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Effect of Elevated Serum Prolactin Concentrations on Cytokine Production and Natural Killer Cell Activity

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Abstract
In vitro and in vivo studies in rodents and human suggested an immunostimulatory effect of prolactin. The aim of the present study was to determine the impact of chronically elevated serum prolactin concentrations on the immune system in patients with prolactinomas. For this purpose parameters of the humoral and cellular immune system were studied in seven patients with prolactinomas on two occasions (1) when their serum prolactin concentration had been normalized through treatment with dopamine agonists and (2) when their serum prolactin concentration was high. Serum concentrations of immunoglobulines, interleukin 1, 3 and 6, TNF-α, interferon-γ and the soluble interleukin 2 receptor, leukocyte subsets and the natural killer cell activity were found to be within the normal range on both occasions, i.e. at normal and at high serum prolactin concentrations. The assumption could be made that long-lasting elevation of serum prolactin concentration induces adaptive changes when the acute stimulatory effects of prolactin on several parameters of the immune system have subsided.

In vitro studies and investigations in animal models suggest that the immune system is an important target tissue for prolactin (PRL), a 24-kD single-chain hormone secreted by the anterior pituitary [1–4]. Impaired humoral and cell-mediated immune responses have been described in hypophysectomized rats and in animals treated with bromocriptine, a dopamine agonist drug inhibiting PRL secretion [5–7]. In all studies the immunocompetence could be restored by treatment with PRL. Furthermore, spleen natural killer (NK) cell activity against target cells was reduced in hypophysectomized mice and a direct stimulation of NK cells by PRL has been reported which was more effective than the known stimulator IL-2 [8]. The aim of the present study was to determine the impact of chronically elevated plasma PRL levels in patients with prolactinomas on the immune system. For this purpose patients with prolactinomas were examined on two occasions: (1) when their serum PRL concentration had been normalized through treatment with dopamine agonists and (2) when their serum PRL concentration was high.

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Table 1. General characteristics of the patients with prolactinomas

Case No	Age years	Sex	Treatment	Substitution therapy	Duration of disease	PRL under therapy µg/l	High PRL µg/l
1	38	f	bromocriptine	–	14 a	6.2	199
2	26	m	CV 205–502	–	2 a	32.9	386
3	43	m	bromocriptine CV 205–502	hydrocortone thyroxin	17 a	30.1	1,641
4	65	m	bromocriptine CV 205–502	thyroxin	4 a	24.5	207
5	49	m	bromocriptine	hydrocortone thyroxin	3 a	35.1	105
6	51	m	bromocriptine CV 205–502	thyroxin	3 a	35.4	88
7	44	f	bromocriptine CV 205–502	–	20 a	28.9	533

Subjects and Methods

Subjects

Seven patients with PRL secreting pituitary tumors were studied after informed consent had been obtained. Characteristics of the patients are given in table 1. All patients were studied on two occasions: with normal or close to normal serum PRL concentrations under therapy with dopamine agonists (mean PRL 27.6 ± 4.15 µg/l) and once more after 3 weeks without therapy (mean PRL 451.5 ± 207 µg/l). In addition to the 7 patients, 3 further subjects were tested; despite therapy their PRL concentrations remained considerably elevated and therefore they were excluded from the study. Regarding the results at these high PRL concentrations all findings were however similar and within the normal range as in the other patients. All patients had a solely PRL secreting adenoma and patients with secondary hypothyroidism (n = 4) or hypocortisolism (n = 2) received adequate substitution therapy. Blood was drawn at 8 a.m. and there was no history of any infections during and 3 weeks prior to the investigation.

Methods

Peripheral blood mononuclear cells (PMNC) were obtained by centrifugation of whole heparinized venous blood over a Ficoll Hypaque (Ficoll-Paque, Pharmacia, Uppsala, Sweden) density gradient. PMNC were subsequently suspended in RPMI 1640 (Gibco, Paisley, Scotland/UK) supplemented with 100 IU penicillin and 100 µg streptomycin (Gibco) per ml and adjusted to 1×10^6 PMNC/ml. NK assays were performed as described previously [9]. Each experiment was done with an at least equal number of control persons as patients tested. Three effector target (E:T) ratios (100:1, 50:1, 25:1) were prepared, in which ^{51}Cr-labeled (Behringwerke AG, Marburg, FRG) in vitro propagated NK-sensitive K562 cells served as targets. Target cells (0.125×10^6/ml) suspended in 100 µl RPMI 1640 were added to 100 µl effector cells in the appropriate dilution. All assays were performed in triplicate for each patient and each E:T cell ratio in serum-free RPMI 1640 supplemented with 100 IU penicillin and 100 µg streptomycin/ml. Before incubation suspensions containing effector and ^{51}Cr-labeled target cells were centrifuged at 100 *g* at room temperature for 3 min. After an incubation period of 4 h at 37 °C in a humidified atmosphere containing 5% CO_2, the resulting radioactivity in the supernatant was counted and the percentage of specific lysis assessed according to the formula:

$$\% \text{ specific lysis} = \frac{\text{experimental lysis-spontaneous lysis}}{\text{maximum lysis-spontaneous lysis}}$$

