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Review

Peritoneal environment, cytokines and angiogenesis in the pathophysiology of endometriosis

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Endometriosis, defined by the presence of viable endometrial tissue outside the uterine cavity, is a common condition affecting 2–3% of women of reproductive age. 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. There is substantial evidence that immunological factors and angiogenesis play a decisive role in the pathogenesis of endometriosis. In women with endometriosis, there appears to be an alteration in the function of peritoneal macrophages, natural killer cells and lymphocytes. Furthermore, growth factors and inflammatory mediators in the peritoneal fluid, produced mainly by peritoneal macrophages, are altered in endometriosis, indicating a role for these immune cells and mediators in the pathogenesis of this disease.

Endometriosis, pathologically defined by the presence of viable endometrial tissue outside the uterine cavity, is one of the most enigmatic and problematic maladies affecting women of reproductive age. It is associated with pain and infertility and although it is not a malignant disorder, endometriosis exhibits cellular proliferation, cellular invasion and neoangiogenesis. Endometriosis is most commonly implanted over visceral and peritoneal surfaces within the female pelvis. Despite being one of the most frequently encountered gynaecological diseases, the pathophysiology of this disease remains controversial.

The local environment that surrounds the endometriotic implant in the peritoneal cavity is a dynamic one. Histologically, the peritoneum consists of a thin layer of loose connective tissue covered by a layer of mesothelium and is the most extensive serous membrane in the body, with a rich supply of subperitoneal blood vessels and lymphatics.

Retrograde menstruation, peritoneal adhesion of endometrial tissue and outgrowth of these endometrial cells, glands and stroma are essential elements in the pathogenesis of endometriosis, according to Sampson's classical implantation theory. Histologically, the reflux implantation theory of Sampson (1940) was supported by the distribution of the lesions in the abdominal cavity (Jenkins *et al.,* 1986), the demonstration of the viability of shed menstrual endometrium in tissue culture (Keettel and Stein, 1951), the high prevalence of pelvic endometriosis in girls with congenital menstrual outflow obstruction (Sanfilippo *et al.,* 1986) and animal experiments in which endometriosis was induced by the creation of utero–pelvic fistulas (TeLinde and Scott, 1950).

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Retrograde menstruation is a universal phenomenon with viable endometrial cells found in peritoneal fluid of 76–90% of women, a prevalence much higher than that of endometriosis (Bartosik *et al.,* 1986). This finding indicates that other factors, such as the amount of retrograde flow or immunological changes, may determine the susceptibility of a woman to endometriosis.

Nevertheless, the stimuli necessary to provide attachment and outgrowth of endometrial cells after arrival in the peritoneal cavity are unknown. This article will 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 peritoneal cavity is normally empty except for a thin film of fluid that keeps surfaces moist. The peritoneal fluid arises primarily from two sources: plasma transudate and ovarian exudate. Other sources of peritoneal fluid are tubal fluid, retrograde menstruation and macrophage secretions (Oral *et al.,* 1996).

Studies in women with endometriosis have demonstrated that, in addition to changes in its volume, there are functional changes in several immunological components of the peritoneal fluid, for example phagocytic macrophages–monocytes, natural killer (NK) cells, cytotoxic T lymphocytes, B cells, or inflammatory mediators such as complements and cytokines (Ho *et al.,* 1997a). However, it must be emphasized that the presence of immune cells, cytokines and growth factors does not prove their role in the development or maintenance of endometriosis.

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Peritoneal fluid volume

The volume of peritoneal fluid is usually 5–20 ml, and varies widely depending on physiological condition (Oral *et al.,* 1996). For example, peritoneal fluid volume is influenced by the stage of the menstrual cycle, increasing from an early proliferative mean value of 0.8 ml to a mean value of 18.7 ml after ovulation and decreasing again to a mean value of 5.4 ml in the late secretory phase (Bouckaert *et al.,* 1986). This variation may be an indication of an oestrogenic effect on the permeability of vascular and peritoneal membranes (Maathuis *et al.,* 1978).

Conflicting results have been reported on the influence of endometriosis on peritoneal fluid volume. Endometriosis may cause an increase in the fluid production by altering mesothelial permeability or increasing colloid osmotic pressure as a result of altered protein content (Oral *et al.,* 1996). Overall, findings indicate that the volume of peritoneal fluid in women with endometriosis may be modestly increased, but this appears to be of little clinical importance.

Cellular constituents of peritoneal fluid

Peritoneal fluid contains a variety of free-floating cells, including macrophages, NK cells, lymphocytes, eosinophils, mesothelial cells and mast cells.

