

Peritoneal Growth Factors and Endometriosis

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Endometriosis is classically defined as the presence of endometrial glands and stroma outside the uterine cavity and musculature. It is a common disease that has puzzled researchers for most of the century and still remains one of the most enigmatic disorders in gynecology. In 1994 alone, more than 270 articles on endometriosis appeared in the world scientific literature, many of them contradictory, reflecting our difficulties in deciphering this disorder.

Numerous theories of the histogenesis of endometriosis have been proposed by many investigators in the field. However, three main theories dominate current thinking. The original theory proposed that endometriosis develops from metaplasia of cells lining the pelvic peritoneum.¹ The basis of the coelomic metaplasia concept derives from the fact that both endometrial and peritoneal cells derive from the same coelomic epithelium. A second theory of histogenesis is transplantation of shed uterine endometrium to ectopic locations, as suggested by Sampson in the 1920s.² Many mechanisms have been proposed, including lymphatic, vascular, and iatrogenic dissemination and retrograde menstruation. Considerable circumstantial evidence supports the role of retrograde menstruation with subsequent endometrial transplantation as an etiological factor. The third theory, also known as the induction theory, is a combination of the first two. It states that unknown substances released from shed endometrium induce undifferentiated mesenchyma to form endometriotic tissue.

Retrograde menstruation is a well-established phenomenon consistent with either the transplanta-

tion or the induction model of endometriosis. Studies of women undergoing peritoneal dialysis and laparoscopy suggest that 76% to 90% have retrograde menstruation, a prevalence much higher than that of endometriosis.³ This suggests that other factors such as the amount of retrograde flow or an impaired peritoneal environment may determine a woman's susceptibility to develop endometriosis. While there is a possibility that the peritoneal environment in some women may be conducive to the development of endometriosis, the environment may also be altered by the disease.

Peritoneal fluid (PF) is an important contributor to the environment in which fertilization and early embryonic development take place. Interest is growing in the cellular and humoral components of PF and their role in both the development of endometriosis and reproductive performance. Growth factors and cytokines found in the PF have been postulated to participate in the pathogenesis of endometriosis (Table 1). There are many potential sources of growth factors and cytokines found in the PF. Mesothelial cells that cover the wide surface of the peritoneal cavity and macrophages that form the cell type found in greatest quantity in the PF are likely sources. Endometrial cells themselves have been shown to produce many of these factors. Thus, the refluxed and/or implanted endometrial tissue may also be a source. Another potential source is the follicular fluid released regularly in reproductive-age women. Investigators have attempted to identify factors present in the peritoneal environment of women with endometriosis that may explain the pathogene-

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Table 1. Growth Factors and Cytokines in Human Peritoneal Fluid of Patients With Endometriosis

Mediator	Level	Author(s)
EGF	ND	De Leon et al ⁹⁶
TGF- β	↑	Oosterlynck et al ¹²
IGFs	↑	Giudice et al ¹³
PDGF	↑	Halme et al ³¹
M-CSF	↑	Fukaya et al ⁹⁷
IL-1	↑	Fakih et al, ⁴⁸ Hill and Anderson ⁴⁹ Taketani et al ⁴⁵
	ND	Awadalla et al, ⁵⁰ Koyama et al ⁹⁸ Keenan et al ⁹⁹
IL-2	ND	Keenan et al ⁹⁹
IL-5	ND	Koyama et al ⁹⁸
IL-6	↑	Rier et al, ⁶² Koyama et al ⁹⁸
	ND	Boutten et al, ⁶⁰ Keenan et al ⁶¹
IL-8	↑	Arici et al, ⁸⁵ Ryan et al ⁸⁴
TNF- α	↑	Eisermann et al, ⁶⁹ Halme ⁷⁰ Taketani et al ⁴⁵
	ND	Vercellini et al, ⁷¹ Keenan et al ⁹⁹
IFN- γ	ND	Khorram et al, ⁷⁸ Keenan et al ⁶¹
MCP-1	↑	Arici et al ⁹⁰
RANTES	↑	Khorram et al ⁷⁸

EGF = epidermal growth factor, ND = no difference, TGF = transforming growth factor, IGFs = insulin-like growth factors, PDGF = platelet-derived growth factor, M-CSF = macrophage colony-stimulating factor, IL = interleukin, TNF = tumor necrosis factor, IFN = interferon, MCP = monocyte chemotactic protein.

sis of endometriosis and the pathophysiology of associated symptoms such as pelvic pain or infertility.^{4,5} There are two potential mechanisms by which these mediators may contribute to the pathogenesis and pathophysiology of endometriosis: (1) enhancing the establishment and/or proliferation of ectopic endometrial implants and (2) adversely affecting fertility via secretion of cytokines by or in response to endometrial implants that may change the chemical, hormonal, or cellular milieu (Fig. 1).

