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Modulation of IL-6 Production during the Menstrual Cycle *in Vivo* and *in Vitro*

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During the menstrual cycle (MC), premenopausal women experience changes in basal temperature and their physical condition and well-being. Premenopausal female patients with chronic inflammatory diseases demonstrate changes in disease activity during the MC. The study was initiated to explore reasons for these phenomena. The sex hormone-modulated lipopolysaccharide (LPS)—induced interleukin-6 (IL-6) secretion in a whole blood assay, serum IL-6 concentration, and serum sex hormone concentrations were studied throughout the MC in five healthy female subjects (median, 28 years; mean, 31.2 ± 2.2 years, 26–38 years). Serum IL-6 concentration demonstrated a significant increase in the luteal phase of the MC and was elevated when serum dehydroepiandrosterone (DHEA) was low and vice versa. DHEA decreased LPS-induced IL-6 secretion at six of seven time points during the MC (DHEA, $p = .047$). In contrast, β-estradiol and testosterone increased LPS-induced IL-6 secretion in six of seven time points during the MC (significant for testosterone, $p = .005$). The study demonstrates oscillation of serum IL-6 concentration during the MC and the marked MC-dependent modulation of IL-6 secretion by sex hormones. These mechanisms may be involved in the changes in the basal temperature, the general condition, and, in patients with chronic inflammatory diseases, of disease activity during the MC.

Key Words: menstrual cycle; IL-6; TNF; dehydroepiandrosterone; testosterone; β-estradiol.

INTRODUCTION

During the menstrual cycle (MC), premenopausal healthy women exhibit significant changes in physical condition and general well-being (Freeman, 1997). With respect to female patients with chronic inflammatory diseases, changes in disease-associated parameters are found during the MC (Steinberg & Steinberg, 1985; McDonagh, Singh, & Griffiths, 1993; Latman, 1983; Goldstein, Duff, & Karsh, 1987; Lahita, 1996; Palnaes-Hansen, Bulow, & Karisen, 1987). As a consequence of hormonal cycles, these changes may be due to differences in proinflammatory mechanisms, such as elevated serum concentrations of tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), or interleukin-6 (IL-6), which are known, e.g., for their pyrogenic properties (Dinarello, Cannon, Wolff, Bernheim, Beutler, Cerami, Figari, Palladino, & O'Connor, 1986). Moreover, TNFα and IL-1 also influence sleep patterns (Kapas & Krueger, 1992), which may lead to sleep disturbances in the different phases of the MC and their negative sequelae (Driver, Dijk, Werth, Biedermann, & Borbely, 1996). Studies in premenopausal healthy female subjects demonstrated an increase of serum IL-1 in the luteal phase of the menstrual cycle (Cannon & Dinarello, 1985; Polan, Kuo, Loukides, & Bottomly, 1990). Human endometrial expression of IL-6 (Tabibzadeh, Kong, Babaknia, & May, 1995a) and TNFα (Tabibzadeh, Zupi, Babaknia, Liu, Marconi, & Romanini, 1995b) is also upregulated in the luteal phase

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of the MC, which may contribute to changes in serum cytokine levels. These changes in cytokine secretion during the MC may be a relevant cofactor in modulation of chronic inflammatory diseases in premenopausal female patients (Steinberg & Steinberg, 1985; McDonagh et al., 1993; Latman, 1983; Goldstein et al., 1987; Lahita, 1996; Palnaes-Hansen et al., 1987) and also in premenopausal healthy female subjects (Arnbjornsson, 1983).

Female healthy subjects are often excluded from experiments in basic immunological research because of the large variation in study responses. We recently demonstrated the dehydroepiandrosterone (DHEA)- induced decrease of IL-6 secretion from peripheral blood mononuclear cells *in vitro* and the negative correlation of serum IL-6 and serum DHEA concentrations (Straub, Konecna, Hrach, Rothe, Kreutz, Scholmerich, Falk, & Lang, 1998). The inhibitory effect of DHEA was observed in about 70% of female healthy subjects whose blood was drawn in the follicular phase of the MC (Straub et al., 1998). Preliminary experiments demonstrated differences in hormone-induced modulation of lipopolysaccharide (LPS)-stimulated IL-6 secretion in blood cells during the MC. These results suggested a closer investigation of hormone-induced modulation of LPS-stimulated IL-6 secretion during the MC. Moreover, we wanted to compare the IL-6-modulating effect of the prehormone DHEA with the active hormones testosterone and β-estradiol during the MC.

