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Prolactin enhances production of interferon-γ, interleukin-12, and interleukin-10, but not of tumor necrosis factor-α, in a stimulus-specific manner

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Abstract

Prolactin, an anterior pituitary hormone, has been shown to have a role in immunomodulation. Some reports have shown the importance of prolactin in activating lymphocytes and macrophages, while in hyperprolactinemia patients, prolactin was found to decrease lymphocyte activation and natural killer function. In the present work, at physiological (15 ng/ml) and stress-induced levels (30 ng/ml) of prolactin, interferon-γ (IFN-γ) and interleukin (IL)-12 p70 levels, but not of IL-10 and tumor necrosis factor-α (TNF-α), increased significantly ($p < 0.05$--0.006) in phytohemeagglutinin (PHA) + lipopolysaccharide (LPS)-stimulated whole blood. However, no such effect was observed at high concentrations of prolactin (100–300 ng/ml). In addition, 15 ng/ml of prolactin reversed hydrocortisone suppressive effect on IFN-γ, IL-12 p70, and IL-10 production in PHA + LPS-stimulated whole blood. On the other hand, in LPS-stimulated whole blood, prolactin enhanced significantly ($p = 0.027$) the production levels of IL-10, but not of IFN-γ, IL-12 p70, and TNF-α, in non-concentration-dependent manner. These results suggest that prolactin modulates cytokine response during antigenic response, and this modulation is stimulus specific.

Keywords: Interferon-γ; Interleukin-10; Interleukin-12; Prolactin; Tumor necrosis factor-α

1. Introduction

The balance between cell-mediated and humoral immunity is maintained by the release of cytokines from the T helper (Th) lymphocytes. Th lymphocytes are divided into two subpopulations, Th1 and Th2 cells, based on their ability to produce specific pattern of cytokines [1,2]. Th1 cells induce cell-mediated immunity via their release of cytokines, such as interleukin (IL)-2 and interferon-γ (IFN-γ), while Th2 cells induce humoral immunity via their release of cytokines, such as IL-4, IL-5, and IL-10. However, naïve T helper cells (Th0) serve as precursors to either Th1 or Th2 cells, depending on the signal of activation. Cytokine, such as IL-12, produced by activated monocytes/macrophages or other antigen-presenting cells, is a major inducer of Th1 cell and its cytokines. Monocytes/macrophages-derived IL-12 and tumor necrosis factor-α (TNF-α), with natural killer (NK) cells and Th1-derived IFN-γ, stimulate the function of T cytotoxic cells, NK cells, and activated macrophages. IL-12, IFN-γ, and TNF-α are considered major inflammatory cytokines because they stimulate the synthesis of nitric oxide and other inflammatory mediators that derive chronically delayed hypersensitivity reactions [1–4]. While IL-12 and IFN-γ can inhibit Th2 response, Th2 cytokines such as, IL-10 and IL-4, inhibit Th1 activity and macrophage activation. In addition, they stimulate differentiation of B cells to antibody-producing cells (especially class switching to IgE) and stimulate the growth and activation of eosinophils and mast cells [1–3]. Therefore, Th1 and Th2 responses are mutually inhibitory [5].

Prolactin, a polypeptide hormone, is synthesized in and secreted from the anterior pituitary gland. It serves not only in reproduction and lactation, but also in homeostasis of the individual, such as in immune

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regulation [6]. When prolactin was depleted in vivo by bromocryptine, a dopamine receptor agonist, a decrease in antibody response following immunization with sheep red blood cells [7] and prolongation of graft survival was observed [8]. The effect of bromocryptine on the immune function was reversed by administration of prolactin. Moreover, prolactin was found to stimulate lymphocytes proliferation [9] and macrophage function [10], maybe through the expression of prolactin receptors on immune cells [6]. It was shown that T lymphocyte activation by IL-2 requires prolactin [11] with which it shares target transcription factors, such as IRF-1. This IRF-1 is one of the first genes activated by prolactin [12]. In addition, prolactin enhanced IFN-γ production from NK cells and T lymphocytes [13,14]. These results suggest that prolactin enhance the function of Th1-mediated response. Other reports, however, showed that NK cell function from hyperprolactinemia patients was suppressed [15], and lymphocytes proliferation to mitogens and IL-2 production were decreased [16].

