Effects of interferon-γ on cytokine production by endometrial stromal cells

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Introduction

Endometrial stromal cells (ESC) have been reported to produce various cytokines, including interleukin (IL)-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and macrophage colony-stimulating factor (M-CSF) in the culture media of normal ESC and an endometrial stromal sarcoma cell line, MaMi, were measured using an enzyme-linked immunosorbent assay. Both non-stimulated ESC and non-stimulated MaMi cells constitutively secrete IL-6, IL-8, MCP-1, and M-CSF. In a dose-dependent manner, IFN-γ increased the concentrations of IL-6, MCP-1, and M-CSF and reduced the concentrations of IL-8 in ESC and MaMi cells. These results suggest that IFN-γ produced by both decidual inflammatory cells and the developing embryo plays a role in the maintenance of early pregnancy by modulating the production of these cytokines by human ESC.

Key words: endometrial stromal cell/interferon-γ/interleukins/macrophage colony-stimulating factor/monocyte chemoattractant protein-1

Material and methods

Cell culture conditions

Normal endometrial specimens were obtained from seven premenopausal patients who had undergone hysterectomies for intramural leiomyoma. All the specimens were diagnosed as late proliferative (days 11–13 of the menstrual cycle) on the basis of standard histological criteria. Normal ESC were separated from epithelial glands by digesting the tissue fragments with collagenase as previously described (Arici et al., 1993). Briefly, tissues were cut into 2–3 mm pieces and incubated with collagenase (200 IU/ml) (Gibco-BRL, Gaithersburg, MD, USA) in Iscove’s modified Dulbecco’s medium (IMDM) (Gibco-BRL) with stirring for 2 h at 37°C. The suspension was then filtered through a 150 μm wire sieve to remove mucus and undigested tissue. The filtrate was then passed through a 80 μm wire sieve, which allowed the stromal cells to pass through while intact glands were retained. After washing three times with serum-free IMDM, cells were transferred to culture flasks (Corning, New York, NY, USA) at a density of 10⁶ cells/ml in IMDM supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco-BRL), streptomycin (100 IU/ml) (Gibco-BRL), and penicillin (100 IU/ml) (Gibco-BRL). Culture medium was replaced every 4 days. After three passages (15–20 days after isolation) by standard methods of...
trypsinization, those cells which had a purity of >95% were used for experiments. These cells maintained their ability to undergo decidualization.

An endometrial stromal sarcoma-derived cell line, MaMi, that constitutively produces IL-6, IL-8, and MCP-1, was previously established (Nasu et al., 1997). The cells were maintained under the conditions described above. The cultures were incubated at 37°C in 5% CO₂ in air.

Detection of IL-6, IL-8, MCP-1, and M-CSF
To study the production of IL-6, IL-8, MCP-1, and M-CSF by normal ESC and MaMi cells, 5 × 10⁵ cells were plated on 6-well culture plates (Coming) in 1 ml of culture medium with 10% FBS and cultured until they were fully confluent. The supernatant was replaced with fresh culture medium containing various amounts of recombinant human IFN-γ (R&D systems, Minneapolis, MN, USA). Under these conditions, the supernatant was collected for 0–24 h after the stimulation and stored at –70°C until assayed. These experiments were performed in triplicate and repeated three times.

IL-6, IL-8, MCP-1, and M-CSF were determined in the supernatants with commercially available enzyme-linked immunosorbent assays (ELISA) (R&D systems). Sensitivities of the assays for IL-6, IL-8, MCP-1, and M-CSF were 0.70, 4.4, 5.0, and 8.0 pg/ml respectively.

Statistical analysis
Data are presented as mean ± SD and were analysed using the Bonferroni/Dunn test with StatView 4.5 (Abacus Concepts, Berkeley, CA, USA). *P < 0.05 was considered to be statistically significant.

