Independent regulation of prostaglandins and monocyte chemoattractant protein-1 by interleukin-1β and hCG in human endometrial cells

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BACKGROUND: Inflammatory mediators such as prostaglandins (PG), chemokines, cytokines and their interactions regulate reproductive functions. The relationship between PG and monocyte chemotactant protein-1 (MCP-1) has not been elucidated in human endometrium. The presence of hCG receptors in the human endometrium suggests that this embryonic signal may exert a local function in this tissue. Our objectives were to investigate the possible association between PG and MCP-1 and to examine the role of hCG in interleukin-1β (IL-1β)-regulated PG and MCP-1 production in human endometrium. METHODS: Primary cell cultures isolated from endometrial biopsies were used as an in vitro model. PG and MCP-1 levels were measured in the culture medium. RESULTS: IL-1β stimulates the production of both PG and MCP-1. Neither COX inhibitors nor direct addition of PG affects MCP-1 production. By contrast, MCP-1 is able to induce PGE2 and PGF2α in a concentration-dependent manner but it does not appear to contribute to the increase in PG accumulation following IL-1β stimulation. hCG inhibits IL-1β-induced PG level. However, hCG has no effect on either basal or IL-1β-mediated MCP-1 level. CONCLUSIONS: PG are not involved in the regulation of MCP-1 production in endometrial cells. hCG appears to play a local function in the endometrium.

Key words: hCG/human endometrium/IL-1β/MCP-1/prostaglandins

Introduction

Menstruation and implantation are two critical reproductive processes involving inflammatory responses in the human endometrium (for a review, see Kelly et al., 2001). Inflammatory mediators such as prostaglandins (PG), chemokines and cytokines have been associated with these events (Jones et al., 1997). Although the precise mechanisms regulating menstruation and implantation are still unknown, increasing evidence suggests the involvement of the interaction between these mediators.

It is now widely acknowledged that PG are important regulators of female reproductive function (for a review, see Poyser, 1995). PG are produced from arachidonic acid (AA) stored in cell membrane phospholipids and liberated by phospholipases (PL). The conversion of AA to PG is catalysed by the rate-limiting enzyme cyclooxygenase (COX). Two isoforms have been identified, a constitutively expressed form COX-1 and an inducible form COX-2 (for a review, see Otto and Smith, 1995). PGE2 and PGF2α are the two most studied prostanoids in human endometrium. The aberrant ratio of PGE2:PGF2α is associated with menstrual disorders such as dysmenorrhoea and menorrhagia (for a review, see Poyser, 1995). In some species, an increase in endometrial prostaglandin production was observed around the peri-implantation period or at the implantation site, suggesting an important role for PG in the implantation process (for a review, see Psychoyos et al., 1995).

Chemokines are increasingly recognized as important regulators of uterine function (for a review, see Kayisli et al., 2002). Monocyte chemoattractant protein-1 (MCP-1), a member of the beta-chemokine family, displays chemotactic activity for monocytes/macrophages, T lymphocytes, basophils and natural killer cells and recruits them into sites of inflammation (for a review, see Leonard and Yoshimura, 1990). MCP-1 can be released by endometrial epithelial and stromal cells in culture (Arici et al., 1995). In human endometrium, its expression was detected in a cyclical pattern during the menstrual cycle (Jones et al., 1997). The highest
level of MCP-1 is detected peri-menstrually (Arici et al., 1999).

It has been demonstrated that in some cell types, interleukin-1 (IL-1) is able to induce both PG and MCP-1 production (Efsen et al., 2001). IL-1β is a pro-inflammatory cytokine expressed in the endometrium throughout the menstrual cycle, particularly around the time of implantation (Simon et al., 1995). It is postulated to mediate the dialogue between the blastocyst and the endometrium in implantation (Ibid, Simon et al., 1995). IL-1β can induce PG production in human endometrial stromal cells and an epithelial cell line (Kniss et al., 1997; Huang et al., 1998; Tamura et al., 2002).