In the present sets of experiments, the influence of physiological, stress-induced [17,18] levels of cortisol, prolactin, high levels of prolactin, and cortisol–prolactin combined on monocyte (IL-12 p70, TNF-α, IL-10), Th1 (IFN-γ and TNF-α), and Th2 (IL-10) cytokines production following stimulation of whole blood with different mitogens were studied. Cortisol is used in the present study because of its known inhibitory action on the production of Th1-derived (IL-12) and Th1 (IFN-γ) cytokines, and, to a lesser extent, on Th2 cytokines [19,20]. This study should give a better understanding as to how prolactin interacts and modulates immune-mediated functions through its role in enhancing or inducing cytokines when combined with T-dependent and T-independent mitogens. Moreover, using whole blood gives more comparable results with in vivo condition by keeping all the the physiological cellular interactions and natural microenvironment intact [21–23].

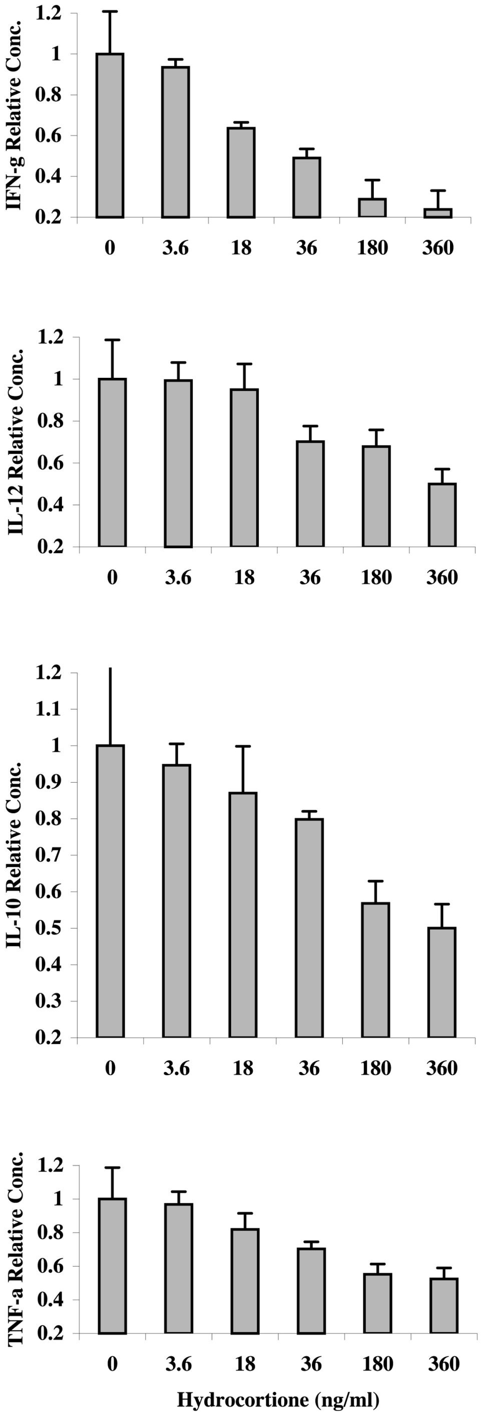

Fig. 1. Effect of hydrocortisone on cytokines production in PHA + LPS-stimulated whole blood from 16 healthy volunteers (four males and 12 females with a mean age of 24.9 ± 4.8). Increasing concentrations of hydrocortisone was added as indicated and it suppressed significantly ($p < 0.001$) the production of all cytokines. Blood samples from males or females behaved similarly, on exposure to hydrocortisone. Data are expressed as relative mean ± SE. (a) Mean PHA + LPS-induced IFN-γ production was 383 ± 68.5 pg/ml, (b) mean PHA + LPS-induced IL-12 p70 production was 15.9 ± 3.2 pg/ml, (c) mean PHA + LPS-induced IL-10 production was 829.9 ± 144.0 pg/ml, and (d) mean PHA + LPS-induced TNF-α production was 802.2 ± 69.5 pg/ml.

