

# The effect of estradiol, but not progesterone, on the production of cytokines in stimulated whole blood, is concentration-dependent

**Khalid Z. Matalka**

Faculty of Pharmacy and Medical Technology, University of Petra, Amman, JORDAN.

*Correspondence to:* Khalid Z. Matalka, Ph.D.  
Faculty of Pharmacy and Medical Technology,  
University of Petra,  
P. O.Box 961343, Amman, JORDAN  
TEL: 962-6-571-5546 FAX: +962-6-571-5570  
EMAIL: kzm@uop.edu.jo  
EMAIL: kzm@go.com.jo

*Submitted:* March 3, 2003

*Accepted:* March 7, 2003

*Key words:* **estradiol; progesterone; TNF- $\alpha$ ; IFN- $\gamma$ ; IL-10; IL-12**

*Neuroendocrinol Lett 2003; 24(3/4):185-191 pii: NEL243403A06 Copyright © Neuroendocrinology Letters www.nel.edu*

## **Abstract**

**OBJECTIVES:** The purpose of this study was to determine the effects of estradiol and progesterone on interferon- $\gamma$  (IFN- $\gamma$ ), interleukin (IL)-12, IL-10 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) productions in polyclonal activators (phytohemagglutinin+lipopolysaccharide)-stimulated whole blood cultures.

**METHODS:** Nineteen healthy males and females volunteered in the study. Blood samples were drawn, diluted, and cultured for 24h with different concentrations of estradiol, progesterone or hydrocortisone and then PHA+LPS was added for another 24 h. The supernatant, then, was harvested and assayed for IL-12 p70, IFN- $\gamma$ , IL-10 and TNF- $\alpha$ .

**RESULTS:** At preovulatory concentrations, estradiol enhanced significantly IFN- $\gamma$ , IL-12 and IL-10, but not TNF- $\alpha$ , production levels and reversed the suppressive effect of hydrocortisone in PHA+LPS stimulated whole blood. While IL-10 levels kept increasing at pregnancy estradiol concentrations, IFN- $\gamma$ , IL-12 levels and IFN- $\gamma$ /IL-10 ratio decreased significantly. No effect of progesterone on IL-12 p70, IFN- $\gamma$ , IL-10 and TNF production levels was observed.

**CONCLUSIONS:** The present study shows that those pregnancy estradiol concentrations (and higher) enhance the production of IL-10 and reduce IL-12, IFN- $\gamma$  levels and IFN- $\gamma$ /IL-10 ratio in stimulated whole blood cells. Because of the known IL-10 inhibitory actions on T helper (Th) 1 cells and monocytes/macrophages, these high IL-10 levels keep Th2 cytokines favored during pregnancy and may be useful in shifting Th1-mediated autoimmune diseases towards non-pathogenic Th2 pathway.

## Introduction

Cytokines produced by leukocytes play an important role in the initiation and regulation of the immune response and basically the balance between cell-mediated and humoral immunity. This balance is initiated and maintained by different pattern of cytokines released from T helper (Th) lymphocytes, CD4+ cells, which are designated into two subpopulations, Th1 and Th2 cells. Th1 cells produce interleukin-2 (IL-2) and interferon- $\gamma$  (IFN- $\gamma$ ) and less specifically tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and thereby stimulating cell mediated immunity whereas Th2 cells produce IL-4, IL-5, and IL-10, which induce humoral immunity [1]. However, naive T helper cells (Th0) serve as precursors to either Th1 or Th2 cells depending on the signal of activation. For instance, IL-12, produced by activated monocytes/macrophages or other antigen presenting cells, is a major inducer of Th1 lymphocyte and its cytokines. IL-12 and TNF- $\alpha$  derived from monocytes/macrophages and IFN- $\gamma$  derived from Th1 and natural killer (NK) lymphocytes stimulate the function of T cytotoxic cells, NK cells, and activated macrophages [1–3]. Cytokines such as, IL-12, IFN- $\gamma$  and TNF- $\alpha$  are considered major inflammatory cytokines because they stimulate the synthesis of nitric oxide and other inflammatory mediators that derive chronic delayed hypersensitivity reactions [1–4]. While IL-12 and IFN- $\gamma$  promote Th1 response, IFN- $\gamma$  blocks Th2 cytokines (IL-4 and IL-5) production. On the other hand, 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 and stimulate the growth and activation of eosinophils and mast cells [1–3]. Therefore, Th1 and Th2 responses are mutually inhibitory [4]. Additionally, CD8+T lymphocytes can secrete Th1 and Th2 cytokines and were designated Tc1 and Tc2, respectively [1].