SUBJECTS AND METHODS

Subjects and Blood Samples

Five healthy premenopausal female subjects were included in the study and their state of health was verified through a 33-item questionnaire. The questionnaire addressed known diseases in the past and at present, current symptoms of diseases, current medication, alcohol intake, smoking habits, family history, and surgery history adapted from the SENIEUR protocol (Ligthart, Corberand, Fournier, Galanaud, Hijmans, Kennes, Muller-Hermelink, & Steinmann, 1984). The subjects were 26, 28, 28, 36, and 38 years of age, and they were not taking contraceptives. The mean body mass index ±SEM was 23.4 ± 0.5 kg/m^2 (range: 22–25 kg/m^2). All subjects were informed about the purpose of the study and gave consent to participate. Blood was drawn between 4.00 and 6.00 PM and serum was immediately stored at −80° C in adequate aliquots. For cell culture experiments, heparinized blood was drawn at the same time.

Laboratory Parameter

Immunometric enzyme immunoassay for the quantitative determination of serum DHEA (DSL, Inc., Webster, TX), serum β-estradiol (IBL, Hamburg, Germany), serum progesterone (IBL), serum follicle-stimulating hormone (IBL), serum luteinizing hormone (IBL), serum IL-6 (high sensitivity Quantikine, R&D Systems, Minneapolis, MN; sensitivity 0.1 pg/ml), and serum TNFα (high sensitivity Quantikine, R&D Systems, Minneapolis, MN; sensitivity 0.2 pg/ml) were used. Interassay and intraassay coefficients of variation were below 10%.

Drugs, Stimulation, and Production of Supernatants in Cell Culture Experiments

Fifteen microliters of whole blood was incubated in 985 μl RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, and 100

μg/ml streptomycin in 1-ml culture plates (all additions from Sigma, Munich, Germany). This yielded about 5000 to 8000 monocytes per well. To study IL-6 production, the whole blood culture was stimulated with 0.1 ng/ml LPS (*Salmonella typhimurium*, Sigma). At the same time, DHEA, β-estradiol, or testosterone was added to final concentrations of 1×10^{-6}M, 5×10^{-7}M, 1×10^{-7}M, 5×10^{-8}M, 1×10^{-8}M, 5×10^{-9}M, or 1×10^{-9}M. The hormones were dissolved in dimethyl sulfoxide (DMSO, Serva, Heidelberg, Germany) and diluted to the final concentrations in culture medium at the day of the experiment. After 24 h, supernatants were harvested for measurement of IL-6 and stored at −80°C until assayed by immunometric enzyme immunoassay (Endogen, Boston, MA). Using this ELISA, sensitivity was <1 pg/ml and intraassay and interassay coefficients of variation were below 10%.

Presentation of the Data and Statistical Analysis

All data are given as means ± SEM. All incubations in cell culture experiments were performed in quintuple. The nonparametric Friedman test for comparison of numerous dependent values (SPSS/PC for Windows 95, SPSS Inc., Chicago) was used to verify changes during the MC. In the Friedman test, $p < .05$ indicated a significant change of the variable during the test procedure, which means an increased, a decreased, or a peak response. Values are expressed as means ± SEM and the significance level is $p < .05$.

RESULTS

Serum Concentration of Sex Hormones, IL-6, and TNF α during the MC

To identify the days of the MC, we received the last five MC reports of the participating subjects and we investigated serum progesterone, serum β-estradiol, serum follicle-stimulating hormone, and serum luteinizing hormone. With respect to the previous cycles all subjects had absolutely normal MCs lasting 26 to 28 days. The results were centralized using the anamnestic and laboratory data (center = time point of ovulation = day 0). The hormones measured demonstrated the typical variation during the MC (Fig. 1). Furthermore, the serum DHEA presented also changes, with a first maximum around the ovulation and a second maximum at day 12 after the ovulation ($p = .032$ in the Friedman test, Fig. 2A). In contrast, serum IL-6 was elevated when serum DHEA was low and vice versa (Figs. 2A and 2B). Serum IL-6 concentration was significantly higher in the luteal phase of the MC than in the follicular phase (Fig. 2B). The TNFα concentration tended to increase during the MC and was highest before the menstruation (day −8, 1.3 ± 0.3; day −5, 1.4 ± 0.3; day 0, 1.4 ± 0.4; day 5, 1.4 ± 0.4; day 8, 1.4 ± 0.3; day 12, 1.4 ± 0.3; day 16, 1.5 ± 0.4; day 20, 1.6 ± 0.4 pg/ml).

Counts of Mononuclear Cells and IL-6 Secretion of LPS-Stimulated Whole Blood

It was ascertained that the monocyte count ($p = .456$ in Friedman's test), granulocyte count ($p = .412$), and lymphocyte count ($p = .601$) in whole blood did not significantly change during the MC. To study the LPS-stimulated IL-6 secretion of whole blood cells, blood samples obtained during various phases of the MC were incubated with LPS and IL-6 secretion was measured by ELISA. IL-6 secretion was significantly increased during the luteal phase of the MC compared to the follicular phase ($p = .021$, Fig. 3).