Macrophages

Macrophages are most abundant type of cell in the peritoneal fluid, and may have a role in the pathogenesis of endometriosis (Ho *et al.,* 1997a). Normally, peritoneal fluid contains $0.5–2.0 \times 10^6$ leukocytes ml^{-1}, of which 85% are macrophages (Van Furth *et al.,* 1979). The concentration of macrophages appears to fluctuate during the menstrual cycle, and is highest during menses (Oral *et al.,* 1996).

As well as increasing in number, peritoneal macrophages are more activated in endometriosis (Oral *et al.*, 1996). Once activated, macrophages may release products such as cytokines, prostaglandins (PGs), complement components and hydrolytic enzymes, regulating events in the peritoneal cavity. Macrophages can remove red blood cells, damaged tissue fragments and, probably, endometrial cells that gain access to the peritoneal cavity. Endometriosis may develop when the 'disposal system' is overwhelmed by high amounts of retrograde menstruation or when a defective peritoneal 'disposal system' permits implantation and growth of the endometrial cells or fragments (Dmowski, 1995).

The higher number and activation of macrophages in the peritoneal cavity is also likely to be accompanied by an increase in macrophage-derived cytokines. Some of these cytokines may stimulate the proliferation and differentiation of T cells and, subsequently, T-cell-derived factors may play a critical role in the activation of B cells (Dmowski, 1995). Moreover, it is possible that these cytokines affect other cells present in the peritoneal cavity, such as ectopic endometrial cells.

Available data indicate that products from peritoneal fluid macrophages play an active role in the initiation, maintenance and progression of endometriosis (Senturk and Arici, 1999). 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 interleukins, tumour necrosis factor α (TNF-α), macrophage-derived growth factor (MDGF) and monocyte chemotactic protein 1 (MCP-1) (Oral *et al.,* 1996), as well as vascular endothelial growth factor (VEGF) (McLaren, 2000).

Natural killer cells

A defect in NK cell activity in women with endometriosis resulting in a decreased cytotoxicity to autologous endometrium was reported by Oosterlynck *et al.* (1991). NK cell activity is decreased in the peripheral blood as well as in the peritoneal fluid of women with endometriosis, and this decrease was significantly related to an increase in disease stage (Oosterlynck *et al.,* 1992). Ho *et al.* (1995) reported that in the peritoneal fluid of women with stage III–IV endometriosis, NK cytotoxicity was significantly lower than it was in women without endometriosis.

These findings have given rise to the NK cell theory of endometriosis, which presupposes that endometriotic cells are natural NK target cells, and that impaired clearance of ectopic endometrium by NK cells in the peritoneal cavity contributes to the development of the disease. However, it can still be argued that alterations in NK cell activity associated with endometriosis can be an effect of the disease. Aberrant NK cell activity may be the result of an imbalance in the immune response to a chronic antigenic stimulus, such as ectopic endometrium, or to a previously initiated autoimmune event unrelated to endometriosis (Hill, 1992). Kikuchi *et al.* (1993) suggested that changes in the differentiation of NK cells were the consequence of the presence of endometriotic implants.

Studies in which human endometrial tissue has been xenotransplanted into immunodeficient mice demonstrated the importance of NK cells *in vivo* for the growth of endometrial tissue on ectopic sites. Indeed, *nude* mice, which have a congenital defect of T and B cells, temporarily accept human endometrial grafts. The same *nude* mice, when treated with NK anticellular serum before xenotransplantation, permanently accept endometrial grafts. Mice deficient in T, B and NK cells do not reject endometrial grafts (Aoki *et al.,* 1994), indicating an important role for NK cells in the pathogenesis of endometriosis.

Lymphocytes

Approximately 30–50% of peritoneal fluid cells are lymphocytes (Oosterlynck *et al.,* 1992) and their total number is higher in women with endometriosis (Badawy *et al.,* 1984).

Because the T-cell-mediated immune system is involved in the rejection of homologous transplants (Bach and Sachs, 1987), some unique alteration in T-cell function with regard

to rejection has long been considered in women with endometriosis. Evidence to date indicates that changes in cell-mediated immunity occur in women with endometriosis (Ho *et al.,* 1997a). An increased T helper (T_h) to T suppressor (T_c) cluster determinant-4 (CD4):CD8 ratio has been noted in peritoneal fluid samples of women with endometriosis, indicating an increased cellular immune activity in the peritoneal environment of these women (Hill *et al.,* 1988). However, Ho *et al.* (1995) reported that there was no specific change of CD4:CD8 in peripheral blood or in peritoneal fluid in women with endometriosis. Significantly higher numbers of T cells and NK cells, but fewer B lymphocytes and no plasma cells have been observed in the peritoneal fluid of women with endometriosis compared with those in women without the disease (Dmowski *et al.,* 1994).