Estrogen and progesterone are steroid hormones that cause definite changes in the endometrium in preparation to receive and implant a fertilized ovum. High levels of both estrogen and progesterone are found during induction of ovulation (preovulation) and following ovulation, respectively, and even higher levels are found during pregnancy. Estrogen role in pregnancy may be to ensure optimum blood flow in the uterus, while progesterone is important to maintain the pregnancy. Furthermore, these hormones and others were found to modulate immune response in several models. For instance, estrogen receptors were found to be expressed in the human thymus and CD8+T cells [5] and then later estrogen receptors alpha were found to be expressed in all lymphocytes subsets [6]. 17 $\beta$ -estradiol (E2), the biological active form of estrogen, enhanced antigen-stimulated IL-10, and to lesser extent IFN- $\gamma$ , secretion in dose dependent manner from T cell clones isolated from normal subjects and from multiple sclerosis patients [7]. Progesterone, on the other hand, was found to favor the development of human Th2 cells by producing IL-4 and IL-5 cytokines from T cell lines [8] and to increase leukemia inhibiting

factor production from T cells obtained from the fetal maternal interface [9]. The above findings and with the clinical observations that during pregnancy some Th1-mediated autoimmune diseases such as rheumatoid arthritis and multiple sclerosis exacerbation attacks are remitted [10–11] suggest a shift towards a Th2 response. This shift toward a Th2 response may be triggered by increase in circulatory levels of 17 $\beta$  estradiol and progesterone during pregnancy. In lipopolysaccharide (LPS)-stimulated whole blood, however, 10<sup>-11</sup>–10<sup>-5</sup> M of estradiol or progesterone did not modulate the production of IL-12, TNF- $\alpha$  or IL-10 [12].

In the present study, the effect of estradiol and progesterone in ex vivo whole blood cytokines assay model using a combination of T-dependent and independent activators was examined [13–15]. This model is more comparable to in vivo condition by keeping all the physiological cellular interactions and natural microenvironment intact [16–17]. Second, the effects of physiological concentrations of estradiol and progesterone found in the peripheral circulation during normal menstrual cycle or during pregnancy, as well as pharmacological estrogen doses on IL-12 p70, TNF- $\alpha$ , IL-10 and IFN- $\gamma$  cytokines production was evaluated. Cortisol is also used in the present study because of it is known inhibitory action on the production of Th1-derived (IL-12) and Th1 (IFN- $\gamma$ ) cytokines, and to lesser extent on Th2 cytokines [18] and its high level during pregnancy [12]. This study should give a better understanding of how estradiol and progesterone modulate immune mediated functions through their role in affecting the induction of cytokines when combined with polyclonal activators.

## Materials and methods

### Reagents

All the following reagents: RPMI 1640, penicillin-streptomycin, L-glutamine, lipopolysaccharide (LPS, L-6143), phytohemagglutinin (PHA-L, L-4144), hydrocortisone, 17- $\beta$  estradiol, and bovine serum albumin (BSA) were purchased from Sigma. Progesterone was a gift from WHO (International Chemical Reference Substances. Soinc 3, Sweden). Endotoxin-free Dulbecco's phosphate buffer (without calcium and magnesium) was obtained from PAA Laboratories GmbH (Linz, Austria). Culture 6-well plates and maxisorp 96-well flat bottom plates were purchased from Nunc International (Denmark).

### Subjects

Nineteen healthy male and female (8 males and 11 females) volunteers with ranging age between 19–38 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 the morning between 8 – 9:30 a.m.

### Whole Blood Culture

Blood was drawn into sterilized sodium heparin tubes (Vacutainer, Becton-Dickinson) and processed within 45 minutes. The production of whole blood cytokines was performed as described elsewhere [15–17] with modifications. The blood was diluted with 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 6-well culture plates, 1.8 ml of the diluted blood was added. Hydrocortisone, estradiol or progesterone 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<sub>2</sub> at 37 C for 24 h. After the first incubation, PHA+LPS in 40 µl volume were added to give a final concentrations of 5 µg/ml and 1 µg/ml for PHA and LPS, respectively, and incubated in 5% CO<sub>2</sub> 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.

### Cytokines Assay

Measurements of IL-12 p70, IL-10, IFN-γ and TNF-α were accomplished by ELISAs using the adapted procedure recommended by the manufacturer (Duo-Set R&D Systems, UK). Briefly, capture 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 µg/ml and 600 ng/ml for IL-10. Standards (human recombinant) for all assays were used in the range of 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. Streptavidin-Horseradish peroxidase conjugate with H<sub>2</sub>O<sub>2</sub>-Tetramethylbenzidine (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. The sensitivities of IL-12 p70, IL-10, IFN-γ and TNF-α assays were 4, 4, 8, and 5 pg/ml, respectively.