2. Results

2.1. Hydrocortisone suppresses phytohemeagglutinin + lipopolysaccharide-induced IFN-γ and, to a lesser extent, IL-10, IL-12 p70, and TNF-α levels

Increasing concentrations of hydrocortisone suppressed significantly the IFN-γ production ($p < 0.001$) in phytohemeagglutinin (PHA) + lipopolysaccharide (LPS)-stimulated whole blood, which was dose-dependent (Fig. 1). The concentration of hydrocortisone to inhibit 50% (CI 50%) of IFN-γ production was 32 ng/ml (89 nmol/l). In addition, IL-12 p70, IL-10, and TNF-α production levels were also significantly reduced ($p < 0.001$) on increasing concentration of hydrocortisone in PHA + LPS-stimulated whole blood. However, CI 50% for IL-12 p70, IL-10, and TNF-α were 357, 498, and 446 ng/ml (991, 1382, and 1239 nmol/l) of hydrocortisone, respectively (Fig. 1).

2.2. Prolactin increases PHA + LPS-induced IFN-γ and IL-12 p70, but not IL-10 or TNF-α levels

Overall, increasing concentrations of prolactin produced significant changes in IFN-γ and IL-12p70 levels ($p < 0.02$ and 0.05, respectively) in PHA + LPS-stimulated whole blood (Fig. 2). At 15 and 30 ng/ml concentration of prolactin, IFN-γ and IL-12p 70 production levels increased significantly ($p = 0.01$ and 0.008 for IFN-γ, 0.05 and 0.006 for IL-12p70, respectively) in PHA + LPS-stimulated whole blood (Fig. 2). This increase in IFN-γ and IL-12 p70 productions, however, was not observed at higher concentrations of prolactin (Fig. 2). No effect of prolactin was observed at the concentrations used for the production of IL-10 or TNF-α in PHA + LPS-stimulated whole blood (Fig. 2).

2.3. Prolactin reverses hydrocortisone suppressive effect on cytokines production in PHA + LPS stimulation

In the preceding experiments, the effect of prolactin to reverse hydrocortisone suppressive effect on IFN-γ, IL-12 p70, IL-10, and TNF-α production in PHA + LPS-stimulated whole blood was studied. At a concentration of 15 ng/ml, it was able to reverse hydrocortisone (36 ng/ml or 100 nmol/l) suppressive effect on the production of IFN-γ, IL-12 p70, and IL-10, but not of TNF-α (Fig. 3). The relative concentrations of IFN-γ, IL-12 p70, and IL-10 went up from 0.51, 0.65, and 0.81 to 1.05, 1.1, and 0.91, respectively ($p < 0.03$, 0.03 and 0.05 to 0.5, 0.5 and 0.8, respectively).

2.4. Prolactin alone increased basal IL-12p70 and, to a lesser extent, IL-10, but not IFN-γ or TNF-α levels

The possibility that prolactin itself, without PHA and LPS, could induce the production of IFN-γ, IL-12 p70, IL-10, and TNF-α, was tested in whole blood from five healthy volunteers. Increasing concentrations of prolactin did not affect the basal (without mitogens) concentrations of IFN-γ and TNF-α. The basal level of IL-12 p70 was within the range from undetectable (<4) to 5 ng/ml and went up to an average of 11.5, 10.1, and 8.8 pg/ml at prolactin concentrations of 5, 15, and 30 ng/ml, respectively. IL-10 relative concentration was 1.35, 1.26, and 1.07 at 5, 15, and 30 ng/ml of prolactin, respectively.

