Role of prostaglandin E2 in bacterial growth in women with endometriosis

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STUDY QUESTION: Can prostaglandin E2 (PGE2) in menstrual and peritoneal fluid (PF) promote bacterial growth in women with endometriosis?

SUMMARY ANSWER: PGE2 promotes bacterial growth in women with endometriosis.

WHAT IS KNOWN ALREADY: Menstrual blood of women with endometriosis is highly contaminated with Escherichia coli (E. coli) compared with that of non-endometriotic women: E. coli-derived lipopolysaccharide (LPS) promotes the growth of endometriosis.

STUDY DESIGN, SIZE AND DURATION: Case-controlled biological research with a prospective collection of body fluids and endometrial tissues from women with and without endometriosis with retrospective evaluation.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: PF and sera were collected from 58 women with endometriosis and 28 women without endometriosis in an academic research laboratory. Menstrual blood was collected from a proportion of these women. Macrophages (MΦ) from PF and stromal cells from eutopic endometria were isolated in primary culture. The exogenous effect of PGE2 on the replication of E. coli was examined in a bacterial culture system. Levels of PGE2 in different body fluids and in the culture media of MΦ and stromal cells were measured by ELISA. The effect of PGE2 on the growth of peripheral blood lymphocytes (PBLs) was examined.

MAIN RESULTS AND THE ROLE OF CHANCE: The PGE2 level was 2–3 times higher in the menstrual fluid (MF) than in either sera or in PF. A significantly higher level of PGE2 was found in the MF and PF of women with endometriosis than in control women (P < 0.05 for each). Exogenous treatment with PGE2 dose dependently increased E. coli colony formation when compared with non-treated bacteria. PGE2-enriched MF was able to stimulate the growth of E. coli in a dilution-dependent manner; this effect was more significantly enhanced in women with endometriosis than in control women (P < 0.05). PGE2 levels in the culture media of LPS-treated MΦ/stromal cells were significantly higher in women with endometriosis than in non-endometriosis (P < 0.05 for each). Direct application of PGE2 and culture media derived from endometrial MΦ or stromal cells significantly suppressed phytohemagglutinin-stimulated growth of PBLs.

LIMITATIONS AND REASONS FOR CAUTION: Further studies are needed to examine the association between PGE2-stimulated growth of E. coli and endotoxin level and to investigate the possible occurrence of sub-clinical infection within vaginal cavity.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings may provide some new insights to understand the physiopathology or pathogenesis of the mysterious disease endometriosis and may hold new therapeutic potential.

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Introduction
Endometriosis is an estrogen-dependent chronic inflammatory disease mostly affecting women of reproductive age. Besides hormonal regulation, both secondary and initial inflammatory mediators are known to be involved in the growth of endometriosis (Attar and Bulun, 2006; Khan et al., 2008, 2009). As a source of an initial inflammatory mediator (lipopolysaccharide, LPS), we recently demonstrated Escherichia coli (E. coli) contamination of menstrual blood with increased levels of bacterial endotoxin (LPS) in the menstrual fluid (MF) and peritoneal fluid (PF) of women with endometriosis compared to that in control women with no endometriosis and an LPS/Toll-like receptor 4 (TLR 4) cascade in the growth of endometriosis (Khan et al., 2010). However, the mechanistic basis of this microbial contamination of menstrual blood is unclear.

LPS-promoted proliferation and invasion of endometriotic stromal cells via up-regulation of cyclooxygenase 2 (COX2) and prostaglandin E2 (PGE2) have been reported (Takenaka et al., 2010). A growing body of evidence demonstrates that PGE2 regulates many pathophysiological processes including cell proliferation, anti-apoptosis, immune suppression and angiogenesis during the development of endometriosis (Wu et al., 2010). It has been previously reported that PGE2-enriched seminal plasma has the capacity to replicate HTLV-II and other human immunodeficiency virus (HIV)-associated viruses (Kuno et al., 1986a). This may explain the increase in HIV viral load in the homosexual population either by the direct action on viral replication or indirect immunosuppressive effect of PGE2 (Kuno et al., 1986a,b).