Maximum lysis was determined by treating ^{51}Cr-labeled K 562 cells with 1% SDS in distilled water. For the quantitative determination of cytokines in the plasma of the patients commercially available assays were used. IL-1, IL-3 and IL-6 were measured by ELISAs (Quantikine, R&D Systems, Minneapolis, Minn., USA) TNF-α was determined by an IRMA (Fa. Medgenix, Brussels, Belgium). IFN-γ was measured by ELISA (Endogen Inc., Boston, Mass., USA). The soluble IL-2 receptor was determined with an immunoenzymometric assay (Immunotech SA, Marseille, France). In addition the quantitative immunglobulin production and lymphocyte subset distribution was determined by flow cytometry of the whole blood sample.

Statistical Analysis

Data are expressed as the mean ± SEM. Means were compared by the Wilcoxon signed rank test and $p < 0.05$ was considered significant.

Table 2. Mean (± SEM) NK activity at different E:T ratios in patients with prolactinomas on two occasions: (1) when serum PRL concentration had been normalized through treatment with dopamine agonists and (2) when serum PRL concentration was high

	NK activity, E:T ratios, %		
Population	100:1	50:1	25:1
Low PRL	41.2 ± 5.9	34.7 ± 4.7	25.8 ± 4.1
High PRL	34.0 ± 7.8	27.0 ± 10.4	17.8 ± 4.8
Controls	43.4 ± 12.2	32.6 ± 13.5	22.3 ± 12.9

Table 3. Mean (± SEM) serum immunoglobulin concentrations in patients with prolactinomas on two occasions: (1) when serum PRL concentration had been normalized through treatment with dopamine agonists and (2) when serum PRL concentration was high

	IgG, mg/dl	IgA, mg/dl	IgM, mg/dl
Low PRL	1,268.4 ± 138.0	220.5 ± 50.4	115.1 ± 19.4
High PRL	1,200.0 ± 151.5	199.0 ± 46.9	104.2 ± 16.7
Controls	800 – 1,800	90 – 450	60 – 250

Table 4. Mean (± SEM) T lymphocyte subset distribution in patients with prolactinomas on two occasions: (1) when serum PRL concentration had been normalized through treatment with dopamine agonists; (2) when serum PRL concentration was high

	T lymphocyte subset distribution				
	CD 3 T cells	CD 4 T helper cells	CD 8 T suppressor cells	CD 19 B cells	CD 56 NK cells
Low PRL	72.6 ± 4.8	49.6 ± 4.9	24.8 ± 3.5	8.5 ± 3.5	12.0 ± 3.9
High PRL	74.6 ± 5.1	51.0 ± 6.8	24.6 ± 3.7	9.0 ± 2.0	11.0 ± 5.6
Controls %	46 – 83	32 – 62	13 – 45	5 – 19	8 – 22

Table 5. Mean (± SEM) serum levels of cytokines (IL-1, IL-3, IL-6, IFN-γ, TNF-α) and the soluble IL-2 receptor in patients with prolactinomas on two occasions: (1) when serum PRL concentration had been normalized through treatment with dopamine agonists; (2) when serum PRL concentration was high

	IL-1β pg/ml	IL2-R pM	IL-3 pg/ml	IL-6 pg/ml	IFN-γ pg/ml	TNF-α pg/ml
Low PRL	ND	76.1 ± 13.5	4.9 ± 2.2	ND	1.9 ± 1.2	3.8 ± 1.6
High PRL	ND	81.5 ± 11.6	2.5 ± 1.1	ND	2.7 ± 1.2	4.7 ± 1.3
Normal range	ND	25 – 115	0 – 30	ND	0 – 10	0 – 10

ND = Not detectable.

Results

Mean NK activity in 7 patients was 41.2 ± 5.9% when their serum PRL had been normalized through treatment with dopamine agonists and 34.0 ± 7.8% when their serum PRL was elevated at an E:T ratio of 100:1. Also, at E:T ratios of 50:1 and 25:1, the NK activity was similar whether or not the patients had normal or elevated serum PRL concentrations (table 2). The NK activity in the seven patients at high and normal PRL concentrations is shown in figure 1. The quantitative immunoglobulin concentrations were within the normal range and similar on both occasions (table 3). The percentage T and B lymphocytes and subsets of T lymphocytes including CD4+ T helper cells and CD8+ T suppressor cells as well as the CD4/CD8 ratio were evaluated and found to be within the normal range and again similar on both occasions tested (table 4). Serum levels of cytokines (IL-1, IL-3, IL-6, IFN-γ, TNF-α) and the soluble IL-2 receptor were normal and did not change between the two time points of investigation (table 5).