The cellular compartment of the peritoneal fluid is mainly composed of macrophages. Macrophages in the peritoneal cavity play a role in maintaining the normal homeostasis by removing the red blood cells, or other damaged cells that gain access to this cavity. In addition to phagocytosis, macrophages maintain this homeostasis by release of cytokines, growth factors, prostaglandins, enzymes, and reactive oxygen metabolites.⁶ The presence of macrophages in the peritoneal cavity may be due to the presence of several physiological sources of inflammatory response. They include retrograde menstruation, spermatozoa, and ruptured follicles. Originally, an increased number of macrophages in the peritoneal cavity of women with endometriosis was reported.⁷ Regardless of the number of macrophages in the PF, it has been suggested that the major difference between normal women and women with endometriosis resides in the activation level of macrophages.^{4,8,9} Several products of activated macrophages or constituents of PF have been shown to exert adverse influence on gametes, fertilization, or embryonic development when tested

in vitro. Such products include interleukin-1 (IL-1), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α).

In this regard, the current knowledge of the peritoneal growth factors and cytokines in endometriosis and their potential role in the development of the disease and associated pathologies are reviewed below.

GROWTH FACTORS

Growth factors are a wide group of proteins produced by a variety of cells and acting in both paracrine and autocrine fashion. These factors are able to affect the growth and differentiation of cells and may act alone or in synergy with other factors. Although many growth factors are named after their originally observed biological action, they are generally involved in a wide range of actions including stimulation of cell growth, inhibition of cellular proliferation, and alterations of cell functions. To exert their effects, growth factors and cytokines interact with specific cell surface receptors that possess protein kinase activity in their cytoplasmic domains.¹⁰ Many of these factors are attached to specific carrier proteins or binding proteins that may target and modulate their action in specific tissues. Tyrosine kinase activity is

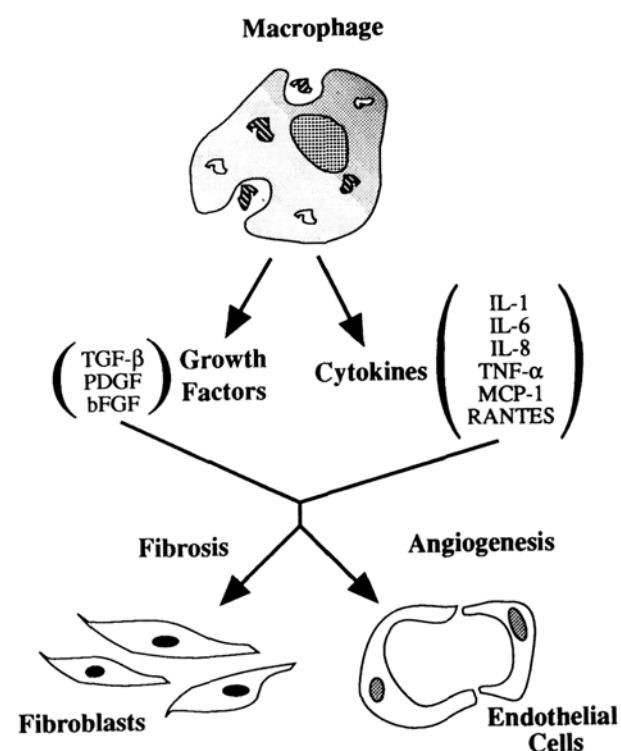


Figure 1. Role of macrophages and macrophage mediators in the pathogenesis of endometriosis.

considered to be the primary effector system in transmembrane signaling processes and results in phosphorylation of intracellular proteins that regulate pathways altering gene expression, cellular metabolism, and cellular division.¹⁰ Some growth factors are responsible for proliferation of endometrium *in vitro*, and they could play a part in the etiology of endometriosis. Growth factors in PF may be derived from several sources, including sloughed endometrium, ovarian follicular fluid, peritoneal cells, ectopic endometrium itself, and the pelvic inflammatory response associated with this disorder. It has been suggested that because macrophage-conditioned medium contains mitogens, growth factors secreted from peritoneal macrophages may also promote endometriosis.¹¹ Several growth factors have been found in the peritoneal environment, including EGF, TGF- β , IGFs, and PDGF.^{12,13}

EGF

Epidermal growth factor (EGF) is perhaps the best characterized of all growth factors. Originally identified as a peptide that stimulates the growth of basal epithelial cells, EGF is a single polypeptide chain of 53 amino acids of a molecular weight of 5 kD. It stimulates proliferation of many cell types including fibroblasts, keratinocytes, and epithelial cells.¹⁴ It is generated from a larger 128-kD precursor by proteolytic cleavage.¹⁴ In the human endometrium, EGF immunoreactivity was first detected in homogenates.¹⁵ Then, Haining et al,¹⁶ using immunohistochemistry, localized EGF to both epithelial and stromal cells. This growth factor is found at similar levels throughout the menstrual cycle.

