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INVASION PROMOTING FACTORS IN ENDOMETRIOTIC AND ENDOMETRIAL TISSUE

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This thesis is dedicated to all these women suffering from endometriosis.

ABSTRACT

Endometriosis is a common gynaecologic disorder, affecting women of fertile age with pelvic pain and infertility, and is characterized by the presence of endometrial glands and stroma outside the uterine cavity. Retrograde shed endometrial fragments are presumed to adhere to pelvic structures and grow invasively, thereby creating endometriotic tissue.

Different factors might participate in the invasive process. The plasminogen activating system (PAs) is involved in tissue degradation and remodelling under both normal and pathological conditions. The activation of plasminogen, leading to the formation of plasmin, is catalysed by tissue-type PA (tPA) or by urokinase plasminogen activator (uPA) when bound to its receptor uPAR. Receptor binding of uPA initiates pericellular proteolysis and cell migration, two processes required for tissue invasion. Two plasminogen activator inhibitors PAI-1 and PAI-2 regulate the plasminogen activating system.Plasmin, a highly potent protease, is able to degrade a broad spectrum of matrix and basement membrane proteins and plasmin also activates zymogens of other matrix-degrading proteases.

In this thesis we hypothesize that the PAs are important contributors both to menstrual shedding, endometrial adhesion and endometriotic invasion.

We investigated endometriotic and endometrial tissue as well as peritoneal fluid (PF) from women with endometriosis and used endometrium and PF from healthy women as controls. We analysed the concentrations of plasminogen activators and inhibitors and the IL-1β, IL-6 and TNFα in homogenates of endometriotic and endometrial tissue as well as in peritoneal fluid and in culture medium from isolated, separated epithelial and stromal cells. With *in situ* hybridisation (ISH) and immunohistochemistry (IHC) we investigated in which type of cells, glandular and/or stromal cells, the mRNA and the protein for uPA, PAI-1 and uPAR were expressed in endometriotic and endometrial tissue samples.

We found an up-regulation of mRNA for uPA, PAI-1 and uPAR and higher concentrations of uPA, and PAI-1 in endometriotic and endometrial tissue from women with endometriosis compared to endometrial tissue from control women.

These findings might be of importance for menstrual shedding and adhesion of viable endometrial fragments to other structures and for the invasive growth of endometriosis. The differences found between the three tissue types concerning IL-1β, IL-6 and TNFα might have importance for the inflammatory reaction at the endometrial adhesion and in the growth regulation of endometriotic tissue. The higher level of PAI-2 in PF from women with endometriosis might be a consequence of the inflammatory process and contribute to the development of adherences in the peritoneal cavity.

Above all the studies have shown, that there is an important difference in the expression of the components of the PAs in endometrium from women with endometriosis compared to healthy endometrium supporting the hypothesis that ectopic endometrial invasion takes place at least partly as a consequence of a disturbed fibrinolytic activity in the eutopic endometrium.

Key words: Endometriosis, endometrium, PF, tPA, uPA, uPAR, PAI-1, PAI-2, IL-1β, IL-6, TNFα.

LIST OF PUBLICATIONS

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Fibrinolytic factors in endometriotic tissue, endometrium, peritoneal fluid, and plasma from women with endometriosis and in endometrium and peritoneal fluid from healthy women.
Fertility and Sterility 1998;70:821-825

II. Christine Bruse, Diana Radu, Agneta Bergqvist
***In situ* localization of mRNA for the fibrinolytic factors uPA, PAI-1 and uPAR in endometriotic and endometrial tissue.**
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Immunohistochemical localization of uPA, PAI-1 and uPAR in endometriotic tissue and endometrium from women with and without endometriosis.
Manuscript

IV. Christine Bruse, Yongmei Guan, Magdalena Carlberg, Kjell Carlström, Agneta Bergqvist
Basal release of uPA, PAI-1, and suPAR from cultured separated endometriotic and endometrial stromal and epithelial cells.
Submitted

V. Agneta Bergqvist, Christine Bruse, Magdalena Carlberg, Kjell Carlström
Interleukin 1β, interleukin-6, and tumor necrosis factor-α in endometriotic tissue and in endometrium.
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CONTENTS

LIST OF ABBREVIATIONS

AEC	3-amino-9-ethyl-carbazole
ATF	aminoterminal fragment
cDNA	complementary DNA
CLSM	confocal laser-scanning microscopy
ECM	extracellular matrix
EEC	endometrial epithelial cell
EGF	epidermal growth factor
ELISA	enzyme linked immunosorbent assay
EMC	uterine endometrium from healthy controls
EMP	uterine endometrium from patients with endometriosis
EOS	endometriotic tissue
ESC	endometrial stromal cell
FBS	fetal bovine serum
FGF	fibroblast growth factor
GF	growth factor
GPI	glycosyl-phosphatidyl-inositol
IGF	insulin-like growth factor
IL-1	interleukin 1
IL-6	interleukin 6
IL-8	interleukin 8
ISH	in situ hybridization
LRP	lipoprotein receptor-related protein
MCP	monocyte chemotactic protein
MEM	modified Eagles medium
MMP	matrix metalloprotein
mRNA	mesenger ribonucleic acid
PA	plasminogen activating
PAI	plasminogen activator inhibitor
PBS	phosphate buffered saline
PC	pheochromocytoma
PF	peritoneal fluid
PMC	peritoineal mesothelial cell
r_s	Spearmans rank correlation coefficient
scuPA	single chain urokinase plasminogen activator
SERPIN	serine protease inhibitor
SPD	serine protease domain
SSC	saline sodium citrate
suPAR	soluble variant of urokinase plasminogen activator
TEM	transmission electron microscopy
TGFα	transforming growth factor α
TGFß	transforming growth factor ß
TNFα	tumor necrosis factor α
tPA	tissue type plasminogen activator
uPA	urokinase plasminogen activator

uPAR	urokinase plasminogen activator receptor
UTP	uridine triphosphate
VEGF	vascular endothelial growth factor
VN	vitronectin

1 INTRODUCTION

1.1 ENDOMETRIOSIS

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus. It is one of the most common benign gynaecologic disorders and affects about 10 % of women in reproductive age (Wheeler, 1989). Endometriosis is associated with abdominal and pelvic pain as well as infertility. The incidence will increase up to 20 % in women with infertility and 25 % in women with pelvic pain (Eskenazi and Warner, 1997). The aetiology of endometriosis is not clear, but there is increasing evidence for a genetic factor in developing endometriosis (Kennedy, 2003). There is a sevenfold increased risk of endometriosis in a first-degree relative of patients with endometriosis (Moen and Magnus, 1993). The histogenesis of endometriosis has been explained by three different theories described below, of which the implantation theory is the most accepted.

1.1.1 Implantation theory

The implantation theory is proposed as a consequence of retrograde menstruation, when endometrial tissue passes through the fallopian tubes into the peritoneal cavity where it attaches and proliferates at ectopic sites (Sampson J, 1927). The viability of sloughed endometrial cells and the capacity of these cells to implant have been demonstrated in studies both in humans and in primates (Ridley and Edwards, 1958, TeLinde and Scott, 1950, D´Hooghe *et al.,* 1995).Since retrograde menstruation occurs in up to 90 % of women, an additional factor is likely to contribute to the development of endometriosis (Halme *et al.,* 1984). Alterations in the immunologic response to refluxed menstrual debris have been implicated in the genesis and maintenance of the endometriotic lesion. This defective immunosurveillance may lead to decreased clearance of sloughed off endometrial fragments from the peritoneal cavity and may allow for attachment of ectopic endometrium to peritoneal surfaces. An abnormal immune response could also promote the persistence and growth of ectopic endometrial tissue (Lebovic *et al.,* 2001).

1.1.1.1 Lymphatic/haemotogenous theory

This theory is proposed by Halban (1925) and might explain endometriosis at extragenital tracts. It is supported by the findings of endometrial fragments in lymph nodules (Lamm *et al.,* 1974, Ueki, 1991).

1.1.1.2 Direct mechanical implantation theory

This theory applies to women with endometriosis in whom it can be shown that a surgical operation, i.e. hysterotomy, may have been responsible for the dissemination or direct ingrowth of endometrial tissue into wounds (Anderson, 1993).

1.1.2 Coelomic metaplasia theory

The theory of coelomic metaplasia proposes that endometriosis may develop from metaplasia of mesothelial cells lining the pelvic peritoneum (Ridley, 1968). An

attractive component of the theory is that it can explain the occurrence of endometriosis anywhere mesothelium is found e.g. in the pleural cavity (Foster *et al.,* 1981).

1.1.3 Induction theory

The induction theory is an extension of the coelomic metaplasia theory. It is based on the proposition that menstrual endometrium produces substances that induce peritoneal tissue or embryonic rests, e.g. Müllerian remnants, to form endometriotic lesions (Fujii, 1991).

1.1.4 Appearance and location of endometriotic lesions

In its earliest stage, peritoneal endometriosismay not always be visible to the eye. Biopsies from normal-appearing peritoneum have detected histological proven endometriosis in 6 % of cases (Nisolle *et al.,* 1990). The first visible sign of endometriosis could be areas of hypervascularization of the peritoneum and/or transparent vesicular lesions easy to miss (Fig 1A). Later the lesions become red because of bleeding since the ectopic lesions respond to cyclic hormonal changes with shedding like the eutopic endometrium (Fig 1B). Debris of hemosiderin will give the lesions their typical appearance with a brownish, blue or black colour (Fig 1B, D). This appearance represents an advanced stage of endometriosis and the lesions will now become fibrotic as a consequence of the chronic inflammatory process that encloses the ectopic lesion (Fig 1C). This scarification process sometimes totally devascularizes the endometriotic foci and the lesion becomes white and is considered as healed endometriosis or quiescent lesions (Nisolle *et al.,* 1993).
Endometriotic cysts, so-called endometriomas are found in the ovaries and can reach a size of ten centimeters or more (Fig 1D). The inside of the cyst is covered with endometriotic tissue that menstruates and an accumulation of menstrual debris together with glandular secretion gives the content its typical appearance and name “chocolate cysts”.
The most common location for endometrial implants in the pelvic cavity is in fossa Douglasi followed by the ovarian fossae, uterosacral ligaments and bladder peritoneum (Stratton *et al.,* 2002). Endometriosis can also be found at extra-genital sites as on the bowel, bladder, lung and in scars (Bergqvist *et al.,* 1993).

1.1.5 Endometrial adhesion

According to the implantation theory of endometriosis, refluxed endometrial cells must have the ability to implant outside the uterus. Models of amniotic membranes to study adhesion of endometrial fragments have been used (van der Linden *et al.,* 1996, Groothuis *et al.,* 1998). An intact layer of amniocytes prevented endometrial attachment and the authors concluded that the initial attachment of menstrual endometrial cells to peritoneum must depend on breaches in the mesothelial layer. Later Groothuis and co-workers (Groothuis *et al.,* 1999) used peritoneal explants to study the adhesion of proliferative phase endometrium from women without endometriosis. Adhesion of endometrial fragments was observed with light field microscopy and transmission electron microscopy (TEM), only at locations where the mesothelium was absent or damaged and the basement membrane was exposed. Similar work by this group using antegrade-shed menstrual tissue also failed to demonstrate

Fig 1A

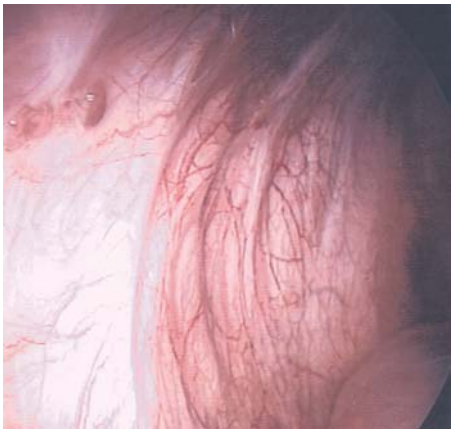

Fig 1B

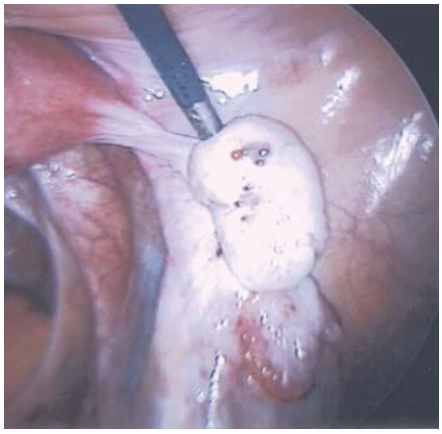

Fig 1. Early stage of endometriosis is demonstrated with hypervascularization of the peritoneum and subtle, transparent vesicles. Sign of a biopsy is seen to the right of the vesicles, taken to verify the diagnosis of endometriosis (A). Endometriotic lesions of mixed colour (red and black) on the surface of the ovary represent different ages of the implants (B).