### Data Analysis

All data in figures 1– 4 are presented as relative concentration of each cytokine (± SE). The relative concentration is a better indicator of change upon 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 (± SE) for each cytokine measured with PHA+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 between two conditions (specific concentration with baseline) when n > 10, whereas Wilcoxin-rank test was performed between two conditions when n < 10.

## Results

### Hydrocortisone suppresses PHA+LPS-induced IFN-γ and to a lesser extent IL-10, IL-12 p70 and TNF-α levels

Increasing concentrations of hydrocortisone suppressed significantly IFN-γ production ( $F(5,90) = 35.2$ ,  $p < 0.001$ ) from PHA+LPS-stimulated whole blood in dose-dependent fashion (*Figure 1*). The concentration of hydrocortisone to inhibit 50% (CI 50%) of IFN-γ production was 89 nmol/l. In addition, IL-12 p70 ( $F(5,90) = 9.98$ ), IL-10 ( $F(5,90) = 13.3$ ) and TNF-α ( $F(5,90) = 21.7$ ) production levels were also significantly reduced ( $p < 0.001$ ) upon increasing concentration of hydrocortisone in PHA+LPS-stimulated whole blood. However, CI 50% for IL-12 p70, IL-10 and TNF-α were 991, 1382 and 1239 nmol/l of hydrocortisone, respectively (*Figure 1*). Furthermore, IFN-γ/IL-10 ratio dropped significantly ( $F(5,90) = 2.97$ ,  $p = 0.016$ ) from 0.75 to 0.295 at the concentrations tested (*Table I*).

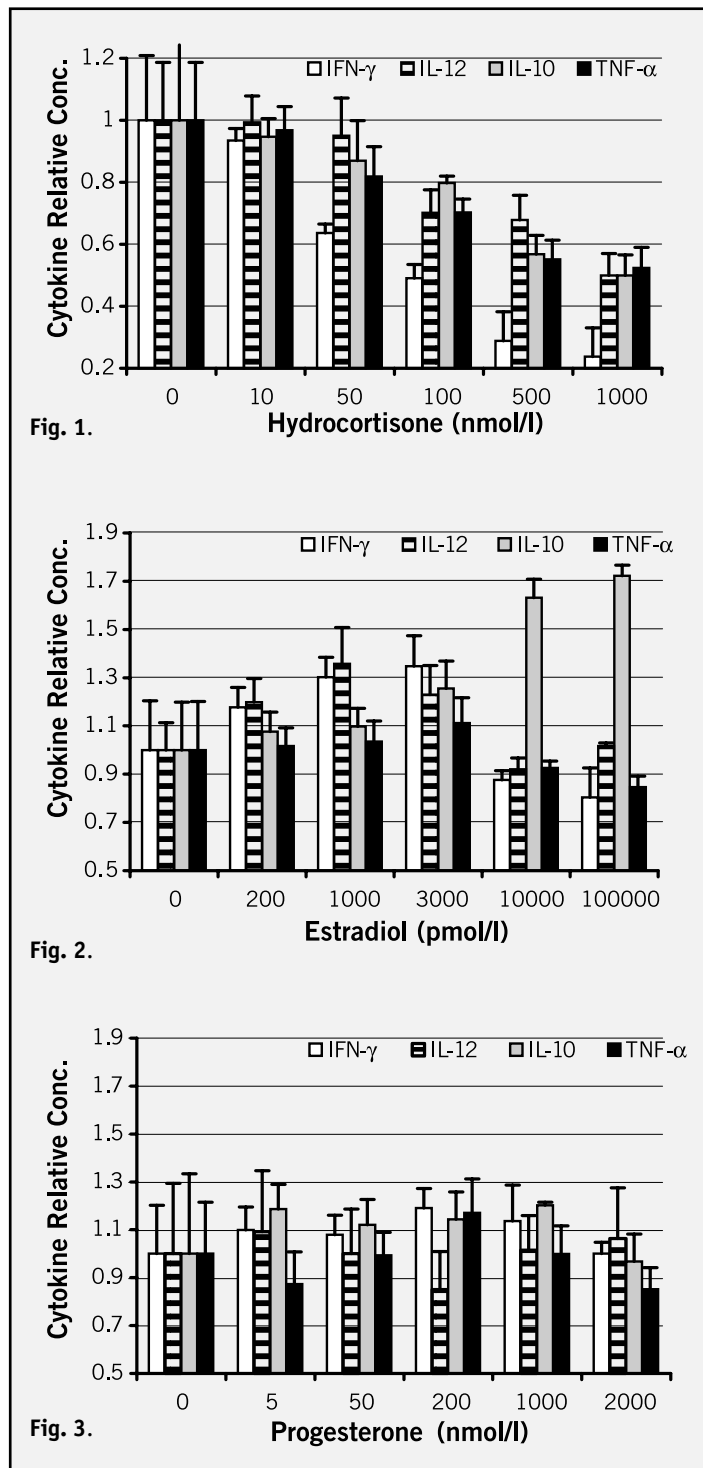
### Estradiol, but not Progesterone, alters PHA+LPS-induced IFN-γ, IL-12 p70 and IL-10 levels in a concentration-dependent manner