The local immunosuppressive effect of PGE2 has also been demonstrated to promote the growth of cancer cells (Kojima et al., 2001). Immune cells of lymphoid lineage that comprise T and B lymphocytes and natural killer cells play essential roles in determining whether to accept or reject survival, and implantation and proliferation of endometrial and endometriotic cells. An aberrant function of these immune cells has been reported in women with endometriosis (Osuga et al., 2011). Information on the immunosuppressive effect of PGE2 on peripheral blood lymphocytes (PBLs) derived from women with endometriosis is limited.

Clinically, women with endometriosis are reported to complain of dysmenorrhea and pelvic pain. An association with elevated levels of PGE2/PGF2α in MF, PF and in tissues derived from the eutopic and ectopic endometria of women with endometriosis has been described (Benedetto, 1989; Koike et al., 1992; Wu et al., 2007). Besides its role in promoting viral replication, information regarding the involvement of PGE2 in the proliferation of bacteria such as E. coli is lacking. We speculated that PGE2 in the MF of women with endometriosis could be responsible for the increased replication of E. coli, either by its direct effect or indirect immunosuppressive effect, once this common vaginal microbial flora transmigrates from the lower genital tract into the uterine cavity.

To address this question, firstly, we measured PGE2 levels in different body fluids; namely MF, PF and sera derived from women with and without endometriosis. Secondly, we investigated whether PGE2 had any direct role on the replication of E. coli in a bacteria culture system. Thirdly, we examined the dilution-dependent effect of MF derived from control women and women with endometriosis on E. coli growth and patterns of bacterial growth after neutralizing PGE2. Finally, we extended our experiment to investigate whether PGE2-exhibited growth suppression effect on PBLs derived from women with and without endometriosis.

Materials and Methods

Patient samples
The subjects in this study were women of reproductive age. PF and sera were collected, during laparoscopy, from 58 women with endometriosis and cycle-matched to 28 women without endometriosis. Women with endometriosis aged between 20 and 42 years were recruited by either elective laparoscopy for infertility or diagnostic laparoscopy for dysmenorrhea and subsequently confirmed by histology. The control group, between 18 and 32 years old, consisted of fertile women without any evidence of endometriosis and were operated on for dermoid cyst by laparoscopy. Neither the endometriotic group nor the control, endometriosis-free group had been on hormonal medication in the 3 months prior to the surgical procedure. All control women and women with endometriosis had regular menstrual cycles (28–32 days). All induced menstrual cycles were excluded from the current study. All body fluid samples and biopsy specimens were collected in accordance with the guidelines of the Declaration of Helsinki and with the approval of the Institutional Review Board of Nagasaki University. An informed consent was obtained from all women.

Collection of menstrual blood
With informed consent and under strict aseptic measure, we collected menstrual blood from 20 women with endometriosis and 15 women without endometriosis on Day 1–Day 3 of the menstrual cycle as described previously (Kamiyama et al., 2004; Khan et al., 2010). Briefly, in the dorsal lithotomy position, women underwent cleansing and disinfection of the vulva and vagina. A Fr. 12-sized plastic catheter connected to a 10-ml plastic syringe was inserted into the uterine cavity transcervically, and intrauterine aspiration was performed under negative pressure induced by the manual suction of a syringe pump. The materials obtained were transferred into heparinized endotoxin-free plastic containers. After serial processing and centrifugation, MF was collected and stored. All subjects underwent a laparoscopy to confirm the presence or absence of endometriosis.

All samples of sera, PF and MF were collected prospectively and stored at $–80^\circ$ C for subsequent analysis.

Isolation of macrophages and endometrial stromal cells
Macrophages (Mφ) from the PF and stromal cells from the eutopic endometria were collected from six women each with or without endometriosis. The detailed procedures of the isolation of Mφ and stromal cells were described previously (Osteen et al., 1989; Rana et al., 1996; Khan et al., 2005a,b). The purity of Mφ and stromal cell preparation was $>95\%$, as judged by positive cellular staining for CD68 and vimentin, respectively and negative staining for CD45, a pan-leukocyte marker, cytokeratin, an epithelial cell marker and von Willebrand factor, a micro-vessel marker.