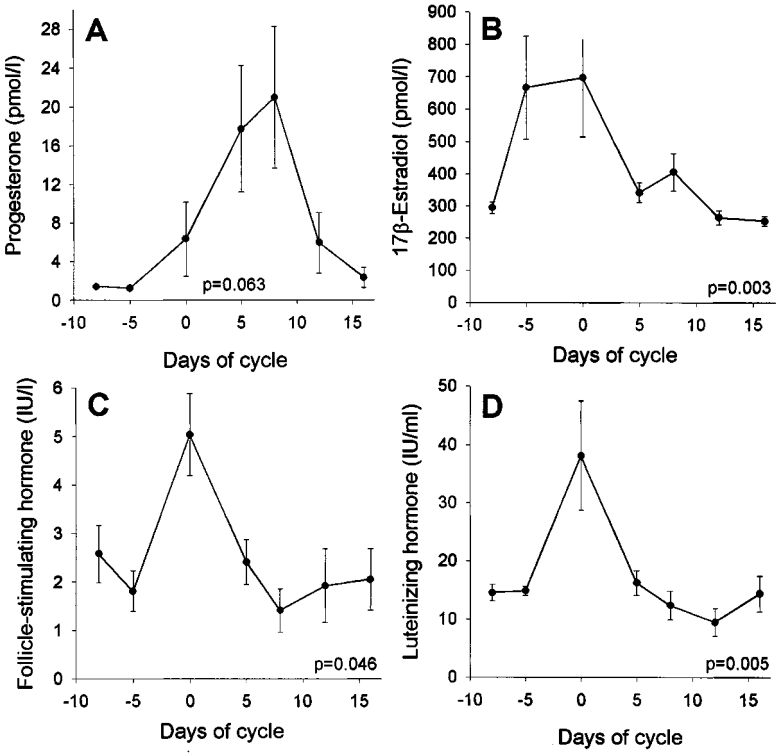

FIG. 1. Changes of serum concentrations of progesterone (A), β-estradiol (B), follicle-stimulating hormone (C), and luteinizing hormone (D) during the menstrual cycle in five premenopausal female women. Serum was obtained at different times during the menstrual cycle and the hormones were measured by ELISA. The data are given in mean values ± SEM. The *p* value of Friedman's test is given in each graph.

Modulation of LPS-Stimulated IL-6 Secretion by DHEA, β-Estradiol, and Testosterone

Figures 4A, 5A, and 6A demonstrate the MC-dependent changes of LPS- stimulated IL-6 secretion by DHEA, β-estradiol, and testosterone. In these experiments we used seven different hormone concentrations, as mentioned under Subjects and Methods. With respect to all hormones tested, a peak response was detected around the ovulation and before the next menstruation (Figs. 4A, 5A, and 6A). A significant downregulation of IL-6 secretion was observed between days 5 and 8 after ovulation (Figs. 4A, 5A, and 6A) which was detected for all hormones tested. Furthermore, at a concentration of about 1 to 5 $\times$ 10^{-8}M we observed a decline in stimulated IL-6 secretion, which was demonstrated to be the best inhibitory concentration for DHEA in an earlier study (Straub et al., 1998).

In each woman, we measured serum hormone concentrations at each time point during the MC. For the calculation and demonstration of hormone-induced modulation of LPS-stimulated IL-6 secretion (Fig. 4B, 5B, and 6B), we used the results of that hormone concentration of the seven different *in vitro* tested concentrations which