Overall, increasing concentrations of estradiol produced significant changes in IFN-γ ( $F(5,108) = 3.56$ ,  $p = 0.006$ ) and IL-10 levels ( $F(5,108) = 5.2$  and  $p < 0.001$ ) in PHA+LPS-stimulated whole blood (*Figure 2*). At 1000 pmol/l of estradiol, both of IFN-γ and IL-12 p70 production levels increased significantly ( $t = 3.73$ ,  $df = 18$ ,  $p = 0.0015$  and  $t = 2.38$ ,  $df = 18$ ,  $p = 0.028$ , respectively), while at 3000 pmol/l of estradiol, IFN-γ level

**Table I.** The effect of hydrocortisone and estradiol on IFN-γ/IL-10 ratio in PHA+LPS stimulated whole blood

	Hydrocortisone (nmol/l)					
	0	10	50	100	500	1000
IFN-γ/IL-10	0.75±0.17	0.76±0.19	0.52±0.11	0.46±0.10*	0.32±0.11*	0.29±0.09*
	Estradiol (pmol/l)					
	0	100	1000	3000	10000	100000
IFN-g/IL-10	0.77±0.15	0.75±0.10	0.89±0.15	0.74±0.10	0.42±0.09*	0.39±0.08*

\*p < 0.02–0.001



**Figure 1.** Effect of hydrocortisone on cytokines production in PHA+LPS-stimulated whole blood from 16 healthy volunteers (4 males and 12 females with a mean age of  $24.9 \pm 4.8$ ). Increasing concentrations of hydrocortisone was added as indicated and suppressed significantly ( $p < 0.001$ ) the production of all cytokines. Blood samples from males or females behaved similarly upon exposure to hydrocortisone. Data are expressed as relative mean  $\pm$  SE. a) Mean PHA+LPS-induced IFN- $\gamma$  production was  $383 \pm 69$  pg/ml, b) Mean PHA+LPS-induced IL-12 p70 production was  $15.9 \pm 3.2$  pg/ml, c) Mean PHA+LPS-induced IL-10 production was  $829 \pm 144$  pg/ml, and d) Mean PHA+LPS-induced TNF- $\alpha$  production was  $802 \pm 70$  pg/ml.

**Figure 2.** Effect of estradiol on cytokines production in PHA+LPS-stimulated whole blood from 19 normal volunteers (8 males and 11 females with a mean age of  $26.5 \pm 4.2$ ). Overall, increasing concentrations of estradiol produced significant changes in IFN- $\gamma$  ( $p=0.006$ ) and IL-10 ( $p < 0.001$ ) levels. At 1000 pmol/l of estradiol, both of IFN- $\gamma$  and IL-12 p70 production levels increased significantly ( $p < 0.00153$  and  $0.028$ , respectively). However, IL-10 levels kept significantly in dose-dependent manner. Blood samples from males or females behaved similarly upon exposure to estradiol. Data are expressed as relative mean  $\pm$  SE. a) Mean PHA+LPS-induced IFN- $\gamma$  production was  $313 \pm 68$  pg/ml, b) Mean PHA+LPS-induced IL-12 p70 production was  $16.0 \pm 1.8$  pg/ml, c) Mean PHA+LPS-induced IL-10 production was  $642 \pm 127$  pg/ml, and d) Mean PHA+LPS-induced TNF- $\alpha$  production was  $717 \pm 144$  pg/ml.

**Figure 3.** Effect of progesterone on cytokines production in PHA+LPS-stimulated whole blood from 9 normal volunteers (3 males and 6 females with a mean age of  $26.0 \pm 2.8$ ). No significant difference in the production of IFN- $\gamma$ , IL12-p70, IL-10 or TNF- $\alpha$  levels was observed. Blood samples from males or females behaved similarly upon exposure to progesterone. Data are expressed as relative mean  $\pm$  SE. a) Mean PHA+LPS-induced IFN- $\gamma$  production was  $134 \pm 43$  pg/ml, b) Mean PHA+LPS-induced IL-12 p70 production was  $14.2 \pm 1.4$  pg/ml, c) Mean PHA+LPS-induced IL-10 production was  $478 \pm 160$  pg/ml, and d) Mean PHA+LPS-induced TNF- $\alpha$  production was  $469 \pm 99$  pg/ml.