Fig 1C

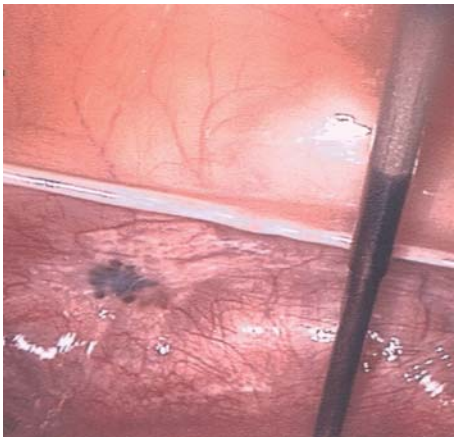

Fig 1 D

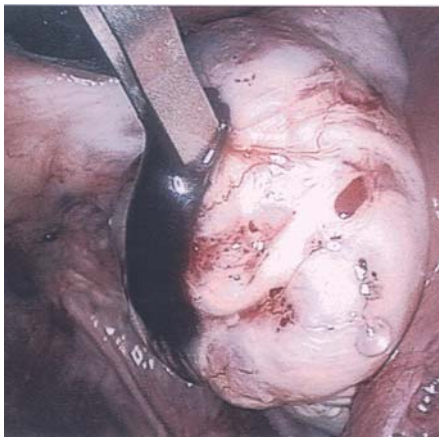

Fig 1. Black endometriotic lesions covered with a fibrous film and surrendered by white lesions i.e. scar formation on the peritoneum (C). Opening and draining of an endometrioma with its typical content of chocolate like fluid that also has given it its name “chocolate cyst”. Brownish-black lesions are also seen in the punch behind the endometrioma (D). Photo Fredrik Nordenskjöld.

adhesion of endometrial fragments to intact mesothelium (Koks *et al.,* 1999). The authors hypothesized that trauma to the mesothelial lining was a prerequisite for endometrial cell adhesion.In contrast to this, Witz and co-workers (Witz *et al.,* 2001, 2002) showed, using confocal laser-scanning microscope (CLSM) and TEM, that both proliferative, secretory and menstrual phase endometrium adhere to peritoneum explants with an intact layer of viable mesothelial cells and that this will occur within one hour.

1.1.6 Invasive capacity of early endometriotic lesions

In vitro studies of collagen gel invasion have shown that the invasive potential of endometriotic cells is comparable to that of cell lines from a metastatic carcinoma (Gaetje *et al.,* 1995), and it has long been recognized that early endometriosis invades the ECM (Spuijbroek *et al.,* 1992). Following endometrial attachment to mesothelium, transmesothelial invasion of endometrial epithelial and stromal cells occurs rapidly (Witz *et al.,* 2002, 2003). Using a technique where fluorescence-labelled peritoneal mesothelial cells (PMCs) were cultured on cover slips and fluorescence-labelled endometrial epithelial cells (EECs) and stromal cells (ESCs) were plated on the PMCs the investigators could follow the transmesothelial invasion through a confocal laser-scanning microscope.

After one hour of culture both epithelial and stromal cells had adhered to PMCs. After three hours both epithelial and stromal cells had migrated to intercellular junctions in PMC and after six hours, extension of pseudopodia from the invading cells was seen under the mesothelial cell layer. By 12 hours, there was vacuolisation and lifting of the mesothelial cell layer that had been undermined by endometrial cells. The invasion was completed after 24–27 hours when there was evidence that PMCs had proliferated and migrated over the invaded endometrial cells (Witz *et al.,* 2003). In a recent study Fasciani and co-workers (Fasciani *et al.,* 2003) demonstrated that cells from endometrial explants could proliferate and invade a fibrin matrix *in vitro* generating new glands, stroma and vessels consistent with endometriosis. This suggests that menstrual endometrial fragments reaching pelvic fibrin adhesions would be able to proliferate as the first step of endometriosis *in vivo*.

1.2 THE PLASMINOGEN ACTIVATING SYSTEM

The plasminogen activating system (PA system), also called the fibrinolytic system, is involved in tissue degradation and remodelling under both physiologic, normal and non neoplastic pathological conditions as well as in neoplastic conditions (Danø et al., 1985). Physiologic, normal conditions include involution of mammary glands after the termination of lactation (Ossowski et al., 1979, Politis, 1996), disruption of the follicle wall involved in ovulation (Strickland et al 1978) as well as blastocyst implantation into the uterus (Strickland et al., 1992). Non-neoplastic, pathological conditions include inflammatory processes (Reich et al., 1978) and wound healing (Schafer et al., 1994). In neoplastic conditions, the PA system plays an important role in tumour invasion and metastasis (Andreasen et al., 2000). Moreover, the system plays a role in cell adhesion,

migration and intracellular signal transduction, through a protease independent function (Irigoyen et al., 1999, Andreasen et al., 2000, de Bock and Wang, 2004).
The PA system is composed of two serine proteases, tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA) and its receptor (uPAR). The PA system also includes two serine proteinase inhibitors (SERPINS), plasminogen activator inhibitor PAI-1 and PAI-2, and the zymogen plasminogen. The proteolytic effect of the PA system is generated through the potent protease plasmin, which is converted from its pro-form plasminogen to plasmin by cleavage catalyzed by uPA or tPA.

1.2.1 Plasminogen activators

1.2.1.1 Tissue type plasminogen activator (tPA)

The serine proteinases tPA is a 70 kDa protein, which is synthesized and secreted as a single-chain precursor mainly by endothelial cells but also by epithelial cells, fibroblasts and macrophages as well as by tumour cells (Rijken and Collen, 1981, Irigoyen et al., 1999, Andreasen et al., 2000). The single-chain precursor can be proteolytically converted to a two-chain form by cleavage of a single polypeptide bond. The plasminogen activation activity of the two-chain tPA is 10- to 50-fold higher than that of the single-chain form (Andreasen et al., 2000).Due to its high affinity for fibrin and its activation by fibrin binding, the main biological role of tPA seems to be associated with fibrinolysis (Irigoyen et al 1999, Andreasen et al., 2000).

1.2.1.2 Urokinase plasminogen activator (uPA)

uPA or urokinase, so named because of its initial early detection in urine, is a serine protease that is highly specific for the activation of plasminogen. It is produced as a single-chain glycoprotein, pro-uPA or scuPA, with a molecular weight around 55 kDa. When secreted, pro-uPA is converted to the active two-chain form uPA by proteolytic cleavage of a single peptide bond, mainly by plasmin. Pro-uPA bound to its receptor, uPAR, is activated by plasmin much faster than when free in the fluid phase (Ellis et al., 1989, Duval-Jobe et al., 1994). Disulfide bridges link the two peptide-chains of uPA, and the molecule contains three functional domains. In the carboxyl-terminal region is a serine protease domain, SPD, also called the B chain. The non-catalytic amino-terminal fragment, ATF, corresponding to the A chain, contains the kringle domain and the growth factor, GF, domain, which is responsible for its interaction with uPAR (Appella et al., 1987). The two-chain uPA has an activity about 250-fold higher than that of pro-uPA (Petersen et al., 1988). Besides the activation of plasminogen to plasmin, uPA directly activates pro-hepatocyte growth factor/scatter factor and macrophage-stimulating protein (Naldini et al., 1992, Mars et al., 1993). uPA also cleaves fibronectin (Keski-Oja and Vaheri, 1982), as well as its own receptor uPAR (Høyer-Hansen et al., 1992, 1997) and inhibitor PAI-1 (Andreasen et al., 1986, Nielsen et al., 1986). uPA activity is controlled by binding of PAIs and by endocytosis (Andreasen et al., 1997, Irigoyen et al., 1999).

1.2.2 Urokinase plasminogen activator receptor (uPAR)

1.2.2.1

The receptor for urokinase plasminogen activator (uPAR/CD87) is a multifunctional cell surface protein, synthesized as a 313 amino acid residue polypeptide that is carboxyl-terminally processed and attached to the cell membrane by a glycosyl-phosphatidyl-inositol (GPI) anchor (Plough et al., 1991, Danø et al., 1994). An anchorless, soluble variant of uPAR (suPAR) may arise by differential splicing, or by different cleavage of the GPI anchor (Pyke et al., 1993). The uPAR consists of three homologous, internally disulphide-bonded domains (D 1-3), numbered from the N-terminus. The amino-terminal domain of uPAR (D1) contains the main uPA-binding site (Behrendt et al., 1991), whereas the next two domains (D2-3) bind the extracellular matrix protein vitronectin (VN) (Wei et al., 1994). uPAR also binds integrins at sites distinguishable from its uPA- and vitronectin-binding sites (Wei et al., 1996). Pro-uPA and active uPA binds with the same affinity to intact uPAR, and binding of uPA promotes besides plasmin generation also non-proteolytic events as cell adhesion by increasing the affinity of the receptor for vitronectin and integrins (Deng et al., 2001). Moreover, the uPA:uPAR binding promotes cell migration, differention and proliferation, that requires intracellular signaling. uPAR lacks a cytosolic domain but transmits intracellular signals through its association with transmembrane proteins, e.g. integrins (Wei et al., 1996).

The region linking D1 and D2D3 in uPAR (and suPAR) is prone to cleavage by several proteases including uPA and this cleavage abolishes the uPA binding. The uPAR fragments D1 and D2D3 have been shown as active inducers of chemotaxis and cell adhesion *in vitro* and both fragments have been identified *in vivo* (Sidenius et al., 2000).Binding of PAI-1 to receptor bound uPA triggers internalization of the complex, mediated by low-density lipoprotein receptor-related protein (LRP). After internalization and clearance of uPA: PAI-1, uPAR recycles back to the cell surface (Cubellis *et al.,* 1990, Nykjaer et al., 1997).

1.2.3 Plasminogen activator inhibitors

Plasminogen activator inhibitors PAI-1 and PAI-2 and α2-antiplasmin, the primary inhibitor of plasmin, are members of the SERPIN family. For theirs inhibitory mechanism is the surface-exposed reactive center loop of importance since the reactive center peptid bond interacts with the active site of the proteinases and stably coupling them in an inactive conformation.

1.2.3.1 Plasminogen activator inhibitor 1 (PAI-1)

PAI-I is a single-chain 50 kDa glycoprotein produced by many cell types, e.g endothelium, platelets, fibroblasts and macrophages. PAI-1 is rapidly secreted after synthesis and reacts quickly with uPA and/or tPA or binds with high affinity to the adhesive glycoprotein vitronectin, a common component of the ECM, to stabilize its active form. PAI-1/vitronectin complex gives PAI-1 other functions not related to its proteinase inhibitory effect i.e. involvement in the regulation of cell adhesion and inhibiting vitronectin/integrin-dependent cell migration (Stahl and Mueller 1997, Kjøller *et al.,* 1997).

When coupled to uPAR-bound uPA, PAI-I inhibits ECM degradation and the uPA: PAI-1 complex is internalized and degraded in lysosomes. Binding to uPAR and the α_2-macroglobulin receptor is required for uPA: PAI-1 internalization.

1.2.3.2 Plasminogen activator inhibitor 2 (PAI-2)

PAI-2 is a more specific inhibitor of uPA but reacts more slowly than PAI-1 with the proteinases. PAI-2 exists in one secreted and one cytosolic form (Astedt *et al.,* 1987). It is rapidly induced in macrophages in response to TNFα and lipopolysaccarid, and most of the newly synthesized PAI-2 remains intracellular with only a fraction secreted. In its cytosolic form, it plays an important role in intracellular proteolysis involved in processes such as apoptosis and inflammation. In its secreted form, PAI-2 participates in control of tissue remodelling and fibrinolysis (Wohlwend *et al.,* 1987, Dickinson *et al.,* 1995).

1.2.4 Plasminogen and plasmin

The one-chain precursor of plasmin, plasminogen, is synthesized in the liver and is present in high concentrations in blood and in extracellular fluids. It converts into the two-chain structure of plasmin by proteolytic cleavage of a single peptide bond. The cleavage is catalysed by uPA or tPA and the conversion is enhanced when plasminogen interacts with its cell surface receptors of various cell types (Ellis *et al.,* 1991).Cell-surface-associated plasmin has broad substrate specificity and catalyses the breakdown of many extracellular matrix proteins, either directly or indirectly via activation of other matrix-degrading proteases. Plasmin also converts latent forms of growth factors and cytokines into active forms (Irigoyen *et al.,* 1999, Andreasen *et al.,* 2000).The primary inhibitor of plasmin is α2-antiplasmin which rapidly inactivates the soluble forms of plasmin, while cell and fibrin bound plasmin is protected from inhibition (Plow *et al.,* 1986).