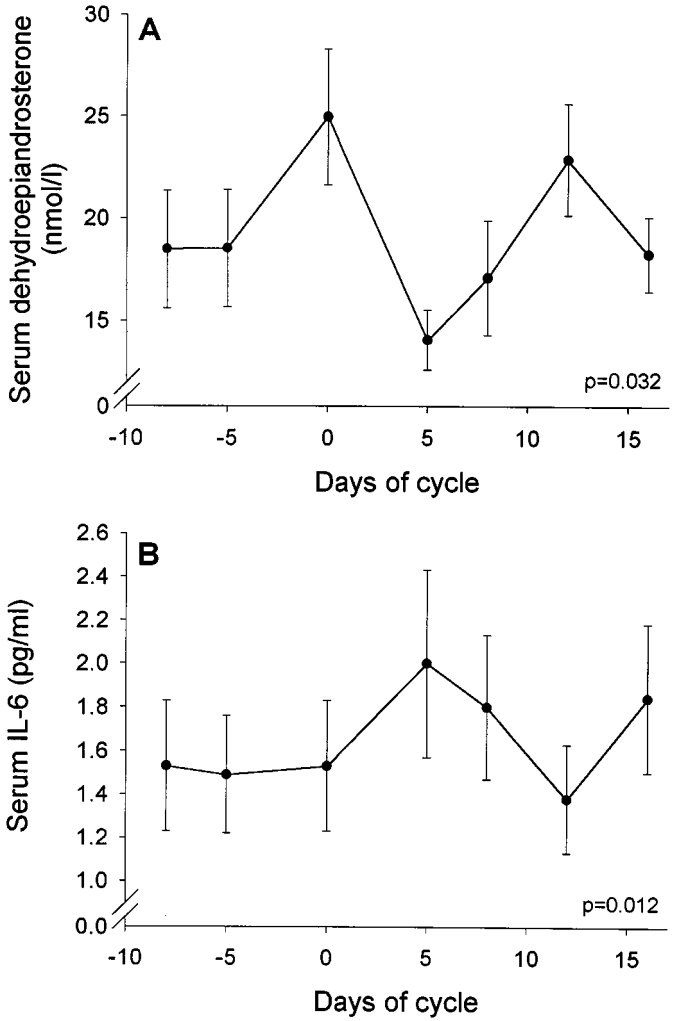

FIG. 2. Changes of serum concentrations of dehydroepiandrosterone (A) and IL-6 (B) during the menstrual cycle in five premenopausal female women. Serum was obtained at different times during the menstrual cycle and the parameters were measured by ELISA. The data are given in mean values ± SEM. The *p* value of Friedman's test is given in each graph.

was nearest to the serum hormone concentration at the time point of the experiment. Since testosterone was low in all subjects, the results with 1×10^{-9}M testosterone were used in all experiments throughout the menstrual cycle (in Fig. 6B). At six of seven time points during the MC, DHEA decreased LPS-induced IL-6 secretion ($p = .047$ in Friedman's test, Fig. 4B). DHEA did not modulate IL-6 secretion at ovulation and in the late luteal phase (Fig. 4B). In contrast, β-estradiol (Fig. 5B) and testosterone (Fig. 6B) increased LPS-induced IL-6 secretion at five of seven time points. However, only the modulation caused by testosterone reached statistical significance ($p = .005$ in Friedman's test, Fig. 6B).

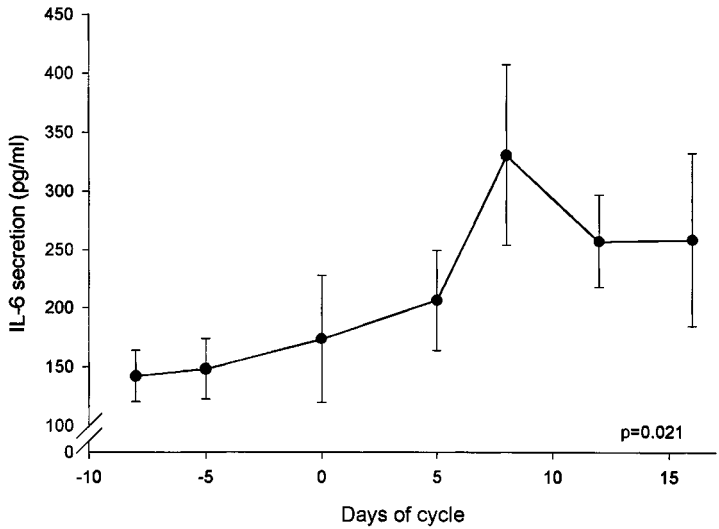

FIG. 3. Lipopolysaccharide (LPS)-stimulated IL-6 secretion of peripheral blood cells during the menstrual cycle in five premenopausal female women. Blood was drawn and incubated for 24 h with 0.1 ng/ml LPS at different time points during the menstrual cycle. IL-6 was detected in supernatants by ELISA. Data are given in mean IL6 values ± SEM. The *p* value of Friedman's test is given.

DISCUSSION

Serum levels of IL-6 fluctuated significantly during the MC with an elevated serum concentration in the luteal phase. LPS-stimulated IL-6 secretion from whole blood cells was also significantly higher during the luteal phase of the MC. Hormone-induced modulation of LPS-stimulated IL-6 secretion *in vitro* demonstrated an increase at ovulation and during the late luteal phase. *In vitro,* DHEA mainly decreased IL-6 secretion, β-estradiol was without significant effect, and testosterone increased *in vitro* IL-6 secretion in these premenopausal women.

