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The peritoneal environment in endometriosis

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TABLE OF CONTENTS

The local environment of peritoneal fluid (PF) surrounding the endometriotic implant is immunologically dynamic and links the reproductive and immune systems. Peritoneal fluid contains a variety of free floating cells, including macrophages, mesothelial cells, lymphocytes, eosinophils and mast cells. Macrophages are attracted to the peritoneal environment more abundantly than any other cell type. These scavengers promote cellular growth and viability through secretion of growth factors and cytokines. It is now becoming evident that cytokines play an important role in reproduction at various levels, including gamete function, fertilization and embryo development, implantation and postimplantation survival of the conceptus. Peritoneal fluid has been shown to affect negatively ovum capture by the fimbria, sperm survival, spermatozoon–oocyte interaction and embryonic development. We have recently identified the presence of two pro-inflammatory chemoattractant cytokines for monocyte/macrophages (MCP-1) and for granulocytes (interleukin-8, IL-8) in the PF. Concentrations of both IL-8 and MCP-1 are not only elevated in PF of women with endometriosis compared to those without endometriosis, but they are related to the severity of the disease. Over the past 70 years, at least a dozen theories have been proposed to explain the histogenesis and aetiology of endometriosis. It appears that the aetiology is multifactorial, and today a composite theory of retrograde menstruation with implantation of endometrial fragments in conjunction with peritoneal factors to stimulate cell growth is the most widely accepted explanation for peritoneal endometriosis.

Key words: cytokines/endometriosis/macrophage/peritoneal fluid

Introduction

Endometriosis, defined by the presence of viable endometrial tissue outside the uterine cavity, is a common condition among women of reproductive age. Over the past 70 years, at least a dozen theories have been proposed to explain the histogenesis and aetiology of endometriosis. None of the theories alone can explain the entire spectrum and anatomical distribution of the lesions. It appears that the aetiology is multifactorial, and today a composite theory of retrograde menstruation with implantation of endometrial fragments in conjunction with peritoneal factors to stimulate cell growth is the most widely accepted explanation for peritoneal endometriosis (Olive and Schwartz, 1993).

The association of endometriosis with infertility has long been noted. It is estimated that 25–50% of infertile women have endometriosis and, among women with endometriosis, 30–50% are infertile (Strathy *et al.*, 1982). In addition to infertility, endometriosis may also cause severe pelvic pain. Dysmenorrhoea, dyspareunia, back and rectal pain are common complaints in women with endometriosis. Investigators have attempted to identify the factors present in the peritoneal environment of patients with endometriosis that may help explain the pathogenesis of endometriosis and the pathophysiology of associated symptoms such as infertility and pain. Many have proposed that patients with endometriosis have an altered peritoneal environment that affects the presenting symptoms of the disease (Halme *et al.*, 1987; Syrop and Halme, 1987a). On the other hand, recently, there has been failure to demonstrate any association between concentrations of cytokines and symptoms

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or severity of disease (Overton *et al.*, 1996). In this article, we review the current understanding of the peritoneal environment in patients with endometriosis as well as its potential role in the development of the disease and its associated pathologies.

Peritoneal fluid in endometriosis

The local environment that surrounds the endometriotic implant in the peritoneal cavity is quite dynamic. Peritoneum is the most extensive serous membrane in the body. The surface area of the peritoneum is generally equal to that of the skin (diZerega and Rodgers, 1992). It consists of two layers: a loose connective tissue layer that contains collagen, elastic fibres, fat cells and macrophages and a mesothelial layer that consists of squamous cells. The peritoneal fluid (PF) arises primarily from two different sources: plasma transudate and ovarian exudate; other sources are tubal fluid, retrograde menstruation and macrophage secretions.

Fallopian tubes and ovaries are bathed in PF. Oocytes are exposed to the peritoneal environment even after they are captured by the fimbria because the Fallopian tube is a conduit freely communicating with the peritoneal cavity. Spermatozoa are exposed to PF factors in the Fallopian tube before and during fertilization. The embryo undergoes early development in the Fallopian tube, where it is also potentially exposed to cellular and soluble components of PF. There is evidence suggesting that at least some of the uterine fluid may be of peritoneal origin (Casslen, 1986).

The role of endometriosis-associated inflammatory changes in the local peritoneal environment has been receiving increasing attention. Changes in fluid volume as well as concentration of a variety of cells, hormones and other compounds have been characterized during normal menstrual cycles and in women with endometriosis (Syrop and Halme, 1987b). Cellular and biochemical constituents of the PF have been suggested to play an important role in the pathogenesis of endometriosis (Halme *et al.*, 1988; Koutsilieris *et al.*, 1991).

PF is an immunologically dynamic environment that links the reproductive and immune systems. The abnormalities of PF seen in patients with endometriosis may also be linked to endometriosis-associated infertility. PF components may adversely interact with ovulatory function, gamete transport or survival, spermatozoon–ovum interaction, early embryonic development and implantation. PF has been shown to affect negatively ovum capture by the fimbria, sperm survival, spermatozoon–oocyte interaction and embryonic development (Ramey and Archer, 1993).

Characteristics of peritoneal fluid

Peritoneal fluid volume

PF is an ultrafiltrate of plasma. The peritoneal cavity of the human usually contains 5–20 ml of serous straw-coloured fluid which varies widely depending on the physiological condition. In the female, this volume changes during the menstrual cycle to reach a maximum level after ovulation. PF volume depends on follicular activity, corpus luteum vascularity and hormone production (Syrop and Halme, 1987b). Endometriosis may alter PF volume by increasing fluid production by altering mesothelial permeability or increasing colloid osmotic pressure as a result of altered protein content. A recent review assessed results from 17 studies that used different times of collection during the menstrual cycle (Hurst and Rock, 1991). Five of those 17 studies showed increased volume in endometriosis, and 11 showed no change. The largest of these suggested increased PF volume in endometriosis throughout the menstrual cycle (Syrop and Halme, 1987b). However, two well-designed studies of PF in endometriosis, performed between cycle days 8–12 and cycle days 13–18, failed to show an increase in PF volume among patients with endometriosis (Rock *et al.*, 1982; Rezai *et al.*, 1987). Cycle day variations, improperly selected control groups and different collection techniques may explain the discrepancies found in most of these studies.