1.2.5 The PA system in adhesion, migration and invasion

The knowledge about the role of the PA system in cell adhesion, migration and invasion is based upon studies on cell lines in different *in vitro* assays.
The plasmin activation of pro-uPA, bound to its cell surface receptor uPAR, proceeds much faster than activation of fluid-phase pro-uPA. Furthermore, uPAR bound uPA catalyses plasminogen activation more efficiently than fluid-phase uPA (Ellis *et al.,* 1989, Andreasen *et al.,* 1997). This enhancement of plasminogen activation seems to be due to co-accumulation of uPA and plasminogen on the same cell surface.Pericellular plasmin generation leads to the degradation of fibrin and ECM proteins and to the activation of latent growth factors and of the zymogen of matrix metalloproteinases (MMPs) (Andreasen *et al.,* 1997, Matrisian, 1992). MMPs are able to degrade native collagen, which is resistant to plasmin (Mignatti and Rifkin, 1993).
The plasmin generation is controlled by PAI-I, which reacts with uPAR bound uPA. The uPAR/uPA/PAI-1 complex is then rapidly endocytosed and degraded (see above *1.1.3.1*).

Binding of uPA to uPAR stimulates intracellular signaling and induces conformational changes in uPAR, which increase its affinity for the adhesive protein vitronectin and

integrins, which promotes cell adhesion (Ossowski and Aguirre-Ghiso, 2000; Kjøller, 2002). PAI-I competes with uPAR for the binding site on vitronectin, and thus the affinity of PAI-I for the somatomedin B domain of vitronectin is much higher than the affinity of uPAR for this domain, PAI-1 can competitively inhibit the uPAR dependent attachment of cells to vitronectin and cells attached to vitronectin through uPAR can be detached by PAI-1 (Deng *et al.,* 1996, 2001).

Cell migration or the locomotion of a cell on a surface involves extension at the leading edge and retraction at the trailing edge of the cell. Extension is associated with adhesion to and retraction involves detachment from the extra cellular matrix. The effects of uPA on cell migration may be caused by proteolytic as well as non-proteolytic mechanisms.

Binding of uPA to uPAR would stimulate cell migration through plasmin generation at focal adhesion sites, which would lead to degradation of extra cellular matrix proteins and adhesion receptors, which would help detachment of the trailing edge.
A non-proteolytic stimulatory effect on cell migration of uPA-uPAR binding would involve stimulating adhesion at the leading edge, through binding of uPAR to vitronectin. uPA may also act by initiation of signal-transduction cascades at the leading edge and/or regulation of integrin-binding activity at both the leading and the trailing edge. It seems possible that both mechanisms are operating simultaneously in individual migrating cells (Andreasen *et al.,* 1997, 2000). PAI-I inhibits cell migration by interfering with the interactions between vitronectin and vitronectin binding integrins (Kjøller *et al.,* 1997).

Invasion involves not only cell migration but also an active penetration of cells into the extra cellular matrix. The plasmin generation catalyzed by uPA upon binding to uPAR on the surface of invading cells, seems to be the rate-limiting factor for invasion. The observed effects of PAI-1on invasion has been variable, but the effect is expected to depend on expression of the other components of the PA system by the invading cell, of endocytosis receptors, integrins and on the composition of the extracellular matrix (Andreasen *et al.,* 1997, 2000).

1.2.6 The PA system in human endometrium.

The fibrinolytic activity, i.e. the result of the action of plasminogen activators and inhibitors, in uterine fluid shows an increasing activity during the proliferative phase to a maximum at midcycle and then falls to its lowest level during the secretory phase and then increases again premenstrually (Casslén, 1981, Casslén and Åstedt, 1981). Casslén (1981) found that in contrast to the activator activity the uPA concentration decreased in the midcycle and increased again in the premenstrual phase in uterine fluid. The plasminogen activator concentrations in endometrial homogenates studied by Rybo (1966) shows a successive increase during the premenstrual days and reach the highest value on the first day of the menstruation. Koh *et al.* (1992) found significantly higher concentrations of tPA and PAI-1 in endometrium in the late secretory phase compared to the proliferative or the early secretory phase. uPA concentration did not differ significantly. The release of uPA but not tPA from endometrial tissue explants varied over the menstrual cycle with a maximum at midcycle, was low in the secretory phase and increased again premenstrually (Casslén and Åstedt, 1983). The number of

receptors for uPA is higher in the secretory than in the proliferative phase (Casslén and Gustavsson, 1991). Progesterone reduces the release of uPA but not of tPA in stromal cell cultures (Casslén *et al.,* 1992) and stimulates degradation of uPA in endometrial stromal cells by increasing PAI-1 and uPAR (Casslén *et al.,* 1995, 1998). Guan and co-workers (Guan *et al.,* 2002) later confirmed this regulatory effect of progesterone on the release of uPA and PAI-1 in stromal cell cultures.

MMPs play a key role in the cyclical breakdown of ECM that leads to menstruation (Rodgers *et al.,* 1994, Salamonsen *et al.,* 1996). Progesterone withdrawal initiates endometrial secretion of pro-MMPs (Hampton and Salamonsen, 1994, Lockwood *et al.,* 1998). MMP-1 and MMP-3 are expressed only at menstruation and it is likely that both have important roles in tissue degradation that initiates menstruation (Hampton and Salamonsen, 1994). Pro-MMPs are activated by proteases and plasmin is a key activator of the zymogens (Carmeliet *et al.,* 1997).

1.3 CYTOKINES IN HUMAN ENDOMETRIUM

Cytokines are multifunctional glycoprotein produced not only by resident leucocytes in endometrial stroma but also by endometrial epithelial and stromal cells (Tabibzadeh 1991). Diverse functions for cytokines in endometrium have been suggested, e.g. as regulators of cell growth, cellular proliferation and differention (Karyia *et al.,* 1991). Cytokines acts in a endocrine, paracrine and autocrine way and their synthesis is under the hormonal control of steroids (Tabibzadeh and Sun, 1992, Kelley *et al.,* 2001).

1.3.1 Interleukin-1

IL-1 comprises of two different molecules, α and β, which bind to the same receptor and mediate similar biological activities (Arai *et al.,* 1990). Il-1α and β are present in endometrium in epithelial, stromal and lymphoid cells but IL-1β appears normally in lower amounts (Tabibzadeh and Sun, 1992). IL-1β mRNA is expressed both in proliferative and secretory phase with its lowest and highest level in the early and late secretory phase respectively (von Wolff *et al.,* 2000). IL-1 is involved in the regulation of immune response and inflammation and also induces secretion of IL-6. IL-1β stimulates endometrial epithelial cells to produce monocyte chemotactic protein-1 (MCP-1) (Akoum *et al.,* 1996). *In vitro* studies on cultured human endometrium stromal cells have shown that both the mRNA expression and protein levels of PAI-1, uPAR and suPAR were up-regulated by IL-1β in a dose-dependent manner and is suggested to play a role in regulating PAI-1 and uPAR during stromal cell decidualization before implantation (Chung *et al.,* 2001).

1.3.2 Interleukin-6

Il-6 is present in human endometrium during the proliferative phase in epithelial cells in low levels, and increases 5-10 fold in the mid- to late secretory phase. IL-6 mRNA is expressed at low levels in the proliferative phase in epithelial, stromal and CD45-positive leucocytes, and the expression increases progressively in the secretory phase. A high concentration of IL-6 in the late secretory phase and its function as an inflammatory cytokine suggests a role in menstrual shedding (von Wolff *et al.,* 2002).

IL-6 secretion is stimulated by IL-1 and by tumour necrosis factor α (TNFα), another cytokine that has been associated with menstrual shedding (Tabibzadeh, 1996).

1.3.3 Tumor necrosis factor α (TNFα)

TNFα exerts a variety of effects ranging from pro-inflammatory and cytotoxic, to growth and immunomodulatory, stimulating angiogenesis and induces apoptosis in host cells (Tabibzadeh, 1996). TNFα mRNA and protein has been localized in endometrium and is expressed, to a large extent, in the endometrial epithelial cells and, to a lesser extent, in the stromal and lymphoid cells (Tabibzadeh, 1991). Both mRNA and the protein are present in the proliferative phase and increase during the secretory phase and are expressed at maximum levels in the late secretory phase (von Wolff *et al.,* 2000). TNFα has been identified as a factor inducing apoptosis and tissue oedema by damaging endothelial cells, which is characteristic for the late secretory phase. Like IL-1β, TNFα stimulates endometrial epithelial cells to produce MCP-1.

2 AIMS OF THE STUDY

The general aim of these studies has been to evaluate the hypothesis that the plasminogen activating system might participate in and promote the invasive growth of endometriotic tissue and that there might be a dysfunction in the regulation of the PA components already in the endometrium in women with endometriosis.

More specific aims has been

♦ To study the concentrations of plasminogen activators and their inhibitors in homogenates from endometriotic and endometrial tissue, as well as in peritoneal fluid and plasma from women with endometriosis, and from control women (*Paper I*).

♦ To study the tissue distribution of the mRNA and the protein for urokinase, urokinase receptor and the inhibitor (PAI-1), in eutopic and ectopic endometrium (*Paper II and III*).

♦ To study *in vitro* the release of uPA, suPAR and PAI-1 in epithelial and stromal cells in endometriotic tissue and endometrium, both from women with and without endometriosis (*Paper IV*).

♦ To study the concentrations of some important growth regulators, as IL-1β, IL-6 and TNF-α, in homogenates from endometriotic tissue and endometrium, both from women with and without endometriosis (*Paper V*).

3 MATERIALS AND METHODS

3.1 ETHICAL CONSIDERATIONS

The local ethics committee at Huddinge University Hospital had approved the studies and the women had given their oral informed consent for the samples to be collected.

3.2 STUDY POPULATION

All women, contributing with endometriotic or endometrial tissue, peritoneal fluid or plasma were regularly menstruating, and none of them had received any hormonal treatment or contraceptives or been pregnant or breast-feeding for at least 2 months before surgery.

3.3 MATERIALS

3.3.1 Endometriotic and endometrial tissue samples

Endometriotic tissue samples were obtained at laparoscopy or laparotomy performed because of ultrasound-verified endometriotic cysts, pelvic pain or dysmenorrhea. The tissue samples were mainly obtained from endometriotic cysts, but also from peritoneal lesions, ovarian lesions, omental endometriosis, a submucous vaginal lesion and from scars in the abdominal wall. From most of the women contributing with endometriotic tissue an endometrial sample could be obtained simultaneously by uterine curettage.

Control endometrium was obtained by uterine curettage mainly from women undergoing laparoscopic sterilization, but also from women undergoing surgery for benign cysts, cone biopsy, myomas and in one case surgery for uterine prolapse.
The endometriotic and endometrial tissue samples were immediately after surgical extirpation rinsed in saline to remove blood and mucous. The endometriotic cysts were scraped inside with the back edge of a knife to obtain endometriotic tissue as pure as possible and endometriotic samples from other locations were separated from inappropriate tissue as thoroughly as possible.
Part of the tissue was thereafter snap frozen in liquid nitrogen or on dry ice and stored at - 70° C (Paper I, II, III and V). The rest was sent for histopathologic diagnose and dating of the endometrial samples, according to Noyes *et al.,* (1950).
Tissue samples for isolation and culture of cells (*Paper IV*) were transported in cold phosphate buffered saline (PBS) to the laboratory for further processing.

3.3.2 Peritoneal fluid

Peritoneal fluid (PF) was obtained from women with endometriosis at laparoscopy for infertility or pelvic pain, and from control women at laparoscopy for sterilization, pelvic pain or benign ovarian cysts. The PF was aspirated from the pouch of Douglas before other surgical interventions were done. The aspiration was performed using of a syringe attached to a baby-feeding catheter through the laparoscope and the fluid was collected in sterile vacuum tubes. PF contaminated with blood was avoided.
The samples were centrifuged and the supernatants were stored in –70° C.

3.3.3 Blood samples

Blood samples were obtained in the morning before surgery. For PA and PAI antigen, blood was collected in citrated vacuum tubes (Diatube H®) and for assays of serum level of estradiol and progesterone blood was collected in sterile vacuum tubes. The samples were centrifuged at 2,000x g for 10 minutes at room temperature and the plasma was stored at -70° C.

3.4 LABORATORY PROCEDURES

3.4.1 Tissue homogenate (*Paper I and V*)

The frozen samples of endometriotic and endometrial tissue were thawed, rinsed in PBS added with 0.5 M sodium chloride and dried on filter-paper before they were weighed. The tissue samples were then homogenized with 1 mL PBS plus 0.5 M sodium chloride by the use of a Polytron homogeniser and centrifuged at 10,000 x g for 10 minutes at 4° C. The supernatants were diluted with PBS plus 0.5 M sodium chloride to contain 0.01 g tissue/mL, and stored at -70° C until assayed. The PBS was added with 0.5 M sodium chloride in order to release the PAs and PAIs from the tissue.