It is puzzling that T cells have specific cytotoxicity toward autologous endometrial cells to which they are exposed regularly during menstruation. When ectopic endometrial cells implant in the peritoneal cavity, they are processed by activated macrophages and presented to T cells. In women with endometriosis, altered macrophages stimulate implantation and proliferation of misplaced, and possibly altered, endometrial cells (Dmowski *et al.,* 1994). Under the influence of macrophage-released cytokines, the different T-cell subsets (Th1 and Th2) can proliferate and differentiate into functionally activated cells. After T-cell activation, two different groups of cytokines, Th1 and Th2 cytokines, are secreted from corresponding cells. Th1 cytokines, including interleukin 2 (IL-2), IL-12, interferon γ, as well as TNF-α and TNF-β, generally result in cellular immunity, whereas Th2 cytokines, IL-4, IL-5, IL-6, IL-10 and IL-13, activate B cells, resulting in their differentiation and proliferation into antibody-secreting plasma cells. Preliminary studies using RT–PCR demonstrated that the peritoneal T cells of women with endometriosis express predominant Th1 cytokine, IL-2 and interferon γ mRNA (Ho *et al.,* 1997b).

Peritoneal fluid soluble constituents

Steroid hormones

The influence of hormones on the development of endometriosis was postulated by Novak (1931). The probability of hormonal modulation of endometriosis is supported by the presence of oestrogen and progesterone receptors in endometriotic lesions (Bergqvist *et al.,* 1993). A distinction should be made between the influence of hormones in initiating and in maintaining endometriosis. The initiation of growth of endometriosis in monkeys has been shown to be independent of oestrogens; however, either oestradiol or progesterone, alone or in combination, is required for maintenance of the long-term viability of endometrial implants (DiZerega *et al.,* 1980). These studies of the influence of oestrogens and progesterone were performed in an animal model with surgically implanted endometrium.

In spontaneous endometriosis, cyclic ovarian hormone secretion seems necessary for the growth or proliferation of ectopic endometrial tissue. However, the exact mechanisms underlying the differentiation in the proliferation of endometriotic deposits are not clear. It is conceivable that the mitogenic effect of oestrogen is modulated by locally produced paracrine and autocrine factors (Tabibzadeh *et al.,* 1988). However, there appears to be no significant difference in the concentrations of oestradiol or progesterone in peritoneal fluid between women with endometriosis and controls free of the disease (Mahmood and Templeton, 1991).

Prostaglandins

Prostaglandins are biosynthesized from polyunsaturated fatty acids (PUFAs), predominantly arachidonic acid. Sources of prostaglandins in peritoneal fluid are the peritoneal macrophages, the peritoneal surface, ovarian follicles and endometriotic implants (Ylikorkala and Viinikka, 1983). In addition, passive diffusion occurs from other organs in the peritoneal cavity.

Prostaglandins may be involved in the pathogenesis of endometriosis (Bulun *et al.,* 2000). Peritoneal macrophages from women with endometriosis release significantly more PGE_2 and $PGF_{2\alpha}$ compared with macrophages from women without endometriosis (Karck *et al.,* 1996). Prostaglandins, which play a prominent role in the physiology of endometrium, are also involved in the regulation of the production and function of cytokines (Graham *et al.,* 1994). PGE_2 is thought to be a potent inducer of aromatase activity in endometriotic stromal cells (Noble *et al.,* 1997; Bulun *et al.,* 2000). Aromatase activity gives rise to local biosynthesis of oestrogen, which in turn stimulates PGE_2 production, thus establishing a positive feedback cycle. The tissue localization of the prostaglandin receptor family in the context of endometriotic tissue remains unclear, but there is evidence that prostaglandin concentrations are increased in the peritoneal fluid of women with endometriosis, indicating their possible importance in the aetiology of the disease.

Cytokines and growth factors

As discussed above, 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; induction of release of hormones, enzymes and acute phase proteins; enhancement of various cytotoxic activities; regulation of immunoglobulin secretion; and chemotaxis. In general, cytokines exert biological effects on a variety of cell types (that is, they are pleiotropic) but, in addition, they may either induce or downregulate the production of other cytokines.