In endometriotic cells, the production of MCP-1 is upregulated by IL-1β (Akomu et al., 1995). However, no information is available regarding the possible link between the regulation of PG and MCP-1 by IL-1β in human endometrium. Previous studies have indicated that COX metabolites up-regulated MCP-1 in hepatic stellate cells (Efsen et al., 2001), but repressed MCP-1 formation in experimental glomerulonephritis (Schneider et al., 1999). These studies infer some cell specificity in the determination of the interaction between PG and MCP-1. It is of interest to study the relationship between PG and MCP-1 in human endometrial cells.

The presence of LH/hCG receptors has been reported for many years in the human endometrium, but their physiological relevance is still questioned (Reshef et al., 1990; Rao, 1999). hCG, the embryonic signal for the maternal recognition of pregnancy in the human, appears to play an important role in blastocyst implantation (for a review, see Srisuparp et al., 2001). It is primarily produced by the trophoblast of the placenta. Besides its well-known luteotrophic function, hCG is able to inhibit apoptosis, promote decidualization (Han et al., 1999) and exert anticarcinogenic and immunomodulatory effects (Russo and Russo, 1994). Up-regulation of COX-2 gene expression in response to hCG has been demonstrated in both human endometrial stromal and epithelial cells (Han et al., 1996; Zhou et al., 1999). In addition, Arici et al. (1997) reported the induction of higher MCP-1 mRNA expression and protein production by LH/hCG in human ovarian follicles.

Thus, the aims of this study are to investigate the possible relationship between PG and MCP-1 and to study the effect of hCG on IL-1β-mediated PG and MCP-1 production. Separated and non-separated human endometrial cell cultures are compared and non-separated human endometrial cells are used in the present study as the primary in vitro model.

Materials and methods

Tissue collection and culture of endometrial cells

Endometrial biopsies were taken from women aged between 31 and 45 years with regular menstrual cycles undergoing gynaecological investigation for benign conditions. Biopsies were dated according to the stated last menstrual period (LMP). A total of 11 biopsies was used, five from proliferative and six from secretory phase. Informed consent for donation of anonymous endometrial samples was obtained before tissue collection. The tissue samples were placed in sterile Hanks’ balanced salt solution (HBSS) containing 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.25 mg/ml amphotericin B at 4°C and transported to the laboratory immediately. Endometrial tissues were placed in a 50 ml conical tube containing trypsin (0.02%; Boehringer Mannheim Corp., USA), collagenase (type II, 0.02%; Sigma, USA) and deoxyribonuclease I (DNase-I, 0.015%; Sigma) in HBSS and incubated at 37°C for 20 min in a shaking water bath. Cells obtained from the digestion were recovered by centrifugation. The cell pellets were resuspended in 15 ml HBSS and deposited on 12.5 ml Ficoll gradient (Amersham Biosciences, Sweden). The gradients were centrifuged at 800 g for 20 min. Endometrial cells were recovered at the interface and washed with HBSS. The cell pellets were resuspended in Roswell Park Memorial Institute (RPMI) 1640 (ICN Biomedicals, Inc., USA) containing 50 ng/ml gentamicin (Sigma), 10 mg/ml insulin (Sigma), 5 mg/ml transferrin (Sigma) and 10% fetal bovine serum (Wisent, Inc., Quebec, Canada) depleted of steroids by dextran-charcoal extraction. Endometrial cells were seeded in 24-well plates and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. It is difficult to use standard haemocytometer or coulter counter techniques to evaluate the total number or the relative proportion of epithelial and stromal cells in the non-separated cell preparation. However, the relative proportion of cells is quite reproducible between experiments as evaluated subjectively by observation under the phase contrast microscope. We have demonstrated previously, using the bovine endometrium, that our method of isolation and purification as well as estimation of cell distribution was highly accurate and reproducible (Asselin et al., 1996). In the present study, when endometrial cells are fully attached and after culture medium replacement, we estimate that the relative proportion of epithelial and stromal cells is ~50% but could vary from 40 to 60% between experiments. As an additional measure, we have evaluated the expression of cytokeratin, an epithelial cell marker, and vimentin, a stromal (mesenchymal) cell marker, in a different set of experiments. Dual labelling with a fluorescence secondary antibody confirmed our previous results claiming a proportion of 50% epithelial (cytokeratin positive) cells (Figure 1F). The presence of contaminating immune cells was evaluated in three parallel cultures. Lymphocytes do not adhere to plastic and those eventually present in suspension were eliminated at the time of medium change. The presence of macrophages was evaluated by Diff-Quick and non-specific esterase staining (Roberge et al., 1994).