3.4.2 Isolation and culture of cells (*Paper IV*)

The following procedures were performed under sterile conditions. The fresh tissue was cut with scissors into small pieces of about 1 mm^3 and rinsed carefully in PBS to remove blood. Digestion of the tissue was performed for 1 hour at 37°C with shaking every 15 minutes with 0.25 % type I A collagenase (Sigma, St Louis, MO) in Dulbecco´s Modified Eagle Medium Nutrient Mixture F-12 (D-MEM/F-12) without phenol red, containing 10 % fetal bovine serum (FBS), 1 % of 1000 IU / mL penicillin, 1000 µg / mL streptomycin, and 1 % glutamine (the medium and its supplements were obtained from Invitrogen, Life Technologies, Carlsbad, CA). Stromal and epithelial cells were separated by a series of filtration and centrifugation steps. The first filtration step was performed through a 100 µm cell stainer (Becton Dickinsson Labware, Franklin Lakes, NJ) to remove mucous material, debris and undigested tissue. The cells were freed from collagenase by centrifugation (5 min, 400 x g). The pellet was then re suspended in D-MEM/F-12. Following centrifugation at 55 x g for 2 min, the supernatant contained the stromal cells, and the epithelial cells were collected in the pellet.

3.4.2.1 Culture of stromal cells

The supernatant containing isolated stromal cells was transferred into a 25 cm^2 plastic culture flask. After one passage for removing macrophages the cells were seeded at 50,000 cells/well (24-well plates), and an adhesion period of one week followed of which the cells were incubated with D-MEM/F-12, containing 10 % FBS. The cells were incubated in an atmosphere of 5 % CO_2 / 95 % air and the medium was changed every second day. After completed adhesion period, the medium was changed and collected after 24 hours. The media were centrifuged at 2860 x g for 5 min to remove dead cells, and stored at –20 °C until assayed for uPA, PAI-1 and suPAR. The stromal cell cultures were 99 % pure when analyzed by immunocytochemistry using antibodies against vimentin and cytokeratin (ZYMED, San Fransisco, CA).

3.4.2.2 Culture of epithelial cells

The epithelial cell pellet was washed again and centrifuged at 55 x g for 2 min and the new pellet was re-suspended in 5 ml D-MEM/F-12. Filtration of the epithelial cell suspension was then carried out through a 40 μm sieve (Becton Dickinson Labware), allowing passage of contaminating stromal cells as well as of single epithelial cells. The retained large glands were backwashed from the sieve with 5 ml D-MEM/F-12 and again centrifuged at 55 x g for 2 min. The final cell pellet was resuspended in defined keratinocyte serum-free medium (Invitrogen, Life Technologies) supplemented with insulin, epidermal growth factor (EGF 0.03 μM), and fibroblast growth factor (FGF). The epithelial cells were seeded with a density of 2-5 x 10^5 cells/cm^2 on a transparent Transwell Clear membrane insert with a membrane poor size of 0.4 μm (Invitrogen, Life Technologies). The membrane insert had previously been coated with 40 μL matrigel (Invitrogen, Life Technologies, Carlsbad, CA, USA) diluted 1:3 with PBS and allowed to gel for one hour at 37 °C. The inserts were placed in a 24-well tissue culture plate, so that a dual-chambered system resulted, which provided access of the medium to both sides of the membrane. The medium was replaced every second day, 0.25 mL in the apical compartment and 0.5 mL in the basal compartment. As this was a short-term study, we did not wait for cells growing into confluence. After an adhesion period of four days in an atmosphere of 5 % CO_2/95% air in 37 °C, the culture medium from the basal compartment was collected, centrifuged at 2860 x g for five min to dispose dead cells, and stored at –20 °C until assayed for uPA, PAI-1 and suPAR. The epithelial cell cultures were 98 % pure when analyzed by immunocytochemistry using antibodies against vimentin and cytokeratin (ZYMED).

3.4.2.3 Macrophage staining

Both stromal and epithelial cell cultures were checked for contamination of macrophages by the use of a primary mouse-antihuman monoclonal CD_{14} antibody (ZYMED) as previously described by Guan *et al.* (2002). Briefly, stromal and epithelial cells were incubated with the antibody in parallel with macrophages obtained from peripheral venous human blood. Following incubation with the primary antibody, a detection kit (ZYMED) including a secondary antibody was used to identify macrophages. 0.1 % of CD_{14} positive staining was obtained from cultured stromal cells and 1 % from cultured epithelial cells compared with cultured macrophages from human peripheral venous blood. The degree of macrophage contamination in the cell cultures was thus very small and seems very unlikely to have affected our results.

3.4.3 Immunoassays (*Paper I, VI and V*)

3.4.3.1 Paper I

The antigen levels of tPA, uPA and PAI-1 in tissue homogenates, PF and plasma were assayed by enzyme linked immunosorbent assay (ELISA) using commercial kits from Biopool AB, Umeå, Sweden. The immunoassay for tPA (IMULYSE tPA) detects free as well as tPA in complex with PAI-1. The uPA assay (TintElize uPA) detects single-chain, high- and low molecular weight forms of uPA. The immunoassay for PAI-1 (IMULYSE PAI-1) detects unbound active and latent PAI-1 and PAI-1 in complex with PAs to>70%. PAI-2 was assayed with the use of an in house ELISA using monoclonal and polyclonal antibodies (Lecander *et al.,* 1987) detecting 60 % of the inhibitor in complex with PAs. Detection limits were for tPA 1.5 μg /L, for uPA 0.1 μg/L, for PAI-1 1 μg/L and for PAI-2 5μg/L respectively.

3.4.3.2 Paper IV

The levels of uPA, PAI-1 and suPAR released from cultured endometriotic and endometrial stromal and epithelial cells, were assayed by ELISA using commercial kits from Biopool AB (uPA) and from American Diagnostica Inc., Greenwich, CT, (Imubind PAI-1 and suPAR). The PAI-1 assay detects latent and active forms of PAI-1 and PAI-1 complexes and the uPAR assay soluble, native and recombinant uPAR as well as uPAR/uPA and uPAR/uPA/PAI-1 complexes. The detection limits were for PAI-1 0.05 µg /L and for uPAR 0.1 µg/L, respectively.

3.4.3.3 Paper V

The levels of IL-β in the tissue homogenates were assayed by ELISA using commercial kit from R&D Systems, Minneapolis, MN and IL-6 and TNFα by immunoradiometric methods using commercial kits from Medgenix SA, Fleurus, Belgium.The detection limits were for IL-1β 1 ng/L, and for IL-6 and TNFα 5ng/L respectively.

3.4.4 *In situ* hybridisation (*Paper II*)

The method is described in detail in *Paper II*. Briefly, to obtain sense and anti sense probes for uPA, PAI-1 and uPAR, cRNA probes were transcribed from DNA templates and labelled with ^{35}S-UTP. Prior to hybridisation, cryostat sections of endometriotic and endometrial tissue were fixed, deproteinated, and dehydrated. The sections were then incubated in a hybridisation buffer with labelled probes over night at 55° C. After hybridisation, the sections were washed, immersed in RNase, de-salted, dehydrated and air-dried. The slides were coated with nuclear track emulsion and after a exposure at 4°C for 3-14 weeks the slides were developed and counterstained with Cresyl Violet. Radiolabelled sense probes served as negative controls. The sections were analysed in a microscope with light- and dark-field illumination. A semi quantitative, four-grade scale was used for evaluation of the mRNA expression in the tissues.

3.4.5 Immunohistochemistry (*Paper III*)

The method is described in detail in *Paper III.* Monoclonal antibodies against human uPA, PAI-1 and uPAR were used (American Diagnostica Inc.). Immunostaining was performed on cryostat sections of endometriotic and endometrial tissue. Briefly, the sections were fixed, and endogenous peroxidase activity blocked. Non-specific staining was blocked with normal horse serum and to reduce background staining a avidin/biotin blocking kit were used. The sections were incubated with primary antibodies overnight at 4° C. The biotinylated second antibody was added followed by the avidin-biotin-peroxidase complex and antibody binding was visualized by the addition of 3-amino-9-ethyl-carbazole (AEC). Repeated rinses in PBS with Triton X-100 were performed during the immunostaining process. Finally, the sections were counterstained with haematoxylin and mounted. PBS replaced the primary antibody on sections that served as negative controls. Slides of human breast cancer served as positive controls. A semi quantitative, four-grade scale was used for evaluation of the staining.

3.4.6 Statistical analyses (*Paper I, II, IV and V*)

The Kruskal-Wallis test, followed by *post hoc* analysis by Mann-Whitney U-test, was used when comparing differences between three different groups and the Mann-Whitney U test to analyse differences between two different groups (*Paper I, II, IV and V*). Differences between paired observations were analysed by Wilcoxons signed rank test or t-test for paired observations (*Paper I and IV*). Correlations were tested by Spearman´s rank correlation test (*Paper IV and* V). The values were not normally distributed and are given as median and range. $P<0.05$ was defined as statistically significant.

4 RESULTS

4.1 PAPER I

To determine whether there is a difference in the components of the PA system, that might be of importance for the invasive growth of endometriosis, homogenates of endometriotic tissue and endometrium from women both with and without endometriosis was assayed for the antigen concentrations of tPA, uPA, PAI-I and PAI-2. Furthermore, the antigen concentrations in PF and plasma from women with endometriosis as well as in PF from women without endometriosis were assayed.

The major differences found in this study were related to uPA and PAI-1 in tissue homogenates and PAI-2 in PF. When comparing the three types of tissue we found that the concentrations of uPA and PAI-I, were significantly higher in endometriotic tissue and in endometrium from women with endometriosis compared to control endometrium ($p<0.001$, $p<0.01$ and $p<0.001$, $p<0.05$ respectively). In paired samples from the same women, we found that the concentration of PAI-1 was significantly higher in endometriotic tissue than in endometrium ($p<0.05$). Later calculations, not shown in the paper, reveal a significantly positive correlation for both PAI-1 and PAI-2 in endometriotic tissue and in endometrium from the same women ($r_s = 0.81$, $p<0.05$ and $r_s = 0.76$, $p<0.05$).

In PF the concentration of PAI-2 was significantly higher in women with endometriosis than in controls ($p<0.01$). In paired samples of PF and plasma from women with endometriosis, the concentration of tPA was significantly higher in PF ($p<0.05$). When all samples of PF and plasma were compared, the concentrations of both tPA and PAI-2 were significantly higher in PF ($p<0.001$ and $p=0.051$, respectively). The only statistically significant difference concerning cycle phases was in the proliferative phase for PAI-2 and in the secretory phase for uPA in PF, where the concentrations of the antigens were higher in women with endometriosis ($p<0.01$, and $p<0.05$, respectively).

4.2 PAPER II

In this study we investigated the expression of mRNA for uPA, PAI-1 and uPAR by *in situ* hybridisation in glandular and stromal cells from endometriotic tissue and endometrium from patients with endometriosis and from control endometrium. We also wanted to see if there were any differences in the mRNA expression related to the menstrual cycle phase.

uPA mRNA was mainly expressed in stromal cells in the three types of tissue, but also in endometriotic glandular cells. Endometrial stromal cells from women with endometriosis, expressed significantly stronger signals for uPA mRNA than stromal cells from control endometrium both in proliferative and secretory phase ($p<0.05$). There were, however, no statistically significant differences in uPA mRNA expression between cycle phases, either in glands or in stroma, in the three types of tissue. In endometriotic glands and stroma and in endometrial stroma from women with

endometriosis, the hybridisation signals were frequently localized in clusters. In endometrial stromal cells from controls, the signal pattern was more dispersed.

PAI-1 mRNA was exclusively expressed in stromal cells in the three types of tissue. Both endometriotic and endometrial stroma from patients with endometriosis expressed significantly stronger signals for PAI-1 mRNA than endometrial stroma from controls ($p<0.01$ and $p<0.05$, respectively). There were no statistically significant differences in the expression for PAI-1 mRNA between cycle phases in the three types of tissue. Like uPA mRNA, PAI-1 mRNA was frequently localized in clusters in endometriotic and endometrial stroma from women with endometriosis, while in endometrial stroma from controls the signal pattern was more dispersed.

uPAR mRNA was expressed both in glands and in stroma in the three types of tissue. In endometrial glandular cells from women with endometriosis the hybridisation signals were expressed with extraordinarily varying intensity, from weak to strong. In endometriotic glands and stroma the uPAR mRNA expression was significantly stronger than in endometrial glands and stroma from women without endometriosis ($p<0.01$ respectively) as well as in endometrial stroma from women with endometriosis ($p<0.05$). There was also a statistically significant difference in uPAR mRNA expression between endometrial glands from women with endometriosis versus controls ($p<0.01$). In endometrial glands from women with endometriosis as well as in controls there was significantly stronger signals for uPAR mRNA in proliferative phase compared to the secretory phase for both ($p<0.05$ respectively). There were however, no statistically significant differences in the expression of hybridisation signals for uPAR between the cycle phases in stroma from the three tissue types.