Ectopic endometrium is known to express estrogen receptors,¹⁷ and its growth and maintenance are dependent upon continued stimulation by estrogen. In recent years it has become apparent that estrogen action in the endometrium may be mediated by the peptide growth factors, in particular EGF.¹⁸ Epidermal growth factor exerts its effects through binding to its cell surface receptor, the EGF receptor. This receptor serves as the common receptor for EGF and transforming growth factor- α (TGF- α).¹⁹ In murine endometrium, EGF receptor and receptor messenger ribonucleic acid (mRNA) have been demonstrated.²⁰ Epidermal growth factor receptors have been described in normal human endometrium by several investigators.²¹ Receptor-binding assays revealed an increased number of EGF receptor sites during the proliferative phase, peaking just before ovulation, abruptly decreasing thereafter, and reaching a nadir just before menses.²² It has been shown using immunohistochemistry that EGF receptor is expressed in the glands and stroma of eutopic and ectopic endo-

metrium of women with endometriosis.²¹ In addition, the presence of EGF and EGF receptor has been reported in endometrial implants in surgically induced endometriosis in the rat.²³

TGF- β

Transforming growth factor- β is a fundamental regulatory peptide of 25 kD that is mainly produced by macrophages, platelets, osteoblasts, and activated lymphocytes.²⁴ In addition to its growth-regulating properties, TGF- β has one of the most potent chemottractants for human monocytes and a fibrogenic and angiogenic factor, indicating that it is an important mediator of tissue repair.²⁵ Furthermore, TGF- β has striking immunological functions and can profoundly inhibit T lymphocyte, B lymphocyte, and natural killer cell functions.²⁶ Recently, Oosterlynck et al¹² demonstrated that TGF- β was increased in the PF of women with endometriosis compared to both fertile and infertile women without endometriosis. They suggested that the decreased natural killer activity of PF in women with endometriosis may be secondary to increased PF TGF- β activity. Both TGF- β mRNA and protein are found in human stromal and epithelial cells.²⁷ Hammond et al²⁸ demonstrated that TGF- β s induced proliferation of human endometrial cells. With immunohistochemistry the presence of TGF- β has been demonstrated in surgically induced endometrial implants in the rat.²⁹ Finally, the increased concentrations of TGF- β in women with endometriosis could explain, at least partially, the angiogenic activity found in the PF of women with endometriosis.

IGFs

The insulin-like growth factors (IGF-I and IGF-II) are mitogens that can also promote differentiation. The IGFs circulate bound to IGF-binding proteins (IGFBPs), which also regulate their actions at target tissues. Giudice et al¹³ have shown that human PF contains IGF-I, IGF-II, IGFBP-1, to IGFBP-4, and the IGFBP-3 protease. Peritoneal fluid IGF levels were approximately 60% of paired serum levels, and PF levels of IGFBP-2 and IGFBP-3 were approximately half of their serum concentrations. In addition, they demonstrated that IGF was mitogenic to endometrial stromal cells in a dose-dependent manner. The IGF system may be one of several growth factor systems in PF that have the capacity to stimulate endometrial cellular proliferation.

PDGF

Platelet-derived growth factor (PDGF) is a well-characterized secretory product of activated macrophages that plays a major role in the inflammatory response serving, in part, as a potent mitogen for fibroblasts and a chemotactic agent for fibroblasts, monocytes, and neutrophils.³⁰ It is a 30-kd protein that consists of two subunits, A and B, connected by interchain disulfide bonds.³⁰ The subunits may be combined as homodimers or heterodimers, giving rise to three dimeric forms of PDGF (PDGF-AA, PDGF-AB, and PDGF-BB). Halme et al³¹ have demonstrated that peritoneal macrophages isolated from patients with endometriosis release growth factor activity *in vitro* to a greater extent than those derived from women without the disease. Recent evidence suggests that this macrophage-derived growth factor may be similar or identical to PDGF.³² In the human endometrium, PDGF-BB mRNA is expressed throughout the menstrual cycle.³³ Platelet-derived growth factor exerts a significant concentration-dependent stimulatory effect on endometrial stromal cell proliferation *in vitro*.³² This effect is enhanced by estradiol (E₂) in an additive manner. In addition, PDGF and EGF mutually enhance their proliferative effects.³⁴

bFGF

Basic fibroblast growth factor (bFGF) is an 18-kd heparin-binding angiogenic protein.³⁵ It is highly mitogenic for capillary endothelial cells *in vitro* and can induce angiogenesis *in vivo*.³⁶ In the endometrium of cycling women, bFGF is present in high concentrations throughout the menstrual cycle and, interestingly, increases in atrophic endometrium.³⁷ Secretion of bFGF by endometrial cells increases in response to 17 β -E₂ and is inhibited by progesterone.³⁸ Basic fibroblast growth factor has been found in endometrial glandular epithelium and is a potent mitogen for endometrial stromal cells in culture.³⁹ It may be one of several growth factors acting in a paracrine fashion contributing to stromal proliferation. This growth factor has not been reported so far in the peritoneal environment of endometriosis patients.