Some earlier studies in premenopausal healthy female subjects demonstrated an increase of serum IL-1 in the luteal phase of the menstrual cycle (Cannon & Dinarello, 1985). In one recent study, IL-6 serum concentration did not change during the MC but C-reactive protein, which is mainly stimulated by IL-6, was significantly higher during the luteal phase of the MC (Jilma, Dirnberger, Loscher, Rumplmayr, Hildebrandt, Eichler, Kapiotis, & Wagner, 1997). Expression of IL-6 (Tabibzadeh et al., 1995a) and TNFα (Tabibzadeh et al., 1995b) in human endometrial epithelium is upregulated in the luteal phase of the MC. Prostaglandin E2, which is a positive stimulus for IL-6 secretion (Bunning, Russell, & Van Damme, 1990), is also upregulated during the luteal phase of the MC (Leslie & Dubey, 1994). Moreover, soluble IL-6 receptor is upregulated preovulatory and during the late luteal phase (Gorai, Taguchi, Chaki, Kikuchi, Nakayama, Yang, Yokota, & Minaguchi, 1998). With respect to these studies and our own data, the increase of serum cytokine levels periovulatory and during the late luteal phase seems to be a general principle. The upregulation of IL-6 and soluble IL-6 receptor coincide with the implantation window of the ovum in the early postovulatory phase, which indicates that IL-6 may be of importance for successful embryonic implantation (Tabibzadeh et al., 1995a). The increase

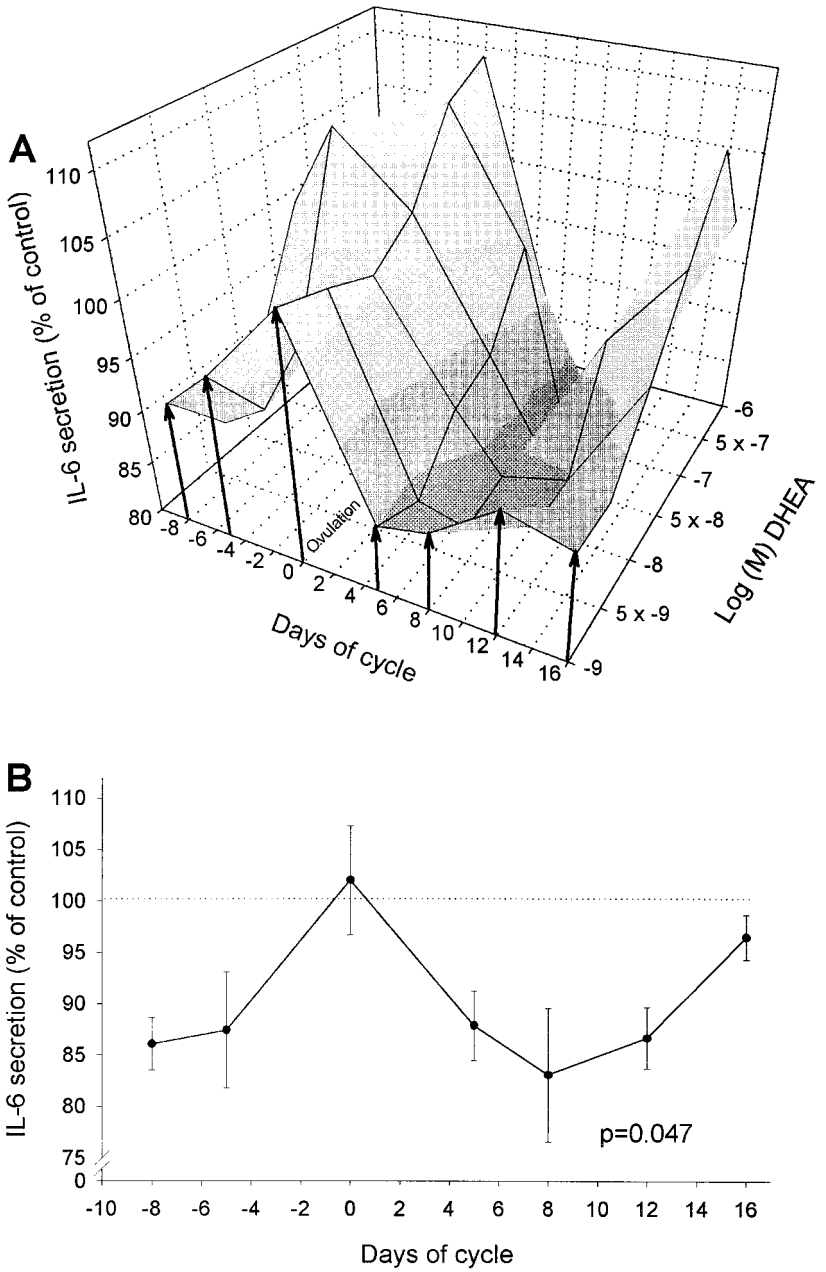

FIG. 4. Modulation of LPS-stimulated IL-6 secretion by dehydroepiandrosterone (DHEA). (A) Three-dimensional mesh graph of IL-6 in percentage of control (control = 100%) versus time and concentration of DHEA. At different time points during the menstrual cycle, peripheral blood cells were incubated for 24 h with 0.1 ng/ml LPS only (control = 100%, dotted line) or with 0.1 ng/ml LPS plus an indicated hormone concentration. IL-6 was detected in supernatants by ELISA. (B) Modulation of LPS-stimulated IL-6 secretion by DHEA. For the calculation of the data for this graph, the result of the hormone concentrations of the seven different *in vitro* tested concentrations was used which was nearest to the serum hormone concentration at the time point of the experiment. For method and determination of IL-6 see legend to A. Data are given in percentages of control (means ± SEM). The *p* value of Friedman's test is given. The time point of ovulation is zero.