4.3 PAPER III

The aim of this study was, following the results from our previous studies (*Paper I and II*), to localize uPA, PAI-1 and uPAR in morphologically intact endometriotic and endometrial tissue slices, and to study the expression of the proteins in situ when stromal and glandular epithelial cells may interact in a paracrine way and all cells are exposed to the variations in the circulating hormonal levels.

uPA and uPAR were localized in both epithelial and stromal cells in the three tissue types, above all to the cytoplasm, but uPA and in a few cases uPAR, was also found in the nuclei. PAI-1 was above all localized in the cytoplasm in stromal cells, but a faint staining was also observed in a few cases in the cytoplasm in epithelial cells in endometriotic and endometrial samples from women with endometriosis.

When comparing the three tissue types, the immunostaining of uPA was most prominent in the proliferative phase in endometrial tissue from women with endometriosis compared to the two other. In the secretory phase, there was a slight tendency to stronger staining in endometriotic tissue compared to endometrial tissue from women with endometriosis, but there was an obvious stronger staining in both endometriotic and endometrial tissue from women with endometriosis for uPA than in endometrial tissue from controls. When comparing the two menstrual phases in each tissue type, there was a tendency to stronger staining in the secretory phase in endometriotic tissue, but in the two endometrial tissue types there were a tendency to stronger staining in the proliferative phase.

Comparing the three tissue types, it seemed to be a slight stronger staining for PAI-1 in endometriotic stroma than in endometrial stroma, both in the proliferative and in the secretory phase. There was no difference between the cycle phases when comparing the proliferative and the secretory phase in each tissue type. But there was an obvious difference in stromal staining in endometrium from two women with endometriosis, whom were in menstrual phase, staining moderate to strong for PAI-1.

The staining for uPAR was most prominent in stromal cells, both in proliferative and secretory phase, in endometrium from women with endometriosis.
In control endometrium it seemed to be a slight stronger staining in glandular cells in proliferative phase compared with endometriotic and endometrial tissue from women with endometriosis. There was a difference in immunostaining in both glandular and stromal cells in endometrium from controls, between the two cycle phases, with stronger staining in the proliferative phase.

4.4 PAPER IV

In this study, we cultured separated and isolated stromal and epithelial cells from endometriotic and endometrial tissue from women with and without endometriosis, to find out what endogenous potential each of these cell types has *in vitro*, to release uPA, PAI-1 and suPAR without any influence of endogenous or exogenous hormones or growth factors or other paracrine interactions.

Both stromal and epithelial cells from endometriotic and endometrial tissue released the three types of antigens, but the release of PAI-1 was significantly higher from stromal cells in the three types of tissue than from epithelial cells ($p<0.01$ respectively). Furthermore, the release of PAI-1 from endometriotic stromal cells was significantly higher than from endometrial stromal cells from women with endometriosis and from controls ($p<0.05$ and $p<0.01$ respectively). A significantly positive correlation between PAI-1 in epithelial and stromal cells from the same patient were found in culture medium from endometrium both from women with endometriosis and controls ($r_s=0.79$, $p<0.05$ and $r_s=0.72$, $p<0.05$).

The release of uPA from stromal cells from control endometrium was significantly higher in the proliferative phase than in the secretory phase ($p<0.05$).

The release of uPAR from epithelial cells from control endometrium was also significantly higher in the proliferative phase compared with the secretory phase ($p<0.05$).

4.5 PAPER V

To obtain more information about the possible roles of cytokines in the autocrine and paracrine growth regulation of endometriosis, we measured the concentrations of IL-1β, IL-6 and TNFα in homogenates from endometriotic tissue and endometrium, both from women with and without endometriosis.

In endometriotic tissue the concentration of IL-1β was significantly higher than in endometrium both from women with and without endometriosis ($p<0.05$ and $p<0.001$ respectively).

In unpaired samples both endometriotic tissue and endometrium from women with endometriosis had significantly higher concentrations of IL-6 compared to control endometrium ($p<0.001$ respectively). In paired samples from women with endometriosis, the concentration of IL-6 was significantly higher in endometriotic tissue than in endometrium from the same women ($p<0.05$).

In contrast to the two other cytokines, the concentration of TNFα was significantly lower in endometriotic tissue compared to control endometrium ($p<0.01$).

When comparing the three types of tissue in each cycle phase, the levels of IL-1β in the proliferative phase was significantly higher in endometriotic tissue than in endometrium, both from women with and without endometriosis ($p< 0.01$ and $p<0.001$ respectively). In secretory phase a significant difference was found for IL-6 in endometrium from women with endometriosis compared with control endometrium ($p<0.01$). When comparing the two cycle phases in each tissue type, the only statistical significant difference found was in endometrium from women with endometriosis where the level of IL-1β was higher in the secretory phase ($p<0.01$).

The tissue concentrations of IL-1β and IL-6 showed significant positive correlations in endometriotic tissue and in endometrium from controls ($r_s =0.65$, $p<0.01$ and $r_s =0.44$, $p<0.05$).

Concerning the type of endometriotic lesion, endometriotic cysts had significantly higher IL-1β content than lesions from other locations ($p<0.01$).

5 DISCUSSION

Endogenous up-regulation of uPA, PAI-1 and uPAR or a product of leukocyte infiltration?

The results show that there is a significant difference in both the mRNA expression (*Paper* II) and the protein levels (*Paper I, III*) for uPA, PAI-1 and uPAR as well as the release of these factors (*Paper IV*) in endometriotic and endometrial tissue from women with endometriosis compared with endometrial tissue from women without endometriosis. The higher levels of uPA, PAI-1 and uPAR found in women with endometriosis, may be due to either an up-regulation of the factors by stromal and/or epithelial cells in endometriotic and endometrial tissue or the factors may be produced by infiltrating inflammatory cells.We observed that the mRNA for uPA and PAI-1 in endometriotic and endometrial stroma from women with endometriosis was more often expressed in clusters compared to controls (*Paper II*). This cluster formation may represent one single inflammatory cell, but the method did not allow discriminating between cells. The cluster formations did not show any cycle variation.

Leukocyte infiltration in normal endometrial stroma begins at the end of the secretory phase, reaches a maximum at the first day of menses and then declines and disappears in early proliferative phase (Noyes *et al.,* 1950). Klentzeris and co-workers (1995) showed that the endometrial leukocyte contents of women with endometriosis did not differ from that of women without endometriosis. In both groups, the number of endometrial granulated lymphocytes and macrophages increased significantly between the early and late secretory phase. Neither Fernández-Shaw and co-workers (1995) nor Jones and co-workers (1996) found any differences in stromal leukocyte infiltration between eutopic endometrium from women with endometriosis and control endometrium.

When studying macrophage infiltration of the endometrial stroma, Braun and co-workers (2002) however saw a reduced number of macrophages during early proliferative and late secretory phases in woman with endometriosis compared to controls. Thus, there is a cyclic variation of infiltrating inflammatory cells in endometrium both from women with and without endometriosis. Accordingly, it is not likely that the cluster formation observed in endometrial stroma from women with endometriosis, not varying during the menstrual cycle, would correspond to uPA and/or PAI-1 mRNA producing inflammatory cells, unless there is a functional difference of endometrial leukocytes between the two groups. However, in ectopic endometrium, the number of leukocytes is within the same range as that of eutopic endometrium in late secretory phase without any obvious cycle variations (Fernández-Shaw *et al.,* 1995, Jones *et al.,* 1996). The percentage of macrophages is significantly reduced in ectopic compared to eutopic endometrium in proliferative and early secretory phase (Jones *et al.,* 1996). Thus it cannot be excluded that the cluster formations observed in endometriotic glands and stroma represent uPA and PAI-1 mRNA producing inflammatory cells.

Cytokines as regulators of PAI-1

In cell culture, however, isolated endometriotic stromal cells released significant amounts of PAI-1 into culture medium without any paracrine influence as *in vivo*. The contamination degree of macrophages in the cell cultures was very low, 0.1 %, why it seems unlikely that macrophages have affected the result. The culture medium we used contained 10 % FBS. It has been reported that PAI-1 mRNA level increases rapidly following serum stimulation (Ryan *et al.,* 1996) but we have shown in another study that PAI-1 is released from endometriotic stromal cells also when serum free culture medium is used (Guan *et al.,* 2003). Thus the addition of FBS to the culture medium does not explain the enhanced release of PAI-1. Taken together, our results suggest an endogenous up-regulation of PAI-1 in endometriotic stromal cells but not in endometrial cells. Known inducers of the gene are different growth factors as TGFβ, EGF, inflammatory cytokines as IL-1, and TNFα.

The level of IL-1β was significantly higher in endometriotic tissue compared to endometrial tissue both from women with and without endometriosis (*Paper V*). The level of IL-1β was significantly higher in proliferative than in secretory phase, indicating that migrating leucocytes including tissue macrophages are presumably not the main source of IL-1β in endometriotic tissue as migrating leukocytes present no cycle phase variation and there is a reduced number of macrophages in proliferative phase (Fernández-Shaw *et al.,* 1995, Jones *et al.,* 1996). Moreover, we have previously in fact shown that both stromal and epithelial cells from endometriotic as well as from endometrial tissue are capable of producing IL-1β *in vitro* (Bergqvist *et al.,* 2000).

The level of TNFα in endometriotic tissue was significantly lower compared to the concentrations in endometrial homogenates from controls (*Paper V*), indicating that TNFα is not the inducer of PAI-1.

TGFβ, which is mainly produced by activated lymphocytes and macrophages (Sporn *et al.,* 1986) but also by epithelial and stromal cells in endometrium (Chegini *et al.,* 1994) and endometriotic tissue (Tamura *et al.,* 1999), induces PAI-1 expression as shown by Lund and co-workers (1987) and Keski-Oja and co-workers (1988) in lung fibroblasts and carcinoma cells *in vitro*. We have shown that addition of TGFβ to the culture medium increased the release of PAI-1 from endometriotic stromal cells but not from endometrial stromal cells (Guan *et al.,* 2003). Others have reported increased release of PAI-1 from endometrial stromal cells stimulated *in vitro* with TGFβ (Sandberg *et al.,* 1998). However, irrespective the fact that exogenous stimulation of TGFβ enhance the production of PAI-1 *in vitro,* the question remains whether endogenous TGFβ in endometriotic stromal cells but not epithelial cells increases PAI-1 release by an autocrine way. The same could be argued for the PAI-1 release from endometrial stromal and epithelial cells.Consequently, IL-1 remains as a possible endogenous inducers of PAI-1 in endometriotic stromal cells.

PAI-1, TNFα and apoptosis

An impaired spontaneous apoptosis in the endometrial glands in women with endometriosis during late secretory/menstrual and early proliferative phases has been

demonstrated by Dmowski and co-workers (2001) and this may contribute to an increased viability of shed endometrial cells in these women. The same group have also shown a decreased apoptosis in ectopic endometrium (Dmowski *et al.,* 1998, Gebel *et al.,* 1998). Addition of PAI-1 inhibits apoptosis of tumour cells *in vitro* (Kwaan *et al.,* 2000). This anti-apoptotic effect of PAI-1 was also observed when PAI-1 was added to non-tumoral cells in culture (Kwaan *et al.,* 2000). The authors suggested that this could explain why high levels of PAI-1 have been found to be a bad prognostic factor in a number of tumours.

It can be hypothesized that the higher levels of PAI-1 in endometriotic and endometrial tissue from women with endometriosis have significance for the impaired apoptosis in endometrial glands and for the invasiveness of endometriotic tissue. Using IHC we demonstrated a stronger expression of PAI-1 in endometriotic tissue, but not in endometrium from women with endometriosis compared to controls.

TNFα is regarded as a primary local signal initiating and modulating apoptosis during menstruation (Tabibzadeh *et al.,* 1996). The lower levels of TNFα in endometriotic and endometrial tissue from women with endometriosis compared to control endometrium (*Paper* V) might also contribute to the impaired spontaneous apoptosis, resulting in survival of the refluxed endometrial tissue, more prone to adhere to other structures in the pelvis and to the invasive property of endometriotic tissue.