CYTOKINES

Cytokines are a heterogeneous group of soluble regulatory polypeptides that are released from cells and regulate cell growth, differentiation, and/or function by binding to specific cellular receptors. Cytokines are small polypeptides generally containing between 100- and 200-amino acid residues, includ-

ing a signal sequence that results in their secretion into the extracellular environment.⁴⁰ The molecular weight is in the range of 10 to 20 kd (Table 2). They may exert autocrine or paracrine effects but, unlike hormones, are produced by a variety of cell types that are not localized in a distinct gland and act on many different types of target cells. Cytokine activities are varied and include proliferation and differentiation of immune cells; growth of connective tissue and endothelial cells; induction of release of hormones, enzymes, and acute phase proteins; enhancement of various cytotoxic activities; regulation of immunoglobulin secretion and isotype; chemotaxis; and direct antiviral and tumoricidal effects.⁴¹ Expression of biological activity is achieved at very low concentrations because of binding by cytokines to a specific, saturable, high-affinity receptor on the plasma membrane of the cell. Additionally, cytokines may either induce or downregulate the production of other cytokines. A subgroup of cytokines, chemokines, are potent leukocyte chemotactic factors, each with a distinct but partially overlapping spectrum of action. Chemokines are further subdivided, according to the position of the first two cysteines, as CXC or CC.⁴² The CXC chemokines are neutrophil chemoattractants (eg, IL-8), whereas the CC chemokines are predominantly monocyte chemoattractants (eg, MCP-1, RANTES).

Much evidence points toward an interaction between the immune system and reproduction. Cytokines play an important role in reproduction at various levels including gonadal function, gamete function, fertilization and embryo development, implantation, and postimplantation survival of the conceptus. Many cytokines such as IL-1 and TNF- α can have detrimental effects on reproductive cells, especially sperm and embryos, when present at an appropriate place, time, and concentration.⁴³ Particular interest has been focused on the cytokine levels in the PF of women with endometriosis. Several cytokines have been shown to affect the growth of endometrial stromal cells in culture.⁴⁴ However, the role of the individual pleiotropic factor in regulating endometrial or endometriotic cell growth is unclear. Taketani et al⁴⁵ have reported that medical treatment of endometriosis not only decreases cytokine levels but also concurrently eliminates embryotoxicity of the peritoneal fluid of women with endometriosis.

IL-1

Interleukin-1 is a pleiotropic, proinflammatory cytokine, secreted by mononuclear phagocytes, endothelial cells, epithelial cells, and fibroblasts. It has stimulatory and regulatory effects on the growth and differentiation of numerous cell types.⁴⁶ The IL-1

Table 2. Molecular and Functional Parameters of Various Cytokines

<i>Cytokine</i>	<i>Molecular Size and Source</i>	<i>Activity</i>
IL-1	17 kd, primarily from activated monocytes or macrophages	Induces lymphokine release by T cells and augments natural killer cells-mediated activity
IL-6	26 kd, monocytes or macrophages, endothelial cells, fibroblasts, T cells	Acts on T cells, B cells, fibroblasts, and hepatocytes
IL-8	8 kd, dimeric, macrophages, neutrophils, fibroblasts, endothelial cells	Induces chemotaxis of neutrophils, angiogenic factor
TNF- α	17 kd, multimeric, produced by activated macrophages	Cytolysis, induces the expression of MHC antigens, angiogenic factor
IFN- γ	40–50 kd, T lymphocytes	Antiviral activity, inhibits cell growth/differentiation, and induces MHC antigens
MCP-1	8 kd, monocytes, endothelial cells, fibroblasts	Monocyte chemoattractant, stimulates the respiratory burst in monocytes
RANTES	8 kd, macrophages, T cells	Chemoattractant for monocytes and T lymphocytes

IL = interleukin, TNF = tumor necrosis factor, MHC = major histocompatibility complex, IFN = interferon, MCP = monocyte chemotactic protein.

system is composed of IL-1 α (159 amino acids), IL-1 β (153 amino acids), and an inhibitor, IL-1 receptor antagonist (152 amino acids). Although IL-1 α and IL-1 β are encoded by different genes and have different amino acid sequences, both are recognized by the same receptor on target cells and produce the same biological effects.⁴⁷