Syrop and Halme (1986) attempted to determine the impact of PF volume on endometriosis-associated infertility. Women who achieved a pregnancy had a significantly lower PF volume than those who did not achieve pregnancy. Thus, PF volume was inversely related to the subsequent pregnancy rate in patients with endometriosis. In addition, Haney and Weinberg (1988) found that the volume of PF decreased in every woman with endometriosis after treatment with medroxyprogesterone acetate.

Overall, there are suggestive findings that the volume of PF in patients with endometriosis may be modestly increased, but this appears to be of little clinical importance and correlates poorly with infertility. Even if the increase in PF volume is proved, its role as a cause-and-effect link between endometriosis and infertility is debatable, as higher volumes are also present in women with unexplained infertility.

Peritoneal fluid cell contents

PF contains a variety of free floating cells, including macrophages, mesothelial cells, lymphocytes, eosinophils and mast cells. Granulocytes, normally present in small numbers, are greatly increased with pelvic inflammation. Hill *et al.* (1988) used monoclonal antibodies rather than

histological morphology to identify cell types in PF, and found that women with early stages of endometriosis had the most pronounced increases of total leukocytes, macrophages, helper T lymphocytes and natural killer cells compared to fertile controls. These findings support an active immunological process. There is compelling evidence for increased white blood cell populations in the PF of women with endometriosis as compared to women without endometriosis. These cells may be chemotactically attracted to the peritoneal cavity in response to the disease, or their increased presence could represent the primary abnormality.

Macrophages

Macrophages are attracted to the peritoneal environment more abundantly than any other cell type. These cells originate in the bone marrow, circulate as monocytes, and then migrate to various body cavities where they function primarily as phagocytes when activated. Macrophage-directed host defence mechanisms resulting in the recognition, phagocytosis, and destruction of micro-organisms are well known. Macrophages digest and process peritoneal debris such as spermatozoa and endometrial tissue and present antigens to the T cells.

Macrophages are capable of secreting various substances, such as growth factors, cytokines, prostanoids, complement components and hydrolytic enzymes (Nathan, 1987). Macrophages also promote cellular growth and viability through secretion of growth factors and cytokines. Furthermore, macrophages release low amounts of reactive oxygen metabolites, such as superoxide anion, hydrogen peroxide and singlet oxygen. Endometriosis produces a localized inflammatory response. 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, these cells extravasate through small pores in the vessel wall and enter the peritoneal cavity to perform their phagocytic and secretory functions. Because of this, the association between macrophages and endometriosis has been investigated extensively (Figure 1).

Haney *et al.* (1981) first reported an increase in peritoneal macrophages in infertile women with endometriosis. Subsequent studies of the PF of women with this disorder have confirmed an increased number, concentration and activation of macrophages (Olive *et al.*, 1985; Dunselman *et al.*, 1988). Normally, PF contains leukocytes in concentration of 0.5–2.0 $\times$ 10^6/ml, of which 85% are macrophages (van Furth *et al.*, 1979). Their concentration appears to fluctuate during the menstrual cycle, being highest at the time of menstruation.

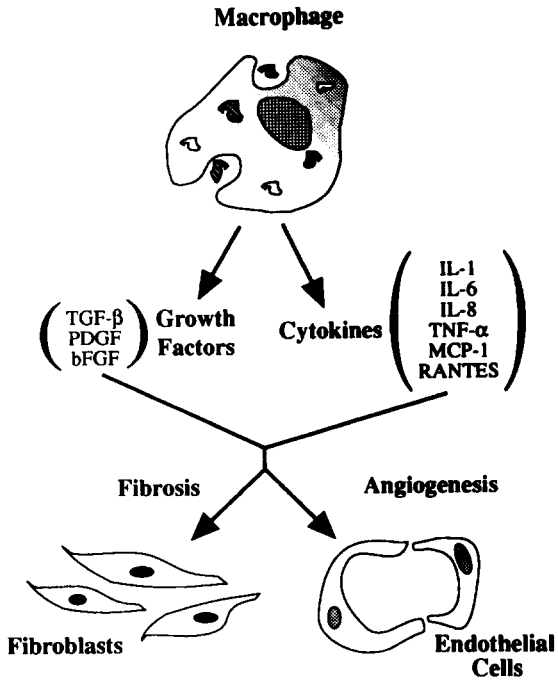

Figure 1. Role of macrophages and macrophage mediators in the pathogenesis of endometriosis. EGF = epidermal growth factor; TGF = transforming growth factor; PDGF = platelet-derived growth factor; bFGF = basic fibroblast growth factor; IL = interleukin; TNF = tumour necrosis factor; MCP-1 = monocyte chemoattractant protein-1; RANTES = regulated on activation normal T expressed and secreted.

The extent of endometriosis generally does not appear to be associated with macrophage count, although a tendency toward higher numbers has been observed in women with minimal to mild stages of disease (Olive *et al.*, 1985). Elevated numbers of macrophages in the peritoneal cavity of women with endometriosis may be the result of chronic stimulation by ectopic endometrial implants or due to excessive reflux of menstrual debris (Bartosik *et al.*, 1986). On the other hand, Haney *et al.* (1991) have demonstrated that an inverse relationship exists between peritoneal inflammation and endometriosis, arguing against the chronic stimulation hypothesis. As the stage of endometriosis advances, the degree of peritoneal inflammation decreases.