Interleukin 1. High concentrations of IL-1 have been found in the peritoneal fluid of women with endometriosis (Ho *et al.,* 1997a). In culture, macrophages from the peritoneal fluid of endometriosis patients produce more IL-1

than do those of controls (Mori *et al.,* 1991). There are several physiological and pathological effects attributed to IL-1 that may relate to endometriosis. IL-1 induces the synthesis of prostaglandins, and stimulates fibroblast proliferation, collagen deposition and fibrinogen formation, which could contribute to the fibrosis and adhesion formation associated with endometriosis. In addition, IL-1 stimulates B-cell proliferation and antibody production, which could be related to the autoantibodies associated with the disease (Senturk and Arici, 1999). IL-1 also stimulates IL-2 secretion by T cells and NK cells, which in turn can induce NK proliferation and T-cell growth (Dilloo *et al.,* 1994).

Interleukin 6. IL-6 is a potent cytokine that has diverse effects, several of which are potentially related to tissue repair, including stimulation of angiogenesis (Le and Vilcek, 1989). IL-6 may be secreted by macrophages in response to a variety of substances found in the peritoneal fluid, including IL-1 (Sironi *et al.,* 1989). IL-6 is an activator of macrophages (Akira *et al.,* 1993) and promotes cellular proliferation of endometrium (Giudice, 1994). Endometriotic stromal cells express IL-6 mRNA and produce IL-6 protein (Tsudo *et al.,* 2000). In addition, increased concentrations of IL-6 have been noted in the ectopic endometrial tissue culture (Keenan *et al.,* 1994), peripheral blood (Koumantakis *et al.,* 1994) and peritoneal fluid of women with endometriosis (Punnonen *et al.,* 1996).

In contrast, the study of Buyalos *et al.* (1992) failed to show a difference in peritoneal fluid IL-6 concentrations between normal women and women with endometriosis. Rapkin *et al.* (2000) also found no significant differences in the peritoneal fluid IL-6 concentrations between women with and without endometriosis, whereas Mahnke *et al.* (2000) reported significantly higher concentrations of IL-6 in the peritoneal fluid of women with more extensive disease. Taken together, the available evidence indicates that IL-6 may be involved in the pathogenesis of endometriosis. However, further research is needed to clarify its exact role in the disease process.

Interleukin 8. IL-8 is a potent angiogenic, proinflammatory, growth-promoting cytokine (Koch *et al.,* 1992). It is a chemoattractant for neutrophils and induces the expression of several cell adhesion molecules (Koch *et al.,* 1992). It can also lead to neutrophil activation (Peveri *et al.,* 1988) and hence may contribute to the pathogenesis of inflammatory diseases such as endometriosis. The presence of inflammation and neovascularization observed in and around ectopic endometrial implants, and the presence of inflammatory neutrophils in these lesions is compatible with the biological actions of IL-8 (Van Deuren *et al.,* 1992).

Rana *et al.* (1996) reported increased concentrations of IL-8 in the peritoneal fluid from women with endometriosis, of which a substantial amount was suggested to be derived from peritoneal macrophages. It has also been suggested that IL-8 stimulates the growth of ectopic (Harada *et al.,* 1999) as well as eutopic endometrial cells and DNA synthesis in a dose-dependent manner (Iwabe *et al.,* 1998). In addition, endometrium produces IL-8 (Arici *et al.,* 1998a), which, in turn, induces the proliferation of endometrial stromal cells (Arici *et al.,* 1998b). This finding may be important since it is possible that excessive endometrial angiogenesis plays a role in the pathogenesis of endometriosis. It appears that IL-8 is involved in the pathogenesis of endometriosis. What is not clear is whether IL-8 is an initiating factor or a consequence of the presence of endometriotic explants within the peritoneal environment.

*Interleukin 10. S*ignificantly higher concentrations of IL-10 are found in women showing early stages of endometriosis compared with normal women (Ho *et al.,* 1997b). IL-10 is also found at higher concentrations in the peritoneal fluid, which may be a result of enhanced macrophage activity in women with endometriosis (Punnonen *et al.,* 1996). High concentrations of both IL-6 and IL-10 may contribute to the disturbed immune regulation observed in women with endometriosis. Punnonen *et al.* (1996) also reported that the activated peritoneal $CD4^+$ Th1 cells from women with endometriosis were decreased in number and suggested that the suppression of T cells is the result of the increased IL-10 in the peritoneal fluid.

