocytes were determined before treatment with PRL-suppressing drugs and while the patients were on therapy resulting in significantly lower serum PRL concentrations.

Immnofluorescence and flow cytometry

Aliquots of 100 μL of heparinized whole blood were stained with different anti-CD monoclonal antibodies (abs) for 20 min at room temperature (RT). Red blood cells were then lysed in 1 mL of FACS-lysing solution (purchased from Becton-Dickinson, San Jose, CA, USA) for 15 min at RT then pelleted at 400 *g* for 6 min. After one washing step leucocytes were resuspended in 250 mL of phosphate-buffered saline (PBS) and 15 000 cells were analysed on a flow cytometer (FACScan; Becton-Dickinson). For two-colour analysis, cells were stained with fluorescein–isothiocyanate (FITC)

Table 1. Characteristics of the patients and serum prolactin levels before and after treatment, as well as treatment and daily dosage of DA

Patient no.	Sex	Age	PRL before treatment (ng mL^{-1})	PRL after treatment (ng mL^{-1})	Dopamine agonist	Daily dose
1	F	43	1785	622	Lisuride	0·40 mg
2	F	46	900	54	Bromocriptine	5·00 mg
3	M	52	52	4	Bromocriptine	5·00 mg
4	M	67	40	23	Quinagolide	0·30 mg
5	M	26	76	21	Bromocriptine	1·25 mg
6	F	29	622	159	Lisuride	0·60 mg
7	M	53	131	80	Bromocriptine	1·25 mg
8	F	38	102	5	Quinagolide	0·07 mg
9	M	61	847	40	Bromocriptine	10·0 mg

Growth hormone, thyroxin, testosterone and oestradiol serum levels were within normal range at both times of investigation in all patients (not shown). Mean serum PRL level of healthy control subjects was 7.3 ng mL^{-1} ± 1·15 (SEM).

and phycoerythrin (PE)-labelled anti-CD45 vs. CD14, anti-CD3 vs. CD19, anti-CD3 vs. -CD4, anti-CD3 vs. CD8 and anti-CD3 vs. CD16/56 (SimultestR IMK Plus reagent kit; Becton-Dickinson). Three-colour staining of CD4, CD8 and CD19 subtypes [stained with peridinine chlorophyll (PerCP) protein-labelled monoclonal antibodies obtained from Becton Dickinson] was performed with anti CD45RA (FITC), CD45RO (PE), HLA-DR (PE), CD25 (FITC), CD28 (PE), CD69 (PE) (all purchased from Becton-Dickinson), CD71 (transferrin reeptor) (FITC labelled-Immunotech, Marseille, France). Lymphocytes were identified by light-scatter characteristics and CD45 expression. Isotype-matched mouse antibodies (IgG1 and IgG2) conjugated with FITC, PE or PerCP (Becton-Dickinson) were used as negative controls.

Mitogen-induced lymphocyte proliferation

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation with Ficoll–Paque (Pharmacia, Uppsala, Sweden). Autologous serum was collected before removing the separated cells from Ficoll. Following two washing steps, cells were resuspended in RPMI-1640 medium (Sigma, St Louis, MO, USA) containing penicillin (50 U L^{-1}) and streptomycin (50 mg L^{-1}), both obtained from IRH Biosciences (Lenexa, KS, USA) and 25% autologous serum. PBMCs were seeded on a 96-well microtitre plate (10^5 cells per well) with different phytohaemagglutinin (PHA) (Sigma) concentrations (12·5, 6·25, 3·12, 1·56, 0·75,0·37 μg mL^{-1}) and incubated for 48 h at 37 °C in a fully humidified air atmosphere containing 5% CO_2. Cells were pulsed with [^{3}H]-thymidine (1 μCi per well) for a further 18 h and then recovered with a cell harvester (1295-001 Cell Harvester, LKB Wallac, Turku, Finland). Incorporated activity was measured with a liquid scintillation counter (1205 Betaplate TM, LKB, Wallac).

Phagocytosis and oxidative burst of polymorphnuclear phagocytes

Phagocytosis of opsonized and FITC-labelled *Escherichia (E.) coli* and oxidative burst after stimulation with formyl–methionine–leucine (fMLP) or *E. coli* were determined with Phago Test and Burst Test (Orpegen, Heidelberg, Germany) according to the manufacturer's instructions.