Menstrual shedding and adhesive properties

A prerequisite for the development of endometriosis is probably the adhesion of viable endometrial fragments to the peritoneum. This has been demonstrated *in vitro* by several studies (van der Linden *et al.,* 1996, Groothuis *et al.,* 1998, Koks *et al.,* 1999, Witz *et al.,* 2001, 2002). Shed fragments of endometrium from women with endometriosis might possess the necessary proteolytic properties for adhesion and implantation. The over expression of uPA and uPAR in endometrial tissue and of uPA and PAI-1 in ectopic implants from women with endometriosis predispose to both. The environment in the peritoneal cavity with activated macrophages producing excess amounts of cytokines and growth factors in the PF might contribute to the adhesion and implantation. Production of fibronectin important for the adhesion process has been reported (Kauma *et al.,* 1988) as well as secretion of IL-1, TNFα, EGF, and TGFα and of the angiogenic cytokines VEGF, TGFβ and IL-8 (Arici *et al.,* 1996, McLaren *et al.,* 1996) contributing to proliferation of the endometrial tissue (Zhang *et al.,* 1991, Braun *et al.,* 1994).

The probably most important consequence of the increased expression of uPA and uPAR in endometrium from women with endometriosis might be an enhanced pericellular proteolysis of the ECM leading to a disturbed menstrual shedding. Also the higher levels of IL-6 in endometrium from women with endometriosis compared to control endometrium might have consequences for the menstrual shedding. IL-6 has been proposed to contribute the maintenance of homeostasis in normal endometrium and that disturbances of IL-6 mediated responses may play a role in disorders of the endometrium in women with endometriosis (Zarmakoupis *et al.,* 1995). This might result in a retrograde transportation of viable endometrial tissue fragments with a higher

potential to implant and invade other structures. Endometrial fragments with an intact microstructure express adhesion molecules (van der Linden, 1995) and invade ECM earlier than isolated endometrial stromal cells (Wild *et al.,* 1994).

Cell adhesion to components of the ECM is largely determined by the integrin composition of the individual cell, but integrin-independent cell adhesion may also be important (Gumbiner 1996, Deng *et al.,* 2001). uPAR can act as a cell adhesion receptor through its interaction with vitronectin in the ECM, and the adhesive properties of cells depend on the relative number of uPAR on their surface (Deng *et al.,* 2001). Binding of uPA to uPAR increases the binding affinity of uPAR to the somato medin B domain of vitronectin, which promotes cell adhesion (Deng *et al.,* 1996). It also stimulates cell migration by plasmin-catalyzed degradation of adhesion receptors, i.e. integrins (Andreasen *et al.,* 2000). The higher expression of uPA and uPAR and the low expression of PAI-1 (Palmieri *et al.,* 2002) in endometrium from women with endometriosis may contribute to adhesion of shed endometrial fragments to other structures and to cell migration.

Over expression of suPAR *in vitro* from endometrial cells from women with endometriosis was reported by Sillem and co-workers (1997, 1998). We found no difference between women with or without endometriosis in the release of suPAR from endometrial stromal and epithelial cells *in vitro*. But they used a different culture technique. They used enzymatically digested endometrial tissue containing mixed cell types, which might result in paracrine influences between the cells, affecting the release. We found that uPAR mRNA was up-regulated in endometrial glands from women with endometriosis compared to control endometrium, and we also saw a difference in protein staining using IHC, with stronger staining in both glands and stroma in the secretory phase in women with endometriosis. We did not assay suPAR in homogenates, but it might be hypothesized that an over expression of cell membrane anchored uPAR also reflects an over expression of suPAR. It has been suggested that suPAR promotes cell adhesion and chemotaxis of monocytotic cells (Sidenius *et al.,* 2000).

Invasion

PAI-1 shares the binding-site on vitronectin with uPAR and since the affinity is higher than that of uPAR, PAI-1 competitively inhibits the attachment of cells to vitronectin (Deng *et al.,* 1996). Moreover, PAI-1 also blocks integrin-mediated cell adhesion and migration (Stefansson and Lawrence, 1996; Kjøller *et al.,* 1997). Furthermore PAI-1 detaches cells by disrupting uPAR-vitronectin and integrin-vitronectin interactions (Czekay *et al.,* 2003). The classic role of PAI-1 is to act as an inhibitor of uPA (and tPA) and a regulator of plasminogen-mediated degradation of ECM in order to inhibit invasion. Contradictory, high levels of PAI-1 in several types of cancer have been reported to relate to poor prognosis for patients (Duffy, 1996). The reason is unclear, but PAI-1 induced cell detachment that facilitates metastatic spreading might be one reason (Deng *et al.,* 1996, Czekay *et al.,* 2003). Another explanation to a high expression of PAI-1 in tumours is that PAI-1 is presumed to protect the cancer tissue itself against the destructive effect of uPA (Pappot *et al.,* 1997). We can only speculate about the significance of the over expression of PAI-1 in endometriotic tissue. However

the simultaneously higher expression of uPA in endometriotic tissue makes it tempting to compare endometriotic tissue with malignant tissue in its capacity to invade surrounding structures.
The higher level of uPA in endometrium from women with endometriosis compared to controls, and the higher level of PAI-1 in endometriotic tissue was recently confirmed by another group (Gilabert-Estellés *et al.,* 2003). They could not find any increased level of uPA in endometriotic tissue, but these diverging results compared to ours might however be referred to the method used.

Production of the angiogenic cytokines VEGF, TGFβ and IL-8 in endometrial tissue has been reported (Arici *et al.,* 1996, McLaren *et al.,* 1996). TGFβ and VEGF are suggested to contribute to the development of endometriosis by promoting neovascularization of endometrial cells attached to the peritoneum (Wieser *et al.,* 2002, Harada *et al.,* 2001). The higher levels of PAI-1 found in endometriotic implants might also contribute to angiogenesis. PAI-1 is highly expressed in angiogenic endothelial cells in neuroblastoma tumours (Sugiura *et al.,* 1999) and PAI-1-deficient mice are resistant to cancer invasion and vascularization but reestablishment of PAI-1 expression in host cells restored tumour vascularization and invasion (Bajou *et al.,* 1998). These data suggest that PAI-1 has a stimulatory function in angiogenesis and Isogai and co-workers demonstrated *in vitro* that PAI-1 promotes angiogenesis by stimulating endothelial cell migration toward fibronectin (Isogai *et al.,* 2001).

PAI-2 in peritoneal fluid

The only significant difference found for fibrinolytic factors in PF was for PAI-2. The higher concentrations of the inhibitor might be a consequence of the inflammatory process related to the acute and chronic inflammation taking place at the adhesion sites in the peritoneal cavity. Recruited, activated macrophages release PAI-2 and high concentrations of PAI-2 have been found in inflamed peritoneal tissue in patients with appendicitis (Whawell *et al.,* 1995). A reduced fibrinolytic activity in peritoneal biopsies from patients with peritonitis, was shown by Ince and co-workers (2002). Reduced fibrinolytic capacity is associated with the development of adhesions (Holmdahl, 1997). IL-1 stimulates fibroblast proliferation, collagen deposition and fibrinogen formation, is elevated in PF in women with endometriosis and is suggested to be responsible for the adhesions and peritoneal fibrosis in women with endometriosis (Haney, 1993). Thus taken together, the higher concentration of PAI-2 in PF and of IL-1β in ectopic implants may reflect the increased adhesion formation in women with endometriosis.

Limitations

The discussion above is an attempt to illustrate the possible significance of the results from the different studies in this thesis and to connect the results for further conclusions. However, it is important to be aware of that the knowledge of processes involving the PA system as adhesion, migration and invasion is entirely based on *in vitro* studies and might only reflect a widely more complex situation *in vivo*. There are also limitations in the methods used to try to identify processes involving the PA

system (Andreasen *et al.,* 1997), e.g. there is not necessarily a correlation between the amount of a certain protein in a certain localization and its functional importance there.

6 CONCLUSIONS AND FUTURE PERSPECTIVE

♦ The results in this thesis have shown that there is a significant difference between endometriotic tissue and endometrium from women with endometriosis compared to endometrium from women without endometriosis concerning the expression of both the fibrinolytic factors uPA, PAI-1 and uPAR and the cytokines IL-1β, IL-6 and TNFα.

♦ The increased expression of uPA and uPAR in endometrium from women with endometriosis will possibly enhance the pericellular proteolysis and degradation of ECM and lead to a disturbed menstrual shedding resulting in a retrograde rejection of intact, viable endometrial tissue fragments with higher potential to implant and invade.

♦ The spontaneous apoptosis in endometrial and endometriotic tissue might be impaired by the over expression of PAI-1. This may lead to an increased survival of shed endometrial fragments with an enlarge potential to implant and invade the peritoneal lining.

♦ The lower levels of TNFα in endometriotic and endometrial tissue from women with endometriosis may also contribute to an impaired spontaneous apoptosis.

♦ The higher levels of PAI-1 in endometriotic implants may contribute to angiogenesis by stimulating endothelial cell migration.

♦ The simultaneously increased expression of both uPA and PAI-1 in endometriotic tissue may contribute to the conflicting result of higher potential to invade surrounding tissue.

♦ IL-1β is a possible endogenous inducers of PAI-1 in endometriotic stromal cells.

♦ The higher concentration of PAI-2 in PF reduces the fibrinolytic capacity and the higher level of IL-1β in ectopic implants stimulates fibroblast proliferation, collagen deposition and fibrinogen formation and this may contribute to the development of adhesions in the pelvis.

Studies by our group and others referred to in this thesis, have demonstrated important differences between eutopic endometrium from women with endometriosis compared to endometrium from healthy women, indicating a disturbance that might be fundamental for the development of endometriosis. Further comparing studies are necessary for the understanding of the pathogenesis of this enigmatic disease. Established methods for cell cultures could be used, as tools to increase our knowledge of how possible therapeutic compounds might act on the release of the PA system components. Especially PAI-1 would be of interest because its possible role in angiogenesis, apoptosis and invasion.

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8 REFERENCES

Akoum A, Lemay A, McColl S, Turcot-Lemay L, Maheux R (1996) Elevated concentration and biologic activity of monocyte chemotactic protein-1 in the peritoneal fluid of patients with endometriosis. Fertil Steril 66: 17-23.

Anderson MC (1993) Mini-symposium:The pathogenesis of endometriosis. Current Obstetrics & Gynaecology 3: 122-126.

Andreasen PA, Egelund R, Petersen HH (2000) The plasminogen activation system in tumor growth, invasion, and metastasis. Cell Mol Life Sci 57: 25-40.

Andreasen PA, Kjoller L, Christensen L, Duffy MJ (1997) The urokinase-type plasminogen activator system in cancer metastasis: a review. Int J Cancer 72: 1-22.

Andreasen PA, Riccio A, Welinder KG, Douglas R, Sartorio R, Nielsen LS, Oppenheimer C, Blasi F, Dano K (1986) Plasminogen activator inhibitor type-1: reactive center and amino-terminal heterogeneity determined by protein and cDNA sequencing. FEBS Lett 209: 213-218.

Appella E, Robinson EA, Ullrich SJ, Stoppelli MP, Corti A, Cassani G, Blasi F (1987) The receptor-binding sequence of urokinase. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440.

Arai KI, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T (1990) Cytokines: coordinators of immune and inflammatory responses. Annu Rev Biochem 59: 783-836.

Arici A, Tazuke SI, Attar E, Kliman HJ, Olive DL (1996) Interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukin-8 expression in human mesothelial cells. Mol Hum Reprod 2: 40-45.

Astedt B, Lecander I (1987) The placental type plasminogen activator inhibitor. Fibrinolysis 1: 203-208.

Bajou K, Noel A, Gerard RD, Masson V, Brunner N, Holst-Hansen C, Skobe M, Fusenig NE, Carmeliet P, Collen D, Foidart JM (1998) Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. Nat Med 4: 923-928.

Behrendt N, Ploug M, Patthy L, Houen G, Blasi F, Dano K (1991) The ligand-binding domain of the cell surface receptor for urokinase-type plasminogen activator. J Biol Chem 266: 7842-7847.

Bergqvist A (1993) Different types of extragenital endometriosis: a review. Gynecol Endocrinol 7: 207-221.

Bergqvist A, Nejaty H, Froysa B, Bruse C, Carlberg M, Sjoblom P, Soder O (2000) Production of interleukins 1beta, 6 and 8 and tumor necrosis factor alpha in separated and cultured endometrial and endometriotic stromal and epithelial cells. Gynecol Obstet Invest 50: 1-6.

Braun DP, Ding J, Shen J, Rana N, Fernandez BB, Dmowski WP (2002) Relationship between apoptosis and the number of macrophages in eutopic endometrium from women with and without endometriosis. Fertil Steril 78: 830-835.

Braun DP, Muriana A, Gebel H, Rotman C, Rana N, Dmowski WP (1994) Monocyte-mediated enhancement of endometrial cell proliferation in women with endometriosis. Fertil Steril 61: 78-84.

Carmeliet P, Moons L, Lijnen R, Baes M, Lemaitre V, Tipping P, Drew A, Eeckhout Y, Shapiro S, Lupu F, Collen D (1997) Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat Genet 17: 439-444.