PGE2 assay in different body fluids
PGE2 level was measured in duplicate by ELISA (Quantikine, R&D system, Minneapolis, MN, USA) in the sera, PF and MF derived from women with and without endometriosis. After the first passage, Mφ and stromal cells ($10^5$ cells per well) were serum-starved for 24 h and cultured for...
PGE$_2$ promotes growth of E. coli

another 24 h with LPS (10 ng/ml) derived from E. coli (serotype 0111:B4; Sigma, St. Louis, MO, USA). The PGE$_2$ level was measured in the culture media derived from basal (LPS non-treated) and LPS-treated Mφ and stromal cells. The antibody used in PGE$_2$ determination did not cross-react with other cytokines. The intra-assay and inter-assay coefficients of variation were <10% with a detection limit of 8.25 pg/ml.

Bioculture of E. coli

We used the similar strain of E. coli (K-12, serotype 0111:B4, Sigma) in a bacteria culture system that we detected in menstrual blood (Khan et al., 2010) and the cell wall extract (LPS) of which was used for our current experiment. We cultured E. coli in Luria-Bertani (LB) agar plates. To induce uniform colony growth, 100 μl of E. coli, dissolved in dimethylsulfoxide (DMSO), was added to each of 5 ml LB liquid media and incubated in a shaking incubator (280 rpm) at 37°C for 2, 3, 4 and 6 h. We found a time-dependent increase in the colony formation of E. coli with a minimal colony formation at 2–3 h and with a dilution of $10^{-4}$ and $10^{-5}$. In our next experiment, we cultured PGE$_2$-treated and non-treated E. coli in LB liquid media for 3 h and then incubated overnight in LB agar plates at a dilution of $10^{-6}$ and $10^{-7}$. Next morning, E. coli growth was counted using a handy colony counter (Shibata, Tokyo, Japan) and expressed as colony forming unit (CFU)/ml. PGE$_2$ was diluted in DMSO and expressed as CFU/ml. All experiments were performed in triplicates for each dilution.

Treatment of E. coli with PGE$_2$

We investigated the dose-dependent effect of PGE$_2$ (#14010, Cayman Chemical, USA) on the growth of E. coli in a bacteria culture system as described above. The serially diluted E. coli in LB liquid media was treated with various doses of PGE$_2$ (1 pg/ml–100 ng/ml) and incubated overnight at 37°C. The colony formation of treated and non-treated E. coli was examined, all colonies were counted in each culture plate and expressed as CFU/ml. All experiments were performed in triplicates for each dose of PGE$_2$.

Treatment of E. coli with MF

In an attempt to examine the direct effect of MF on E. coli growth, diluted E. coli ($10^{-7}$) in LB liquid media was treated with serially diluted of MF (1:100, 1:200, 1:300, 1:500 and 1:1000) collected from women with endometriosis and incubated overnight at 37°C. In separate experiments, E. coli in LB liquid media was pre-treated with anti-PGE$_2$ antibody (10 μg/ml) (Sigma Chemical Co. USA), maintained in culture for 20 min and then further co-treated with serial dilution of MF and incubated overnight, as above. We also performed similar experiments on the effect of MF (1:100 dilution) derived from women with and without endometriosis on the pattern of E. coli growth. The colony formation of MF-treated, anti-PGE$_2$ antibody pre-treated and MF non-treated E. coli was counted and expressed as CFU/ml. All experiments were performed in triplicates for each dilution of MF.

Treatment of lymphocytes with PGE$_2$

Total population of lymphocytes was collected from the peripheral blood of six women each with and without endometriosis by Ficoll-Paque separation (Pharmacia Biotechnology, USA). The lymphocytes (10$^7$ cells/ml) were treated with 0, 1, 10, 30, 50 and 100 μg/ml of phytohemagglutinin (PHA), a potent mitogen for lymphocytes and then co-treated with PHA (50 μg/ml) and PGE$_2$ (10, 50 and 100 pg/ml). Phosphate-buffered saline was used as a vehicle treatment for control experiments and to standardize lymphocyte count. The pattern in the changes of lymphocyte growth was examined by counting the number of lymphocytes for each treatment and expressed as fold increase in the growth of lymphocytes compared with non-treated cells.