A macrophage is activated before it becomes a phagocytic scavenger. Activation is manifested by several changes: increased activities of enzymes such as acid phosphatase, myeloperoxidase, leucine aminopeptidase, enhanced phagocytic ability and cellular enlargement. Several studies have examined the level of macrophage activation associated with endometriosis and infertility and have suggested that macrophage number and concentration may be less important than macrophage activation. Halme *et al.* (1983) showed that macrophages harvested

from the peritoneal cavity of infertile women with endometriosis are larger and more activated (46 versus 15%) than macrophages from fertile women. Subsequent studies by this group confirmed an increased number of large, mature macrophages in the peritoneal cavity of women with endometriosis, with increased ability to phagocytose opsonized zymosan (Halme *et al.*, 1984a, 1987). These data also suggest that macrophages from patients with endometriosis are more differentiated than controls.

Macrophages can induce proliferation of cells, such as fibroblasts and endothelial cells, that are involved in inflammation, tissue repair and neovascularization through secretion of factors such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor-α (TNFα), macrophage-derived growth factor (MDGF) and monocyte chemoattractant protein-1 (MCP-1). Studies have shown that increased amounts of these factors are associated with endometriosis (Fakih *et al.*, 1987; Halme *et al.*, 1988; Halme, 1989).

Macrophage products may be responsible for growth or maintenance of ectopic endometrium. Surrey and Halme (1990) demonstrated that the PF from women with endometriosis stimulated cell proliferation in primary endometrial stromal cells in culture. Recently, using a unique co-culture system of immune cells and endometrial cells, significant differences between the effects of peripheral blood monocytes in women with and without endometriosis were observed (Braun *et al.*, 1994). Blood monocytes from patients with endometriosis enhance autologous endometrial cell proliferation, whereas blood monocytes from fertile patients suppress endometrial cell proliferation. Further support for this concept is demonstrated by studies showing that peritoneal macrophages from women with endometriosis produce substantially more fibronectin, a growth factor for fibroblasts, than macrophages from healthy women (Kauma *et al.*, 1988).

Increased numbers of macrophages in women with endometriosis and without mechanical infertility may also have an impact on oviductal transport of spermatozoa or their survival within the peritoneal compartment. Macrophage-mediated sperm phagocytosis has been noted quantitatively in the mouse (Olive *et al.*, 1987a) and the human (Muscato *et al.*, 1982). This concept is supported by the observation that ethiodiol, having been associated with increased fertility after hysterosalpingography, inhibits macrophage phagocytosis (Boyer *et al.*, 1986, Johnson *et al.*, 1992).

On the basis of the data from studies on mice embryos (Umesaki *et al.*, 1992), peritoneal macrophages may also have an adverse effect on fertilization and embryonic development. These adverse effects may result from direct cell-mediated damage or from humoral factors derived from macrophages. Possible humoral factors include prostaglandins, cytokines and growth factors.

Endometrial cells

The histogenesis of endometriosis is classically explained by the endometrial implantation theory of Sampson (1927) as being the consequence of retrograde seeding of endometrial cells during menstruation. Initially, this theory was questioned because retrograde menstruation was thought to be a rare phenomenon. However, investigation has shown that retrograde menstruation is in fact quite common. Polishuk and Sharf (1965), performing culdoscopy during the menstrual period, found blood-stained PF in 50% of the patients. Others have found that ~90% of normal women experience retrograde menstruation, with 70% exhibiting grossly bloody PF during menstruation (Halme *et al.*, 1984b). However, the occurrence of red blood cells in PF during menstruation is not necessarily related to retrograde transport of viable endometrial cells.

Studies analysing the incidence of endometrial cells in PF are conflicting; the incidence varying from 0 to 59% (Kruitwagen *et al.*, 1991). Several investigators have found endometrial tissue in the PF of women with or without endometriosis with equal frequency (Koninckx *et al.*, 1980; Bartosik *et al.*, 1986). Only one study has identified endometrial tissue more frequently in the PF of women with endometriosis (Badawy *et al.*, 1984a). In addition, the incidence of endometriosis is high among women with excessive retrograde flow due to anomalies of Mullerian-duct development with outflow obstruction (Olive and Henderson, 1987; Golan *et al.*, 1989). These data suggest that retrograde transport of viable endometrial cells during menstruation occurs in most women with patent tubes, suggesting that another factor aside from the presence of endometrial cell reflux is critical for the pathogenesis of endometriosis.

Mesothelial cells

The mesothelium, a simple squamous epithelium, lines the peritoneal cavity. It has been shown that human peritoneal cells are capable of producing haematopoietic growth factors, either constitutively (IL-1, IL-6, IL-8, MCP-1, granulocyte colony-stimulating factor, macrophage colony-stimulating factor) or in response to a variety of stimuli, including TNFα, IL-1 and epidermal growth factor (EGF) (Lanfrancone *et al.*, 1992). In addition, these cells secrete CA-125 from their apical surfaces (Zeillemaker *et al.*, 1994). These findings suggest that mesothelial cells play an important role in the regulation of peritoneal inflammation and tissue regeneration.

Lymphocytes (alterations in cell-mediated immunity)

An increased T-helper to T-suppressor ratio has been noted in PF samples from women with endometriosis, suggesting cellular immune activity in the peritoneal environment of these women (Hill *et al.*, 1988). Significantly higher numbers of T cells and natural killer (NK) cells, but few B lymphocytes and no plasma cells have also been observed in the PF of women with endometriosis as compared to those without disease (Dmowski *et al.*, 1994). NK cells represent a cell population whose functional activities arise from the basic responsibility of safeguarding the biological system from foreign antigen invasion. The local NK-mediated cytotoxicity in PF might be important in pathogenesis of endometriosis by preventing implantation of regurgitated endometrial cells. Oosterlynck *et al.* (1992) demonstrated significantly decreased NK activity of PF mononuclear cells in women with endometriosis. In PF of women with stage III–IV endometriosis, there was a significant decrease of NK cytotoxicity compared with those without endometriosis (Ho *et al.*, 1995).

It is interesting to note, however, that in endometriosis, despite decreased NK cell activity, the percentages of peripheral NK cells are unchanged (Oosterlynck *et al.*, 1991) or increased (Hill *et al.*, 1988).