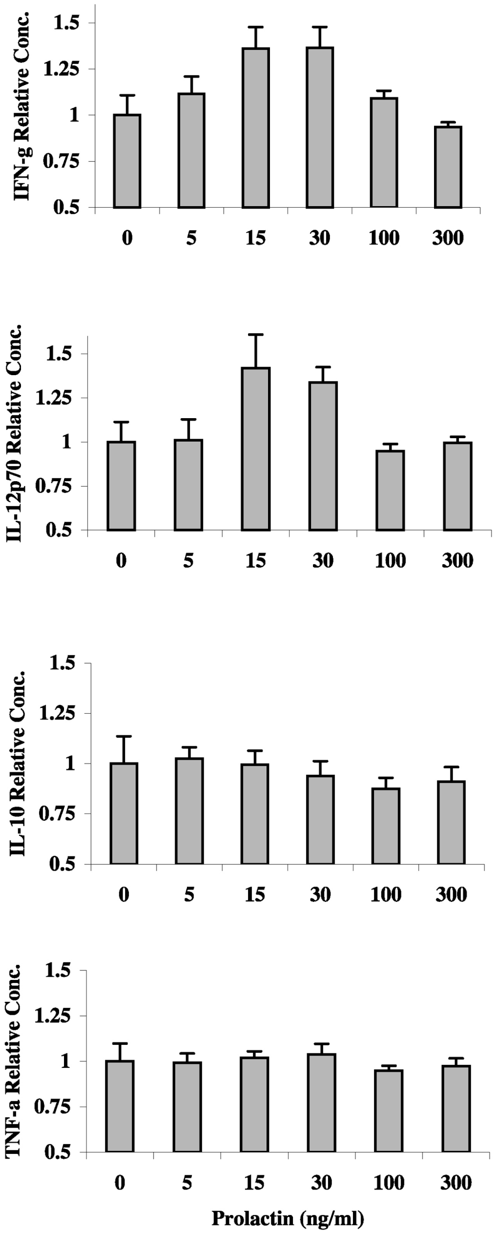

Fig. 2. Effect of prolactin on cytokines production in PHA + LPS-stimulated whole blood from 15 normal volunteers (four males and 11 females with a mean age of 24.4 ± 4.7). At prolactin concentration of 15 and 30 ng/ml, IFN-γ and IL-12 p70 production levels significantly increased ($p = 0.01$ and 0.008 for IFN-γ, 0.05 and 0.006 for IL-12p70, respectively), but no effect on IL-10 and TNF-α levels was observed. Data are expressed as relative mean ± SE. (a) Mean PHA + LPS-induced IFN-γ production was 345.7 ± 74.6 pg/ml, (b) mean PHA + LPS-induced IL-12 p70 production was 18.8 ± 2.9 pg/ml, (c) mean PHA + LPS-induced IL-10 production was 776.4 ± 141.6 pg/ml, and (d) mean PHA + LPS-induced TNF-α production was 823.7 ± 162.3 pg/ml.

2.5. Prolactin increases LPS-induced IL-10, but not TNF-α, IFN-γ or IL-12 p70 levels

Increasing concentrations of prolactin significantly enhanced IL-10 production in LPS-stimulated whole blood ($p = 0.027$) (Fig. 4). The increase was highest at 5 ng/ml of prolactin and remained high even at higher prolactin concentrations. In addition, TNF-α production slightly increased at 5 ng/ml prolactin in LPS-stimulated whole blood (Fig. 4), but with no statistical significance. However, no effect of prolactin on IFN-γ and IL-12p70 production in LPS-stimulated whole blood was observed.

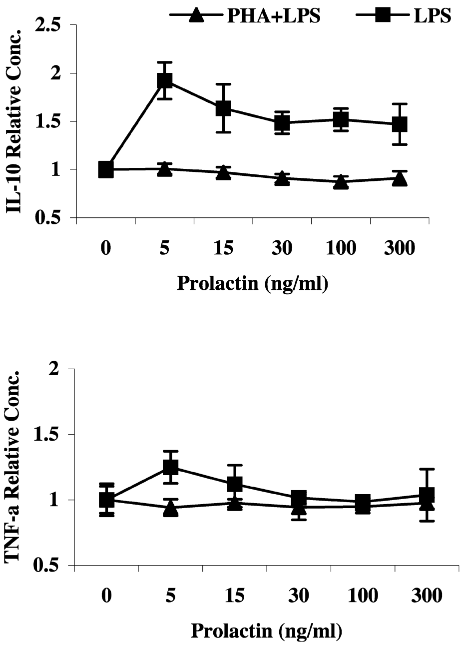

Fig. 4. Prolactin increases IL-10 production in LPS-stimulated whole blood from six healthy volunteers (three males and three females with a mean age of 24.0 ± 4.1). Increasing concentrations of prolactin was added as indicated and IL-10 production was significantly increased ($p = 0.027$). Data are expressed as relative mean ± SE. (a) Mean LPS-induced IL-10 production was 149.4 ± 43.4 pg/ml and (b) mean LPS-induced TNF-α production was 365.8 ± 99.0 pg/ml.