Results
Concentrations of IL-6, IL-8, MCP-1, and M-CSF in the culture media without cells were below the detection levels. Small amounts of IL-6, MCP-1, and M-CSF were detected in the supernatant of non-stimulated ESC after 24 h incubation (Figure 1). Considerable amounts of IL-8 were detected in the supernatant of non-stimulated ESC after 24 h incubation (Figure 2). The concentrations of IL-6, MCP-1, and M-CSF were increased by recombinant human IFN-γ in a dose-dependent manner, while IL-8 production was suppressed, also in a dose-dependent manner, by IFN-γ. The production of these cytokines in response to IFN-γ was similar in ESC and MaMi cells.

Discussion
It has been suggested that cultured ESC produce various kinds of cytokines (Casey et al., 1989; Tabibzadeh et al., 1989; Daiter et al., 1992; Arici et al., 1993; Dudley et al., 1993; Hatayama et al., 1994). These production of cytokines by ESC is regulated by a cytokine network through interaction with other surrounding cells. In the present study, we examined the effects of IFN-γ on the production of IL-6, IL-8, MCP-1, and M-CSF by ESC. The production of IL-6, MCP-1, and M-CSF were up-regulated with increasing concentrations of IFN-γ. These observations are consistent with previous reports on the effect of IFN-γ on the production of cytokines by ESC (Tabibzadeh et al., 1989; Daiter et al., 1993; Dudley et al., 1993; Martin and Dorf, 1991; Hamilton et al., 1993). The production of these cytokines was similar in ESC and MaMi cells.

ESC (Tabibzadeh et al., 1989) and other cell types (Rollins et al., 1990; Martin and Dorf, 1991; Hamilton et al., 1993; Brown et al., 1994; Akoum et al., 1996).

Concerning the regulation of IL-8 production, most attention has been paid to inducers and/or enhancers of IL-8 production. In fact, cytokines such as IL-1 and TNF-α are known to enhance the production of IL-8 in various cell types, including endothelial cells (Strieter et al., 1989), human dermal fibroblasts and keratinocytes (Larsen et al., 1989), gastric cancer cells (Yasumoto et al., 1992), and endometrial stromal sarcoma cells (Nasu et al., 1997). On the other hand, an inhibitor of IL-8 production has not been fully defined. It was recently reported that progesterone suppresses the production of IL-8 by human chorioddecidual cells (Kelly et al., 1994) and rabbit uterine cervical fibroblasts (Ito et al., 1994). Dexamethasone and IL-10 were also reported to suppress the production of IL-8 by polymorphonuclear leukocytes (Tobler et al., 1992; Cassatella et al., 1993; Kasama et al., 1994). IFN-γ exerts opposite effects on IL-8 gene expression and protein secretion, depending on the lineage of the target cells: an inhibitory effect of IFN-γ on IL-8 production was reported in fibroblasts (Oliveira et al., 1992; Tobler et al., 1992), polymorphonuclear leukocytes (Cassatella et al., 1993), monococytes (Gusella et al., 1993; Schneider-Candrian et al., 1995), and renal proximal tubular epithelium (Gerritsma et al., 1996), whereas a stimulatory effect has been shown in keratinocytes (Barker et al., 1990), and gastric cancer cell lines (Yasumoto et al., 1992).
Macrophages, natural killer (NK) cells, and lymphocytes are commonly observed in the decidua in normal early pregnancy (Haller et al., 1993; Hunt, 1994). However, neutrophils are rarely observed in the decidua. Our observation that IFN-γ enhances production of IL-6, M-CSF and MCP-1 and that it inhibits production of IL-8 by ESC may provide an explanation for the recruitment of these inflammatory cells. IL-8, originally described as a neutrophil chemotactic and activating cytokine for the recruitment of these inflammatory cells. IL-8, originally described as a neutrophil chemotactic and activating cytokine for the recruitment of these inflammatory cells. IL-8, originally described as a neutrophil chemotactic and activating cytokine for the recruitment of these inflammatory cells.

In conclusion, we demonstrated for the first time that IFN-γ enhances the production of IL-6, MCP-1, and M-CSF by ESC and inhibited the production of IL-8 by these cells. It is suggested that IFN-γ produced by both decidual inflammatory cells and the developing embryo plays a role in the maintenance of early pregnancy by regulating both the functions and distribution of immune-effector cells at the maternal–fetal interface.

References


Cytokine regulation by IFN-γ in endometrial stroma


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