Why NK activity is decreased in the PF of women with endometriosis remains to be clarified. One possibility is that this effect is a result of soluble factors contained in the sera and PF of affected women. NK cell activity can be decreased *in vitro* by addition of sera or PF from women with endometriosis (Kanzaki *et al.*, 1992).

Soluble substances

Prostaglandins

Prostaglandins (PG) are the most extensively studied compounds in PF to date (Rock and Hurst, 1990). There are several sources of PG in the peritoneal cavity, including macrophages, the peritoneal surface, ovarian follicles and endometriotic implants (Ylikorkala and Viinikka, 1983). Due to their short half-life, physiologically active PG are infrequently measured; instead, prostaglandin metabolites have been studied: PGEM, PGFM, 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane (TX)B_2 have been measured as representatives of their active precursors PGE, $PGF_{2\alpha}$, PGI_2 and TXA_2 respectively. Under physiological conditions, no significant cyclic variation of PG concentrations has been found (Syrop and Halme, 1987b).

The accumulated data on PG in endometriosis remain hard to interpret. There are contradictory reports regarding levels of $PGF_{2\alpha}$ and PGE_2 in PF of women with or without endometriosis. Some of these have failed to show any difference (Chacho *et al.*, 1986), whereas others have found higher concentrations throughout the menstrual cycle (Badawy *et al.*, 1985) or only in the secretory phase (De Leon *et al.*, 1986). Moon *et al.* (1981) documented the presence of $PGF_{2\alpha}$ in ectopic endometrium. Drake *et al.* (1981) measured stable metabolites of TXA_2 and prostacyclin in PF and observed an increase in patients with endometriosis compared with controls. They suggested that these mediators of smooth muscle contraction might cause infertility by altering tubal function. On the other hand, Badawy *et al.* (1982) and Dawood *et al.* (1984) could not demonstrate cycle-dependent changes in the PF concentrations of PG, nor were there differences between patients with unexplained infertility and those with endometriosis. The concentration of 6-keto-PGF_1 was elevated in patients with endometriosis, but no increase in total 6-keto-PGF_1 was observed (Dawood *et al.*, 1984). In animal studies, moderate and severe endometriosis are associated with increased PF concentrations of $PGF_{2\alpha}$, with no change in PGE_2 (Schenken *et al.*, 1984). Interestingly, $PGF_{2\alpha}$ has also been implicated in endometrial cell proliferation (Orlicky *et al.*, 1986).

PG may also affect embryo implantation (Hahn *et al.*, 1986). A possible mechanism is increased uterine contractility with resultant expulsion of the embryo. Increased PF prostaglandins have been related to ovulation interference as well as to increase in tubal motility, such that the embryo arrives in the uterine cavity at a suboptimum time for implantation.

In summary, data on the amounts of TXB_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ in the PF of women with endometriosis are conflicting, but suggest that they may be raised. The question of whether these compounds can act as markers or even act as causal agents of reduced fertility is unknown.

Cytokines and growth factors

As discussed previously, macrophages in the peritoneal cavity release cytokines and growth factors in response to a variety of inflammatory stimuli. Cytokine activities are varied and include the following: 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 chemotaxis. Cytokines generally exert biological effects on a variety of cell types, i.e. they are pleiotropic. Additionally, cytokines may either induce or down-regulate the production of other cytokines.

It is now becoming evident that cytokines play an important role in reproduction at various levels, including gamete function, fertilization and embryo development,

implantation and postimplantation survival of the conceptus. Furthermore, growth factors and inflammatory mediators produced by peritoneal leukocytes have recently been postulated to participate in the pathogenesis of endometriosis (Table I). IL-1, IL-2, IL-6, IL-8 and TNFα are all increased in the PF of women with endometriosis, suggesting that these cytokines may be involved in the pathogenesis and pathophysiology of disease (Eisermann *et al.*, 1988; Rier *et al.*, 1994; Arici *et al.* 1996).

Table I. Growth factors and cytokines in the peritoneal fluid of women with endometriosis compared to those without the disease

Mediator	Level[a]	Reference
EGF	ND	De Leon *et al.* (1986)
TGFβ	=	Oosterlynck *et al.* (1994b)
IGF	=	Giudice *et al.* (1994)
PDGF	=	Halme *et al.* (1988)
M-CSF	=	Fukaya *et al.* (1994)
IL-1	=	Fakih *et al.* (1987), Hill and Anderson (1989), Taketani *et al.* (1992)
	ND	Awadalla *et al.* (1987), Koyama *et al.* (1993), Keenan *et al.* (1995)
IL-2	ND	Keenan *et al.* (1995)
IL-5	ND	Koyama *et al.* (1993)
IL-6	=	Rier *et al.* (1995), Koyama *et al.* (1993)
	ND	Boutten *et al.* (1992), Keenan *et al.* (1994)
IL-8	=	Arici *et al.* (1996), Ryan *et al.* (1995)
TNFα	=	Eisermann *et al.* (1988), Halme (1989), Taketani *et al.* (1992)
	ND	Vercellini *et al.* (1993), Keenan *et al.* (1995)
IFNγ	ND	Khorram *et al.* (1993), Keenan *et al.* (1994)
MCP-1	=	Arici *et al.* (1995b), Akoum *et al.* (1996)
RANTES	=	Khorram *et al.* (1993)

[a]↑ = increased; ND = no difference.
EGF = epidermal growth factor; TGFβ = transforming growth factor β; IGF = insulin-like growth factor; PDGF = platelet-derived growth factor; M-CSF = macrophage–colony stimulating factor; IL = interleukin; TNF = tumour necrosis factor; IFN = interferon; MCP-1 = monocyte chemoattractant protein-1; RANTES = regulated on activation normal T expressed and secreted.