2.6. PHA + LPS versus LPS-stimulated whole blood on IL-12 p70, IL-10, IFN-γ, and TNF-α production

At zero hormone concentration, 1 μg/ml of LPS-stimulated whole blood did not induce IFN-γ production more than unstimulated whole blood (basal level), and PHA + LPS-stimulated whole blood produced more IL-10 and TNF-α levels than LPS alone ($p < 0.03$ and 0.03, respectively) (Fig. 5). These results suggest that: (1) 1 μg/ml of LPS has no effect on IFN-γ (and probably other cytokines) production from T lymphocytes, and (2) in PHA + LPS-stimulated whole blood, both monocytes

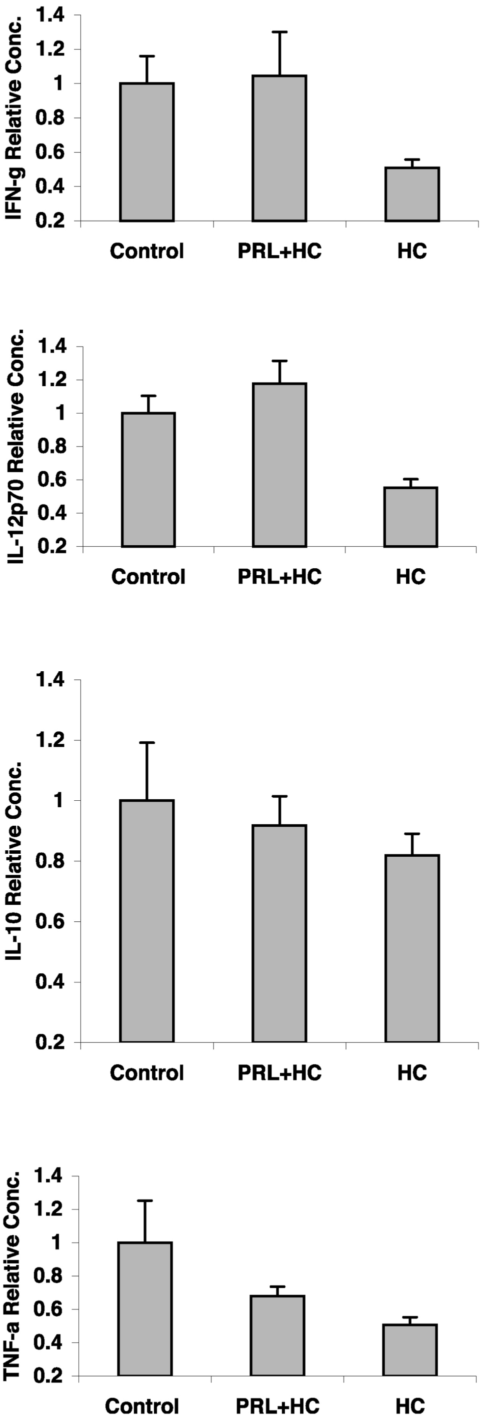

Fig. 3. Prolactin reverses hydrocortisone suppressive effect on cytokines production in PHA + LPS-stimulated whole blood from five healthy volunteers (two males and three females with a mean age of 23.0 ± 5.2). Blood was incubated with no hormone, or with prolactin (15 ng/ml) and hydrocortisone (36 ng/ml) or hydrocortisone (36 ng/ml) alone for 24 h, then blood was stimulated with PHA + LPS for another 24 h. Addition of prolactin reversed the hydrocortisone suppressive effect on IFN-γ, IL-12 p70, and IL-10 production (i.e. relative concentrations and p values became insignificant, see text for details), but not on TNF-α. Data are expressed as relative mean ± SE. (a) Mean PHA + LPS-induced IFN-γ production was 576.1 ± 94.7 pg/ml, (b) mean PHA + LPS-induced IL-12 p70 production was 15.2 ± 1.6 pg/ml, (c) mean PHA + LPS-induced IL-10 production was 969.8 ± 186.3 pg/ml, and (d) mean PHA + LPS-induced TNF-α production was 1251.2 ± 316.7 pg/ml.