Although some investigators have shown that IL-1 is present in higher concentrations in PF of patients with endometriosis,^{48,49} results obtained are not consistent.⁵⁰ Fakih et al,⁴⁸ using a bioassay system, found elevated IL-1 activity in PF from patients with mild endometriosis. Hill and Anderson,⁴⁹ who used radioimmunoassay, observed elevated levels of IL-1 β in PF from 5 of 26 patients with endometriosis. On the contrary, Awadalla et al⁵⁰ demonstrated that PF IL-1 activity determined with a bioassay system did not differ between patients with or without endometriosis. In addition, peritoneal macrophages express higher levels of IL-1 receptor antagonist mRNA rather than IL-1 β mRNA with progression of endometriosis.⁵¹ The expression of mRNA and proteins of IL-1 α , IL-1 β , and IL-1 receptor antagonist in human endometrium has been reported.⁵² Experiments utilizing radioiodinated IL-1 α reveal that the plasma membranes prepared from human endometrial epithelium and stromal cells possess high-affinity receptors for IL-1.⁵³ Interleukin-1 β inhibits growth of normal human endometrial stromal cells in vitro.⁵⁴ Interestingly, endometrial stromal cells have often been compared to fibroblasts, but fibroblasts proliferate when exposed to IL-1. In view of the inhibitory effect of IL-1 on stromal cell proliferation, one might hypothesize that this cytokine is counterbalancing effects of other exogenous growth stimulatory substances associated with endometriosis-related macrophages.

There are conflicting observations as to the effect of IL-1 on early reproductive events in vitro.^{43,48,55} Interleukin-1 inhibits mouse embryo development in vitro,^{43,48} but only at very high concentrations (>10⁶

U/mL). This effect, however, is specific for IL-1 β , since neutralization experiments with anti-IL-1 β antibody abrogates the embryotoxic effect.⁵⁶ Interleukin-1 also impairs the oocyte-penetrating capacity of the sperm, both in the hamster and the human.⁵⁷ On the other hand, Hill et al reported no significant effect of IL-1 on sperm motion parameters.⁵⁵ It has been demonstrated that IL-1 and TNF- α levels are markedly low in the PF from women who have undergone medical treatment for endometriosis as compared to women with untreated endometriosis. Finally, PF of women with treated endometriosis is much less embryotoxic than PF of women with untreated disease.⁴⁵

IL-6

Interleukin-6 is a cytokine produced by macrophages, T and B cells, fibroblasts, endothelial cells, endometrial stromal cells, and several other cell types.⁵⁸ The biological activities of IL-6 are numerous and include regulation of immunocompetent cell growth and differentiation, induction of acute phase proteins, and stimulation or inhibition of cell growth, depending upon the cell type.⁵⁸ Importantly, recent evidence suggests that this cytokine plays an active role in reproductive physiology, including regulation of ovarian steroid production and early implantation events.⁵⁹ Conflicting results have been reported for the levels of immunoreactive IL-6 detected in human PF. Although IL-6 is found in the PF of patients with mild endometriosis and infertile or fertile controls, the difference is not significant.⁶⁰ Furthermore, IL-6 concentrations in PF of women with minimal or mild endometriosis are much lower than those generally observed during acute inflammation. In a recent study, although there was not significant difference in the PF levels of IL-6 in women with or without endometriosis, levels of IL-6 were signifi-

cantly higher in the macrophage-conditioned media of women with endometriosis compared to those without endometriosis.⁶¹ Rier et al⁶² have shown that the severity of endometriosis correlates with the increased level of IL-6 accompanied by a decrease of IL-6 soluble receptor level in the peritoneal fluid. It is likely that these inconsistent findings are related to the antibody specificity. In the rat model, serum IL-6 level increases at 4 weeks but returns to normal level at 8 weeks after the surgical induction of endometriosis.⁶³ The sources of the increased serum IL-6 concentration remain to be established. Endometrial stromal cells are known to produce IL-6 in vitro that is regulated by 17β -E₂, and it is possible that IL-6 secreted from ectopic endometrial tissue is responsible for the increased serum levels.⁶⁴ Recently, it has been shown that ectopic endometrial cells secrete higher levels of IL-6 in vitro compared to eutopic endometrial cells.⁶² Alternatively, immunocompetent or other cells outside the peritoneal cavity may contribute to the increased serum IL-6 levels.

There are no conclusive data on the effect of IL-6 on endometrial cell growth, but IL-6 may inhibit endometrial epithelial and stromal cell proliferation.⁶⁴ Furthermore, ectopic endometrial cells appear to be resistant to this IL-6-induced growth inhibition, and this response correlates with the weak expression of IL-6 receptor on these cells.⁶² Because IL-1 is known to enhance IL-6 production and IL-1 has been shown to inhibit endometrial stromal cell proliferation in vitro, it is possible that the antiproliferative effect of IL-6 is due to other related cytokines.⁵⁴ Interestingly, if IL-6 is inhibitory to endometrial cell growth, an increased circulating level may actually be a protective mechanism to prevent the development of endometriosis.