was significantly higher ( $t=2.82$ ,  $df=18$ ,  $p < 0.011$ ) but not IL-12 level ( $t=1.92$ ,  $df=18$ ,  $p=1.92$ ). This increase in IFN- $\gamma$  and IL-12 p70 productions, however, was not observed at higher concentrations of estradiol (Figure 2). At 10,000 and 100,000 pmol/l of estradiol, IL-10 levels kept increasing significantly indicating a dose-dependent effect with a significant decrease in IFN- $\gamma$ /IL-10 ratios ( $F(5,108) = 3.38$ ,  $p = 0.007$ ) (Table 1). No significant effect of estradiol was observed at the concentrations used on the production of TNF- $\alpha$  ( $F(5,108)=0.532$ ,  $p=0.75$ ) in PHA+LPS-stimulated whole blood (Figure 2).

Progesterone, on the other hand, did not cause any significant changes in the production of IFN- $\gamma$ , IL-12 p70, IL-10 and TNF- $\alpha$  from PHA+LPS-stimulated whole blood (Figure 3).

*Estradiol reverses hydrocortisone suppressive effect on IL-10, IFN- $\gamma$  and IL-12 p70 production in PHA+LPS stimulation*

In the preceding experiments, the effect of estradiol levels at preovulatory phase to reverse hydrocortisone suppressive effect on IFN- $\gamma$ , IL-12 p70, IL-10 and TNF- $\alpha$  production in PHA+LPS-stimulated whole blood

**Figure 4.**

Estradiol reverses hydrocortisone suppressive effect on cytokines production in PHA+LPS-stimulated whole blood from 5 healthy volunteers (2 males and 3 females with a mean age of  $23.0 \pm 5.2$ ). Blood was incubated with no hormone, or with estradiol (1000 or 3000 pmol/l) and hydrocortisone (100 nmol/l) or hydrocortisone (100 nmol/l) alone for 24 h then blood was stimulated with PHA+LPS for another 24 h. Addition of estradiol reversed significantly hydrocortisone suppressive effect on IFN- $\gamma$ , IL-12 p70 and IL-10 production (*i.e.* relative concentrations increased and p values became insignificant, see text for details) but not TNF- $\alpha$ . Data are expressed as relative mean  $\pm$  SE.

**a)** Mean PHA+LPS-induced IFN- $\gamma$  production was  $576 \pm 95$  pg/ml, **b)** Mean PHA+LPS-induced IL-12 p70 production was  $16.8 \pm 1.6$  pg/ml, **c)** Mean PHA+LPS-induced IL-10 production was  $970 \pm 186$  pg/ml, and **d)** Mean PHA+LPS-induced TNF- $\alpha$  production was  $1251 \pm 317$  pg/ml.

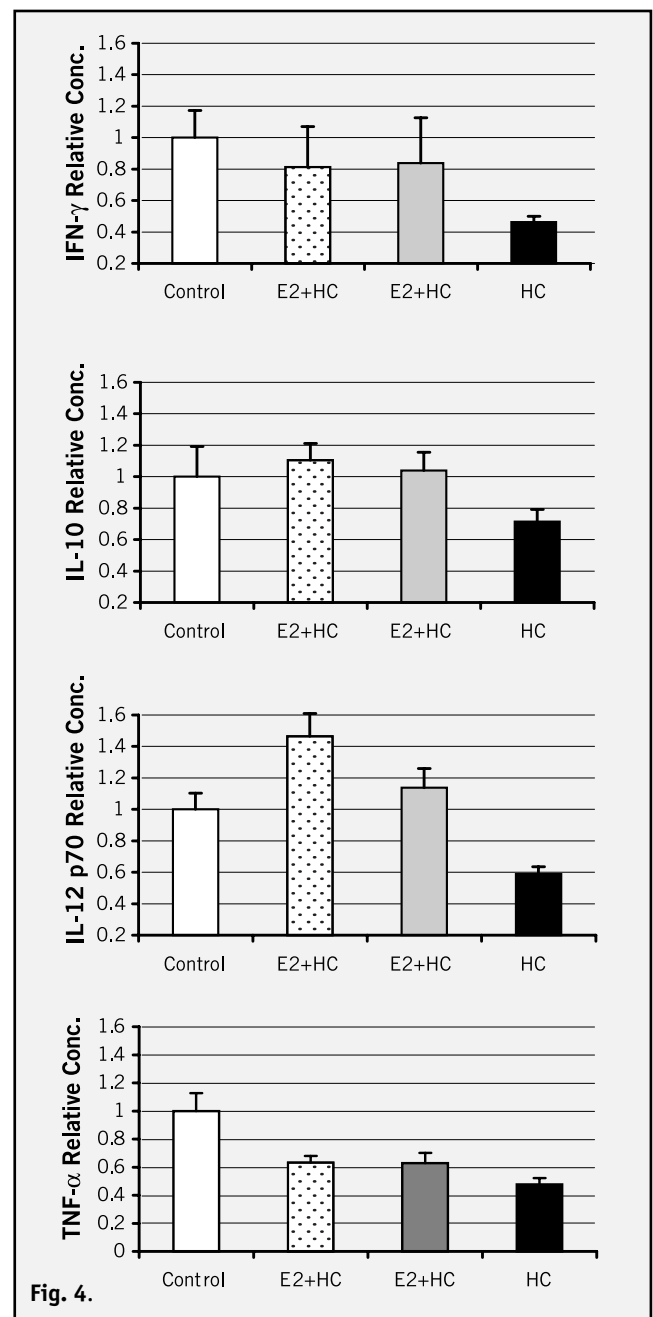
was performed. At 1000 and 3000 pmol/l of estradiol, was able to reverse hydrocortisone (100 nmol/l) suppressive effect on the production of IL-10 ( $p > 0.3$ ), IFN- $\gamma$  ( $p > 0.25$ ) and IL-12 p70 ( $p > 0.5$ ) but not TNF- $\alpha$  ( $p < 0.05$ ) (Figure 4).