However, in a conflicting study (McLaren *et al.,* 1997), no differences with respect to IL-10 concentrations were observed in the peritoneal fluid of women with or without endometriosis. In agreement with the findings of Punnonen *et al.* (1996), Wu *et al.* (1999) reported increased production of IL-10 and IL-6 by the peritoneal macrophages from women with endometriosis, confirming observations that the peritoneal macrophages are the principal source of these cytokines in peritoneal fluid. Given that both IL-10 and IL-6 are potent modulators of inflammatory responses, B-cell and macrophage functions in particular, it is likely that increased IL-10 and IL-6 production is responsible, in part, for the disturbed immune regulation observed in patients with endometriosis.

Interleukin 13. IL-13 is a macrophage-inhibiting cytokine. McLaren *et al.* (1997) reported that women with endometriosis had significantly lower concentrations of IL-13 in peritoneal fluid, compared with women without endometriosis. Immunolocalization of IL-13 indicates that glandular epithelial cells and stromal cells in both eutopic and ectopic endometrium are immunopositive for IL-13. Therefore, the reduced amounts of IL-13 in the peritoneal fluid of women with endometriosis may lead to a lack of suppression of macrophage activation, thereby contributing to the overall pathogenesis of the disease. Further work is needed to confirm these findings and clarify the role of IL-13 in the pathogenesis of endometriosis.

Tumour necrosis factor α. The concentration of TNF-α, a cytokine with a wide range of biological effects, is also increased in the peritoneal fluid of women with endometriosis (Richter *et al.,* 1998) and its concentrations may correlate with the stage of the disease (Richter *et al.,* 1998). TNF-α is made by a wide variety of cells, including

fibroblasts, macrophages and T and B cells, and has a biphasic effect on the growth of human endometrial adenocarcinoma cells *in vitro* (Ininns *et al.,* 1992). TNF-α also enhances the adhesion of endometrial stromal cells to mesothelial cells when included in the culture medium (Zhang *et al.,* 1993). The results of a study carried out in a rat model of endometriosis support the role of TNF in the development of endometriosis and provide evidence of the potential effectiveness of recombinant human TNF binding protein 1 (rhTBP-1) in its treatment (D'Antonio *et al.,* 2000). Iwabe *et al. (*2000) demonstrated that TNF-α stimulates proliferation of endometriotic stromal cells through induction of IL-8 gene and protein expression and concluded that the TNF-α may be one of the essential factors for the pathogenesis of endometriosis.

Intercellular adhesion molecule 1. Intercellular adhesion molecule 1 (ICAM-1) is a soluble molecule that can interfere with immunological functions, and may play important roles in the initiation and regulation of endometriotic lesions. ICAM-1-mediated cell–cell adhesion is essential for various immunological functions, including NK cell-mediated cytotoxicity against endometrium. In patients with endometriosis, concentrations of soluble ICAM-1 in peritoneal fluid are increased and this interferes with the activity of NK cells. This finding implies that the increased ICAM-1 impairs NK cell activity and accelerates the progression of the disease (Koninckx *et al.,* 1998). Endometriotic cells may show significant over-expression of ICAM-1 protein compared with eutopic endometrium (Vigano *et al.,* 1998). Therefore, the release of higher concentrations from ectopic samples may be the mechanism by which ectopic endometrial cells escape immunosurveillance (Somigliana *et al.,* 1996). Significantly high concentrations of soluble ICAM-1 have also been found in the sera of patients with endometriosis, especially those with advanced stages of the disease (Wu *et al.,* 1998). Available data on ICAM-1 is in agreement with the contention that this cytokine plays a role in the pathophysiology of endometriosis.

Monocyte chemotactic protein 1. Peritoneal fluid from patients with endometriosis has increased chemotactic activity for macrophages (Leiva *et al.,* 1993). MCP-1 is a potent chemotactic and activating factor specific for monocytes (Oral *et al.,* 1996). MCP-1 is secreted by a number of cell types, including endothelial cells, fibroblasts (Yoshimura and Leonard, 1990) and leukocytes (Yoshimura *et al.,* 1989). Concentrations of MCP-1 are high in the peritoneal fluid of women with endometriosis (Arici *et al.,* 1997), and are correlated with the severity of the disease. However, in women who have undergone medical treatment with gonadotrophins, the concentrations of MCP-1 are suppressed (Arici *et al.,* 1997).

