Perimenstrual alterations in type-1/type-2 cytokine balance of normal women

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Background: Perturbations of the type-1/type-2 cytokine balance play a role in the pathogenesis of many diseases. Several immune-based diseases, such as asthma, have significant clinical exacerbations during specific intervals of the menstrual cycle and are associated with oral contraceptive pills (OCRs). The mechanism for these changes is not known, but may involve alterations in the type-1/type-2 cytokine balance.

Objective: To determine if the type-1/type-2 cytokine balance in healthy women changes during a regular menstrual cycle.

Methods: Peripheral blood mononuclear cells from 14 healthy women (seven taking monophasic OCPs) obtained during the perimenstrual interval (3 days prior to 4 days after the onset of menses) and the mid-cycle interval (days 13 to 16) were stimulated with PHA. Supernatants were analyzed for type-1 (IFN-γ) and type-2 (IL-10) cytokines.

Results: During the perimenstrual interval PBMC produced less IFN-γ and more IL-10, resulting in a decreased IFN-γ: IL-10 ratio compared with the mid-cycle interval. The perimenstrual decrease in the IFN-γ: IL-10 ratio was observed in women not taking OCP, but not in women taking OCP. Furthermore, the OCP group had a lower mid-cycle IFN-γ: IL-10 ratio compared with the control group. Finally, subjects reported increased levels of distress during the perimenstrual interval compared with the mid-cycle interval.

Conclusions: These data suggest that healthy women have a perimenstrual shift in the type-1/type-2 cytokine balance toward a type-2 response that is blunted in women taking OCP.

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INTRODUCTION

Type-1 (interferon-γ, IFN-γ) and type-2 (interleukin-4, IL-5, and IL-10) cytokines play a critical role in the regulation of cellular versus humoral immune response of healthy humans.[1] The balance of type-1 and type-2 cytokines is determined by a number of factors including the cytokine milieu of the microenvironment,[1] the nature and dose of the antigen,[2] the antigen presenting cell directing the response,[3] and the genetics of the host.[4] Perturbations in the type-1/type-2 cytokine balance may contribute to the pathogenesis of immune-based diseases such as increased infections,[5,6] asthma,[7,8] and certain autoimmune diseases.[9]

Patients with many of these immune-based diseases may have exacerbations during specific periods of the menstrual cycle.[10,11] During the perimenstrual interval (3 days prior to 4 days after the onset of menses)[11] some women report a worsening of their asthma symptoms confirmed by a decrease in peak expiratory flow rate.[12] Furthermore, increased emergency department visits of females for asthma exacerbation during the perimenstrual interval have been reported.[11] The mechanisms of the perimenstrual changes disease pattern are not known but may involve direct sex hormone-induced and/or perimenstrual distress-associated alterations in type-1/type-2 cytokine expression.

A recent case report described a woman with stable perimenstrual asthma that deteriorated when she began taking oral contraceptive pills (OCPs) and recovered when the OCPs were discontinued.[13] A similar case report was published 30 years ago.[14] The mechanism of these changes was not investigated but was thought to involve sex hormone-mediated changes in immune function. Indeed, OCPs have been reported to alter natural killer cell activity[15] and lymphocyte proliferative responses;[16] however the effects of OCPs on the type-1/type-2 cytokine balance is poorly understood.

Several in vitro assessments of immune function fluctuate during a normal menstrual cycle in healthy women. Previous reports have demonstrated a perimenstrual decrease in natural killer cell cytotoxicity,[17] phagocyte activity,[18] and antigen-specific immune responses.[19] In addition, expression of type-2 cytokines was increased in endometrial samples obtained during the mid-luteal phase of the menstrual cycle.[20] Initial reports have suggested that peripheral blood mononuclear cells (PBMC) obtained during the perimenstrual period have a cytokine balance favoring a type-2 response after antigen stimulation.[21] This perimenstrual type-2 cytokine shift could potentially predispose susceptible individuals to exacerbations of diseases such as asthma in which type-2 cytokines are involved in the pathogenesis.

This study was undertaken to determine whether the type-1/type-2 cytokine balance is altered during the perimenstrual period compared with the mid-cycle period of a normal menstrual cycle in healthy women. Furthermore, we sought to investigate the

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effect of monophasic OCPs on the catamenial cytokine alterations and whether perimenstrual distress was associated with the alterations in type-1/type-2 cytokine production.