Isolation of separated epithelial and stromal cells

The separation of epithelial and stromal cells was performed as described previously (Akomu et al., 1995). Briefly, the pellets of non-separated endometrial cells obtained above were resuspended in 10 ml of culture medium. Unit gravity sedimentation of the cell suspension (Freshney, 2000, p. 222) was conducted at room temperature for 1 h. The top 8 ml of medium was defined as the stromal-enriched fraction and the bottom 2 ml as the epithelial-enriched fraction. The stromal fraction was transferred to 24-well plates and incubated at 37°C in an atmosphere of 5% CO₂ in air for 30 min. The medium containing floating cells and debris was replaced and the adherent stromal cells were maintained in culture. The epithelial fraction was diluted with culture medium and placed into 75 cm² culture dishes for a preincubation process, after which the contaminating stromal cells were eliminated by selective adhesion (Freshney, 2000, p. 211). Epithelial cells were recovered and seeded into 24-well plates and incubated at 37°C in a humidified atmosphere of 5% CO₂:95% air.
**Treatments**

The medium was changed every 2 days. After confluence, the cells were treated with fresh, serum-free RPMI 1640 medium containing different concentrations of IL-1β (0–1 ng/ml) (Sigma), IL-1β (1 ng/ml) plus indomethacin (10 μM) (Sigma), IL-1β (1 ng/ml) plus NS-398 (10 μM) (Cayman, USA), hCG (0.1 μg/ml, 10000 IU/mg; Sigma), IL-1β (1 ng/ml) plus hCG (0.1 μg/ml), PGE₂ (0–10 μM)), PGF₂α (0–10 μM)) or MCP-1 (0–50 ng/ml) in triplicate at 37°C in an atmosphere of 5% CO₂/95% air. After 24 h, the culture medium was collected and kept at −20°C for prostaglandin and MCP-1 measurement. Cells were recovered for protein assay.

**Immunofluorescence microscopy analysis**

Primary separated and non-separated human endometrial cells were grown on Lab Tek 4-well chamber slides (Nalge Nunc International, USA). Cells were fixed with cold methanol for 6 min at room temperature and then rinsed with phosphate-buffered saline (PBS). Slides were then blocked in PBS containing 10% goat serum for 1 h at room temperature. After washing in PBS (three times, 5 min each), slides were incubated with mouse monoclonal antibodies against cytokeratin (recognizing 4, 5, 6, 8, 10, 13 and 18 isoforms) or vimentin (Sigma) at a dilution of 1:200 in PBS containing 0.5% goat serum for 1 h at room temperature. Immunofluorescence was obtained by reaction with a fluorescent secondary antibody (Alexa Fluor® 488 goat anti-mouse IgG; Molecular Probes, Inc., USA) at a dilution of 1:300 in PBS containing 0.5% goat serum for 1 h at room temperature. Slides were then rinsed three times with PBS. Fluorescence was visualized using a Zeiss Axiolab microscope (Zeiss, Germany) and images captured and integrated using the Northern Exposure program (Empix Imaging Inc., Canada).

**Enzyme immunoassays (EIA) for PG**

An EIA technique was used for PGE₂ and PGF₂α measurement as described previously (Asselin et al., 1996), which utilized acetylcholinesterase-linked PG tracers (Cayman Chemical Company, USA). The inter- and intra-assay coefficients of variation (n = 12) were 16 and 10% respectively.