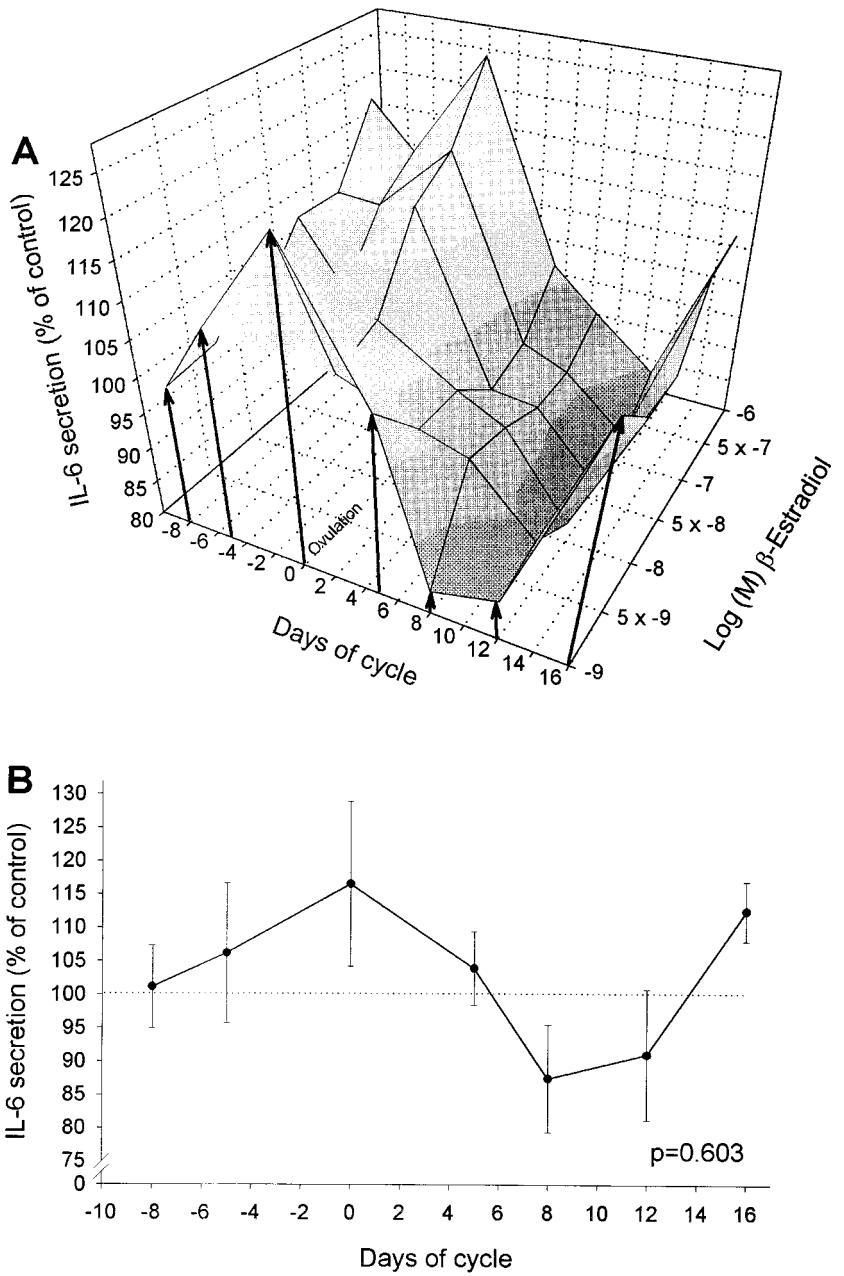

FIG. 5. Modulation of LPS-stimulated IL-6 secretion by β-estradiol. (A) Three-dimensional mesh graph of IL-6 in percentage of control (control = 100%) versus time and concentration of β-estradiol. For method and determination of IL-6 see legend to Fig. 4A. (B) Modulation of LPS-stimulated IL-6 secretion by β-estradiol. For the calculation of the data for this graph, the result of the hormone concentration of the seven different *in vitro* tested concentrations was used which was nearest to the serum hormone concentration at the time point of the experiment. For method and determination of IL-6 see legend to Fig. 4A. Data are given in percentages of control (means ± SEM). The *p* value of Friedman's test is given. The time point of ovulation is zero.