Co-treatment of lymphocytes with PHA, culture media and PGE$_2$ inhibitor

We co-treated lymphocytes with PHA and 10% culture media (100 μl) derived from separately cultured Mφ and stromal cells collected from women with endometriosis. In a separate experiment, the lymphocytes were pre-treated with anti-PGE$_2$ antibody (10 μg/ml), maintained in culture for 20 min and then further co-treated with 10% culture media derived from each of Mφ and stromal cells to prove that it is the PGE$_2$ in the culture media causing suppression in the growth of PHA-stimulated lymphocytes.

Statistical analysis

The clinical characteristics of the subjects were evaluated by one-way analysis of variance. The data are expressed either as mean $\pm$ SEM or mean $\pm$ SD. The Mann–Whitney U-test and Student’s t-test were used to analyze differences between groups and the Kruskal–Wallis test was used to analyze differences among groups. A box plot analysis of PGE$_2$ levels in different body fluids was performed using the medians and inter-quartile range (IQR). Tukey’s post hoc test was also used to exclude bias with multiple comparisons. A P-value of $<0.05$ was considered statistically significant.

Results

The clinical profiles of women with and without endometriosis are shown in Table I. There were no significant differences in the mean age or other clinical characteristics between women with and without endometriosis. The distribution of peritoneal lesions in women with revised-ASRM stage I–II endometriosis and stage III–IV endometriosis are shown in Table I. We found a predominance of mixed peritoneal lesions in both stage I–II and stage III–IV.

| Table I Clinical profiles of patients with and without endometriosis. |
|-----------------|-----------------|-------------------|
|                 | Controls (n = 28) | Endometriosis (n = 58) |
| Age in years (mean $\pm$ SD) | 28.4 $\pm$ 3.9 | 30.2 $\pm$ 3.5 |
| Range of age in years | 18–32 | 20–42 |
| r-ASRM staging: I–II/III–IV | 18/12 | 25/23 |
| Distribution of peritoneal lesions | Stage I–II: red/black/white/mixed: 8/9/6/12 | Stage III–IV: red/black/white/mixed: 4/5/4/8 |
| Menstrual cycle: P/S/M/A | 10/15/3/0 | 20/32/6/0 |

The results are expressed as mean $\pm$ SD. P, proliferative phase; S, secretory phase; M, menstrual phase; r-ASRM, revised staging of the American Society of Reproductive Medicine.
endometriosis. Two cases in stage III–IV endometriosis had a chocolate cyst only without any coexistent peritoneal lesions.

**PGE2 levels in different body fluids**

Kruskal–Wallis testing indicated that the PGE2 level was highest in the MF among the three body fluids examined \((P < 0.001\) versus PF and sera) (Fig. 1). We found a 2–3 times higher PGE2 level in the MF of women with and without endometriosis compared with PGE2 levels in either PF or in sera (Fig. 1). A significantly higher level of PGE2 was found in the MF of women with endometriosis than in women without endometriosis \((P < 0.05\) for each, Fig. 1). No apparent difference in PGE2 levels in sera was found between women with and without endometriosis.

**PGE2-stimulated growth of E. coli**

PGE2 appears to promote a dose-dependent increase in E. coli colony formation (Fig. 2A). Some colonies of E. coli were highly responsive to growing after treatment with lower doses of PGE2, while other colonies grew in response to higher doses of PGE2 when compared with colony growth in the control (DMSO) LB agar plate \((P < 0.05,\) DMSO versus PGE2 1 pg/ml and 10 pg/ml; \(P < 0.05,\) DMSO versus PGE2 10, 50 and 100 ng/ml, Fig. 2A). We performed a similar experiment in another bacteria culture plate (McConkey agar plate) to confirm the validity of our results and we found a similar pattern of E. coli growth in response to PGE2.

**MF-stimulated growth of E. coli**

MF from women with endometriosis was found to significantly stimulate the growth of E. coli in a dilution-dependent fashion when

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**Figure 1** PGE2 levels in the sera, PF and MF derived from women with (hatched box) and without (white box) endometriosis. PGE2 levels were found to be highest in the MF, intermediate in the PF and the lowest in the sera, whether derived from women with and without endometriosis. Although no group difference was observed in serum, PGE2 levels were significantly higher in the PF and MF of women with endometriosis than in control women \((P < 0.05\) for each). Boxes represent the distance (IQR) between the first (25%) and third (75%) quartiles, and horizontal lines in the boxes represent median values.