**MCP-1 ELISA**

MCP-1 in the culture medium was measured using a previously reported sandwich enzyme-linked immunosorbent assay (Akoum et al., 1996). Briefly, 250 ng/well of mouse monoclonal anti-human MCP-1 antibody (R & D systems, USA) and a 1:1000 dilution of rabbit polyclonal anti-human MCP-1 antibody (a generous gift from Dr Paul H. Naccache) were used for detection. TMB (3,3',5,5'-tetramethylbenzidine; Bio-Rad Laboratories Ltd, Canada) was used as the substrate. The enzymatic reaction was stopped by the addition of sulphuric acid. The optical density was determined at 450 nm. MCP-1 concentration was interpolated from the standard curve using recombinant human MCP-1 (R & D systems). The sensitivity for MCP-1 was 50 pg/ml. The intra- and inter-assay coefficients of variation were <6%.

**Western blot analysis**

Proteins from cultured human non-separated endometrial cells were extracted and quantified as described recently (Chapdelaine et al., 2001). Aliquots of 10 μg protein of each sample were separated on 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes. The membranes were blocked overnight at 4°C in PBS containing 5% fat-free dry milk and 0.05% Tween 20. After 1 h of incubation at room tempera-

**Prostaglandin accumulation in the culture medium of human endometrial epithelial, stromal and non-separated cells**

PG accumulation in the culture medium of epithelial, stromal and non-separated cells following treatment with IL-1β (1 ng/ml) for 24 h was illustrated in Figure 2. IL-1β significantly increased both PGE₂ and PGF₂α in stromal cells. In epithelial cells, IL-1β induced a significant up-regulation of PGE₂, but not PGF₂α. Basal PG levels were comparable in stromal and epithelial cells. However, the effect of IL-1β in stromal cells was significantly higher than in epithelial cells.

**Statistical analysis**

Data are presented as the mean ± SEM. Statistical analysis was performed using analysis of variance (ANOVA) followed by Fisher’s protected least significant difference, Duncan’s new multiple range and Student–Newman–Keuls multiple comparison tests (Super ANOVA; Abacus Concepts, USA). The interaction between the effect of IL-1β and cell types and the interaction between hCG and IL-1β were tested using two-way ANOVA. P < 0.05 was considered statistically significant.

**Results**

**Characteristics of primary human endometrial cell cultures**

Separated human endometrial cells were prepared from the biopsies and morphological characteristics of epithelial, stromal and non-separated cells were examined under a phase-contrast microscope. Epithelial cells exhibited a typical cuboidal morphology (Figure 1A), stromal cells an elongated fibroblast morphology (Figure 1B) and non-separated cell cultures a combination of both morphologies (Figure 1C). There was no difference in gross morphology between cells obtained from the proliferative or the secretory phase of the cycle. The presence of contaminating monocytes was found to be <0.02%, thus ruling out their contribution to the present findings on PG and MCP-1 production. Epithelial and stromal cells in separated and non-separated cells were identified by cell-specific staining of intermediate filament proteins. Epithelial cells were positively labelled with the epithelium-specific cytokeratin antibody (Figure 1D and F), whereas stromal cells did not react with this antibody. By contrast, stromal cells exhibited a strong vimentin staining (Figure 1E) and epithelial cells weak background staining (data not shown).

**Prostaglandin accumulation in the culture medium of human endometrial epithelial, stromal and non-separated cells**

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for both PGF$_{2\alpha}$ and PGE$_2$. In non-separated cells, IL-1$\beta$ stimulated significantly PGE$_2$ and PGF$_{2\alpha}$ ($P < 0.05$).

**Time-course of the effect of IL-1$\beta$ on COX proteins, prostaglandin and MCP-1 levels**

The effect of IL-1$\beta$ (1 ng/ml) on COX-1 and COX-2 protein expression from 0 to 24 h was illustrated in Figure 3. The increase in COX-2 protein level was already noticeable at 2 h (3.7-fold as time 0) and proceeded progressively up to 24 h (4.8-fold as time 0) (Figure 3A). There was no detectable change in COX-1 protein expression. A significant stimulation of PG accumulation was detectable after 2 h, whereas the up-regulation of MCP-1 was evident after 4 h (data not shown).