Statistics

Statistical analysis of data before and after treatment was performed by paired *t*-test; significance of data comparing patients and healthy control subjects was performed by unpaired *t*-test. Data were analysed using StatWorks software running on an Apple Macintosh IIvx computer.

Results

Patients and healthy control subjects

Dopamine agonist therapy resulted in a prompt decrease in serum PRL concentration in all patients. The mean serum PRL concentration before treatment was 506·1 ng mL^{-1} ± 210·41 standard error of mean (SEM); 2 weeks after therapy with DA mean levels were 112·0 ng mL^{-1} ± 69·73 SEM ($P = 0·03$). In all patients PRL levels declined markedly. However, serum PRL concentrations were able to be suppressed to normal range in only four patients; five patients revealed decreased but still elevated PRL serum concentrations (Table 1). At both times of investigation, growth hormone, thyroxine, testosterone and oestradiol serum levels were within normal range in all patients (data not shown).

The mean serum PRL level of healthy control subjects was 7·3 ng mL^{-1} ± 1·15 SEM.

Immunophenotype of peripheral blood lymphocytes

The distribution of T and B lymphocytes, as well as of NK cells, showed no significant differences before (pre) and after (post) treatment and compared with healthy control subjects (HC): T lymphocytes (CD3), 67% ± 3·99 SEM (pre), 69% ± 2·21 SEM (post), 63% ± 3·32 SEM (HC); B lymphocytes (CD19), 17% ± 1·48 SEM (pre), 17% ± 1·75 SEM (post), 18% ± 1·35 SEM (HC); NK cells (CD16/16), 16% ± 3·80 SEM (pre), 14% ± 1·75 SEM (post), 15% ± 2·82 SEM (HC).

Only T-helper lymphocytes were significantly elevated compared with healthy control subjects and the CD4/CD8 ratio also remained high after treatment: T-helper lymphocytes (CD4), 72% ± 2·62 SEM (pre), 73% ± 2·63 SEM (post), 60% ± 3·68 SEM (HC); T-suppressor lymphocytes (CD8), 28% ± 2·45 SEM (pre), 27% ± 1·99 SEM (post), 40% ± 2·40 SEM (HC); $P = 0{\cdot}01$ (Fig. 1).

No significant differences in the expression of CD45RA (resting T cells), CD45RO (activated or memory T cells), CD25 [interleukin (IL)-2 receptor], CD28 (receptor for CD80), CD57 (NK cells), CD71 (transferrin receptor), CD69 (activation induced molecule AIM), and HLA-DR (MHC class II) on $CD4^+$ (not shown) and $CD8^+$ lymphocytes was observed (not shown). B ($CD19^+$) lymphocytes revealed a significantly higher expression of CD69 in patients with low serum PRL concentrations than before treatment ($P = 0{\cdot}01$) as well as healthy individuals ($P = 0{\cdot}00$): 4% ± 0·81 SEM (pre), 14% ± 2·95 SEM (post), 2% ± 0·63 SEM (HC). But no significant difference could be detected between untreated patients and healthy control subjects in this respect ($P = 0{\cdot}16$) (Fig. 2). All other activation-associated molecules (HLA-DR, CD25, CD71, CD28) on B lymphocytes showed no difference between patients and healthy control subjects (not shown).

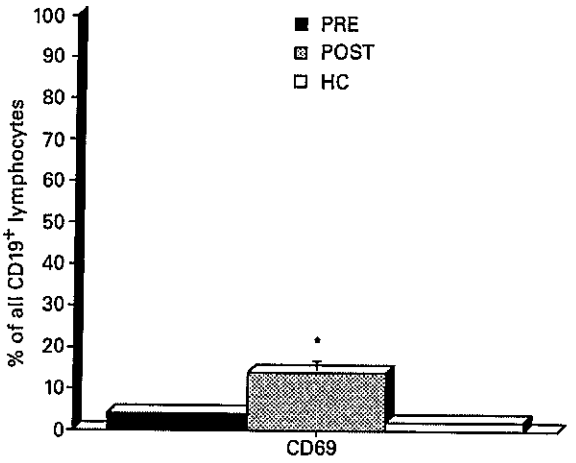

Figure 2. Expression of CD69 on B lymphocytes of patients before (pre) and after treatment (post) and healthy control subjects (HC) with SEM.