TNF- α

Tumor necrosis factor- α is a secretory protein that has been increasingly recognized as an important biological mediator. It is synthesized as a 26-kd membrane-bound precursor form that is cleaved proteolytically to a mature 17-kd form,⁶⁵ is primarily secreted by macrophages and monocytes, and mediates macrophage cytotoxicity against susceptible cells.⁶⁶ It is a pleiotropic factor that exerts a variety of effects including proinflammation, growth promotion, growth inhibition, immunomodulation, angiogenesis, and cellular toxicity.⁶⁵ It is able to elicit hemorrhagic necrosis of tumors in animals and is cytotoxic for a variety of cell lines in vitro. It is known to stimulate the growth of human fibroblasts in vitro,⁶⁷ to activate neutrophil granulocytes,⁶⁸ and to stimulate fibrin deposition in tumor vasculature.

Eisermann et al first documented the presence of

TNF- α in human PF.⁶⁹ In that study, the mean concentration of TNF- α in patients with extensive endometriosis was elevated, but the highest values were seen in women with acute pelvic inflammatory disease. This finding supports the concept that an inflammatory process may be linked to the increase in activated macrophages seen in the PF of women with endometriosis. This could result an elevation of TNF- α in proportion to the degree of inflammation present.

Halme⁷⁰ showed a significant increase in the level of cytotoxicity in the PF of patients with mild endometriosis as compared to fertile women. In addition, the macrophage-conditioned medium of women with endometriosis exhibited significantly more activity than those of fertile women and women with unexplained infertility without endometriosis. However, Vercellini et al could not find any difference in the plasma and PF TNF- α levels in infertile women with or without endometriosis.⁷¹ Recently, it has been shown that TNF- α promotes the adherence of human endometrial cells to peritoneal mesothelial cells in a dose-dependent fashion.⁷² There are two possible explanations for this effect of TNF- α . First, it might affect the proliferation of mesothelial cells for endometrial stromal cells to adhere. Second, TNF- α might induce adhesion molecules on the surface of mesothelial cells.

Tumor necrosis factor- α has been linked to a variety of additional reproductive effects. This factor significantly affects sperm motility in vitro, but only at very high concentrations.⁵⁵ In addition, Hill et al⁴³ tested various concentrations of cytokines and found significant embryotoxicity only by TNF- α and INF- γ . In conclusion, TNF- α might be involved in the pathogenesis of the disease, acting as a growth factor on fibroblasts, and in the associated pathophysiology by inducing luteolysis and eliciting cytotoxicity against spermatozoa.

IFN- γ

Interferon- γ is a 23-kd homodimer that is produced primarily by activated T lymphocytes.⁷³ It is toxic to a variety of virally infected and neoplastic cells and can interfere with cell growth by disrupting the structural organization of the plasma membrane cytoskeletal complex.⁷⁴ It also adversely affects sperm motility, fertilization, early embryonic development, and trophoblast proliferation in vitro.^{43,55,56} This cytokine was detected in the supernatant of endometrial cell cultures and the receptor for IFN- γ was demonstrated in eutopic endometrium.⁷⁵ A local antiproliferative effect of IFN- γ is supported by the finding that IFN- γ inhibits in vitro proliferation of endometrial epithelial cells.⁴⁴ This cytokine also

significantly inhibits blastocyst implantation in vitro.⁷⁶ This inhibition is observed at concentrations as low as 10^3 U/mL. Hill et al have demonstrated that TNF- α and INF- γ adversely affect many reproductive processes including sperm motility,⁵⁵ fertilization,⁷⁷ and embryo development.⁴³ Interferon- γ concentrations do not differ in the PF of women with or without endometriosis.^{61,78}

Chemoattractant Cytokines

IL-8

Interleukin-8 is a chemoattractant and activating cytokine for neutrophils⁷⁹ and a potent angiogenic agent.⁸⁰ It is produced by a number of cell types, including monocytes,⁸¹ endothelial cells, fibroblasts, mesothelial cells, and endometrial stromal cells.⁸² Recently PF of women with endometriosis was shown to have increased neutrophil chemotactic activity.⁸³ However, the nature and source of this chemotactic factor remain to be determined. One of the candidates is IL-8. In a case-control study, IL-8 was detectable in the PF of 67% of cycling women.⁸⁴ We first investigated IL-8 levels in the PF of women with or without endometriosis, then assessed peritoneal mesothelial cells as a potential source of PF IL-8.⁸⁵ Mean concentration of IL-8 in PF samples obtained from control patients ($n = 28$) was 4.8 ± 0.5 pg/mL (\pm SE); from patients with minimal to mild endometriosis ($n = 24$), 28 ± 3 pg/mL; and from patients with moderate to severe endometriosis ($n = 21$) was 530 ± 65 pg/mL ($P = .023$) (Fig. 2, panel A). We found that cultured mesothelial cells constitutively express IL-8 mRNA and secrete IL-8 protein. Mesothelial cells were incubated with IL-1 α (10 U/mL) or TNF- α (10 ng/mL) for 6 hours (Fig. 2, panel B). Both IL-1 α and TNF- α induced higher levels of IL-8 mRNA and protein. We speculate that the regulated expression of this angiogenic and chemoattractant factor may play a role in the pathogenesis of endometriosis.