Human endometrial tissue expresses MCP-1 and this expression is regulated by IL-1, TNF-α, platelet-derived growth factor (PDGF), and interferon γ in endometrial cell culture (Arici *et al.,* 1995). Secretion of MCP-1 is upregulated in cytokine-stimulated endometrial cells of women with endometriosis but not in those of normal women (Akoum *et al.,* 1995).

Increased concentrations of MCP-1 may play a role in the growth and maintenance of ectopic endometrial tissue by not only stimulating macrophages to secrete growth factors and cytokines, but also by stimulating endometrial cell proliferation directly (Arici *et al.,* 1997). Whether the increased MCP-1 in peritoneal fluid is a cause or consequence of the disease is not known. Taken together, these findings make it plausible that MCP-1 is involved in the pathogenesis of endometriosis and support the contention that pathophysiological changes are present in the eutopic endometrium of patients with endometriosis.

RANTES. RANTES (regulated upon activation, normal T cell expressed and secreted) is a cytokine with monocyte, macrophage, T-lymphocyte, and eosinophil attractant and activating properties discovered in the early 1990s (Schall *et al.,* 1990). The peritoneal fluid concentrations of RANTES are increased in women with endometriosis and concentrations are related to the severity of the disease (Khorram *et al.,* 1993). The exact role of this cytokine in the pathophysiology of endometriosis is not yet clearly understood.

Vascular endothelial growth factor. VEGF is a potent angiogenic factor involved in both physiological and pathological angiogenesis. Sources of VEFG include the eutopic endometrium, ectopic endometriotic tissue and peritoneal fluid macrophages. There is increasing evidence to indicate that the VEGF family is involved with both the aetiology and maintenance of peritoneal endometriosis (McLaren, 2000). Peritoneal fluid from patients with endometriosis contains significantly greater amounts of VEGF than controls, and there are increased VEGF concentrations in peritoneal fluid from women with more advanced endometriosis (Mahnke *et al.,* 2000). These findings indicate that the inflammation associated with endometriosis may promote angiogenesis for the progressive growth of the disease through increased VEGF concentrations (McLaren, 2000). Treatment of women with endometriosis with a GnRH agonist resulted in significant decreases in mean peritoneal fluid VEGF concentrations (Kupker *et al.,* 1998), indicating a role for VEGF in the establishment and maintenance of endometriosis.

Insulin-like growth factor. Insulin-like growth factor (IGF) is another well-known mitogenic peptide, and has a possible role as one of several mediators of oestrogen and other growth factors in various body tissues (Yap *et al.,* 1998). IGF-I concentrations in peritoneal fluid are significantly higher (Kim *et al.,* 2000) and IGF-binding protein-3 (IGFBP-3) concentrations and the relative proportion of IGFBP-2 are significantly lower, in patients with endometriosis than they are in women without endometriosis, indicating the involvement of the IGF system in the pathophysiology of endometriosis. The IGF peptides and their receptors have also been demonstrated