Phagocytosis and metabolic burst

Phagocytic activity of granulocytes tested with opsonized *E. coli* revealed no significant difference in the patients with considerably elevated serum PRL concentrations and after treatment with DA. The percentage of phagocytizing granulocytes was similar to that observed in healthy control subjects: 54% ± 15·33 SEM (pre), 53% ± 16·01 SEM (post), 51% ± 13·48 SEM (HC) (not shown).

Metabolic burst of granulocytes also exhibited no difference among all three groups, tested after stimulation by fMLP [14% ± 3·17 SEM (pre), 10% ± 4·02 SEM (post), 11% ± 2·22 SEM (HC)] or by opsonized *E. coli*: [90% ± 5·45 SEM (pre), 92% ± 4·38 SEM (post), 94% ± 3·29 SEM (HC)] (not shown).

Mitogen-induced lymphocyte proliferation

After stimulation with different concentrations of PHA, peripheral blood lymphocytes (PBLs) showed an equal proliferation rate before and after DA treatment, except at the highest concentration tested (12·5 $\mu g\,mL^{-1}$), which led to an increased [^{3}H]-thymidine incorporation in lymphocytes of those patients with lower serum PRL levels: 146 668 cpm ± 21181·55 SEM (pre); 222 704 cpm ± 35109·84 SEM (post); $P = 0{\cdot}05$ (Fig. 3).

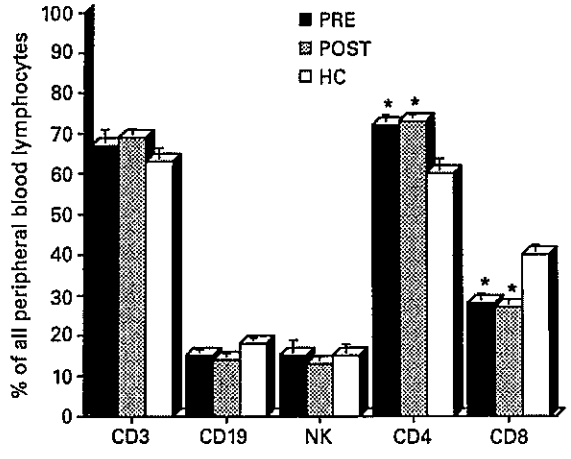

Figure1. Distribution of T lymphocytes (CD3), B lymphocytes (CD19), NK cells (CD16/56), and T-helper (CD4) and T-suppressor/cytotoxic lymphocytes (CD8) in patients before treatment (pre), after administration of dopamine agonists (post) and of healthy control subjects (HC) with SEM.

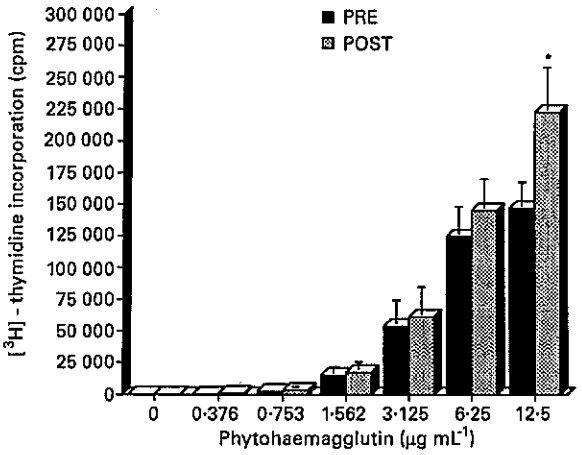

Figure 3. PHA-induced lymphocyte proliferation in patients before treatment (pre), after administration of dopamine agonists (post) with SEM.

Discussion

It is evident from *in vivo* and *in vitro* studies in rodents that the immune system is an important target of pituitary PRL and that the hormone plays a physiological role in the regulation of humoral and cell-mediated immune response. Hypophysectomy results in depressed antibody titres against sheep red blood cells and delayed hypersensitivity reactions [7]. Hartmann *et al.* [9] showed an inhibition of lymphocyte proliferation by anti-PRL antibodies *in vitro,* and receptors for PRL on human lymphocytes have been identified on T cells and B cells. Most *in vivo* studies have investigated the lack of PRL, which leads to immunodeficiency. In contrast, no significant influence of chronically elevated serum PRL on serum concentrations of different cytokines and immunoglobulins, as well as on natural killer cell activity could be detected [8].