Cytokines Interleukin-1: The IL-1 system is composed of IL-1α (159 amino acids), IL-1β (153 amino acids) and an inhibitor, IL-1 receptor antagonist (152 amino acids). Although IL-1α and IL-1β 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 (Dower et al., 1986). Although some investigators could not observe a difference in IL-1 activity in the PF or in the in-vitro rate of production of IL-1 between individuals with and without endometriosis (Awadalla et al., 1987), others have shown that it is raised in endometriosis (Fakih et al., 1987). IL-1 induces prostaglandin synthesis, T-cell proliferation, and stimulation of B-lymphocyte immunoglobulin production. The increased level of this cytokine in the PF of patients with endometriosis may have a stimulatory effect on fibroblast proliferation and collagen deposition, suggesting a role in the pathogenesis of adhesions and peritoneal fibrosis in endometriosis. There are conflicting observations as to the effect of IL-1 on early reproductive events in vitro (Fakih et al., 1987; Hill et al., 1987a,b). IL-1 inhibits mouse embryo development in vitro (Fakih et al., 1987; Hill et al., 1987b), but only at very high concentrations (>10^6 U/ml). IL-1 also impairs the oocyte penetrating capacity of the spermatozoon, both in the hamster and the human (Sueldo et al., 1990). On the other hand, Hill et al. (1987a) reported no significant effect of IL-1 on sperm motion parameters. It has been demonstrated that IL-1 and TNFα concentrations were markedly low in the PF from women who had 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 (Taketani et al., 1992).

Interleukin-2: IL-1 stimulates IL-2 secretion by T cells. IL-2 was found to abrogate the deficient cytolytic and lymphokine-activated killer cell activity in peripheral blood monocytes of endometriosis patients (Oosterlynck et al., 1994a). However, IL-2 was not detectable in either PF or macrophage-conditioned media of women with endometriosis (Keenan et al., 1995). This finding suggests that there is no obvious role of IL-2 in either the pathogenesis or pathophysiology of endometriosis.

Interleukin-6: IL-6 is a potent cytokine that has diverse effects, several of which are potentially related to tissue repair, including stimulation of acute-phase reactant synthesis and angiogenesis (Le and Vilcek, 1989). IL-6 is secreted by monocytes/macrophages and has been noted to be raised in women with endometriosis (Buyalos et al., 1991). Rier et al. (1995) have shown that the severity of endometriosis is correlated with the increased concentration of IL-6 accompanied by a decrease of IL-6 soluble receptor concentration in the PF.

Interleukin-8: This cytokine is a chemoattractant for neutrophils and a potent angiogenic factor. It is produced by a number of cell types, including monocytes, endothelial cells, fibroblasts, mesothelial cells and endometrial stro-

mal cells (Arici et al., 1993). PF of women with endometriosis has been shown to have increased neutrophil chemotactic activity (Leiva et al., 1993). Recently, it has been identified that IL-8 concentrations are higher in the PF of women with endometriosis than of controls. Furthermore, the IL-8 concentrations seem to be related to the stage of the disease (Ryan et al., 1995; Arici et al., 1996).

Tumour necrosis factor-α: The concentration of TNFα, another cytokine with a wide range of biological effects, is known to be increased in PF of women with endometriosis (Eisermann et al., 1988). Human peritoneal macrophages have been noted to secrete TNFα in vitro, with those collected from women with endometriosis having the greatest production rate (Halme, 1989). TNFα also enhances the adhesion of endometrial stromal cells to mesothelial cells when included in the culture medium (Zhang et al., 1993). TNFα has been linked to a variety of additional reproductive effects. It significantly affects sperm motility in vitro, but only at very high concentrations (Hill et al., 1987a). In addition, Hill et al. (1987b) tested various concentrations of cytokines and found significant embryotoxicity only by TNFα and IFNγ.

Interferon-γ: IFNγ 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 (Wang et al., 1981). It also adversely affects sperm motility, fertilization, early embryonic development and trophoblast proliferation in vitro (Hill et al., 1987a,b; Berkowitz et al., 1988). A local antiproliferative effect of IFNγ is supported by the finding that IFNγ inhibits in-vitro proliferation of endometrial epithelial cells (Tabibzadeh et al., 1988). This cytokine also significantly inhibits blastocyst implantation in vitro (Haimovici et al., 1991). This inhibition is observed at concentrations as low as 10^3 U/ml. Hill et al. have demonstrated that TNFα and IFN-γ adversely affect many reproductive processes including sperm motility (Hill et al., 1987a), fertilization (Hill et al., 1989) and embryo development (Hill et al., 1987b). IFNγ concentrations do not differ in the PF of women with or without endometriosis (Khorram et al., 1993; Keenan et al., 1994).

Monocyte chemotactic protein-1: This cytokine is a potent chemotactic and activating factor specific for monocytes. PF from patients with endometriosis has increased chemotactic activity for macrophages (Leiva et al., 1993). We have previously shown that human endometrial tissue expresses MCP-1 (Arici et al., 1995a). Recently, it has been shown that MCP-1 secretion is up-regulated in cytokine-stimulated endometrial cells of women having endometriosis as compared with normal women (Akoum et al., 1995). We find that MCP-1 concentrations are raised in PF from women with endometriosis. In addition, amounts of MCP-1 were significantly higher in the PF from women who had untreated endometriosis than in that from women who had undergone medical treatment with gonadotrophin-releasing hormone analogue (A.Arici et al., unpublished results).

RANTES: RANTES is a newly discovered T-cell-specific cytokine of the platelet factor 4 gene superfamily (Schall, 1991). RANTES is a selective chemoattractant for monocytes and T lymphocytes (Schall et al., 1990). Recently, PF concentrations of RANTES have been found to be increased in women with endometriosis and concentrations are related to the severity of the disease (Khorram et al., 1993).