Monocyte Chemotactic Protein-1 (MCP-1)

Peritoneal fluid from patients with endometriosis has increased chemotactic activity for macrophages.⁸³ The presence of such a chemotactic stimulus will increase macrophage number and activation, resulting in secretion of a variety of cytokines. There are several candidate chemoattractants that may recruit macrophages. One of them is MCP-1, a 76-amino acid basic protein that chemoattracts and activates monocytes/macrophages. It is secreted by a number of cell types including endothelial cells, fibroblasts, monocytes, lymphocytes, mesengial cells,

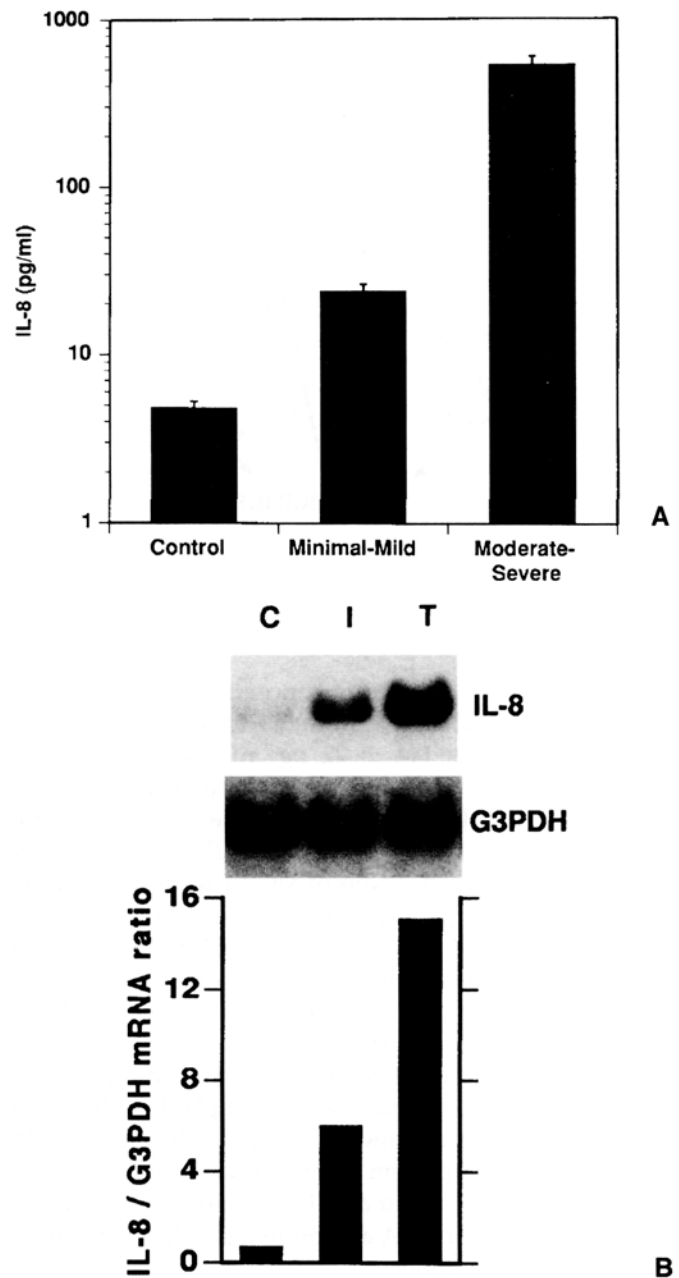


Figure 2. A: Immunoreactive interleukin-8 (IL-8) levels according to endometriosis: control, minimal to mild, and moderate to severe. Values are mean \pm SE. B: Northern analysis of IL-8 mRNA in mesothelial cells treated with IL-1 α and tumor necrosis factor- α (TNF- α). Confluent mesothelial cells in culture were incubated for 6 hours with IL-1 α (10 U/mL) and TNF- α (10 ng/mL). Total RNA (10 μ g per lane) was evaluated. C-control, I-IL-1 α , T-TNF- α .

and endometrial cells.^{86–88} It can be regulated by IL-1, TNF- α , and IFN- γ . In a recent study, endometriotic cells have been shown to secrete MCP-1 in vitro upon stimulation with IL-1 β and TNF- α .⁸⁹ We first investigated MCP-1 levels in the peritoneal fluid of women with or without endometriosis, then investigated mesothelial cells as a potential source of this