*Estradiol alone increased basal IL-12 p70, IL-10 and to lesser extent IFN- $\gamma$  but not TNF- $\alpha$  levels*

The possibility that estradiol at preovulatory concentrations, without PHA+LPS, could induce IFN- $\gamma$ , IL-12 p70, IL-10 and TNF- $\alpha$  was tested in whole blood from 5 healthy volunteers. At 1000 and 3000 pmol/l of estradiol, IFN- $\gamma$  went up  $\sim 1.2$  times the basal value but no change was observed with TNF- $\alpha$  level. The basal level of IL-12 p70 was within the range of undetectable ( $< 4$ ) to 6 pg/ml and went up to an average of 10 pg/ml at concentrations of estradiol 200, 1000, or 3000 pmol/l, respectively. However, these IL-12 values were not enough to increase IFN- $\gamma$  levels more than basal values (data not shown), indicating that T cells at these IL-12 concentrations need another stimulus to secrete IFN- $\gamma$ . Furthermore, IL-10 relative concentration was 1.52, 1.87 and 1.78 at 200, 1000 and 3000 pmol/l of estradiol, respectively.

## Discussion

The present work showed clearly that the effect of estradiol on the production of cytokines in whole blood assay is concentration-dependent. Estradiol up-regulates the secretion of IL-12, IFN- $\gamma$  and IL-10 at preovulatory estradiol levels but IFN- $\gamma$ /IL-10 ratios kept unchanged. At high estradiol levels, such as in pregnancy, IL-10 production levels kept increasing while IFN- $\gamma$ , IL-12 and IFN- $\gamma$ /IL-10 ratio decreased significantly. The data, here, supports that those high levels of estradiol shift cytokine levels towards Th2 response. In the present study, however, no estradiol effect on TNF- $\alpha$  production was observed. In similar experimental study, estradiol also did not affect TNF-



$\alpha$  level in LPS-stimulated whole blood [12]. Although *in vivo* results demonstrated that estrogen decreases endotoxin-induced TNF- $\alpha$  levels [19], this might indicate that estrogen induces the secretion of other hormones *in vivo* and thereby reduces the production of TNF- $\alpha$  [20]. Furthermore, no effect of progesterone on the production of IL-12, IFN- $\gamma$ , IL-10 and TNF- $\alpha$  in whole blood assay could be established. In addition, progesterone failed to modulate the same cytokines above, but increased IL-4 from isolated clones of T cells from normal subjects and multiple sclerosis patients [21], IL-4 and IL-5 from T cell lines [8] and leukemia inhibiting factor from T cells obtained from the fetal maternal interface [9]. Although, the above results might indicate the progesterone induces its modulatory effect on Th0 and Th2 cells only, it seems more likely that progesterone and estrogen operate at the

level of individual cytokine gene expression regardless of the cell type [21].