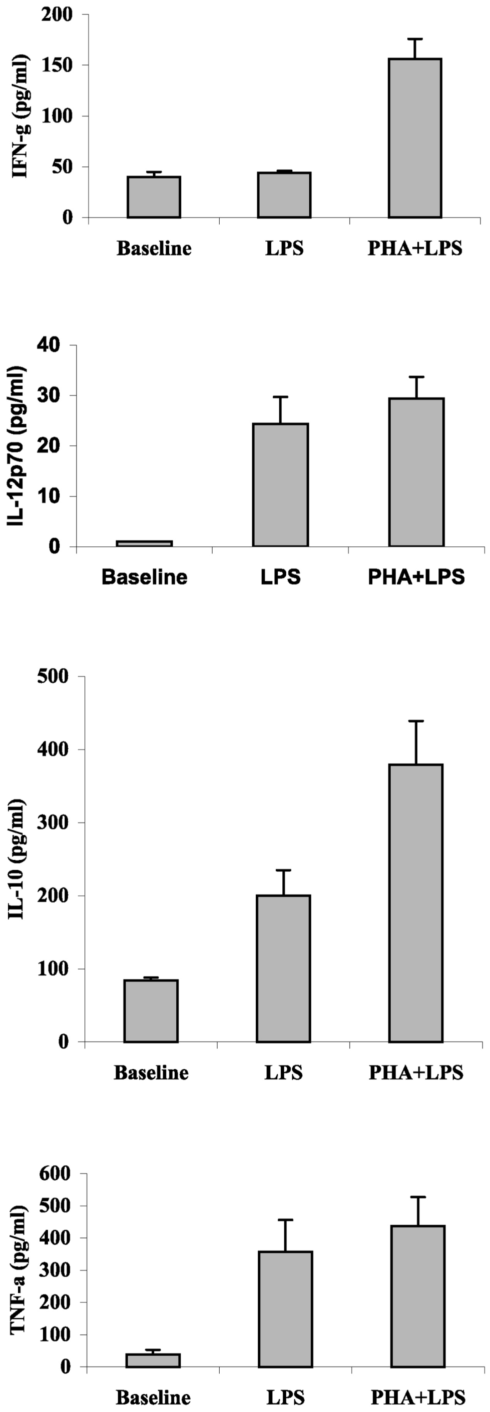

Fig. 5. PHA + LPS versus LPS stimulation of whole blood to induce cytokines from four healthy volunteers (four males and four females with a mean age of (21.5 ± 0.6). PHA + LPS-stimulated whole blood produced significantly more IFN-γ ($p < 0.03$), IL-10 ($p < 0.03$), and TNF-α ($p < 0.03$), but not IL-12 p70, than LPS-stimulated whole blood.

and T lymphocytes are responsible for producing IL-10 and TNF-α. The first observation is similar and supported by Kwak et al. study [24] and the second is supported by the data of Elenkov et al. [25,26] that monocytes are the primary source of IL-12, IL-10, and TNF-α in LPS-stimulated whole blood assay.