**Effect of IL-1$\beta$ and COX inhibitors on prostaglandin level**

The effects of IL-1$\beta$ and the combination of IL-1$\beta$ and COX inhibitors on COX protein and prostaglandin levels were determined in non-separated endometrial cells. Confluent cells were incubated with different doses of IL-1$\beta$ and IL-1$\beta$ plus COX inhibitors for 24 h. Western blot analysis showed that IL-1$\beta$ increased COX-2 protein level in a concentration-dependent manner (1.4-, 2.7-, 6.3-fold versus...
control respectively) (Figure 3B). There was a significant increase in COX-2 protein level by 1 ng/ml IL-1β compared with control. No difference was observed between IL-1β alone and the combination of IL-1β and COX inhibitors on either COX-1 or COX-2 protein levels.

Both PGE₂ and PGF₂α levels were increased in a concentration-dependent manner by IL-1β (Figure 3C). Co-treatment of cells with indomethacin (10 μmol/l), a non-specific COX inhibitor, or NS-398 (10 μmol/l), a COX-2 specific inhibitor, significantly reduced IL-1β-induced prostaglandin secretion.

Effects of IL-1β and COX inhibitors on MCP-1 secretion by human endometrial cells

MCP-1 concentration was measured in the culture medium from the same experiments used for determination of PG production. MCP-1 was detectable in the medium of all cultures but in variable levels. IL-1β enhanced MCP-1 production in a concentration-dependent manner (Figure 3D). Co-treatment of COX inhibitors with IL-1β did not have any effect on MCP-1 levels compared with IL-1β alone.

Effect of prostaglandins on MCP-1 level

Non-separated endometrial cells in culture were incubated with different concentrations of PGE₂ (0–10 μmol/l) or PGF₂α (0–10 μmol/l) for 24 h. Table I showed that PGF₂α and PGE₂ did not have any significant effect on MCP-1 levels (P > 0.05).

Effect of MCP-1 on prostaglandin secretion

After reaching confluence, cells were treated with different concentrations of MCP-1 (0, 0.5, 5 and 50 ng/ml) for 24 h.

Table I. Effects of prostaglandin (PG) on monocyte chemoattractant protein-1 (MCP-1) production by human non-separated endometrial cells; data are expressed as mean ± SEM (n = 3 for PGE₂ and n = 2 for PGF₂α).

<table>
<thead>
<tr>
<th>Concentration (μmol/l)</th>
<th>MCP-1 (ng/ml)</th>
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<tbody>
<tr>
<td>PGE₂</td>
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<tr>
<td>0</td>
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<tr>
<td>0.01</td>
<td>1.72 ± 0.49</td>
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<tr>
<td>0.1</td>
<td>1.89 ± 0.72</td>
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<tr>
<td>1</td>
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<tr>
<td>10</td>
<td>0.98 ± 0.38</td>
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<tr>
<td>PGF₂α</td>
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<tr>
<td>0</td>
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<tr>
<td>0.01</td>
<td>2.65 ± 1.18</td>
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<tr>
<td>0.1</td>
<td>2.63 ± 1.12</td>
</tr>
<tr>
<td>1</td>
<td>2.49 ± 1.19</td>
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<tr>
<td>10</td>
<td>2.57 ± 1.15</td>
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</table>

Figure 4. Effect of monocyte chemoattractant protein-1 (MCP-1) on prostaglandin (PG) secretion by human non-separated endometrial cells. Cells were incubated with different doses of MCP-1 (0, 0.5, 5 and 50 ng/ml) for 24 h. The culture media were collected for PG measurement by enzyme immunoassay. The results are presented as mean ± SEM of three experiments. *Significantly different compared with control (P < 0.05).
As illustrated in Figure 4, MCP-1 induced a significant increase of PGE$_2$ compared with control ($P < 0.05$).