The role of cytokines and growth factors in the pathophysiology of endometriosis-associated infertility is also actively under investigation. Peritoneal macrophages, through IL-1 secretion, may modulate human granulosa–luteal cell progesterone production (Halme *et al.*, 1985). Hill *et al.* (1987b) tested various concentrations of IL-1, IL-2, IFNγ and TNFα and found significant embryotoxicity only by the latter two cytokines. Similarly, Schneider *et al.* (1989) did not find that IL-1 and IL-2 exhibited embryotoxic effects. Thus, IFNγ and TNFα remain the two cytokines found in the PF of patients with endometriosis consistently exhibiting gamete toxicity. Cytokine concentrations in PF from women with endometriosis have also been reported to be reduced after medical treatment (Taketani *et al.*, 1992). A reduction in embryotoxicity was associated with this drop in cytokine concentrations as determined by using mouse 2-cell embryos.

Growth factors Platelet-derived growth factor: PDGF is a well-characterized secretory product of activated macrophages, playing a major role in the inflammatory response as a potent mitogen for fibroblast and angiogenic precursor cells. PDGF was previously identified in the PF of women with endometriosis (Halme et al., 1988). This was originally thought to be unique to the macrophage and was termed 'macrophage-derived growth factor'. Subsequently, some of this molecular activity was found to be identical to PDGF (Surrey and Halme, 1991). PDGF has a significant dose-dependent proliferative effect on endometrial stromal and epithelial cells (Surrey and Halme, 1991).

Epidermal growth factor: EGF acts as a mitogen for most epithelial cells. Its concentration in the PF is positively correlated with the day of the cycle. Highest concentrations are seen in the luteal phase (De Leon et al., 1986). Ectopic endometrium is known to express oestrogen receptors (Prentice et al., 1992a), and its growth and maintenance are dependent on continued stimulation by oestrogen. In recent

years it has become apparent that oestrogen action in the endometrium may be mediated by the peptide growth factors, in particular EGF (Mellor and Thomas, 1994). EGF exerts its effects through binding to its cell surface receptor, the EGF receptor. EGF receptors have been described in normal human endometrium by several investigators (Prentice et al., 1992b). It has been shown using immunohistochemistry that EGF receptor is expressed in the glands and stroma of eutopic and ectopic endometrium of women with endometriosis (Prentice et al., 1992b).

Macrophage-colony stimulating factor (M-CSF): This growth factor has been identified in the PF of women at consistently higher concentrations than in plasma (Weinberg et al., 1991). Furthermore, the concentration of M-CSF was significantly higher in the patients with endometriosis than in those without endometriosis (Fukaya et al., 1994). It is involved in the differentiation of monocytes to become phenotypically activated macrophages and can serve as a chemotactic factor for blood monocytes.

Transforming growth factor-β (TGFβ): Beside its growth-regulating properties, TGFβ is one of the most potent chemoattractants for human monocytes and is an inducer of fibrosis and angiogenesis, indicating that it is an important mediator of tissue repair. Furthermore, TGFβ has striking immunological functions and can profoundly inhibit T lymphocyte, B lymphocyte and NK cell functions (Rook et al., 1986). Increased TGFβ activity in the PF of women with endometriosis has been demonstrated by Oosterlynck et al. (1994b). They suggested that the decreased NK activity of PF in women with endometriosis may be secondary to increased TGFβ activity in PF.

Insulin-like growth factors: IGF-I and IGF-II are mitogens that can also promote differentiation. The IGF circulate bound to IGF-binding proteins (IGFBP), which also regulate their actions at target tissues. Giudice et al. (1994) have shown that human PF contains IGF-I, IGF-II, IGFBP-1, -2, -3, and -4; and the IGFBP-3 protease. 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 has the capacity to stimulate endometrial cellular proliferation.

Basic fibroblast growth factor: bFGF, a heparin-binding angiogenic protein, is highly mitogenic for capillary endothelial cells in vitro and can induce angiogenesis in vivo (Folkman and Klagsbrun, 1987). Secretion of bFGF by endometrial cells increases in response to 17β-oestradiol and is inhibited by progesterone (Presta, 1988). bFGF has been found in endometrial glandular epithelium and is a potent mitogen for endometrial stromal cells in culture (Irwin et al., 1991). bFGF may be one of several growth factors acting in a paracrine fashion that is contributing to stromal proliferation. This growth factor has not been reported so far in the peritoneal environment of endometriosis patients.

An uncharacterized macrophage-derived growth factor has been described in an animal model which functions as a competence factor, allowing endometrial stromal cells to respond to mitogenic stimuli such as oestrogen (Olive *et al.*, 1991).

Angiogenic factors Angiogenic factors released from peritoneal macrophages may also play a role in the development of endometriosis. By now, several angiogenic agents have been purified, including bFGF, angiogenin, TGFα and TGFβ, TNFα, EGF and IL-8 (Folkman and Klagsbrun, 1987). PF of women with endometriosis displays greater angiogenic activity than PF obtained from women without the disease (Oosterlynck *et al.*, 1993). Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that is present in increased amounts in the PF of women with endometriosis (McLaren *et al.*, 1996). Also, the concentration of VEGF in the proliferative phase is significantly higher than in the secretory phase. These angiogenic factors in the PF of women with endometriosis could be produced by retrograde menstruation of endometrial cells or by the ectopic endometriotic lesions themselves. The growth of endometriosis requires an accessible blood supply. It could be postulated that the release of angiogenic factors into the peritoneal compartment produces an increased microvascularization of the parietal peritoneum.

CA-125

Endometrial cells express the antigen CA-125, an epitope that has been demonstrated to be associated with coelomic epithelium and its neoplastic derivatives. Since Barbieri *et al.* (1986) demonstrated raised serum concentrations of CA-125 in patients with advanced endometriosis, this marker has been suggested as a means of diagnosing or following the disease (Pittaway and Fayez, 1986). Concentrations of CA-125 in PF are higher than in serum, but originally no significant difference was observed in the PF concentrations of CA-125 of women with and without endometriosis (Williams *et al.*, 1988; Moen *et al.*, 1991). More recently, Barbati *et al.* (1994), using a two-step immunoradiometric assay, found concentrations of CA-125 in PF to be a more sensitive indicator of disease than those in serum. CA-125 is not likely to play a direct role in the pathophysiology of endometriosis, but serum concentrations may provide a useful indicator of the extent of disease or response to therapy.