3. Discussion

The present work showed that prolactin enhanced the production of IFN-γ, IL-12 p70, IL-10, but not of TNF-α, from whole blood in a stimulus-specific manner. Prolactin enhanced production of IFN-γ and IL-12 p70 in PHA + LPS, but not of LPS-stimulated whole blood. This enhancement was seen at physiological and stress-induced prolactin concentrations (15–30 ng/ml) [17,18]. At high prolactin concentrations, however, this increase in IFN-γ and IL-12 p70 levels was not observed. At physiological concentrations of prolactin, activation of prolactin receptor involves prolactin-induced sequential receptor dimerization by a single hormone molecule, whereas in high concentrations, every receptor becomes engaged and the cross-linking cannot occur [6]. This may explain as to why at high prolactin concentrations, IFN-γ and IL-12 p70 production levels in PHA + LPS-stimulation were not increased. However, in LPS stimulation, IL-10 concentration remained elevated even at high concentrations of prolactin. The latter observation may question the role of prolactin receptors in immune cells. Recently, Bouchard et al. [27] showed that prolactin receptors-deficient mice have normal immune function and prolactin receptor pathways were not essential for immunomodulation.

At physiological concentration (15 ng/ml), prolactin was able to reverse hydrocortisone inhibitory effect on IFN-γ, IL-12 p70, and IL-10 production. In these experiments, prolactin and cortisol were simultaneously added to cultured cells for 24 h before mitogen stimulation. This was done to ensure maximum effect of each hormone and to resemble the single-hormone experiments. Recently, it has been shown that in order to induce the maximum suppressive effect of cortisol on cytokines production, the immune cells should be exposed to cortisol for a minimum of 24 h [19]. On the other hand, following brief in vitro exposure to glucocorticoids, mitogen-stimulated peripheral blood mononuclear cells (PBMC) increased IFN-γ and IFN-γ/IL-10 ratio [19]. In addition, brief in vivo exposure to cortisol (2 h) increased delayed-type hypersensitivity (DTH) reaction [28] and is found to be mediated by local increase in IFN-γ production [29].

Recently, Matalka et al. [18] have shown that a stress (e.g. academic examination) induces reactivation of latent Epstein-Barr virus (EBV), which was indicated by increasing levels of EBV IgG. This increase in EBV IgG levels was not correlated with the increase in stress-cortisol levels, but was negatively correlated with the increase in prolactin levels. In other words, if no increase in prolactin levels following stress to counter the effect of higher levels of cortisol occurred, T cytotoxic lymphocytes control over EBV is reduced and, therefore, EBV genes are reactivated. In addition, elevated prolactin and prolactin to cortisol ratio was examined in Th1-mediated autoimmune diseases. It was observed that prolactin and prolactin to cortisol ratio was higher in active rheumatoid arthritis (RA) at times when proinflammatory cytokines were elevated too [30].

Prolactin enhanced production of IL-10 in LPS-stimulated whole blood. This increase was highest at 5 ng/ml of prolactin and it remained elevated at high concentrations of prolactin. This increase in IL-10 following prolactin and LPS stimulation was not accompanied with any change in basal IFN-γ levels. These observations suggest: first, prolactin and LPS produced higher levels of IL-10 due to nil activation of Th1 or NK cells as they are the only sources of IFN-γ, and second, the present IL-12-induced concentrations do not induce IFN-γ by themselves, and may be higher IL-12 concentration [14] or another T cell stimulus is needed.

In a number of autoimmune diseases, such as systemic lupus erythematosus (SLE), RA, multiple sclerosis (MS), and autoimmune thyroiditis, hyperprolactinemia was observed [31–34]. However, the role of prolactin in the pathogenesis of such autoimmune diseases was difficult to establish. In SLE, T cell response is mediated by Th2 dominance with excessive production of IL-10, while IL-12 and TNF-α productions appear to decrease [35–37]. In RA and MS, Th1 is more pronounced and excess of IL-12, IFN-γ and TNF-α levels were observed while IL-10 level decreased [38–40]. The present study may explain, in part, the role of prolactin in Th1- and Th2-mediated autoimmune diseases—that enhancing cytokines production by prolactin is based on the type of stimulus that activates the immune cells. Therefore, this study suggests that reducing prolactin to minimal levels (<5 ng/ml) in Th1- or Th2-mediated autoimmune diseases might help the outcome of the disease [15].