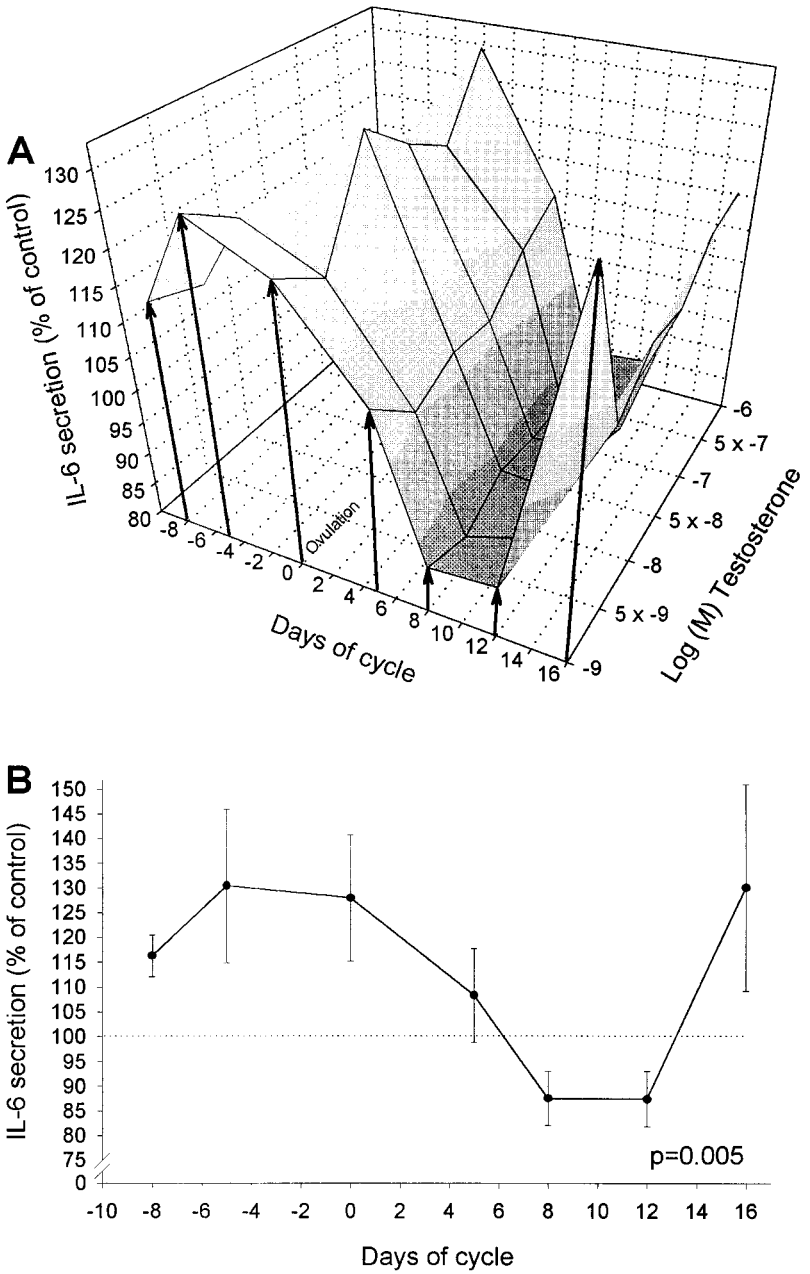

FIG. 6. Modulation of LPS-stimulated IL-6 secretion by testosterone. (A) Three-dimensional mesh graph of IL-6 in percentage of control (control = 100%) versus time and concentration of testosterone. For method and determination of IL-6 see legend to Fig. 4A. (B) Modulation of LPS-stimulated IL-6 secretion by testosterone. For the demonstration of the data, results of experiments with 1×10^{-9}M testosterone were used in all experiments throughout the menstrual cycle. For method and determination of IL-6 see legend to Fig. 4A. Data are given in percentages of control (means ± SEM). The *p* value of Friedman's test is given. The time point of ovulation is zero.