Table II. Effect of the peritoneal fluid on sperm parameters

Reference	No. of patients		Model	Effect of endometriosis
	Control	Endometriosis		
Halme & Hall (1982)	84	65 (Mi)	fertilization	no effect
		85 (Mo)		
Muscato *et al.* (1982)	22	10	phagocytosis by macrophages	increased
Oak *et al.* (1985)	20	20 (MM)	motility	decreased
Stone & Himsl (1986)	77	29 (Mi)	survival	no effect
Chacho *et al.* (1986)	34	25 (Mn)	fertilization	decreased
		8 (Mi), 2 (Mo)		
Burke (1987)			velocity	decreased
Awadalla *et al.* (1987)	20	20	phagocytosis by macrophages	no effect
Sueldo *et al.* (1987)	21	16 (MM)	fertilization	decreased
Leach *et al.* (1990)	10	26 (MM)	motility	no effect
Coddington *et al.* (1992)	3	7 (Mn)	fertilization	decreased
		6 (Mi), 3 (Mo)		
Curtis & Jackson (1993)	36	19 (MM)	motility	decreased
Bielfeld *et al.* (1993)	4	4 (Mi)	capacitation	no effect
		4 (MS)		
Drudy *et al.* (1994)	9	9 (Mn)	motility	decreased
		9 (Mn, treated)		
Arumugam *et al.* (1994)	25	11 (Mi)	acrosome	decreased
		14 (MS)	reaction	

Mn = minimal stage; Mi = mild stage; MM = minimal–mild stage; Mo = moderate stage; MS = moderate–severe stage.

Effects of peritoneal fluid on reproductive events

On sperm survival

It is clear that macrophages are primarily responsible for physiological phagocytosis of cellular debris, including spermatozoa, in the pelvis (Olive *et al.*, 1987a). To date, only one study has shown increased sperm phagocytosis in PF of patients with infertility and endometriosis. In a landmark study, Muscato *et al.* (1982) demonstrated that peritoneal macrophages phagocytosed spermatozoa *in vitro*, and that macrophages from women with endometriosis were more active than those from women without disease. In patients with antisperm antibodies, increased numbers of macrophages could promote sperm phagocytosis (London *et al.*, 1985). Olive *et al.* (1985) suggested that this increase reflects the presence of infertility without a mechanical cause.

The issue of sperm toxicity in patients with endometriosis has been addressed by many investigators. Sperm motility (Curtis and Jackson, 1993; Drudy *et al.*, 1994), capacitation (Bielfeld *et al.*, 1993), acrosome reaction (Arumugam, 1994) and gamete interaction (Coddington *et al.*, 1992) have been studied. A summary of the findings concerning sperm parameters is presented in Table II. Stone and Himsl (1986) inseminated patients just prior to laparoscopy and then collected cul-de-sac fluid. No difference in motile sperm count could be found between those with and without mild endometriosis. However, Burke (1987) reported reduced sperm velocity *in vitro* when PF from patients with endometriosis was added to the medium. Oak *et al.* (1985) incubated normal spermatozoa in PF and found a significantly reduced proportion of motile spermatozoa in the presence of endometriosis when compared to controls. Using a computerized semen analysis system, Leach *et al.* (1990) did not find an effect of PF from patients with endometriosis on sperm velocity after up to 6 h of incubation.

On spermatozoon–oocyte interactions (fertilization)

Studies to evaluate the effect of PF on sperm penetration of oocytes revealed conflicting results. PF, in general, reduces fertilization of murine oocytes *in vitro* when added to the medium, but this effect is enhanced when the PF is from women with endometriosis (Sueldo *et al.*, 1987). When spermatozoa are exposed to macrophage-conditioned medium, a decrease in hamster egg penetration assay

scores is noted (Chacho *et al.*, 1986). Halme and Hall (1982) were unable to demonstrate any effect of PF from patients with endometriosis on penetration of zona-free hamster ova.

Table III. Effect of peritoneal fluid on mouse embryo development *in vitro*

Reference	No. of patients		Effect of
	Control	*Endometriosis*	*endometriosis*
Marcos *et al.* (1985)	10	18	embryotoxic
Awadalla *et al.* (1987)	20	20	no effect
Steinleitner *et al.* (1990)	12	12 (MM)	embryotoxic
Prough *et al.* (1990)	7	10 (MM)	embryotoxic
Taketani *et al.* (1992)	21	19 (untreated)	embryotoxic
		10 (treated)	no effect
Dodds *et al.* (1992)	10	10	no effect
Tzeng *et al.* (1994)	6	12 (MS)	embryotoxic
		6 (MM)	no effect

MM = minimal–mild stage; MS = moderate–severe stage.

On early embryonic development

The availability of murine embryos has made it possible to test the effect of PF or macrophage-conditioned media on the development of mouse embryos *in vitro*. Steinleitner *et al.* (1990a) found that PF from women with endometriosis injected i.p. into hamsters caused a decrease in oocyte recovery and embryo development. The causative agent proved to be heat labile. Similarly, Prough *et al.* (1990) found that a heat-labile substance in the PF of women with endometriosis, obtained during follicular phase laparoscopy, inhibited growth of the 2-cell mouse embryo. In an elegant study by Steinleitner *et al.* (1990b), activated, deactivated and non-activated macrophages were injected into the peritoneal cavity of ovarian-stimulated mice about to mate. The number of oocytes and embryos recovered was depressed by activated macrophages, but this effect was not apparent with deactivated macrophages or macrophages in the basal state. It has been reported that PF from women with endometriosis induces decreased fertilization in the mouse model (Sueldo *et al.*, 1987) and also causes a decrease in mouse embryo cleavage rate (Marcos *et al.*, 1985). Recently, in two studies, medical treatment of endometriosis eliminated the embryotoxicity of PF. The activity of embryotoxic factor(s) was directly related to the clinical stage of endometriosis (Taketani *et al.*, 1992; Tzeng *et al.*, 1994). Other reports, however, have failed to demonstrate an adverse effect of PF from patients with endometriosis on fertilization and embryo development in mice (Awadalla *et al.*, 1987; Dodds *et al.*, 1992).