Casslen B (1981) Proteinases and proteinase inhibitors in uterine fluid with special reference to IUD-users. Acta Obstet Gynecol Scand Suppl 98: 5-37.

Casslen B, Astedt B (1981) Fibrinolytic activity of human uterine fluid. Acta Obstet Gynecol Scand 60: 55-58.

Casslen B, Astedt B (1983) Occurrence of both urokinase and tissue plasminogen activator in the human endometrium. Contraception 28: 553-564.

Casslen B, Gustavsson B, Angelin B, Gafvels M (1998) Degradation of urokinase plasminogen activator (UPA) in endometrial stromal cells requires both the UPA receptor and the low-density lipoprotein receptor-related protein/alpha2-macroglobulin receptor. Mol Hum Reprod 4: 585-593.

Casslen B, Gustavsson B, Astedt B (1991) Cell membrane receptors for urokinase plasminogen activator are increased in malignant ovarian tumours. Eur J Cancer 27: 1445-1448.

Casslen B, Nordengren J, Gustavsson B, Nilbert M, Lund LR (1995) Progesterone stimulates degradation of urokinase plasminogen activator (u-PA) in endometrial stromal cells by increasing its inhibitor and surface expression of the u-PA receptor. J Clin Endocrinol Metab 80: 2776-2784.

Casslen B, Urano S, Ny T (1992) Progesterone regulation of plasminogen activator inhibitor 1 (PAI-1) antigen and mRNA levels in human endometrial stromal cells. Thromb Res 66: 75-87.

Chegini N, Zhao Y, Williams RS, Flanders KC (1994) Human uterine tissue throughout the menstrual cycle expresses transforming growth factor-beta 1 (TGF beta 1), TGF beta 2, TGF beta 3, and TGF beta type II receptor messenger ribonucleic acid and protein and contains. Endocrinology 135: 439-449.

Chung HW, Wen Y, Ahn JJ, Moon HS, Polan ML (2001) Interleukin-1beta regulates urokinase plasminogen activator (u-PA), u-PA receptor, soluble u-PA receptor, and plasminogen activator inhibitor-1 messenger ribonucleic acid expression in cultured human endometrial stromal cells. J Clin Endocrinol Metab 86: 1332-1340.

Cubellis MV, Wun TC, Blasi F (1990) Receptor-mediated internalization and degradation of urokinase is caused by its specific inhibitor PAI-1. EMBO J 9: 1079-1085.

Czekay RP, Aertgeerts K, Curriden SA, Loskutoff DJ (2003) Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. J Cell Biol 160: 781-791.

Dano K (1994) The urokinase receptor, protein structure and role in plasminogen activation and cancer invasion. Fibrinolysis 8: 189-203.

Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L (1985) Plasminogen activators, tissue degradation, and cancer. Adv Cancer Res 44: 139-266.

de Bock CE, Wang Y (2004) Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. Med Res Rev 24: 13-39.

Deng G, Curriden SA, Hu G, Czekay RP, Loskutoff DJ (2001) Plasminogen activator inhibitor-1 regulates cell adhesion by binding to the somatomedin B domain of vitronectin. J Cell Physiol 189: 23-33.

Deng G, Curriden SA, Wang S, Rosenberg S, Loskutoff DJ (1996) Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? J Cell Biol 134: 1563-1571.

D'Hooghe TM, Bambra CS, Raeymaekers BM, De Jonge I, Lauweryns JM, Koninckx PR (1995) Intrapelvic injection of menstrual endometrium causes endometriosis in baboons (Papio cynocephalus and Papio anubis). Am J Obstet Gynecol 173: 125-134.

Dickinson JL, Bates EJ, Ferrante A, Antalis TM (1995) Plasminogen activator inhibitor type 2 inhibits tumor necrosis factor alpha-induced apoptosis. Evidence for an alternate biological function. J Biol Chem 270: 27894-27904.

Dmowski WP, Ding J, Shen J, Rana N, Fernandez BB, Braun DP (2001) Apoptosis in endometrial glandular and stromal cells in women with and without endometriosis. Hum Reprod 16: 1802-1808.

Dmowski WP, Gebel H, Braun DP (1998) Decreased apoptosis and sensitivity to macrophage mediated cytolysis of endometrial cells in endometriosis. Hum Reprod Update 4: 696-701.

Duffy MJ (1996) Proteases as prognostic markers in cancer. Clin Cancer Res 2: 613-618.

Duval-Jobe C, Parmely MJ (1994) Regulation of plasminogen activation by human U937 promonocytic cells. J Biol Chem 269: 21353-21357.

Ellis V, Behrendt N, Dano K (1991) Plasminogen activation by receptor-bound urokinase. A kinetic study with both cell-associated and isolated receptor. J Biol Chem 266: 12752-12758.

Ellis V, Scully MF, Kakkar VV (1989) Plasminogen activation initiated by single-chain urokinase-type plasminogen activator. Potentiation by U937 monocytes. J Biol Chem 264: 2185-2188.

Eskenazi B, Warner ML (1997) Epidemiology of endometriosis. Obstet Gynecol Clin North Am 24: 235-258.

Fasciani A, Bocci G, Xu J, Bielecki R, Greenblatt E, Leyland N, Casper RF (2003) Three-dimensional in vitro culture of endometrial explants mimics the early stages of endometriosis. Fertil Steril 80: 1137-1143.

Fernandez-Shaw S, Clarke MT, Hicks B, Naish CE, Barlow DH, Starkey PM (1995) Bone marrow-derived cell populations in uterine and ectopic endometrium. Hum Reprod 10: 2285-2289.

Foster DC, Stern JL, Buscema J, Rock JA, Woodruff JD (1981) Pleural and parenchymal pulmonary endometriosis. Obstet Gynecol 58: 552-556.

Fujii S (1991) Secondary mullerian system and endometriosis. Am J Obstet Gynecol 165: 219-225.

Gaetje R, Kotzian S, Herrmann G, Baumann R, Starzinski-Powitz A (1995) Invasiveness of endometriotic cells in vitro. Lancet 346: 1463-1464.

Gebel HM, Braun DP, Tambur A, Frame D, Rana N, Dmowski WP (1998) Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis. Fertil Steril 69: 1042-1047.

Gilabert-Estelles J, Estelles A, Gilabert J, Castello R, Espana F, Falco C, Romeu A, Chirivella M, Zorio E, Aznar J (2003) Expression of several

components of the plasminogen activator and matrix metalloproteinase systems in endometriosis. Hum Reprod 18: 1516-1522.

Groothuis PG, Koks CA, de Goeij AF, Dunselman GA, Arends JW, Evers JL (1998) Adhesion of human endometrium to the epithelial lining and extracellular matrix of amnion in vitro: an electron microscopic study. Hum Reprod 13: 2275-2281.

Groothuis PG, Koks CA, de Goeij AF, Dunselman GA, Arends JW, Evers JL (1999) Adhesion of human endometrial fragments to peritoneum in vitro. Fertil Steril 71: 1119-1124.

Guan YM, Carlberg M, Bruse C, Bergqvist A (2003) Impact of epidermal growth factor and transforming growth factor beta-1 on the release of fibrinolytic factors from cultured endometrial and ovarian endometriotic stromal cells. Gynecol Obstet Invest 55: 7-13.

Guan YM, Carlberg M, Bruse C, Carlstrom K, Bergqvist A (2002) Effects of hormones on uPA, PAI-1 and suPAR from cultured endometrial and ovarian endometriotic stromal cells. Acta Obstet Gynecol Scand 81: 389-397.

Gumbiner BM (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84: 345-357.

Halban J (1925) Hysteroadenosis Metastatica. Arch Gynaecol 124: 457-468.

Halme J, Becker S, Wing R (1984) Accentuated cyclic activation of peritoneal macrophages in patients with endometriosis. Am J Obstet Gynecol 148: 85-90.

Hampton AL, Salamonsen LA (1994) Expression of messenger ribonucleic acid encoding matrix metalloproteinases and their tissue inhibitors is related to menstruation. J Endocrinol 141: R1-3.

Haney AF (1993) Endometriosis, macrophages, and adhesions. Prog Clin Biol Res 381: 19-44.

Harada T, Iwabe T, Terakawa N (2001) Role of cytokines in endometriosis. Fertil Steril 76: 1-10.

Holmdahl L (1997) The role of fibrinolysis in adhesion formation. Eur J Surg Suppl: 24-31.

Hoyer-Hansen G, Ploug M, Behrendt N, Ronne E, Dano K (1997) Cell-surface acceleration of urokinase-catalyzed receptor cleavage. Eur J Biochem 243: 21-26.

Hoyer-Hansen G, Ronne E, Solberg H, Behrendt N, Ploug M, Lund LR, Ellis V, Dano K (1992) Urokinase plasminogen activator cleaves its cell surface receptor releasing the ligand-binding domain. J Biol Chem 267: 18224-18229.

Ince A, Eroglu A, Tarhan O, Bulbul M (2002) Peritoneal fibrinolytic activity in peritonitis. Am J Surg 183: 67-69.

Irigoyen JP, Munoz-Canoves P, Montero L, Koziczak M, Nagamine Y (1999) The plasminogen activator system: biology and regulation. Cell Mol Life Sci 56: 104-132.

Isogai C, Laug WE, Shimada H, Declerck PJ, Stins MF, Durden DL, Erdreich-Epstein A, DeClerck YA (2001) Plasminogen activator inhibitor-1 promotes angiogenesis by stimulating endothelial cell migration toward fibronectin. Cancer Res 61: 5587-5594.

Jones RK, Bulmer JN, Searle RF (1996) Immunohistochemical characterization of stromal leukocytes in ovarian endometriosis: comparison of eutopic and ectopic endometrium with normal endometrium. Fertil Steril 66: 81-89.

Kariya M, Kanzaki H, Takakura K, Imai K, Okamoto N, Emi N, Kariya Y, Mori T (1991) Interleukin-1 inhibits in vitro decidualization of human endometrial stromal cells. J Clin Endocrinol Metab 73: 1170-1174.

Kauma S, Clark MR, White C, Halme J (1988) Production of fibronectin by peritoneal macrophages and concentration of fibronectin in peritoneal fluid from patients with or without endometriosis. Obstet Gynecol 72: 13-18.

Kelly RW, King AE, Critchley HO (2001) Cytokine control in human endometrium. Reproduction 121: 3-19.

Kennedy S (2003) Genetics of endometriosis: a review of the positional cloning approaches. Semin Reprod Med 21: 111-118.

Keski-Oja J, Raghow R, Sawdey M, Loskutoff DJ, Postlethwaite AE, Kang AH, Moses HL (1988) Regulation of mRNAs for type-1 plasminogen activator inhibitor, fibronectin, and type I procollagen by transforming growth factor-beta. Divergent responses in lung fibroblasts and carcinoma cells. J Biol Chem 263: 3111-3115.

Keski-Oja J, Vaheri A (1982) The cellular target for the plasminogen activator, urokinase, in human fibroblasts - 66 000 dalton protein. Biochim Biophys Acta 720: 141-146.

Kjoller L (2002) The urokinase plasminogen activator receptor in the regulation of the actin cytoskeleton and cell motility. Biol Chem 383: 5-19.

Kjoller L, Kanse SM, Kirkegaard T, Rodenburg KW, Ronne E, Goodman SL, Preissner KT, Ossowski L, Andreasen PA (1997) Plasminogen activator inhibitor-1 represses integrin- and vitronectin-mediated cell migration independently of its function as an inhibitor of plasminogen activation. Exp Cell Res 232: 420-429.

Klentzeris LD, Bulmer JN, Liu DT, Morrison L (1995) Endometrial leukocyte subpopulations in women with endometriosis. Eur J Obstet Gynecol Reprod Biol 63: 41-47.

Koh S (1992) Concentrations of plasminogen activators and inhibitors in the human endometrium at different phases of the menstrual cycle. J Reprod Fertil 96: 407-413.

Koks CA, Groothuis PG, Dunselman GA, de Goeij AF, Evers JL (1999) Adhesion of shed menstrual tissue in an in-vitro model using amnion and peritoneum: a light and electron microscopic study. Hum Reprod 14: 816-822.

Kwaan HC, Wang J, Svoboda K, Declerck PJ (2000) Plasminogen activator inhibitor 1 may promote tumour growth through inhibition of apoptosis. Br J Cancer 82: 1702-1708.

Lamm DL, Gittes RF, Benirschke K (1974) Ectopic endometrial glands in lymph nodes masquerading as metastatic adenocarcinoma. J Urol 111: 770-772.

Lebovic DI, Mueller MD, Taylor RN (2001) Immunobiology of endometriosis. Fertil Steril 75: 1-10.