In the present study, lymphocyte subsets and proliferation as well as phagocytic activity of PMNCs were investigated, but again chronic hyperprolactinaemia appears to have only minor effects on the immune system. An increase in relative T-helper ($CD4^+$) lymphocyte count and in the CD4/CD8 ratio have been observed, similar to the report of Lopez-Karpovitch *et al.* [10]. It is well known that pituitary hormones are required for haemo- and lymphopoiesis [11,12]. Bellone *et al.* [13] demonstrated that PRL potentiates the proliferative effect of IL-3 and granulocyte–macrophage colony-stimulating factor (GM-CSF) on haemopoietic stem cells and that this phenomenon is a direct effect of PRL. As PRL stimulates the secretion of thymulin by thymic epithelial cells, a protein that induces $CD8^+$ T-lymphocyte proliferation in the murine system, our observation of an increased CD4/CD8 ratio is unexpected [2,14]. There was also no correlation between CD4/CD8 ratio and elevation of PRL. Thus, the CD4 and CD8 counts are not directly related to PRL levels *per se.* In addition, the immunophenotype of T-helper lymphocytes showed a normal distribution of memory cells and the expression of different surface molecules such as HLA-DR. It has been reported that PRL induces IL-2 receptor formation on rat splenic lymphocytes *in vitro* [15]. However, CD25 in hyperprolactinaemic patients did not differ from that in healthy control subjects. Even the percentage of $CD4^+$ lymphocytes expressing transferrin receptors (CD71), which reflects proliferation rate, was not affected by high prolactin levels. Furthermore, hypoprolactinaemia has been reported to be associated with impaired lymphocyte mitogenesis [7]. In the present study, *ex vivo* derived PBLs from patients with prolactinomas leading to chronically elevated hormone levels showed no significant enhancement of mitogen-induced proliferation when cultured in autologous serum. In contrast, we observed a reduction in the proliferative response at the highest PHA concentration tested in patients with high PRL levels. This confirms the results obtained by Vidaller *et al.* [16] in four patients with hyperprolactinaemia, whose PBMCs showed a decreased proliferation on exposure to concanavalin A, pokeweed mitogen and PHA. Although bromocriptine revealed an influence on T-lymphocyte proliferation and IL-2 production *in vitro* [17], administration of bromocriptine *in vivo* did not alter cytotoxic T-lymphocyte activity in the murine system [18]. After treatment with DAs, B lymphocytes from patients revealed an increased expression of CD69 compared with healthy control subjects. As no difference could be observed between untreated patients and healthy control subjects, this phenomenon could be an effect of DA binding to B lymphocytes. Resting PBLs do not express CD69, but it is quickly induced in B cells following stimulation by cross-link of surface immunoglobulins or by IL-2. Existing data also suggest that CD69 molecules may act as a trigger for haemopoietic cells at different stages. However, the biological significance of CD69-induced cell activation is still unresolved [19].

Somatolactogens have been reported to be particularly effective in modulating the effector functions in phagocytic cells, including the production of reactive oxygen intermediates and the oxygen-dependent killing of bacteria [20,21]. Prolactin-primed monocytes for enhanced hydrogen peroxide production in response to phorbol 12-myristate 13-acetate (PMA) *in vitro* [22]. Growth hormone induces activation of neutrophils for enhanced respiratory burst *in vitro* [23]. This effect is supposed to be mediated via the PRL receptor and can be blocked by octreotide, a somatostatin analogue [24]. We could not observe increased metabolic and phagocytic activity of peripheral neutrophilic leucocytes in whole blood containing high PRL serum levels. Again, DA treatment did not alter the respiratory burst and phagocytosis in neutrophils. One reason could be that cells are adapted to elevated PRL concentrations under circulating conditions, for instance by down-regulation of their PRL receptors. Furthermore, leucocytes from healthy donors used in other studies had been incubated at higher PRL concentrations than under *in vivo* conditions.

We therefore conclude that PRL is required for lymphocyte maturation to achieve normal immune function but chronically high PRL levels do not lead to an 'overstimulation' of the immune system.

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