METHODS

Subjects

A total of 14 healthy female volunteers (mean age 28.9 ± 6.7, range 20 to 44 years) with a history of normal menstrual cycles were studied during the perimenstrual interval (PERI, 3 days prior to 4 days after the onset of menses) and mid-cycle interval (MID, days 13 to 16) of a normal menstrual cycle. Subjects were further divided into two groups. Volunteers taking combination estradiol and progesterone monophasic OCPs for contraceptive purposes only were classified into the oral contraceptive group (n = 7). Subjects not having taken OCPs for more than 1 year were classified into the control group (n = 7). All blood samples were collected between 7 and 9 AM to control for diurnal variation. The study was approved by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center-Houston. Informed consent was obtained from each subject prior to enrollment in the study.

Isolation of PBMC

Peripheral blood mononuclear cells were isolated from heparinized venous blood using Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation as previously described.[22] PBMC were subsequently resuspended at 1×10^6 viable cells per milliliter of RPMI-1640 containing 10% human AB serum (Biocell, Rancho Dominguez, CA), 90 U/mL penicillin, 90 μg/mL streptomycin, and 2 mM L-glutamine (all from Sigma St. Louis, MO). Viability of PBMC was determined using trypan blue (Sigma) exclusion.

Mitogen-Stimulated Cultures

Cultures of 1×10^6 PBMC per milliliter were stimulated with 10 μg/mL phytohemagglutinin (PHA, Sigma) for 72 hours at 37°C and 5%CO_2. Supernatants were harvested and stored at −70°C for subsequent interferon-gamma (IFN-γ) and interleukin-10 (IL-10) determination.

Cytokine Analyses

Cytokine levels in culture supernatants were determined by ELISA using commercial paired monoclonal antibodies specific for human IFN-γ and IL-10 (R&D Systems, Minneapolis, MN). Concentrations were calculated based on standard curves using recombinant human cytokine standards (rhIFN-γ and rhIL-10, both from R&D Systems). Sensitivities of the ELISAs were 6.0 pg/mL and 6.3 pg/mL, respectively.

Self-Report Measures

At each visit all subjects were asked to fill out several questionnaires assessing general health, diet including recent alcohol use, sleep, and physical activity. To determine the amount of perimenstrual distress, subjects were asked to complete Form T of the Menstrual Distress Questionnaire (MDQ, Western Psychological Services) at each visit. The MDQ is a commonly utilized self-report instrument that measures the degree and nature of distress during the menstrual cycle.[23]

Statistics

All data are displayed as mean ± standard error of the mean (SEM). Comparisons between the perimenstrual and mid-cycle samples utilized the Wilcoxin signed rank test. Comparisons between the control and OCP group utilized the Mann-Whitney test. The Spearman nonparametric correlation test was utilized to determine if the self-report measures correlated with the cytokine data.

RESULTS

Perimenstrual Alterations in the Type-1/Type-2 Cytokine Balance

To investigate the type-1/type-2 cytokine balance during the menstrual cycle, PBMC isolated from healthy women during the perimenstrual and mid-cycle intervals were stimulated with PHA. All fourteen subjects were initially included in these analyses. Culture supernatants were assessed for production of type-1 (IFN-γ) and type-2 (IL-10) cytokines. As shown in Figure 1, PBMC obtained during the perimenstrual interval produced less IFN-γ than the mid-cycle period (PERI 2213 ± 418 pg/mL; MID 3255 ± 605 pg/mL, $P = 0.05$). However, production of IL-10 was increased during the perimenstrual interval compared with the mid-cycle interval (PERI 1939 ± 234 pg/mL; MID 1605 ± 181 pg/mL; $P = .02$). There were no differences observed in PHA-stimulated PBMC proliferation as measured by ^{3}H-thymidine uptake (data not shown).

The relative balance of type-1 and type-2 cytokine, rather that the absolute production of a single cytokine, may play a more significant role in determining the balance of the immune response in situ.[24] The type-1/type-2 cytokine ratio therefore has frequently been utilized to represent the relative type-1/type-2 cytokine balance.[25] The IFN-γ: IL-10 ratio during the perimenstrual interval was decreased compared with the ratio during the midcycle interval (PERI 1.3 ± 0.3; MID 2.2 ± 0.5; $P = .0031$). These data indicate a perimenstrual shift in the type-1/type-2 cytokine balance toward a type-2 response in healthy women during a normal menstrual cycle.