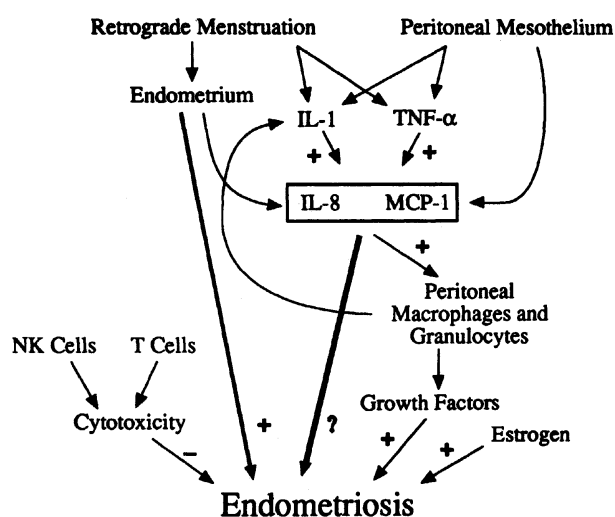


Figure 3. The role of interleukin-8 and monocyte chemoattractant protein-1 in pathogenesis of endometriosis.

chemokine.⁹⁰ The mean concentration of MCP-1 in the peritoneal fluid of women without endometriosis ($n = 18$) was 144 ± 22 pg/mL (mean \pm SE); of women with moderate endometriosis ($n = 24$), 547 ± 244 pg/mL; and of those with severe endometriosis ($n = 8$), 1258 ± 392 pg/mL ($P = .036$, $P = .05$, respectively). The levels of MCP-1 were significantly higher in the peritoneal fluid from women who had untreated endometriosis (861 ± 254 pg/mL) than in women who had undergone medical treatment with gonadotropin-releasing hormone agonist (GnRHa) (122 ± 37 pg/mL) ($P = .05$).⁹⁰ This finding may be due to the lessened inflammatory reaction caused by the reduction of endometriotic implants rather than a direct effect of GnRHa. In mesothelial cells, MCP-1 mRNA and protein were detectable constitutively; however, both IL-1 α and TNF- α induced higher levels of MCP-1 mRNA and protein in a dose- and time-dependent manner.

RANTES

RANTES is a newly discovered 8-kd T cell-specific cytokine of the platelet factor-4 gene superfamily.⁹¹ RANTES is a selective chemoattractant for monocytes and T lymphocytes.⁹² Recently, PF concentrations of RANTES have been found elevated in women with endometriosis and levels correlate with the severity of disease.⁷⁸

SUMMARY

Because endometriosis is associated with a localized inflammatory response, there is growing interest in the cellular immune system that could be im-

portant to the pathogenesis of endometriosis. The role of PF components and macrophage-derived secretory products in the development of endometriosis has been studied for several years. Since the macrophage is the predominant nucleated cell in the PF it probably represents the first-line host response to an inflammatory stimulus. Attracted by chemotaxis, macrophages extravasate through small pores in the vessel wall and enter the peritoneal cavity to perform their phagocytic and secretory functions. There is evidence to suggest that peritoneal macrophages from women with endometriosis possess accentuated activation characteristics resulting in enhanced phagocytic activity and secretion of several soluble substances. Macrophage-derived growth factors and cytokines as well as PF from patients with endometriosis promote the growth of endometrial stromal cells, endometrial epithelial cell lines, and endometrial carcinoma cell lines.^{28,93} In addition, growth factors such as TGF- β , bFGF, and PDGF provide mitogenic stimuli to fibroblastic cells. Peritoneal fluid of women with endometriosis displays greater angiogenic activity than PF obtained from women without the disease.⁹⁴ The increased concentration of substances present in PF of women with endometriosis, such as bFGF, TGF- β , TNF- α , EGF, and IL-8, which are also known angiogenic factors, may explain outgrowth of endometriotic lesions and associated adhesion formation.

A relationship between infertility and endometriosis is well documented in the literature.⁹⁵ We believe that the peritoneal environment in these women plays a role in endometriosis-associated infertility. Endometriosis-derived PF has been shown to have a toxic effect on sperm motility/survival, sperm-oocyte interaction, and embryonic development.^{55,57,76,77} The mechanism by which endometriosis-associated PF reduces fertility remains unknown. It seems possible that soluble factors produced by activated macrophages and T lymphocytes may adversely affect fertilization and early embryo development, resulting in reproductive failure. Conflicting results may be attributable to differences in sample size, lack of patient homogeneity, individual menstrual cycle variation, and bioassay variability.

In summary, there is convincing evidence that increased concentrations of growth factors/cytokines found in the PF of patients with endometriosis display a dual effect; while inducing proliferation of the endometrial implants, they may be inhibiting early reproductive events. We have studied IL-8 and MCP-1 because they are two of the most cell-specific chemoattractants. Our investigations have shown that concentrations of MCP-1 and IL-8 are not only elevated in PF of women with endometriosis, but the levels also correlate with the severity of the disease. Our hypothesis is that elevated levels of peritoneal

IL-8 and MCP-1 may also play a role in the growth and maintenance of ectopic endometrial tissue not only by stimulating leukocytes to secrete cytokines and growth factors, but also by directly stimulating endometrial cell proliferation (Fig. 3). In the future, specific antagonists to individual cytokines that are shown to stimulate the growth of endometriosis and to interfere with fertilization and implantation in women with endometriosis may offer a possibility of prevention and treatment of this disorder.

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