**Effect of hCG on IL-1β-induced prostaglandin levels**

We investigated the effect of hCG on IL-1β-induced prostaglandin levels. Cells were exposed to hCG (0 and 0.1 µg/ml), IL-1β (1 ng/ml) and IL-1β (1 ng/ml) plus hCG (0.1 µg/ml) for 24 h. Western blot analysis did not detect any difference of either COX-2 or COX-1 protein between IL-1β and IL-1β plus hCG (Figure 5A).

As illustrated in Figure 5B, 0.1 µg/ml hCG did not have any appreciable effect on either PGE$_2$ or PGF$_{2\alpha}$ levels compared with control. However, the addition of 0.1 µg/ml hCG to 1 ng/ml IL-1β inhibited IL-1β-induced PG accumulation. A significant reduction was observed only for PGF$_{2\alpha}$ based on two-way ANOVA ($P < 0.05$).

![Figure 5A](https://academic.oup.com/humrep/article-abstract/19/11/2465/2356427/42x154)

**Figure 5.** Effect of hCG on interleukin-1β (IL-1β)-induced prostaglandin (PG) and monocyte chemoattractant protein-1 (MCP-1) production by human non-separated endometrial cells. After reaching confluence, cells were incubated with hCG (0 and 0.1 ng/ml), IL-1β (1 ng/ml) and IL-1β (1 ng/ml) plus hCG (0.1 µg/ml) for 24 h. Proteins were extracted for COX-1 and COX-2 assay (A). The culture medium was collected for PG (B) and MCP-1 measurement (C). The results are presented as mean ± SEM of four experiments. *Significantly different compared with the other groups ($P < 0.05$).

**Effects of hCG on IL-1β-induced MCP-1 production**

As illustrated in Figure 5C, no change was observed in either basal or IL-1β-induced MCP-1 levels in response to 0.1 µg/ml hCG.

**Discussion**

In the present study, we have used an *in vitro* system based on human primary endometrial cell cultures to study the interactions between cytokines and PG. First we have compared the characteristics of separated and non-separated cell cultures. In agreement with other studies (Matthews *et al.*, 1992), epithelial cells, but not stromal cells, were positive for cytokeratin in our culture system. By contrast, expression of vimentin is much higher and consistent in stromal cells than in epithelial cells. Using these characteristics, the relative proportion of epithelial cells could easily be identified with cytokeratin in the non-separated cell cultures (Figure 1F).

We next describe the pattern of PG synthesis in separated epithelial and stromal cultures and compare them with non-separated cell cultures. Most studies on human endometrial cells *in vitro* are based on the use of stromal cells. This phenomenon may result from the tendency of epithelial cells to be less viable, more difficult to isolate and grow slower. The presence of IL-1 receptor type I (IL-1R tI) mRNA has been reported in the human endometrium (Simon *et al.*, 1993), especially in the epithelium. However, in the present study stromal cells are more responsive to IL-1β than epithelial cells (Figure 2). One reason may be that epithelial cells, when losing the three-dimensional structure occurring *in vivo*, also lose functional characteristics (for a review, see Fleming, 1999). It has been shown by others that the interactions between endometrial epithelial cells (EEC) and stromal cells (ESC) do influence their specific characteristics *in vitro* (Osteen *et al.*, 1994; Pierro *et al.*, 2001). In this study, epithelial and stromal cells were seeded together after enzymatic digestion. Several factors have motivated the choice of this model. First, the co-culture approach is much more efficient in terms of yield of viable cells and allows an optimal use of the precious human endometrial samples. Second, although the three-dimensional structure is probably not maintained with the non-separated cell model, it does allow physical and paracrine interactions between stromal and epithelial cells. This is strongly suggested by the pattern of PG accumulation found in non-separated cultures (Figure 2). Indeed, the level of PG accumulation and the response to IL-1β is lower or equal to that observed for epithelial cells which represent ~50% of the cell population in confluent non-separated cultures. Although less complete than the use of separated and non-separated endometrial cell cultures in parallel, we believe that co-culture represent an interesting compromise for *in vitro* study of endometrial function. No significant difference in basal PG accumulation was observed between cultures prepared from biopsies taken at different stages of the cycle.