Effect of Oral Contraceptive Pills on the Perimenstrual Cytokine Alterations

To determine if the use of OCPs altered the perimenstrual changes in the type-1/type-2 cytokine balance, subjects were grouped into those currently taking monophasic OCPs and control subjects not having taken OCPs for at least 1 year (Fig 2). Peripheral blood mononuclear cells from the control group produced slightly less IFN-γ (PERI 2184 ± 531 pg/mL; MID 4173 ± 950 pg/mL, $P = .16$) and more IL-10 (PERI 1989 ± 423 pg/mL; MID 1632 ± 341 pg/mL, $P = .22$) during the perimenstrual interval compared with the mid-cycle interval. There was a significant perimenstrual decrease in the IFN-γ: IL-10 ratio in control subjects (PERI 1.5 ± 0.5; MID 2.9 ± 0.8,

P = .03); however, PBMC from the OCP group produced similar levels of IFN-γ (PERI 2242 ± 689 pg/mL; MID 2234 ± 628 pg/mL, P = .38) and IL-10 (PERI 1889 ± 238 pg/mL; MID 1557 ± 156 pg/mL, P = .11) during both the perimenstrual and mid-cycle intervals. Furthermore, the change in the IFN-γ: IL-10 ratio (PERI 1.1 ± 0.3; MID 1.5 ± 0.4, P = .16) was decreased in the OCP group compared with the control group. These data suggest that subjects taking OCP have a blunted perimenstrual shift in the type-1/type-2 cytokine balance toward a type-2 response compared with subjects not taking OCP.

To determine if the absence of a perimenstrual cytokine shift was due to a persistent type-2 cytokine response throughout the menstrual cycle, we compared the control group with the OCP group during the mid-cycle and the perimenstrual period (Fig 3). During the perimenstrual interval IFN-γ and IL-10 production, as well as the IFN-γ: IL-10 ratios were similar in both the control and OCP groups. During the mid-cycle interval there was a trend toward a decrease in IFN-γ production but no change in IL-10 production. The overall effect was a trend toward a decreased IFN-γ: IL-10 ratio in the OCP group compared with the control group suggesting a type-1/type-2 cytokine balance favoring a type-2 cytokine response. Therefore, the type-1/type-2 cytokine balance in the OCP group was shifted toward a type-2 cytokine response during both the mid-cycle and perimenstrual period.

Self-Report Measures

There were no reported significant changes in sleep patterns, physical activity, general health status, and dietary intake including alcohol consumption during the perimenstrual period compared with the mid-cycle period (data not shown).

To assess the levels of perimenstrual distress, the subjects were asked to complete Form T of the Menstrual Distress Questionnaire. As shown in Table 1, the perimenstrual period was associated with an increase in the scores on the pain, water retention, and negative affect scales compared with the mid-cycle interval. These data indicate higher levels of distress during the perimenstrual interval compared with the mid-cycle period. Mid-cycle scores from the control and the OCP group were similar (data not shown). During the perimenstrual interval the OCP group tended to report lower scores on the pain, water retention, and negative affect scales, suggesting a lower level