The reason for these contradictory findings is unclear, and doubts have been raised about the lack of sensitivity of the murine embryo model for predicting toxicity toward human embryos. In summary, PF from patients with endometriosis has been demonstrated frequently to be toxic to embryos *in vitro* (Marcos *et al.*, 1985) and *in vivo* (Steinleitner *et al.*, 1990a) (Table III), but the underlying mechanism and clinical significance remain unknown.

Other studies

An unknown PF factor has been found to interfere with ovum capture (Suginami *et al.*, 1986). Oocyte capture was significantly diminished by incubating the fimbria with native or cell-free PF of women with endometriosis. The source of this activity remains obscure, but the substance that diminishes oocyte capture is water soluble with a molecular size of >100 kDa. Danazol appears to decrease the amount of this substance in the PF of women with endometriosis.

PF oestrogen and progesterone concentrations are not different in endometriosis when compared with controls in the proliferative or secretory phase (De Leon *et al.*, 1986). PF total protein content is unchanged by the presence of endometriosis (Halme *et al.*, 1984a). The activity of PF lysozyme, a ubiquitous product of macrophages, is not different in endometriosis when compared with controls (Olive *et al.*, 1987b). Halme and Mathur (1987) documented anti-endometrial antibodies in the PF of 22 patients and found 0.96 specificity, 0.23 sensitivity. Complement components C_{3c} and C_4, mediators of host response to inflammation, are also increased in the PF of patients with endometriosis (Badawy *et al.*, 1984b). Integrins, important cell adhesion molecules, are expressed in endometriotic lesions and in epithelial cells in PF (Van der Linden *et al.*, 1994). These cell adhesion molecules could be involved in the attachment of endometrial tissue fragments to the peritoneum after the shedding of endometrial tissue during menstruation. PF of patients with minimal to moderate endometriosis demonstrates a significantly higher chemotactic activity for granulocytes than that of patients without endometriosis or with medical suppression (Leiva *et al.*, 1993). The amounts and activity of numerous other compounds have been measured in PF, but concentrations of these compounds do not appear to be raised in patients with endometriosis. They include fibronectin, plasminogen activator activity, substance P, cholesterol, inhibin and albumin (Ramey and Archer, 1993).

Conclusion

Endometriosis stands as one of the most investigated disorders of gynaecology, with >4500 articles published on the subject in the past 25 years. Yet, despite this intense

academic interest, there remain basic holes in our understanding of this enigmatic disease. The importance of understanding the peritoneal microenvironment in order to decipher the pathogenesis of endometriosis has long been recognized. However, it is still unknown whether the increased activity of peritoneal inflammatory cells and their mediators is due to the presence of endometriotic implants or whether the endometriosis is resulting from an occult systemic immune disease. There are two hypotheses: the ectopic endometrium incites an intraperitoneal inflammatory response, resulting in recruitment and differentiation of peritoneal macrophages; the alternative is the converse, that a primary disorder of macrophage number or function may contribute directly to ectopic endometrial growth.

Intense research continues to improve our understanding of the PF environment. This will enable us to offer better and more effective modes of treatment. Findings summarized above are compelling evidence suggesting that the PF in women with endometriosis is pro-inflammatory. While this fact is now widely accepted, investigators have remained divided on whether these changes precede the disease or follow endometriosis as a consequence. We have recently identified the presence of two pro-inflammatory chemoattractant cytokines for monocyte/macrophages (MCP-1) and for granulocytes (IL-8) in the PF. Concentrations of both IL-8 and MCP-1 are not only raised in PF of women with endometriosis compared to those without endometriosis, but are correlated with the severity of the disease. In the peritoneal cavity, several tissues can account for the increased concentrations of IL-8 and MCP-1. Aside from mesothelial cells, which form the majority of the cells in the peritoneal cavity, macrophages and endometrial cells themselves are potential sources of these chemoattractant cytokines. On the basis of these findings it is tempting to postulate that IL-8 and MCP-1 are mediators in the pathogenesis of endometriosis.

What role PF plays in the resulting infertility in patients with endometriosis remains to be clarified. Some existing data suggest that changes in the peritoneal environment may play an important part in the interruption of the process of normal folliculogenesis, ovulation, conception and implantation. Conflicting reports concerning adverse effects of PF on reproductive processes also exist; these differences may be due to variations in study design. Small sample size and inadequate controls have also contributed to this confusion.

The studies on PF characteristics, such as PF volume, macrophages and prostaglandins also have limitations. Cycle timing, control group selection and collection techniques have varied from one study to another, making it difficult to interpret the data. Cycle day at the time of recovery is rarely controlled, and such variation may have a profound effect. Selection of control groups has also been a major source of criticism.

In summary, the PF is a dynamic environment that links the reproductive and immune systems. It appears likely that endometriotic tissue is influenced by this environment, and in turn the PF is altered by the presence of endometriosis. The result is a cellular soup rich in stimuli ready to assist in the growth and maintenance of endometrial implants as well as inhibition of fertility. Macrophages appear to play a leading role in this drama, with contributions from other cell types such as peritoneal mesothelium, lymphocytes and even endometrium itself. Accumulated soluble substances such as prostaglandins, cytokines and growth factors probably act as mediators to induce both endometriotic support and interference with fertility/early embryonic development. To date, studies have been limited by inadequate methodology and a failure to test hypotheses *a priori*. Further basic research into the specific roles of these cells and soluble factors may offer the possibility of more effective prevention and treatment of endometriosis.

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