In addition to increase in IL-10 levels, Gilmore et al. [7] have shown that estradiol at concentrations (3700–370,000 pmol/l), significantly increased IFN- $\gamma$  in a dose dependent manner from proteolipid protein specific T cell clones. In the present study, IFN- $\gamma$  levels decreased at 10,000–100,000 pmol/l of estradiol but IL-10 levels became even higher. The difference in the two studies is that Gilmore et al. used CD4+ clones i.e. they studied the effect of estradiol on CD4+ T cells only, while in the present study the effect was on all types of stimulated blood cells. The latter exhibits more cellular interactions and the natural microenvironment was kept intact. For instance, IL-10 produced from LPS-stimulated monocytes or LPS-stimulated whole blood, might contribute to a more inhibitory production of IFN- $\gamma$  as seen in the present study. It is known that two of IL-10 inhibitory actions are on IFN- $\gamma$  production from T lymphocytes and on IL-12 mediated IFN- $\gamma$  pathway [22,23].

Using whole blood model, Elenkov et al. [12] did not show effect of estradiol nor progesterone on the production of IL-10, IL-12 or TNF- $\alpha$  in LPS-stimulated whole blood. Nonetheless, they showed that LPS-stimulated whole blood from pregnant women produces less IL-12 and TNF- $\alpha$  from postpartum counterparts. In the present study, a combination of PHA+LPS was used to stimulate whole blood and revealed higher IL-12, IFN- $\gamma$  and IL-10 production levels at preovulatory levels of estradiol and even higher IL-10 levels at estradiol concentrations during pregnancy. It was observed by the author [15] and others that: (1) 1  $\mu$ g/ml of LPS did not induce IFN- $\gamma$  production more than unstimulated whole blood (basal level)[13,15], (2) monocytes are the primary source of IL-12, IL-10 and TNF- $\alpha$  in LPS-stimulated whole blood assay [14], and (3) PHA+LPS-stimulated whole blood produced significantly more IL-10 and TNF- $\alpha$  levels than LPS alone [15]. These observations with the data from the present study suggest that estradiol increased IL-10 production was mainly from stimulated lymphocytes but not monocytes. Furthermore, PHA is a monocyte-dependent T cell stimulus where it acts on both monocytes and lymphocytes and probably an enhanced signal from estradiol-stimulated lymphocyte increased the production of IL-12. It is known that IFN- $\gamma$  acts as a positive feedback on monocytes/macrophages to secrete more IL-12 [23]. A recent work by Maruyama et al have shown that induction of IL-12 p40 gene (but not IL-12 p35 gene) is regulated by NF-kappa B in the presence of IFN- $\gamma$ , in which IFN induces IFN-regulatory factor which in turn induces the transcription of IL-12 p40 gene [24]. Therefore, estradiol (at preovulatory concentrations) enhances the production of IFN- $\gamma$  from stimulated T cells, which could be through estrogen response enhancer at the promoter region [25], and thereby it stimulates IL-12 production from monocytes/macrophages [23]. It has been shown that IL-12 can prime T cells for high IL-10 production [26], which

then inhibits IL-12 induced T cell responses. However, what kept IL-10 increasing in high estrogen-stimulated blood cells is still to be identified.

The present study showed that hydrocortisone suppressed the production of Th1 more than Th2 cytokines as it showed more inhibitory action towards the production of IFN- $\gamma$ . This was also seen in other studies [18]. However, the present study also showed that estradiol at preovulatory concentrations can reverse the suppressive effect of hydrocortisone on IFN- $\gamma$ , IL-12 and IL-10 productions. The data here add that the balance between hormones also regulate cytokines production from stimulated leukocytes [27]. In pregnancy, however, cortisol and estradiol levels are increased [12]. The present study showed that these hormones cause a shift towards Th2 cytokine response by decreasing IFN- $\gamma$ /IL-10 ratio and therefore keeping Th2 cytokines favored during pregnancy.

In summary, the present study shows that those pregnancy estradiol concentrations (and higher) enhance and reduce IL-12 and IFN- $\gamma$  levels from stimulated whole blood cells. Because of the known IL-10 inhibitory actions on Th1 cells and monocyte/macrophages, these high IL-10 levels keep Th2 cytokines favored during pregnancy. Although, progesterone did not affect IL-10 levels, it was recently shown that it promotes the production of IL-4 [19]. Therefore, these two hormones keep Th2 cytokines more dominating during pregnancy and might be useful in shifting Th1-mediated autoimmune diseases towards non-pathogenic Th2 pathway.

### Acknowledgments

This work is supported by a grant # 5/3/2000 from the Deanship of Research at University of Petra-Amman, Jordan. The author wishes to thank Ms. Dalia Ali for her excellent technical assistance.