**Figure 2** Colony forming units (CFU/ml) of E. coli in response to variable doses of PGE2 (A). A fluctuating growth pattern of E. coli was observed in response to a lower dose to higher dose of PGE2. Multiple comparison analysis revealed that from a lower dose to higher dose, PGE2 was able to significantly stimulate the growth of E. coli \((\text{**P} < 0.05\) for each indicated dose) when compared with non-treated (DMSO only) bacteria. (B) The colony formation (CFU/ml) of E. coli was significantly increased in response to PGE2-enriched MF derived from women with endometriosis in a dilution-dependent fashion comparing with DMSO-treated E. coli (white bar, \(\text{**P} < 0.05\) for each indicated dilution). The MF-stimulated growth of E. coli was significantly suppressed after pretreatment of E. coli with anti-PGE2 antibody (black bar) \((\text{**P} < 0.05\) versus anti-PGE2 antibody-non-treated bacteria for each dilution). (C) The colony formation of E. coli was significantly higher in response to MF (diluted 1:100) collected from women with endometriosis than in control women \((\text{**P} < 0.05\) for each). MF-stimulated E. coli growth (white bar) was equally suppressed in both control women and in women with endometriosis after pretreatment of bacteria with anti-PGE2 antibody (black bar) \((\text{**P} < 0.05\) for each). DMSO, dimethylsulfoxide; anti-PGE2 (−) denotes colonies without pretreatment with anti-PGE2 antibody; anti-PGE2 (+) denotes colonies with pretreatment with anti-PGE2 antibody. The results are expressed as mean ± SD of triplicate experiments for each indicated dose of PGE2 or for each dilution of MF.
compared with DMSO-treated E. coli (P < 0.05 for each dilution of MF except 1:1000, Fig. 2B). We found a higher colony formation of E. coli in response to low dilutions of MF (1:100 and 1:200) and lower E. coli growth in response to higher dilution of MF (1:300 and 1:500). We did not find any growth of E. coli with further dilution of MF (1:1000), it was similar to DMSO-treated E. coli growth. This MF-stimulated E. coli growth was significantly suppressed after pretreatment of E. coli with anti-PGE2 antibody (P < 0.05 for each dilution of MF except 1:1000, Fig. 2B).

We also found a significantly higher colony formation of E. coli in response to MF (1:100) derived from women with endometriosis compared with the effect of MF (1:100) derived from control women (P < 0.05, Fig. 2C). This MF (1:100)-stimulated E. coli growth was significantly suppressed for both control and endometriotic MF after pretreatment of E. coli with anti-PGE2 antibody (P < 0.05 for each, Fig. 2C).

**PGE2 levels in the culture media of Mφ and stromal cells**

LPS treatment significantly increased the secretion of PGE2 in the culture media of Mφ derived from women with and without endometriosis when compared with non-treated cells (P < 0.05 for each group) (Fig. 3A). Compared with non-treated cells, LPS-treated PGE2 levels were significantly higher in the culture media of stromal cells derived from women with endometriosis (P < 0.05) but not in...
control women (Fig. 3B). We also found a more substantial and significant increase in PGE2 levels in the culture media of LPS-non-treated cells derived from women with endometriosis than in control women (P < 0.05 versus control, Fig. 3A and B). LPS-treated PGE2 levels in the culture media of Mφ and stromal cells were significantly higher in women with endometriosis than in control women (P < 0.05 for each cells, Fig. 3A and B).

**The effect of PGE2 on the growth of lymphocytes**

Figure 4A shows a dose-dependent growth of lymphocytes, in response to PHA, derived from women with and without endometriosis with no significant difference between control and endometriotic lymphocytic growth. Co-administration of PGE2 dose-dependently (10–100 pg/ml) decreased PHA-stimulated growth of lymphocytes derived from women with endometriosis (P < 0.05 for 10 and 50 pg/ml; P < 0.01 for 100 pg/ml). This growth suppression effect of PGE2 on PHA-stimulated lymphocytes was only observed at a dose of 100 pg/ml in control women (Fig. 4A). In our preliminary experiment examining the dose-dependent effect of PGE2 (10 pg to 10 ng/ml) on lymphocyte growth, we found that there was no further decrease of lymphocyte growth in response to PGE2 with a dose beyond 100 pg/ml (data not shown).