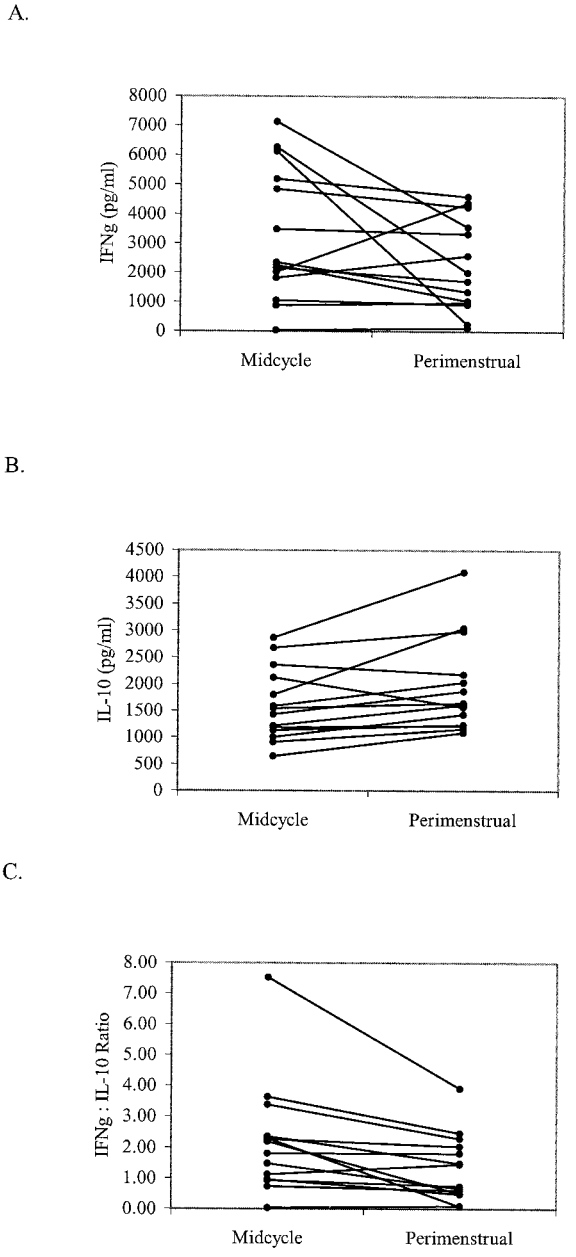

Figure 1. Perimenstrual type-1/type-2 cytokine imbalances in healthy women. IFN-γ and IL-10 production by PHA-stimulated PBMC isolated from healthy women during the MID and PERI intervals was determined. Data are given for each subject individually. During the PERI interval compared with the MID interval, (A) IFN-γ production was decreased (2213 ± 418 pg/mL versus 3255 ± 605 pg/mL; P = .05), (B) IL-10 production was increased (1939 ± 234 pg/mL versus 1605 ± 181 pg/mL, P = .02), and (C) the IFN-γ: IL-10 ratio was decreased (1.3 ± 0.3 versus 2.2 ± 0.5; P = .0031). N = 14 subjects.

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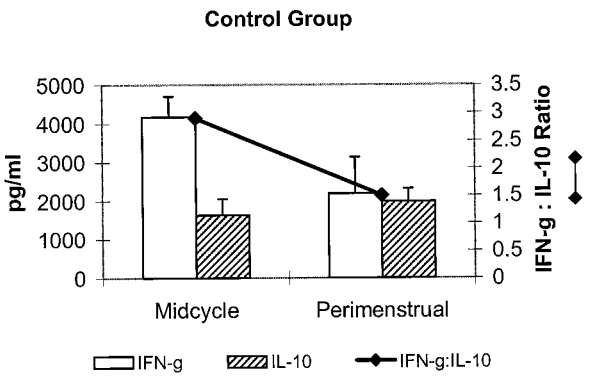

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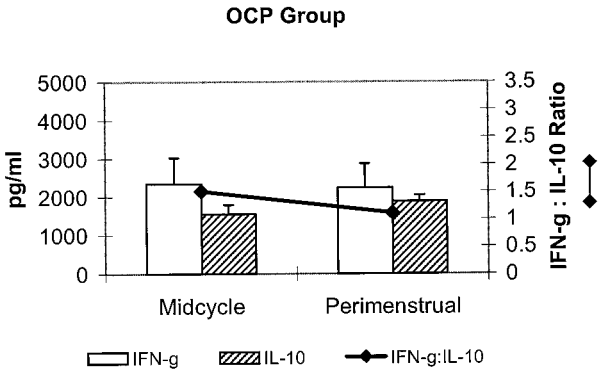

Figure 2. The effect of monophasic OCP on perimenstrual type-1/type-2 cytokine alterations. IFN-γ and IL-10 production by PHA-stimulated PBMC isolated from the control group (n = 7 subjects) and the OCP group (n = 7 subjects) was determined. (A) Control: During the PERI interval compared with the MID interval, IFN-γ production was decreased (P = ns), IL-10 production was increased (P = ns), and IFN-γ: IL-10 ratio was decreased (P = .03). (B) OCP: During the PERI interval compared to the MID interval, IFN-γ production, IL-10 production, and IFN-γ: IL-10 ratio were unchanged (P = NS). Open bars represent IFN-γ production. Striped bars represent IL-10 production. Solid line represents IFN-γ: IL-10 ratio. Y-error bars represent SEM.

of perimenstrual distress. No statistically significant correlations between any of the eight MDQ scales and perimenstrual type-1/type-2 cytokine alterations were found.