Table 1. Peritoneal factors suggested to be related to endometriosis

Peritoneal factor	Activity	Origin of evidence	Reference
Steroid hormones	Maintenance of endometriosis	*In vitro*, animal and human	DiZerega *et al.*, 1980; Mahmood and Templeton, 1991; Berqvist *et al.*, 1993
Prostaglandins	Induce aromatase, modulate cytokine production and function	*In vitro* and animal	Graham *et al.*, 1994; Bulun *et al.*, 2000
IL-1	Induce prostaglandin synthesis, collagen deposition, stimulate IL-2 and IL-6 as well as autoantibody production	*In vitro*	Dilloo *et al.*, 1994; Senturk and Arici, 1999
IL-6	Stimulate angiogenesis, promote cellular proliferation	*In vitro*	Le and Vilcek, 1989; Giudice *et al.*, 1994
IL-8	Stimulate angiogenesis, inflammation and cell proliferation	*In vitro*, animal and human	Koch *et al.*, 1992; Van Deuren *et al.*, 1992; Rana *et al.*, 1996; Arici *et al.*, 1998a,b
IL-10	Modulate inflammatory response	*In vitro*	Punnonen *et al.*, 1996; Wu *et al.*, 1999
IL-13	Inhibit macrophages	*In vitro*	McLaren *et al.*, 1997
TNF-α	Stimulate cell proliferation and adhesion	*In vitro* and animal	Ininns *et al.*, 1992; Zhang *et al.*, 1993; D'Antonio *et al.*, 2000; Iwabe *et al.*, 2000
ICAM-1	Mediate cell adhesion, impair natural killer cell activity	*In vitro*	Somigliana *et al.*, 1996; Koninckx *et al.*, 1998
MCP-1	Activate macrophages, stimulate cell proliferation and maintain ectopic endometrium	*In vitro*	Oral *et al.*, 1996; Arici *et al.*, 1997
RANTES	Attract cytokine, macrophage and T lymphocytes	*In vitro*	Schall *et al.*, 1990
VEGF	Stimulate angiogenesis	*In vitro*, animal and human	Kupker *et al.*, 1998; McLaren, 2000; Mahnke *et al.*, 2000
IGF	Stimulate mitosis, mediate oestrogen and other growth factors	*In vitro*	Giudice *et al.*, 1994; Yap *et al.*, 1998
PDGF	Potentiate mitosis	*In vitro*	Surrey and Halme, 1991; Chegini *et al.*, 1992
EGF	Stimulate mitosis, mediate oestrogen action	*In vitro*	Chegini *et al.*, 1992; Mellor and Thomas, 1994
bFGF	Induce angiogenesis and mitosis	*In vitro*, animal	Folkman and Klagsbrun, 1987; Irwin *et al.*, 1991
M-CSF	Stimulate proliferation and growth	*In vitro*	Weinberg *et al.*, 1991; Stanley *et al.*, 1997
TGF-β	Induce angiogenesis, chemoattract monocytes, inhibit natural killer cell, T and B lymphocyte function	*In vitro*	Rook *et al.*, 1986; Oral *et al.*, 1996
In vitro	Stimulate proliferation and mitosis	*In vitro*	Fukaya *et al.*, 1999; Laping, 1999

bFGF: basic fibroblast growth factor; EGF: epidermal growth factor; ICAM-1: intercellular adhesion molecule 1; IGF: insulin-like growth factor; IL: interleukin; M-CSF: macrophage-colony stimulating factor; MCP-1: monocyte chemotactic protein 1; RANTES: regulated upon activation, normal T cell expressed and secreted; TGF-β: transforming growth factor β; TNF-α: tumour necrosis factor α; VEGF: Vascular endothelial growth factor.

immunohistochemically in ectopic endometrial tissues (Chang and Ho, 1997). In addition, IGFs are mitogenic to endometrial stromal cells cultured *in vitro* (Giudice *et al.,* 1994), indicating a role for this group of peptides in the pathogenesis of endometriosis.

Platelet-derived growth factor. PDGF is a well-characterized secretory product of activated macrophages and plays a major role in the inflammatory response as a potent mitogen for fibroblasts and angiogenic precursor cells as well as endometrial cells (Chegini *et al.,* 1992). PDGF has been identified in the peritoneal fluid of women with endometriosis (Halme *et al.,* 1988) and has a significant dose-dependent proliferative effect on endometrial stromal and epithelial cells (Surrey and Halme, 1991). Further work is needed to clarify the exact role of PDGF in endometriosis.

Epidermal growth factor. Epidermal growth factor (EGF) is mitogenic for human endometrial cells (Chegini *et al.,* 1992). EGF concentrations in the peritoneal fluid are high in women with endometriosis (Simms *et al.,* 1991). EGF concentrations are also positively correlated with the day of the cycle, and are highest during the luteal phase (DeLeon *et al.,* 1986).

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, which is expressed in the glands and stroma of eutopic and ectopic endometrium of women with endometriosis (Prentice *et al.,* 1992). However, in a somewhat conflicting report, Huang *et al.* (1996) have suggested that there is no difference in the peritoneal fluid concentrations of EGF between women with and without endometriosis. The role of EGF in the pathogenesis of endometriosis remains unclear.

Basic fibroblast growth factor. Basic fibroblast growth factor (bFGF) is a heparin-binding angiogenic protein that 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 oestradiol and is inhibited by progesterone (Presta, 1988). bFGF is present in endometrial glandular epithelium and is a potent mitogen for endometrial stromal cells in culture (Irwin *et al.,* 1991). Peritoneal fluid concentrations of bFGF do not differ significantly between women with and without endometriosis (Seli *et al.,* 1998). Although findings *in vitro* indicate that bFGF may be involved in endometriosis, there is as yet insufficient evidence to support this theory.

Macrophage-colony stimulating factor. Peritoneal fluid concentrations of the growth factor, macrophage-colony stimulating factor (M-CSF), correlate with the total number of macrophages (Weinberg *et al.,* 1991). M-CSF has been identified at significantly higher concentrations in the peritoneal fluid of women with endometriosis (Weinberg *et al.,* 1991) and is involved in the differentiation of monocytes to become phenotypically activated macrophages in addition to serving as a chemotactic factor for blood monocytes. M-CSF may regulate the proliferation of endometrial tissue (Stanley *et al.*, 1997) and hence have a role in the development of endometriosis.