We further used 10% culture media (10 μl) from each of unstimulated Mφ and stromal cells derived from women with endometriosis. We found that PHA-promoted growth of lymphocytes was significantly suppressed after application of 10% culture media derived from the Mφ and stromal cell cultures (P < 0.05 for both, Fig. 4B). Pretreatment of lymphocytes with anti-PGE2 antibody (10 μg/ml) abrogated the growth-suppressing effect of culture media on these cells (Fig. 4B).

**Discussion**

We demonstrated for the first time that higher PGE2 levels in the MF of women with endometriosis was involved in the bacterial growth of *E. coli* in a bacteria culture system. This effect of PGE2 on bacteria may be contributed by its direct growth-promoting effect on E. coli or by its indirect immunosuppression effect on PBLs. In fact, we found significantly higher levels of PGE2 in the MF and PF of women with endometriosis than in control women. The experimental doses of PGE2 stimulating E. coli growth were within the levels found in MF and PF.

The direct effect of PGE2-enriched MF in promoting the growth of *E. coli* was supported by the findings that pretreatment of MF with anti-PGE2 antibody significantly suppressed MF-stimulated replication of *E. coli* in a dilution-dependent fashion. The growth-promoting effect of MF on *E. coli* was more prominent in women with endometriosis than in control women. This indicates that among different macromolecules in menstrual blood (Badawy et al., 1985; Zhou et al., 1989), PGE2 is one of the components that may be involved in bacterial growth. The indirect effect of PGE2 on the growth of *E. coli* can be explained by the significant suppression of PHA-stimulated growth of PBL in response to PGE2. This immunosuppressive effect of PGE2 was observed for PBL derived from women with and without endometriosis. An unexpected observation was that we did not find any significant difference in lymphocyte growth in response to PHA between women with and without endometriosis. This can be explained by the fact that we collected total population of PBLs (CD3+) from these two groups of women and not individual population of lymphocytes such as CD4+ or CD8+ lymphocytes. Further studies may clarify this issue using individual subsets of lymphocytes.

We recently demonstrated that menstrual blood of women with endometriosis was highly contaminated with a non-pathogenic strain of *E. coli* with significantly higher levels of endotoxin (LPS) in both MF and PF in women with endometriosis compared with controls (Khan et al., 2010). The colony formation of *E. coli* and endotoxin levels in MF was markedly higher in women with red lesion containing r-ASRM stage I–II endometriosis than in women with endometrioma containing stage III–IV endometriosis. We also reported that LPS was involved in the TLR 4-mediated pro-inflammatory response by Mφ and growth of both eutopic and ectopic endometrial cells (Khan et al., 2010). However, the mechanistic basis of the bacterial growth in menstrual blood was unclear. Here, we report that PGE2-enriched MF as well as PGE2 itself might play a role in bacterial growth such as *E. coli*. Although we could not investigate the association between PGE2-stimulated growth of *E. coli* and endotoxin levels in bacteria culture media in our current study, we can at least speculate that PGE2-enriched MF may be involved in *E. coli* replication by its direct growth promoting effect.

Mφ and stromal cells are the predominant endometrial cell types producing PGE2 (Herath et al., 2006). We detected higher levels of basal and LPS-treated PGE2 in the culture media of both Mφ and endometrial stromal cells derived from women with endometriosis than in control women. We found that in addition to suppressing PHA-stimulated growth of lymphocytes by PGE2, the application of PGE2 containing culture media derived from both of these cells was able to significantly suppress PHA-promoted growth of lymphocytes. This growth-inhibiting effect of culture media on lymphocytes was lost after pretreatment of cells with anti-PGE2 antibody. Information regarding PGE2-mediated suppression of T-cell function has previously been reported by other investigators (Benedetto, 1989; Koike et al., 1992). Here, we reported that PGE2 has a similar capacity to suppress immune cell function in women with endometriosis. This finding is biologically important. First, during the menstrual period, higher levels of PGE2 are produced and released into the menstrual blood by both endometrial Mφ and stromal cells in the intrauterine environment and may directly or indirectly enhance *E. coli* growth. Second, as an anti-apoptotic agent, higher levels of PGE2 in MF and PF may facilitate the survival of ectopic endometrial cells in the pelvic environment. Third, PG-suppressing agents, such as current hormonal therapies for endometriosis including non-steroidal anti-inflammatory drugs, may be clinically useful not only for suppressing the growth of endometriosis, but also for protecting against bacterial growth in women with endometriosis. The relationship between PGE2 levels and numbers of immune cells in menstrual blood is unknown. Further studies are needed to clarify the growth-suppressing effect of PGE2 on lymphocytes and other immune cells, described herein.