DISCUSSION

Once believed to be completely autonomous, there is substantial evidence that the immune system is bi-directionally connected with neuroendocrine networks.[26] For example, through activation of the hypothalamic-pituitary-axis, psychological stress has demonstrable affects on the immune system and in some instances may significantly contribute to the onset or exacerbation of clinical illness.[26–30] The associations of the menstrual cycle and the female sex hormones with clinical illness continue to generate intense interest. Certain diseases, especially those associated with perturbation of the type-1/type-2 cytokine balance, often have clinically significant exacerbations reported by patients during the perimenstrual interval of an otherwise normal menstrual cycle.[10,11] The mechanisms that regulate the type-1/type-2 cytokine balance during the menstrual cycle and its potential contribution to disease exacerbations have not been clearly defined.

In the current investigation, the type-1/type-2 cytokine balance in PHA-stimulated PBMC was determined during the mid-cycle and the perimenstrual intervals of a regular menstrual cycle in healthy women. During the perimenstrual interval, production of IFN-γ was decreased, production of IL-10 was increased, and the IFN-γ: IL-10 ratio was decreased compared with the mid-cycle interval. In contrast, perimenstrual alterations in the IFN-γ: IL-10 ratio was blunted in women taking OCPs. It is not clear whether the mid-cycle or the perimenstrual interval represents a baseline type-1/type-2 cytokine balance. The overall type-1/type-2 cytokine balance, rather than a change in only one cytokine, is believed to be important in determining the outcome of the immune response in situ.[24] Therefore it is, the relative change in the cytokine balance during the menstrual cycle that may predispose women to an increase in the susceptibility and/or severity of a variety of immune-based diseases. Further, it is the perimenstrual interval of the cycle most associated with disease exacerbation, suggesting that the mid-cycle period may be the baseline immune status.

The mechanisms responsible for these changes are poorly understood but may involve a direct effect of the sex hormone-induced alterations on immune function, perimenstrual distress-associated immune alterations, or a combination of the two. Women often report increased levels of psychological and physical distress during the perimenstrual interval compared with other times of their normal menstrual cycle.[23] Previous reports have demonstrated that increased psychological stress results in a suppression of cellular immune function[30] and a shift in the

type-1/type-2 cytokine balance toward a type-2 response.[26] The association of distress and type-1/type-2 cytokine alterations in the current investigation is similar to previous reports of stress-associated dysregulation of the type-1/type-2 cytokine balance.[26] Subjects in the current investigation reported significant elevations in perimenstrual distress as determined by the MDQ, which may be sufficient to induce a type-2 cytokine shift. Therefore it is possible that a stress-distress-immune link could, at least in part, explain the increased disease susceptibility and/or exacerbations of many women during the perimenstrual interval.

Another potential mechanism of the altered cytokine production is a direct effect of sex hormones such as estradiol, progesterone, luteinizing hormone, follicle-stimulating hormone and/or prolactin, on immune cells. Progesterone has been reported to increase production of type-2 cytokines and decrease production of type-1 cytokines in both in vitro and in vivo studies.[31] Plasma estrogen levels have been found to correlate with perimenstrual asthma exacerbations.[11] The use of OCPs has been associated with both increase susceptibility and/or severity of immune-based diseases[13,32,33] as well as alterations in immune function.[15,16] Oral contraceptive pill use in healthy women has been associated with a suppression of natural killer cell cytotoxicity[15] and mitogen-induced lymphocyte proliferation.[16] The decrease in the perimenstrual cytokine alterations and the trend toward a type-2 cytokine response in the OCP group suggests an effect of pharmacologic levels of estrogen and progesterone on the type-1/type-2 cytokine balance. It is important to note that the oral OCPs taken in this study contained both estradiol and progesterone during the mid-cycle period and placebo during the perimenstrual interval. Consistent with this, both groups had a similar type-1/type-2 cytokine balance during the perimenstrual interval (placebo pill). However, during the mid-cycle interval, however, the OCP group (estradiol/progesterone pill) was shifted toward a type-2 cytokine response compared to the control group. The cyclical changes in the concentrations of pituitary and/or gonadal hormones may therefore directly influence the balance of type-1 and type-2 cytokines during the menstrual cycle. Both in vitro and in vivo studies are underway investigating the