The first indication of the induction of PG by IL-1β in human endometrium dates back to 1990 (Mitchell *et al.*, 1990). In stromal cells, it was shown that IL-1β can elevate...
both PGE_2_ and PGF_{2\alpha} levels by increasing COX-2 mRNA and protein levels (Tamura et al., 2002). This effect involves protein kinase A (PKA), nuclear factor kappa B (NF-KB), and/or the extracellular signal-regulated kinase 1 and 2 (ERK1/2) signalling pathways (Tamura et al., 2002). In our study, similar induction of PG by IL-1\(\beta\) is observed in non-separated endometrial cells and correlated with a concentration-dependent increase of COX-2 but not COX-1 protein levels (Figure 3). The time-course of COX-2 induction with a significant increase at 2h shows a much faster expression than reported before in stromal cells (Huang et al., 1998). The difference may be due to the presence of epithelial cells.

MCP-1 can be released by many reproductive tissues. A variety of growth factors and cytokines such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), IL-1 and interferon-\(\gamma\) (IFN-\(\gamma\)) stimulates MCP-1 secretion. In human endometrium, MCP-1 level is the lowest around the time of ovulation when estrogen is high (Arici et al., 1999). In glandular epithelial cells, MCP-1 mRNA level is higher than in stromal cells, but not regulated (Arici et al., 1995). No increase in MCP-1 mRNA levels was found in glandular epithelium treated with IL-1 (Arici et al., 1995). The mechanisms by which MCP-1 gene transcription is regulated are agonist specific. NF-KB pathway has been suggested for the regulation of MCP-1 by mediators such as IL-1, glucocorticoid, lipopolysaccharide (LPS) and TNF-\(\alpha\) (King et al., 2001; Ueda et al., 1997). Our data show that in human non-separated endometrial cells, IL-1\(\beta\) stimulation of MCP-1 production is concentration-dependent and highly reproducible, regardless of the possible difference in the proportion of epithelial and stromal cells in each confluent culture (Figure 3D). This indicates that the proportion of epithelial and stromal cells was fairly similar between experiments or that interactions between these cell types make them equivalent in terms of MCP-1 production in response to IL-1\(\beta\). The increase of MCP-1 in response to IL-1\(\beta\) in the endometrium may be correlated with the activated NF-KB observed during menstruation.

Previous studies have shown that PG can modulate other inflammatory mediators including MCP-1. In ovine and bovine corpus luteum (CL), administration of PGF_{2\alpha} induces MCP-1 mRNA expression (Tsai et al., 1997). PGE_2, an important immunomodulator and mediator of inflammation within the uterus, stimulates MCP-1 release from placenta (Denison et al., 1998). Additionally, COX inhibitors modulate MCP-1 levels in different ways depending on cell types (Kyrkanides et al., 2002). In human endometrium, high expression of MCP-1 and COX-2 is observed before the initiation of menstruation, coinciding with leukocyte accumulation in the uterus (Jones et al., 1997). Collectively, this led us to hypothesize that there could be a relationship between PG and MCP-1 in the endometrium. We show that treatment with indomethacin, a non-specific COX inhibitor, or NS-398, a COX-2-specific inhibitor, markedly reduces PG levels stimulated by IL-1\(\beta\) (Figure 3C). COX inhibitors alone, especially indomethacin, also decreased basal PG accumulation in the culture media (data not shown). However, neither indomethacin nor NS-398 has any effect on either IL-1\(\beta\)-induced (Figure 3D) or basal MCP-1 level (data not shown). Comparing our data with those obtained in hepatic stellate cells and glomerular mesangial cells (Schneider et al., 1999; Efsen et al., 2001) confirms that the interaction between PG and MCP-1 varies depending on tissues or cell types. We next investigated MCP-1 production in response to direct addition of PG and found that MCP-1 secretion is not sensitive to PGF_{2\alpha} or PGE_2 (Table I). Recent studies demonstrated the presence of PGF_{2\alpha} receptor as well as PGE_2 receptors in human endometrium (Milne et al., 2001; Milne and Jabbour, 2003). Thus, the lack of response is likely due to the inability of PG to modulate MCP-1 level in endometrial cells. Globally, neither COX inhibitors nor direct addition of PG had any effect on MCP-1 level, suggesting that PG do not play a role in the regulation of MCP-1 synthesis in human endometrial cells. Interestingly, the incubation of cells with MCP-1 gave rise to a significant concentration-dependent increase in PGE_2, but not PGF_{2\alpha} production (Figure 4). A time-course study was then conducted to test whether MCP-1 could contribute to PG production induced by IL-1\(\beta\). The results revealed that a significant increase in PG production was already detectable 2h following exposure of cells to IL-1\(\beta\) (1 ng/ml), while the up-regulation of MCP-1 was evident only after 4h (data not shown). Moreover, IL-1\(\beta\) stimulates both PGE_{2\alpha} and PGE_2 whereas MCP-1 preferentially stimulates PGE_2. These observations suggest that PG production in response to IL-1\(\beta\) stimulation is independent of MCP-1.