Our findings provide some evidence that in addition to viral replication, PGE2 has the capacity to stimulate bacterial growth. The fluctuating pattern in the growth of *E. coli* in response to PGE2 may be due to the polyclonal nature of *E. coli*. It means that some clones of *E. coli*
are more responsive to grow at lower doses and some clones at higher doses of PGE2. This can also be explained by the differential binding affinity of PGE2 for its receptors, EP2/EP4, as reported for different epithelial cells and microbial pathogens (George et al., 2010; Goldman et al., 2010; Wu et al., 2010). A strong receptor-ligand binding affinity in lower and higher doses of PGE2 and decreased functional affinity by the intermediate dose of PGE2 may explain our current findings. This variation in the growth pattern of E. coli in response to variable doses of PGE2 remains to be clarified in further experiments.

The optimal concentrations of PGE2 and PGF2α in MF and PF correspond to 1–100 pg/ml for women who complain of mild-to-moderate pelvic pain and 10–100 ng/ml for women with severe pelvic pain associated with endometriosis (unpublished data). In fact, during menstruation, higher levels of PGE2, in association with PGF2α, may be involved in uterine contraction with consequent pain symptoms (Benedetto, 1989; Koike et al., 1992; Wu et al., 2007). Although we did not investigate endotoxin (LPS) in our current study, it may also have an effect on uterine contraction in women with endometriosis via COX2 up-regulation and production of PG (Takenaka et al., 2010). Therefore, women with endometriosis who complain of a variable severity of pelvic pain or menstrual pain might be susceptible to an ongoing growth of migrating E. coli in their menstrual blood.

Our findings have some clinical and biological implications. First, higher PGE2 levels in PF after menstrual reflux may be involved in the growth of endometriosis, because PGE2 acts as a regulator of COX2/P450 aromatase activation resulting in tissue accumulation of estrogen (Attar and Bulun, 2006; Bulun, 2009). As a master molecule, PGE2 has a multi-functional role including the regulation of cell proliferation, anti-apoptosis, immune suppression and angiogenesis during the development of endometriosis (Wu et al., 2010). Second, the role of PGE2 in bacterial growth such as E. coli by its direct and indirect effect may partly explain the mechanistic basis of E. coli contamination of menstrual blood in women with endometriosis as we reported recently (Khan et al., 2010).

In fact, we demonstrated that a higher colony formation of E. coli in menstrual blood with consequent higher endotoxin (LPS) levels in MF and PF of women with endometriosis significantly stimulated the growth of eutopic and ectopic endometrial cells via LPS/TLR 4 cascade (Khan et al., 2010). When we tried to link our current findings with our previous experiment on LPS/TLR 4 system, we found a parallel increase of both LPS and PGE2 in the menstrual blood collected from women with endometriosis. LPS is one of the mediators stimulating overexpression of COX2 with consequent production of different PGs including PGE2. LPS-mediated COX2 expression and PGE2 production have already been reported (Takenaka et al., 2010; Liu et al., 2011). If we consider the inflammatory condition of the intrauterine or pelvic environment, we can collectively make an association between LPS/TLR 4/COX2 and PGE2 with the growth of endometrial cells as well as growth of bacteria.

After similar ascending migration of bacteria from the vaginal cavity into the uterine cavity of all women, differences in PGE2 levels of MF between women with and without endometriosis may be involved in higher colony formation of E. coli in the menstrual blood of women with endometriosis. Further studies are needed to investigate subclinical infection within the vaginal cavity and to clarify our current findings.

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Authors’ roles

K.N.K. was involved in the study concept, study design, experiments, data analysis and manuscript draft; M.K. and A.F. contributed equally to sample collection; N.Y. was involved in experimental assistance; M.N., T.I. and H.M. were equally involved in draft advice.

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Conflict of interest

None declared.

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