Transforming growth factor β. In addition to its growth regulating properties, transforming growth factor-β (TGF-β) is one of the most potent chemoattractants for human monocytes and is an inducer of fibrosis and angiogenesis (Oral *et al.,* 1996). Furthermore, TGF-β has striking immunological effects and can profoundly inhibit T-lymphocyte, B-lymphocyte and NK cell functions (Rook *et al.,* 1986). The peritoneal fluid of women with endometriosis contains increased TGF-β activity, and Oosterlynck *et al.* (1994) suggested that the decreased NK activity of peritoneal fluid in women with endometriosis is secondary to increased TGF-β activity. Women with stage III and IV endometriosis have higher concentrations of TGF-β compared with women with milder endometriosis, and a significant decrease in concentrations was achieved after treatment with a GnRH agonist (Kupker *et al.,* 1998), indicating a role for paracrine activity in endometriosis.

Hepatocyte growth factor. Hepatocyte growth factor (HGF) is an injury-released growth factor with diverse effects on epithelial and endothelial cells. These effects include proliferation, migration, extracellular matrix production and tubulogenesis (Laping, 1999). HGF concentrations in the peritoneal fluid of women with stage III–IV endometriosis are significantly higher than those from women without endometriosis. The concentrations from women with stage I–II endometriosis appear to be intermediate (Osuga *et al.,* 1999). HGF secretion is significantly increased in cultured endometrial stromal cells (Fukaya *et al.,* 1999), and HGF stimulates the proliferation and migration of, and morphogenic changes in, endometrial epithelial cells (Fukaya *et al.,* 1999). Given the known mitogenic properties of HGF, its increased secretion by eutopic endometrial stromal cells and higher peritoneal fluid concentrations in advanced endometriosis imply that it may play a role in the progression of endometriosis.

Conclusions

Discrepancies among studies about the changes in peritoneal immunology in women with endometriosis remain as a result of differences in the methodologies used and in the severity of endometriosis studied. However, in general, it is agreed that a local, sterile inflammation occurs in the peritoneal cavity and there is substantial evidence that immunological factors and angiogenesis play a decisive role in the pathogenesis of the disease. The reported data on various peritoneal factors included in this review are summarized (Table 1).

Peritoneal macrophages play a pivotal role in the intra-abdominal environment. They increase in number and are activated in women with endometriosis. Endometrial tissue, after arrival in the peritoneal cavity, is likely to adhere to the mesothelial lining if the regurgitated amount of tissue is too great or if the capacity of the intra-abdominal cells to clear the abdominal cavity is impaired. The adherence may be mediated by cell adhesion molecules and soluble factors produced by peritoneal macrophages in an advanced stage of differentiation. After adherence, endometrial tissue growth is promoted by steroids, growth factors and angiogenic factors present in the peritoneal fluid, in a paracrine and autocrine fashion.

The development of ectopic endometrium requires an accessible blood supply. The peritoneal fluid of women with endometriosis displays greater angiogenic activity than does fluid obtained from women without the disease (Oosterlynck *et al.,* 1993). Angiogenic and growth stimulating factors in the peritoneal fluid, peritoneum and endometriotic implants of women with endometriosis have been studied extensively. It may be postulated that the release of angiogenic factors into the peritoneal compartment produces an increased microvascularization of the parietal peritoneum. However, it must be emphasized that the mere presence of these cytokines, growth factors and macrophage attractants and the production *in vitro* of growth-promoting factors by macrophages do not prove their involvement in the development or maintenance of the disease. In addition, the factors discussed were detected in women who had already developed endometriosis, and may therefore have been a consequence rather than a cause of the disease.

Progress made recently in studies of peritoneal fluid regarding the pathogenesis of endometriosis sheds new light on the fundamental quandaries of this mysterious disease. Although cellular and chemical alterations in the peritoneal fluid are apparent, many questions remain to be answered. It is unlikely that immunological changes in isolation will explain the pathophysiology of endometriosis. The effects of various cytokines and growth factors in the peritoneal fluid of endometriosis are complicated, and a clear delineation of the roles of each of these factors is lacking. Much more work is needed in this area to clarify the role of each individual cytokine and growth factor in the pathogenesis of endometriosis.

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