The present data support the fact that prolactin alone is not highly effective on immune regulation, but it modulates cytokine response during an antigenic response. This modulation of prolactin seems to be stimulus-specific; prolactin enhances IL-10 production with T-independent mitogens, while it enhances IFN-γ and IL-12 with T-dependent mitogens. Further studies in the latter direction are mandatory to explain the role of prolactin in the complex cytokine network, stress-induced, and autoimmune conditions in a better way.

4. Materials and methods

4.1. Reagents

All the following reagents, RPMI 1640, penicillin–streptomycin, L-glutamine, LPS (L-6143), PHA-L (L-4144), hydrocortisone, and bovine serum albumin (BSA), were purchased from Sigma. Endotoxin-free Dulbecco's phosphate buffer (without calcium and magnesium) was obtained from PAA Laboratories Gmbh (Linz, Austria). Recombinant human prolactin was obtained from R&D systems UK. Culture six-well plates and maxisorp 96-well flat bottom plates were purchased from Nunc International (Denmark).

4.2. Subjects

Twenty-two healthy male and female (nine males and 13 females) volunteers with ranging age between 19 and 37 years old enrolled in this study. All volunteers signed an informed consent. All females participated in the study were in the early to mid follicular phase (days 3–9). None of the volunteers have taken any medication for at least a week before the blood sample was drawn. All blood samples were drawn in between 8:00 and 9:30 a.m.

4.3. Whole blood culture

Blood was drawn into sterilized sodium heparin tubes (Vacutainer, Becton-Dickinson) and processed within 45 min. Whole-blood cytokines productions were performed as described elsewhere [21–23] with modifications. The blood was diluted to 1 : 9 with RPMI 1640, supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, without exogenous serum. To each well of the six-well culture plates, 1.8 ml of the diluted blood was added. Hydrocortisone or prolactin was added to each well in 0.2 ml volumes of sterilized phosphate buffer, giving a final dilution of the blood 1:10. The plates then were incubated in 5% CO_2 at 37 °C for 24 h. After the first incubation, PHA + LPS, or LPS alone, in 40 μl volume was added to give a final concentrations of 5 and 1 μg/ml for PHA and LPS, respectively, and incubated in 5% CO_2 at 37 °C for another 24 h. After the second incubation, the blood was collected from wells into sterilized tubes and each well was washed with 0.5 ml of RPMI to ensure removal of all well content. Tubes were centrifuged and supernatant was separated, aliquoted and stored in sterilized tubes at −30 °C until assayed.

4.4. Cytokine assays

Measurements of IL-12 p70, IL-10, IFN-γ, and TNF-α were accomplished by ELISAs developed by the author using the adapted procedure recommended by the manufacturer (DuoSet R&D Systems, UK). Briefly, captures antibodies for all cytokines were coated at 4 μg/ml in PBS, pH 7.2–7.4, and anti-cytokine-biotinylated detector antibodies for IL-12 p70, IFN-γ, and TNF-α were used at 175 and 600 ng/ml for IL-10. Standards (human recombinant) for all assays were used in the range 15.6–1000 pg/ml, except for IL-12 p70 (7.8–500 pg/ml) with 7 points of standard curve and the zero standard. Strepavidinx–Horseradish peroxidase conjugate with H_2O_2–Tetramethylbenzidene (R&D, UK) substrate was used. Plates were read by Wellscan Denley ELISA plate reader and absorbance was transformed to cytokine concentrations (pg/ml) using a standard curve computed on Excel developed by the author. The sensitivities of IL-12 p70, IL-10, IFN-γ, and TNF-α assays were 4, 4, 8, and 5 pg/ml, respectively.

4.5. Data analysis

The entire data in Figs. 1–4 are presented as relative concentration of each cytokine ($\pm$SE). The relative concentration is a better indicator of change on stimulation of hormone at any given concentration to that of hormone-free condition (own control) in any blood sample from one individual. The mean of actual concentration ($\pm$SE) for each cytokine measured with PHA and LPS is also given in figure legends. Comparisons between different conditions of hormone concentrations/conditions were analyzed by one-way ANOVA. Paired *t*-test was used to compare the two conditions (specific concentration with baseline) when $n > 10$, whereas Wilcoxin-rank test was performed when $n < 10$.

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