### REFERENCES

- 1 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; **17**:138–146.
- 2 Fearon DT, Locksley RM. The intrinsic role of innate immunity in the acquired immune response. *Science* 1996; **272**:50–53.
- 3 Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 1995; **13**:251–276.
- 4 Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends in Endocrinol Metab* 1999; **10**:359–368.
- 5 Cohen JH, Danel L, Cordier G, Saez S, Revillard JP. Sex steroid receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. *J Immunol* 1983; **131**:2767–2771.
- 6 Tornwall J, Carey AB, Fox RI, Fox HS. Estrogen in autoimmunity: expression of estrogen receptors in thymic and autoimmune T cells. *J Gend Specif Med* 1999; **2**:33–40.
- 7 Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* 1997; **158**:446–451.

- 8 Piccinni MP, Guidizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, et al. Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established TH1 cell clones. *J Immunol* 1995; **155**:128–133.
- 9 Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S. Defective production of both leukemia inhibitory factor and type-2 T helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med* 1998; **4**:1020–1024.
- 10 Confavreux C, Hutchinson M, Hours MM, Cortinovios-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. *N Engl J Med* 1998; **339**: 285–291.
- 11 Silman A, Kay A, Brennan P. Timing of pregnancy in relation to onset of rheumatoid arthritis. *Arthritis Rheum* 1992; **35**: 152–159.
- 12 Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, Crane M, Kanik KS, Chrousos GP. IL-12, TNF- $\alpha$ , and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin Endocrinol Metab* 2001; **86**:4933–4938.
- 13 Kwak DJ, Augustine NH, Borges WG, Joyner JL, Green WF, Hill HR. Intracellular and extracellular cytokine production by human mixed mononuclear cells in response Group B streptococci. *Infect Immun* 2000; **68**:320–327.
- 14 Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. *J Immunol* 1998; **161**:2586–2593.
- 15 Matalka KZ. Prolactin enhances the production of interferon- $\gamma$ , interleukin-12, and interleukin-10, but not of tumor necrosis factor- $\alpha$ , in a stimulus specific manner. *Cytokine* 2003; **21**: 187–194.
- 16 Lyte M. Generation and measurement of interleukin-1, interleukin-2, and mitogen levels in small volumes of whole blood. *J Clin Lab Anal* 1987; **1**:83–88.
- 17 Matalka KZ. Pathogenic mechanism in Crohn's disease: Immunological alterations measured by whole blood mitogen responses, IL-1, and IL-2 (Thesis). Milwaukee (WI): University of Milwaukee Wisconsin:1988.
- 18 Agarwal SK, Marshall GD. Glucocorticoid-induced type 1/type 2 cytokine alterations in humans: A model for stress-related immune dysfunction. *J Interferon Cytokine Res* 1998; **18**: 1059–1068.
- 19 Puder JJ, Freda PU, Goland RS, Wardlaw SL. Estrogen modulates the hypothalamic-pituitary-adrenal and inflammatory cytokine responses to endotoxin in women. *J Clin Endocrinol Metab* 2001; **86**:2403–2408.
- 20 Chrousos GP, Torpy, DJ, Gold PW. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. *Ann Intern Med* 1998; **129**:229–240.
- 21 Correale J, Arias M, Gilmore W. Steroid hormone regulation of cytokine secretion by proteolipid protein-specific CD4+ T cells clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* 1998; **161**:3365–3374.
- 22 Snijders A, Hilkens CM, van der Pouw Kraan TC, Engel M, Aarden LA, Kapsenberg ML. Regulation of bioactive IL-12 production in lipopolysaccharide-stimulated human monocytes is determined by the expression of the p35 subunit. *J Immunol* 1996; **156**:1207–1212.
- 23 Retini C, Kozel TR, Pietrella D, Monari C, Bistoni F, Vecchiarelli A. Interdependency of interleukin-10 and interleukin-12 in regulation of T-cell differentiation and effector function of monocytes in response to stimulation with *Cryptococcus neoformans*. *Infect Immun* 2001; **69**:6064–6073.
- 24 Maruyama S, Sumita K, Shen H, Kanoh M, Xu X, Sato M, et al. Identification of IFN regulatory factor-1 binding site in IL-12 p40 gene promoter. *J Immunol* 2003; **170**:997–1001.
- 25 Fox HS, Bond BL, Parslow TG. Estrogen regulates the IFN- $\gamma$  promoter. *J Immunol* 1991; **146**:4362–4367.
- 26 Meyaard L, Hovenkamp E, Otto AS, Miedema F. IL-12-induced IL-10 production by human T cell as a negative feedback for IL-12-induced immune responses. *J Immunol* 1996; **156**: 2776–2782.
- 27 Matalka KM, Sidki, A, Adul-Malik SM, Thewaini AJ. Academic Stress-Influence on Epstein-Barr virus and cytomegalovirus reactivation, cortisol and prolactin. *Lab Med* 2000; **31**: 163–168.