of TNFα concentration paralleled by an increase in IL-6 concentration during the late luteal phase of the MC may be an important feature for endometrial apoptosis. It has been suggested that upregulation of proinflammatory cytokines such as TNFα plays a role in the induction of programmed cell death in the late luteal phase (Tabibzadeh et al., 1995b) and, in addition, TNFα is important for the induction of endothelial injury in the endometrium (Shalaby, Laegreid, Ammann, & Liggitt, 1989). Taken together, increased levels of TNFα may be necessary to induce menstrual bleeding (Philippeaux & Piguet, 1993). However, these changes of cytokine expression are not restricted to the uterus but were also found in peripheral blood mononuclear cells in our study. At least DHEA, β-estradiol, and testosterone seem to play important roles. In a more general view, the upregulation of several hypothalamus-stimulating cytokines may induce the change in basal temperature (Dinarello et al., 1986) and sleep abnormalities in the luteal phase (Kapas & Krueger, 1992) and may therefore contribute to the premenstrual syndrome. Furthermore, the increase of cytokine secretion may change the disease activity of chronic inflammatory diseases in the luteal phase (Steinberg & Steinberg, 1985; McDonagh et al., 1993; Latman, 1983; Goldstein et al., 1987; Lahita, 1996; Palnaes-Hansen et al., 1987). However, the measured change of serum IL-6 of ± 1 pg/ml around a mean level of about 1.5 pg/ml needs careful consideration because much more serum IL-6 seems to be necessary to stimulate the hypothalamus (Tsigos, Papanicolaou, Defensor, Mitsiadis, Kyrou, & Chrousos, 1997). Nevertheless, this low cytokine concentration may reflect a continuous production of IL-6 in the tissue. In the tissue, there may be a much higher concentration which may be significant, at least at the local site. Such a place could be the hypothalamus, the adrenal glands (Gonzalez-Hernandez, Bomstein, Ehrhart-Bornstein, Gschwend, Gwosdow, Jirikowski, & Scherbaum, 1995), the liver, the spleen, or the lymph nodes. Hence, in endocrine organs, local IL-6 secretion may change hormone secretion and vice versa.

In a recent study, we demonstrated a DHEA-induced decrease of LPS-stimulated IL-6 secretion in peripheral blood mononuclear cells and monocytes (Straub et al., 1998). Only 70% of female subjects demonstrated the typical decrease at a concentration of 1 to 5×10^{-8}M DHEA (Straub et al., 1998). The present study offers an explanation because DHEA-modulated IL-6 secretion varied during the MC. In the follicular and early luteal phase there is an apparent decrease by DHEA of IL-6 secretion from peripheral blood cells. At 85% of all time points DHEA decreased IL-6 secretion. However, β-estradiol and testosterone increased IL-6 secretion at 71% of all time points, which indicates that the DHEA-induced decrease of IL-6 secretion is not due to synthesis of downstream hormones such as β-estradiol or testosterone. Therefore, DHEA may exert its effect on IL-6 secretion of peripheral blood mononuclear cells via other downstream hormones, such as androstenediol or androstentriol, which were found to be important modulators of immune functions (Padgett & Loria, 1994). In the literature, the regulation of IL-6 production by β-estradiol and testosterone varies significantly depending on the conditions used, such as cell type, species, stimulus, and simultaneous administration of other modulatory factors such as cytokines or hormones (Kutteh, Rainey, & Carr, 1991; Girasole, Jilka, Passeri, Boswell, Boder, Williams, & Manolagas, 1992; Li, Danis, & Brooks, 1993; Pottratz, Bellido, Mocharla, Crabb, & Manolagas, 1994; Ray, Prefontaine, & Ray, 1994; Bellido, Jilka, Boyce, Girasole, Broxmeyer, Dalrymple, Murray, & Manolagas, 1995; Kassem, Harris, Spelsberg, & Riggs, 1996). In general, β-estradiol decreases IL-6 secretion,

whereas the situation is not clear with respect to testosterone. However, under certain conditions, β-estradiol can even increase IL-6 secretion (Guerne, Carson, & Lotz, 1990). In all mentioned studies, the phase of the MC was not taken into account at the time of blood sampling. Therefore, the varying conditions do not allow a comparison of the different studies. From the present point of view, modulation of IL-6 secretion depends on gender, reproductive status, and MC, which all modulate the intracellular concentration of steroid hormone receptors. Comparative validation of data concerning endocrine–immune interaction often also obtained from different species has to be done with caution.

In conclusion, there are marked changes of unstimulated and stimulated IL-6 secretion during the menstrual cycle. The periovulatory rise of IL-6 and the late luteal rise of IL-6 and TNFα may be cofactors in the rise in basal temperature or the premenstrual syndrome. Changes in disease activity in chronic inflammatory diseases may reflect the oscillating modulation of immune function due to sex hormone rhythms. Since IL-6 plays an important role in the development of a functional T helper lymphocyte phenotype (Rincon, Anguita, Nakamura, Fikrig, & Flavell, 1997), Th1/Th2 balance may be directly related to hormonal changes. DHEA, β-estradiol, and testosterone are modulators of IL-6 secretion from peripheral blood cells, and DHEA seems to be the main inhibitory signal for IL-6 secretion. Future studies have to demonstrate the effects of downstream hormones of DHEA, such as androstenediol and androstentriol, on cytokine secretion from peripheral blood cells. In the light of the cyclic changes of DHEA-modulated IL-6 secretion the uncontrolled administration of DHEA in therapy of chronic inflammatory diseases needs further discussion.

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