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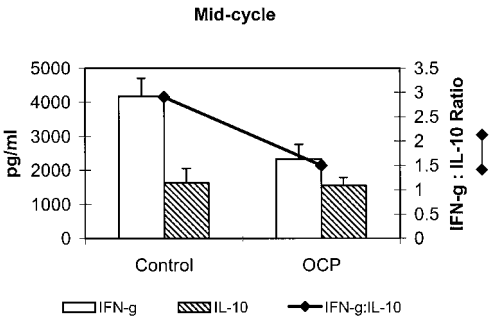

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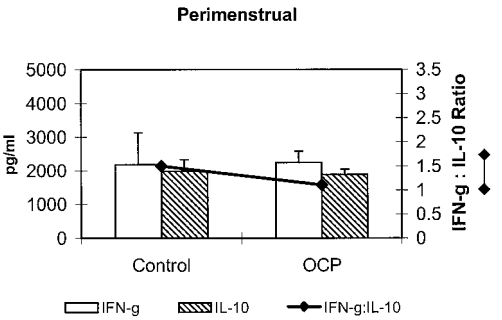

Figure 3. Type-1/type-2 cytokine balance in women not taking and taking OCP. PHA stimulated IFN-g and IL-10 production by PBMC isolated form the control and OCP group during the MID (A) and PERI (B) intervals were compared. (A) During the MID interval, the control group tended to have higher IFN-g levels, lower IL-10 levels, and a lower IFN-γ: IL-10 ratio compared with the OCP group. (B) During the PERI interval, the control group had similar IFN-g levels, IL-10 levels and the IFN-g: IL1-0 ratio compared with the OCP group. Open bars represent IFN-γ production. Striped bars represent IL-10 production. Solid line represents IFN-γ: IL-10 ratio. Y-error bars represent SEM.

Table 1. Form T of the Menstrual Distress Questionnaire*

	Perimenstrual	Mid-cycle	*P* Value
Pain	54.4 ± 14.5	41.6 ± 1.3	.0066
Water retention	64.1 ± 5.9	41.3 ± 1.4	.0015
Autonomic reactions	46.9 ± 2.2	45.5 ± 0.7	>.9999
Negative affect	43.8 ± 2.7	38.7 ± 1.3	.0444
Impaired concentration	41.3 ± 1.9	41.3 ± 1.8	.9375
Behavior change	40.6 ± 0.7	42.5 ± 1.6	.4688
Arousal	37.5 ± 2.6	42.4 ± 4.8	.1934
Control	45.9 ± 1.9	45.9 ± 1.4	>.9999

* All subjects (n = 14) completed the Form T of the MDQ at each visit. Data are represented as mean ± SEM.

relative roles of perimenstrual distress and sex hormones on the type-1/type-2 cytokine balance.

Imbalances in the type-1/type-2 cytokine production play an important role in the pathogenesis of infectious, asthmatic, and certain autoimmune diseases.[5–7,9] Type-2 cytokines are believed to play a central role in the pathogenesis of asthma. Interleukin-4 and IL-5 are elevated in bronchial biopsies,[34] bronchoalveolar lavage cells,[7] and the blood of patients with asthma.[34] These data are provocative in that the prominent type-2 cytokine response observed during the perimenstrual period may be an important mediator of the perimenstrual exacerbations of asthma in susceptible women.[11] Furthermore, the persistent type-2 cytokine response in women taking OCP may explain, at least in part, the worsening of asthma in susceptible women who take OCP.[13,14] Future studies will investigate the perimenstrual and OCP alterations in type-1/type-2 cytokine balance in asthmatic subjects and correlate these changes with disease specific outcomes.

In summary, these data demonstrate that the type-1/type-2 cytokine balance is shifted toward a type-2 response during the perimenstrual interval compared with the mid-cycle interval in healthy women. Women taking OCPs have a cytokine response shifted toward a type-2 response throughout the menstrual cycle. These studies provide a potential mechanism for the known clinical exacerbations of immune-based pathology such as asthma during the perimenstrual period. If confirmed with additional studies, such mechanisms should serve as a target for the development interventions to treat or even prevent the perimenstrual associated disease exacerbations.

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