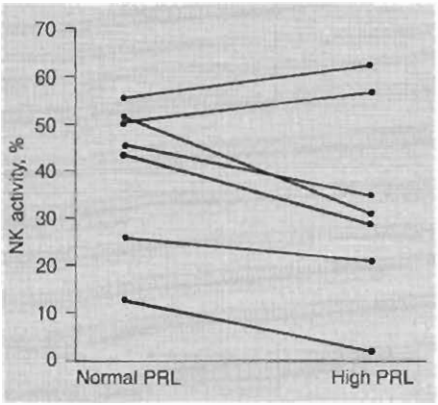

Fig. 1. NK activity in the prolactinoma patients at an E:T ratio of 100:1 at low and high plasma PRL concentrations.

Discussion

Investigations in animal models and in vitro studies have suggested a direct involvement of PRL in the regulation of the immune system. These findings have been further substantiated by the detection of PRL receptors on T and B lymphocytes [10–12]. In addition, PRL is required for the production of white blood cells in the bone marrow [13]. Furthermore, a rat lymphoid tumor cell line (Nb2) has been discovered which is dependent on PRL for growth [14], and antibodies to PRL have been reported to inhibit lymphocyte proliferation [15]. Preliminary data also suggest a beneficial effect of bromocriptine in the treatment of autoimmune diseases such as endogenous iridocyclitis and endocrine orbitopathy [16, 17] as well as adjuvant therapy after heart transplantation [18]. However, until now there has been no study where the relevance of these in vitro findings and of the indirect evidence of a possible influence of PRL on the immune system has been tested. Therefore, the present study was made in an effort to test for the physiological or pathophysiological relevance of prolactin on several parameters of the immune system in humans.

To our surprise PRL had no effect on any parameter of cellular and humoral immunity determined in this study. The present data demonstrate that patients with high PRL levels produced by a pituitary adenoma had no increased NK cell activity when compared to the NK activity at low PRL levels or of normal control persons. Rather, a tendency to an increase in NK activity was noted when serum PRL concentrations were suppressed by treatment with bromocriptine. It cannot be totally ruled out that an immunostimulating effect of PRL remained unmasked in the hyperprolactinemic patients because of an additional immunostimulating effect of bromocriptine in the treated bromocriptine patients with normal or near normal PRL levels. In this case the suppression of elevated PRL levels and the associated normalization of the stimulated immune system would be counteracted by an immunoenhancing effect of bromocriptine itself. However, because of the findings in animal models where cellular and humoral immunity were suppressed by bromocriptine this possibility seems unlikely. A different explanation for the findings of the present study is the report of a dose dependency of the PRL effect on NK activity of large granular lymphocytes (LGL) [8]. There, physiological to slightly supraphysiological concentrations of PRL enhanced NK activity of LGLs whereas higher concentrations inhibited NK activity. Furthermore, prolactin in high doses induced also NK inhibiting factors in unseparated peripheral blood lymphocytes [8]. Thus, the immunoenhancing effects of PRL observed in vitro could be abolished by simultaneously occurring immunosuppressive effects in vivo. The PRL effect on the immune system could also be time-dependent. Thus chronic PRL elevation could lead to adaptive changes where the acute immunomodulatory properties of PRL would be abolished.

Recent reports suggest that IL 6 is produced by the normal pituitary gland and a subset of pituitary adenomas and thus possible changes could be presumed in patients with prolactinomas. Furthermore several reports demonstrated an influence of various cytokines on the regulation of PRL release [19–23] and because of the bidirectional nature of the relationship between the endocrine and immune systems an effect in the opposite direction – i.e. from the endocrine system towards the immune system – seemed possible. Thus, in addition to plasma concentrations of immunoglobulins and subsets of peripheral blood mononuclear cells, the plasma concentrations of the cytokines IL-1, IL-3, IL-6, IFN-γ and TNF-α and of the soluble IL-2 receptor were measured. Surprisingly – like the NK activity – neither the leukocyte subsets nor the plasma concentrations of immunoglobulins, or any cytokine or the soluble IL-2 receptor differed whether or not the patients had markedly elevated PRL concentrations or normal PRL concentrations. On the other hand these findings fit well with the previously reported lack of considerable changes of the immune system during pregnancy [24, 25] where serum prolactin levels are physiologically elevated. This seems reasonable, since a substantial stimulation of the immune system during pregnancy would render mammalian reproduction impossible. The

in vitro observed immunostimulatory effects of PRL could also be counteracted in pregnancy by simultaneously occurring changes in immunosuppressive hormones such as progesterone, CRH and glucocorticoids [26]. In the prolactinoma patients either a concentration dependency or adaptive changes with a time factor can be considered responsible for the lack of observable change in the immune system. In addition, local changes of cytokine concentrations without alteration to the circulating concentrations cannot be ruled out. The fact that the incidence of infections or malignant diseases appears to be unchanged in prolactinoma patients indicates that the immune system is functioning normally.

In summary, chronically elevated serum PRL concentrations in patients with prolactinomas appear not to stimulate the immune system. The abolished effect of acutely elevated PRL levels could either be due to a concentration dependency of the PRL effect on the immune system or by adaptive changes during prolonged elevation of PRL concentration.

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