Lecander I, Astedt B (1987) Specific plasminogen activator inhibitor of placental type PAI 2 occurring in amniotic fluid and cord blood. J Lab Clin Med 110: 602-605.

Lockwood CJ, Krikun G, Hausknecht VA, Papp C, Schatz F (1998) Matrix metalloproteinase and matrix metalloproteinase inhibitor expression in endometrial stromal cells during progestin-initiated decidualization and menstruation-related progestin withdrawal. Endocrinology 139: 4607-4613.

Lund LR, Riccio A, Andreasen PA, Nielsen LS, Kristensen P, Laiho M, Saksela O, Blasi F, Dano K (1987) Transforming growth factor-beta is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. EMBO J 6: 1281-1286.

Mars WM, Zarnegar R, Michalopoulos GK (1993) Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. Am J Pathol 143: 949-958.

Matrisian LM (1992) The matrix-degrading metalloproteinases. Bioessays 14: 455-463.

McLaren J, Prentice A, Charnock-Jones DS, Smith SK (1996) Vascular endothelial growth factor (VEGF) concentrations are elevated in peritoneal fluid of women with endometriosis. Hum Reprod 11: 220-223.

Mignatti P, Rifkin DB (1993) Biology and biochemistry of proteinases in tumor invasion. Physiol Rev 73: 161-195.

Moen MH, Magnus P (1993) The familial risk of endometriosis. Acta Obstet Gynecol Scand 72: 560-564.

Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, Daikuhara Y, Tsubouchi H, Blasi F, Comoglio PM (1992) Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. EMBO J 11: 4825-4833.

Nielsen LS, Andreasen PA, Grondahl-Hansen J, Skriver L, Dano K (1986) Plasminogen activators catalyse conversion of inhibitor from fibrosarcoma cells to an inactive form with a lower apparent molecular mass. FEBS Lett 196: 269-273.

Nisolle M, Casanas-Roux F, Anaf V, Mine JM, Donnez J (1993) Morphometric study of the stromal vascularization in peritoneal endometriosis. Fertil Steril 59: 681-684.

Nisolle M, Paindaveine B, Bourdon A, Berliere M, Casanas-Roux F, Donnez J (1990) Histologic study of peritoneal endometriosis in infertile women. Fertil Steril 53: 984-988.

Noyes R (1950) Dating the endometrial biopsy. Fertil Steril 1: 3-25.

Nykjaer A, Conese M, Christensen EI, Olson D, Cremona O, Gliemann J, Blasi F (1997) Recycling of the urokinase receptor upon internalization of the uPA:serpin complexes. EMBO J 16: 2610-2620.

Ossowski L, Aguirre-Ghiso JA (2000) Urokinase receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. Curr Opin Cell Biol 12: 613-620.

Ossowski L, Biegel D, Reich E (1979) Mammary plasminogen activator: correlation with involution, hormonal modulation and comparison between normal and neoplastic tissue. Cell 16: 929-940.

Palmieri D, Lee JW, Juliano RL, Church FC (2002) Plasminogen activator inhibitor-1 and -3 increase cell adhesion and motility of MDA-MB-435 breast cancer cells. J Biol Chem 277: 40950-40957.

Pappot H, Skov BG, Pyke C, Grondahl-Hansen J (1997) Levels of plasminogen activator inhibitor type 1 and urokinase plasminogen activator receptor in non-small cell lung cancer as measured by quantitative ELISA and semiquantitative immunohistochemistry. Lung Cancer 17: 197-209.

Petersen LC, Lund LR, Nielsen LS, Dano K, Skriver L (1988) One-chain urokinase-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. J Biol Chem 263: 11189-11195.

Ploug M, Ronne E, Behrendt N, Jensen AL, Blasi F, Dano K (1991) Cellular receptor for urokinase plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. J Biol Chem 266: 1926-1933.

Plow EF, Freaney DE, Plescia J, Miles LA (1986) The plasminogen system and cell surfaces: evidence for plasminogen and urokinase receptors on the same cell type. J Cell Biol 103: 2411-2420.

Politis I (1996) Plasminogen activator system: implications for mammary cell growth and involution. J Dairy Sci 79: 1097-1107.

Pyke C, Eriksen J, Solberg H, Nielsen BS, Kristensen P, Lund LR, Dano K (1993) An alternatively spliced variant of mRNA for the human receptor for urokinase plasminogen activator. FEBS Lett 326: 69-74.

Reich E (1978) In "Molecular Basis of Biological Degradative Processes" pp. 155-169. New York: Academic Press.

Ridley JH (1968) The histogenesis of endometriosis: A review of facts and fancies. Obstet Gynecol Surv 23: 1-35.

Ridley JH, Edwards IK (1958) Experimental endometriosis in the human. Am J Obstet Gynecol 76: 783-789; discussion 789-790.

Rijken DC, Collen D (1981) Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. J Biol Chem 256: 7035-7041.

Rodgers WH, Matrisian LM, Giudice LC, Dsupin B, Cannon P, Svitek C, Gorstein F, Osteen KG (1994) Patterns of matrix metalloproteinase expression in cycling endometrium imply differential functions and regulation by steroid hormones. J Clin Invest 94: 946-953.

Ryan MP, Kutz SM, Higgins PJ (1996) Complex regulation of plasminogen activator inhibitor type-1 (PAI-1) gene expression by serum and substrate adhesion. Biochem J 314 (Pt 3): 1041-1046.

Rybo G (1966) Plasminogen activators in the endometrium. II. Clinical aspects. Variation in the concentration of plasminogen activators during the menstrual cycle and its relation to menstrual blood loss. Acta Obstet Gynecol Scand 45: 429-450.

Salamonsen LA, Woolley DE (1996) Matrix metalloproteinases in normal menstruation. Hum Reprod 11 Suppl 2: 124-133.

Sampson JA (1927) Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 14: 422-469.

Sandberg T, Casslen B, Gustavsson B, Benraad TJ (1998) Human endothelial cell migration is stimulated by urokinase plasminogen activator:plasminogen activator inhibitor 1 complex released from endometrial stromal cells stimulated with transforming growth factor beta1; possible mechanism for paracrine stimulation of endometrial angiogenesis. Biol Reprod 59: 759-767.

Schafer BM, Maier K, Eickhoff U, Todd RF, Kramer MD (1994) Plasminogen activation in healing human wounds. Am J Pathol 144: 1269-1280.

Sidenius N, Sier CF, Blasi F (2000) Shedding and cleavage of the urokinase receptor (uPAR): identification and characterisation of uPAR fragments in vitro and in vivo. FEBS Lett 475: 52-56.

Sillem M, Prifti S, Monga B, Buvari P, Shamia U, Runnebaum B (1997) Soluble urokinase-type plasminogen activator receptor is over-expressed in uterine endometrium from women with endometriosis. Mol Hum Reprod 3: 1101-1105.

Sillem M, Prifti S, Neher M, Runnebaum B (1998) Extracellular matrix remodelling in the endometrium and its possible relevance to the pathogenesis of endometriosis. Hum Reprod Update 4: 730-735.

Sporn MB, Roberts AB, Wakefield LM, Assoian RK (1986) Transforming growth factor-beta: biological function and chemical structure. Science 233: 532-534.

Spuijbroek MD, Dunselman GA, Menheere PP, Evers JL (1992) Early endometriosis invades the extracellular matrix. Fertil Steril 58: 929-933.

Stahl A, Mueller BM (1997) Melanoma cell migration on vitronectin: regulation by components of the plasminogen activation system. Int J Cancer 71: 116-122.

Stefansson S, Lawrence DA (1996) The serpin PAI-1 inhibits cell migration by blocking integrin alpha V beta 3 binding to vitronectin. Nature 383: 441-443.

Stratton P, Winkel CA, Sinaii N, Merino MJ, Zimmer C, Nieman LK (2002) Location, color, size, depth, and volume may predict endometriosis in lesions resected at surgery. Fertil Steril 78: 743-749.

Strickland S (1978) In "Regulatory Proteolytic Enzymes and Their Inhibitors" pp. 181-185. Oxford: Pergamon.

Strickland S, Richards WG (1992) Invasion of the trophoblasts. Cell 71: 355-357.

Sugiura Y, Ma L, Sun B, Shimada H, Laug WE, Seeger RC, DeClerck YA (1999) The plasminogen-plasminogen activator (PA) system in neuroblastoma: role of PA inhibitor-1 in metastasis. Cancer Res 59: 1327-1336.

Tabibzadeh S (1991) Human endometrium: an active site of cytokine production and action. Endocr Rev 12: 272-290.

Tabibzadeh S (1996) The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodelling. Mol Hum Reprod 2: 77-92.

Tabibzadeh S, Sun XZ (1992) Cytokine expression in human endometrium throughout the menstrual cycle. Hum Reprod 7: 1214-1221.

Tamura M, Fukaya T, Enomoto A, Murakami T, Uehara S, Yajima A (1999) Transforming growth factor-beta isoforms and receptors in endometriotic cysts of the human ovary. Am J Reprod Immunol 42: 160-167.

TeLinde RW, Scott RB (1950) Experimental endometriosis. Am J Obstet Gynecol 60: 1147-1173.

Ueki M (1991) Histologic study of endometriosis and examination of lymphatic drainage in and from the uterus. Am J Obstet Gynecol 165: 201-209.

van der Linden PJ, de Goeij AF, Dunselman GA, Erkens HW, Evers JL (1995) Expression of cadherins and integrins in human endometrium throughout the menstrual cycle. Fertil Steril 63: 1210-1216.

van der Linden PJ, de Goeij AF, Dunselman GA, Erkens HW, Evers JL (1996) Endometrial cell adhesion in an in vitro model using intact amniotic membranes. Fertil Steril 65: 76-80.

Wei Y, Lukashev M, Simon DI, Bodary SC, Rosenberg S, Doyle MV, Chapman HA (1996) Regulation of integrin function by the urokinase receptor. Science 273: 1551-1555.

Wei Y, Waltz DA, Rao N, Drummond RJ, Rosenberg S, Chapman HA (1994) Identification of the urokinase receptor as an adhesion receptor for vitronectin. J Biol Chem 269: 32380-32388.

Whawell SA, Thompson EM, Fleming KA, Thompson JN (1995) Plasminogen activator inhibitor-2 expression in inflamed appendix. Histopathology 27: 75-78.
Wheeler JM (1989) Epidemiology of endometriosis-associated infertility. J Reprod Med 34: 41-46.

Wieser F, Fabjani G, Tempfer C, Schneeberger C, Zeillinger R, Huber JC, Wenzl R (2002) Tumor necrosis factor-alpha promotor polymorphisms and endometriosis. J Soc Gynecol Investig 9: 313-318.

Wild RA, Zhang RJ, Medders D (1994) Whole endometrial fragments form characteristics of in vivo endometriosis in a mesothelial cell co-culture system: an in vitro model for the study of the histogenesis of endometriosis. J Soc Gynecol Investig 1: 65-68.

Witz CA, Allsup KT, Montoya-Rodriguez IA, Vaughn SL, Centonze VE, Schenken RS (2002) Culture of menstrual endometrium with peritoneal explants and mesothelial monolayers confirms attachment to intact mesothelial cells. Hum Reprod 17: 2832-2838.

Witz CA, Cho S, Centonze VE, Montoya-Rodriguez IA, Schenken RS (2003) Time series analysis of transmesothelial invasion by endometrial stromal and epithelial cells using three-dimensional confocal microscopy. Fertil Steril 79 Suppl 1: 770-778.

Witz CA, Thomas MR, Montoya-Rodriguez IA, Nair AS, Centonze VE, Schenken RS (2001) Short-term culture of peritoneum explants confirms attachment of endometrium to intact peritoneal mesothelium. Fertil Steril 75: 385-390.

Wohlwend A, Belin D, Vassalli JD (1987) Plasminogen activator-specific inhibitors produced by human monocytes/macrophages. J Exp Med 165: 320-339.

von Wolff M, Thaler CJ, Strowitzki T, Broome J, Stolz W, Tabibzadeh S (2000) Regulated expression of cytokines in human endometrium throughout the menstrual cycle: dysregulation in habitual abortion. Mol Hum Reprod 6: 627-634.

von Wolff M, Thaler CJ, Zepf C, Becker V, Beier HM, Strowitzki T (2002) Endometrial expression and secretion of interleukin-6 throughout the menstrual cycle. Gynecol Endocrinol 16: 121-129.

Zarmakoupis PN, Rier SE, Maroulis GB, Becker JL (1995) Inhibition of human endometrial stromal cell proliferation by interleukin 6. Hum Reprod 10: 2395-2399.

Zhang RJ, Wild RA, Medders D, Gunupudi SR (1991) Effects of peritoneal macrophages from patients with endometriosis on the proliferation of endometrial carcinoma cell line ECC-1. Am J Obstet Gynecol 165: 1842-1846.