Despite its important role in implantation (Simon et al., 1994), IL-1\(\beta\) can disrupt blastocyst implantation by inhibiting decidualization of endometrial stromal cells (Kariya et al., 1991; Frank et al., 1995). hCG, a heterodimeric glycoprotein hormone required to maintain pregnancy, exerts its effects through LH/hCG receptors by stimulating progesterone secretion from the corpus luteum in the first trimester. Moreover, hCG is suggested to have important roles within the uterus through an effect on cytokine production (Uzumcu et al., 1998). IL-6 and TNF-\(\alpha\) are stimulated by hCG in human endometrial cells (Uzumcu et al., 1998). We have tested the possibility that hCG could have an effect on IL-1\(\beta\) via modulation of PG and/or MCP-1 production. We found that at the concentration of 0.1 \(\mu\)g/ml, hCG did not have any effect on basal PG secretion (Figure 5B). The co-treatment of hCG (0.1 \(\mu\)g/ml) with IL-1\(\beta\) (1 ng/ml), however, reduced PG production induced by IL-1\(\beta\). A significant reduction was observed only for PGF_{2\alpha} (\(P < 0.05\)). The mechanism by which hCG reduced IL-1\(\beta\)-induced PG is unknown. In gonads, LH/CG receptors are coupled to cAMP generation and act through PKA. This would, however, decrease NF-KB and inhibit MCP-1 production, which is not the case here. In primary baboon endometrial epithelial cells and a human endometrial cell line, hCG does not induce cAMP generation, but rather a PKA-independent ERK1/2 cascade that would also decrease MCP-1 formation (Srisuparp et al., 2001). Therefore, according to the literature, all mechanisms of hCG action that could decrease PG production would also lead to a reduction of MCP-1 (Tamura et al., 2002). Therefore, further investigation of this particular action must be conducted to elucidate the mechanisms involved. As indicated in
previous studies, suppression of PG synthesis is required for successful implantation (Smith and Kelly, 1988). This finding in our study supports the local role of hCG in the endometrium. hCG may indirectly modulate the receptive endometrium to facilitate the implantation process. In contrast, hCG did not have any effect on either basal or IL-1β-induced MCP-1 level (Figure 5C). Human CG receptors have been well characterized on human endometrial epithelial and stromal cells (Reshef et al., 1990). The failure of hCG to suppress IL-1β-induced MCP-1 is surprising because hCG can inhibit various cellular responses mediated by NF-KB. Inhibition of NF-KB activation by hCG is not cell type specific (Manna et al., 2000). Given the range of hCG concentrations tested (from 0.1 to 10 μg/ml), it seems unlikely that the effective dose was missed. Therefore, MCP-1 levels do not appear to be directly modulated by hCG in human endometrial cells.

In conclusion, IL-1β stimulates the production of both PG and MCP-1 in non-separated human endometrial cells by apparently distinct and independent mechanisms. hCG, added in combination with IL-1β, is able to block the induction of PG synthesis but not that of MCP-1. These results support the role for cytokines in the regulation of endometrial cell function and of a local action of hCG in the uterus.

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